

STANDARD OPERATING PROCEDURES

SECTION 7 – ORGANIC

SECTION 8 – INORGANIC

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

EPA Method 524.2 Rev. 4.3

DETERMINATION OF VOLATILE ORGANIC COMPOUNDS IN WATER BY PURGE AND TRAP AND CAPILLARY COLUMN GAS CHROMATOGRAPHY/ MASS SPECTROMETRY

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1. SUMMARY OF METHOD

EPA method 524.2 is used in the District's monitoring of volatile organic compounds (VOCs) throughout the basin. The method is used as the primary source of data in the analysis of VOCs for the District's main laboratory. Measurement of low levels of VOCs in finished drinking water requires an extensive QA/QC procedure. VOCs are purged from a 20ml sample and trapped onto an absorbent material. This material is then rapidly heated to desorb the VOCs into the system. The column is temperature programmed to separate the target analytes required of the method. Analytes are detected using a mass spectrometer. A data system is used to convert responses into actual concentrations of all analytes. Identification is based on the comparison of the mass spectra and retention time of an unknown to a library of reference mass spectral data of the target analytes. Quantification is based on internal standard. The District's laboratory uses spike samples for additional QA/QC documentation for this method.

2. ANALYTES

- 2.1 This is a gas chromatographic mass spectrometry (GC/MS) method, applicable to the determination of a wide range of volatile organic compounds. The following compounds can be determined using this method:

<u>Lims code</u>	<u>Analyte</u>
BENZ	Benzene
BRBENZ	Bromobenzene
CH2BrC	Bromochloromethane
CHBrCl	Bromodichloromethane
CHBr3	Bromoform
CH3Br	Bromomethane
nBBENZ	n-Butylbenzene
sBBENZ	sec-Butylbenzene
tBBENZ	tert-Butylbenzene
CCl4	Carbon tetrachloride

CLBENZ	Chlorobenzene
CIETHA	Chloroethane
CHCl3	Chloroform
CH3Cl	Chloromethane
2CITOL	2-Chlorotoluene
4CITOL	4-Chlorotoluene
CHBr2C	Dibromochloromethane
DBCP	1,2-Dibromo-3-chloropropane
EDB	1,2-Dibromoethane
CH2Br2	Dibromomethane
12DCB	1,2-Dichlorobenzene
13DCB	1,3-Dichlorobenzene
14DCB	1,4-Dichlorobenzene
CCl2F2	Dichlorodifluoromethane
11DCA	1,1-Dichloroethane
12DCA	1,2-Dichloroethane
11DCE	1,1-Dichloroethene
c-12DCE	cis-1,2-Dichloroethene
t-12DCE	trans-1,2-Dichloroethene
12DCP	1,2-Dichloropropane
13DCP	1,3-Dichloropropane
22DCP	2,2-Dichloropropane
11DCP	1,1-Dichloropropene
c13DCP	cis-1,3-Dichloropropene
t13DCP	trans-1,3-Dichloropropene
EtBENZ	Ethylbenzene
HClBut	Hexachlorobutadiene
ISPBENZ	Isopropylbenzene
4IPTOL	4-Isopropyltoluene
CH2Cl2	Methylene chloride
NAP	Naphthalene
NBENZ	Nitrobenzene
PRBNZ	Propylbenzene
STYR	Styrene
1112PC	1,1,1,2-Tetrachloroethane
1122PC	1,1,2,2-Tetrachloroethane
PCE	Tetrachloroethene
TOLU	Toluene
123TCB	1,2,3-Trichlorobenzene
124TCB	1,2,4-Trichlorobenzene
111TCA	1,1,1-Trichloroethane
112TCA	1,1,2-Trichloroethane
TCE	Trichloroethene
CCl3F	Trichlorofluoromethane
123TCP	1,2,3-Trichloropropane
124TMB	1,2,4-Trimethylbenzene
135TMB	1,3,5-Trimethylbenzene
VNYLCL	Vinyl chloride

o-XYL	o-Xylene
mp-XYL	m,p-Xylene
Cl3F3E	Trichlorotrifluoroethane
TOTALX	Total Xylenes
THMS	Total THMs
MEK	MEK (2-Butanone)
MIBK	MIBK (4-Methyl 2-pentanone)
2ClEVE	2-chloroethyl vinyl ether
B2CLEE	bis(2-Chloroethyl)ether
MTBE	Methyl-t-butyl ether
DIPE	Diisopropyl Ether
TAME	Tert Amyl Methyl Ether
ETBE	Ethyl tert Butyl Ether
TBA	Tert-butyl alcohol
ACETON	Acetone
ACRNTR	Acrylonitrile
CS2	Carbon disulfide
2HEXON	2-Hexanone

(targets in bold represent the custom "EPA-100" mix)

The following analytes do not have a LIMS code but can be analyzed by 524.2.

Allyl chloride
Chloroacetonitrile
trans-1,4-Dichloro-2-butene
1,1-Dichloropropanone
Diethyl ether
Ethyl methacrylate

Methacrylonitrile
Methyl acrylate
Methyl iodide
Methylmethacrylate
2-Nitropropane
Pentachloroethane
Propionitrile
Tetrahydrofuran

3. APPARATUS AND EQUIPMENT

- 3.1 Sample Bottles - 250 ml amber glass bottles & 40 ml amber vials - fitted with an open top screw cap lined with Teflon.
- 3.2 Purge and Trap unit – Varian Archon Autosampler and Tekmar 3000/3100 Purge and Trap. Use a Vocab 4000 trap – "I" trap from Supelco or Vocab 3000 trap – "K" trap from Supelco.

- 3.3 Varian Model 3400/ 3800 gas chromatograph with a (Varian) Saturn-3/ Saturn 2000 GC/MS system.
- 3.4 Column: Fused Silica Capillary column, 60 meter x 0.32 mm ID DB-VRX with 1.8 micron film thickness.
- 3.5 Volumetric flasks (1000ml, 500ml, 200ml, 100ml, and 10ml), and Hamilton micro syringes - 10ul to 250ul.

4. REAGENTS AND CONSUMABLE MATERIALS

- 4.1 Reagent Water - Millipore Milli-Q System or equivalent.
- 4.2 Standard stock VOCs - 200 ug/ml volatile Aromatics and Haloalkanes mix (Ultra Scientific, Accustandard) and a Custom EPA-100 Mix (Accu-std), TBA Custom std mix, Custom - MEK mix MEK, MIBK, B2C1EE, Custom-Oxy std mix - (Ultra Scientific).
- 4.3 Internal and Surrogate 1 & 2 mixture (Ultra Scientific) 2000 ug/ml.
- 4.4 Tune standard - 4-Bromofluorobenzene -diluted to give a 25ng/ul solution. Used to pass EPA tune specifications.
- 4.5 GC² Methanol - Burdick and Jackson.
- 4.6 Ascorbic acid - ACS grade - if source water is chlorinated.
- 4.7 UHP grade Helium carrier gas.
- 4.8 Hydrochloric acid (1+1) - carefully add a measured volume of concentrated HCl to an equal volume of reagent water.

5. SAMPLE COLLECTION, PRESERVATION AND HANDLING

- 5.1 All samples must be collected in four amber 40ml vials while spikes are collected in 250 mL amber bottles. The 40ml vials and the 250ml amber glass bottles should be filled just to overflowing. If the concentration of the Trihalomethanes is important and the sample is known to be from a chlorinated source, ascorbic acid (25mg/40ml vial) must be added to the sample prior to collection. Do not flush out the rapidly dissolving ascorbic acid. Adjust the pH of all samples to <2 by carefully adding two drops of 1:1 HCl to each 40 ml vial of sample. Mix the sample for 1 min. All samples must be chilled to 4°C at the time of collection, and they must be maintained at that temperature until ready for analysis. Samples must be stored

away from all contaminating organic solvent vapors. Total hold time from sample collection to analysis is 14 days. If the samples are not analyzed by this period, they must be discarded. The Water Quality Department must be informed in order to resample the site. A travel blank of the volatile free reagent water must accompany each set of samples brought into the laboratory.

6. ANALYSIS

6.1 Tuning with BFB:

The Saturn 2000 must meet the BFB criteria before analyses are performed. Inject 1.0 ul of a 25ng/ul Bromofluorobenzene (BFB) standard directly into the column. Obtain a background corrected mass spectrum of BFB peak and confirm that all the key M/Z criteria in Table A are achieved. If the tune does not pass, adjust the tune parameters and rerun BFB. Hardcopy the BFB report.

6.2 Calibration Curve:

A three to five point standard calibration curve must be run containing all method analytes, depending upon the concentration range desired. Examples of concentrations used in the curve are: 0.5, 2.0, 5.0, 10.0, 20.0, and 30.0 ppb. You may increase the range of the calibration curve, however a 0.5 ppb standard must be run to confirm the RDL of all analytes. A concentration of 2.0 ppb of the internal is injected into every sample and standard via the Archon/Tekmar purge/trap unit. The recoveries and area counts are tracked to insure a properly running instrument. The standard calibration curve for each analyte must be within a +/- 20% relative standard deviation. If not, the analytes which failed (or the entire calibration curve) should be re-analyzed. Once the calibration curve has been established, it must be verified on each working day by analyzing the continuous calibration check standard. Typically, a standard calibration curve can last approximately 2 months.

6.3 Standard Preparation:

Calibration standards are prepared from separate stock solutions. Three separated calibration mixture are prepared based on the co-elution problems with each other.

1. VOC Cali include: the Aromatic mix, the Haloalkane mix, and the custom MEK mixes are combined into one solution.
2. Oxy Cali include: Oxy mix, TBA and Nitrobenzene.
3. TIC Cali include: Acetone, Acrolein, Acrylonitrile, Carbon disulfide, 2-Hexanone and Vinyl acetate.

The calibration check solution is analyzed at 2.0ppb – it is the second source standard from Supelco. The custom EPA-100 mix with MTBE added is used for spikes, low and high LFB check solutions – contains 20 targets which are both

regulated by the state and have been detected within the District's basin. Working standards shall be made up in batches and verified against the working calibration. Working standards are valid for 14 days from the day they are made. Stock standards are good for one month or when QA/QC data shows they need to be replaced.

Preparing internal and surrogate standards for the Archon:

The internal, surrogate standard mix from Ultra Scientific – is at 2000 ug/mL. Add 250uL of this to a 10 ml volumetric flask containing GC grade methanol. Bring to a final volume of 10mls of GC² methanol. We have found that this internal is stable and does not interfere with any of the other targets.

Baking the column:

Bake the column whenever any changes are made to the system that introduces air into the system such as cutting the ends of the column or installing a new column, or any work done on the Archon autosampler or Tekmar purge and trap. The system – both purge & trap and the GC/MS, should be periodically baked to remove water vapor and organic interferences.

Notes:

1. Scan numbers of the key targets and the internal standard should be documented so as to monitor the life of the column. The column should be replaced when resolution has dropped below an acceptable level. The early gases are good indicators of the column's age and performance.
2. Data is collected for each run under specific file names within the software system. Mass spectral data are obtained with electron impact (EI) ionization at 70 eV electron energy. For samples that have ion abundance over the system's working range, a dilution with reagent water is necessary. Tentatively identify samples by comparison of mass spectrum (after background subtraction) to a reference spectrum in a user library. Ions above 10% relative abundance in the mass spectrum of the standard must be present in the spectrum of the component and should agree within absolute 10%. The GC retention time of the sample component must be within 10 scans of the time observed for that same compound when a calibration solution was analyzed.
3. Samples should be analyzed as soon as possible after collection –but have a 14 day holding time. Communication between the lab and the water quality department is important to understand each sample. There may be specific conditions or problems associated with each sample – an example would be frothing or very high levels. The more a chemist knows about the sample, the better he or she can provide quality assurance and processes, which can produce reliable results. If data shows that the

value of the result is outside the calibration range -- the sample must be diluted or additional standards analyzed to bracket the value within +/-20% of the value.

TABLE A

BFB KEY M/Z ABUNDANCE CRITERIA

MASS	CRITERIA
50	15 to 40% of mass 95
75	30 to 80% of mass 95
95	base peak, 100% relative abundance
96	5 to 9% of mass 95
173	<2% of mass 174
174	>50% of mass 95
175	5 to 9% of mass 174
176	>95% but <101% of mass 174
177	5 to 9% of mass 176

In order to achieve proper results, a system must be within target analyte contamination or interferences. To this goal, it is mandatory that both a travel blank and a reagent water blank be run with each set of samples. Conditions for the GC/MS system are as follows:

MS and GC Conditions:

- | | | | | |
|-----|-----------------------------|---------------------------------|-----------|----------|
| 1. | Initial column temperature: | 35°C | | |
| 2. | Hold time: | 2 minutes | | |
| 3. | Final temperature: | 82°C | 178°C | 220°C |
| 4. | Rate: | 4°C/min | 4.5°C/min | 50°C/min |
| 5. | Hold time: | 0 min. | 0 min. | 5 min. |
| 6. | Helium flow rate: | 1.0 ml/minute | | |
| 7. | Total run time: | 41 minutes | | |
| 8. | Head pressure: | 5 PSI | | |
| 9. | Injector temperature: | 220°C | | |
| 10. | Transfer line GC/MS: | 220°C | | |
| 11. | Trap GC/MS: | 150°C | | |
| 12. | Manifold GC/MS: | 80°C | | |
| 13. | Total scan time | 0.7 seconds | | |
| 14. | Mass range: | 46 to 260 AMU and 35 to 260 AMU | | |
| 15. | Fil/Mult delay: | 3.00 minute | | |

Tekmar ALS/3000/3100 Purge and Trap Conditions:

- | | | |
|----|-------------|---------------|
| 1. | Purge Time: | 11.00 minutes |
| 2. | Bake Time: | 10.00 minutes |
| 3. | Pre-Heat: | 245°C |
| 4. | Desorb: | 250°C |
| 5. | Bake: | 260°C |

Archon Conditions:

- | | | |
|----|--------------------|----------|
| 1. | Internal Standard: | 1 ul |
| 2. | Sample Volume | 20 ml |
| 3. | Desorb Time: | 1 minute |
| 4. | Syringe Flush: | 2 times |
| 5. | Rinse Volume: | 10 ml |

7. QA/QC REQUIREMENTS

- 7.1 Laboratory Reagent Blank - Run before samples. Use to demonstrate that all glassware and reagent interference are under control. If any contamination peaks are produced, determine source of contamination and eliminate interference.
- 7.2 Laboratory Fortified Blank - The fortification concentration of each analyte should confirm the ability to detect at the reportable level. If the recovery of any analyte falls outside the control limits, +/-30%, the source of the problem must be identified and corrected.
- 7.3 Standards - Verify calibration standards quarterly by analyzing a standard prepared from reference material obtained from an independent or second source. EPA performance evaluations are an excellent process to determine the validity of the method. Results must be within +/- 30% of those used to routinely check calibration. Daily, run a low level standard to check the reportable detection level, RDL. % RSD of each calibration curve should be less than 20%. If one or more calibration curve has more than 20% RSD, re-integrate the peak and verify peak integration. If the problems are not solved by reprocess, rerun new calibration curve using freshly made standards to meet the 20% requirement.
- 7.4 Samples - Samples must be analyzed within 14 days after collection. Samples must be stored at 4°C or below until ready for analysis. Duplicates are run on 5% of samples, or once during run, whichever is greater. Results should be within +/- 20%.
- 7.5 Spike Recoveries - The laboratory must add a known concentration of spike solution, the same as used for LFB, to at least 5% of samples or once per analytical run, whichever is greater. Recoveries should be within the acceptable range, +/- 30%. Wherever possible, run a second source standard for spikes.
- 7.6 QC Requirements - Analyze EPA QC check sample with known values if available. The results for each analyte must be within the EPA acceptance criteria. Semi-annually, analyze EPA Performance Evaluation samples. Analyze additional check samples whenever major maintenance to the system occurs to ensure the validity of the method.
- 7.7 Continuous Calibration Check Standard: Daily analyze a 2 ppb

continuous calibration check standard. Also, confirm the RDL. The concentration measured using the calibration curve must be within $\pm 30\%$ of the true value of the concentration in the calibration solution. If this condition is not met, recalibration may be required.

- 7.8 If samples fail any of the above QC requirements, resample request will be followed to re-analyze the sample. Also verify tuning compound, BFB and proceed to the system diagnostics to investigate any malfunction of the system

8. PREVENTIVE MAINTENANCE AND CORRECTIVE ACTIONS

- 8.1 Glassware - Glassware must be carefully cleaned. Do not heat volumetric glassware above 220°C.
- 8.2 Reagents - The use of high purity solvents and reagents will help to minimize contamination problems.
- 8.3 Carryover - Contamination carryover may occur when a sample containing a low concentration of analytes is analyzed immediately following a sample containing a high concentration. Use volatile free reagent water rinses between samples to minimize carryover.
- 8.4 All reagents and apparatus must be routinely demonstrated to be free from interference under the conditions of the analysis by running laboratory method blanks. Minimize contact of the samples and reagents with solvent vapors (methylene chloride). This will help reduce contamination.
- 8.5 A refrigerator blank should be run at least once a month. This blank, volatile free reagent water, is sealed in a 40 ml vial and placed in the VOC storage refrigerator for one month. Analyzed each month, it should be free of any organic contamination. Freons are the most likely interference to be picked up by this blank.
- 8.6 Record all corrective actions in the maintenance log book. Include a complete description of the problem and what action was taken to correct it.

SOP PROCEDURE CHANGE

For EPA Method 524.2

<u>CHANGE</u>	<u>REASON</u>	<u>DATE</u>	<u>INITIALS</u>
--GC program	Shorter run time and better separation for some targets at the end.	8/2004	LV
--Change of sample volume from 25 ml to 20 ml.		03/06/08	GGA
--Addition of reported analytes.	Update target list	04/17/09	GGA
--Addition of LIMS codes, added additional analytical trap, and changed Archon conditions.	Update analyte list, equipment used, and Archon settings.	04/17/09	GGA

ORANGE COUNTY WATER DISTRICT

EPA METHOD 524.2

PREPARATION OF STANDARDS AND QUALITY CONTROLS

CALIBRATION STANDARD MIX: (See Next Page)

2ppb CHECK STANDARD & 2nd SOURCE STANDARD: (See Next Page)

SAMPLE SPIKING:

Spike 200 ml sample with 2 µl of Custom EPA 100 Conc. - 2.0 µg/L Low Spike

***INTERNAL & SURROGATE (1&2) STANDARD:

Internal Standard Mix 524 Ultra Sci. = 2,000 µg/ml STS - 160
(Fluorobenzene)

Dilute 250 µl of stock in 10 ml Methanol - conc. = 1000 µg/L

BFB TUNNING

Stock: 2000 ug/mL in MeOH

Working Std - dilute 12.5 ul of Stock to 1mL MeOH

524 Calibrations (in 200ml)

VOC CALI

Stock Conc.- Ultra Sci	ARO (200ug/ml)_DWM-570	HALO (200ug/ml)_DWM-540	MEK (200ug/ml)_CUS-3154
A - 0.5ppb	0.5 ul	0.5 ul	2.5 ul
B - 2ppb	2 ul	2 ul	10 ul
C - 5ppb	5 ul	5 ul	25 ul
D - 10ppb	10 ul	10 ul	50 ul
E - 20ppb	20 ul	20 ul	100 ul
F - 30ppb	30 ul	30 ul	150 ul

OXY CALI

Stock Conc.- Ultra Sci	OXY (200ug/ml)_CUS-3153	TBA (200ug/ml)_CUS-2298	NBENZ (100ug/ml)_NAI-130
A - 0.5ppb	0.5 ul	2 ul	10 ul
B - 2ppb	2 ul	5 ul	20 ul
C - 5ppb	5 ul	10 ul	50 ul
D - 10ppb	10 ul	20 ul	100 ul
E - 20ppb	20 ul	40 ul	200 ul
F - 30ppb	30 ul	-----	-----

TIC CALI

Stock Conc.- Ultra Sci	TIC (200ug/ml)_CUS-10327	Vinyl Acetate (200ug/ml)_CUS-10326	ACRQ (100ug/ml)_AMN-603
A - 10ppb	10 ul	10 ul	20 ul
B - 20ppb	20 ul	20 ul	40 ul
C - 40ppb	40 ul	40 ul	80 ul
D - 80ppb	80 ul	80 ul	160 ul

524 QC Standards (stock std conc. : 200ug/ml & MTBE AT 100 ug/ml)
 (*Various concentrations for Oxy Mix (Spex) 2nd source)

Low MTBE	0.2 ppb	500 mL
	MTBE (Ultra Sci, NV-250-1,)	1 ul

VOC	2ppb	1L
	Supelco VOC Mix, (4-7932)	10 ul
	MEK (Ultra Sci, CUS-3154)	50 ul

RDL	0.5ppb	1L
	Supelco VOC Mix, (4-7932)	2.5 ul
	MEK (Ultra Sci, CUS-3154)	12.5 ul

OXY/TBA	1 / 2ppb	500mL
	OXY Mix (Spex_XQ-4347)*	5 ul

TIC	10ppb	500mL
	VINYL ACETATE (SPEX_S-3800-200)	25 ul
	TIC (SPEX_XQ-4091)	25 ul
	ACRQ (SPEX_XQ-4092)	25 ul

EPA 100	10ppb	1L
	EPA Custom Mix w/ MTBE	50 ul
	Accustandard, (S-6259-R2)	

Low Spike	2ppb	200mL Sample
	EPA Custom Mix w/ MTBE	2 ul
	Accustandard, (S-6259-R2)	

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

EPA Method 525.2, Rev. 2.0

DETERMINATION OF ORGANIC COMPOUNDS IN WATER BY LIQUID-SOLID EXTRACTION AND CAPILLARY COLUMN GAS CHROMATOGRAPHY/ MASS SPECTROMETRY

File Name: M:\SOP\Organic\Epa Method SOP\525_113006.doc Effective Date: 11/30/2006
Revision: 5 Supersedes: 4 (7/6/2006)

1. SUMMARY OF METHOD

A measured volume of sample of approximately 1 liter is extracted using a C18 disk. Manual SPE is used. The organic compounds are eluted from the disk with small quantities of ethyl acetate and methylene chloride. The extract is dried with sodium sulfate and concentrated to 1 ml using the Zymark concentrator - using gentle heat and UHP nitrogen gas. The final extract is injected into a GC/MS system for separation, identification and quantitation. Internal and surrogate standards are added to every sample. The method is applicable to a wide range of organic compounds. This method is used primarily as a conformational tool. Results should confirm both qualitative as well as quantitative data from specific detector methods.

2. ANALYTES

2.1 This is a gas chromatographic mass spectrometry (GC/MS) method, applicable to the determination of a wide range of organic compounds. The following compounds can be determined using this method. They are separated based on the Calibration Standards used for this method – Ultra Scientific:

Semi- Volatiles – M-525.2-SV-ASL Analytes (@ 100 ug/ml in acetone)

<u>Target</u>	<u>MW</u>	<u>Quan Mass</u>	<u>CAS#</u>	<u>LIMS ID</u>
Acenaphthylene	152	152	208-96-8	ACENAP
Anthracene	178	178	120-12-7	ANTHRA
Benz[a]anthracene	228	228	56-55-3	BaANTH
Benzo[b]fluoranthene	252	252	205-82-3	BbFLUR
Benzo[ghi]perylene	276	276	191-24-2	BghiPR
Benzo[a]pyrene	252	252	50-32-8	BaPYRE
Butyl Benzyl phthalate	312	149	85-68-7	BBP
2-chlorobiphenyl	188	188	2051-60-7	CBP
Chrysene	228	228	218-01-9	CHRYN
Dibenz[a,h]anthracene	278	278	53-70-3	DBahAN
2,3-dichlorobiphenyl	222	222/152	16605-91-7	DCBP
Bis(2-ethylhexyl) adipate	370	129	103-23-1	DEHA

Bis(2-ethylhexyl) phthalate	390	149	117-81-7	DEHP
Diethyl phthalate	222	149	84-66-2	DEP
Dimethyl phthalate	194	163	131-11-3	DMP
Di-n-butyl phthalate	278	149	84-74-2	DnBP
2,4-dinitrotoluene	182	165	121-14-2	24DNT
2,6-dinitrotoluene	182	165	606-20-2	26DNT
Fluorene	166	166	86-73-7	FLUOR
Hexachlorobenzene	282	284	118-74-1	HEXCLB
2,2',4,4',5,6'-hexachlorobiphenyl	358	360	60145-22-4	HCBP
2,2',3,3',4,4',6-heptachlorobiphenyl	392	394/396	52663-71-5	HPCIBP
Hexachlorocyclopentadiene	270	237	77-47-4	HCICPD
indeno [1,2,3-cd] pyrene	276	276	193-39-5	INDPYR
Isophorone	138	82	78-59-1	IPHOR
2,2',3,3',4,5',6,6'-octachlorobiphenyl	426	430/428	40186-71-8	OCBP
2,2',3',4,6-pentachlorobiphenyl	324	326	60233-25-2	PCBP
pentachlorophenol (400 ug/ml)	264	266	87-86-5	PCP
Phenanthrene	178	178	85-01-8	PHENAN
Pyrene	202	202	129-00-0	PYRENE
2,2',4,4'-tetrachlorobiphenyl	290	292	2437-79-8	TECBP
2,4,5-trichlorobiphenyl	256	256	15862-07-4	TCBP

Organochlorine Pesticides – M-525.2-CP-ASL 29 Analytes (@ 100 ug/ml in acetone)

<u>Target</u>	<u>MW</u>	<u>Quan Mass</u>	<u>CAS#</u>	<u>LIMS ID</u>
Alachlor	269	160	15972-60-8	ALACHL
Aldrin	362	66	309-00-2	ALDRIN
Atrazine	215	200/215	1912-24-9	ATRAZ
α-BHC	288	181	319-84-6	BHCa
β-BHC	288	181	319-85-7	BHCb
γ-BHC	288	181	58-89-9	LINDNE
ζ-BHC	288	181	319-86-8	BHCd
Chlorobenzilate	324	139	510-15-6	CLBZLA
Chlorothalonil	264	266	1897-45-6	CLTNIL
Chloroneb	206	191	2675-77-6	CLNEB
Dacthal (DCPA)	330	301	1861-32-1	DCPA
4,4'-DDD	318	235/165	72-54-8	DDD
4,4'-DDT	352	235/165	50-29-3	DDT
4,4'-DDE	316	246	72-55-9	DDE
Dieldrin	378	79	60-57-1	DIELDR
Endosulfan I	404	195	959-98-8	ENDOI
Endosulfan II	404	195	33213-65-9	ENDOI
Endosulfan sulfate	420	272	1031-07-8	ENDOSL
Endrin	378	67/81	72-20-8	ENDRIN
Endrin Aldehyde	378	67	7421-93-4	ENDR-A
Etridazole	246	211/183	2593-15-9	ETRDZL
α-Chlordane	406	375/373	5103-71-9	CLDA
γ-Chlordane	406	373	5103-74-2	CLDG
Heptachlor	370	100	76-44-8	HEPTA
Heptachlor Epoxide (B)	386	81	1024-57-3	HEPEPX
Methoxychlor	344	227	72-43-5	METHOX
Permethrin, mixed isomers (200ug/ml)	390	183	c54774-45-7 t51877-74-8	PMTHRN
Simazine	201	201/186	122-34-9	SIMAZ
Trans-nonachlor	440	409	39765-80-5	t-NONA

Nitrogen/Phosphorus Pesticides – M-525.2-NPI-ASL 40 Analytes (@ 100 ug/ml in acetone)

<u>Target</u>	<u>MW</u>	<u>Quan Mass</u>	<u>CAS#</u>	<u>LIMS ID</u>
Alachlor	269	160	15972-60-8	ALACHL
Atraton%	211	196/169	1610-17-9	ATRATN
Atrazine	215	200/215	1912-24-9	ATRAZ
Bromacil	260	205	314-40-9	BROMAC
Butachlor	311	176/160	23184-66-9	BUTACL
Butylate	217	57/146	2008-41-5	BTYATE
Chloropropham	213	127	101-21-3	CPRPHM
Chloropyrifos	349	197/97	2921-88-2	CIPYRI
Cycloate	215	83/154	1134-23-2	CYCATE
Cyfluthrin	240	225/68	21725-46-2	CYANAZ
Dichlorvos	220	109	62-73-7	DCLVOS
Diphenamid	239	72/167	957-51-7	DPHNMD
EPTC	189	128	759-94-4	EPTC
Ethoprop	242	158	13194-48-4	ETHPRP
Fenarimol	330	139	60168-88-9	FNAIML
Fluridone	328	328	59756-60-4	FLRDNE
Hexazinone	252	171	51235-04-2	HEXZON
Methyl paraxon	247	109	950-35-6	MPRXON
Metolachlor	283	162	51218-45-2	METOCL
Mevinphos	224	127	7786-34-7	MVNPHS
MGK-264, mixed isomers	275	164/66	113-48-4	MGK264
Molinate	187	126	2212-67-1	MOLINT
Napropamide	271	72	15299-99-7	NPRMDE
Norflurazon	303	145	27314-13-2	NORFLR
Pebulate	203	128	1114-71-2	PBUATE
Prometon%	225	225/168	1610-18-0	PROMTN
Prometryn	241	241/184	7287-19-6	PROMET
Pronamide	255	173	23950-58-5	PROAMD
Propachlor	211	120	1918-16-7	PROPCL
Propazine	229	214/172	139-40-2	PROPAZ
Simetryn	213	213	1014-70-6	SIMETY
Stirofos	364	109	22248-79-9	STRFOS
Tebuthiuron	228	156	34014-18-1	TBTURN
Terbacil	216	161	5902-51-2	TRBACL
Tertbutryn	241	226/185	886-50-0	TRBURN
Triadimefon	293	57	43121-43-3	TRDMFN
Tricyclazole	189	189	41814-78-2	TRCZOL
Trifluralin	335	306	1582-09-8	TRFLRN
Vernolate	203	128	1929-77-7	VRNLTE
Ametryn	227	227/170	834-12-8	AMERYN

Nitrogen/Phosphorus Pesticides – M-525.2-NP2-ASL 6 Analytes (@ 100 ug/ml in acetone)

<u>Target</u>	<u>MW</u>	<u>Quan Mass</u>	<u>CAS#</u>	<u>LIMS ID</u>
Carboxin	235	143	5234-68-4	CRBXIN
Diazinon	304	137/179	333-41-5	DIAZI
Disulfoton	274	88	298-04-4	DISLFN
Fenamiphos	303	303/154	22224-92-6	FNAPHS
Merphos	298	209/153	150-50-5	MERPHS
Terbufos	288	57	13071-79-9	TRBFOS

Custom Mix (Accustandard) – S-12600 3 Analytes (@ 100 ug/ml in acetone

<u>Target</u>	<u>MW</u>	<u>Quan Mass</u>	<u>CAS#</u>	<u>LIMS ID</u>
Captan	299	79	133-06-2	CAPTAN
Ethion	384	231	563-12-2	ETHION
Trithion (Carbofenotion)	342	157	786-19-6	TRTION

Custom Mix (Restek) – 557399 5 Analytes (@ 100 ug/ml in acetone

<u>Target</u>	<u>MW</u>	<u>Quan Mass</u>	<u>CAS#</u>	<u>LIMS ID</u>
Acetochlor	269	146/224	34256-82-1	ACETOC
Caffeine	194	194	58-08-2	CAFFEI
Dimethoate	229	125 / 87	60-51-5	DMTH
Terbufos Sulfone	320	199	56070-16-7	TERSUL
Thiobencarb	257	100 / 72 / 125	28249-77-6	THIO

Di-n-octylsebacate	342	149 / 167 / 108	2743-50	DIOL
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1 **1** – if this target is to be reported - a separate sample must be collected – which does not contain either of the preservatives – sodium sulfite or the 1+1 HCl. This must be scheduled with the water quality department under special sample conditions and arrangements. This requires pre-notification and arrangements of reporting formats within the LIMS system.

% **Atraton** and **Prometon** are not extracted efficiently from water at pH of <2. In order to report these targets a separate bottle with just sodium sulfite – no 1+1 HCl addition. This must be scheduled with the water quality department under special sample conditions and arrangements. This requires pre-notification and arrangements of reporting formats within the LIMS system.

QA/QC Targets

Targets are separated based on Calibration standards – Accustandard

GC/MS Calibration Tune Standard – IST-341-1 1 Analytes (@ 100 ug/ml in Methylene Chloride) – Ultra Scientific

Decafluorotriphenylphosphine (DFTPP)

GC/MS Performance Check Solution – GCM-160-1 3 Analytes (@ 1000 ug/ml in Acetone) – Ultra Scientific

Decafluorotriphenylphosphine (DFTPP)

Endrin

4,4'-DDT

GC Performance Check Solution – Custom Mix 4175 – Ultra Scientific

Anthracene

Phenanthrene

Ben(a) anthracene

Chrysene

Internal & Surrogate Standards – ISM-510 7 Analytes (@ 500 ug/ml in Acetone)

Acenaphthene-d10

Phenanthrene-d10

Chrysene-d12

1,3-dimethyl-2-nitrobenzene

Perylene-d12

Triphenylphosphate

Pyrene-d10

Fortification Solution – ATS-161-1 1 Analyte (@ 500 ug/ml in Methylene Chloride) – Ultra Scientific

p-terphenyl-d14

3. APPARATUS AND EQUIPMENT

- 3.1 Sample Bottles – Two-liter amber glass bottles fitted with a screw cap lined with teflon.
- 3.2 Autosampler vials - equipped with Teflon-lined septum.
- 3.3 Concentrator Tube - Zymark 250 mL tubes used with the Zymark Turbo-Vap.
- 3.4 Analytical Balance: Capable of weighing accurately to nearest 0.0001 g.
- 3.5 Zymark Turbo-Vap - used to concentrate extracts.
- 3.6 3800 Varian gas chromatograph with a Saturn Ion Trap Mass Spectrometer and a data workstation. Equipped with an 8400 autosampler for injecting samples into the GC.
- 3.7 Column: Fused Silica Capillary column, Varian – Factor Four VF-5MS + 5M EZ Guard: 30 meters long x 0.25 mm I.D. with a 0.25 micron film thickness, with 5 meters of guard column.
- 3.8 Disposable pasteur pipets, graduated cylinders (1000ml, 100ml, and 10ml), and Hamilton micro syringes - 10 ul to 100 ul.

4. REAGENTS AND CONSUMABLE MATERIALS

- 4.1 Reagent Water - Millipore Milli-Q System or equivalent.
- 4.2 Methylene Chloride, Ethyl Acetate, Acetone: Burdick & Jackson - capillary GC² solvent.
- 4.3 Hydrochloric Acid: ACS reagent grade 6N.
- 4.4 Methanol: Burdick & Jackson - capillary GC² solvent.
- 4.5 Sodium Sulfate: (ACS) Granular, anhydrous. Heat sodium sulfate at 400°C four hours, and store sodium sulfate at 130°C.
- 4.6 Sodium sulfite, anhydrous : ACS grade.
- 4.7 Empore disks: 0.5 grams of 8 um octadecyl bonded silica in 47 mm x 0.5 mm disk.
- 4.8 Helium carrier gas: UHP grade. Liquid carbon dioxide: technical grade.
Nitrogen gas: UHP grade.

5. SAMPLE COLLECTION, PRESERVATION AND HANDLING

- 5.1 Samples are collected in amber 2-liter amber bottles, fitted with a screw cap lined with teflon. Keep samples sealed from collection time until analysis. Sampling equipment must be free of plastic tubing, gaskets, and other parts that may leach interfering analytes into the water sample.
- 5.2 All samples should be iced or refrigerated at 4 °C and kept in the dark from the time of collection until extraction. Residual chlorine should be reduced at the sampling site by addition of 200 mg of sodium sulfite to each sample. After addition to the sample, seal the bottle and shake for one minute. The sample must be dechlorinated prior to adding acid to lower the pH of the sample. Adding sodium sulfite and HCl to the sample bottles prior to shipping to the sampling site is not permitted. Hydrochloric acid should be used at the sampling site to retard the microbiological degradation of some analytes in water. The sample pH is adjusted to <2 with 1+1 HCl at the field. Store all samples at 4°C until ready for extraction. All samples must be extracted within 14 days after collection (see 5-4 below for special targets). The extract should be analyzed within 30 days after sample extraction.
- 5.3 Not all targets can be determined with the same preservation techniques and reagents. Cyanazine, Atraton, and Prometon all require special sampling and reporting procedures. These targets are not normally reported by this method, but may be requested under special circumstances. Cyanazine – if this target is to be reported - a separate sample must be collected – which does not contain either of the preservatives – sodium sulfite or the 1+1 HCl. This must be scheduled with the water quality department under special sample conditions and arrangements. This requires pre-notification and arrangements of reporting formats within the LIMS system. Atraton and Prometon are not extracted efficiently from water at pH of <2. In order to report these targets a separate bottle with just sodium sulfite – no 1+1 HCl addition. This must be scheduled with the water quality department under special sample conditions and arrangements. This requires pre-notification and arrangements of reporting formats within the LIMS system.
- 5.4 If the following analytes are to be determined, the samples cannot be held for 14 days but must be extracted immediately after collection and preservation: Carboxin, diazinon, disulfoton, disulfutonsulfoxide, fenamiphos, and terbufos. These targets are not normally reported by this method, but may be requested under special circumstances. This must be scheduled with the water quality department under special sample conditions and arrangements. This requires pre-notification and arrangements of reporting formats within the LIMS system.
- 5.5 The processing of a field blank (travel blank - TB) is recommended along with each sample set. A sample bottle for the TB is filled at the laboratory, with the same preservation reagents added, and shipped to the site(s) along with the other sample bottles. The TB is processed in the same manner as the samples.

6. EXTRACTION

- 6.1 Check pH of samples. If sample pH is > 2, request a request.
- 6.2 Assemble C-18 Empore disk and filtration apparatus.
- 6.3 Add 10 mL of acetone to the disk. Apply low vacuum, and let disk soak for 3 minutes, then apply vacuum to remove the solvent completely.
- 6.4 Repeat the above step with 5 ml of a 1:1 mixture of ethyl acetate and methylene chloride
- 6.5 Apply 10 mL of methanol and allow it to soak into the disk for 3 minutes. Do not allow the disk to run dry until the end of the extraction step.
- 6.6 Add 10 ml of DI water and soak for 3 minutes.
- 6.7 Then add 1 liter of sample into the filtration reservoir and apply vacuum (actual sample volume is 995-ml and 5-ml of methanol). Inject surrogate standard and spike standards. Process sample.
- 6.8 After the sample is processed, pull air through disk for 7 minutes to remove residual water from the disk.
- 6.9 Remove the filter base and place the vial into the receiver. Add 5 ml of ethyl acetate to the reservoir and allow disk to soak for 3 minutes before applying vacuum.
- 6.10 Add 5 ml of methylene chloride to the reservoir and allow to disk soak for 3 minutes before applying vacuum.
- 6.11 Add 5 ml of 1:1 ethyl acetate:methylene chloride mixture to the reservoir and allow to soak for 3 minutes before applying vacuum. Dry disk completely. Wait 15 minutes before proceeding. Make certain the extract is dry.
- 6.12 Transfer extract to a Turbo-Vap concentrator tube, rinsing the collection vial with two 3 ml portions of 1:1 ethyl acetate and methylene chloride.
- 6.13 Evaporate the eluant with a Turbo-Vap concentration workstation to a volume of 1.0 mL. Inject 10ul of 500ug/ml recovery standard solution terphenyl-d14 (5 ppb) to monitor the recovery of the internal standards in the extraction process. Transfer to 2 GC autosampler vials.

7. ANALYSIS

- 7.1 The first step for the instrumental analysis of this method is the mass calibration of the system. Inject into the GC/MS system a 1ul aliquot of the 5 ng/ul solution of DFTPP, Endrin, and 4,4'-DDT. The Endrin and DDT degradation checks may be performed simultaneously with the DFTPP check or in a separate injection. Acquire a mass spectrum that includes data for m/z 45-450. The DFTPP mass spectrum must meet all the criteria in Table 1. A single spectrum or an average spectrum across the GC peak may be used to evaluate the performance of the system. This criterion must be met for each day's analytical run. If the system repeatedly fails the criteria – retune the system until it passes.
- 7.2 Verify that the Endrin and DDT degradation products are within acceptable limits: <20% degradation. Fill out the system performance daily checklist and file for reference. A copy will be included in the raw data packet. If the degradation for these targets are greater than the acceptable level – system maintenance must be performed – usually this will mean replacement of GC injection port liner – Siltek

liner.

- 7.3 Once the system's daily performance checks have passed, inject a midlevel standard STD-D (2.0 ppb). This standard must meet all of the following criteria:
- Anthracene and Phenanthrene should be separated by baseline.
 - Benz [a]anthracene and chrysene should be separated by a valley - whose height is less than 25% of the average peak height of these two compounds.
 - The GC/MS software system should be able to identify each of the compounds in the CCC. >99% of the compounds should be identifiable.
- 7.4 Initial Calibration: A six point calibration curve is required for this method; using standards STD-A (0.1ppb), B (0.5ppb), C (1.0ppb), D (2.0ppb), E (5.0ppb), F (10.0ppb). At least five calibration curve points must be utilized for each target compound. For each analyte and surrogate, calculate the mean RF and the relative standard deviation (RSD). The %RSD of any analyte or surrogate within the calibration range must be less than 30%. If not – inject other standards or perform system maintenance and begin the system analysis and performance check over.
- 7.5 Continuous Calibration check: Check the system tune and performance check for each 12-hour work shift. A six point calibration curve is required for this method; using standards STD-A (0.1ppb), B (0.5ppb), C (1.0ppb), D (2.0ppb), E (5.0ppb), F (10.0ppb). At least five calibration curve points must be utilized for each target compound. For each analyte and surrogate, calculate the mean RF and the relative standard deviation (RSD). The %RSD of any analyte or surrogate within the calibration range must be less than 30%. If not – inject other standards or perform system maintenance and begin the system analysis and performance check over.
- EPA method 525 is used to monitor for low-level organics in ground water. Because of these low levels, any type of contamination or interference can cause analytical problems. Thus, reagent blanks must be monitored for every extraction run - monitoring of reagent blanks is essential to the success of this method. If results for reagent blanks rise above this level corrective actions must be performed. Analyze a 6-point calibration once a month and the average response factor is used to quantify the unknowns (0.1, 0.5, 1.0, 2.0, 5.0, and 10.0 ppb). Toxaphene standards must be run at higher levels - 2.0, 5.0, 10.0, 20.0, 50.0, and 100 ppb. Verify the calibration by measurement of two mid-point calibration check standards, one at the beginning and one at the end of the run. The check standard area counts must be within +/- 30% of the standard curve. LFBs (laboratory fortified blanks) should also be analyzed with each extraction run. Standards used for these QA/QC samples must be ordered from a second source whenever possible. For extended runs, check standards should be interspersed with samples at regular intervals.

If the response of any analyte varies from the predicted response by more than +/- 20%, test must be repeated using fresh calibration standards. Data is collected and processed by Saturn GC/MS software. A calibration file must be created by analyzing the six point calibration curve and entering these values into the cali file. An unknown is identified by comparing its mass spectra with the NIST92 library. Its purity must exceed 800 for positive identification. Any results above the highest calibration standard must be confirmed with a standard that is within +/- 20% of the actual result or the sample must be diluted to within the calibration range.

GC Conditions:

- | 1. | Seg | Temp | Rate | Hold Time | Total |
|----|-----|------|------|-----------|-------|
| | 1 | 35 | 0 | 0.5 | 0.5 |
| | 2 | 120 | 30 | 1.0 | 4.33 |
| | 3 | 174 | 8 | 1.0 | 12.08 |
| | 4 | 200 | 6 | 1 | 17.42 |
| | 5 | 216 | 5 | 0.5 | 21.12 |
| | 6 | 270 | 6 | 1.0 | 31.12 |
| | 7 | 295 | 5 | 5 | 41.12 |
- Helium linear velocity: 30 cm/sec
 - Splitless injection with 3.5 minute delay
 - Injector temperature (1079): Initial 40°C, then 40°C to 300°C @ 100°C/minute hold for 25.00 minutes
 - Transfer Line Temp: 280 °C
 - 2 ul sample is injected at 10 ul/sec

MS Conditions :

- | | | |
|-----|--------------------|---------------|
| 1. | Mass range : | 45 to 450 amu |
| 2. | Seconds/scan : | 0.800 |
| 3. | Acquire time : | 41.12 minutes |
| 4. | Fil/Mul delay : | 5 minutes |
| 5. | Peak threshold : | 1 count |
| 6. | Mass defect : | 0 mmn/100 amu |
| 7. | Background mass : | 45 amu |
| 8. | Ionization mode : | EI |
| 9. | Auto ion control : | ON |
| 10. | Cal gas : | OFF |

5 ng of DFTPP is injected (1ul of a 5ug/ml solution) before every run into the GC/MS to determine whether the MS is "in tune". Using the GC method DFTPP, MS method DFTPP and AS method DFTPP, the ion abundance of the mass spectra of DFTPP can be compared to the following set of criteria.

Mass

Relative Abundance Criteria

54	10-80% of the base peak
68	<2% of mass 69
70	<2% of mass 69
127	10-80% of the base peak
197	<2% of mass 198
198	base peak of >50% of 442
199	5-9% of mass 198
275	10-60% of the base peak
365	>1% of the base peak
441	Present and < mass 443
442	base peak of >50% of 198
443	15-24% of mass 442

EnviroPro software is used to determine whether the ion abundance criteria is met. Use file EP to get into EnviroPro and type in the file name, type the correct forms output, 6B, and the califile DFTPP. From the main menu type R for run procedure and DFT525 to get the printout of the relative ion abundance of the DFTPP injection. This printout is kept with each data package.

8. QA/QC REQUIREMENTS

- 8.1 Laboratory Reagent Blank - Run before samples. Use to demonstrate that all glassware and reagent interferences are under control. If any contamination peaks are produced, determine source of contamination and eliminate interference.
- 8.2 Laboratory Fortified Blank - Must analyze at least two laboratory fortified blanks (LFBs) with each set of extractions. Run a LFB-low and an LFB-high with each analytical run. The fortification concentration of each analyte should confirm the ability to detect at the reportable level. If the recovery of any analyte falls outside the control limits, the source of the problem must be identified and corrected.
- 8.3 Standards - Verify calibration standards quarterly by analyzing a standard prepared from reference material obtained from an independent or second source. EPA performance evaluations are an excellent process to determine the validity of the method. Results must be within +/- 20% of those used to routinely check calibration. Daily, run a low level standard to check the reportable detection level, RDL.
- 8.4 Samples - Samples must be extracted within 14 days after collection – unless specific targets need to be determined. Samples must be stored at 4°C until ready for extraction. Duplicates are run on 10% of samples, or once during run, whichever is greater. Results should be within +/- 20%. Extracts should be analyzed within 30 days after sample extraction.
- 8.5 Spike Recoveries - The laboratory must add a known concentration of spike solution, the same as used for LFB, to at least 10% of samples or once per

analytical run, whichever is greater. Recoveries should be within the acceptable range. Wherever possible, run a second source standard for spikes.

- 8.6 QC Requirements - Analyze EPA QC check sample with known values if available. The results for each analyte must be within the EPA acceptance criteria. Annually, analyze EPA Performance Evaluation samples. Analyze these check samples whenever major maintenance to the system occurs to ensure the validity of the method.
- 8.7 Anthracene and phenanthrene peaks must be separated by baseline. Benz-[a]-anthracene and chrysene must be separated by a valley that is less than 25% of the average peak height of the two compounds. Endrin breakdown must not exceed 20%. The area counts of mass 67 at the elution time of endrin aldehyde must not exceed 10% of the area counts of mass 67 from endrin. The % RSDs for all analytes in the calibration curve must not exceed 30%. The % RSD for PCP - pentachlorophenol may not exceed 60%.
- 8.8 When running the daily midpoint standard, ensure that the absolute areas of the quantitation ions of the internal and surrogate standards have not decreased by more than 30% of the continuing calibration check or more than 50% of the initial calibration curve.
- 8.9 Request a resample if sample failed QA/QC, or has unusual hits.

9. PREVENTIVE MAINTENANCE AND CORRECTIVE ACTIONS

- 9.1 Glassware - Glassware must be carefully cleaned. Clean all glassware as soon as practical after use by thoroughly rinsing with last solvent used in it. Follow by washing with hot water and 10% HCl and thoroughly rinsing with tap water followed by reagent water. After drying, heat in oven. Do not heat volumetric glassware above 220°C. Thorough rinsing with acetone may be substituted for heating.
- 9.2 Reagents - The use of high purity solvents and reagents will help to minimize contamination problems.
- 9.3 Carryover - Contamination carryover may occur when a sample containing a low concentration of analytes is analyzed immediately following a sample containing a high concentration. Use solvent rinses between samples to minimize carryover.
- 9.4 All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory method blanks. Minimize contact of the samples, reagents, or solvents with any plastics. This will help reduce contamination. Interferences by phthalate esters can pose a major problem in pesticide analysis.
- 9.5 PAHs are susceptible to degradation by residual chlorine - thus samples containing residual chlorine must be dechlorinated. Phthalate and adipate esters

may be difficult to quantify at levels below 2 ppb because of contamination from glassware and the air.

- 9.6 Record all corrective actions in the maintenance log book. Include a complete description of the problem and what action were taken to correct it.

[illegible]

ORANGE COUNTY WATER DISTRICT

EPA METHOD 525.2

PREPARATION OF STANDARDS AND QUALITY CONTROLS

1. Calibration Standard: **AccuStandard** - Stock = 100 µg/ml
Working Standard: dilute 100µl of stock to 1ml with Methanol - conc. = 10µg/ml
2. 2nd Source Standard: **Ultra Scientific/ Restec** - Stock = 100 ug/ml
Working Standard: dilute 100µl of stock to 1ml with Methanol - conc. = 10µg/ml

A. CALIBRATION STANDARD: (dilute in Ethyl Acetate)

Mix A - Std A, C, Custom 3 (Accustandard S-12600)

Mix B - Std B, F, Custom 5 (Restek 557399)

Mix C - BDE (at 20 ug/mL)

<u>Calib. Std. Conc.</u>	<u>Volume of Std.</u>	<u>Final Dil. Vol.</u>
Std A - 0.1 µg/L	10 µl of 10 µg/mL Std.	1.0 ml
Std B - 0.5 µg/L	50 µl of 10 µg/mL Std.	1.0 ml
Std C - 1.0 µg/L	10 µl of 100 µg/mL Std.	1.0 ml
Std D - 2.0 µg/L	20 µl of 100 µg/mL Std.	1.0 ml
Std E - 5.0 µg/L	50 µl of 100 µg/mL Std.	1.0 ml
Std F - 10.0 µg/L	100 µl of 100 µg/mL Std.	1.0 ml

B. INTERNAL & SURROGATE STANDARD SOLUTION:

Stock Solution - 500 µg/ml in Acetone

Spike Calibration Standard and Samples with 10µl of stock standard - conc. = 5µg/L

Recovery Standard: Terphenyl-d14

Stock Solution - 500 µg/ml in Methylene Chloride

Spike Calibration Standard and Samples (after extraction) with 10µl of stock standard

C. PERFORMANCE CHECK STANDARD (DFTPP)

Ultra Scientific

Stock Solution - 1000 µg/ml in Acetone

Dilute 5µl of stock to 1mL with CH₂Cl₂ - Inject 1µl = conc. 5 ng/mL (5ug/L)

D. LAB PERFORMANCE CHECK STANDARD (LPC)

(Endrin, DDT)

Stock Solution - 1000 µg/ml in Acetone

E. GC PERFORMANCE CHECK STANDARD (GCP)

(Custom Mix)

Stock Solution - Custom PAH - CUS 4175 - 100 µg/ml in Acetone

****Dilute 5µl LPC stock and 50 ul of Custom PAH to 1mL with CH₂Cl₂ - conc. 5ug/L

ORANGE COUNTY WATER DISTRICT

EPA METHOD 525.2

PREPARATION OF STANDARDS AND QUALITY CONTROLS

F. CHECK STANDARD:

- a. 0.1ppb, 1ppb, 2ppb and 5ppb Mix with std A, B, C, F, Cust 3 and Cust 5
- b. 2ppb Cust UCMR Mix - Dilute 10ul of cust UCMR mix to 1 ml in Ethyl Acetate.

G. LFB & SPIKE:

525.2 Targets

Low LFB = 0.1µg/L Spike 1-L DI with 10 µl of 10 µg/mL: Std A, C, B, F, Cust 5, Cust 3 & BDE

LFB = 1.0 µg/L Spike 1-L DI with 10 µl of 100 µg/mL: Std A, C, B, F, Cust 5, Cust 3 & BDE

Spike = 1.0 µg/L Spike 1-L sample with 10 µl of 100 µg/mL Std A, C, B, F, Cust 5, Cust 3 & BDE

UCMR Targets

(Varies Concentration)

Low C = 2ppb	Spike 1000 ml sample with 10 ul of custom mix std
LFB C = 5ppb	Spike 1000 ml sample with 25 ul of custom mix std
Spike / Spike Dup	Alternate spiking each batch of sample with 10 and 25 ul of custom mix

ORANGE COUNTY WATER DISTRICT

EPA METHOD 525.2

STANDARDS

A	Ultra Scientific	SVM - 525 - 1	Semi Volatile Mix
B	Ultra Scientific	PPM - 525E - 1	Organochlorine Pesticides
C	Ultra Scientific	NPM - 525C - 1	Nitrogen/Phosphorus Pesticide Mix
D	Ultra Scientific	PPS - 240 - 1	Toxaphene
E	Ultra Scientific	NPM - 108B - 1	DEF
F	Ultra Scientific	NPM - 525B - 1	Nitrogen/Phosphorus Pesticide Mix
G	Ultra Scientific	GCM - 160A - 1	GC/MS Performance Check
H	Ultra Scientific	ISM - 510 - 1	Internal and Surrogate Std Fortification Solution
I	Restek	557399	Custom mix of 5 new targets
J	Accustandard	S-12600	Custom mix of captan, ethion, trithion
K	Accustandard	BDE-CSM	8 BDE Compounds Mix
L	Ultra Scientific	CUS 6875 (LFB/SPK)	Custom mix of 8 new targets (combined of Cus 3 & 5)

STANDARD A	SVM - 525 - 1	Semi Volatile Mix
acenaphthylene	di-n-butylphthalate	2,2',4,4',5,6'-hexachlorobiphenyl
anthracene	2,3-dichlorobiphenyl	hexachlorocyclopentadiene
benzo [a] anthracene	di (2-ethylhexyl) adipate	indeno [1,2,3-cd] pyrene
benzo [b] fluoranthene	di (2-ethylhexyl) phthalate	isophorone
benzo [k] fluoranthene	diethylphthalate	2,2',3,3',4,5',6,6'- octachlorobiphenyl
benzo [g,h,i,] perylene	dimethylphthalate	2,2',3',4,6- pentachlorobiphenyl
benzo [a] pyrene	2,4-dinitrotoluene	pentachlorophenol
butylbenzylphthalate	2,6-dinitrotoluene	phenanthrene
2-chlorobiphenyl	fluorene	pyrene
chrysene	2,2',3,3',4,4',6-heptachlorobiphenyl	2,2',4,4'-tertachlorobiphenyl
dibenzo [a,h] anthracene	hexachlorobenzene	2,4,5-trichlorobiphenyl

STANDARD B	PPM - 525E - 1	Organochlorine Pesticides
alachlor	4,4'-DDD	HCH, alpha
aldrin	4,4'-DDE	HCH, beta
chlordane, (alpha-chlordane)	4,4'-DDT	HCH, delta
chlordane, (gamma-chlordane)	dieldrin	HCH, gamma (Lindane)
chlordane, (trans-nonachlor)	endosulfan I	heptachlor
chlornel	endosulfan II	heptachlor epoxide
chlorobenzilate	endosulfan sulfate	methoxychlor
chlorothalonil	endrin	permethrin, cis
DCPA	endrin aldehyde	permethrin, trans
	etridiazole	simazine

ORANGE COUNTY WATER DISTRICT

EPA METHOD 525.2

STANDARDS

A	Ultra Scientific	SVM - 525 - 1	Semi Volatile Mix
B	Ultra Scientific	PPM - 525E - 1	Organochlorine Pesticides
C	Ultra Scientific	NPM - 525C - 1	Nitrogen/Phosphorus Pesticide Mix
D	Ultra Scientific	PPS - 240 - 1	Toxaphene
E	Ultra Scientific	NPM - 108B - 1	DEF
F	Ultra Scientific	NPM - 525B - 1	Nitrogen/Phosphorus Pesticide Mix
G	Ultra Scientific	GCM - 160A - 1	GC/MS Performance Check
H	Ultra Scientific	ISM - 510 - 1	Internal and Surrogate Std Fortification Solution
I	Restek	557399	Custom mix of 5 new targets
J	Accustandard	S-12600	Custom mix of captan, ethion, trithion
K	Accustandard	BDE-CSM	8 BDE Compounds Mix
L	Ultra Scientific	CUS 6875 (LFB/SPK)	Custom mix of 8 new targets (combined of Cus 3 & 5)

STANDARD C	NPM - 525C - 1	Nitrogen/Phosphorus Pesticide Mix
ametryn	fenarimol	prometryn
atraton	fluoridone	pronamide
atrazine	hexazinone	propachlor
bromacil	methyl paraoxon	propazine
butachlor	metolachlor	simetryn
butylate	metribuzin	stirofos
chlorpropham	mevinphos	tebuthiuron
chlorpyrifos	MGK 264 - isomer a	terbacil
cyanazine	MGK 264 - isomer b	terbutryn
cycloate	molinate	triademefon
dichlorvos	napropamide	tricyclazole
diphenamid	norflurazon	trifluralin
EPTC	pebulate	vernolate
ethoprop	prometon	

STANDARD F	NPM - 525B - 1	Nitrogen/Phosphorus Pesticide Mix
carboxin	disulfoton	merphos
diazinon	fenamiphos	terbufos

STANDARD G	GCM - 160A - 1	GC/MS Performance Check
decafluorotriphenylphosphine (DFTPP)		
endrin		
4,4'-DDT		

STANDARD H	ISM - 510 -1	Internal and Surr Std Fortification Sol
acenaphthene-d10	1,3-dimethyl-2-nitrobenzene	
phenanthrene-d10	perylene-d12	
chrysene-d12	triphenylphosphate	
	pyrene-d10	

ORANGE COUNTY WATER DISTRICT

EPA METHOD 525.2

STANDARDS

A	Ultra Scientific	SVM - 525 - 1	Semi Volatile Mix
B	Ultra Scientific	PPM - 525E - 1	Organochlorine Pesticides
C	Ultra Scientific	NPM - 525C - 1	Nitrogen/Phosphorus Pesticide Mix
D	Ultra Scientific	PPS - 240 - 1	Toxaphene
E	Ultra Scientific	NPM - 108B - 1	DEF
F	Ultra Scientific	NPM - 525B - 1	Nitrogen/Phosphorus Pesticide Mix
G	Ultra Scientific	GCM - 160A - 1	GC/MS Performance Check
H	Ultra Scientific	ISM - 510 - 1	Internal and Surrogate Std Fortification Solution
I	Restek	557399	Custom mix of 5 new targets
J	Accustandard	S-12600	Custom mix of captan, ethion, trithion
K	Accustandard	BDE-CSM	8 BDE Compounds Mix
L	Ultra Scientific	CUS 6875 (LFB/SPK)	Custom mix of 8 new targets (combined of Cus 3 & 5)

STANDARD I	Custom 5: Restek - 557399
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acetochlor
 dimethoate
 benthocarb
 terbufos sulfone
 caffeine

STANDARD J	Custom 3: Accustandard - S-12600
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Captan
 Ethion
 Trithion

STANDARD K	8 - BDE mix:
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mix of 10 BDE's, 20ug/mL in iso-octane, except for deca-bde, @ 200 ug/mL

2,4,4'-Tribromodiphenyl ether
 2,2',4,4'-Tetrabromodiphenyl ether
 2,2',4,4',5-Pentabromodiphenyl ether
 2,2',4,4',6-Pentabromodiphenyl ether
 2,2',4,4',5,5'-Hexabromodiphenyl ether
 2,2',4,4',5',6-Hexabromodiphenyl ether
 2,2',3,3',4,4',5,5',6'-Decabromdiphenyl ether

Method 525.2 Rev. 1.1 (LIGHTS OUT)

Add 10 ml of acetone to the assembled SPE apparatus (with C-18 disk).

Turn the vacuum pump on and off quickly, allowing a small amount of solvent to drip through.

Let the disk soak for 3 minutes.

Aspirate the remaining solvent, followed by drying under vacuum for 3 mins.

Insert a clear 40-mL collection vial. Add 5-ml of 1:1 ethyl acetate:CH₂Cl₂

Turn the vacuum pump on and off quickly, allowing a small amount of solvent to drip through.

Let the disk soak for 3 minutes.

Aspirate the remaining solvent, then dry under vacuum for 3 mins. Remove collection vial and transfer solvent to waste bottle

Add 10-ml of methanol to the disk.

Aspirate a small amount of the solvent, leaving some on top of the disk.

Let the solvent soak the disk for 3 min, then apply vacuum.

LEAVE A LAYER OF SOLVENT ON TOP OF THE DISK, KEEP DISK WET!

DO NOT LET THE DISK GO DRY!!!

Add 10-ml of D.I. to reservoir to keep disk wet.

Add 5 ml of methanol to 1000 ml of sample, then add the sample to disk.

Add the INT/SURR 10 μ l, spike & High LFB 10 μ l of 100 μ g/ml and Low LFB 10 μ l of 10 μ g/ml working std

Add BDE low 5 μ L, High LFB & spike 50 μ L of 20

Turn on vacuum, adjust the pressure to 50 ml/min flow rate (<10 in-hg).

The sample should be processed for at least 20 mins but no longer than 25mins.

After sample is processed pull air through to dry disk for 7mins.

Insert a clear 40-ml tube containing 9g of baked Na₂SO₄.

Add 5 ml of ethyl acetate to disk, rinsing the sides of the reservoir.

Let some of the solvent drip through the disk, then let it soak for 3 minutes.

Pull the remaining solvent through the disk.

Repeat the above procedure with a 5-ml aliquot of CH₂Cl₂.

Repeat the eluting step again with 5-ml aliquots of Ethyl Acetate:CH₂Cl₂ (1:1) mixture.

Add baked Na₂SO₄ until all excess water is removed.

Wait 15 minutes before proceeding.

Transfer extract to a large Zymark tube and rinse the tube with two 3-ml portions of Ethyl Acetate: CH₂Cl₂ (1:1) mixture. Add rinses to the Zymark tube.

Zymark settings: Temp: 35, Pressure: 7 psi

Concentrate the extract to 1mL, if below 1mL add ethyl acetate to bring to volume
add 10 μ l of 500 μ g/ml p-Ternphenyl d10

Transfer to two amber autosampler vials (gc) with inserts.

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

EPA Method 548.1

DETERMINATION OF ENDOTHALL IN DRINKING WATER BY ION-EXCHANGE EXTRACTION, ACIDIC METHANOL METHYLATION AND GAS CHROMATOGRAPHY/MASS SPECTROMETRY

File Name: M:\SOP\Organic\epa method sop\548_102309.doc
Revision: 7

Effective Date: 10/23/2009
Supersedes: 7 (10/23/2009)

1. SUMMARY OF METHOD

EPA Method 548.1 determines Endothall using gas chromatography/mass spectrometry (GC/MS). The sample is diluted 1:10 with Millipore reagent water to a final volume of 100 ml and extracted with anion exchange disks mounted on a 6-position manifold. Alternatively, 8 ml cartridges are filled with Bio-Rex 5 anion exchange resin and used to extract endothall from the sample or pre-assembled 6ml cartridges with BIO-REX 5 sorbent are used instead. Endothall is eluted from the anion exchange disk with acidic methanol and methylene chloride. The dimethyl ester is formed upon heating this extract at 50°C. Salted reagent water is added to the extract and the endothall ester is partitioned into the methylene chloride. The extract volume is then reduced to 1 ml and analyzed by GC/MS.

2. ANALYTES

Endothall is the only analyte analyzed by this method.

LIMS code	analyte
ENDOTH	Endothall

3. APPARATUS AND EQUIPMENT

- 3.1 Sample bottles - 40 ml vials, fitted screw cap with teflon.
- 3.2 Vials - 40 ml amber glass, screw cap with teflon septa and limited volume insert.
- 3.3 Six station extraction manifold with 47 mm glassware sets (Varian 1214-6001 or equivalent).
- 3.4 Turbo-Vap concentration workstation (Zymark ZW8003)

- 3.5 Concentrator tubes - 50 ml Turbo-Vap tubes with 1.0 ml end-point (Zymark - ZA2039)
- 3.6 Gas Chromatograph/Mass Spectrometer - Varian 3800, Varian 8200 Autosampler, Varian Saturn Ion Trap mass spectrometer, and data system (using Varian GC/MS software).
- 3.7 100, 1000 and 2000 ml volumetric flasks.
- 3.8 Nitrogen Evaporation Apparatus.
- 3.9 Balance - analytical, capable of accurately weighing 0.0001g.
- 3.10 125 ml separatory funnels
- 3.11 10, 100, 250 uL Hamilton syringes
- 3.12 Brinkmann Autopipettors - 2-10 ml and 10-50 ml capacity with 1 L amber glass bottles
- 3.13 Vacuum pump
- 3.14 Explosion proof refrigerator

4. REAGENTS AND CONSUMABLE MATERIALS

- 4.1 Reagent Water - Millipore. Milli-Q system or equivalent filtered through a 0.45 micron filter.
- 4.2 Methylene Chloride - pesticide quality or equivalent. HPLC quality.
- 4.3 Sodium Sulfate - (ACS) granular. Prepare a 10% w/v solution in reagent water.
- 4.4 Sulfuric Acid - (ACS) reagent grade. Prepare a 10% v/v acidic methanol solution by adding 100 ml sulfuric acid to 1 L of methanol.
- 4.5 Endothall and Acenaphthene d-10 from Ultra Scientific and/or Absolute standard.
- 4.6 Sodium Hydroxide - (ACS) pellets
- 4.7 Methanol - pesticide quality or equivalent
- 4.8 Anion Exchange Disks by Empore - 47 mm
- 4.9 Bio-Rex 5 anion exchange resin
- 4.10 8 ml solid phase extraction cartridge

- 4.11 6 ml solid phase extraction cartridge with BIO-REX 5 sorbent
- 4.12 Disodium EDTA - (ACS) granular
- 4.13 Decafluorotriphenylphosphine (DFTPP)
- 4.14 Disposable glass pipettes
- 4.15 GC autosampler vials

5. SAMPLE COLLECTION, PRESERVATION AND HANDLING

- 5.1 Collect samples in 40 ml vial. Prepare 4 vials for spike and duplicated samples. Do not pre-rinse the bottle before collecting the sample. If residual chlorine is present add 100 mg sodium thiosulfate per liter of sample and mix well. Endothall should be kept in the dark away from heat. All samples must be iced and refrigerated at 4°C from the time of sampling until extraction. Furthermore, all samples must be extracted within 7 days of collection and analyzed within 14 days of extraction.

6. EXTRACTION

- 6.1 Liquid - Solid phase extraction (SPE):
 - 6.1.1 Place an anion exchange Empore disk onto the filtration apparatus. Use the larger vacuum pump when conditioning the disks. A stronger vacuum is required to draw Millipore water through the disk once the disk is saturated with methanol.
 - 6.1.2 Add 10 ml of MEOH, then 10 ml Milli Q, then 10 ml acidic methanol, then 10 ml Milli Q, then 20 ml 1N NaOH, then 20 ml Milli Q water being careful not to let the disk dry between conditioning steps. Let each solvent soak for 3 minutes before drawing it off and adding the next solvent. This is very important.
 - 6.1.3 Sample Preparation:
 - 6.1.3.1 Dilute each sample 1:10 with Millipore water. Dilution is necessary to reduce matrix effects due to the presence of calcium, magnesium and sulfate.
 - 6.1.3.2 Spikes are prepared by adding 10uL of a 100 ug/ml second source std (such as from Absolute Std) to 100 ml of 1:10 diluted sample. This is equivalent to a 10 ppb spike.
 - 6.1.3.3 LFBs are prepared by adding 4 uL of a 100 ug/ml second source std. to 100 ml of Millipore water. This is equivalent to a 4 ppb LFL.
 - 6.1.4 Add 100 ml of sample into the filtration reservoir and apply a vacuum.

The sample should flow through the disk without applying any vacuum. If a vacuum is required use the small vacuum pump. Low recoveries will result if flow rates are too high.

- 6.1.5 Do not let the sample flow exceed 3 ml per minute; it should take at least 30 minutes to pass the sample through the disk.
- 6.1.6 After the sample is processed, draw air through disk for 1 minute, and then add 1 ml of methanol to remove all traces of water. Then draw air through the disk for 5 minutes at 10 psi.
- 6.1.7 Remove the filter base and place a 40 ml receiving vial (VOC sample vial) in the manifold. Add 8 ml of acidic methanol, draw this eluant through the disk for 3 minutes. Repeat process. Then draw 6 ml of methylene chloride through the disk over 1 minute. Repeat process. Use the small vacuum pump to draw solvents through the disk.
- 6.1.8 Sample Derivatization: Cap the vial and place in a water bath at 50°C for 1 hour. Allow the vial to cool for ten minutes before proceeding. Note that all standards must be derivatized. Place 16 ml of acidic methanol and 12 ml of methylene chloride in a 40 ml receiving vial and add the required amount of standard. For example, a 10 ppb calibration standard will require addition of 1 uL of a 100 ug/ml standard.
- 6.1.9 Sample Partition:
 - 6.1.9.1 Pour the contents of the vial into a 125 ml separatory funnel. Rinse the vial twice with 1 ml of methylene chloride and add the rinse to the separatory funnel.
 - 6.1.9.2 Add 40 ml of 10% sodium sulfate solution to the separatory funnel.
 - 6.1.9.3 Shake the funnel, vent and then shake vigorously for 30 seconds (shake 70 times). Place the lower phase into a Zymark tube. Repeat the extraction procedure with 2 ml aliquots of methylene chloride twice and add the organic phase to the Zymark tube.
- 6.1.10 Turbo-Vap concentration workstation:
 - 6.1.10.1 Reduce the extract to a volume of 1.0 ml. The temperature of the water bath is 35°C and the nitrogen is set on the lowest flow possible that does not set off the alarm.
 - 6.1.10.2 Add 5 uL of the internal std. (This volume is for a 500 ug/ml stock standard. Addition of 5 uL of internal std. to 1 ml of extract is equivalent to a final concentration of 5 ppb in a 100 ml sample volume).

- 6.1.10.3 Transfer this extract into GC autosampler vials with disposable glass pipettes. Refrigerate extracts if they will not be analyzed immediately. Extracts should be stored in the dark at 4°C or less.

6.2. Cartridge - Solid phase extraction (SPE):

- 6.2.1. Assemble a 6ml SPE cartridge onto the filtration apparatus. Use the larger vacuum pump when conditioning the cartridges. A stronger vacuum is required to draw Millipore water through the cartridge once the cartridge is saturated with methanol.
- 6.2.2. Drain packing fluid from cartridge. Add 5ml of Acetone, let solvent soak for 3 minutes, and completely dry cartridge as vacuum is applied for 3 minutes. Add 10 ml of MEOH, then 10 ml Mili Q, then 10 ml acidic methanol, then 20 ml Milli Q, then 20 ml 1N NaOH, then 20 ml Milli Q water being careful not to let the cartridge dry between conditioning steps. Let each solvent soak for 3 minutes before drawing it off and adding the next solvent. This is very important.
- 6.2.3. Sample Preparation:
- 6.2.3.1. Dilute each sample 1:10 with Millipore water. Dilution is necessary to reduce matrix effects due to the presence of calcium, magnesium and sulfate.
- 6.2.3.2. Spikes are prepared by adding 10uL of a 100 ug/ml second source std. (such as from Abs.std) to 100 ml of 1:10 diluted sample. This is equivalent to a 10 ppb spike.
- 6.2.3.3. LOW LFBs are prepared by adding 4 uL of a 100 ug/ml second source std. to 100 ml of Millipore water. This is equivalent to a 4 ppb LFL.
- 6.2.4. Add 100 ml of sample into the filtration reservoir and apply a vacuum. The sample should flow through the cartridge without applying any vacuum. If a vacuum is required use the small vacuum pump. Low recoveries will result if flow rates are too high.
- 6.2.5. Do not let the sample flow exceed 1.5 ml per minute; it should take at least 60 minutes to pass the sample through the cartridge.
- 6.2.6. After the sample is processed, draw air through cartridge for 1 minute, and then add 10 ml of methanol to remove all traces of water. Let soak for 1 minute. Then draw air through the cartridge for 10 minutes at 10 psi.
- 6.2.7. Place a 10 ml receiving tube in the manifold. Add 4 ml of acidic methanol; let soak cartridge for 3 minutes. Draw elute into vial at low vacuum. Then add 3 ml of methylene chloride; let soak cartridge for 3 minutes. Draw elute into vial at low vacuum. Transfer elute from the 10 ml vial to a 40 ml vial and reinsert 10 ml vial back into SPE. Repeat solvent rinsing process, but have vacuum on high and dry cartridge completely after each elution. Transfer elute to the same 40 ml vial. Rinse the 10 ml receiving tube twice with 1mL of CH₂CL₂. Add these rinses to the 40ml vial.

6.2.8. Sample Derivatization:

Cap the vial and place in a water bath at 50°C for 1 hour. Allow the vial to cool for ten minutes before proceeding. Note that all standards must be derivatized. Place 8 ml of acidic methanol and 6 ml of methylene chloride in a 40 ml receiving vial and add the required amount of standard for the calibration and calibration check standards. For example, a 10 ppb calibration standard will require addition of 10 uL of a 100 ug/ml standard.

6.2.9. Sample Partition:

6.2.9.1. Pour the contents of the vial into a 125 ml separatory funnel. Rinse the vial twice with 1 ml of methylene chloride and add the rinse to the separatory funnel.

6.2.9.2. Add 40 ml of 10% sodium sulfate solution to the separatory funnel.

6.2.9.3. Shake the funnel, vent and then shake vigorously for 30 seconds (shake ~70 times). Place the lower phase into a Zymark tube. Repeat the extraction procedure with 2 ml aliquots of methylene chloride twice and add the organic phase to the Zymark tube.

6.2.10. Turbo-Vap concentration workstation:

6.2.10.1. Reduce the extract to a volume of 1.0 ml. The temperature of the water bath is 35°C and the nitrogen is set on the lowest flow possible that does not set off the alarm (9 psi).

6.2.10.2. Add 5 uL of the internal std. (This volume is for a 500 ug/ml stock standard. Addition of 5 uL of internal std. to 1 ml of extract is equivalent to a final concentration of 5 ppb in a 100 ml sample volume).

6.2.10.3. Transfer this extract into GC autosampler vials with disposable glass pipettes. Refrigerate extracts if they will not be analyzed immediately. Extracts should be stored in the dark at 4°C or less.

7. ANALYSIS

7.1

Instrumentation: Gas chromatograph/Mass Spectrometer - Varian 3800 gas chromatograph, Varian 8200 autosampler, Varian Saturn Ion Trap and computer data system. The column used is a Varian factor four column, 30 m x 0.25 mm, 0.25 micron film

GC Conditions:

1.	Seg	Temp	Rate	Hold Time	Total
	1	100	0	2	2.00
	2	200	12	0	10.33
	3	300	60	2	14.00

2. Helium linear velocity: 75 cm/sec
3. Splitless injection with 3.5 minute delay
4. Injector temperature (1079): Initial 50°C to 250°C @ 180 °C/minute then hold for 10.00 minutes
5. Transfer Line Temp: 280 °C
6. 2 ul sample is injected at 10 ul/sec - sandwich technique is used with a 0.2 ul solvent plug

MS Conditions :

1. Mass range : 45 to 450 amu
2. Seconds/scan : 0.80
3. Acquire time : 33.0 minutes
4. Fil/Mul delay : 10 minutes
5. Peak threshold : 1 count
6. Mass defect : 0 mmn/100 amu
7. Background mass : 45 amu
8. Ionization mode : EI
9. Auto ion control : ON
10. Cal gas : OFF

5 ng of DFTPP is injected (1ul of a 5ug/ml solution) before every run into the GC/MS to determine whether the MS is "in tune". Using the GC method DFTPP, MS method DFTPP and AS method DFTPP, the ion abundance of the mass spectra of DFTPP can be compared to the following set of criteria.

<u>Mass</u>	<u>Relative Abundance Criteria</u>
54	10-80% of the base peak
68	<2% of mass 69
70	<2% of mass 69
127	10-80% of the base peak
197	<2% of mass 198
198	base peak of >50% of 442
199	5-9% of mass 198
275	10-60% of the base peak
365	>1% of the base peak
441	Present and < mass 443
442	base peak of >50% of 198
443	15-24% of mass 442

7.2 EnviroPro software is used to determine whether the ion abundance criteria is met. Use file EP to get into EnviroPro and type in the file name, type the correct

forms output, 6B, and the califile DFTPP. From the main menu type R for run procedure and DFT525 to get the printout of the relative ion abundance of the DFTPP injection. This printout is kept with each data package.

- 7.3 A Laboratory Fortified Blank (LFB) is run with each set of samples at a concentration of 10 ppb. A Low LFB is also run at a concentration of 4 ppb. Spikes are run every 10 samples or at least once per set of samples at a concentration of 10 ppb. Duplicates are run at least once per set of samples or every 10 samples.
- 7.4 DFTPP is run before every analysis. DFTPP must pass the ion abundance criteria set forth in the method. The ion abundance criteria is the same as the ion abundance criteria in EPA Method 525.1 therefore, software provided for 525.1 will be used to judge whether DFTPP passes the ion abundance criteria for this method. Refer to Exhibit 5 for specific ion abundance criteria values. The external calibration used is 5-point calibration ranging from 2 to 50 ppb refer to standard preparation for directions on how to prepare calibration curve.
- 7.5 In addition a calibration check standard at 10ppb must also be analyzed right after the 5-pt calibration. The *Front* standard should be right after the 5-point curve and the *Back* should be the last sample analyzed. If run is greater than 8 hours, then check standard must be analyzed every 10 samples.
- 7.6 The amount of endothall in a sample is calculated using the internal standard method. Acenaphthene d-10 is the internal standard (m/z 164). Endothall (m/z 183, 155 and 123) is calculated using three ions. The Varian software compares the response factors of the sample to the response factors in the calibration curve.

8. QA/QC REQUIREMENTS

- 8.1 Method blanks (reagent blanks) in order to demonstrate that glassware and reagents are free of any interference, a reagent blank must be analyzed at the beginning of each analysis. If the blank produces any interference, determine the source of the interference and eliminate that interference before proceeding.
- 8.2 Laboratory Fortified Blank - Analyze at least one laboratory-fortified blank, LFB, with every 10 samples or one per run, whichever is greater. The fortification concentration of each analyte should be 10 times the MDL, whichever is less - in this method 10 ppb will be used. If the recovery of any analyte falls outside the control limits, the source of the problem must be identified and corrected.
- 8.3 Verify calibration standard by analyzing a standard prepared from reference material obtained from an independent or second source (continuing calibration check standard), immediately after the calibration. Run one at the end also. Results must be within +/- 30% of those used to routinely check calibration. Weekly, run a low level standard to check the reportable detection level, RDL.
- 8.4 Samples must be extracted within 7 days after collection. Duplicates are run on 10% of samples, or once during run, whichever is greater. Results should be

within +/- 30%.

- 8.5 The laboratory must add a known concentration of spike solution, the same as used for LFB, at least 10% of samples or once per analytical run, whichever is greater. Recoveries should be within +/- 30%.

9. PREVENTIVE MAINTENANCE AND CORRECTIVE ACTIONS

- 9.1 Glassware - Glassware must be carefully cleaned. Clean all glassware as soon as practical after use by thoroughly rinse. Follow by washing with hot water and 10% HCl and thoroughly rinsing with tap water followed by reagent water. After drying, heat in oven. Do not heat volumetric glassware. Thorough rinsing with acetone may be substituted for heating.
- 9.2 Reagents - The use of high purity solvents and reagents will help to minimize contamination problems.
- 9.3 Carryover - Contamination carryover may occur when a sample containing a low concentration of analytes is analyzed immediately following a sample containing a high concentration. Use solvent rinses between samples to minimize carryover.
- 9.4 Minimize contact of the samples, reagents, or solvents with any plastics. This will help reduce contamination.
- 9.5 Record all corrective actions in the maintenance log book. Include a complete description of the problem and what action was taken to correct it.
- 9.6 No or Poor Chromatography-
- 9.6.1 Check all the standards. If calibration and/or QC lie outside the acceptable limits we must first re-run the standards to assure that the injection procedure is working properly. After the re-run if standards still lie outside the acceptable limits we must make a new standard or conduct instrument maintenance to assure that calibration and QC lie with acceptable parameters. For elevated spike recoveries make sure that the sample matrix is clean. If sample matrix is altering recoveries then a new matrix must be selected. If instrument performance is acceptable the samples must be re-extracted. If the sample lies outside allowable holding time they must be re-sampled.
- 9.6.2 Request re-sample if it's necessary.
- 9.6.3 If there are hits, request resample

[illegible]

Method 548.1 Cartridge Rev 1.3 -- 10/23/09

(pg 1 of 2)

Turn on the H2O bath - use MTBE cap for all vials

Samples and QCs Prep

QCs - 100ml volumetric flask w/ Millipore water
add the spikes (LOW: 4uL; LFB: 10uL & Spike : 10uL of 100ug/ml Absolute Std)
Samples - in a 100ml volumetric flask, dilute each sample 1:10 with Millipore water

Cartridge Conditioning

548 Cartridge: UCT Bio Rex 5, Part# EC548006.

Attach assembled cartridge to SPE manifold and drain packing fluid from cartridge.
Add 5-ml of Acetone to the assembled SPE cartridge with reservoir.

aspirate a small amount of the solvent.
Let the solvent soak for 3 min, then apply vacuum for 3 min. Completely dry the cartridge.

FROM THIS STEP ON - DO NOT LET THE CARTRIDGE GO DRY!!!
LEAVE A THIN LAYER OF SOLVENT ON THE TOP LAYER OF THE CARTRIDGE!

add 10-ml of MeOH to the assembled SPE cartridge
aspirate a small amount of the solvent. Soak the cartridge for 3 min.
apply vacuum, leave a thin layer of solvent on the top most layer of the cartridge.
DO NOT LET THE CARTRIDGE GO DRY!!

add 10-ml of D. I., aspirate a small amount, then soak for 3 min.
apply vacuum, leave a thin layer of water on the top most layer of the cartridge.
DO NOT LET THE CARTRIDGE GO DRY!!

add 10-ml of 10% acidic Methanol
aspirate a small amount, soak for 3 min., apply vacuum, leave a thin layer of solvent on top.
DO NOT LET THE CARTRIDGE GO DRY!!

Rinse the cartridge and reservoir with 10 mL of D.I. water. Let water drip through.
Add an additional 10 mL of D.I. water. Aspirate a small amount and let soak for 3 minutes.
apply vacuum, leave a thin layer of water on top layer of cartridge.
make sure all traces of acidic MeOH are gone

add 20 ml of 1 N NaOH to the cartridge with reservoir.
aspirate a small amount, soak for 3 min., apply vacuum, leave a thin layer of solvent on top.
DO NOT LET THE CARTRIDGE GO DRY!!

Rinse the cartridge and reservoir with 10 mL of D.I. water. Let water drip through.
Add an additional 10 mL of D.I. water. Aspirate a small amount and let soak for 3 minutes.
apply vacuum, leave a thin layer of water on top layer of cartridge.
DO NOT LET THE CARTRIDGE GO DRY!!
make sure all traces of NaOH are gone

add the diluted QCs and samples

DO NOT USE THE VACUUM PUMP AT ALL ! (Total time should be at least 60 minutes)
(while samples drip - prepare 125ml sep funnel & 40ml clear vials)

after the sample has been processed, pull air through the cartridge for 1 min. -- FULL VACUUM

add 10-ml of methanol to the cartridge to remove all traces of water
Let soak for 1 minute
pull air through the cartridge for 10 additional minutes at **10 psi. (low PSI on vacuum)**

Method 548.1 Cartridge Rev 1.3 -- 10/23/09

(pg 2 of 2)

CARTRIDGE ELUTION

-- Insert a clear 10-ml glass vial to collect the elute--

Keep the PSI of the vacuum below 10.
add 4-ml of 10% acidic methanol
trigger vacuum to allow a small amount to drip through, soak for 3 min.
Trigger the vacuum on and off to allow the elute to exit the cartridge in a dropwise fashion.
Continue until all solvent has been drained. **Do not dry cartridge completely.**

Add 3 ml of CH₂Cl₂
trigger vacuum to allow a small amount to drip through, soak for 3 min.
Trigger the vacuum on and off to allow the elute to exit the cartridge in a dropwise fashion.
Continue until all solvent has been drained. **Do not dry cartridge completely.**

Transfer elute to a 40 ml glass vial
Insert same 10 ml collection vial back into SPE.

Increase to full vacuum.

Repeat the two elution steps again. Dry cartridge completely for each solvent.

Transfer elute to the 40 ml vial.
Rinse the 10 mL collection vial twice with 1mL of CH₂Cl₂. **Add these rinses to the 40 mL vial.**
Cap 40 mL vial and place in a 50 degree water bath for 1 hour

Standards: add 8 mL of 10% acidic MeOH & 6 mL of CH₂Cl₂ to a clear 40ml vial - use MTBE caps
add the appropriate amount of the standard (2ul, 5ul, 10ul, 25ul & 50ul of 100ug/ml Crescent)
cap vial, and place in a 50 degree water bath for 1 hour
***Cal. check is spiked w/10ul of 100ug/ml Absolute Standard ***

Remove the sample from the water bath
wait for the samples to cool to room temp (about 10min.) before proceeding

add 40-ml of 10% Na₂SO₄ solution to the sep. funnel

pour the content of the vial into separatory funnel w/ added 10% Na₂SO₄
rinse the vial twice with 1-ml portion of CH₂Cl₂, add the rinse to the funnel

cap funnel, vent & shake vigorously for 30 sec

wait 10 min. for the layers to separate

drain the bottom organic layer into a Zymark tube

repeat the shaking and draining process 2 more times
each time, adding 2-ml of CH₂Cl₂ to the funnel, and combine all of the extracts

Turbo Vap settings: Temp: 35, Pressure: 9 psi

concentrate the extract to 1ml

remove the Zymark from the turbo vap - let it cool (it takes < 1min)
rinse the lower 25% of the Zymark 8-10 times (1mL will evaporated to <0.6ml)

add CH₂Cl₂ to bring the sample to 1ml
add Internal - 5ul (of conc. 500 ug/mL) --- mix well
transfer to 2 amber autosampler vials; **1 w/ insert and 1 w/out**
Store in refrigerator 4°C until analysis.

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

EPA Method 504.1.1

Rev 2

1,2-DIBROMOETHANE (EDB), 1,2-DIBROMO-3-CHLOROPROPANE (DBCP), AND 1,2,3-TRICHLOROPROPANE (123TCP) IN WATER BY MICROEXTRACTION AND GAS CHROMATOGRAPHY

File Name: M:\SOP\Organic\epa method sop\504_0101.doc
Revision: 2

Effective Date: 11/01/2001
Supersedes: 1 (01/01/2001)

1. SUMMARY OF METHOD

35 ml of sample is extracted with 2.0 ml of hexane. 4 uL of the extracted sample is then injected into a GC equipped with an electron capture detector. Calibration and check standards are extracted in a manner similar to that used for the samples. Analysis time is approximately 23 minutes. At low concentrations, EDB may be masked by dibromochloromethane - a THM. Results must be confirmed by a second column. When high enough concentrations of any analytes are detected, confirm by GC/MS.

1.1 Sample Collection

Samples for the analysis of EDB, DBCP and 1,2,3-TCP are collected in 40 ml clear VOA vials. Four vials for each site are taken so that spikes and/or duplicates can be processed. When sampling, allow the tap to flush for several minutes so that the water temperature stabilizes. Adjust the flow to 500 ml/minute and collect the sample. All samples must be chilled and delivered to the laboratory at 4°C. Any chlorinated samples, must have the dechlorinating agent (sodium thiosulfate) added to the sample bottle. 3mg of sodium thiosulfate crystals are added to the empty vial - just prior to shipping to the sampling site. A travel blank of the reagent water must accompany each set of samples brought into the laboratory. Store all samples at 4°C until extraction – all samples must be analyzed within 14 days of collection. All extracts should be analyzed immediately after each extraction.

1.2 Reagents, Equipment & Apparatus

1.2.1 Reagent water

1.2.2 Hexane extraction solvent - B & J GC² grade.

1.2.3 Methanol - ACS reagent grade.

1.2.4 EDB, DBCP, and 123TCP standards - Method 504 mix from Ultra Scientific.

- 1.2.5 Sample containers – 40 ml screw cap VOA vials, each equipped with a PTFE septum.
- 1.2.6 Vials - autosampler screw-cap with PTFE faced septa.
- 1.2.7 Micro syringes - 10 uL, 25 uL, and 50 uL gas-tight Hamilton Syringes.
- 1.2.8 Volumetric flasks -100 mL glass with stoppers.
- 1.2.9 GC columns DB-1 or Durawax-DX3: DB-1 0.32 mm 10 X 30 meter long fused silica capillary column. Durawax-DX3 is the confirmatory column.
- 1.2.10 GC equipped with ECDs able to do temperature programming.
- 1.2.11 Sodium chloride - NaCl - ACS grade. Treat each batch (lot-#) of NaCl with a muffle furnace, bake at 400°C for 30min. Store the treated NaCl in a glass container – log the treatment batch into reagent logbook – note the original lot # on the bottle and extraction log.

1.3 Reagent Blank Water

Reagent blank water is free of any organic contaminants. Boil a volume of 18 megohm water for approximately 15 minutes. While maintaining a temperature of 90°C, purge the water with UHP He for 1 hour. Reagent water may also be prepared using the Millipore milli-Q water system.

1.4 Glassware Washing

Wash glassware in the same manner as with volatile glassware. Soak glassware in soap solution and rinse with 10% HCl, tap water, and deionized water. Allow glassware to bake at 220°C overnight.

1.5 GC parameters

Activate the proper method, build the autosampler table (if needed) and then activate method. The linear velocity of the carrier gas is set at 25 cm/second.

GC Parameters:

GC 3800s

Initial Column Temperature:	35°C
Initial Column Hold Time:	4.00 minutes
Final Column Temperature:	190°C
Column Rate in °C/minute:	8°
Final Column Hold Time:	3.00 minutes
Flow A & B in mL/minute:	1.5
Pressure A & B in PSI:	10
Column Velocity in cm/second:	25.0
Injector A & B Temperature:	200°C
Detector Temperature:	300°C
ECD Initial Attenuation:	32 range 10

(Autozero on)

1.6 Extractions

Let sample containers warm up to room temperature; use a transfer pipette to remove 5mls of sample from each VOA vial. Replace the cap and weigh the vial to the nearest 0.1g – note the weight onto the extraction log sheet. For calibration standards, check standards, laboratory fortified samples, and reagent blanks use a 50ml-graduated cylinder to transfer the 35ml sample volume into the vial. Spike the QA/QC samples according to the appropriate level. After weighing the samples, remove the container's cap and add 6 g of the muffle treated NaCl to each vial. Recap the sample container and dissolve the NaCl by shaking for about 20 sec. Add 2.0 ml of hexane to each sample vial by using a dispenser – pre-measure to ensure that 2.0 mls of hexane is delivered. Recap and shake vigorously for 1 minute. Allow the water and hexane phases to separate for at least 10 minutes. Draw off an aliquot (0.5mls) of hexane into the auto-sampler vial using a Pasteur pipette, making sure that none of the water is drawn into the vial. If possible, make-up a second vial in the same manner for back-up analysis. Dispose of the vials contents properly and re-weigh the vial with the correct cap to attain the sample volume. Again, weigh to the nearest 0.1g and note this on the extraction log sheet. The net weight of the sample will be inputted into the chromatography software to adjust for the correct volume for each sample. The extracts should be analyzed as soon as possible after extraction – same day if possible. The extracts should be stored at 4°C until the analytical system is ready for analysis.

1.7 Calibration/Standardization

Prepare secondary dilution standards by preparing a 10ug/ml standard mix (STD#1) and from this a 0.35ug/ml standard mix (STD#2) from the stock standards for both Ultra Scientific (Calibration) and Accu Standard (LFBs and Spikes) sources - prepared with methanol. These two diluted standards will allow for further setup of calibration and QA/QC standards. Prepare a calibration curve that consists of 0.01 ppb, 0.05 ppb, 0.1 ppb, 0.2 ppb, and 0.5 ppb using STD#2. Follow the same extraction procedures for samples when extracting these standards. Each analysis run contains this 5-point curve. At this point, the chemist can prepare a calibration curve of concentration versus peak area; or, if the ratio to response to concentration is a constant over the calibration range, linearity through the origin can be assumed and a calibration factor can be used. With each run two LFBs are analyzed, low – 0.01ppb and high – 0.10ppb. The low level LFB is used to ensure that the RDL can be achieved for this analytical run.

1.8 Spikes/Duplicates

Each analysis of a set of samples contains at least 10% of the total samples as spikes. The spiking concentration is 0.1 ppb. Duplicate analysis has a frequency of at least 10% over the course of a daily run. All values from the spike sample are documented; and, after enough data are acquired, a control chart with upper and

lower warning and control limits is graphed. The allowable acceptance criterion is 60% to 140% of the expected true value – Conifer is set to a +/-30% range to flag for problems. Report all spike data on Control Chart Sheet. Once a month, the values from these sheets will be entered into a computer for control chart generation.

2. ANALYTES

- 2.1 1,2 - Dibromoethane (EDB)
- 2.2 1,2 - Dibromo-3-Chloropropane (DBCP)
- 2.3 1,2,3-Trichloropropane (123TCP)
- 2.4 The established method detection limit for this method is <0.01 ppb. The reportable detection limit (RDL) is 0.01 ppb.

3. QA/QC REQUIREMENTS

- 3.1 Laboratory Reagent Blank - Run before samples. Use to demonstrate that all glassware and reagent interferences are under control. If any contamination peaks are produced, determine source of contamination and eliminate interference.
- 3.2 Laboratory Fortified Blank - Must analyze at least two laboratory fortified blank (LFB) one after every calibration and one at the end of the analysis. With each run two LFBs are analyzed, low – 0.01ppb and high – 0.10ppb. The low level LFB is used to ensure that the RDL can be achieved for this analytical run (the fortification concentration of each analyte should be 10 times the EDL or the MCL, whichever is less). If the recovery of any analytes falls outside the control limits, the source of the problem must be identified and corrected.
- 3.3 Standards - Verify calibration standards quarterly by analyzing a standard prepared from reference material obtained from an independent or second source. Results must be within +/- 30% of those used to routinely check calibration. Weekly, run a low level standard to check the reportable detection level, RDL.
- 3.4 Samples - Samples must be analyzed within 14 days after collection. Samples must be stored at 4°C until ready for analysis. Duplicates are run on 10% of samples, or once during run, whichever is greater.
- 3.5 Spike Recoveries - The laboratory must add a known concentration of spike solution, the same as used for LFB, to at least 10% of samples or once per analytical run, whichever is greater. Recoveries should be within the acceptable range (+/- 30%).
- 3.6 QC Requirements - Monthly, analyze EPA QC check sample with known values if available. The results for each analyte must be within the EPA acceptance

criteria. Semi-annually, analyze EPA Performance Evaluation samples.

- 3.7 To establish the ability to achieve low detection levels and to generate accurate and precise chromatography, the analyst must analyze seven low level replicates (0.04 ppb or lower). The MDL is then calculated based on the precision and accuracy of these results. The acceptance criterion calls for a recovery of +/- 20% and an MDL that does not exceed 0.01 ppb. Anytime major maintenance to the system occurs a new MDL determination must be performed. Compliance to this shows that system performance is acceptable and sample analysis can begin.

4. REPORTING REQUIREMENTS

- 4.1 For any positive hit, a confirmation must be performed. If in high enough concentrations a GC/MS analysis can be performed. If not, the sample must be re-analyzed using the confirmation column. If any result is within 75% of an action level, the water quality (WQ) department must be notified immediately. Sample results will be carried through the LIMs system. The raw data will be written-up in a standard data package - with QA/QC recoveries of spikes and standards noted. This data package will then be reviewed, by either the senior chemist or the supervising chemist, for completeness. Once approved the final form will be generated and approved by the laboratory director. Any abnormalities will be noted on the data package sign-off sheet (corrective actions will also be noted). Specific attention must be given to interference peaks, which may cause false positive results. This is especially true for EDB, which can be masked or misidentified with the THM – dibromochloromethane.

5. CORRECTIVE ACTIONS

- 5.1 Calibration Standards/QC standards fail-
 - 5.1.1 Check all the standards. If calibration and/or QC lie outside the acceptable limits we must first re-run the standards to assure that the injection procedure is working properly.
 - 5.1.2 If standards are still outside the acceptable limits, re-extract a new standard or conduct instrument maintenance.
 - 5.1.3 For elevated spike recoveries make sure that the sample matrix is clean. If sample matrix is altering recoveries then a new matrix must be selected. If LFB's fail, check standard. Conduct a method review in both cases. The method review would include verification of standard lots and actual process steps. All staff members involved in the processing of samples would be included in this process.
 - 5.1.4 If instrument performance is acceptable the samples must be re-extracted. If the sample lie outside allowable holding time they must be re-sampled.
 - 5.1.5 If front check standard fails, check standards, make up new dilutions and re-analyzed.
 - 5.1.6 Request re-sample if it's necessary.

5.1.7 If there're analytical hits, request for resample.

5.2 All corrective actions will be noted in the maintenance logbook and the data review sign-off sheet. Solvent blanks must be analyzed with each run in order to monitor any interferences. Reagent water must also be monitored, on every analytical run, for contamination.

6. PREVENTIVE MAINTENANCE

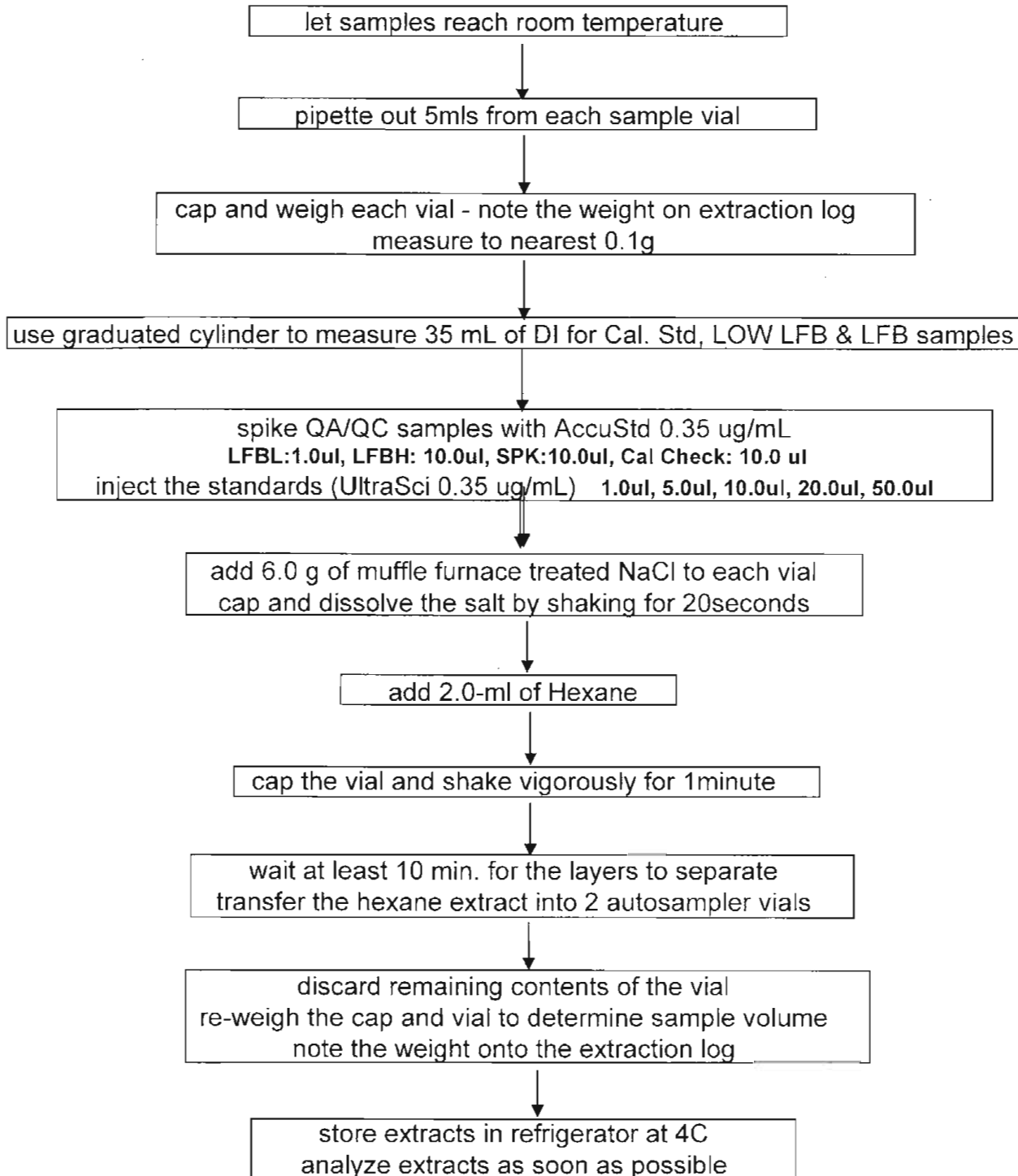
6.1 Changing of septum and gas cylinders requires that the column oven, detectors and injectors be cooled down. Additionally, detectors need to be turned off after cooling. When injector and column ovens have been brought up to temperature and allowed to equilibrate, turn on detector and activate the method. Check the syringe to ensure that it is being inserted properly and solvent/sample is unrestricted. Retention times must be monitored to ensure they remain consistent throughout the run. If retention times do vary, check septum and column for leaks and deterioration. The wash reservoir must contain the proper solvent (hexane / 3/4 full).

SOP PROCEDURE CHANGE

FOR EPA Method 504.1

[illegible]

METHOD 504.1, Revision 1.1



ORANGE COUNTY WATER DISTRICT

EPA METHOD 504.1

PREPARATION OF STANDARDS AND QUALITY CONTROLS

Stock Concentration for all these standards are 200 ug/ml or 200,000 ug/L

A. CALIBRATION STANDARD - ULTRA SCIENTIFIC

Intermediate Standards:

- 1) Dilute 50 ul of stock to 1.0 ml with methanol. ----- **STD #1 conc.** 10 µg/ml
- 2) Dilute 35 ul of **STD#1** to 1.0 ml with methanol.----- **STD #2 conc.** 0.35 µg/ml

Working Standards:

<u>Calib. Std. Conc.</u>	<u>Volume of Standard Use</u>	<u>Final Dil. Vol.</u>
STD A - 0.01 µg/L	1.0 µl of STD #2	35 ml
STD B - 0.05 µg/L	5.0 µl of STD #2	35 ml
STD C - 0.10 µg/L	10.0 µl of STD #2	35 ml
STD D - 0.20 µg/L	20.0 µl of STD #2	35 ml
STD E - 0.50 µg/L	50.0 µl of STD #2	35 ml
STD F - 1.00 µg/L	100.0 µl of STD #2	35 ml
STD G - 2.00 µg/L	200.0 µl of STD #2	35 ml

B. CALIBRATION CHECK STANDARD: (Accustandard)

Spike sample with 10 µl of STD # 2 - conc. = 0.10ug/L

C. LFB & SPIKE (Accustandard):

Low LFB = 0.01 µg/L Spike QC sample with 1 µl of STD #2
LFB = 0.10 µg/L Spike QC sample with 10 µl of STD #2

Sample Spk = 0.10 µg/L Spike sample with 10 µl of STD #2

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ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

EPA Method 505

DETERMINATION OF ORGANOHALIDE PESTICIDES IN WATER BY MICROEXTRACTION AND GAS CHROMATOGRAPHY

File Name: M:\SOP\Organic\epa method sop\505_0500.doc
Revision: 3

Effective Date: 05/2000
Supersedes: 2 (10/1996)

1. SUMMARY OF METHOD

- 1.1 A measured volume of sample of approximately 50 milliliters is solvent extracted with 3.0 ml of hexane. 2ul of the extracted sample is then injected into a GC equipped with an electron capture detector (ECD). Calibration standards are extracted in a manner similar to that which is used to extract the samples. Analysis time is approximately 30 minutes in length. Results must be confirmed by: using a second column, another specific detector method which includes the target of interest, or GC/MS method 525.

2. ANALYTES

- 2.1 This is a gas chromatographic (GC) method applicable to the determination of certain chlorinated pesticides in groundwater and finished drinking water. The following compounds can be determined using this method:

<u>LIMS code</u>	<u>analyte</u>
ALACHL	Alachlor
ALDRIN	Aldrin
CLTNIL	Chlorothalonil
DIELDR	Dieldrin
ENDRIN	Endrin
ENDR-A	Endrin Aldehyde
ENDR-K	Endrin Ketone
LINDNE	HCH-gamma (Lindane)
HEPT	Heptachlor
HEPEPX	Heptachlor epoxide
HCICPD	Hexachlorocyclopentadiene
METHOX	Methoxychlor
TOXA	Toxaphene
CIDANE	Chlordane

3. APPARATUS AND EQUIPMENT

- 3.1 Sample Bottles - 250 ml amber glass bottles fitted with a screw cap lined with teflon.
- 3.2 Autosampler vials - equipped with Teflon-lined septum.
- 3.3 3500 Varian gas chromatograph with dual columns - electron capture detectors (ECD). Equipped with an 8200 autosampler for injecting samples into the GC.
- 3.4 Column: Fused Silica Capillary column, DB-1 30 meters long x 0.32 mm I.D. with a 0.25 micron film thickness. Alternate column - Durawax-DX3 30 meters long x 0.32 mm I.D. with a 0.25 micron film thickness.
- 3.5 Disposable pasteur pipets, 80ml test tubes (50ml) with teflon stoppers, and micro syringes - 10ul, 25ul, and 50ul gas-tight Hamilton syringes.

4. REAGENTS AND CONSUMABLE MATERIALS

- 4.1 Reagent Water - Millipore Milli-Q System or equivalent.
- 4.2 Hexane: Burdick & Jackson - capillary GC² solvent.
- 4.3 Methanol: Burdick & Jackson - capillary GC² solvent.
- 4.4 Sodium Thiosulfate (Na₂S₂O₃) : ACS grade.
- 4.5 Stock standards of target pesticides - two vendors - Ultra Scientific as primary - Supelco.
- 4.6 Sodium Chloride - ACS grade - stored in a glass container

5. SAMPLE COLLECTION, PRESERVATION AND HANDLING

- 5.1 Samples are collected in amber 250ml bottles, fitted with a screw cap lined with teflon. If the samples contain residual chlorine add 60 mg/L of sodium thiosulfate. All samples must be chilled and delivered to the laboratory at 4°C. All samples must be extracted within 7 days after collection. The extract should be analyzed within 14 days after extraction.

6. EXTRACTION

- 6.1 Pre-rinse the 80ml test tube with approximately 2 mls of hexane - discard and let dry. Rinse the volumetric flask with three small aliquots of sample. After allowing the sample to come to room temperature, transfer 50 mls of the sample to a

volumetric flask. Adjust to the volumetric line. Add 8.6 grams of sodium chloride to the flask. Add 3.0 mls of GC² grade hexane to the top of the volumetric flask. There should be enough head space to provide adequate mixing. Shake the flask vigorously for 2 minutes. Allow the phases to separate (15 minutes) and then transfer the hexane layer to two autosampler system.

7. ANALYSIS

- 7.1 EPA method 505 is used to monitor for low level of chlorinated pesticides in ground water. Because of these low levels, any type of contamination or interferences can cause analytical problems. Thus, reagent blanks must be monitored for every extraction run -monitoring of reagent blanks is essential to the success of this method. If results for reagent blanks rise above this level, corrective actions must be performed. Analyze a 5-point calibration at the beginning of each analytical run. Verify the calibration by measurement of two calibration check standards, one at the beginning and one at the end of the run. These check standards should be at two different concentration levels to verify the calibration curve. LFBs (laboratory fortified blanks) should also be analyzed with each extraction run. Standards used for these QA/QC samples must be ordered from a second source whenever possible. For extended runs, check standards should be interspersed with samples at regular intervals. If the response of any analyte varies from the predicted response by more than +/- 20%, test must be repeated using fresh calibration standards. The five point calibration should be 0.01, 0.10, 0.20, 0.50, and 1.0 ppb. Any results above 1 ppb must be confirmed with a standard that is within +/- 20% of the actual result. For Toxaphene, a separate calibration curve must be analyzed at 1.00, 2.00, 4.00, 6.00, 8.00 ppb.

Instrument Conditions:

1. Initial column temperature: 180°C
2. Hold time: 1 minute
3. Final temperature: 260°C
4. Temperature rate: 4°C/minute
5. Hold time: 9 minutes
6. Helium linear velocity: 25 cm/sec
7. Splitless injection with 45 second delay
8. Injector temperature: 200°C
9. Detector temperature: 300°C

8. QA/QC REQUIREMENTS

- 8.1 Laboratory Reagent Blank - Run before samples. Use to demonstrate that all glassware and reagent interferences are under control. If any contamination peaks are produced, determine source of contamination and eliminate interference.
- 8.2 Laboratory Fortified Blank - Must analyze at least two laboratory fortified blank

(LFB) one after every calibration and one at the end of the analysis. The fortification concentration of each analyte should confirm the ability to detect at the reportable level. If the recovery of any analyte falls outside the control limits, the source of the problem must be identified and corrected.

- 8.3 Standards - Verify calibration standards quarterly by analyzing a standard prepared from reference material obtained from an independent or second source. EPA performance evaluations are an excellent process to determine the validity of the method. Results must be within $\pm 20\%$ of those used to routinely check calibration. Daily, run a standard to check the reportable detection level, RDL. Endrin's area count should be monitored closely in order to control breakdown products. This will be a good indicator of the column's condition as well as the cleanliness of the glass insert area.
- 8.4 Samples - Samples must be extracted within 7 days after collection. Samples must be stored at 4°C until ready for extraction. Duplicates are run on 10% of samples, or once during run, whichever is greater. Results should be within $\pm 20\%$. Extracts should be analyzed within 14 days after extraction. All positive identifications must be confirmed using the confirmation column or GC/MS method 525.
- 8.5 Spike Recoveries - The laboratory must add a known concentration of spike solution, the same as used for LFB, to at least 10% of samples or once per analytical run, whichever is greater. Recoveries should be within the acceptable range. Wherever possible, run a second source standard for spikes.
- 8.6 QC Requirements - Analyze EPA QC check sample with known values if available. The results for each analyte must be within the EPA acceptance criteria. Semi-annually, analyze EPA Performance Evaluation samples. Analyze these check samples whenever major maintenance to the system occurs to ensure the validity of the method.
- 8.7 Endrin and DDT - Track the degradation of both targets on the correct % degradation form - for each analytical system. Breakdown must be adequately consistent during the analysis run. Monitor all spikes and standards for degradation. When consistency is lost - service the injection port of the instrument.

9. PREVENTIVE MAINTENANCE AND CORRECTIVE ACTIONS

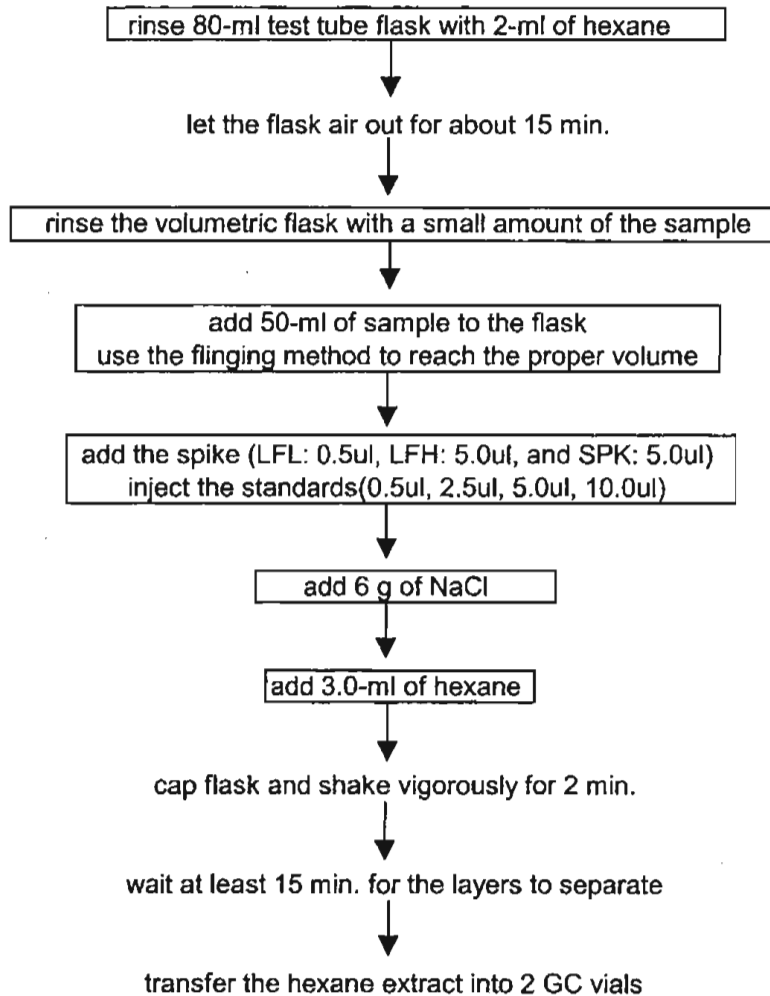
- 9.1 Glassware - Glassware must be carefully cleaned. Clean all glassware as soon as practical after use by thoroughly rinsing with last solvent used in it. Follow by washing with hot water and detergent and thoroughly rinsing with tap water followed by reagent water. After drying, heat in oven. Do not heat volumetric glassware above 220°C . Thorough rinsing with acetone may be substituted for heating.
- 9.2 Reagents - The use of high purity solvents and reagents will help to minimize

contamination problems. The most common problems with this method are largely due to any impurities in the extracting solvent, which act as interferences. Always try to batch order the hexane by lot number. Each time a new batch of hexane is received a complete QA/QC analysis must be performed.

- 9.3 Carryover - Contamination carryover may occur when a sample containing a low concentration of analytes is analyzed immediately following a sample containing a high concentration. Use solvent rinses between samples to minimize carryover.
- 9.4 All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory method blanks. Minimize contact of the samples, reagents, or solvents with any plastics. This will help reduce contamination. Interferences by phthalate esters can pose a major problem in pesticide analysis when using the electron capture detector.
- 9.5 Record all corrective actions in the maintenance log book. Include a complete description of the problem and what action were taken to correct it.

[illegible]

METHOD 505



ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

EPA Method 506

DETERMINATION OF PHTHALATE AND ADIPATE ESTERS IN DRINKING WATER BY LIQUID-LIQUID OR LIQUID SOLID EXTRACTION AND GAS CHROMATOGRAPHY WITH PHOTOIONIZATION DETECTION

File Name: M:\SOP\Organic\epa method sop\506_1101_SPE.doc
Revision: 2

Effective Date: 11/01/2001
Supersedes: 1 (10/01/1995)

1. SUMMARY OF METHOD

EPA method 506 is used for the determination of phthalates and adipate esters. A one liter sample is extracted using a C18 disk. An automated extraction system is used – Horizon Technology (SPE-DEX 4700). The organic compounds are eluted from the disk with acetonitrile and methylene chloride. The eluant is then concentrated to 1 mL and analyzed on a Varian 3400 gas chromatograph with a Photoionization Detector (PID).

2. ANALYTES

<u>LIMS code</u>	<u>Analyte</u>
DEHP	Bis (2-ethylhexyl) phthalate
BBP	Butylbenzyl phthalate
DnBP	Di-n-butyl phthalate
DEP	Diethyl phthalate
DMP	Dimethyl phthalate
DEHA	Bis (2-ethylhexyl) adipate
DnOP	Di-n-octyl phthalate

3. APPARATUS AND EQUIPMENT

- 3.1 Sample Bottles - Two liter amber glass bottles fitted with a screw cap lined with teflon.
- 3.2 Autosampler vials - equipped with Teflon-lined septum.
- 3.3 Concentrator Tube - Zymark 50 mL tubes used with the Zymark Turbo-Vap.
- 3.4 Analytical Balance: Capable of weighing accurately to nearest 0.0001 g.

- 3.5 Zymark Turbo-Vap - used to concentrate extracts.
- 3.6 3400 Varian gas chromatograph with dual columns - photoionization detectors. Equipped with an 8200 autosampler for injecting samples into the GC column.
- 3.7 Column: Fused Silica Capillary column, DB-5 30 meters long x 0.32 mm I.D. with a 0.25 micron film thickness. Alternate column - DB-1 30 meters long x 0.32 mm I.D. with a 0.25 micron film thickness.
- 3.8 Disposable Pasteur Pipets and graduated cylinders (1000ml, 100ml, and 10ml).

4. REAGENTS AND CONSUMABLE MATERIALS

- 4.1 Reagent Water - Millipore Milli-Q System or equivalent.
- 4.2 Methylene Chloride: Burdick & Jackson - capillary GC² solvent.
- 4.3 Acetonitrile: Burdick & Jackson - capillary GC solvent.
- 4.4 Methanol: Burdick & Jackson - capillary GC² solvent.
- 4.5 Sodium Sulfate: (ACS) Granular, anhydrous. Heat sodium sulfate at 752°F four hours, and store sodium sulfate at 130°C.
- 4.6 Sodium Thiosulfate (Na₂S₂O₃) : ACS grade.
- 4.7 Liquid-Solid Extraction (LSE) disks : Empore 3M, C18 47mm disks
- 4.8 Acetone: Burdick & Jackson - GC grade

5. SAMPLE COLLECTION, PRESERVATION AND HANDLING

- 5.1 Samples are collected in amber two liter bottles, fitted with a screw cap lined with teflon. If the samples contain residual chlorine add 80 mg/L of sodium thiosulfate. After the sample is collected in a bottle containing preservative, seal the bottle and shake for one minute. Store samples at 4°C. All samples must be extracted within 14 days after collection. The extract should be analyzed within 14 days after extraction.

6. EXTRACTION

- 6.1 The District uses the automated extraction unit (Horizon Technology – SPE-DEX 4700). A separate SOP has been developed to utilize this system for 506 samples. Please refer to that SOP for more details. The automated system's extractors are purged before each run to eliminate any type of contamination or carryover between samples. The disk is placed in the automated unit and the system will

automatically condition each disk by the approved EPA methodology.

- 6.2 Transfer one liter of sample to the specified auto-extractor 1-liter bottles. Add 5 mls of methanol per liter of sample to be extracted (0.5% methanol in sample). Process the sample based on the automated method. The automated system is setup to process all samples in the same manner.
- 6.3 The automated system will process each sample – rinsing the sample bottle during the elution step. Dry the extract with 5 grams of anhydrous sodium sulfate. Rinse the drying tube and sodium sulfate with two 5 ml portions of CH₃CN and transfer into a Zymark concentrator tube.
- 6.4 Concentrate the sample to between 0.5 ml and 1.0 mls – do not concentrate the extract to less than 0.5 ml. Make any volume adjustments with CH₃CN – to the 1.0 ml marked level. Transfer the 1.0 ml extract into a autosampler vial and store in the sample extract refrigerator at 4 °C.

7. ANALYSIS

- 7.1 Phthalates are common laboratory contaminants and this method is very susceptible to high backgrounds of specific targets. Monitoring of reagent blanks is essential to the success of this method. The reporting detection level of all targets for this method is 2.0 ug/L. Background levels should never exceed 1.0 ppb. If results for reagent blanks rise above this level corrective actions must be performed. Do a Laboratory Reagent Blank and a 5-point calibration at the beginning of each analytical run. Verify the calibration by measurement of two calibration check standards, one at the beginning and one at the end of the run. These check standards should be at two different concentration levels to verify the calibration curve. For extended runs, check standards should be interspersed with samples at regular intervals. If the response of any analyte varies from the predicted response by more than +/- 20%, test must be repeated using fresh calibration standards. The five point calibration should be 1.0, 2.0, 5.0, 7.5 and 10 ppb. Any results above 10 ppb must be confirmed with a standard that is within +/- 20% of the actual result.

Instrument Conditions:

1. Initial column temperature: 100°C
2. Hold time: 1 minute
3. Final temperature: 270°C
4. Temperature rate: 10°C/minute
5. Hold time: 8 minutes
6. Helium linear velocity: 29 cm/sec
7. Splitless injection with 45 second delay
8. Injector temperature: 275°C
9. Detector temperature: 275°C

8. QA/QC REQUIREMENTS

- 8.1 Laboratory Reagent Blank - Run before samples. Use to demonstrate that all glassware and reagent interferences are under control. If any contamination peaks are produced, determine source of contamination and eliminate interference.
- 8.2 Laboratory Fortified Blank - Must analyze at least two laboratory fortified blank (LFB) one after every calibration and one at the end of the analysis. The fortification concentration of each analyte should confirm the ability to detect at the reportable level. If the recovery of any analyte falls outside the control limits, the source of the problem must be identified and corrected.
- 8.3 Standards - Verify calibration standards quarterly by analyzing a standard prepared from reference material obtained from an independent or second source. EPA performance evaluations are an excellent process to determine the validity of the method. Results must be within +/- 20% of those used to routinely check calibration. Daily, run low and high level standards to check the reportable detection level, RDL.
- 8.4 Samples - Samples must be extracted within 14 days after collection. Samples must be stored at 4°C until ready for extraction. Duplicates are run on 10% of samples, or once during run, whichever is greater. Results should be within +/- 20%. Extracts should be analyzed within 14 days after extraction.
- 8.5 Spike Recoveries - The laboratory must add a known concentration of spike solution, the same as used for LFB, to at least 10% of samples or once per analytical run, whichever is greater. Recoveries should be within the acceptable range. Wherever possible, run a second source standard for spikes.
- 8.6 QC Requirements - Analyze EPA QC check sample with known values if available. The results for each analyte must be within the EPA acceptance criteria. Semi-annually, analyze EPA Performance Evaluation samples. Analyze these check samples whenever major maintenance to the system occurs to ensure the validity of the method.

9. PREVENTIVE MAINTENANCE AND CORRECTIVE ACTIONS

- 9.1 Glassware - Glassware must be carefully cleaned. Clean all glassware as soon as practical after use by thoroughly rinse. Follow by washing with hot water and 10% HCl and thoroughly rinsing with tap water followed by reagent water. After drying, heat in oven. Do not heat volumetric glassware. Thorough rinsing with acetone may be substituted for heating.
- 9.2 Reagents - The use of high purity solvents and reagents will help to minimize contamination problems.
- 9.3 Carryover - Contamination carryover may occur when a sample containing a low concentration of analytes is analyzed immediately following a sample containing a high concentration. Use solvent rinses between samples to minimize carryover.
- 9.4 Minimize contact of the samples, reagents, or solvents with any plastics. This will help reduce contamination.
- 9.5 Record all corrective actions in the maintenance log book. Include a complete description of the problem and what action were taken to correct it.
- 9.6 No or Poor Chromatography-
 - 9.6.1 Check all the standards.
 - 9.6.2 Request re-sample if it's necessary.
 - 9.6.3 If there's hits, confirm with GC/MS using second Column.

SOP PROCEDURE CHANGE

[illegible]

METHOD 506, Revision 1.1 (REVISED 09/15/04)

Refrigerate D.I water overnight in 4L bottle

•

Purge each extractor unit using **purge method 550.9**

•

Transfer samples into a 1L amber bottle with 5mL of methanol

•

Add the spike(LFL: 2ul, LFH: 5ul, Spike: 5ul [concentration 1000ug/ml]

Cover bottle with foil and adaptor

•

Assemble C-18 Empore disk

•

load bottles into each extractor

•

Start extracting using **extract method 550.1**

•

after the sample has been extracted, transfer to a 125 ml erl-flask with 10g of Na₂SO₄

rinse the tube with 2ml of Acetonitrile twice

•

repeat the above rinse step with 2ml aliquot of CH₂CL₂ two more times

•

after 4th rinse, wait for 15 minutes.

•

transfer to small Zymark tube

rinse the erl-flask with two-2 ml of acetonitrile, add the rise to the Zymark tube

•

repeat the above rinse with two 2ml aliquots of CH₂CL₂ 2 more times

•

Zymark settings: Temp: 40, Pressure: 9 psi

•

concentrate the extract to 1ml and transfer to an amber autosampler vial

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

EPA Method 507 - Revision 2.3

DETERMINATION OF NITROGEN AND PHOSPHOROUS CONTAINING PESTICIDES IN WATER BY GAS CHROMATOGRAPHY WITH A NITROGEN- PHOSPHOROUS DETECTOR

File Name: M:\SOP\Organic\epa method sop\507_110409.doc Effective Date: 11/04/2009
Revision: 9 Supersedes: 8 (10/15/2009)

1. SUMMARY OF METHOD

This method is used to determine nitrogen and phosphorus containing pesticides in groundwater. A one-liter volume of sample is extracted with methylene chloride in a separatory funnel. The methylene chloride extract is then transferred to a KD apparatus, where it is then exchanged into MTBE. The final extract is brought to a volume of 2 mL and analysis is performed by a GC equipped with a nitrogen-phosphorus detector.

2. ANALYTES

2.1 This is a gas chromatographic (GC) method applicable to the determination of certain nitrogen-phosphorus containing pesticides in groundwater and finished drinking water. The following compounds are determined using this method:

<u>LIMS code</u>	<u>analyte</u>
ATRAZ	Atrazine
BROMAC	Bromacil
DIAZI	Diazinon
MOLINT	Molinate
PROMET	Prometryn
PROPAZ	Propazine
MALATH	Malathion
SIMAZ	Simazine
BUTACL	Butachlor
METOCL	Metolachlor
MTRBZN	Metribuzin
PROPCL	Propachlor
MPARA	methyl-Parathion
THIO	Thiobencarb
PARA	Parathion
DMTH	Dimethoate

PROMTN	Prometon
CAFFEI	Caffeine
ALACHL	Alachlor
EPTC	EPTC
NORFLR	Norflurazon
TRBACL	Terbacil
ETHION	Ethion

3. APPARATUS AND EQUIPMENT

- 3.1 Sample Bottles - Two liter amber glass bottles fitted with a screw cap lined with Teflon.
- 3.2 Autosampler vials - equipped with Teflon-lined septum.
- 3.3 KD - concentrators & three ball Snyder tubes.
- 3.4 Analytical Balance: Capable of weighing accurately to nearest 0.0001 g.
- 3.5 Volumetric flasks.
- 3.6 3500 Varian gas chromatograph with dual columns - Nitrogen-Phosphorus detector (NPD). Equipped with an 8100 autosampler for injecting samples into the GC.
- 3.7 Column: Fused Silica Capillary column, DB-5 30 meters long x 0.25 mm I.D. with a 0.25 micron film thickness. Alternate column - DB-1701 30 meters long x 0.25 mm I.D. with a 0.25 micron film thickness.
- 3.8 Disposable Pasteur Pipettes and graduated cylinders (1000ml, 100ml, and 10ml).

4. REAGENTS AND CONSUMABLE MATERIALS

- 4.1 Reagent Water - Millipore Milli-Q System or equivalent.
- 4.2 Methylene Chloride: Burdick & Jackson - capillary GC² solvent.
- 4.3 Methyl tert-Butyl ether (MTBE): Burdick & Jackson - capillary GC² solvent.
- 4.4 Methanol: Brudick & Jackson - capillary GC² solvent.
- 4.5 Sodium Sulfate: (ACS) Granular, anhydrous. Heat sodium sulfate at 400°C four hours, and store the sodium sulfate in a glass container when cooled.
- 4.6 Sodium Thiosulfate (Na₂S₂O₃): ACS grade.

- 4.7 Acetone: Burdick & Jackson - capillary GC solvent.
- 4.8 Phosphate buffer, pH 7 - Prepare by dissolving 45.76 g of K₂HPO₄ in some DI water. Add 12.4 ml of HCl and dilute mixture to 2-L
- 4.9 Sodium chloride, crystal, ACS grade - Heat treat in a shallow tray at 450°C for a minimum of 4 hours to remove interfering organic substances.
- 4.10 Sodium thiosulfate, granular, anhydrous, ACS grade.
- 4.11 2-Nitrotoluene solution (22.5 ul /100ml acetone) - for use as internal standard.
- 4.12 2-Nitro-m-xylene solution (22.5 ul/100ml acetone) - for use as surrogate standard.

5. SAMPLE COLLECTION, PRESERVATION AND HANDLING

- 5.1 Samples are collected in amber 2.5 liter bottles, fitted with a screw cap lined with Teflon. If the samples contain residual chlorine add 60 mg/L of sodium thiosulfate. Store samples at 4°C. All samples must be extracted within 14 days after collection. The extract should be analyzed within 14 days after extraction.

6. EXTRACTION

- 6.1 In a 2-L separatory funnel, add 100 g of NaCl and 25 ml of phosphate buffer. Transfer one liter of the sample to a separatory funnel. Shake the sample to dissolve the salt. Spike the sample with the surrogate standard spiking solution; spike the QC with the appropriate standards. Add 60 ml of methylene chloride to the separatory funnel that contained the sample, and extract the sample by vigorously shaking the funnel for 2 minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration through glass wool, centrifugation, or other physical methods. Collect the methylene chloride extract in a 500 ml Erlenmeyer flask containing approximately 7 grams of anhydrous sodium sulfate.
- 6.2 Add a second 60 ml volume of methylene chloride to the separatory funnel and repeat the extraction procedure a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner. Swirl flask to dry extract; allow flask to sit for 15 minutes. Assemble a K-D concentrator by attaching a 25 ml concentrator tube to a 500 ml evaporative flask. Decant methylene chloride extract into the K-D concentrator. Rinse the remaining sodium sulfate with two 25

ml portions of methylene chloride and decant rinses into the K-D concentrator. Add 1 to 2 clean boiling stones to the evaporative flask and attach a macro Snyder column. Pre-wet the Snyder column by adding about 1 ml of methylene chloride to the top. Place the K-D apparatus on a hot water bath (65 to 70°C) so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation the balls of the column will actively chatter, but the chambers will not flood. When the apparent volume of liquid reaches 2 ml, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.

- 6.3 Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 ml of MTBE. Add 10 ml of MTBE and a fresh boiling stone. Attach a micro-Snyder column to the concentrator tube and pre-wet the column by adding about 0.5 ml of MTBE to the top. Place the micro K-D apparatus on the water bath so that the concentrator tube is partially immersed in hot water. Adjust the vertical position of the apparatus and the water temperature as required to complete concentration in 5 to 10 minutes. When the apparatus volume of liquid reaches 1 ml, remove the micro K-D from the bath and allow it to drain and cool. Repeat this process once. After the second MTBE exchange, remove the micro-Snyder column, and rinse the walls of the concentrator tube while adjusting the volume to 2.0 ml with MTBE. Add the appropriate amount of internal standard spiking solution to the sample extract, seal, and shake to distribute the internal standard. Transfer the extract to an appropriate sized TFE-fluorocarbon-sealed, screw-cap vial and store. Refrigerate at 4°C until analysis by GC-NPD.

7. ANALYSIS

- 7.1 EPA method 507 is used to monitor for low levels of nitrogen-phosphorus containing pesticides in ground water. Because of these low levels, any type of contamination or interferences can cause analytical problems. Thus, reagent blanks must be monitored for every extraction run - monitoring of reagent blanks is essential to the success of this method. If results for reagent blanks rise above this level corrective actions must be performed. Analyze a 5-point calibration at the beginning of each analytical run. Verify the calibration by measurement of two calibration check standards, one at the beginning and one at the end of the run. These check standards should be at two different concentration levels to verify the calibration curve. LFBs (laboratory fortified blanks - (low:0.1 ppb and high:1.0 ppb)) should also be analyzed with each extraction run. Standards used for these QA/QC samples must be ordered from a second source whenever possible. For extended runs, check standards should be interspersed with samples at regular intervals. If the response of any analyte varies from the predicted response by more than +/- 30%, test must be repeated using fresh calibration standards. The five point calibration should be 0.1, 0.25, 0.5, 1.0, and 2.0 ppb. Any results above 2 ppb must

be confirmed with a standard that is within +/- 20% of the actual result.

Instrument Conditions:

1. Column temperature:

<u>Temp (°C)</u>	<u>Rate (°C)</u>	<u>Hold (min.)</u>	<u>Total (min.)</u>
60	----	0.5	0.5
160	25	5	9.5
188	3	0	18.83
207	20	0	19.78
300	50	2	23.64

2. Helium linear velocity: 38 cm/sec

3. Injection: Inject with 2ul

<u>Time</u>	<u>Split Status</u>	<u>Split Ratio</u>
Initial	ON	20
0	OFF	OFF
0.7	ON	100
3	ON	20

4. Injector temperature: 220°C

5. Detector: NPD

6. Hydrogen Flow: 4.5 ml/minute

7. Air: 175 ml/minute

8. QA/QC REQUIREMENTS

8.1 A Laboratory Performance Check solution (LPC) – shall be analyzed before each analytical run to check that the system is within specification. The LPC for 507 – checks for the following criteria; Sensitivity: $S/N > 3$, Chromatographic performance: $0.80 < PGF < 1.20$, and Column performance: $resolution > 0.7$ - by using a mixture of the following targets – Vernolate, Bromacil, Prometon, and Atrazine – from Ultra Scientific. All criteria must be within specifications before analysis can be performed. See LPC form for calculations. A Laboratory Reagent Blank (RB) shall also be analyzed before samples. Use the RB to demonstrate that all glassware and reagent interferences are under control. If any contamination peaks are produced, determine source of contamination and eliminate interference.

8.2 A Calibration Check sample (second source) shall be run immediately after the

calibration curve to ensure the calibration curve is valid. A Laboratory Fortified Blank - Must analyze at least two laboratory fortified blank (LFB) one after every calibration. Two standards (at the low lfb and lfb level) must be analyzed at the end of the analysis. The fortification concentration of each analyte should confirm the ability to detect at the reportable level. If the recovery of any analyte falls outside the control limits, the source of the problem must be identified and corrected.

- 8.3 Standards - Verify calibration standards quarterly by analyzing a standard prepared from reference material obtained from an independent or second source. EPA performance evaluations are an excellent process to determine the validity of the method. Results must be within +/- 20% of those used to routinely check calibration. Daily, run a low level standard to check the reportable detection level, RDL.
- 8.4 Samples - Samples must be extracted within 14 days after collection. Samples must be stored at 4°C until ready for extraction. Duplicates are run on 10% of samples, or once during run, whichever is greater. Results should be within +/- 30%. Extracts should be analyzed within 14 days after extraction. All positive identifications must be confirmed using the confirmation column or GC/MS method 525.
- 8.5 Spike Recoveries - The laboratory must add a known concentration of spike solution @ 1.0 ppb, the same as used for LFB, to at least 10% of samples or once per analytical run, whichever is greater. Recoveries should be within the acceptable range +/- 30%. Wherever possible, run a second source standard for spikes.
- 8.6 QC Requirements - Analyze EPA QC check sample with known values if available. The results for each analyte must be within the EPA acceptance criteria. Annually, analyze EPA Performance Evaluation samples. Analyze these check samples whenever major maintenance to the system occurs to ensure the validity of the method.

9. PREVENTIVE MAINTENANCE AND CORRECTIVE ACTIONS

- 9.1 Glassware - Glassware must be carefully cleaned. Clean all glassware as soon as practical after use by thoroughly rinsing with last solvent used in it. Follow by washing with hot water and 10% HCl and thoroughly rinsing with tap water followed by reagent water. After drying, heat in oven. Do not heat volumetric glassware above 220°C. Thorough rinsing with acetone may be substituted for heating.
- 9.2 Reagents - The use of high purity solvents and reagents will help to minimize contamination problems.
- 9.3 Carryover - Contamination carryover may occur when a sample containing a low

concentration of analytes is analyzed immediately following a sample containing a high concentration. Use solvent rinses between samples to minimize carryover.

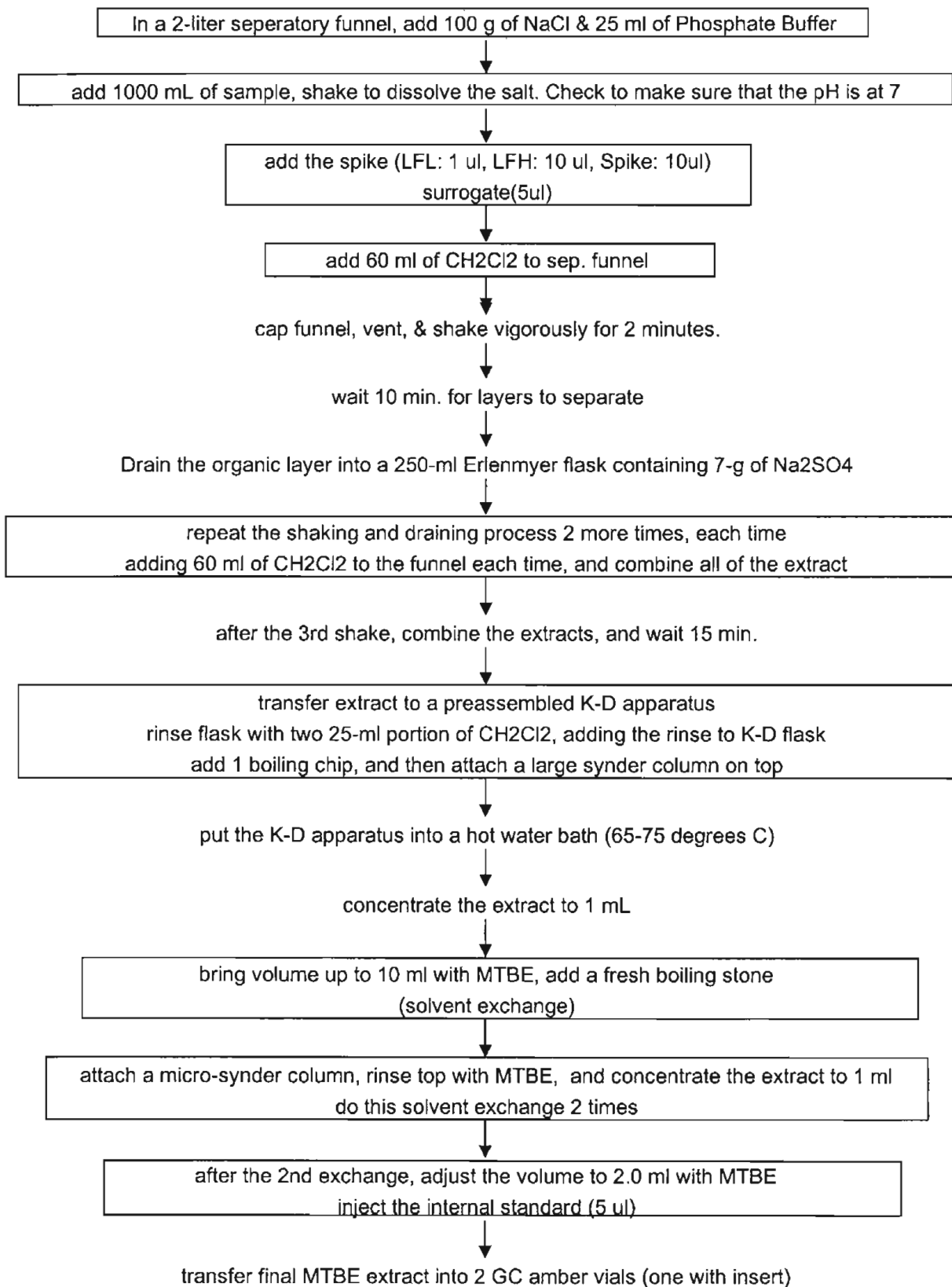
- 9.4 All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory method blanks.
- 9.5 Record all corrective actions in the maintenance log book. Include a complete description of the problem and what action were taken to correct it.

10. NO OR POOR CHROMATOGRAPHY

- 10.1 Check all the standards. If calibration and/or QC lie outside the acceptable limits we must first re-run the standards to assure that the injection procedure is working properly. After the re-run if standards still lie outside the acceptable limits we must make a new standard or conduct instrument maintenance to assure that calibration and QC lie with acceptable parameters. For elevated spike recoveries make sure that the sample matrix is clean. If sample matrix is altering recoveries then a new matrix must be selected. If instrument performance is acceptable the samples must be re-extracted. If the sample lies outside allowable holding time they must be re-sampled.
- 10.2 Request a re-sample if sample has unusual hits.

[illegible]

METHOD 507, Rev. 2.1



ORANGE COUNTY WATER DISTRICT

EPA METHOD 507

Standard Preparation and Quality Control

Final Solvent ---- MTBE

The following analytes and concentrations apply to the calibration standards.

1A. Calibration Standard: Ultra Scientific, Catalog # CUS-11111 - Conc. Varies (see below)

<u>Analytes</u>	<u>Concentration</u> <u>ug/mL</u>	<u>Analytes</u>	<u>Concentration</u> <u>ug/mL</u>	<u>Analytes</u>	<u>Concentration</u> <u>ug/mL</u>
Alachlor	100	EPTC	100	Parathion	100
Atrazine	100	Ethion	100	Prometon	100
Benthiocarb	100	Malathion	300	Prometryne	100
Bromacil	300	Methyl Parathion	100	Propachlor	100
Butachlor	300	Metolachlor	300	Propazine	100
Caffeine	100	Molinate	100	Simazine	100
Diazinon	100	Norflurazon	100	Terbacil	100
Dimethoate	300				

1B. Calibration Standard: Ultra Scientific, Catalog # PST-1535M100A01 - Analyte: Metribuzin; Conc: 100ug/mL

<u>Analytes</u>	<u>Concentration</u> <u>ug/mL</u>
Metribuzin	100

The following analytes and concentrations apply to the 2nd source standard.

2. 2nd Source Standard: SPEX, Catalog # XQ-1315 - Conc. Varies (see below)

<u>Analytes</u>	<u>Concentration</u> <u>ug/mL</u>	<u>Analytes</u>	<u>Concentration</u> <u>ug/mL</u>	<u>Analytes</u>	<u>Concentration</u> <u>ug/mL</u>
Alachlor	100	EPTC	100	Parathion	100
Atrazine	100	Ethion	100	Prometon	100
Benthiocarb	100	Malathion	300	Prometryne	100
Bromacil	300	Methyl Parathion	100	Propachlor	100
Butachlor	300	Metolachlor	300	Propazine	100
Caffeine	100	Metribuzin	100	Simazine	100
Diazinon	100	Molinate	100	Terbacil	100
Dimethoate	300	Norflurazon	100		

3. Surrogate Standard: Aldrich, Catalog # N2, 835-3

Working Standard: Dilute 43 uL of 2-Nitro-m-xylene in 10 mL acetone.

4. Internal Standard: Aldrich, Catalog # N2, 730-6

Working Standard: Dilute 22.5 uL of 2-Nitrotoluene in 10 mL acetone.

A. Calibration Standards ---- Ultra Scientific

<u>Standard</u> <u>Concentration</u>	<u>Volume of</u> <u>CAL std 1A</u>	<u>Volume of</u> <u>CAL std 1B</u>	<u>Volume of</u> <u>Surrogate</u>	<u>Volume of</u> <u>Internal Std</u>	<u>Final Volume</u>	<u>Surrogate</u> <u>Conc. ug/L</u>
STD A - 0.10 ug/L	1.0 uL	1.0 uL	1.0 uL	5 uL	2 mL	2.5
STD B - 0.25 ug/L	2.5 uL	2.5 uL	2.5 uL	5 uL	2 mL	6.25
STD C - 0.50 ug/L	5.0 uL	5.0 uL	5.0 uL	5 uL	2 mL	12.5
STD D - 1.0 ug/L	10.0 uL	10.0 uL	10.0 uL	5 uL	2 mL	25
STD E - 2.0 ug/L	20.0 uL	20.0 uL	20.0 uL	5 uL	2 mL	50

B. Calibration Check Standard --- SPEX

<u>Standard</u> <u>Concentration</u>	<u>Volume of</u> <u>Standard</u>	<u>Volume of</u> <u>Surrogate</u>	<u>Volume of</u> <u>Internal Std</u>	<u>Final Volume</u>	<u>Surrogate</u> <u>Conc. ug/L</u>
1.0 ug/mL	10.0 uL	5.0 uL	5 uL	2 mL	12.5

C. Low LFB, LFB, Spike, and Spike Duplicate --- SPEX

<u>Standard</u> <u>Concentration</u>	<u>Volume of</u> <u>Standard</u>	<u>Volume of</u> <u>Surrogate</u>	<u>Volume of</u> <u>Internal Std</u>	<u>Final Volume</u>	<u>Surrogate</u> <u>Conc. ug/L</u>
Low LFB - 0.1 ug/L	1.0 uL	5.0 uL	5 uL	2 mL	12.5
LFB - 1.0 ug/L	10.0 uL	5.0 uL	5 uL	2 mL	12.5
Spike - 1.0 ug/L	10.0 uL	5.0 uL	5 uL	2 mL	12.5
Spike Dup - 1.0 ug/L	10.0 uL	5.0 uL	5 uL	2 mL	12.5

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

EPA Method 508

DETERMINATION OF CHLORINATED PESTICIDES IN WATER BY GAS CHROMATOGRAPHY WITH AN ELECTRON CAPTURE DETECTOR

File Name: M:\Sop\Organic\epa method sop\508_012908.doc Effective Date: 01/29/08
Revision: 9 Supersedes: 8 (10/09/2007)

1. SUMMARY OF METHOD

A measured volume of sample of approximately 1 liter is solvent extracted with methylene chloride by shaking in a separatory funnel. The Methylene chloride extract is isolated, dried, and concentrated to a volume of 5 ml after exchanging into methyl tert-butyl ether (MTBE). Chromatographic conditions are described which permit the separation and measurement of the analytes in the extract by GC with an electron capture detector (ECD). 2.0 ul of concentrated sample is injected to the ECD for analysis. EPA method 508 is used for the determination of chlorinated pesticides in groundwater and finished drinking water.

2. ANALYTES

2.1 This is a gas chromatographic (GC) method applicable to the determination of certain chlorinated pesticides in groundwater and finished drinking water. The following compounds can be determined using this method:

<u>LIMS code</u>	<u>Analyte</u>
ALACHL	Alachlor
ALDRIN	Aldrin
CIPYRI	Chlorpyrifos
CLTNIL	Chlorothalonil
DDD	4,4'-DDD
DDE	4,4'-DDE
DDT	4,4'DDT
DIELDR	Dieldrin
ENDOI	Endosulfan I
ENDOSL	Endosulfan sulfate
ENDRIN	Endrin
ENDR-A	Endrin Aldehyde
ENDR-K	Endrin Ketone
LINDNE	HCH-gamma (Lindane)
HEPTA	Heptachlor

HEPEPX	Heptachlor epoxide
METHOX	Methoxychlor
TOXA	Toxaphene
CIDANE	Chlordane
BHCa	HCH-alpha(Alpha-BHC)
BHCb	HCH-beta(Beta-BHC)
BHCd	HCH-delta(Delta-BHC)
ENDOI	Endosulfan II
CLDA	Chlordane - alpha
CLDG	Chlordane - gamma
CLBZLA	Chlorobenzilate
CLNEB	Chloroneb
DCPA	DCPA - Dacthal
ETRDZL	Etridiazole
PROPCL	Propachlor
TRFLRN	Trifluralin
PMTHRN	Permethrin - (total of cis/trans)
HEXCLB	Hexachlorobenzene
HCLCPD	Hexachlorocyclopentadiene
PCB16	PCB-1016
PCB21	PCB-1221
PCB32	PCB-1232
PCB42	PCB-1242
PCB48	PCB-1248
PCB54	PCB-1254
PCB60	PCB-1260
CAPTAN	Captan (added 9/2004)
TRITON	Trithion (added 9/2004)

3. APPARATUS AND EQUIPMENT

- 3.1 Sample Bottles – 2.5 liter amber glass bottles fitted with a screw cap lined with teflon.
- 3.2 Brown autosampler vials - equipped with Teflon-lined septum.
- 3.3 Concentrator Tube - Zymark 200 mL tubes used with the Zymark Turbo-Vap.
- 3.4 Analytical Balance: Capable of weighing accurately to nearest 0.0001 g.
- 3.5 Zymark Turbo-Vap - used to concentrate extracts.
- 3.6 CP 3800 Varian gas chromatograph with dual columns - electron capture detectors (ECD). Equipped with an CR 8400 autosampler for injecting samples into the GC.
- 3.7 Column: Fused Silica Capillary column, DB-5 30 meters long x 0.32 mm I.D. with a 0.25 micron film thickness. Alternate column - DB-1701 30 meters long x 0.32 mm I.D. with a 0.25 micron film thickness.

- 3.8 Disposable Pasteur Pipettes and graduated cylinders (1000ml, 100ml, 10ml and 5ml).

4. REAGENTS AND CONSUMABLE MATERIALS

- 4.1 Reagent Water - Millipore Milli-Q System or equivalent.
- 4.2 Methylene Chloride: Burdick & Jackson - capillary GC² solvent.
- 4.3 Methyl tert-Butyl ether (MTBE): Burdick & Jackson - capillary GC² solvent.
- 4.4 Methanol: Burdick & Jackson - capillary GC² solvent.
- 4.5 Sodium Sulfate: (ACS) Granular, anhydrous. Heat sodium sulfate at 400°C four hours, and store sodium sulfate at 130°C.
- 4.6 Sodium Thiosulfate (Na₂S₂O₃) : ACS grade.
- 4.7 Acetone: Burdick & Jackson - capillary GC solvent.
- 4.8 Phosphate buffer, pH 7 – In a 2L volumetric flask add and dissolve 45.76g K₂HPO₄ (dipotassium phosphate) in DI water, add 12.4ml HCl and dilute to 2L. Transfer to a 2.5L brown bottle for storage.
- 4.9 Sodium chloride, crystal, ACS grade - Heat treat in a shallow tray at 400°C for a minimum of 4 hours to remove interfering organic substances.
- 4.10 Sodium thiosulfate, granular, anhydrous, ACS grade.
- 4.11 Pentachloronitrobenzene (PCNB) - 100µg/ml from Ultra Scientific (Cat # PPS-130), for use as INTERNAL STANDARD.
- 4.12 4,4'-Dichlorobiphenyl (DCB) - 500µg/ml from Ultra Scientific (Cat #PPS-120), for use as SURROGATE STANDARD.

5. SAMPLE COLLECTION, PRESERVATION AND HANDLING

- 5.1 Samples are collected in amber 2.5 liter bottles, fitted with a screw cap lined with teflon. If the samples contain residual chlorine add 80 mg/L of sodium thiosulfate. After addition to the sample, seal the bottle and shake for one minute. Store samples at 4°C. All samples must be extracted within 7 days after collection. The extract should be analyzed within 14 days after extraction.

6. EXTRACTION

- 6.1 In a 2-L separatory add 100g of NaCl, 25ml of phosphate buffer and 1 liter of sample. Seal and shake the separatory to dissolve the salt. Spike the samples with 10 ul of the surrogate standard spiking solution. Add spiking solution at this time. Check pH. Add 60 ml of methylene chloride to the separatory funnel contained the sample. Extract the sample by vigorously shaking the funnel for 2 minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration through glass wool, centrifugation, or other physical methods. Collect the methylene chloride extract in a 250 ml Erlenmeyer flask containing approximately 7 grams of anhydrous sodium sulfate.
- 6.2 Add a second 60 ml volume of methylene chloride to the separatory funnel and repeat the extraction procedure a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner. Swirl flask to dry extract; allow flask to sit for 15 minutes. Transfer the extract to the 200 ml concentrator tube. Rinse the remaining sodium sulfate with one 25 ml portions of methylene chloride and decant rinses into the concentrator tube.
- 6.3 Setting the Turbo-Vap to 40 degrees and 12 psi, concentrate the extract to between 2-3 ml. Add 10 ml of MTBE and reduce down to between 2-3 ml. Repeat with two more 10 ml of MTBE to completely exchange the solvents – final volume 1ml (adjust the volume to 1ml with MTBE if needed). Transfer extract to a 5ml volumetric flask. Add 3ml MTBE to concentrator tube and rinse the tube thoroughly. Add the rinse to the volumetric flask, bring up the final volume to 5ml, and then add 5 ul of internal standard solution to the sample extract. Mix well and transfer the extract to 2 brown auto sampler vials and store. Refrigerate at 4°C until analysis by GC-ECD.

7. ANALYSIS

- 7.1 EPA method 508 is used to monitor for low level of chlorinated pesticides in ground water. Because of these low levels, any type of contamination or interferences can cause analytical problems. Thus, reagent blanks must be monitored for every extraction run - monitoring of reagent blanks is essential to the success of this method. If results for reagent blanks rise above this level corrective actions must be performed. Analyze a 5-point calibration at the beginning of each analytical run. Verify the calibration by measurement of two calibration check standards, one at the beginning and one at the end of the run. These check standards should be at two different concentration levels to verify the calibration curve. LFBs (laboratory fortified blanks) should also be analyzed with each extraction run. Standards used for these QA/QC samples must be ordered from a second source whenever possible. For extended runs, check standards should be interspersed with samples at regular intervals. If the response of any analyte varies from the predicted response by more

than +/- 20%, test must be repeated using fresh calibration standards. The five point calibration should be 0.01, 0.05, 0.10, 0.15 and 0.20 ppb. Any results above 0.20 ppb must be confirmed with a standard that is within +/- 20% of the actual result. 2 ul of sample is injected for analysis.

Instrument Conditions: **GC Method 1 for 508 Compounds.**

1. Column Temp: Initial column temperature: 60°C
Hold time: 0 min
Final temperatures: 140°C 225°C 280°C
Rate °C / min: 35 3 20
Hold times: 1 min 1 min 1 min
Total Run Time 3.29 32.62 36.37 min
2. Flow: Helium linear velocity: 45 cm/sec
Flow 2.2 ml/min
3. Injection: Injector Program: Initial On 10
0 Off Off
0.75 On 100
1 On 10

Injector temperature: 250°C hold 1 min.
4. Detector Temp: 310°C

Instrument Conditions: **GC Method 7 for BDE Compounds.**

1. Column Temp: Initial column temperature: 100°C
Hold time: 0 min
Final temperatures: 160°C 265°C 315°C
Rate °C / min: 35 13 60
Hold times: 0 min 1 min 19.38 min
Total Run Time 1.71 10.79 31 min
2. Flow: Helium linear velocity: 45 cm/sec
Flow 2.2 ml/min
3. Injection: Injector Program: Initial On 10
0 Off Off
0.75 On 100
1 On 10

Injector temperature: Initial 280°C hold 10 min.
Final 310°C hold 10 min
4. Detector Temp: 315°C

8. QA/QC REQUIREMENTS

- 8.1 Laboratory Reagent Blank - Run before samples. Use to demonstrate that all glassware and reagent interferences are under control. If any contamination peaks are produced, determine source of contamination and eliminate interference.
- 8.2 Laboratory Fortified Blank - Must analyze at least two laboratory fortified blank (LFB) one after every calibration and one at the end of the analysis. The fortification concentration of each analyte should confirm the ability to detect at the reportable level. If the recovery of any analyte falls outside the control limits, the source of the problem must be identified and corrected.
- 8.3 Standards -After calibrate the system with 5 point calibration standards, verify calibration standards by analyzing a standard prepared from reference material obtained from an independent or second source for daily analysis, one from the beginning and one at the end. EPA performance evaluations are an excellent process to determine the validity of the method. Results must be within +/- 30% of those used to routinely check calibration. Daily, run a low level standard to check the reportable detection level, RDL.
- 8.4 Samples - Samples must be extracted within 7 days after collection. Samples must be stored at 4°C until ready for extraction. Duplicates are run on 10% of samples, or once during run, whichever is greater. Results should be within +/- 20%. Extracts should be analyzed within 14 days after extraction. All positive identifications must be confirmed using the confirmation column or GC/MS method 525.
- 8.5 Spike Recoveries - The laboratory must add a known concentration of spike solution, the same as used for LFB, to at least 10% of samples or once per analytical run, whichever is greater. Recoveries should be within the acceptable range. Wherever possible, run a second source standard for spikes.
- 8.6 QC Requirements - The system must pass the Sensitivity, chromatographic, and column performance criteria. Before the sample analysis, inject 1 µl of Laboratory performance check solution from Ultra Scientific Cat # PPM-508 to verify the system suitability ; EPA Method 508, Revision 3.1. Analyze EPA QC check sample with known values if available. The results for each analyte must be within the EPA acceptance criteria. Annually, analyze EPA Performance Evaluation samples. Analyze these check samples whenever major maintenance to the system occurs to ensure the validity of the method.
- 8.7 Endrin and DDT degradation - Monitor the degradation of both Endrin and DDT by filling out the correct form for each instrument. Breakdown must be adequately consistent during the analysis run. Monitor all spikes and standards for degradation.

When consistency is lost - service the injection port of the instrument - (greater than 20% on either target).

9. PREVENTIVE MAINTENANCE AND CORRECTIVE ACTIONS

- 9.1 Glassware - Glassware must be carefully cleaned. Clean all glassware as soon as practical after use by thoroughly rinsing with last solvent used in it. Follow by washing with hot water and 10% HCl and thoroughly rinsing with tap water followed by reagent water. After drying, heat in oven. Do not heat volumetric glassware above 220°C. Thorough rinsing with acetone may be substituted for heating.
- 9.2 Reagents - The use of high purity solvents and reagents will help to minimize contamination problems.
- 9.3 Carryover - Contamination carryover may occur when a sample containing a low concentration of analytes is analyzed immediately following a sample containing a high concentration. Use solvent rinses between samples to minimize carryover.
- 9.4 All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory method blanks. Minimize contact of the samples, reagents, or solvents with any plastics. This will help reduce contamination. Interferences by phthalate esters can pose a major problem in pesticide analysis when using the electron capture detector.
- 9.5 Record all corrective actions in the maintenance logbook. Include a complete description of the problem and what action was taken to correct it.
- 9.6 No or Poor Chromatography-
 - 9.6.1 Check all the standards. If calibration, LPC, Degradation check and/or QC lie outside the acceptable limits we must first re-run the standards to assure that the injection procedure is working properly. After the re-run if standards still lie outside the acceptable limits we must make a new standard or conduct instrument maintenance to assure that calibration and QC lie with acceptable parameters. For elevated spike recoveries make sure that the sample matrix is clean. If sample matrix is altering recoveries then a new matrix must be selected. If instrument performance is acceptable the samples must be re-extracted. If the sample lies outside allowable holding time they must be re-sampled.
 - 9.6.2 Request re-sample if it is necessary.
 - 9.6.3 If there are hits, confirm with GC/MS

SOP PROCEDURE CHANGE
FOR EPA Method 508

<u>CHANGE</u>	<u>REASON</u>	<u>DATE</u>	<u>INITLS</u>
Laboratory performance check Solution (Ultra Scientific Cat # PPM-508)	Verify system suitability	01/02/01	LY
Calibration Check Standard	Checking the calibration Curve	01/02/01	LY
<hr/>			
--Added 9.6.1-----	audit suggestion-----	2/19/03-----	
<hr/>			
GC Temperature program & LVS---	Added BDEs compounds	9/15/04	
Injector Temperature	(Brominated Diphenyl Ethers)		
<hr/>			
Section 9.1 replace washing from detergent-with 10% HCL	We do not use detergent in washing glassware for this method.	9/15/04	lvs
<hr/>			
2 different GC methods for 508 compounds and BDEs and run them separated. -----	Get better QC results in shorter run for 508 compounds	6/13/05	lvs
<hr/>			
-Surrogate spiking solution concentration changed to 250ug/ml. 10 ul is now injected into the sample. - To bring the concentration of surrogate in the sample to the middle of the curve.			
<hr/>			
GGA-----		10/09/07	
-Surrogate five point calibration curve now has new concentrations- 0.5, 1.0, 2.5, 4.0, and 5.0 ppb. - Curve was adjusted due to a change in the concentration of the surrogate spiking solution.			
<hr/>			
-Internal spike concentration changed to 20ug/ml. 5 ul is now injected into the sample. -To allow an easier volume to measure by syringe.			
<hr/>			
- For the concentration step, the volume was changed from 1ml to 2-3ml. - Change was to help optimize concentration step, especially to aid in the recovery of early targets that may be more volatile.			
<hr/>			
--- Surrogate spiking solution concentration changed to 500ug/ml. 5ul is now injected into the sample. - Stock could be used instead of having to make a dilution. ----			
<hr/>			
08/25/08 GGA-----			
<hr/>			

EPA Method 508.1 Rev. 1.0 - Cartridge (pg 1 of 2)

Sample Volume: 1000 ml

QA/QC Sample Prep:

Prepare two 2.5L ambers of Millipore water preserved/w sodium sulfite and refrigerate.

Extraction Day - Transfer the preserved water to 1L bottles for QCs

Adjust to pH ≤ 2 for all samples by adding 6N HCl

Add 5 mL MeOH to each 1L sample

Add the Surrogate - 5ul of NDMA-d6 - Mix immediately until homogeneous

Low spk: 2 uL. - LFB,SPIKE,SPIKE DUP: 10 uL

Please read THIS NOTE before proceed w/ the extraction:

EPA - 508.1- Section 11.2.2

Cartridge Conditioning - This conditioning step is critical for recovery of analytes and have a marked effect on method precision and accuracy. If the cartridge goes dry during the conditioning phase, the conditioning must be started over. Once the conditioning has begun, the cartridge must not go dry until the last portion of the sample passes because analyte and surrogate recoveries may be affected.

CARTRIDGE CLEAN UP

Insert waste collection vials into the manifold

Add 5 ml of EtAc to the assembled SPE cartridge

soak for 1 minute, dry completely

Add 5 ml of CH₂Cl₂ to the assembled SPE cartridge

soak for 1 minute, dry completely

Remove waste collection vials from manifold. Pour waste into EtAc:CH₂Cl₂ waste bottle.

CARTRIDGE CONDITIONING - SAMPLE EXTRACTING

Add 10 mL of MeOH - soak for 30 seconds

**DO NOT let the methanol elute below the top of the cartridge packing
FROM THIS POINT - DO NOT LET THE CARTRIDGE GO DRY**

Rinse the cartridge with 10 mL DI water

Turn off the vacuum before the water level drops below the top edge of the packing

Fill the reservoirs w/ sample before turning on the vacuum

Adjust the pressure to 10 mL/min flow rate (50min/sample)

After the entire sample has been processed, pull air through for 10 minutes @ **full vacuum**

DO NOT let the cartridge dry longer the 10 minutes

CARTRIDGE ELUTION

Place the collection tubes into the extraction tank

Rinse the sample bottles with 5 mL EtAc and add to cartridge

Pulse vacuum to pull the solvent through the cartridge until it begins to drip

soak cartridge for 1 min.



EXTRACT CONCENTRATION



Zymark settings: Temp: 45, Pressure: 15 psi

concentrate the extract to 1-ml

Make sure the final volume is 1ml - if not bring up the volume w/ CH₂Cl₂

Inject 5 uL of Internal Standard (NDMA-d14) to extract

Transfer to 1 GC amber autosampler vial

Red

ORANGE COUNTY WATER DISTRICT

EPA METHOD 508

Prepare calibration and check standards for 508 mix & CAP/TRI separately

Final Solvent - MTBE

1. Calibration Standard: ULTRA SCI - Custom Mix: Catalog # CUS-6751

2. 2nd Source Standard: Spex - Custom Mix: Catalog # XQ-1701

Working Standard: dilute 200µl of stock to 1ml with MTBE - conc. = 2µg/ml

The following analytes & concentrations apply to both calibration and 2nd source standard - except

Endrin Aldehyde & Endrin Ketone are not in 2nd source (Spex) mix

Analyze	Conc. ug/mL	Analyze	Conc. ug/mL	Analyze	Conc. ug/mL
Alachlor	50	Endosulfan sulfate	10	Methoxychlor	10
Aldrin	10	Endosulfan II	10	HCH-alpha(alpha-HBC)	10
Chlorpyrifos	10	Etridiazole	10	HCH-beta(Beta-BHC)	10
Chlorothalonil	10	Endrin	10	HCH-delta(Delta-BHC)	10
4,4'-DDD	10	Endrin Aldehyde	10	Hexachlorocyclopentadiene	10
4,4'-DDE	10	Endrin Ketone	10	Propachlor	50
4,4'-DDT	10	HCH-gamma (Lindane)	10	Chlordane - alpha	10
Dieldrin	10	Heptachlor	10	Chlordane - gamma	10
Endosulfan I	10	Heptachlor epoxide	10	Chlorobenzilate	50
Chloroneb	50	Trifluralin	10	Hexachlorobenzene	10
DCPA - Dacthal	10	Permethrin(cis/trans)	50		

3. Captan and Trithion Standards: Restek, Catalog # 557836

Analyte	Conc. ug/mL
Captan	10 (added 9/2004)
Trithion	10 (added 9/2004)

Working Standard: Dilute 200 uL of stock to 1 mL with MTBE, concentration = 2.0 ug/mL

4. Surrogate Standard: Ultra Scientific, Catalog # PPS-120

4,4'-dichlorobiphenyl ---- stock concentration 500ug/mL

5. Internal Standard: Ultra Scientific, Catalog # PPS-130

PCNB8 ---- stock concentration 100 ug/mL

Working Standard: Dilute 200 uL of stock to 1 mL methanol, concentration = 20 ug/mL

A. CALIB. STANDARD: Ultra Scientific & Restek

Standard Conc.	Vol. of 508 Mix (10ug/mL)	Vol. of Cap/Tri (10ug/mL)	Vol. of Internal (20ug/mL)	Vol. of Surrogate (500ug/mL)	Final Dil. Vol.	Surrogate Conc. ug/L
Std A - 0.01 µg/L	1 µl	1 µl	5 µl	1 µl	5 mL	0.5
Std B - 0.05 µg/L	5 µl	5 µl	5 µl	2 µl	5 mL	1.0
Std C - 0.10 µg/L	10 µl	10 µl	5 µl	5 µl	5 mL	2.5
Std D - 0.15 µg/L	15 µl	15 µl	5 µl	8 µl	5 mL	4.0
Std E - 0.20 µg/L	20 µl	20 µl	5 µl	10 µl	5 mL	5.0

B. CALIBRATION CHECK: SPEX and Restek

Standard Conc.	Vol. of 508 Mix (10ug/mL)	Vol. of Cap/Tri (10ug/mL)	Vol. of Internal (20ug/mL)	Vol. of Surrogate (500ug/mL)	Final Dil. Vol.	Surrogate Conc. ug/L
0.10 µg/L	10 µl	10 µl	5 µl	5 µl	5 mL	2.5

C. LFBs & SPIKES:

Standard Conc.	Vol. of 508 Mix (10ug/mL)	Vol. of Cap/Tri (10ug/mL)	Vol. of Internal (20ug/mL)	Vol. of Surrogate (500ug/mL)	Final Dil. Vol.	Surrogate Conc. ug/L
Low LFB - 0.01 µg/L	5 µl of 2ug/mL	5 µl of 2ug/mL	5 µl	5 µl	5 mL	2.5
LFB - 0.10 µg/L	10 µl of 10ug/mL	10 µl of 10ug/mL	5 µl	5 µl	5 mL	2.5
Spike - 0.10 µg/L	10 µl of 10ug/mL	10 µl of 10ug/mL	5 µl	5 µl	5 mL	2.5
Spike Dup - 0.10 µg/L	10 µl of 10ug/mL	10 µl of 10ug/mL	5 µl	5 µl	5 mL	2.5

D. DEGRADATION CHECK (DDT & ENDRIN) - Ultra Scientific, Catalog # ISM-451

Stock concentration = 1ug/mL

Spike 20µl of stock to 1.0ml MTBE - conc. 0.1µg/ml

E. LAB PERFORMANCE CHECK (LPC) - Ultra Scientific - Catalog # PPM-508

Directly transfer the stock solution into a vial with a limited volume insert and run the analysis

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

EPA Method 510.1

DETERMINATION OF MAXIMUM TOTAL TRICHALOMETHANE FORMATION POTENTIAL

File Name: M:\SOP\Organic\epa method sop\510_1195.doc
Revision: 1

Effective Date: 11/01/1995
Supersedes: N/A

1. SUMMARY OF METHOD

This method is used to determine the maximum total trihalomethane potential in groundwater. Studies were conducted with the Mesa Consolidated Water District to determine THM (trihalomethanes) formation potential in groundwater after treatment with hydrogen peroxide and ozone. Six samples are taken per site in 300 ml narrow mouth amber glass bottles. They are collected free of headspace and stored at 4°C in the dark in an area free of THM contamination. All samples are processed within fourteen days of collection. The samples are chlorinated with a combined buffer/hypochlorite reagent then stored in the dark at 25°C for seven days. The free chlorine should initially be approximately 20 mg/L, and the free chlorine - after incubation - must be at least 0.2 mg/L. The samples are then dechlorinated and analyzed using EPA method 601/602 to determine trihalomethane concentration.

2. ANALYTES

2.1 The four trihalomethanes are listed below:

Chloroform
Bromodichloromethane
Dibromochloromethane
Bromoform

3. PRE-START UP

3.1 Reagents Needed

Sodium sulfite - ACS reagent grade
3,5-dihydroxybenzoic acid (DHBA) - 97% pure
Reagent Water - Millipore Super-Q Water or equivalent

Boric Acid - anhydrous - ACS reagent grade
Sodium Hydroxide - ACS reagent grade
Sodium Hypochlorite solution - 5.25%
See reagent list for EPA method 601/602

3.2 Chlorine Solution 5 g/L.

In an amber glass bottle, add 100 mL of a 5.25% NaOCl solution to 1000 ml of reagent water. Place this solution in an oven overnight at the boiling point of water, but do not allow the solution to boil. Analyze for free chlorine using Standard Method 408 D (DPD Ferrous Titrimetric Method). All analysis for free chlorine in this method will use STD Method 408 D. Cool and store at 4°C, wrapped in foil.

3.3 Blank Water.

To every liter of reagent water, add 1 mL of 1.0 N sodium hydroxide solution and 1 mL of a 5 g/L free chlorine solution. Alternatively, add enough of the chlorine solution to produce a blank water containing at least 5 mg/L free chlorine.

Place the blank water in an oven overnight at the boiling point of water, but do not allow the blank water to boil. Cool the blank water and neutralize with 1.0 N sulfuric acid. Analyze for free chlorine. Add more chlorine solution and repeat the boiling procedure if the concentration of free chlorine is below 0.2 mg/L. Dechlorinate a sample of blank water and analyze for trihalomethanes using EPA Method 601/602. If the total THM concentration is above 2.0 ug/L, boil and purge the blank water again. Repeat this step until the concentration of total THMs is less than 2 ug/L.

3.4 Combined Buffer/Hypochlorite Reagent

- 3.4.1 Dissolve 23.3 grams of dry NaOH in 2500 mL of reagent water.
- 3.4.2 Add 66.7 grams of boric acid.
- 3.4.3 Add 200 mL of a 5 g/L free chlorine solution or enough chlorine solution to produce a combined buffer/hypochlorite reagent solution containing approximately 500 mg/L free chlorine.
- 3.4.5 Place the reagent in an oven overnight at the boiling point of water, but do not allow the reagent to boil. Approximately 12 mL of this reagent added to 300 mL of sample should yield a pH of 9.2 and a free chlorine value of approximately 20 mg/L.

3.5 Dechlorinating Agent

- 3.5.1 Dissolve 2.0 grams of sodium sulfite in 100 ml of blank water.
- 3.5.2 Discard after 1 week.

3.6 **3,5-dihydroxybenzoic Acid Stock Solution**

3.6.1 Dissolve 0.154 grams of DHBA in 1000 mL of reagent water.

3.7 **Chlorination**

3.7.1 Pour 20 mL of sample out of the 300 mL bottle into a beaker.

3.7.2 Add 12 mL of combined buffer/hypochlorite reagent to the sample bottle (1 mL of reagent to 25 mL of sample).

3.7.3 Check the pH with narrow range pH paper by touching the pH paper to a rod wetted with the sample. the acceptable range is pH 9.0 to 9.5. If necessary, adjust the pH with 1.0 N NaOH or 1.0 N sulfuric acid.

3.7.4 Fill the sample bottle with as much of the 20 mL aliquot that was initially removed from the sample bottle.

3.7.5 Seal the bottle and shake for 1 minute.

3.8 **Incubation.**

Store the chlorinated samples in dark oven at 25°C for 7 days. After the 7 day incubation period, analyze for free chlorine. If the free chlorine is not at least 0.2 mg/L, the test is invalid and must be repeated.

3.9 **Dechlorination**

3.9.1 Add 0.2 mL of dechlorinating agent to a 25 mL screw cap vial.

3.9.2 Fill at least 3 vials per incubated sample.

3.9.3 Store all vials at 4°C.

3.9.4 Analyze all samples within 14 days of collection.

3.10 **Added Precursor Test**

3.10.1 Fill a 300 mL sample bottle with blank water.

3.10.2 Add 120 uL of the DHBA stock solution to the 300 mL sample bottle.

3.10.3 Process the added precursor sample with the same procedure (chlorination, incubation, dechlorination, and analysis), and do this at the same time as the other samples.

3.10.4 A 100% recovery corresponds to 46 ug/L total THM. This data may be corrected for the laboratory reagent blank test.

3.11 **Laboratory Reagent Blank Test.**

Fill a sample bottle with blank water, and process this sample with the same procedure (chlorination, incubation, dechlorination, and analysis) at the same time as the other samples. The laboratory reagent blank should contain less than 5 ug/L total THM.

4. ANALYSIS

- 4.1 Refer to the Standard Operating Procedure for 601/602. There are several modifications to EPA Method 601/602 required for use with EPA method 510.1. Calibrate the gas chromatograph with a standard containing only the four trihalomethanes. run a calibration curve to determine the linear range. Use concentrations of 2, 10, 20, 40, 50, 60, and 80 ug/L for the standard curve. Dilute the samples as needed to bring the concentration of each analyte within the linear range. Reduce the run time from 45 minutes to 30 minutes. All duplicates must be within 20% at concentrations above 50 ug/L.

5. QA/QC REQUIREMENTS

5.1 Samples.

Samples must be chlorinated and incubated within 14 days of collection. They must be maintained at 4°C until they are ready to be incubated. After incubation, the samples must be analyzed within 14 days.

5.2 QC Requirements.

With each daily set of samples, a known EPA check sample is run. The results for each analyte must be within the EPA's acceptance criteria of 80% to 120% recovery. Duplicates and spikes are run on at least one out of 10 samples. Spikes are run in duplicate. Each analyte must fall within the linear range as determined by the calibration curve. Dilutions are usually required, and serial dilutions are highly recommended.

It is very important that the residual free chlorine be at least 0.2 mg/L after incubation. This amount of residual free chlorine is necessary to ensure that the chlorine demand was satisfied.

The blank water should have a total THM concentration of less than 2 ug/L, and levels approaching 1 ug/L should be attainable. The laboratory reagent blank should have a total THM concentration of no more than 5 ug/L. The recovery of the added precursor test should be between 90% and 110% after subtracting the laboratory reagent blank. If any of these acceptance criteria are not met, corrective action must be taken. There are many preventive measures that improve the quality of data. The most important acceptance criteria that must be met - is the recovery of total THM in the added precursor test. Any corrective action taken must be documented.

6. PREVENTIVE MAINTENANCE AND CORRECTIVE ACTIONS

6.1 Summary.

There are many problems which can evolve when using this method. This section gives a few preventive maintenance and corrective action tips that will reduce such problems. Record all corrective actions in the maintenance log book. Include a complete description of the problem and what actions were taken to correct it.

6.2 Interferences.

Interference problems can be detected when the laboratory reagent blank yields total THM values of greater than 5 ug/L. In some instances, when the total THM values in the sample are a large amount (on the order of 400 ug/L), a laboratory reagent blank value of less than 10 ug/L can be acceptable. If lower total THM values are expected or if the laboratory reagent blank exceeds 10 ug/L, the sources of interferences must be located. If the total THM value for the blank water is greater than 2 ug/L, see the section on blank water below. If the total THM value of the blank water is less than 2 ug/L, the next possible source is the reagents.

6.3 Glassware.

Glassware must be clean of any contaminant that could be a THM precursor. Clean all glassware with a suitable detergent, rinsing twice with reagent water, 10% HCl and methanol. Soak all glassware, including the spargers, beakers, pipets, volumetric flasks, and graduated cylinders for 24 hours in a 10% (v/v) bleach solution. Use of commercial bleach is recommended. Rinse with reagent water then dry at 220°C.

6.4 Blank Water.

If placing reagent water overnight in the oven at the boiling point of water does not produce a total THM value of less than 2 ug/L, purging with helium on a hot plate for a day might produce the desired results. Be sure that the flow rate of helium is very slow. The sparger used in this analysis must be cleaned in the bleach solution along with all other glassware.

As a precaution, dechlorinate the sample of blank water used in this analysis. There is the possibility that the sparger could be contaminated with THM precursors, and these precursors will be chlorinated by the free chlorine in the blank water to produce abnormally high total THM values. If purging with helium does not produce the desired results, the problem could be a contaminated cylinder of helium. A switch to another tank of helium or even to nitrogen might solve the problem.

6.5 Non-linear Response of Analytes.

Chloroform was found to be non-linear at concentrations above 50 ug/l. In the samples analyzed, the chloroform concentrations were in the 300 ug/L range. In all

instances, it was necessary to take dilutions of 1:20, 1:10, and 1:5. All samples were run in duplicate at each dilution, and spikes were done on all dilutions.

[illegible]

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

EPA Method 515.4 Rev. 3.0

DETERMINATION OF CHLORINATED ACIDS IN DRINKING WATER BY LIQUID-LIQUID MICROEXTRACTION, DERIVATIZATION, AND GAS CHROMATOGRAPHY WITH ELECTRON CAPTURE DETECTION

File Name: M:\SOP\Organic\epa method sop\515.4_020409.doc Effective Date: 02/04/2009
Revision: 3 Supersedes: 01/24/2008

1. SUMMARY OF METHOD

Chlorinated phenoxy acids and their respective esters are extracted from an acidified sample with methyl t-butyl ether. At this point the acids are converted to methyl esters by using diazomethane - micro generated. Excess derivatizing reagent is removed by silicic acid. The sample is analyzed for methyl esters by way of gas chromatography equipped with a capillary column and an electron capture detector. EPA method 515.4 is used for the determination of chlorinated herbicides in groundwater and finished drinking water.

2. ANALYTES

- 2.1 This is a gas chromatographic (GC) method applicable to the determination of certain herbicides in groundwater and finished drinking water. The following compounds can be determined using this method:

<u>LIMS code</u>	<u>Analyte</u>
BENTAZ	Bentazon
24-D	2,4-D
DALAPN	Dalapon
DINOSB	Dinoseb
PCP	Pentachlorophenol (PCP)
PICLOR	Picloram
245-TP	2,4,5-TP (Silvex)
DICAMB	Dicamba
tDCPA	DCPA
ACIFEN	Acifluorfen

3. APPARATUS AND EQUIPMENT

- 3.1 Sample Containers – 40 mL amber glass vials fitted with a screw cap lined with teflon.
- 3.2 Extraction Vials – 60 mL clear glass vials with PTFE lined screw cap.
- 3.3 Autosampler vials - equipped with Teflon-lined septum.
- 3.4 Conical Glass Tube - 25 mL tubes.
- 3.5 Analytical Balance: Capable of weighing accurately to nearest 0.0001 g.
- 3.6 CP 3800 Varian gas chromatograph with dual columns - electron capture detectors (ECD). Equipped with a CP 8400 autosampler for injecting samples into the GC.
- 3.7 Column: Fused Silica Capillary column, DB-5 30 meters long x 0.25 mm I.D. with a 0.25 micron film thickness. Alternate column - DB-1, 30 meters long x 0.32 mm I.D. with a 0.25 micron film thickness.
- 3.8 Disposable Pasteur Pipets and graduated cylinders (1000ml, 100ml, and 10ml).
- 3.9 Micro-diazomethane generator.

4. REAGENTS AND CONSUMABLE MATERIALS

- 4.1 Reagent Water - Millipore Milli-Q System or equivalent.
- 4.2 Methyl tert-Butyl ether (MTBE): Burdick & Jackson - capillary GC² solvent.
- 4.3 Acetone: Burdick & Jackson - capillary GC² solvent.
- 4.4 Hexane: Brudick & Jackson - capillary GC² solvent.
- 4.5 Hexane: MTBE (90:10, v/v) Wash Solvent: prepared before extraction.
- 4.5 Sodium Sulfate: (ACS) Granular, anhydrous. Heat sodium sulfate at 400°C four hours.
- 4.6 Acidified Sodium Sulfate: acidified, by Fisher. ACS grade
- 4.7 Copper II Sulfate Pentahydrate, (CuSO₄·5H₂O): ACS grade.
- 4.8 4N NaOH Solution: Dissolve 16g of sodium hydroxide in reagent water and dilute to 100 mL.
- 4.9 Sodium Sulfite (Na₂SO₃) - ACS grade, used as a dechlorinating agent in this method.

- 4.12 Diazald: Aldrich. (for diazomethane generation) Micro-generation kit for

diazomethane.

- 4.13 4,4-Dibromooctafluorobiphenyl (DBOB) - for use as internal standard.
- 4.14 2,4-Dichlorophenylacetic acid (DCPAA) - for use as surrogate standard.
- 4.15 Silicic Acid - ACS Reagent Grade

5. SAMPLE COLLECTION, PRESERVATION AND HANDLING

- 5.1 Samples are collected in 40 mL amber glass vial with PTFE inerd screw cap.
- 5.2 Add sodium sulfite crystal (2 mg/40 mL) to the sample container prior to collecting the sample. This reagent eliminates residual chlorine in the sample.
- 5.3 After addition to the sample, seal the bottle and shake for one minute. Store samples at 4°C. All samples must be extracted within 14 days after collection. The extract should be analyzed within 21 days after extraction.

6. EXTRACTION

- 6.1 Let sample container warm up to room temperature. Transfer 40 mL of sample to the clear 60 mL glass tube. Add surrogate standard, spike DI with calibration standards, and spike samples with second source standard.
- 6.2 Adjust the pH of the sample to be at least or greater than 12 by adding 1 mL of 4N NaOH. Cap vial and gently mix content. Check pH. Add more NaOH if necessary. Note pH on extraction log. Let sample sit at room temperature for 1 hour. Mix content periodically.
- 6.3 Following the hydrolysis step, add 5 mL of Hexane: MTBE (90:10, v:v) to each sample. Cap and shake for 3 minutes. Allow the phases to separate for 5 minutes, then discard the top hexane: MTBE layer.
- 6.4 Adjust the pH to ~1 by adding at least 2 mL of concentrated sulfuric acid. Check pH. Add more acid if necessary. Note pH on extraction log.
- 6.5 Quickly add 2 g of copper II sulfate pentahydrate and 16 g of baked sodium sulfate and shake until it is dissolved.
- 6.6 Add 4 mL of MTBE extraction solvent and then shake for 3 min. Allow the phases to separate for approximately 5 min. Transfer 3 ml of sample extract to a conical vial.
- 6.7 Add 0.1 g of acidified sodium sulfate to each sample. Gently mix.
- 6.8 Transfer the extract to a second conical vial. Add 500 uL of the diazomethane. Let extract sit for 0.5 hour. Remove any unreacted diazomethane by adding 0.1g of

silicic acid. Add more silicic acid if needed. Allow extract to sit for at least 0.5 hour. Extracts are ready to be transferred to gc autosampler vials when no more bubbles are present.

6.9 Generation of diazomethane

Assemble MNNG - Diazomethane Kit . Fill the inner reaction tube of the generator 2/3 full below the expanded portion of the tube with diazald. Care must be taken to insure that none of the diazald enters the outside tube. Add 2 ml of methanol to each reaction tube. Secure the reaction tube with septum and caps. Approximately 5 ml of MTBE is added to the outside tube and the two parts are clamped together with a butyl O-ring and a pinch clamp. Place the tube in an ice bath and add 1.5 ml of 6 N NaOH. The hydroxide solution is added using a syringe through the silicone rubber septum. The diazomethane will co-distill with the MTBE in about 45 minutes. Be sure the tube remains in the ice bath. A yellow solution should be the end product. If no coloration is present, more than likely the procedure did not work and no diazomethane was generated. The diazomethane distilled should be stored in an amber bottle, refrigerated, and used immediately. The holding time for the diazomethane reagent is about 4 days.

7. Standard Preparation

Primary source for standards Ultra Scientific (varied conc). Secondary - Protocol Standards (various conc). DCPA (Accustandard 100ug/mL)

Open standards should be kept at 4°C in Teflon-sealed, screw cap vials. Stock standards are diluted into two working standards (#1 and #2).

1. Calibration Standard:

- a. Ultra Scientific - Custom Mix = varies conc. (18 - 200 µg/ml)

***Working Std #1: dilute 200 µl of Custom Mix to 1ml with Acetone, varies conc.

***Working Std #2: dilute 100 µl of Working #1 to 1ml with Acetone, varies conc.

2. 2nd Source Standard: - Custom Mix = varies conc. (18 - 200µg/ml)

- a. Protocol (Spex) - Custom Mix = varies conc. (18 - 200 µg/ml)

***Working Std #1: dilute 200µl of Custom Mix to 1ml with Acetone, varies conc.

***Working Std #2: dilute 100µl of Working Std #1 to 1ml with Acetone, varies conc.

3. DCPA Diacid - conc. 100 µg/ml

- b. Accustandard - DCPA Diacid - conc. 100 µg/ml

***Working Std #1: dilute 40 µl of DCPA to 1ml w/ Acetone, (4ug/mL)

***Working Std #2: dilute 100 µl of Working Std #1 to 1ml w/ Acetone, (0.4ug/mL)

Standards are extracted in the same manner as samples.

Ultra Scientific

<u>Calib. Std. Conc.</u>	<u>Vol. of Working Stds.</u>	<u>Vol. of Surrogate</u>	<u>Surr. Conc.</u>
Std A - 0.1 µg/L	10 µl of std # 2 & DCPA #2	2.5 ul	Std A - 6.25 µg/L
Std B - 0.25 µg/L	25µl of std # 2 & DCPA #2	5.0 ul	Std B - 12.5 µg/L
Std C - 0.5 µg/L	5µl of std # 1 & DCPA #1	7.5 ul	Std C - 18.75 µg/L
Std D - 1.0 µg/L	10µl of std # 1 & DCPA #1	10.0 ul	Std D - 25.0 µg/L
Std E - 1.5 µg/L	15µl of std # 1 & DCPA #1	15.0 ul	Std E - 37.5 µg/L
Std F - 2.0 µg/L	20µl of std # 1 & DCPA #1	20.0 ul	Std F - 50.0 µg/L

8. ANALYSIS

8.1 EPA method 515.4 is used to monitor for low level of chlorinated herbicides in groundwater. Because of these low levels, any type of contamination or interferences can cause analytical problems. Thus, reagent blanks must be monitored for every extraction run - monitoring of reagent blanks is essential to the success of this method. If results for reagent blanks rise above this level corrective actions must be performed. Analyze a 6-point calibration at the beginning of each analytical run. Verify the calibration by measurement of two calibration check standards, one at the beginning and one at the end of the run. These check standards should be at two different concentration levels to verify the calibration curve. LFBs (laboratory fortified blanks) should also be analyzed with each extraction run. Standards used for these QA/QC samples must be ordered from a second source whenever possible. For extended runs, check standards should be interspersed with samples at regular intervals. If the response of any analyte varies from the predicted response by more than +/- 30%, test must be repeated using fresh calibration standards. The six point calibration should be 0.1, 0.25, 0.5, 1.0, 1.5 and 2.0ppb. Any results above 2 ppb must be confirmed with a standard that is within +/- 20% of the actual result.

Instrument Conditions:

1. GC Column Program:

<u>Rate °/Min</u>	<u>Temp</u>	<u>Hold Time</u>	<u>Total Time</u>
Initial	40	10	10
80	152	0.2	11.6
3	172	0.1	18.37
15	230	0.1	22.34
30	300	1	25.67

2. Helium linear velocity:

30 cm/sec

3. Injection Program:

<u>Time (Min)</u>	<u>Split State</u>	<u>Split Ratio</u>
Initial	On	10
0	Off	0
0.6	On	50
1.5	On	100

4. Injector temperature: 250°C
5. Detector temperature: 320°C

8. QA/QC REQUIREMENTS

- 8.1 Laboratory Reagent Blank - Run before samples. Use to demonstrate that all glassware and reagent interferences are under control. If any contamination peaks are produced, determine source of contamination and eliminate interference.
- 8.2 Laboratory Fortified Blank - Must analyze at least two laboratory fortified blank (LFB) one after every calibration and one at the end of the analysis. The fortification concentration of each analyte should confirm the ability to detect at the reportable level. If the recovery of any analyte falls outside the control limits, the source of the problem must be identified and corrected.
- 8.3 Standards - Verify calibration standards quarterly by analyzing a standard prepared from reference material obtained from an independent or second source. EPA performance evaluations are an excellent process to determine the validity of the method. Results must be within +/- 30% of those used to routinely check calibration. Daily, run a low level standard to check the reportable detection level, RDL.
- 8.4 Surrogate Standard Recoveries - Surrogate recovery from a sample or method blank should be within +/- 30%. If it is outside of the acceptable range, identify the problem (calculations error, degradation of standard, contamination, or instrument performance). Re-analyze extract, or request for resample.
- 8.5 Samples - Samples must be extracted within 14 days after collection. Samples must be stored at 4°C until ready for extraction. Duplicates are run on 10% of samples, or once during run, whichever is greater. Results should be within +/- 30%. Extracts should be store at 0°C or less and analyzed within 21 days after extraction. All positive identifications must be confirmed using the confirmation column or GC/MS method 525.
- 8.6 Spike Recoveries - The laboratory must add a known concentration of spike solution, the same as used for LFB, to at least 10% of samples or once per analytical run, whichever is greater. Recoveries should be within the acceptable range. Wherever possible, run a second source standard for spikes.
- 8.7 QC Requirements - Spikes must be analyzed at a frequency of at least 10% of the sample load. A low level standard must be run at 0.10 ppb to ensure that the system is capable of low level analysis - RDL is confirmed. A Continuous Calibration Check sample should also be included on each analytical run for validation of the calibration curve. Each day a reagent water blank must be analyzed to demonstrate that interferences from the analytical system are under control. To establish the ability to achieve low detection levels and to generate accurate and precise chromatography, the analyst must analyze seven low level replicates (0.10 ppb).

The MDL is then calculated based on the precision and accuracy of these results. The acceptance criterion calls for a recovery of +/- 20% and an MDL that does not exceed 0.05 ppb. Anytime major maintenance to the system occurs a new MDL determination must be performed. Compliance to this shows that system performance is acceptable and sample analysis can begin.

9. PREVENTIVE MAINTENANCE AND CORRECTIVE ACTIONS

- 9.1 Glassware - Glassware must be carefully cleaned. Clean all glassware as soon as practical after use by thoroughly rinsing with hot tap water. Follow by washing with hot water. Acid wash all glassware with a 10% HCl solution and thoroughly rinsing with tap water followed by reagent water. After drying, heat in oven. Do not heat volumetric glassware above 220°C. Thorough rinsing with acetone may be substituted for heating.
- 9.2 Reagents - The use of high purity solvents and reagents will help to minimize contamination problems.
- 9.3 Carryover - Contamination carryover may occur when a sample containing a low concentration of analytes is analyzed immediately following a sample containing a high concentration. Use solvent rinses between samples to minimize carryover.
- 9.4 All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory method blanks.
- 9.5 Septa for ECD injectors need to be changed every 100 to 200 injections. The GC column temperature and the temperature of the injectors and detectors must be lowered to ambient. **Turn off the detectors before opening the system!**
- 9.6 No or Poor Chromatography-
 - 9.6.1 Check all the standards. If calibration curve (30 % RSD or less) and/or QC lie outside the acceptable limits we must first re-run the standards to assure that the injection procedure is working properly. After the re-run if standards still lie outside the acceptable limits we must make a new standard or conduct instrument maintenance to assure that calibration and QC lie with acceptable parameters. Verify the Calibration curve by analyzing a Continuous Calibration Check Standard (front Check Standard) immediately after the completion of the calibration standards. If front check standard fails, check standards, make up new dilutions and re-analyzed. For elevated spike recoveries make sure that the sample matrix is clean. If sample matrix is altering recoveries then a new matrix must be selected. If instrument performance is acceptable the samples must be re-extracted. If the samples lie outside allowable holding time they must be re-sampled.
 - 9.6.2 Request re-sample if it's necessary.
 - 9.6.3 If there're analytical hits, confirm with GC/MS, or use a second column.

Request for resample if possible.

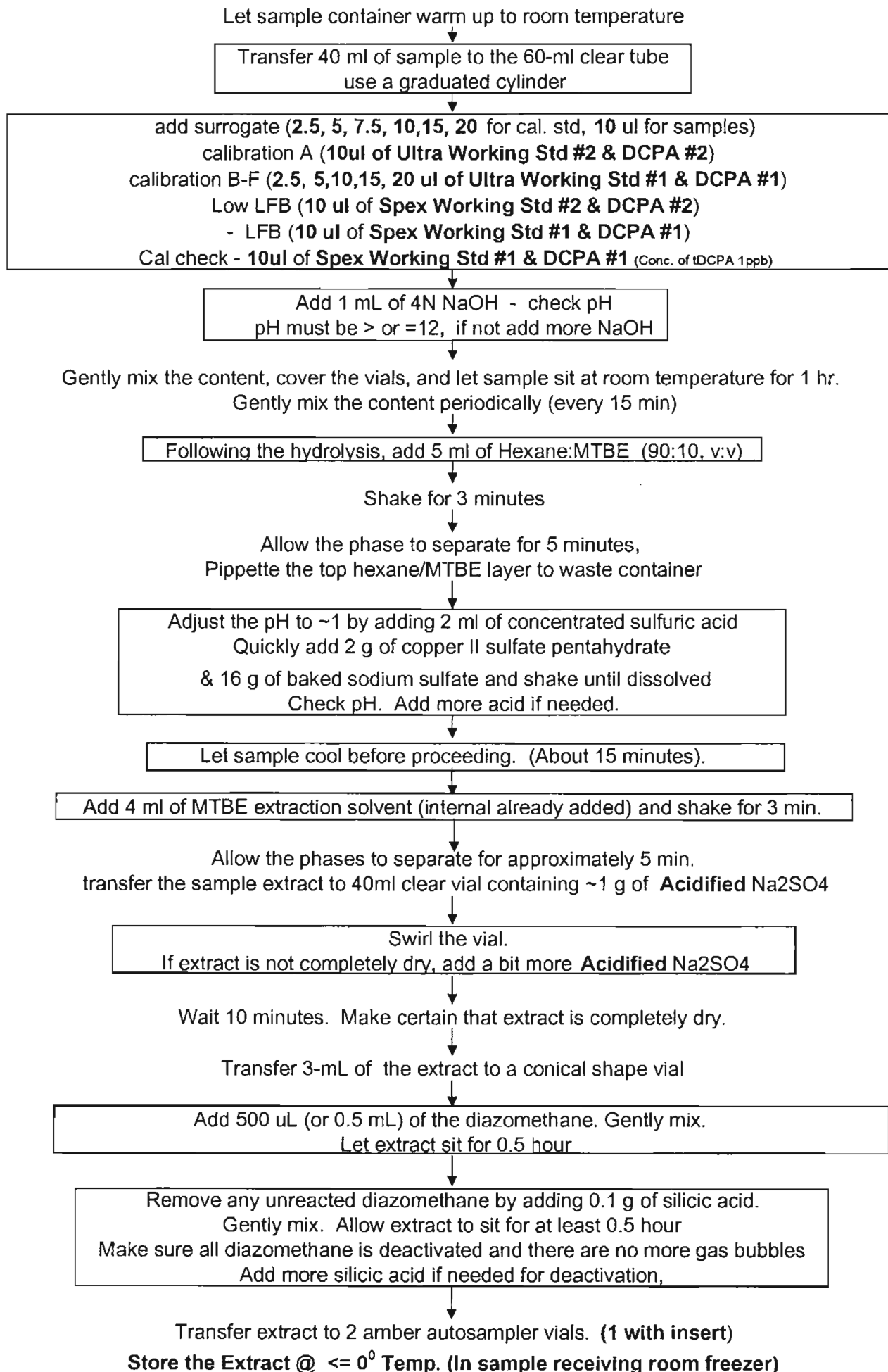
- 9.6.1 Record all corrective actions in the maintenance logbook. Include a complete description of the problem and what action was taken to correct it.

SOP PROCEDURE CHANGE

For EPA Method 515.1

<u>CHANGE</u>	<u>REASON</u>	<u>DATE</u>	<u>INITIALS</u>
Changed preservative amount to 80 mg/L---EPA recommendation-		10/11/04	JRB---
Added Acifluorfen-----	-in method-----	10/20/04	jrb ---
Increased the amount of H2SO4 to 2mL lvs	better respond	2/10/06	
Modified GC Program lvs	Shorter run time	2/24/06	
Silica Gel to Silicic Acid -----	works the same (talked to Mr. Dave Munch (@ EPA)	3/6/06	lvs ----
Removed LPC requirement	Not in EPA method 515.4	01/24/08	ATL

METHOD 515.4, OCWD Rev. 9 (03/10/09)



ORANGE COUNTY WATER DISTRICT

EPA METHOD 515.4 (Rev 1.0)

1. Calibration Standard:

- a. Ultra Scientific - Custom Mix = varies conc. (18 - 200 µg/ml)

***Working Std #1: dilute 200 µl of Custom Mix to 1ml with Acetone, varies conc.

***Working Std #2: dilute 100 µl of Working #1 to 1ml with Acetone, varies conc.

2. 2nd Source Standard: - Custom Mix = varies conc. (18 - 200 µg/ml)

- a. Protocol (Spex) - Custom Mix = varies conc. (18 - 200 µg/ml)

***Working Std #1: dilute 200µl of Custom Mix to 1ml with Acetone, varies conc.

***Working Std #2: dilute 100µl of Working Std #1 to 1ml with Acetone, varies conc.

3. DCPA Diacid - conc. 100 µg/ml

- b. Accustandard - DCPA Diacid - conc. 100 µg/ml

***Working Std #1: dilute 40 µl of DCPA to 1ml w/ Acetone, (4ug/mL)

***Working Std #2: dilute 100 µl of Working Std #1 to 1ml w/ Acetone, (0.4ug/mL)

<u>Analyze</u>	<u>LIMS ID</u>	<u>Stock Conc.</u> <u>µg/ml</u>	<u>Analyze</u>	<u>LIMS ID</u>	<u>Stock Conc.</u> <u>µg/ml</u>
Dicamba	DICAMB	16	Dinoseb	DINOSB	100
Pentachlorophenol	PCP	20	Picloram	PICLOR	100
Dalapon	DALAPN	200	2,4-D	24D	100
Bentazon	BENTAZ	200	Acifluorfen	Acifluorfen	100
DCPA Diacid	DCPA	100	2,4,5-TP (Silvex)	245TP	100

A. CALIB. STANDARD:

Ultra Scientific

<u>Calib. Std. Conc.</u>	<u>Vol. of</u> <u>Working Stds.</u>	<u>Vol. of</u> <u>Surrogate</u>	<u>Surr. Conc.</u>	<u>Std A Conc.</u>	
Std A - 0.1 µg/L	10 µl of std # 2 & DCPA #2	2.5 ul	Std A - 6.25 µg/L	PCP, DCPA	0.1ppb
Std B - 0.25 µg/L	2.5µl of std # 1 & DCPA #1	5.0 ul	Std B - 12.5 µg/L	Dicamba	0.08ppb
Std C - 0.5 µg/L	5µl of std # 1 & DCPA #1	7.5 ul	Std C - 18.75 µg/L	Dalapn, Bentaz	1.0ppb
Std D - 1.0 µg/L	10µl of std # 1 & DCPA #1	10.0 ul	Std D - 25.0 µg/L	Others	0.5ppb
Std E - 1.5 µg/L	15µl of std # 1 & DCPA #1	15.0 ul	Std E - 37.5 µg/L		
Std F - 2.0 µg/L	20µl of std # 1 & DCPA #1	20.0 ul	Std F - 50.0 µg/L		

(Cali. Std Conc. are based on the stock conc of PCP 20ug/ml, others will have diff conc.depend on their stock conc.)

B. LFBs (also Check Std) & SPIKE:

Protocol (Spex)

(old mix)

Low LFB = 0.1 µg/L Spike 40ml DI water with 10µl of Working Standard # 2 & 10 ml of DCPA #2

LFB = 1.0 µg/L Spike 40ml DI water with 10 µl of Working Standard # 1& 10 ml of DCPA #1

Spk/Spk Dup = 1.0 µg/L

Spike 40ml sample with 10 µl of Working Standard # 1 & 10 ml of DCPA #1

C. SURROGATE STANDARD SOLUTION (S.S.):

Stock: Dissolve 0.0100 g of DCAA (2,4-Dichlorophenylacetic Acid) in 10ml acetone

Working: Add 1 mL of Stock S.S. solution to 10 mL of acetone

****Spike samples with 10µl of Working S.S. to give conc. of 25 µg/L

D. INTERNAL STANDARD SOLUTION (I.S.):

Stock: Dissolve 0.01g of DBOB (4,4-Dibromooctafluorobiphenyl) in 10ml MTBE (conc. = 1.0mg/mL)

Primary Dilution: Add 25 uL of Stock I.S. solution to 10 mL of MTBE (conc. = 2.5 ug/mL)

*** MTBE Extraction Solvent with Internal Standard:

add 1 mL of Primary Dilution to 99 mL of MTBE

(conc. = 25 ng/mL)

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

EPA Method 526

DETERMINATION OF SELECTED SEMIVOLATILE ORGANIC COMPOUNDS IN DRINKING WATER BY SOLID PHASE EXTRACTION AND CAPILLARY COLUMN GAS CHROMATOGRAPHY/ MASS SPECTROMETRY (GC/MS)

File Name: M:\SOP\Organic\epa method sop\526_0208.doc
Revision: 3

Effective Date: 2/2008
Supersedes: 09/2004

1. SUMMARY OF METHOD

A measured volume of sample of approximately 1 liter is extracted using a SDVB (polystyrene divinyl benzene) disk or cartridge. Target analytes and surrogate compounds are extracted by passing the measured volume of sample thru the solid phase extraction (SPE) SDVB disk/cartridge. The organic compounds are eluted from the disk/cartridge with small quantities of ethyl acetate and methylene chloride. The extract is dried with anhydrous sodium sulfate and concentrated to 1 ml using the Zymark concentrator - using gentle heat and UHP nitrogen gas. Internal standards are added to every sample after concentration. The final extract is injected into a GC/MS system for separation, identification, and quantitation. The method is applicable to a variety of semivolatile organic compounds which are efficiently extracted from the water sample onto a polystyrene divinylbenzene solid phase sorbent, and sufficiently volatile and thermally stable for gas chromatography.

2. ANALYTES

This is a gas chromatographic mass spectrometry (GC/MS) method, applicable to the determination of selected semivolatile organic compounds in finished drinking water. The following compounds can be determined using this method. They are separated based on the Calibration Standards used for this method – Ultra Scientific:

Target	MW	Quan Mass	CAS#	LIMS ID
Acetochlor	269	146	34256-82-1	ACETOC
Cyanazine	240	225	21725-46-2	CYZINE
Diazinon	304	179	6179-53-2	DIAZI
2,4-Dichlorophenol	162	162	120-83-2	24DCPH
1,2-Diphenylhydrazine	184	182	122-66-7	12DPH
Disulfoton	274	88	298-04-4	DSULTN
Fonofos	246	246	944-22-9	FONOF
Nitrobenzene	123	77	98-95-3	NBENZ
Prometon	225	225	1610-18-0	PROMTN
Terbufos	288	231	13071-79-9	TRBUFS

Internal Standards (@ 500 ug/ml in Acetone)

Acenaphthene-d₁₀

Phenanthrene-d₁₀

Chrysene-d₁₀

Surrogate Standards (@500 ug/ml in Acetone)

1,3-dimethyl-2-nitrobenzene

triphenylphosphate

3. APPARATUS AND EQUIPMENT

- 3.1 Sample Bottles - Two liter amber glass bottles fitted with a screw cap lined with polypropylene.
- 3.2 Autosampler vials – amber vials equipped with PTEE-lined septum.
- 3.3 Concentrator Tube - Zymark 50 mL tubes used with the Zymark Turbo-Vap.
- 3.4 Analytical Balance: Capable of weighing accurately to nearest 0.0001 g.
- 3.5 SPE disk extraction: for manual system: vacuum manifold. For automatic system: automatic extractors by Horizon Technology.
- 3.6 SPE cartridge extraction: for manual system: vacuum manifold. For automatic system: automatic extractors by Horizon Technology.
- 3.7 Zymark Turbo-Vap - used to concentrate extracts.
- 3.8 CP-3800 Varian gas chromatograph with a Saturn 2000 Ion Trap Mass Spectrometer and a data workstation. Equipped with a CP-8200 autosampler for injecting samples into the GC.
- 3.9 Column: Fused Silica Capillary column, Varian Factor Four with 5m guard column 30meters long x 0.25 mm I.D. with a 0.25 micron film thickness, or equivalent column.
- 3.10 Disposable pasteur pipets, graduated cylinders (1000ml, 100ml, 10ml and 1ml) and Hamilton micro syringes – various sizes

4. REAGENTS AND CONSUMABLE MATERIALS

- 4.1 Reagent Water - Millipore Milli-Q System or equivalent.
- 4.2 Methylene Chloride, Methanol, Acetone: Burdick & Jackson - capillary GC² solvent.
- 4.3 Sodium Sulfate: (ACS) Granular, anhydrous. Heat sodium sulfate at 400°C four hours.
- 4.4 SPE disk: 47mm diameter and 0.5 mm thick, manufactured with a polystyrene divinylbenzene (SDVB) sorbent phase.
- 4.5 SPE cartridge: Varian Bond Elute PPL cartridge, 500 mg, 6-mL, or equivalent cartridge.
- 4.6 Helium carrier gas: UHP grade. Nitrogen gas: UHP grade.

5. SAMPLE COLLECTION, PRESERVATION AND HANDLING

- 5.1 Samples are collected in two liter amber bottles, fitted with a screw cap lined with polypropylene. Keep samples sealed from collection time until analysis. Sampling equipment must be free of plastic tubing, gaskets, and other parts that may leach interfering analytes into the water sample.
- 5.2 Preservation reagents are added to each sample bottle prior to shipment to the field.

Compound	Amount	Purpose
L-Ascorbic Acid	0.10 g/L	Dechlorination
Ethylenediaminetetracetic acid trisodium salt	0.35 g/L	Inhibit metal-catalyzed hydrolysis of targets
Diazolidinyl Urea	1.0 g/L	Microbial inhibitor
Tris(hydroxymethyl)aminomethane	7.75 g/L	pH buffer mix

- 5.3 All samples should be iced or refrigerated at 4°C and kept in the dark from the time of collection until extraction. Residual chlorine must be reduced at the time of sample collection with 100 mg of ascorbic acid per liter. Sodium sulfite and sodium thiosulfate cannot be used because they were found to degrade target analytes. After addition to the sample, seal the bottle and shake until contents dissolve. Store all samples at 4°C until ready for extraction. All samples must be extracted within 14 days after collection. The extract should be stored at 0°C or less and analyzed within 28 days after sample extraction.

6. EXTRACTION:

6.1 Manual Liquid - Solid phase extraction (SPE): disk and cartridge

See the attach SPE Flow Charts.

- 6.1 The District uses the automated extraction unit (Horizon Technology – SPE-DEX 4700). A separate SOP has been developed to utilize this system for 526 samples. Please refer to that SOP for more details. The automated system's extractors are purged before each run to eliminate any type of contamination or carryover between samples. The disk/cartridge is placed in the automated unit and the system will automatically condition each disk/cartridge by the approved EPA methodology. The disk/cartridge is washed with 5 ml of ethyl acetate and methylene chloride. The disk/cartridge is soaked for a set period of time and then pre-wet with 5mls of methanol. At this step it is critical to not allow the disk to go dry until the entire volume of sample is processed.
- 6.2 Transfer one liter of sample to the specified auto-extractor 1-liter bottles. Add 5 mls of methanol per liter of sample to be extracted (0.5% methanol in sample. Preservatives will need to be added to the water for all QA/QC samples (RB, LFB's). Failure to add preservatives will lead to complete loss of recovery of some targets. Add 10ul of the surrogate standard (IS) and fortification solution – 500 ug/ml. Results in a 5 ug/L concentration of the IS and SS targets in water. Process the sample based on the automated method. The automated system is setup to process all samples in the same manner.
- 6.3 The automated system will process each sample – rinsing the sample bottle during the elution step. Dry the extract with 15 grams of anhydrous sodium sulfate. Rinse the drying tube and sodium sulfate with two 3 ml portions of 1:1 ethyl acetate and methylene chloride and transfer into a Zymark concentrator tube.
- 6.4 Concentrate the sample to between 0.7 ml and 1.0 mls – do not concentrate the extract to less than 0.7 ml. Make any volume adjustments with ethyl acetate -- to the 1.0 ml marked level. Inject 10ul of 500ug/ml internal standard solution. Transfer the 1.0 ml extract into 2 amber autosampler vials and store the in the freezer at 0 °C or less.

7. ANALYSIS

- 7.1 The first step for the instrumental analysis of this method is the mass calibration of the system. Inject into the GC/MS system a 1ul aliquot of the 5 ng/ul solution of DFTPP. Acquire a mass spectrum that includes data for m/z 45-450. The DFTPP mass spectrum must meet all the criteria in Table 1. A single spectrum at the apex of the DFTPP peak, an average spectrum of the three highest points of the peak, or an average spectrum across the entire peak to may be used to evaluate the performance of the system. If the system repeatedly fails the criteria – retune the system until it passes.

In this method daily DFTPP analysis is not required (Sect. 9.7, p. 18). However, verification of the mass spectrometer tune must be repeated each time a major

instrument modification is made, or maintenance is performed, and prior to analyte calibration (Sect. 10.2.1, p.21).

- 7.2 Initial Calibration: A six point calibration curve is required for this method; using standards STD-A (0.5ppb), B (1.0 ppb), C (2.0ppb), D (4.0ppb), E (8.0ppb), F (10.0ppb). At least five calibration curve points must be utilized for each target compound. For each analyte and surrogate, calculate the mean RF and the relative standard deviation (RSD). The %RSD of any analyte or surrogate within the calibration range must be less than 30%. If not – inject other standards or perform system maintenance and begin the system analysis and performance check over.

- 7.3 **Continuous Calibration check:** Check the system tune and performance check for each 12 hour work shift. LFBs (laboratory fortified blanks) should also be analyzed with each extraction run. Standards used for these QA/QC samples must be ordered from a second source whenever possible. For extended runs, check standards should be interspersed with samples at regular intervals. If the response of any analyte varies from the predicted response by more than +/- 30%, test must be repeated using fresh calibration standards.

Data is collected and processed by Saturn GC/MS software. A calibration file must be created by analyzing the six point calibration curve and entering these values into the cali file. An unknown is identified by comparing its mass spectra with the NIST98 library. Its purity must exceed 800 for positive identification. Any results above the highest calibration standard must be confirmed with a standard that is within +/- 20% of the actual result or the sample must be diluted to within the calibration range.

GC Conditions:

1.	Seg	Temp	Rate	Hold Time	Total	
	1	55	0	0.5	0.5	
	2	150	10	10	10.5	
	3	240	10	0	19	
	4	300	25	3.2	24.6	
2.	Helium linear velocity:		30 cm/sec			
	Constant Column Flow:		1.4 ml/min			
3.	Split injection program:		Time	Split Rate	Split Ratio	
			Initial	On	20	
			0.01	Off	Off	
			1.50	On	150	
			5.00	On	20	
4.	Injector temperature	:	Temp	Rate	Hold	Total
			35	0	0	0

5. Transfer Line Temp: 280 °C
Trap Temp: 225 °C
Manifold: 80 °C
6. 2 ul sample is injected at 0.2 ul/sec - sandwich technique is used with a 0.5 ul solvent plug

MS Conditions :

1. Mass range : 45 to 450 amu
2. Seconds/scan : 0.80
3. Acquire time : 24.6 minutes
4. Fil/Mul delay : 6 minutes
5. Peak threshold : 1 count
6. Mass defect : 0 mmn/100 amu
7. Background mass : 45 amu
8. Ionization mode : EI
9. Auto ion control : ON
10. Cal gas : OFF

5 ng of DFTPP is injected (1ul of a 5ug/ml solution) before every run into the GC/MS to determine whether the MS is "in tune". Using the GC method DFTPP, MS method DFTPP and AS method DFTPP, the ion abundance of the mass spectra of DFTPP can be compared to the following set of criteria.

<u>Mass</u>	<u>Relative Abundance Criteria</u>
51	10-80% of the base peak
68	<2% of mass 69
69	Present
70	<2% of mass 69
127	10-80% of the base peak
197	<2% of mass 198
198	base peak of >50% of 442
199	5-9% of mass 198
275	10-60% of the base peak
365	>1% of the base peak
441	Present and < mass 443
442	base peak of >50% of 198
443	15-24% of mass 442

EnviroPro software is used to determine whether the ion abundance criteria is met. For DFTPP check – Click on “Custom Report” icon. Choose “dftpp.swt”. Select the correct method. Go to “Tune Report Setup”. Select file. Change Retention time if needed. Choose “Tune Report”. Use print icon to print.

8. QA/QC REQUIREMENTS

- 8.1 Laboratory Reagent Blank - Run before samples. Use to demonstrate that all glassware and reagent interferences are under control. If any contamination peaks are produced, determine source of contamination and eliminate interference.
- 8.2 Laboratory Fortified Blank - Must analyze at least two laboratory fortified blanks (LFBs) with each set of extractions. Run a LFB-low and an LFB-high with each analytical run. The fortification concentration of each analyte should confirm the ability to detect at the reportable level. If the recovery of any analyte falls outside the control limits, the source of the problem must be identified and corrected.
- 8.3 Standards - Verify calibration standards quarterly by analyzing a standard prepared from reference material obtained from an independent or second source. EPA performance evaluations are an excellent process to determine the validity of the method. Results must be within +/- 20% of those used to routinely check calibration. Daily, run a low level standard to check the reportable detection level, RDL.
- 8.4 Samples - Samples must be extracted within 14 days after collection – unless specific targets need to be determined. Samples must be stored at 4°C until ready for extraction. Duplicates are run on 10% of samples, or once during run, whichever is greater. Results should be within +/- 20%. Extracts should be analyzed within 28 days after sample extraction.
- 8.5 Spike Recoveries - The laboratory must add a known concentration of spike solution, the same as used for LFB, to at least 10% of samples or once per analytical run, whichever is greater. Recoveries should be within the acceptable range. Wherever possible, run a second source standard for spikes.
- 8.6 QC Requirements - Analyze EPA QC check sample with known values if available. The results for each analyte must be within the EPA acceptance criteria. Annually, analyze EPA Performance Evaluation samples. Analyze these check samples whenever major maintenance to the system occurs to ensure the validity of the method.
- 8.7 The analyst must monitor the peak area of each internal standard in all injections during each analysis day. The IS peak area for any chromatographic run must not deviate by more than $\pm 50\%$ from the average area measured during the initial calibration for that IS. If it deviates more than 50% re-inject a second aliquote of extract to determine whether the failure is due to poor injection or instrument response drift. If the continuing calibration check fails the criteria, recalibration is in order per section 10 of EPA method.
- 8.8 The analyst must also monitor recovery of the surrogate standards. Surrogate recoveries must be between 70 to 130 %. If it fails, check for possible errors, standard solutions for degradation, contamination, and instrument performance.

Re-analyze extract, or re-extract sample if appropriate.

9. PREVENTIVE MAINTENANCE AND CORRECTIVE ACTIONS

- 9.1 Glassware - Glassware must be carefully cleaned. Clean all glassware as soon as practical after use by thoroughly rinsing with last solvent used in it. Follow by washing with hot water and 10% HCl and thoroughly rinsing with tap water followed by reagent water. After drying, heat in oven. Do not heat volumetric glassware above 220°C. Thorough rinsing with acetone may be substituted for heating.
- 9.2 Reagents - The use of high purity solvents and reagents will help to minimize contamination problems.
- 9.3 Carryover - Contamination carryover may occur when a sample containing a low concentration of analytes is analyzed immediately following a sample containing a high concentration. Use solvent rinses between samples to minimize carryover.
- 9.4 All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory method blanks. Minimize contact of the samples, reagents, or solvents with any plastics. This will help reduce contamination. Interferences by phthalate esters can pose a major problem in pesticide analysis.
- 9.5 Record all corrective actions in the maintenance log book. Include a complete description of the problem and what actions were taken to correct it.

SOP PROCEDURE CHANGE
For EPA Method 526

<u>CHANGE</u>	<u>REASON</u>	<u>DATE</u>	<u>INITIALS</u>
Injector from SPI to 1079	Changed Instrument		
GC Program	Shorter run time	7/2004	LVS
Injection Program	To match with the instrument's injection port	7/2004	LVS
Section 9.1 from detergent to 10% HCl	We do not wash our glass wares with detergent	7/2004	LVS
Added cartridge to SOP	Currently using cartridge	02/08	asl
GC oven and Injector Program	Current method	02/08	asl
QC requirements: Internal Area, Surrogate Recoveries, DFTPP requirements	Follow EPA requirements	02/08	asl
Remove section on Field Blanks	Do not analyze FB.	02/08	asl

EPA Method 526 Rev. 1.0 - Cartridge (pg 1 of 2)

Sample Volume: 1000 ml

QA/QC Sample Prep:

Prepare DI water with 526 preservative the day before and keep them in the refrigerator

Extraction Day - Transfer the preserved DI to 1L bottles for QCs

Add the Surrogate - 10ul of (500ug/ml) - Mix immediately until homogeneous

Low spk: 2.5uL. - LFB,SPIKE,SPIKE DUP: 20 uL (of 200ug/ml)

Please read THIS NOTE before proceed w/ the extraction:

EPA - 526- Section 11.3.2

Cartridge Conditioning - This conditioning step is critical for recovery of analytes and have a marked effect on method precision and accuracy. If the cartridge goes dry during the conditioning phase, the conditioning must be started over. Once the conditioning has begun, the cartridge must not go dry until the last portion of the sample passes because analyte and surrogate recoveries may be affected.

CARTRIDGE CLEAN UP

Add 5 ml of EtAc to the assembled SPE cartridge

soak for 1 minutes, collect waste

Add 5 ml of CH₂Cl₂ to the assembled SPE cartridge

soak for 1 minutes, collect waste

Vacuum the cartridge dry after each flush

CARTRIDGE CONDITIONING - SAMPLE EXTRACTING

Add 5 mL of MeOH - soak for 30 seconds

Repeat this step one more time

**DO NOT let the methanol to elute below the top of the cartridge packing
FROM THIS POINT - DO NOT LET THE CARTRIDGE TO GO DRY**

Rinse the Cartridge with 5mL water

Repeat this step one more time

Turn off the vacuum before the water level drops below the top edge of the packing

Fill the cartridge to the top w/ DI H₂O & attach reservoirs and transfer tubes

Fill the reservoirs w/ sample before turn on the vacuum

Adjust the pressure to 20 mL/min flow rate (50min/sample)

After the entire sample has been processed, pull air through for 10 minutes **@ full vacuum**

DO NOT let the cartridge dry longer the 10 minutes

EPA Method 526 - Cartridge (pg 2 of 2)

CARTRIDGE ELUTION

Place the collection tubes into the extraction tank

Rinse the sample bottle with 4 mL EtAc

Turn on vacuum to pull the solvent through the transfer tube and through the cartridge

soak 30 seconds

Reduce the vacuum power so that the solvent exits the cartridge in a dropwise fashion

Repeat rinse the sample bottle with 4 mL CH₂Cl₂

DRYING OF THE EXTRACT

Packed the drying column with 7g Na₂SO₄

Pre-rinse the column with 6 mLs of 1:1 CH₂Cl₂ : EtAc - **Discard this solution**

Pass the extract through the drying column and collect the extract in clean Zymark tubes

Follow with TWO 3mLs of 1:1 CH₂Cl₂ : EtAc

EXTRACT CONCENTRATION

Zymark settings: Temp: 40, Pressure: 10 psi

concentrate the extract to 1-ml, cool down, then add internal (10 ul) of 500ug/ml

Make sure the final volume is 1ml - if not bring up the volume w/ EtAc

Transfer to 2 GC amber autosampler vials

ORANGE COUNTY WATER DISTRICT EPA METHOD 526

1. Calibration Standard: Ultra Scientific: SVM-526 - Stock =200 µg/ml
2. 2nd Source Standard: Protocol Custom Mix - Stock =200 µg/ml

Analytes

NBENZ	Nitrobenzene	CYZINE	Cyanazine (Blade X)
24DCPH	2,4-dichlorophenol	DIAZI	Diazinon
246TCP	2,4,6-trichlorophenol	DSULTN	Disulfoton
12DPH	1,2-diphenylhydrazine	FONOF	Fonofos
PROMTN	Prometon	ACETOC	Acetochlor
TRBUFS	Terbufos		

A. CALIB. STANDARD: *Ultra Scientific* *Final Solvent: Ethyl Acetate*

<u>Calib. Std. Conc.</u>	<u>Volume of Std.</u>	<u>Surrogate</u>	<u>Internal</u>	<u>Final Dil. Vol.</u>	
Std A - 0.5 µg/L	2.5 µl of stock	1.0 ul	10.0 ul	1.0 ml	
Std B - 1.0 µg/L	5.0 µl of stock	2.0 ul	10.0 ul	1.0 ml	
Std C - 2.0 µg/L	10.0 µl of stock	4.0 ul	10.0 ul	1.0 ml	
Std D - 4.0 µg/L	20.0 µl of stock	10.0 ul	10.0 ul	1.0 ml	Surr = 5ppb
Std E - 8.0 µg/L	40.0 µl of stock	16.0 ul	10.0 ul	1.0 ml	
Std F - 10.0 µg/L	50.0 µl of stock	20.0 ul	10.0 ul	1.0 ml	

B. CALIBRATION CHECK: *Protocol Custom Mix* *Final Solvent: Ethyl Acetate*

<u>Check Std. Conc.</u>	<u>Volume of Std.</u>	<u>Surrogate</u>	<u>Internal</u>	<u>Final Dil. Vol.</u>
Low - 0.5 µg/L	2.5 µl of stock	10.0 ul	10.0 ul	1.0 ml
High - 4.0 µg/L	20.0 µl of stock	10.0 ul	10.0 ul	1.0 ml

C. LFB & SPIKE: *Protocol Custom Mix*

Low LFB = 0.50 µg/L Spike 1,000ml DI water with 2.5 µl of stock
 LFB = 4.0 µg/L Spike 1,000ml DI water with 20 µl of stock.

Low Spk/Spk Dp = 0.5 µg/L Spike 1,000ml sample with 2.5 µl of stock
 Spk/Spk Dp = 4.0 µg/L Spike 1,000ml DI water with 20 µl of stock.

D. SURROGATE STANDARD SOLUTION:

Ultra Scientific: 500µg/ml - Spike samples, RB, Low LFB & LFB w/ 10 µl = conc. 5.0 µg/L

E. INTERNAL STANDARD SOLUTION:

Ultra Scientific: 500µg/ml - Spike samples w/ 10µl = conc. 5.0 µg/L

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

EPA Method 528

DETERMINATION OF PHENOLS IN DRINKING WATER BY SOLID PHASE EXTRACTION AND CAPILLARY COLUMN GAS CHROMATOGRAPHY/ MASS SPECTROMETRY (GC/MS)

File Name: M:\SOP\Organic\epa method sop\528_102104.doc
Revision: 1

Effective Date: 10/21/2004
Supersedes: 04/03/2002

1. SUMMARY OF METHOD

A measured volume of sample of approximately 1 liter is extracted using a solid phase extraction (SPE) cartridge containing 0.5g of a modified polystyrene divinyl benzene copolymer. The organic compounds are eluted from the cartridge with small quantities of methylene chloride. The extract is dried with sodium sulfate and concentrated to 1 ml using the Zymark concentrator - using gentle heat and UHP nitrogen gas. The final extract is injected into a GC/MS system for separation, identification, and quantitation. Internal and surrogate standards are added to every sample. The method is applicable to a variety of phenols that are efficiently partitioned from the water sample onto a modified polystyrene divinylbenzene solid phase sorbent, and sufficiently volatile and thermally stable for gas chromatography.

2. ANALYTES

2.1 This is a gas chromatographic mass spectrometry (GC/MS) method, applicable to the determination of phenols in finished drinking water. The following compounds can be determined using this method. They are separated based on the Calibration Standards used for this method – Ultra Scientific:

<u>Target</u>	<u>MW</u>	<u>Quan Mass</u>	<u>CAS#</u>	<u>LIMS ID</u>
Phenol	94	94	108-95-2	PHENOL
2-chlorophenol	128	128	95-57-8	2CIPNL
2-methylphenol (o-cresol)	108	107	95-48-7	oCRESL
2-nitrophenol	139	139	88-75-5	2NPNL
2,4-dimethylphenol	212	107	105-67-9	24DMP
2,4-dichlorophenol	162	162	120-83-2	24DCPH
4-chloro-3-methylphenol	142	142	59-50-7	43CMP
2,4,6-trichlorophenol	196	97	88-06-2	245TCP
2,4-dinitrophenol	184	154,184	51-28-5	24DNP
4-nitrophenol	139	139	93951-79-2	4NPNL
2-methyl-4,6-dinitrophenol	198	121,198	534-52-1	246TBP
Pentachlorophenol	266	266	87-86-5	PCP

Internal Standards (@ 500 ug/ml in Methylene Chloride)

1,2-Dimethyl-3-nitrobenzene

Surrogate Standards (@ varies concentration)

2-Chlorophenol-3,4,5,6-d₄ 400 g/mL in methanol

2,4-dimethylphenol-3,5,6-d₃ 400 g/mL in acetone

2,4,6-Tribromophenol 1000 g/mL in methanol

3. APPARATUS AND EQUIPMENT

- 3.1 Sample Bottles – 2.5 liter amber glass bottles fitted with a screw cap lined with polypropylene.
- 3.2 Autosampler vials – amber vials equipped with PTEE-lined septum.
- 3.3 Concentrator Tube - Zymark 50 mL tubes used with the Zymark Turbo-Vap.
- 3.4 Analytical Balance: Capable of weighing accurately to nearest 0.0001 g.
- 3.5 Zymark Turbo-Vap - used to concentrate extracts.
- 3.6 3800 Varian gas chromatograph with a Saturn Ion Trap Mass Spectrometer and a data workstation. Equipped with an 8200 autosampler for injecting samples into the GC.
- 3.7 Column: Fused Silica Capillary column, VF-5MS 30 meters long x 0.25 mm I.D. with a 0.25 micron film thickness, 5 m EZ guard from Varian Inc.
- 3.8 Disposable pasteur pipets, graduated cylinders (1000ml, 100ml, and 10ml), and Hamilton micro syringes – various sizes
- 3.9 Drying Column: Any small tube such as syringe barrel, a glass dropper....

4. REAGENTS AND CONSUMABLE MATERIALS

- 4.1 Reagent Water - Millipore Milli-Q System or equivalent.
- 4.2 Methylene Chloride, Methanol, Acetone: Burdick & Jackson - capillary GC² solvent.
- 4.3 Hydrochloric Acid: ACS reagent grade 6N and 0.05N.
- 4.4 Sodium Sulfate: (ACS) Granular, anhydrous. Heat sodium sulfate at 400°C four hours.
- 4.5 SPE Cartridges: Varian Bond Elut PPL. The polypropylene cartridges (6mL

volumn) are packed with 0.5g highly cross-linked, and chemically modified styrene divinyl benzene copolymer.

- 4.6 Helium carrier gas: UHP grade. Liquid carbon dioxide: technical grade.
Nitrogen gas: UHP grade.

5. SAMPLE COLLECTION, PRESERVATION AND HANDLING

- 5.1 Samples are collected in two liter amber bottles, fitted with a screw cap lined with polypropylene. Keep samples sealed from collection time until analysis. Sampling equipment must be free of plastic tubing, gaskets, and other parts that may leach interfering analytes into the water sample.
- 5.2 All samples should be iced or refrigerated at 4 °C and kept in the dark from the time of collection until extraction. Residual chlorine should be reduced at the sampling site by addition of 40-50 mg of sodium sulfite to each sample. After addition to the sample, seal the bottle and shake for one minute. The sample must be dechlorinated prior to adding acid to lower the pH of the sample. Adding sodium sulfite and HCl to the sample bottles prior to shipping to the sampling site is **not** permitted. Hydrochloric acid should be used at the sampling site to retard the microbiological degradation of some analytes in water. The sample pH is adjusted to <2 with 6N HCl. Store all samples at 4°C until ready for extraction. All samples must be extracted within 14 days after collection. The extract should be stored at 0°C or less and analyzed within 30 days after sample extraction.
- 5.3 The processing of a field blank (travel blank - TB) is recommended along with each sample set. A sample bottle for the TB is filled at the laboratory, with the same preservation reagents added, and shipped to the site(s) along with the other sample bottles. The TB is processed in the same manner as the samples.

6. EXTRACTION

- 6.1 Transfer one liter of sample to 1-liter bottles. All samples will be at a pH of less than 2. All QA/QC samples must be added sodium sulfite and adjusted to this pH using 6N HCl solution. Add 5ul of the surrogate (SS) compound fortification solution – varies conc. Results in a 2-5 ug/L concentration of the SS targets in water.
- 6.2 Cartridge Clean Up and Conditioning: Rinse each cartridge with three, 3mL methylene chloride. Let the cartridge drain dry after each flush. Then rinse the cartridge with three, 3mL methanol, **DO NOT** allow the methanol to elute below the top of the cartridge packing. **From this point, do not allow the cartridge packing to go dry.** Rinse with three, 3mL 0.05N HCl, turn off the vacuum before the dilute acid level drops below the top edge of the packing. Add 3mL additional 0.05N HCl to the cartridge, attach the transfer tube, and turn on the vacuum, and begin adding sample to the cartridge.

Adjust the vacuum so that the approximate flow rate is 20mL/min (50min for a 1L sample) After all of the sample has passed through the SPE cartridge, draw air or nitrogen through the cartridge for 15-30 min at high vacuum (10-15 in Hg). The cartridge should appear dry (light tan color) before continuing with the elution steps.

Rinse the inside of each sample bottle with 8-10 mL methylene chloride and use vacuum to pull the solvent through the transfer tube and through the cartridge, collecting the solvent in a collection tube. Remove the transfer tubing from the top of the cartridge. Add 2-3mL methylene chloride to the top of the cartridge with a disposable pipette. Pull this solvent through the cartridge at low vacuum, such that the solvent exits the cartridge in a dropwise fashion. Pass the eluate through the drying column, which is packed with approximately 5-7 g of anhydrous sodium sulfate, and collect in a clean collection tube. Wash the sodium sulfate with at least 2 mL methylene chloride and collect in the same tube.

- 6.3 Concentrate the sample to approximately 0.9 ml – do not concentrate the extract to less than 0.5 ml. Add 5 ul of the internal standard. Make any volume adjustments with methylene chloride – to the 1.0 ml marked level. Transfer the 1.0 ml extract into a autosampler vial and store the sample extract in the refrigerator at 0°C or below.

7. ANALYSIS

- 7.1 The first step for the instrumental analysis of this method is the mass calibration of the system. Inject into the GC/MS system a 1ul aliquot of the 5 ng/ul solution of DFTPP. Acquire a mass spectrum that includes data for m/z 45-450. The DFTPP mass spectrum must meet all the criteria in Table 1. A single spectrum or an average spectrum across the GC peak may be used to evaluate the performance of the system. This criterion must be met for each day's analytical run. If the system repeatedly fails the criteria – retune the system until it passes.
- 7.2 Initial Calibration: A five point calibration curve is required for this method; using standards STD-A (1.0ppb), B (2.0ppb), C (5.0ppb), D (7.0ppb), E (10 ppb). At least five calibration curve points must be utilized for each target compound. For each analyte and surrogate, calculate the mean RF and the relative standard deviation (RSD). The %RSD of any analyte or surrogate within the calibration range must be less than 20%. If not – inject other standards or perform system maintenance and begin the system analysis and performance check over.
- 7.3 Continuous Calibration check: Check the system tune and performance check for each 12 hour work shift. For each analyte and surrogate, calculate the mean RF and the relative standard deviation (RSD). The %RSD of any analyte or surrogate

within the calibration range must be less than 20%. If not – inject other standards or perform system maintenance and begin the system analysis and performance check over.

- LFBs (laboratory fortified blanks) should also be analyzed with each extraction run. Standards used for these QA/QC samples must be ordered from a second source whenever possible. For extended runs, check standards should be interspersed with samples at regular intervals. If the response of any analyte varies from the predicted response by more than +/- 20%, test must be repeated using fresh calibration standards. Data is collected and processed by Saturn GC/MS software. A calibration file must be created by analyzing the five point calibration curve and entering these values into the cali file. Do this by typing Q,A, and F2 at the main menu for each calibration level. The percent RSD can be obtained by running EnviroPro. An unknown is identified by comparing its mass spectra with the NIST92 library. Its purity must exceed 800 for positive identification. Any results above the highest calibration standard must be confirmed with a standard that is within +/- 20% of the actual result or the sample must be diluted to within the calibration range.

GC Conditions:

- | | | | | | |
|----|-----|------|------|-------|-------|
| 1. | Seg | Temp | Rate | Time | Total |
| | 1 | 35 | 0 | 6.0 | 6.0 |
| | 2 | 250 | 8 | 26.87 | 32.87 |
2. Helium linear velocity: 30 cm/sec
 3. Splitless injection with 1.5 minute delay
 4. Injector temperature (SPI): Initial 25°C to 200°C @ 200 °C/minute hold for 1 minute
 5. Transfer Line Temp: 280 °C
 6. 2 ul sample is injected at 0.2 ul/sec - sandwich technique is used with a 0.5 ul solvent plug

MS Conditions :

1. Mass range : 45 to 450 amu
2. Seconds/scan : 0.810
3. Acquire time : 32.87 minutes

- | | | |
|-----|--------------------|---------------|
| 4. | Fil/Mul delay : | 10 minutes |
| 5. | Peak threshold : | 1 count |
| 6. | Mass defect : | 0 mmn/100 amu |
| 7. | Background mass : | 43 amu |
| 8. | Ionization mode : | EI |
| 9. | Auto ion control : | ON |
| 10. | Cal gas : | OFF |

5 ng of DFTPP is injected (1ul of a 5ug/ml solution) before every run into the GC/MS to determine whether the MS is "in tune". Using the GC method DFTPP, MS method DFTPP and AS method DFTPP, the ion abundance of the mass spectra of DFTPP can be compared to the following set of criteria.

<u>Mass</u>	<u>Relative Abundance Criteria</u>
54	10-80% of the base peak
68	<2% of mass 69
70	<2% of mass 69
127	10-80% of the base peak
197	<2% of mass 198
198	base peak of >50% of 442
199	5-9% of mass 198
275	10-60% of the base peak
365	>1% of the base peak
441	Present and < mass 443
442	base peak of >50% of 198
443	15-24% of mass 442

EnviroPro software is used to determine whether the ion abundance criteria is met. Use file EP to get into EnviroPro and type in the file name, type the correct forms output, 6B, and the califile DFTPP. From the main menu type R for run procedure and DFT525 to get the printout of the relative ion abundance of the DFTPP injection. This printout is kept with each data package.

8. QA/QC REQUIREMENTS

- 8.1 Laboratory Reagent Blank - Run before samples. Use to demonstrate that all glassware and reagent interferences are under control. If any contamination peaks are produced, determine source of contamination and eliminate interference.
- 8.2 Laboratory Fortified Blank - Must analyze at least two laboratory fortified blanks (LFBs) with each set of extractions. Run a LFB-low and an LFB-high with each analytical run. The fortification concentration of each analyte should confirm the ability to detect at the reportable level. If the recovery of any analyte falls outside the control limits, the source of the problem must be identified and corrected.

- 8.3 Standards - Verify calibration standards quarterly by analyzing a standard prepared from reference material obtained from an independent or second source. EPA performance evaluations are an excellent process to determine the validity of the method. Results must be within +/- 20% of those used to routinely check calibration. Daily, run a low level standard to check the reportable detection level, RDL.
- 8.4 Samples - Samples must be extracted within 14 days after collection – unless specific targets need to be determined. Samples must be stored at 4°C until ready for extraction. Duplicates are run on 10% of samples, or once during run, whichever is greater. Results should be within +/- 20%. Extracts should be analyzed within 30 days after sample extraction.
- 8.5 Spike Recoveries - The laboratory must add a known concentration of spike solution, the same as used for LFB, to at least 10% of samples or once per analytical run, whichever is greater. Recoveries should be within the acceptable range. Wherever possible, run a second source standard for spikes.
- 8.6 QC Requirements - Analyze EPA QC check sample with known values if available. The results for each analyte must be within the EPA acceptance criteria. Semi-annually, analyze EPA Performance Evaluation samples. Analyze these check samples whenever major maintenance to the system occurs to ensure the validity of the method.
- 8.7 When running the daily midpoint standard, ensure that the absolute areas of the quantitation ions of the internal and surrogate standards have not decreased by more than 30% of the continuing calibration check or more than 50% of the initial calibration curve.

9. PREVENTIVE MAINTENANCE AND CORRECTIVE ACTIONS

- 9.1 Glassware - Glassware must be carefully cleaned. Clean all glassware as soon as practical after use by thoroughly rinsing with last solvent used in it. Follow by washing with hot water and detergent and thoroughly rinsing with tap water followed by reagent water. After drying, heat in oven. Do not heat volumetric glassware above 220°C. Thorough rinsing with acetone may be substituted for heating.
- 9.2 Reagents - The use of high purity solvents and reagents will help to minimize contamination problems.
- 9.3 Carryover - Contamination carryover may occur when a sample containing a low concentration of analytes is analyzed immediately following a sample containing a high concentration. Use solvent rinses between samples to minimize carryover.

- 9.4 All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory method blanks. Minimize contact of the samples, reagents, or solvents with any plastics. This will help reduce contamination. Interferences by phthalate esters can pose a major problem in pesticide analysis.
- 9.5 Record all corrective actions in the maintenance logbook. Include a complete description of the problem and what action was taken to correct it.

SOP PROCEDURE CHANGE
For EPA Method 528

CHANGE

Splitless time 1.5 min from 36 sec.

REASON

Increase response of most phenols

DATE INITIALS

10/20/04, ly

Injection volume 2 ul from 1ul

reproducible response and linear calibration.

10/20/04, ly

ORANGE COUNTY WATER DISTRICT

EPA METHOD 528

PREPARATION OF STANDARDS AND QUALITY CONTROLS

1. Calibration Standard: **Protocol** - Stock = 100 ug/ml in Methylene Chloride
2. 2nd Source Standard: **Restek** - Stock = 100 ug/ml in Methylene Chloride

A. CALIBRATION STANDARD: (dilute in Methylene Chloride)

<u>Calib. Std. Conc.</u>	<u>Volume of Std.</u>	<u>surrogate std</u>	<u>Dil. Vol.</u>
Std A - 1.0 µg/L	5 µl of 100 µg/mL Std.	1.25 µl	1.0 ml
Std B - 2.0 µg/L	10 µl of 100 µg/mL Std.	2.50 µl	1.0 ml
Std C - 3.0 µg/L	15 µl of 100 µg/mL Std.	3.75 µl	1.0 ml
Std D - 5.0 µg/L	25 µl of 100 µg/mL Std.	5.00 µl	1.0 ml
Std E - 6.0 µg/L	30 µl of 100 µg/mL Std.	7.50 µl	1.0 ml

Add 5 ul of internal std for each dilution.

B. LFB & SPIKE:

Low LFB = 1 µg/L Spike 500ml DI water with 5 µl of 100 µg/mL Std.
LFB = 5 µg/L Spike 500ml DI water with 25 µl of 100 µg/mL Std.

Sample Spk = 1.0 µg/L Spike 500ml sample with 5 µl of 100 µg/mL Std.

C. SURROGATE STANDARD SOLUTION:

Stock Solution - varies concentration
Spike Calibration Standards and Samples with 5µl of stock standard

D. INTERNAL STANDARD SOLUTION:

Stock Solution - 500 µg/ml in Methylene Chloride
Spike Calibration Standard and Samples (after extraction) with 5µl of stock standard

E. PERFORMANCE CHECK STANDARD (DFTPP) Ultra Scientific

Stock Solution - 1000 µg/ml in Acetone
Dilute 5µl of stock to 1mL with CH₂Cl₂ - Inject 1µl = conc. 5 ng/mL (5ug/L)

EPA Method 528 Rev. 3.0

QA/QC Sample Prep:

Add 20-25 mg of Na Sulfite to 500 mL DI water - shake well
Make sure the Na Sulfite is dissolved before acidify the sample to pH 2 with 6N HCl
Add 5ul surrogate - Mix immediately until homogeneous
Low LFB & Spk/Sp Dup: 5uL. LFB: 25 uL

EXTRACTION

Add 3 ml of methylene chloride to the assembled SPE cartridge (UCT DVB 500mg-6mL)
soak for 2 minutes, collect waste. Repeat this step 2 more times

Let the cartridge drain dry after each flush

Add 3 mL of methanol, soak for 2 minutes
Repeat this step 2 more times

Do not let the methanol to elute below the top of the cartridge packing

FROM THIS POINT - DO NOT LET THE CARTRIDGE TO GO DRY

Add 3mL of 0.05N HCl, soak for 2 minutes
Repeat this 2 more times

Turn off the vacuum before the dilute acid level drops below the top edge of the packing

Add 3mL additional 0.05N HCl

Attach transfer tube, turn on vacuum and begin adding sample to the cartridge

Adjust the pressure to 8 mL/min flow rate (70min/sample)

After the entire sample has been processed, pull air through for 10 minutes.

Rinse the sample bottle with 6 mL methylene chloride, add rinse to transfer tube

Turn on vacuum to pull the solvent through the transfer tube and through the cartridge
let solvent soak for 2 minutes then dry completely

Collecting the solvent in a collection tube

Add 2mL of methylene chloride to the top of the cartridge soak for 2 minutes and dry completely

repeat above step one more time

Reduce the vacuum power so that the solvent exits the cartridge in a dropwise fashion

Pass the eluate through a drying column packed with 7g Na₂SO₄ (rinse tube with 2, 2mL of MeCl₂)

Collect the eluate in a clean zymark tube

Wash the Na₂SO₄ with 3mL methylene chloride and collect in the same zymark

Zymark settings: Temp: 35, Pressure: 9 psi

concentrate the extract to 1-ml, then add internal (5 ul) of 500ug/ml

Transfer to an amber autosampler vial (gc)

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

EPA Method 531.2

Rev. 1.1

DETERMINATION OF CARBAMATES IN WATER BY HPLC WITH POST-COLUMN DERIVATIZATION

File Name: M:\Sop\Organic\epa method sop\531_2_1109.doc
Revision: 1.1

Effective Date: 11/01/2009
Supersedes: Rev. 1.0, 05/27/2009

1. SUMMARY OF METHOD

This method uses high performance liquid chromatography (HPLC) with post-column derivatization and fluorescence detection to determine eleven carbamate compounds in groundwater and finished drinking water. A filtered water sample is injected into a reverse phase HPLC column. Gradient elution chromatography is used for separation of the analytes. After elution, the analytes are hydrolyzed with a sodium hydroxide solution at 80°C. The methyl amine formed during hydrolysis is reacted with o-phthalaldehyde (OPA) and 2-mercaptoethanol to form a highly fluorescent derivative, which is detected by a fluorescence detector.

2. ANALYTES

<u>ANALYTE</u>	<u>LIMS code</u>
Aldicarb	ALDI
Aldicarb sulfoxide	ALDISX
Carbaryl	CARBAR
3-Hydroxycarbofuran	HYDCFR
Methomyl	MTHOMY
Adicarb sulfone	ALDISN
Baygon	BAYGON
Carbofuran	CARBOF
Methiocarb	MTHCRB
Oxamyl	OXAMYL
1-Naphthol	NPTHOL

3. APPARATUS AND EQUIPMENT

3.1 Sample Bottles - 250mL, amber glass, screw cap with septum.

- 3.2 Vials - 2mL, amber glass, screw cap with septum.
- 3.3 Filtration Apparatus - 47mm membrane filters (Millipore HATF 04700 or equivalent) are used to filter mobile phase solvents and post-column reagents used in HPLC.
- 3.4 Ultrasonicator - to degas solvents and reagents.
- 3.5 HPLC - Waters Carbamate Analysis System, 150mm long x 3.9mm ID stainless steel Waters Carbamate Analysis Column (part no. 35577), OPA post-column derivatization, fluorescence detection.

4. REAGENTS AND CONSUMABLE MATERIALS

- 4.1 Reagent Water - Millipore Milli-Q System or equivalent.
- 4.2 HPLC Mobile Phase Solvents:
 - 4.2.1 Water - HPLC grade
 - 4.2.2 Methanol - HPLC grade
 - 4.2.3 Acetonitrile - HPLC grade
- 4.3 Post-Column Derivatization Reagents:
 - 4.3.1 Sodium Hydroxide, 0.075N - Dissolve 6.0 grams sodium hydroxide in reagent water. Dilute to 2.0L. Filter and degas.
 - 4.3.2 Sodium Borate, 0.05N - Dissolve 38.2 grams sodium borate, $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$, in reagent water. Dilute to 2.0L. The sodium borate will completely dissolve at room temperature if prepared the day before use. After dissolution, filter with a 0.45 micron filter, and sonicate.
 - 4.3.3 OPA Reaction Solution - Dissolve 400 +/- 40mg of o-phthal-aldehyde in methanol and dilute to 25.0mL. Add the OPA solution and 2 ml of 2-mercaptoethanol to 2.0L of 0.05N sodium borate and mix. Once 2-mercaptoethanol has been added, do not degas. Prepare fresh daily and store in amber glass reservoir, wrapped with aluminum foil to protect from light.
- 4.4 Sample Preservation Reagents:
 - 4.4.1 Potassium dihydrogen citrate ($\text{C}_6\text{H}_7\text{KO}_7$, CAS #: 866-83-1) – Added to adjust sample pH to ~3.8
 - 4.4.2 Sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$, CAS #: 7772-98-7) – Use as dechlorinating agent.

4.5 Stock Standard Solutions, 100µg/mL - Acquired from Accu Standard, Cat# M-531M, contains mixture of 11 components in acetonitrile solution. Transfer stock standard solutions to teflon-sealed vials. Store at 4°C and protect from light. Stock standards should be replaced after three months, or when QC indicates a problem.

4.6 Surrogate Analyte Standard Solution, 4-bromo-3,5-dimethylphenyl n-methyl carbamate (BDMC, CAS #: 672-99-1) – Acquired from Accustandard, 0.1 mg/mL in acetonitrile. Stock standard should be replaced after three months, or when QC indicates a problem.

5. SAMPLE COLLECTION, PRESERVATION AND HANDLING

5.1 Collect samples in 250mL amber glass bottles containing 2.3g of potassium dihydrogen citrate and 20 mg of sodium thiosulfate. After sample is collected, seal bottle and shake vigorously for one minute. Sample pH should be ~ 3.8. Samples must be iced or refrigerated from time of collection until storage. Samples must be stored at 4°C until analyzed. Samples are stable for 28 days from time of collection.

6. PRE-START UP

- 6.1 Prepare, filter and degas mobile phase eluants and fresh reagents.
- 6.2 Turn on LC, Temperature Control Module (TCM) and fluorescence detector. Make sure all lines are properly connected. Open Quickstart in software.
- 6.3 Prime HPLC pump if necessary. Make sure the lc column is endcapped in order to prevent any solvent degassing within the column.
- 6.4 Prime HPLC OPA/NaOH pumps manually. Set flow to 0.5ml for both reagents.
- 6.5 Activate method. Once all flows have been established, let the system equilibrate for a minimum of one hour.
- 6.6 Prepare samples for analysis by filtering through 0.45µm filter disks and an AP prefilter

7. ANALYSIS

7.1 Prior the analysis, the resolution of peaks in a calibration standard or CCC near the mid level of calibration must be monitored in each analytical batch. Closely eluting peaks that are not baseline resolved must have a resolution (R_s) of 1.0 or greater. Although resolution may be monitored any time during the 24-hour analytical batch, if resolution check fails, all samples must be reanalyzed after problem is corrected.

It is therefore recommended to monitor this prior to sample analysis.

- 7.2 Do a Laboratory Reagent Blank and a 5-point calibration at the beginning of each analytical run. When quantitated using the initial calibration curve, each calibration point, except the lowest point, for each analyte should calculate to be 70-130% of its true value. The lowest point should be 50-150%.
- 7.3 Verify the calibration by measurement of two calibration check standards (CCC), one at the beginning and one at the end of the run, and after every 10 samples during analyses (RB, LFB's, spikes and spike duplicates are not considered as a "sample"). The beginning CCC each day must be at or below MRL. Subsequent CCC should alternate between a mid and high level. If the response of any analyte varies from the predicted response by more than +/- 30% for mid and high level, and +/- 50% for low level, test must be repeated. If these conditions are not met, any field or QC samples that have been analyzed since the last acceptable calibration verification should be reanalyzed after adequate calibration has been restored.

Conditions for the HPLC Carbamate Analysis System are as follows:

Run Time	24.00 min and then 4 min injection delay
Acquisition delay	none
Spurge Rate:	Continuous
Flow Rate:	1.5 mL/min, mobile phase.
Post column reagents:	0.5 ml/min
Initial Mobile Phase:	88% water 12% methanol 0% acetonitrile
Post-Column Oven Temp:	80°C
Fluorescence Detector:	Excitation 339nm Emission 445nm
Sample Inject Volume:	100µl on e2695

Gradient Program:

<u>Time</u>	<u>Flow</u>	<u>%A (water)</u>	<u>%B (MeOH)</u>	<u>%C (ACN)</u>
0.00	1.50	88	12	0
5.30	1.50	88	12	0
5.40	1.50	68	16	16
14.00	1.50	68	16	16
16.10	1.50	50	25	25
20.00	1.50	50	25	25

24.00	1.50	30	35	35
25.00	1.50	88	12	0
31.00	0.50	10	90	0
32.00	0.50	10	90	0

8. QA/QC REQUIREMENTS

- 8.1 Laboratory Reagent Blank - Run before samples. Use to demonstrate that all glassware and reagent interferences are under control. If any contamination peaks are produced, determine source of contamination and eliminate interference.
- 8.2 Laboratory Fortified Blank - Must analyze at least two laboratory fortified blank (LFB) one after every calibration and one at the end of the analysis. The fortification concentration of each analyte should be 10 times the EDL or the MCL, whichever is less. If the recovery of any analyte falls outside the control limits, the source of the problem must be identified and corrected.
- 8.3 Standards - Verify calibration standards quarterly by analyzing a standard prepared from reference material obtained from an independent or second source. Results must be within +/- 30% of those used to routinely check calibration. Daily run a low level standard to check the reportable detection level, RDL.
- 8.4 Samples - Samples must be analyzed within 28 days after collection. Samples must be stored at 4°C until ready for analysis. Duplicates are run on 10% of samples, or once during run, whichever is greater. Results should be within +/- 30%.
- 8.5 Spike Recoveries - The laboratory must add a known concentration of spike solution, the same as used for LFB, to at least 10% of samples or once per analytical run, whichever is greater. Recoveries should be within the acceptable range.
- 8.6 QC Requirements - Monthly, analyze EPA QC check sample with known values if available. The results for each analyte must be within the EPA acceptance criteria. Semi-annually, analyze EPA Performance Evaluation samples.

9. PREVENTIVE MAINTENANCE AND CORRECTIVE ACTIONS

- 9.1. Glassware - Glassware must be carefully cleaned. Clean all glassware as soon as practical after use by thoroughly rinsing. Follow by washing with hot water and detergent and thoroughly rinsing with tap water followed by reagent water. After drying, heat in oven. Do not heat volumetric glassware. Thorough rinsing with acetone may be substituted for heating.
- 9.2. Reagents - The use of high purity solvents and reagents will help to minimize contamination problems.

9.3. Carryover - Contamination carryover may occur when a sample containing a low concentration of analytes is analyzed or filtered immediately following a sample containing a high concentration. Use DI water rinses between samples to minimize carryover.

9.4. No or Poor Chromatography -

- 9.4.1. Check that all flows are at the proper flows. Post column flows can be checked where the OPA/MERC and NaOH flow into the post column reaction coil. Mobile phase flows can be best checked from the exiting waste line from the detector. Loss of mobile phase flow would indicate a leak somewhere within the system.
- 9.4.2. Look to see that post-column flows are actually flowing. If air bubbles are present, prime the necessary lines. Make sure that helium is purging through the eluant containers. Check for leaks around all pump heads.
- 9.4.3. Perform a daily injection purge.
- 9.4.4. Replace standards.
- 9.4.5. Remove column and replace with a union. Inject a standard and begin a normal analysis. If a peak appears on the chromatogram, then the system is considered fine. The problem lies somewhere within the chemistry and/or column.
- 9.4.6. If high pressure exists at a flow of 1.5ml/min and at 88/12 CH₃OH/H₂O, a problem with column frits may exist. Reduce flow slowly (0.5ml increments), remove column and remove column frits. Sonicate column frits in CH₃OH for 15 minutes and put back into column. Equilibrate column with mobile phase. If the pressure still high, then clean the column using the following procedure:

Flow 1ml/min

Column volumes (50ml)

CH₃OH

CHCl₃

CH₃OH

H₂O

Mobile phase

- 9.4.7. If high pressure still exists, reverse column direction and run a standard.
- 9.4.8. Even if high pressure exists, keep using column until tailing or double peaks occur. As long as a RDL can be achieved, use the column.

9.5. Calibration Standards/QC standards fail-

- 9.5.1. Check all the standards. If calibration and/or QC lie outside the acceptable limits we must first re-run the standards to assure that the injection procedure is working properly. After the re-run if standards still lie outside the acceptable limits we must

make a new standard or conduct instrument maintenance to assure that calibration and QC lie with acceptable parameters. For elevated spike recoveries make sure that the sample matrix is clean. If sample matrix is altering recoveries then a new matrix must be selected. If instrument performance is acceptable the samples must be re-extracted. If the samples lie outside allowable holding time they must be re-sampled. If resolution fails, check the standard, and re-analyze. If resolution still fails, stop the instrument and perform the necessary maintenance. If front check standard fails, check standards, make up new dilutions and re-analyzed.

9.5.2. Request re-sample if it's necessary.

9.5.3. If there're analytical hits, request for resample.

10. SOP PROCEDURE CHANGE
For EPA Method 531.2

<u>CHANGE</u>	<u>REASON</u>	<u>DATE</u>	<u>INITIALS</u>
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Gradient flow-----inconsistent retention times---	01/19/06-----	al	
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-HPLC conditions, gradient program, amount of OPA to add, and LIMS codes of anayltes. -			
-Update of SOP ---	11/01/09----	GGA	

ORANGE COUNTY WATER DISTRICT

EPA METHOD 531.2

PREPARATION OF STANDARDS AND QUALITY CONTROLS

*Use HPLC grade or Mili-Q water.

Add 9.2 TO 9.5 g of potassium dihydrogen citrate
and 80 - 300 mg of Sodium Thiosulfate to 1 L of water*.
to make Reagent Water. Shake well to dissolve.
Use this Reagent Water for all calibration standards & QC samples.

Accustandard Mix & Ultra Scientific (100µg/ml)

- | | |
|------------------------|----------------|
| 1. Aldicarb sulfoxide | 6. Aldicarb |
| 2. Aldicarb sulfone | 7. Propoxur |
| 3. Oxamyl | 8. Carbofuran |
| 4. Methomyl | 9. Carbaryl |
| 5. 3-Hydroxycarbofuran | 10. 1-Naphthol |
| | 11. Methiocarb |

Surrogate: Accustandard, 100 µg/mL in Acetonitrile

4-bromo-3,5-dimethylphenyl n-methylcarbamate - BDMC

A. CALIBRATION STANDARD (Accustandard)

Std. Conc.	Volume Use	Surr Conc.	Volume Use	Final Vol.
1.0 µg/L	1.0 µl of stock	2.0 µg/L	2.0 µl of surr	100 ml
2.0 µg/L	2.0 µl of stock	4.0 µg/L	4.0 µl of surr	100 ml
5.0 µg/L	5.0 µl of stock	5.0 µg/L	5.0 µl of surr	100 ml
10.0 µg/L	10.0 µl of stock	8.0 µg/L	8.0 µl of surr	100 ml
20.0 µg/L	20.0 µl of stock	10.0 µg/L	10.0 µl of surr	100 ml

B. Continuous Calibration Check Standard: Accustandard

Std. Conc.	Volume Use	Surr Conc.	Volume Use	Final Vol.
"W" -1.0 µg/L	1.0 µl of stock	5.0 µg/L	5.0 µl of surr	100 ml
"T" - 5 µg/L	5.0 µl of stock	5.0 µg/L	5.0 µl of surr	100 ml

C. Second Source Check Standard (Quality Control Sample QCS):

- ***LFB Low - Conc.= 1.0 µg/L Spike 100 ml of Reagent Water with 1 µl of stock & 5 µl of Surr.
***LFB - Conc. = 10 µg/L Spike 100 ml of Reagent Water with 10 µl of stock & 5 µl of Surr.
***Sample Spk - Conc.= 10 µg/L Spike 100 ml of sample with 10 µl of stock & 5 µl of Surr.

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

EPA Method 532, Rev. 2.0

DETERMINATION OF PHENYLUREA COMPOUNDS IN WATER BY HPLC

1. SUMMARY OF METHOD

EPA Method 532 determines certain phenylurea pesticides using high performance liquid chromatographic (HPLC) with a UV detector. A 500ml sample is passed through a disk containing a C18 organic phase to extract the phenylurea pesticides and surrogate compounds. Identification of target and surrogate analyses and quantitation is accomplished by comparison of retention times and analyte responses using external standard procedures.

2. ANALYTES

This is high performance liquid chromatographic (HPLC) method for the determination of phenylurea compounds in drinking waters.

Analyte	LIMS Code	CAS#	MW	RDL
Diflubenzuron	DFLBNZ	35367-38-5	310	1.0 ug/L
Diuron	DIURON	330-54-1	232	1.0 ug/L
Fluometuron	FLMTRN	2164-17-2	232	1.0 ug/L
Linuron	LINURN	330-55-2	248	1.0 ug/L
Propanil	PRPANL	709-98-8	217	1.0 ug/L
Siduron	SIDURN	1982-49-6	232	1.0 ug/L
Tebuthiuron	TBTURN	34014-18-1	228	1.0 ug/L
Thidiazuron	THDURN	51707-55-2	220	1.0 ug/L

3. APPARATUS AND EQUIPMENT

- 3.1 Sample bottles – 2.5L amber bottles, fitted screw cap with Teflon.
- 3.2 Vials – 1 mL amber glass, screw cap with Teflon septa.
- 3.3 Six station extraction manifold with 47 mm glassware sets (Varian 1214-6001 or equivalent) .
- 3.4 Turbo-Vap concentration workstation (Zymark ZW8003)
- 3.5 200 mL Turbo-Vap tubes with 1.0 mL end-point (Zymark - ZA2039)
- 3.6 High performance liquid chromatograph (HPLC) - quaternary pump, autosampler,

switching valve, column oven, UV, column and computer data system.

3.7 10mL, 5 mL volumetric flasks.

3.8 Balance - analytical, capable of accurately weighing 0.0001g.

4. REAGENTS AND CONSUMABLE MATERIALS

4.1 Reagent Water - Millipore. Milli-Q system or equivalent filtered through a 0.45 micron filter.

4.2 Acetonitrile - pesticide quality or equivalent. HPLC quality.

4.3 Methanol – High purity, demonstrated to be free of analytes and interference.

4.4 Phosphate Buffer Solution, 25 mM – HPLC mobile phase

In a 2L flask, dissolve 3.4 g of KH_2PO_4 (Monobasic Potassium Phosphate) in approximate 1L of HPLC water, add 50 ml of 0.5M phosphoric acid solution, dilute to 2L. The pH should be about 2.4

Filter with 0.45-micron filter. Store in a 2L brown bottle and degas.

Phosphoric Acid Stock Solution (0.5M):

17.0 mL of phosphoric acid diluted to 500ml with HPLC water, mix well. Store in refrigerator.

4.5 Standard solutions-

Calibration standard: Absolute Standards Inc.

2nd Source Standard: Ultra Scientific

Surrogate analytes (SUR): Carbazole (CAS#86-74-8)

5. SAMPLE COLLECTION, PRESERVATION AND HANDLING

5.1 Collect samples in 2.5 liter amber bottles with dry chemical preservative added- 1.25 grams cupric sulfate and 11.75 grams of TRIS HCl, and 0.75 TRIS Base. Prepare bottles for spike and spike duplicate sample.

5.2 After collecting the sample, cap carefully to avoid spillage, and agitate by hand for 1 min. Keep samples sealed from collection time until extraction.

5.3 All samples must be iced and refrigerated at 4°C from the time of sampling until extraction. Furthermore, all samples must be extracted within 14 days of collection and analyzed within 21 days after extraction.

6. EXTRACTION

Liquid - Solid phase extraction (SPE):

- 6.1 An extraction batch: A set of up to 20 field samples extracted together by the same person(s) during a work day using the same lot of solid phase extraction devices and solvents, surrogate solution, and fortifying solution.
- 6.2 Assemble C-18 Empore disk and filtration apparatus. Rinse each disk with two, 10 mL aliquots of methanol, allowing the solvent to soak for about 1 minute by pulling approximately 1 mL through the disk and turning off the vacuum temporarily during the first rinsing. Draw the methanol through the disk until 3-5 mm above the disk surface. Follow the methanol rinse with two, 10 mL aliquots of reagent water being careful to keep the water level at 3-5 mm above the disk surface. Turn off the vacuum. Do not let disk go dry during these steps.
- 6.3 Prepare samples, including QC samples. Fill the extraction reservoirs containing the conditioned disk with sample and turn on the vacuum. The flow rate should be approximately 50 mL/min or about 10 minutes to pull the entire sample through the disk. After the entire sample has passed through the SPE disk, draw air through the disk for 10 minutes at full vacuum.
- 6.4 Insert collection tubes, add approximately 5 mL of methanol to the top of each disk. Pulse the vacuum to pull the methanol through and soak the disk. Allow the solvent to soak the disk for 2 minutes. Start the vacuum and pull the methanol through. Attempt to pull methanol through in a dropwise fashion into the collection tube by pulsing the vacuum. Repeat this elution a second time with approximately 4 mL of methanol, and then a third time with approximately 3 mL.
- 6.5 Concentrate the extract to about 5 mL in water bath (at about 40 c) under a gentle stream of nitrogen. Transfer to 5 mL volumetric flasks. Adjust final volume with methanol if needed. Transfer the extract to an amber GC vial. Store extracts in freezer.

7. ANALYSIS

7.1 Instrumentation:

- 7.1.1 High performance liquid chromatograph - quaternary pump, auto sampler, switching valve, column oven, Photo Diode Array (PDA) detector, column and computer data system.
- 7.1.2 Primary Column – Water's Sunfire or Varian's Pursuit HPLC column (4.6x 150 mm), 5u C18 column, or equivalent
- 7.1.3 Confirmation Column – An HPLC column (4.6x 150 mm) packed with 5 dp cyanopropyl stationary phase (Supelco cat. #58221-U).

7.1.4 HPLC Data system- The Waters "Empower2" software system was used to generate all primary column data.

7.2 HPLC Start-up Conditions:

7.2.1 Prepare the phosphate buffer mobile phase following the directions from 4.5 above.

Photo Diode Array (PDA) detector settings preset within Empower.

Solvent Line C: CH₃CN

Solvent Line D: 25mM phosphate buffer mobile phase

Flow rate: 1.5 mL/min

<u>Time</u>	<u>%C (ACN)</u>	<u>%D (Buffer)</u>	<u>Curve</u>
--	40	60	--
7.5 min	40	60	6
10 min	50	50	6
14 min	60	40	6
15 min	40	60	6
20 min	40	60	6

Column Oven Temp: 25°C

Sample Injected Vol.: 30µL

Detector: λ range: 210 – 400 nm (PDA 2998)

7.3 Stock Standard Solutions & External Calibration:

7.3.1 Use the Absolute Standard as the primary standard, the concentration of these analytes is at 200 µg/mL.

7.3.2 The external calibration is a 5 point calibration ranging from 1.0 – 20.0 ppb.

7.3.3 In addition to calibration curve, daily Continuous Calibration Checks (CCC) standards must also be analyzed. CCC must be analyzed at the beginning and the end of each run, and after every tenth sample during analyses ("sample" is considered to be a field sample. RBs, LFBs, Spikes, Spike Dups, other CCC are not considered to be a "sample". The beginning CCC each day should be analyzed right after the 5-point curve, at or below the MRL in order to verify instrument sensitivity. Subsequent CCCs should alternate between a medium and high concentration standard. The back CCC should be the last sample analyzed.

7.3.4 If a cleanup procedure is needed to extract samples, then the same process

must be applied to the calibration curve in order to show validation of the cleanup step.

8. QA/QC REQUIREMENTS

- 8.1 Method blanks (reagent blanks) in order to demonstrate that glassware and reagents are free of any interference, a reagent blank must be analyzed at the beginning of each analysis. If the blank produces any interference, determine the source of the interference and eliminate that interference before proceeding.
- 8.2 Laboratory Fortified Blank - Analyze at least one laboratory-fortified blank, LFB, with every 20 samples or one per run, whichever is greater. The low LFB must be as near as practical to, but no more than two times the MRL. The high concentration should be near the high end of the calibration range. If the recovery of any analyte falls outside the control limits, the source of the problem must be identified and corrected.
- 8.3 Verify calibration standards quarterly by analyzing a standard prepared from reference material obtained from an independent or second source. Results must be within $\pm 20\%$ of those used to routinely check calibration. For each analytical batch, run a low level standard to check the reportable detection level, RDL.
- 8.4 Samples must be extracted within 14 days after collection. Duplicates are run on 10% of samples, or once during run, whichever is greater. Results should be within $\pm 30\%$.
- 8.5 The laboratory must add a known concentration of spike solution, the same as used for LFB, at least 10% of samples or once per analytical run, whichever is greater. Recoveries should be within $\pm 30\%$.

9. PREVENTIVE MAINTENANCE AND CORRECTIVE ACTIONS

- 9.1 Glassware - Glassware must be carefully cleaned. Clean all glassware as soon as practical after use by thoroughly rinsing. Follow by washing with hot water and 10% HCl and thoroughly rinsing with tap water followed by reagent water. After drying, heat in oven. Do not heat volumetric glassware. Thorough rinsing with acetone may be substituted for heating.
- 9.2 Reagents - The use of high purity solvents and reagents will help to minimize contamination problems.
- 9.3 Carryover - Contamination carryover may occur when a sample containing a low concentration of analytes is analyzed immediately following a sample containing a high concentration. Use solvent rinses between samples to minimize carryover.
- 9.4 Minimize contact of the samples, reagents, or solvents with any plastics. This will help reduce contamination.

- 9.5 Record all corrective actions in the maintenance log book. Include a complete description of the problem and what actions were taken to correct it.
- 9.6 No or Poor Chromatography-
- 9.6.1 Check all of the standards. If calibration and/or QC lie outside the acceptable limits we must first re-run the standards to assure that the injection procedure is working properly. After the re-run if standards still lie outside the acceptable limits we must make a new standard or conduct instrument maintenance to assure that calibration and QC lie with acceptable parameters. Any field sample extracts that have been analyzed since the last acceptable calibration verification should be reanalyzed after adequate calibration has been restored. For elevated spike recoveries make sure that the sample matrix is clean. If sample matrix is altering recoveries then a new matrix must be selected. If instrument performance is acceptable the samples must be re-extracted. If the samples lie outside allowable holding time they must be re-sampled.
- 9.6.2 Request re-sample if necessary.
- 9.6.3 If there are hits, confirm by spiking sample. Analytical hits will be increased by the spike amount.

SOP PROCEDURE CHANGE
For EPA Method 532.1

<u>CHANGE</u>	<u>REASON</u>	<u>DATE</u>	<u>INITIALS</u>
Added section 9	audit request	02/18/03	jrb
Gradient program	shorter run time	09/2005	LVS
Changed extraction procedures	Follow EPA Protocol	08/17/07	AL
Updated SOP	fixed errors and discrepancies	01/13/09	mah
Updated SOP, QC requirements	New instrument method	05/13/09	al

Method 532

Sample Volume: 500 mL

Use Millipore water to prepare a 2.5L bottle for RB, LOW LFB, and LFB.

Check pH = 7.

Assemble SPE apparatus with **C-18 Disk**.



Rinse with 10mL of MeOH

Let soak for 1 min. Aspirate remaining solvent. **Do not dry.**

(Leave small layer of solvent, add more methanol if needed to keep the disk from going dry)



Repeat the above step once more.



Rinse with 10mL of H₂O (Millipore Water)

Let soak for 1 min. Aspirate remaining water. **Do not dry.**

(Leave small layer of water, add more H₂O if needed to keep the disk from going dry)



Repeat the above step once more.



Add 500mL of sample (*Use prepared water for RB, LOW LFB, LFB*).



Inject Low LFB with 2.5 uL of 200 ug/mL Ultra stock.

Inject LFB, sample Spike, and Spike Dup with 25 uL of 200 ug/mL Ultra stock.



Inject all QCs and samples with 25 uL of 100 ug/mL Ultra surrogate.



Turn vacuum on (50mL/min).

Sample should take about 10 minutes to pull through disk.



When sample is done, dry disk for 10 minutes at full vacuum.

Insert a collection tube.



Add 5 mL of Methanol using a pipet to rinse the sides of the reservoir. Let soak for 2 minutes.

Slowly pull the methanol in a dropwise fashion into collection tube using minimal vacuum.



Repeat the step above with 4 mL of Methanol



Repeat the step above with 3 mL of Methanol



Completely dry disk.



Remove the collection tube and transfer extracts to Zymark tube.

Rinse collection tube with 2 mL of Methanol -- **twice**. Adding each rinse to Zymark tube.



Zymark settings: Temp 40, Pressure 6psi.

Concentrate the extract to 5-mL line.

Transfer extract to 5mL volumetric flask, rinsing the zymark tube with small amounts of Methanol.

Adjust final volume to 5mL with the Methanol rinse if needed. Transfer to 2 GC amber vials.

Store extracts in freezer.

ORANGE COUNTY WATER DISTRICT

EPA METHOD 532 (revised 08/05/09)

Sample Volume: 500mL

1. Calibration Standard: Absolute Standards, Inc., Catalog # 30196

Stock = 200 µg/mL in Methanol

2. 2nd Source Standard: Ultra Scientific, Catalog # PPM-532

Stock = 200 µg/mL in 60 Methanol: 40 Acetone

3. Surrogate Standard: Ultra Scientific, Catalog # NH-310

Stock = 100 µg/mL in Methanol

<u>Analyte</u>	<u>LIMS Code</u>
Diflubenzuron	DFLBNZ
Diuron	DIURON
Fluometuron	FLMTRN
Linuron	LINURN
Propanil	PRPANL
Siduron	SIDURN
Tebuthiuron	TBTURN
Thidiazuron	THDURN

A. Calibration Standard:

(Dilute with Methanol)

Absolute Std

	<u>Std. Concentration</u>	<u>Volume of Standard</u>	<u>Surrogate</u>	<u>Final Volume</u>	<u>Surrogate Concentration</u>
	STD A - 1.0 µg/L	2.5 µL Stock	5 µL	5.0 mL	1.0 ug/L
	STD B - 2.0 µg/L	5 µL Stock	10 µL	5.0 mL	2.0 ug/L
Mid Check	STD C - 4.0 µg/L	10 µL Stock	25 µL	5.0 mL	5.0 ug/L
	STD D - 10.0 µg/L	25 µL Stock	40 µL	5.0 mL	8.0 ug/L
	STD E - 20.0 µg/L	50 µL Stock	50 µL	5.0 mL	10.0 ug/L

B. Continuous Calibration Check (CCC): Absolute Standard (same source as calib)

Conc. 1 µg/L

2.5uL of Stock, 25 uL Surrogate. Dilute to 5.0 mL in Methanol.

Front and Back Check

Continuous Calibration Check (CCC): Ultra Standard

Conc. 10 µg/L

25uL of Stock, 25 uL Surrogate. Dilute to 5.0 mL in Methanol.

Front and Back Check

C. Low LFB, LFB, Spike, and Spike Dup: Ultra Scientific

Low LFB = 1.0 µg/L

Spike 500 mL millipore water with 2.5 uL Ultra Scientific Stock

LFB = 10.0 µg/L

Spike 500 mL millipore water with 25 µL Ultra Scientific Stock

Spike, Spike Dup = 10.0 µg/L

Spike 500 mL millipore water with 25 µL Ultra Scientific Stock

D. Surrogate Standard: Ultra Scientific, Carbazole Solution,

**** Inject samples with 25 uL of stock - concentration = 5.0 ug/L**

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

EPA Method 547

DETERMINATION OF GLYPHOSATE IN WATER BY HPLC WITH POST-COLUMN DERIVATIZATION

File Name: M:\SOP\Organic\epa method sop\547_040909_1c3.doc Effective Date: 10/27/2009
Revision: 9 Supersedes: 8 (04/09/2009)

1. SUMMARY OF METHOD

This method uses high performance liquid chromatography (HPLC) with post-column derivatization and fluorescence detection to identify and measure glyphosate (N-Phosphonomethyl-glycine) in groundwater and finished drinking water. A filtered water sample is injected into a cation exchange HPLC column. The analyte is then separated by isocratic elution at a column temperature between 50-55°C. Glyphosate is then oxidized with sodium hypochlorite. The product, glycine, is then reacted with ortho-phthalaldehyde in the presence of mercaptoethanol to give fluorophors which can be detected with a fluorescence detector at an excitation of 350nm and an emission of 440nm.

2. ANALYTES

<u>LIMS code</u>	<u>analyte</u>
GLYPHO	Glyphosate

3. APPARATUS AND EQUIPMENT

- 3.1 Sample Bottles - 250mL, Nalgene plastic with screw cap.
- 3.2 Vials – 2 mL, glass, screw cap with septum.
- 3.3 Filtration Apparatus - 47mm membrane filters (Millipore HATF 04700 or equivalent) are used to filter mobile phase solvents, post-column reagents used in HPLC, all QA/QC as well as Calibration and all samples to be analyzed.
- 3.4 Ultrasonicator - to degas solvents and reagents.
- 3.5 HPLC - Waters Carbamate Analysis System - with column switching valve, autosampler, quaternary pump, fluorescence detector and column oven.

- 3.6 Analysis Columns - 250mm long x 4.6mm ID stainless steel column , Water's Fast Juice KCl column, or equivalent.

4. REAGENTS AND CONSUMABLE MATERIALS

- 4.1 Reagent Water - Millipore Milli-Q System or equivalent.

4.2 HPLC Mobile Phase Solvents:

For the Water's column, 0.05% H₃PO₄ solution. In 2 liters of millipore water, pipette 1.2 ml of the concentrated Phosphoric acid and mix thoroughly. Sonicate for 15-20 minutes afterward.

- 4.2.1 Water - HPLC grade

- 4.2.2 Methanol - HPLC grade

4.3 Post-Column Derivatization Reagents:

- 4.3.1 Sodium Hypochlorite (oxidative solution) - Dissolve in 1L, 1.36g KH₂PO₄ , 11.6g of NaCl and 0.4g NaOH. Add 2.0mls of Clorox Bleach. This will create a 5.24% solution of sodium hypochlorite oxidative buffer. Filter the solution with a 0.45 micron filter and sonicate for 15 minutes. When not in use, place solution in refrigerator. This solution is generally good for 2-3 analysis days.

- 4.3.2 O-Phthalaldehyde/mercaptoethanol solution -

Water's Fast Juice Column

In 1 liters of HPLC grade water, dissolve 19.1g of sodium borate(Na₂B₄O₇*10H₂O). Filter the solution with a 0.45 micron filter. Sonicate for 15 minutes. To prepared borate solution add 0.8g o-phthaladhyde (OPA) dissolved in 10mL of HPLC grade methanol. Add 1mL of mercaptoethanol, shake to mix. DO NOT SONICATE AGAIN.

5. SAMPLE COLLECTION, PRESERVATION AND HANDLING

- 5.1 Collect samples in duplicate in a 250ml nalgene bottle. To each sample bottle add 20mg sodium thiosulfate so as to destroy any chlorine that may be present. When filling the sample bottle, fill to half full to allow for sample expansion. After filling

the sample bottle, seal and shake thoroughly to dissolve dechlorinating agent. Samples should be stored at 4°C. A travel blank of the reagent water must accompany each set of samples brought into the laboratory. If the holding time is greater than two weeks, then the sample should be frozen. When frozen, the sample should be stable for up to 18 months.

6. PRE-START UP

- 6.1 Prepare, filter and degas mobile phase eluents and fresh reagents.
- 6.2 Using a filtration apparatus - 47mm membrane filters (Millipore HATF 04700 or equivalent), filter all QA/QC, Calibration and all samples to be analyzed.
- 6.3 Turn on LC, Temperature Control Module (TCM) and fluorescence detector. Make sure all lines are properly connected. Open Quickstart in software.
- 6.4 Prime HPLC pump if necessary. Column heater should have been set to 55°C (Waters). Switching valve should be set for glyphosate. Make sure the carbamate column is endcapped in order to prevent any solvent degassing within the column..
- 6.5 Prime OPA & NAOCL pump manually. Set flow to 0.5ml for both reagents.
- 6.6 Activate method. Once all flows have been established, let the system equilibrate for a minimum of one hour.

7. ANALYSIS

- 9.3 Do a Laboratory Reagent Blank and a 5-point calibration at the beginning of each analytical run. Verify the calibration by measurement of two calibration check standards, one at the beginning (second source) and one at the end of the run (primary source). These check standards should be at two different concentration levels to verify the calibration curve. For extended runs, check standards should be interspersed with samples at regular intervals. If the response of any analyte varies from the predicted response by more than +/- 20%, test must be repeated using fresh calibration standards.
- 7.2 Analyze each calibration standard and generate a calibration curve. If the ratio of response to concentration (response factor) is constant over the working range (<10% deviation), linearity through the origin can be assumed. If it is >10%, check instrument, reagents and stock standards. Prepare new calibration standard and generate a new calibration.

Conditions for the HPLC Glyphosate System are as follows:

Run Time 15 minutes for the Water's column.

Flow Rate: 1.0 mL/min, mobile phase.
For the Water's column, OPA/Merc flow = 0.50ml/min and NaOCl = 0.50ml/min.

Oven Temp: 55°C - Water's column.

Post-Column Oven Temp: 38°C - maximum efficiency for NaOCl.

Fluorescence Detector: Excitation 350nm
Emission 440nm

Sample Inject Volume: 100ul

Switching Valve: Position for glyphosate either manually or by setting position =5 on the e2795, or computer software (preset when EPA547.mth called up on computer).

8. QA/QC REQUIREMENTS

- 8.1 Laboratory Reagent Blank - Run before samples. Use to demonstrate that all glassware and reagent interferences are under control. If any contamination peaks are produced, determine source of contamination and eliminate interference.
- 8.2 Laboratory Fortified Blank - Must analyze at least two laboratory fortified blank (LFB) after every calibration. The fortification concentration of each analyte should be 10 times the EDL or the MCL, whichever is less. If the recovery of any analyte falls outside the control limits, the source of the problem must be identified and corrected.
- 8.3 Standards - Verify calibration standards quarterly by analyzing a standard prepared from reference material obtained from an independent or second source. Daily, run a Continuous Calibration Curve sample (at the same level as the LFB) to validate calibration curve. Results must be within +/- 20% of those used to routinely check calibration. With every run, analyze a low level standard to check the reportable detection level, RDL.
- 8.4 Laboratory Fortified Sample Matrix (LFSM) - A sample is spiked with a known concentration of glyphosate, 100 ppb. For each sample set, 10% of routine samples should be spiked. The acceptable recovery for the LFSM is 30%. Prepare spikes in a 10ml volumetric flask and retain prepared spiked sample if a problem arises with QA/QC. Percent recoveries and Shewhart Control Charts are generated every twenty data points so as to monitor QA/QC.
- 8.5 Low level check standards (RDL check) - Each analysis is to also include a 20 ppb standard (allowable recovery 30% of known value). The data gathered from low level analysis is also used to generate a percent recovery and Shewhart Control Chart to also monitor that QA/QC is being met. The RDL must be confirmed with each run.

- 8.6 MDL check - At least annually verify the MDL of glyphosate by analyzing 7 replicates of a known spike, either 20 or 25 ppb. Recoveries should be within 30%. From here, calculate the mean recovery and standard deviation. Follow procedures as prescribed in the Federal Register 40 CFR part 136 pages 198-199.
- 8.7 QC Requirements - Analyze ERA QC performance check samples whenever they become available. The results for glyphosate must be within the EPA acceptance criteria. Semi-annually, analyze EPA Performance Evaluation samples.

9. PREVENTIVE MAINTENANCE AND CORRECTIVE ACTIONS

- 9.1 Glassware - Glassware must be carefully cleaned. Clean all glassware as soon as possible after use by thoroughly rinsing with hot water. Follow by washing with hot water and 10% HCl (glassware) and thoroughly rinsing with hot tap water followed by reagent water. After drying, heat in oven.
- 9.2 Reagents - The use of high purity solvents and reagents will help to minimize contamination problems.
- 9.3 No or Poor Chromatography -
- 9.3.1 Check that all flows are at the proper values - check flows from the exit waste line from the detector. When the Waters's column is being used, flows should be 0.88 ml/min +/- 5%.
- 9.3.2 Look to see that post-column flows are actually flowing. If air bubbles are present, prime the necessary lines. The Waters's column requires that the post column reaction reaction flows be at 0.50 ml/min OPA and 0.50 ml/min NaOCl +/- 0.02 ml/min.
- 9.3.3 Perform daily injection purge of the system either through the e2795 controller. Verify the pH of the mobile phase and samples - depending on which column is being used.
- 9.3.4 Check all pump heads for leaks. If a leak is present refer to instrument manual on how to change piston/ plunger seals.
- 9.3.5 Remove column and replace with union. Inject a normal standard and begin analysis. If a peak occurs with no column present, then the system is fine. The problem lies within the chemistry or the actual column. To do this step, the system will have to be shut down slowly. The column oven will have to be cooled below 50°C. At ambient temperatures, do not operate the flow greater than 0.2 ml/min.
- 9.3.6 If the ratio of response to concentration (response factor) is >10%, a new calibration curve needs to be generated. Check instrument, check stock standards and reagents. Prepare and analyze new calibration standards.

9.4 High pressure build up: If pressure reaches beyond 1300 psi, depending on age and condition of column, regenerate the column or replace it. To regenerate the column, prepare a 5mM solution of K_2HPO_4 & 4% methanol (v/v). No pH adjustment is

required. Reverse the flow of the column, remove the guard column and replace it with a union. Set the flow to 0.2 ml/min and set temperature of the column heater at 50°C. Let the system run a minimum of 16 hours, preferably overnight. The Water's max column pressure is 1100psi. If high pressure and or poor chromatography result, flush column with 10% ACN for about 10 column volumes. If the same problems still exist, reverse column and flush again with 10% ACN. The head of the column may be clogged and/or dirty. After cleaning the reversed column, it may become necessary to leave the column reversed for any further analysis.

9.5 The Bio-Rad column should be stored in its mobile phase as well as the Water's column. The maximum organic solvent tolerance for the Water's column is 10%. Beyond 10% organics, will ruin the column.

Record all corrective actions in the maintenance logbook. Include a complete description of the problem and what action(s) were taken to correct it.

SOP PROCEDURE CHANGE
For EPA Method 547

<u>CHANGE</u>	<u>REASON</u>	<u>DATE</u>	<u>INITIALS</u>
Addition of section 7.2 & 9.37 suggestion	Addition of calibration requirements per ELAP audit	12/22/05	AL
Changed flow rate to 1.0 ml/min 1/19/2006	Allow for sharper peak and better resolution		FJD
Decreased run time to 15mins from 20mins	New retention time is at about 10mins from previous retention time of 17mins		FJD 1/19/2006
Changed Na ₂ B ₄ O ₇ *10H ₂ O amount from 16.0g to 19.1g (page2, Sec. 4.3.2)	possible typo	3/11/2008	JP
Change to new HPLC-3 instrument parameter	new instrument	04/09/09	al
Update LFB and QC requirement	update to current practice	04/09/09	al
Added travel blank to sample collection requirements		10/27/09	FJD
Changed post column flow rates to .50mL/min to reflect new post column Parameters.		10/27/09	FJD
Added to section 6 startup to reflect that all QA/QC, Calibration and All samples to analyzed must be pre-filtered.		10/27/09	FJD
Changed directions for the post column reagent in 4.3.2 to sonicate Before adding opa and mercapethanol not after.		10/27/2009	FJD
Deleted line 4.4 Stock standard solution not included in SOP		10/27/2009	FJD
Added to 3.3 to reflect filtering of QA/AC Calibration and samples		10/27/2009	FJD

ORANGE COUNTY WATER DISTRICT

EPA METHOD 547

Standard Preparation and Quality Control

All calibration standards, QCs and samples are filtered.

Extraction Solvent ----- HPLC Water

1. Calibration Standard: Ultra Scientific, Catalog # PPS-190, stock concentration = 100 ug/mL

2. 2nd Source Standard: Accustandard, Catalog # M-547, stock concentration = 100 ug/mL

Analytes

Glyphosate

A. Calibration Standards, Ultra Scientific

<u>Standard Concentration</u>	<u>Volume of Standard</u>	<u>Final Volume</u>
STD A - 20 ug/L	2.0 uL	10 mL
STD B - 50 ug/L	5.0 uL	10 mL
STD C - 100 ug/L	10.0 uL	10 mL
STD D - 150 ug/L	15.0 uL	10 mL
STD E - 200 ug/L	20.0 uL	10 mL

B. Check Standard, Accustandard

<u>Standard Concentration</u>	<u>Volume of Standard</u>	<u>Final Volume</u>
100 ug/L	10.0 uL	10 mL

C. Low LFB, LFB, Spike, and Spike Duplicate, Accustandard

<u>Standard Concentration</u>	<u>Volume of Standard</u>	<u>Final Volume</u>
Low LFB - 20 ug/L	2.0 uL	10 mL
LFB - 100 ug/L	10.0 uL	10 mL
Spike - 100 ug/L	10.0 uL	10 mL
Spike Dup - 100 ug/L	10.0 uL	10 mL

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

EPA Method 548.1

DETERMINATION OF ENDOTHALL IN DRINKING WATER BY ION-EXCHANGE EXTRACTION, ACIDIC METHANOL METHYLATION AND GAS CHROMATOGRAPHY/MASS SPECTROMETRY

File Name: M:\SOP\Organic\epa method sop\548_102309.doc Effective Date: 10/23/2009
Revision: 7 Supersedes: 7 (10/23/2009)

1. SUMMARY OF METHOD

EPA Method 548.1 determines Endothall using gas chromatography/mass spectrometry (GC/MS). The sample is diluted 1:10 with Millipore reagent water to a final volume of 100 ml and extracted with anion exchange disks mounted on a 6-position manifold. Alternatively, 8 ml cartridges are filled with Bio-Rex 5 anion exchange resin and used to extract endothall from the sample or pre-assembled 6ml cartridges with BIO-REX 5 sorbent are used instead. Endothall is eluted from the anion exchange disk with acidic methanol and methylene chloride. The dimethyl ester is formed upon heating this extract at 50°C. Salted reagent water is added to the extract and the endothall ester is partitioned into the methylene chloride. The extract volume is then reduced to 1 ml and analyzed by GC/MS.

2. ANALYTES

Endothall is the only analyte analyzed by this method.

LIMS code	analyte
ENDOTH	Endothall

3. APPARATUS AND EQUIPMENT

- 3.1 Sample bottles - 40 ml vials, fitted screw cap with teflon.
- 3.2 Vials - 40 ml amber glass, screw cap with teflon septa and limited volume insert.
- 3.3 Six station extraction manifold with 47 mm glassware sets (Varian 1214-6001 or equivalent).
- 3.4 Turbo-Vap concentration workstation (Zymark ZW8003)

- 3.5 Concentrator tubes - 50 ml Turbo-Vap tubes with 1.0 ml end-point (Zymark - ZA2039)
- 3.6 Gas Chromatograph/Mass Spectrometer - Varian 3800, Varian 8200 Autosampler, Varian Saturn Ion Trap mass spectrometer, and data system (using Varian GC/MS software).
- 3.7 100, 1000 and 2000 ml volumetric flasks.
- 3.8 Nitrogen Evaporation Apparatus.
- 3.9 Balance - analytical, capable of accurately weighing 0.0001g.
- 3.10 125 ml separatory funnels
- 3.11 10, 100, 250 uL Hamilton syringes
- 3.12 Brinkmann Autopipettors - 2-10 ml and 10-50 ml capacity with 1 L amber glass bottles
- 3.13 Vacuum pump
- 3.14 Explosion proof refrigerator

4. REAGENTS AND CONSUMABLE MATERIALS

- 4.1 Reagent Water - Millipore. Milli-Q system or equivalent filtered through a 0.45 micron filter.
- 4.2 Methylene Chloride - pesticide quality or equivalent. HPLC quality.
- 4.3 Sodium Sulfate - (ACS) granular. Prepare a 10% w/v solution in reagent water.
- 4.4 Sulfuric Acid - (ACS) reagent grade. Prepare a 10% v/v acidic methanol solution by adding 100 ml sulfuric acid to 1 L of methanol.
- 4.5 Endothall and Acenaphthene d-10 from Ultra Scientific and/or Absolute standard.
- 4.6 Sodium Hydroxide - (ACS) pellets
- 4.7 Methanol - pesticide quality or equivalent
- 4.8 Anion Exchange Disks by Empore - 47 mm
- 4.9 Bio-Rex 5 anion exchange resin
- 4.10 8 ml solid phase extraction cartridge

- 4.11 6 ml solid phase extraction cartridge with BIO-REX 5 sorbent
- 4.12 Disodium EDTA - (ACS) granular
- 4.13 Decafluorotriphenylphosphine (DFTPP)
- 4.14 Disposable glass pipettes
- 4.15 GC autosampler vials

5. SAMPLE COLLECTION, PRESERVATION AND HANDLING

- 5.1 Collect samples in 40 ml vial. Prepare 4 vials for spike and duplicated samples. Do not pre-rinse the bottle before collecting the sample. If residual chlorine is present add 100 mg sodium thiosulfate per liter of sample and mix well. Endothall should be kept in the dark away from heat. All samples must be iced and refrigerated at 4°C from the time of sampling until extraction. Furthermore, all samples must be extracted within 7 days of collection and analyzed within 14 days of extraction.

6. EXTRACTION

- 6.1 Liquid - Solid phase extraction (SPE):
 - 6.1.1 Place an anion exchange Empore disk onto the filtration apparatus. Use the larger vacuum pump when conditioning the disks. A stronger vacuum is required to draw Millipore water through the disk once the disk is saturated with methanol.
 - 6.1.2 Add 10 ml of MEOH, then 10 ml Milli Q, then 10 ml acidic methanol, then 10 ml Milli Q, then 20 ml 1N NaOH, then 20 ml Milli Q water being careful not to let the disk dry between conditioning steps. Let each solvent soak for 3 minutes before drawing it off and adding the next solvent. This is very important.
 - 6.1.3 Sample Preparation:
 - 6.1.3.1 Dilute each sample 1:10 with Millipore water. Dilution is necessary to reduce matrix effects due to the presence of calcium, magnesium and sulfate.
 - 6.1.3.2 Spikes are prepared by adding 10uL of a 100 ug/ml second source std (such as from Absolute Std) to 100 ml of 1:10 diluted sample. This is equivalent to a 10 ppb spike.
 - 6.1.3.3 LFBs are prepared by adding 4 uL of a 100 ug/ml second source std. to 100 ml of Millipore water. This is equivalent to a 4 ppb LFL.
 - 6.1.4 Add 100 ml of sample into the filtration reservoir and apply a vacuum.

The sample should flow through the disk without applying any vacuum. If a vacuum is required use the small vacuum pump. Low recoveries will result if flow rates are too high.

- 6.1.5 Do not let the sample flow exceed 3 ml per minute; it should take at least 30 minutes to pass the sample through the disk.
- 6.1.6 After the sample is processed, draw air through disk for 1 minute, and then add 1 ml of methanol to remove all traces of water. Then draw air through the disk for 5 minutes at 10 psi.
- 6.1.7 Remove the filter base and place a 40 ml receiving vial (VOC sample vial) in the manifold. Add 8 ml of acidic methanol, draw this eluant through the disk for 3 minutes. Repeat process. Then draw 6 ml of methylene chloride through the disk over 1 minute. Repeat process. Use the small vacuum pump to draw solvents through the disk.
- 6.1.8 Sample Derivatization: Cap the vial and place in a water bath at 50°C for 1 hour. Allow the vial to cool for ten minutes before proceeding. Note that all standards must be derivatized. Place 16 ml of acidic methanol and 12 ml of methylene chloride in a 40 ml receiving vial and add the required amount of standard. For example, a 10 ppb calibration standard will require addition of 1 uL of a 100 ug/ml standard.
- 6.1.9 Sample Partition:
 - 6.1.9.1 Pour the contents of the vial into a 125 ml separatory funnel. Rinse the vial twice with 1 ml of methylene chloride and add the rinse to the separatory funnel.
 - 6.1.9.2 Add 40 ml of 10% sodium sulfate solution to the separatory funnel.
 - 6.1.9.3 Shake the funnel, vent and then shake vigorously for 30 seconds (shake 70 times). Place the lower phase into a Zymark tube. Repeat the extraction procedure with 2 ml aliquots of methylene chloride twice and add the organic phase to the Zymark tube.
- 6.1.10 Turbo-Vap concentration workstation:
 - 6.1.10.1 Reduce the extract to a volume of 1.0 ml. The temperature of the water bath is 35°C and the nitrogen is set on the lowest flow possible that does not set off the alarm.
 - 6.1.10.2 Add 5 uL of the internal std. (This volume is for a 500 ug/ml stock standard. Addition of 5 uL of internal std. to 1 ml of extract is equivalent to a final concentration of 5 ppb in a 100 ml sample volume).

- 6.1.10.3 Transfer this extract into GC autosampler vials with disposable glass pipettes. Refrigerate extracts if they will not be analyzed immediately. Extracts should be stored in the dark at 4°C or less.

6.2. Cartridge - Solid phase extraction (SPE):

- 6.2.1. Assemble a 6ml SPE cartridge onto the filtration apparatus. Use the larger vacuum pump when conditioning the cartridges. A stronger vacuum is required to draw Millipore water through the cartridge once the cartridge is saturated with methanol.
- 6.2.2. Drain packing fluid from cartridge. Add 5ml of Acetone, let solvent soak for 3 minutes, and completely dry cartridge as vacuum is applied for 3 minutes. Add 10 ml of MEOH, then 10 ml Milli Q, then 10 ml acidic methanol, then 20 ml Milli Q, then 20 ml 1N NaOH, then 20 ml Milli Q water being careful not to let the cartridge dry between conditioning steps. Let each solvent soak for 3 minutes before drawing it off and adding the next solvent. This is very important.
- 6.2.3. Sample Preparation:
- 6.2.3.1. Dilute each sample 1:10 with Millipore water. Dilution is necessary to reduce matrix effects due to the presence of calcium, magnesium and sulfate.
- 6.2.3.2. Spikes are prepared by adding 10uL of a 100 ug/ml second source std. (such as from Abs.std) to 100 ml of 1:10 diluted sample. This is equivalent to a 10 ppb spike.
- 6.2.3.3. LOW LFBs are prepared by adding 4 uL of a 100 ug/ml second source std. to 100 ml of Millipore water. This is equivalent to a 4 ppb LFL.
- 6.2.4. Add 100 ml of sample into the filtration reservoir and apply a vacuum. The sample should flow through the cartridge without applying any vacuum. If a vacuum is required use the small vacuum pump. Low recoveries will result if flow rates are too high.
- 6.2.5. Do not let the sample flow exceed 1.5 ml per minute; it should take at least 60 minutes to pass the sample through the cartridge.
- 6.2.6. After the sample is processed, draw air through cartridge for 1 minute, and then add 10 ml of methanol to remove all traces of water. Let soak for 1 minute. Then draw air through the cartridge for 10 minutes at 10 psi.
- 6.2.7. Place a 10 ml receiving tube in the manifold. Add 4 ml of acidic methanol; let soak cartridge for 3 minutes. Draw elute into vial at low vacuum. Then add 3 ml of methylene chloride; let soak cartridge for 3 minutes. Draw elute into vial at low vacuum. Transfer elute from the 10 ml vial to a 40 ml vial and reinsert 10 ml vial back into SPE. Repeat solvent rinsing process, but have vacuum on high and dry cartridge completely after each elution. Transfer elute to the same 40 ml vial. Rinse the 10 ml receiving tube twice with 1mL of CH₂CL₂. Add these rinses to the 40ml vial.

6.2.8. Sample Derivatization:

Cap the vial and place in a water bath at 50°C for 1 hour. Allow the vial to cool for ten minutes before proceeding. Note that all standards must be derivatized. Place 8 ml of acidic methanol and 6 ml of methylene chloride in a 40 ml receiving vial and add the required amount of standard for the calibration and calibration check standards. For example, a 10 ppb calibration standard will require addition of 10 uL of a 100 ug/ml standard.

6.2.9. Sample Partition:

6.2.9.1. Pour the contents of the vial into a 125 ml separatory funnel. Rinse the vial twice with 1 ml of methylene chloride and add the rinse to the separatory funnel.

6.2.9.2. Add 40 ml of 10% sodium sulfate solution to the separatory funnel.

6.2.9.3. Shake the funnel, vent and then shake vigorously for 30 seconds (shake ~70 times). Place the lower phase into a Zymark tube. Repeat the extraction procedure with 2 ml aliquots of methylene chloride twice and add the organic phase to the Zymark tube.

6.2.10. Turbo-Vap concentration workstation:

6.2.10.1. Reduce the extract to a volume of 1.0 ml. The temperature of the water bath is 35°C and the nitrogen is set on the lowest flow possible that does not set off the alarm (9 psi).

6.2.10.2. Add 5 uL of the internal std. (This volume is for a 500 ug/ml stock standard. Addition of 5 uL of internal std. to 1 ml of extract is equivalent to a final concentration of 5 ppb in a 100 ml sample volume).

6.2.10.3. Transfer this extract into GC autosampler vials with disposable glass pipettes. Refrigerate extracts if they will not be analyzed immediately. Extracts should be stored in the dark at 4°C or less.

7. ANALYSIS

7.1

Instrumentation: Gas chromatograph/Mass Spectrometer - Varian 3800 gas chromatograph, Varian 8200 autosampler, Varian Saturn Ion Trap and computer data system. The column used is a Varian factor four column, 30 m x 0.25 mm, 0.25 micron film

GC Conditions:

1.	Seg	Temp	Rate	Hold Time	Total
	1	100	0	2	2.00
	2	200	12	0	10.33
	3	300	60	2	14.00

- Helium linear velocity: 75 cm/sec
- Splitless injection with 3.5 minute delay
- Injector temperature (1079): Initial 50°C to 250°C @ 180 °C/minute then hold for 10.00 minutes
- Transfer Line Temp: 280 °C
- 2 ul sample is injected at 10 ul/sec - sandwich technique is used with a 0.2 ul solvent plug

MS Conditions :

- Mass range : 45 to 450 amu
- Seconds/scan : 0.80
- Acquire time : 33.0 minutes
- Fil/Mul delay : 10 minutes
- Peak threshold : 1 count
- Mass defect : 0 mmn/100 amu
- Background mass : 45 amu
- Ionization mode : EI
- Auto ion control : ON
- Cal gas : OFF

5 ng of DFTPP is injected (1ul of a 5ug/ml solution) before every run into the GC/MS to determine whether the MS is "in tune". Using the GC method DFTPP, MS method DFTPP and AS method DFTPP, the ion abundance of the mass spectra of DFTPP can be compared to the following set of criteria.

<u>Mass</u>	<u>Relative Abundance Criteria</u>
54	10-80% of the base peak
68	<2% of mass 69
70	<2% of mass 69
127	10-80% of the base peak
197	<2% of mass 198
198	base peak of >50% of 442
199	5-9% of mass 198
275	10-60% of the base peak
365	>1% of the base peak
441	Present and < mass 443
442	base peak of >50% of 198
443	15-24% of mass 442

7.2 EnviroPro software is used to determine whether the ion abundance criteria is met. Use file EP to get into EnviroPro and type in the file name, type the correct

forms output, 6B, and the califile DFTPP. From the main menu type R for run procedure and DFT525 to get the printout of the relative ion abundance of the DFTPP injection. This printout is kept with each data package.

- 7.3 A Laboratory Fortified Blank (LFB) is run with each set of samples at a concentration of 10 ppb. A Low LFB is also run at a concentration of 4 ppb. Spikes are run every 10 samples or at least once per set of samples at a concentration of 10 ppb. Duplicates are run at least once per set of samples or every 10 samples.
- 7.4 DFTPP is run before every analysis. DFTPP must pass the ion abundance criteria set forth in the method. The ion abundance criteria is the same as the ion abundance criteria in EPA Method 525.1 therefore, software provided for 525.1 will be used to judge whether DFTPP passes the ion abundance criteria for this method. Refer to Exhibit 5 for specific ion abundance criteria values. The external calibration used is 5-point calibration ranging from 2 to 50 ppb refer to standard preparation for directions on how to prepare calibration curve.
- 7.5 In addition a calibration check standard at 10ppb must also be analyzed right after the 5-pt calibration. The *Front* standard should be right after the 5-point curve and the *Back* should be the last sample analyzed. If run is greater than 8 hours, then check standard must be analyzed every 10 samples.
- 7.6 The amount of endothall in a sample is calculated using the internal standard method. Acenaphthene d-10 is the internal standard (m/z 164). Endothall (m/z 183, 155 and 123) is calculated using three ions. The Varian software compares the response factors of the sample to the response factors in the calibration curve.

8. QA/QC REQUIREMENTS

- 8.1 Method blanks (reagent blanks) in order to demonstrate that glassware and reagents are free of any interference, a reagent blank must be analyzed at the beginning of each analysis. If the blank produces any interference, determine the source of the interference and eliminate that interference before proceeding.
- 8.2 Laboratory Fortified Blank - Analyze at least one laboratory-fortified blank, LFB, with every 10 samples or one per run, whichever is greater. The fortification concentration of each analyte should be 10 times the MDL, whichever is less - in this method 10 ppb will be used. If the recovery of any analyte falls outside the control limits, the source of the problem must be identified and corrected.
- 8.3 Verify calibration standard by analyzing a standard prepared from reference material obtained from an independent or second source (continuing calibration check standard), immediately after the calibration. Run one at the end also. Results must be within +/- 30% of those used to routinely check calibration. Weekly, run a low level standard to check the reportable detection level, RDL.
- 8.4 Samples must be extracted within 7 days after collection. Duplicates are run on 10% of samples, or once during run, whichever is greater. Results should be

within +/- 30%.

- 8.5 The laboratory must add a known concentration of spike solution, the same as used for LFB, at least 10% of samples or once per analytical run, whichever is greater. Recoveries should be within +/- 30%.

9. PREVENTIVE MAINTENANCE AND CORRECTIVE ACTIONS

- 9.1 Glassware - Glassware must be carefully cleaned. Clean all glassware as soon as practical after use by thoroughly rinse. Follow by washing with hot water and 10% HCl and thoroughly rinsing with tap water followed by reagent water. After drying, heat in oven. Do not heat volumetric glassware. Thorough rinsing with acetone may be substituted for heating.
- 9.2 Reagents - The use of high purity solvents and reagents will help to minimize contamination problems.
- 9.3 Carryover - Contamination carryover may occur when a sample containing a low concentration of analytes is analyzed immediately following a sample containing a high concentration. Use solvent rinses between samples to minimize carryover.
- 9.4 Minimize contact of the samples, reagents, or solvents with any plastics. This will help reduce contamination.
- 9.5 Record all corrective actions in the maintenance log book. Include a complete description of the problem and what action was taken to correct it.
- 9.6 No or Poor Chromatography-
- 9.6.1 Check all the standards. If calibration and/or QC lie outside the acceptable limits we must first re-run the standards to assure that the injection procedure is working properly. After the re-run if standards still lie outside the acceptable limits we must make a new standard or conduct instrument maintenance to assure that calibration and QC lie with acceptable parameters. For elevated spike recoveries make sure that the sample matrix is clean. If sample matrix is altering recoveries then a new matrix must be selected. If instrument performance is acceptable the samples must be re-extracted. If the sample lies outside allowable holding time they must be re-sampled.
- 9.6.2 Request re-sample if it's necessary.
- 9.6.3 If there are hits, request resample

[illegible]

ORANGE COUNTY WATER DISTRICT

EPA METHOD 548.1

Standard Preparation and Quality Control

1. Calibration Standard: Cresent Chemical Co., Inc.; Catalog # CC2095R; stock concentration = 100 ug/mL in MeOH

2. 2nd Source Standard (LFB/Spk): AccuStandard, Catalog # P-183S; stock concentration = 100 ug/mL in MeOH

3. Internal Standard (IS): Accustandard, Catalog # M-548.1-IS; stock concentration = 500 ug/mL in MeOH

Analytes
ENDOTHALL

LIMS Code
ENDOTL

4. DFTPP (Decafluorotriphenylphosphine solution): Ultra Sci, Catalog # 47995N; stock conc. = 1000 ug/mL in Acetone

Methylene Chloride (CH₂Cl₂) is the final solvent used for the 548.1 extraction.

A. Calibration Standards, Accustandard

Calibration curve points are extracted just like samples (see 548.1 flowchart for procedure).

<u>Standard Concentration</u>	<u>Volume of Standard</u>	<u>Final Volume before extraction</u>
STD A - 2 ug/mL	2.0 uL of Calib. Std	14 mL (8 ml 10% acidic acid & 6 ml CH ₂ Cl ₂)
STD B - 5 ug/mL	5.0 uL of Calib. Std	14 mL (8 ml 10% acidic acid & 6 ml CH ₂ Cl ₂)
STD C - 10 ug/mL	10 uL of Calib. Std	14 mL (8 ml 10% acidic acid & 6 ml CH ₂ Cl ₂)
STD D -20 ug/mL	25 uL of Calib. Std	14 mL (8 ml 10% acidic acid & 6 ml CH ₂ Cl ₂)
STD E - 50 ug/mL	50 uL of Calib. Std	14 mL (8 ml 10% acidic acid & 6 ml CH ₂ Cl ₂)
STD F -100 ug/mL	100 uL of Calib. Std	14 mL (8 ml 10% acidic acid & 6 ml CH ₂ Cl ₂)

B. Calibration Check Standard, Ultra Scientific

Calibration check is extracted just like samples (see 548.1 flowchart for procedure).

<u>Standard Concentration</u>	<u>Volume of Standard</u>	<u>Final Volume before extraction</u>
100 ug/mL	10.0 uL of LFB/Spk std (Accustandard)	14 mL (8 ml 10% acidic acid & 6 ml CH ₂ Cl ₂)

C. Low LFB, LFB, Spike, and Spike Duplicate, Ultra Scientific

QCs are extracted just like samples (see 548.1 flowchart for procedure).

<u>Concentration</u>	<u>Volume of Standard</u>	<u>Final Volume before extraction</u>
Low LFB - 4 ug/mL	4.0 uL of LFB/Spk std (Accustandard)	100 mL Millipore water
LFB - 10 ug/mL	10.0 uL of LFB/Spk std (Accustandard)	100 mL Millipore water
Spike - 10 ug/mL	10.0 uL of LFB/Spk std (Accustandard)	10 mL sample and 90 mL Millipore water
Spike Duplicate -10 ug/mL	10.0 uL of LFB/Spk std (Accustandard)	10 mL sample and 90 mL Millipore water

D. PERFORMANCE CHECK STANDARD (DFTPP), Ultra Scientific

Stock Solution - 1000 ug/ml in Acetone

Dilute 5µl of stock to 1mL with CH₂Cl₂ - Inject 1µl = conc. 5 ng/mL (5ug/L)

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Turn on the H2O bath - use MTBE cap for all vials

Samples and QCs Prep

QCs - 100ml volumetric flask w/ Millipore water
add the spikes (LOW: 4uL; LFB: 10uL & Spike : 10uL of 100ug/ml Absolute Std)
Samples - in a 100ml volumetric flask, dilute each sample 1:10 with Millipore water

Cartridge Conditioning

548 Cartridge: UCT Bio Rex 5, Part# EC548006.

Attach assembled cartridge to SPE manifold and drain packing fluid from cartridge.
Add 5-ml of Acetone to the assembled SPE cartridge with reservoir.

aspirate a small amount of the solvent.
Let the solvent soak for 3 min, then apply vacuum for 3 min. Completely dry the cartridge.

FROM THIS STEP ON - DO NOT LET THE CARTRIDGE GO DRY!!!
LEAVE A THIN LAYER OF SOLVENT ON THE TOP LAYER OF THE CARTRIDGE!

add 10-ml of MeOH to the assembled SPE cartridge
aspirate a small amount of the solvent. Soak the cartridge for 3 min.
apply vacuum, leave a thin layer of solvent on the top most layer of the cartridge.
DO NOT LET THE CARTRIDGE GO DRY!!

add 10-ml of D. I., aspirate a small amount, then soak for 3 min.
apply vacuum, leave a thin layer of water on the top most layer of the cartridge.
DO NOT LET THE CARTRIDGE GO DRY!!

add 10-ml of 10% acidic Methanol
aspirate a small amount, soak for 3 min., apply vacuum, leave a thin layer of solvent on top.
DO NOT LET THE CARTRIDGE GO DRY!!

Rinse the cartridge and reservoir with 10 mL of D.I. water. Let water drip through.
Add an additional 10 mL of D.I. water. Aspirate a small amount and let soak for 3 minutes.
apply vacuum, leave a thin layer of water on top layer of cartridge.
make sure all traces of acidic MeOH are gone

add 20 ml of 1 N NaOH to the cartridge with reservoir.
aspirate a small amount, soak for 3 min., apply vacuum, leave a thin layer of solvent on top.
DO NOT LET THE CARTRIDGE GO DRY!!

Rinse the cartridge and reservoir with 10 mL of D.I. water. Let water drip through.
Add an additional 10 mL of D.I. water. Aspirate a small amount and let soak for 3 minutes.
apply vacuum, leave a thin layer of water on top layer of cartridge.
DO NOT LET THE CARTRIDGE GO DRY!!
make sure all traces of NaOH are gone

add the diluted QCs and samples

DO NOT USE THE VACUUM PUMP AT ALL ! (Total time should be at least 60 minutes)
(while samples drip - prepare 125ml sep funnel & 40ml clear vials)

after the sample has been processed, pull air through the cartridge for 1 min. -- FULL VACUUM

add 10-ml of methanol to the cartridge to remove all traces of water
Let soak for 1 minute
pull air through the cartridge for 10 additional minutes at 10 psi. (low PSI on vacuum)

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CARTRIDGE ELUTION

-- Insert a clear 10-ml glass vial to collect the elute--

Keep the PSI of the vacuum below 10.
add 4-ml of 10% acidic methanol
trigger vacuum to allow a small amount to drip through, soak for 3 min.
Trigger the vacuum on and off to allow the elute to exit the cartridge in a dropwise fashion.
Continue until all solvent has been drained. **Do not dry cartridge completely.**

Add 3 ml of CH₂Cl₂
trigger vacuum to allow a small amount to drip through, soak for 3 min.
Trigger the vacuum on and off to allow the elute to exit the cartridge in a dropwise fashion.
Continue until all solvent has been drained. **Do not dry cartridge completely.**

Transfer elute to a 40 ml glass vial
Insert same 10 ml collection vial back into SPE.

Increase to full vacuum.

Repeat the two elution steps again. Dry cartridge completely for each solvent.

Transfer elute to the 40 ml vial.
Rinse the 10 mL collection vial twice with 1mL of CH₂Cl₂. Add these rinses to the 40 mL vial.
Cap 40 mL vial and place in a 50 degree water bath for 1 hour

Standards: add 8 mL of 10% acidic MeOH & 6 mL of CH₂Cl₂ to a clear 40ml vial - use MTBE caps
add the appropriate amount of the standard (2ul, 5ul, 10ul, 25ul & 50ul of 100ug/ml Crescent)
cap vial, and place in a 50 degree water bath for 1 hour
***Cal. check is spiked w/10ul of 100ug/ml Absolute Standard ***

Remove the sample from the water bath
wait for the samples to cool to room temp (about 10min.) before proceeding

add 40-ml of 10% Na₂SO₄ solution to the sep. funnel

pour the content of the vial into separatory funnel w/ added 10% Na₂SO₄
rinse the vial twice with 1-ml portion of CH₂Cl₂, add the rinse to the funnel

cap funnel, vent & shake vigorously for 30 sec

wait 10 min. for the layers to separate

drain the bottom organic layer into a Zymark tube

repeat the shaking and draining process 2 more times
each time, adding 2-ml of CH₂Cl₂ to the funnel, and combine all of the extracts

Turbo Vap settings: Temp: 35, Pressure: 9 psi
concentrate the extract to 1ml
remove the Zymark from the turbo vap - let it cool (it takes < 1min)
rinse the lower 25% of the Zymark 8-10 times (1mL will evaporated to <0.6ml)

add CH₂Cl₂ to bring the sample to 1ml
add Internal - 5ul (of conc. 500 ug/mL) — mix well
transfer to 2 amber autosampler vials; 1 w/ insert and 1 w/out
Store in refrigerator 4°C until analysis.

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

EPA METHOD 549.2

DETERMINATION OF DIQUAT AND PARAQUAT IN DRINKING WATER BY LIQUID-SOLID EXTRACTION AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH ULTRAVIOLET DETECTION

File Name: M:\Sop\Organic\epa method sop\549.2_110609.doc Effective Date: 11/06/2009
Revision: 1 Supersedes: 549_0101

1. SUMMARY OF METHOD

EPA Method 549.2 determines diquat and paraquat using high performance liquid chromatographic (HPLC) with a photodiode array detector. A 250 ml sample is extracted with a C-8 disk specially prepared for the reversed-phase, ion-pair mode. An ion-pair reagent is added to the eluate to a final volume of 10 ml. Diquat is measured at 308 nm and paraquat at 257 nm.

2. ANALYTES

Diquat and paraquat are the analytes determined by this method.

lims code	analyte
DIQUAT	Diquat (1,1'-ethylene-2,2'-bipyridilium dibromide salt)
PARAQU	Paraquat (1,1'-dimethyl-4,4'-bipyridilium dichloride salt)

3. APPARATUS AND EQUIPMENT

- 3.1 Sample bottles - 1 L plastic bottles, fitted screw cap with teflon
- 3.2 Vials - 1ml polypropylene, screw cap with teflon septa
- 3.3 Six station extraction manifold with 47 mm glassware sets (Varian 1214-6001 or equivalent)
- 3.4 High performance liquid chromatograph (HPLC) - quaternary pump, autosampler, switching valve, column oven, photodiode array detector.
- 3.5 Waters Carbamate Analysis System, 150 mm long x 3.9 mm ID stainless steel and computer data system

- 3.6 Balance - analytical, capable of accurately weighing 0.0001g
- 3.7 pH meter - capable of measuring pH to 0.1 units
- 3.8 15 ml graduated polypropylene collection vials

4. REAGENTS AND CONSUMABLE MATERIALS

- 4.1 Reagent Water – Deionized Water
- 4.2 Methanol - HPLC grade or higher purity
- 4.3 Sodium Thiosulfate - (ACS) granular
- 4.4 Orthophosphoric Acid - 85% (w/v) - reagent grade
- 4.5 Standard - Ultra Scientific and Accustandard
- 4.6 Diethylamine - reagent grade
- 4.7 Concentrated Sulfuric Acid - ACS reagent grade
- 4.8 Sodium Hydroxide - reagent grade
- 4.9 Concentrated Hydrochloric Acid - ACS reagent grade
- 4.10 Cetyl Trimethyl Ammonium Bromide, 95% - Aldrich Chemical
- 4.11 1-Hexanesulfonic Acid, sodium salt, 98%, Aldrich Chemical
- 4.12 1-Heptanesulfonic Acid, sodium salt, 98%, Aldrich Chemical
- 4.13 Ammonium Hydroxide, ACS, Concentrated
- 4.14 Reagent Solutions
 - 4.14.1 Conditioning solution A - Dissolve 0.5 g of cetyl trimethyl ammonium bromide and 5 mLs of concentrated ammonium hydroxide into 500 mLs of DI water and dilute to 1000 mLs in volumetric flask

- 4.14.2 Conditioning solution B - Dissolve 20 g of 1-hexanesulfonic acid, sodium salt and 20 mLs of concentrated ammonium hydroxide in 500 mLs of DI water and dilute to 1000 mLs in a volumetric flask
- 4.14.3 Sodium hydroxide solution, 10% w/v - dissolve 50 g NaOH into 400 mL of DI water and dilute to 500 mLs in a volumetric flask
- 4.14.4 Hydrochloric Acid 10% v/v – Add 50 mLs of concentrated hydrochloric acid to 400 mLs of DI water and dilute to 500 mL in a volumetric flask
- 4.14.5 Disk Eluting Solution - Add 13.5 mLs of orthophosphoric acid (phosphoric acid) and 10.3 mLs of diethylamine to 500 mLs DI water and dilute to 1000 mLs in a volumetric flask
- 4.14.6 Ion-pair concentrate - Dissolve 3.75 g of 1-hexanesulfonic acid in 15 mLs of disk eluting solution and dilute to 25 mLs in a volumetric flask
- 4.15 Mobile Phase
 - 4.15.1 Waters Cabarmate Analysis Column - Add 13.5 mLs of orthophosphoric acid (phosphoric acid), 10.3 mLs of diethylamine, and 3 g of 1-hexanesulfonic acid, sodium salt, to 500 mLs DI water and dilute to 1000 mLs in a volumetric flask
- 4.16 Liquid solid 47 mm (C-8) extraction disks by 3M

5. SAMPLE COLLECTION, PRESERVATION AND HANDLING

- 5.1 Collect samples in 1L plastic bottles. Do not pre-rinse the bottle before collecting the sample. If residual chlorine is present add 100mg/L sodium thiosulfate per liter of sample and mix well. If biological activity is suspected, add sulfuric acid to pH 2.
- 5.2 Diquat and paraquat are light sensitive compounds - therefore samples, extracts and standards must be kept in amber bottles to minimize photolytic decomposition.
- 5.3 All samples must be iced and refrigerated at 4°C from the time of sampling until extraction. Furthermore, all samples must be extracted within 7 days of collection and analyzed within 21 days of extraction.

6. **EXTRACTION: Liquid - Solid phase extraction (SPE)**

- 6.1 In order to closely match calibration standards to samples, prepare standards by extracting preserved DI water using the same procedure as samples. Before transferring to the vial, fortify the eluted extract with the appropriate amount of working standard.
- 6.2 Sample may require clean up prior to extraction, which involves passing the sample through a 0.45-micron filter.
- 6.3 Check the pH of the sample or reagent water (QC and calibration) and verify the pH is between 7.0 and 9.0. If the pH of the sample does not fall within this range, adjust the pH with 10% w/v NaOH or 10% v/v HCl.
- 6.4 Assemble C-8 disk and filtration apparatus. Condition the disks. Note: do not let the disks run dry during any conditioning steps.
 - 6.4.1 Add 10 ml of Methanol - soak for 2 minutes - apply vacuum
 - 6.4.2 Add (2) 10 ml aliquots of DI water - apply vacuum
 - 6.4.3 Add 10 ml of conditioning solution A - soak for 2 minutes - apply vacuum
 - 6.4.4 Add (2) 10 ml aliquots of DI water - apply vacuum
 - 6.4.5 Add 10 ml of conditioning solution B - soak for 2 minutes - apply vacuum
 - 6.4.6 Add 250 ml of sample or preserved DI water (for QC and calibration) to the reservoir using a polypropylene graduated cylinder
 - 6.4.7 Add spike and/or calibration standard to samples and reagent water (for QC and calibration)
 - 6.4.8 Apply vacuum - continue applying vacuum for 5 minutes after the sample is processed to dry disk
- 6.5 Elute the extracted samples:
 - 6.5.1 Add 200ul of Ion pair solution to the 15 ml graduated polypropylene collection vial before placing it in the extraction manifold receiver
 - 6.5.2 Add 1 ml of Methanol to the disk - soak for 1 minute

- 6.5.3 Add 4 ml of disk eluting solvent - soak for 2 minutes - apply vacuum to draw the eluting solvent through the disk, leaving a layer of solvent on the disk
- 6.5.4 Add 4ml of disk eluting solvent - soak for 2 minutes - apply vacuum to dry the disk completely
- 6.5.5 Fortify the calibration standards following instructions in section 9.1
- 6.5.6 Remove the collection vial from the extraction manifold and bring the volume to 10 ml with disk eluting solution
- 6.5.7 Transfer the extract to (2) 1 ml polypropylene vials, cap and store. Refrigerate at 4 degrees C until analysis by HPLC

7. ANALYSIS

7.1 Instrumentation:

- 7.1.1 High Performance Liquid Chromatograph - quaternary pump, auto sampler, switching valve, column oven, photodiode array detector, column and computer data system
- 7.1.2 Waters Carbamate Analysis Column - C-18, 3.9 mm x 150 mm

7.2 HPLC Start-up Conditions:

- 7.2.1 Run time for each injection is 6 minutes. Therefore, this method should not be run overnight.
- 7.2.2 Photodiode array detector settings preset within Empower software

7.3 Instrument Conditions

Spurge Rate:	Continuous degas for 2695
Flow rate:	1.2 ml/min isocratic of mobile phase
Column Oven Temp:	25°C
Sample Injected Vol.:	50 µl
Detector:	257 nm for paraquat (Waters 996) 308 nm for diquat

7.4 HPLC Shut Down Procedure:

- 7.4.1 Rinse the column with 90:10 / Acetonitrile:Water with a flow rate of 1.0 ml/min for at least 15 minutes.
- 7.4.2 Gradually reduce the flow rate to 0 ml/min before shutting down the instrument.
- 7.5 Stock Standard Solutions & Calibration:
 - 7.5.1 Use the Ultra Scientific Standard to prepare the calibration standards. Begin by making a 100 ug/mL working standard by adding 100 uL to 1 mL of HPLC water. Use this working standard to fortify the samples. If a problem arises with QA/QC, check the standards and replace if necessary.
 - 7.5.2 The 5-point calibration should be 2, 4, 8, 12, and 16 ppb. Refer to Section I for standards and QC preparation.
 - 7.5.3 In addition to the calibration curve, prepare daily calibration check standards. These check standards should be at two different concentration levels (4 and 8 ppb) to verify the calibration curve.

8. QA/QC REQUIREMENTS

- 8.1 RB - Method blanks (reagent blanks) in order to demonstrate that glassware and reagents are free of any interference. A reagent blank must be analyzed at the beginning of each analysis. If the blank produces any interference, determine the source of the interference and eliminate that interference before proceeding.
- 8.2 LFB - Laboratory Fortified Blank - Analyze at least one laboratory fortified blank, with every 10 samples or one per run, whichever is greater. The fortification concentration of each analyte should be 10 times the EDL or BCL, whichever is less. If the recovery of any analyte falls outside the control limits, the source of the problem must be identified and corrected.
- 8.3 Standard - Verify calibration standards by analyzing a standard prepared from reference material obtained from an independent or second source, immediately after the calibration curve. Results must be within +/- 30% of those used to routinely check calibration. Weekly, run a low level standard to check the reportable detection level, RDL.
- 8.4 Samples - Samples must be extracted within 7 days after collection. Duplicates are run on 10% of samples, or once during run, whichever is greater. Results should be within +/- 30%.
- 8.5 Spike - The laboratory must add a known concentration of spike solution, the same as used for LFB, at least 10% of samples or once per analytical run, whichever is greater. Recoveries should be within +/- 30%.

9. PREPARATION OF STANDARDS AND QUALITY CONTROLS

- 9.1 Calibration Stock Standard: Diquat and Paraquat mix, 1.0 mg/mL in DI
Working Standard: dilute the stock standard 1/10 with DI = 100 µg/mL

Std A - spike 10 mL disk eluting sol'n w/ 5 µl of working standard - 2.0 ppb
Std B - spike 10 mL disk eluting sol'n w/ 10 µl of working standard - 4.0 ppb
Std C - spike 10 mL disk eluting sol'n w/ 20µl of working standard - 8.0 ppb
Std D - spike 10 mL disk eluting sol'n w/ 30µl of working standard - 12.0 ppb
Std E - spike 10 mL disk eluting sol'n w/ 40µl of working standard - 16.0 ppb

- 9.2 LFB and Spike: Diquat and Paraquat mix, 1.0 mg/mL in DI
Working Standard: dilute the stock standard 1/10 with DI = 100 µg/mL

Low LFB - spike 250 ml DI water w/ 5 µl of working standard – 2.0 ppb
LFB - spike 250 ml DI water w/ 20 µl of working standard – 8.0 ppb
Sample Spiking - spike 250 ml sample w/ 20µl of working standard – 8.0 ppb

10. PREVENTIVE MAINTENANCE AND CORRECTIVE ACTIONS

- 10.1 Glassware - Glassware must be carefully cleaned. Clean all glassware as soon as practical after use by thoroughly rinsing. Follow by washing with hot water and detergent and thoroughly rinsing with tap water followed by reagent water. After drying, heat in oven. Do not heat volumetric glassware. Thorough rinsing with acetone may be substituted for heating.
- 10.2 Reagents - The use of high purity solvents and reagents will help to minimize contamination problems.
- 10.3 Carryover - Contamination carryover may occur when a sample containing a low concentration of analytes is analyzed immediately following a sample containing a high concentration. Use solvent rinses between samples to minimize carryover.
- 10.4 Minimize contact of the samples, reagents, or solvents with any plastics. This will help reduce contamination.
- 10.5 Record all corrective actions in the maintenance logbook. Include a complete description of the problem and what action was taken to correct it.
- 10.6 No or Poor Chromatography:
- 10.6.1 Check all of the standards. If calibration and/or QC lie outside the acceptable limits, standards must be re-injected to verify that there are no problems with the instrument. If standards still lie outside of the acceptable

limits, a new standard must be made or instrument maintenance should be conducted to assure that calibration and QC lie within acceptable parameters. If instrument performance is acceptable then the samples must be re-extracted. If spike recoveries are high, make sure that the sample matrix is clean. If sample matrix is altering recoveries then a new matrix must be selected. If the samples are past the holding time then they must be re-sampled.

10.6.2 Request re-sample if necessary.

10.6.3 If there are hits, confirm using the spectra library.

SOP PROCEDURE CHANGE

For EPA Method 549

<u>CHANGE</u>	<u>REASON</u>	<u>DATE</u>	<u>INITIALS</u>
Added "QC and" to 6.1 to better indicate we extract the calibration		2/11/03	jrb
6.3.6 Added reagent water (for QC and calibration)		2/11/03	jrb
6.2 Added "reagent water"		2/11/03	jrb
Added lines 6.3.6 and 6.3.7 to also better represent that we extract calibration using pH adjusted reagent water			
recommended by audit		2/11/03	jrb
Added section 9	Audit request	2/18/03	jrb
Changed HPLC model to 2695	Upgraded HPLC	10/15/04	jrb
Changed PDA model to 996	Different detector	10/15/04	jrb
Changed "UV" to photodiode array	be more specific	10/15/04	jrb
Removed the Hamilton Column from SOP	Don't use that column	10/15/04	jrb
Removed "sparge mobile phase at 100ml/min..."	2695 has continuous degasser	10/19/04	jrb
Updated directions for mobile phase and the amount of aliquots in section 6			
Reason: to update to method 549.2 from 549.1		01/21/09	GGA
Finalized the 549.2 on 11/06/09 by mah			

EPA Method 549.2

make sure pH of the QC water w/ preservative and samples are between **7 and 9**
if pH lies outside of range, adjust with 10% NaOH or 10% HCl

add 10 mL of Methanol to the assembled SPE apparatus (with C-8 disk)

aspirate a small amount of the solvent, keeping the the disk wet
let the solvent soak through disk for 2 min, then apply vacuum
LEAVE A LAYER OF SOLVENT ON TOP OF THE DISK, KEEP DISK WET

add 10 mL of DI water to rinse disk and aspirate, keeping the disk wet—**repeat step again**

add 10 ml of conditioning solution A
aspirate a small amount, soak for 2 min, then apply vacuum
MAKE SURE THE DISK IS STILL WET, leaving a layer of liquid on top

add 10 mL of DI water to rinse disk and aspirate, keeping the disk wet—**repeat step again**

add 10 mL of conditioning solution B
aspirate a small amount, soak for 2 min, then apply vacuum
MAKE SURE THE DISK IS STILL WET, leaving a layer of liquid on top

add the 250 mL samples to the reservoirs using plastic cyclinders

Process 250 mL QC water for each calibration point and for both high and low checks (7 total)
DO NOT SPIKE UNTIL END OF EXTRACTION

add the spike (**LFL: 10 uL, LFB: 20 uL, SPK/SPK DUP: 20 uL of 100 ug/mL Accustd**) to reservoirs

apply vacuum and adjust the pressure to 50 mL/min flow rate for a total of 5 min for entire sample

after the sample has been processed, pull air through the disk for 5 min to dry completely

dry base and insert a 15 mL collection vial containing 200 uL of Ion-pair concentrate

add 1 mL of Methanol to the disk and let soak for 1 min

add 4 mL of Disk Eluting Solution to the disk
apply the vacuum to let a small amount drip through and soak for 2 min
apply vacuum and aspirate the remaining solvent without drying the disk

repeat the above eluting step with another 4 mL of the Disk Eluting Solution
soak for 2 min and completely dry disk

bring the final volume of extract to 10 mL with the Disk Eluting Solution
add the standard to the collection vials to prepare the calibration and check standards
Calibration: A 5 uL, B 10 uL, C 20 uL, D 30 uL, E 40 uL of 100 ug/mL Ultra Sci
Checks: LOW 10 uL, HIGH 20 uL of 100 ug/mL Accustd

mix and transfer the sample extracts to (2) 2 mL polypropylene vials using disposable plastic pipets

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURES

EPA METHOD 550.1

DETERMINATION OF POLYAROMATIC HYDROCARBONS IN WATER USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) WITH PDA(UV) & FLUORESCENCE DETECTION IN SERIES

File Name: M:\Sop\Organic\epa method sop\550_1_0904.doc
Revision: 7

Effective Date: 08/29/2005
Supersedes: 5 (09/01/2004)

1. SUMMARY OF METHOD

EPA method 550.1 uses HPLC with Photodiode Array (PDA) and fluorescence detection in series to determine 16 polycyclic aromatic hydrocarbons (PAH) in groundwater and finished drinking water. A one-liter volume of sample is extracted using C18 disk. Automated extraction system is used – Horizon Technology (SPE-DEX 4700). The targets are then eluted with a mixed solvent of methylene chloride and acetonitrile. The extract is exchanged and concentrated into acetonitrile and then separated by HPLC analysis. Analyte analysis is performed using isocratic/gradient elution in series with ultra violet/visible and fluorescence detection. The PAHs are measured and identified using both ultraviolet and fluorescence detectors.

2. ANALYTES

The following compounds can be determined using this method.

LIMS code	Analyte
ANTHRA	Anthracene
ACNAPE	Acenaphthene
ACENAP	Acenaphthylene
BaANTH	Benzo-(a)-anthracene
BaPYRE	Benzo-(a)-pyrene
BbFLUR	Benzo-(b)-fluoranthene
BghiPR	Benzo-(g,h,i)-perylene
BkFLUR	Benzo-(k)-fluoranthene
CHRYSE	Chrysene
DBaHAN	Dibenzo-(a,h)-anthracene
FLANTH	Fluoranthrene
FLUOR	Fluorene
INDPYR	Indeno-(1,2,3-cd)-pyrene
NAP	Naphthalene
PHENAN	Phenanthrene

3. APPARATUS AND EQUIPMENT

- 3.1 Sample bottles – 2.5 liter amber bottles, fitted screw cap with Teflon.
- 3.2 Vials - 2ml amber glass autosampler vial.
- 3.3 Horizon automated disk extraction system – SPE-DEX 4700 system.
- 3.4 TurboVap concentration workstation (Zymark ZW8003).
- 3.5 Concentrator tube - 50ml TurboVap tubes with 1.0 ml endpoint (Zymark ZA2039)
- 3.6 Balance - analytical, capable of accurately weighing 0.0001g.
- 3.7 High performance liquid chromatograph - quaternary pump, auto sampler, switching valve, column oven, Photodiode Array (PDA) and fluorescence detector, column and computer data system.
- 3.8 Reverse phase C18 column - Vydac, 5-micron particle diameter. 25cm x 4.6 ID stainless steel column. Cat # 201TP54

4. REAGENTS AND CONSUMABLE MATERIALS

- 4.1 Reagent Water - Millipore. Milli-Q system or equivalent filtered through a 0.45-micron filter.
- 4.2 Methylene Chloride - pesticide quality or equivalent. HPLC quality.
- 4.3 Acetonitrile - pesticide quality of equivalent. HPLC quality.
- 4.4 Sodium Thiosulfate - (ACS) granular.
- 4.5 Anhydrous Sodium Sulfate - (ACS) granular.
- 4.6 Stock Standard Solutions:
 - 4.6.1 Ultra Scientific - Use for Calibration
Custom mixed solutions (varying concentrations).
Dilute stock standard 1/10 with Acetonitrile.
 - 4.6.2 Absolute Standards, Inc. - Use for LFB & Spike
Custom mixed solution (varying concentrations)
Dilute stock standard 1/10 with Acetonitrile

If using a PAH mix from Ultra Scientific and/or Absolute Standards, Inc. make a diluted stock standard from which all standards will be made from. All constituents store standards in amber bottle and at 4°C. Whenever a problem arises with QA/QC check standards and if necessary replace standards.

Laboratory Control Check Sample Concentrate:

Prepare a solution of all 16 analytes at a level of about 10 times of Method Detection Limit (MDL).

5. SAMPLE COLLECTION, PRESERVATION AND HANDLING

- 5.1 Collect samples in 2.5- liter amber bottles. Do not pre-rinse the bottle before collecting sample. If residual chlorine is present add 100mg sodium thiosulfate per liter of sample and mix well.
- 5.2 PAH'S are extremely light sensitive compounds - therefore - samples, extracts, and standards must be kept in amber or foil wrapped bottles to minimize photolytic decomposition.
- 5.3 All samples must be iced and refrigerated at 4°C from the time of sampling until extraction. Furthermore, all samples must be extracted within 7 days of collection and analyzed within 40 days of collection.

6. EXTRACTION: Automated Liquid - Solid phase extraction (SPE)

- 6.1 The District uses the automated extraction unit (Horizon Technology – SPE-DEX 4700). A separate SOP has been developed to utilize this system for 550 samples. Please refer to that SOP for more details. The automated system's extractors are purged before each run to eliminate any type of contamination or carryover between samples. The disk is placed in the automated unit and the system will automatically condition each disk by the approved EPA methodology.
- 6.2 Transfer one liter of sample to the specified auto-extractor 1-liter bottles. Add 5 mls of methanol per liter of sample to be extracted (0.5% methanol in sample). Process the sample based on the automated method (method 550.1). The automated system is setup to process all samples in the same manner. The method is as follows: 1st: soak and dry disk with acetone, 2nd: soak disk with methanol, 3rd: soak disk with DI, 4th: process sample, 5th: dry disk for 10 min, 6th: elute sample with acetonitrile twice and then methylene chloride twice.
- 6.3 The automated system will process each sample – rinsing the sample bottle during the elution step. Dry the extract with 10 grams of anhydrous sodium sulfate. Rinse the collection vial with 2-ml of CH₃CN twice, and 2-ml of CH₂Cl₂ twice. Transfer into a Zymark concentrator tube. Rinse the sodium sulfate with two 4

- ml portions of CH₃CN and 4-ml portions of CH₂Cl₂ twice. Add rinses to Zymark tube.
- 6.4 Concentrate the sample to 1.0 ml – do not concentrate the extract to less than 0.5 ml. Make up any volume adjustments with CH₃CN – to the 1.0 ml marked level.
- 6.5 Transfer the 1.0 ml extract into a autosampler vial and store in the sample extract refrigerator at 4 °C until analyzed by HPLC.

7. STOCK STANDARD SOLUTION AND EXTERNAL CALIBRATION:

- 7.1 The external calibration used is a 5-point calibration ranging from 0.05 to 5 ppb. Please refer to standard preparation for directions on how to prepare calibration curve.
- 7.2 In addition to calibration curve, daily calibration check standards (front & back standards) must also be analyzed. The front standard should be right after the 5-point curve and the back should be the last sample analyzed. This requirement is for analysis time less than 8 hours. If a run is greater than 8 hours, then check standard must be analyzed every 10 samples.
- 7.3 If a cleanup procedure is needed to extract samples, then the same process must be applied to the calibration curve in order to show validation of the cleanup step.

8. PREPARATION OF STANDARDS:

- 8.1 Use Ultra Scientific for the calibration Standard. Use Absolute Standards, Inc. for the LFB and spike.
- 8.2 Stock Concentration for Ultra Scientific and Absolute Standards, Inc. is as follows. Dilute the stock standard 1/10 ml with acetonitrile to create the intermediate std.

Analyte	(µg/ml)	Analyte	(µg/ml)
Naphthalene	1,000.00	Benzo(a)anthracene	100.00
Acenaphthylene	2,000.00	Chrysene	100.00
Acenaphthene	1,000.00	Benzo(b)fluoranthene	200.00
Fluorene	200.00	Benzo(k)fluoranthene	100.00
Phenanthrene	100.00	Benzo(a)pyrene	100.00
Anthracene	100.00	Dibenzo(a,h)anthracene	200.00
Fluoranthene	200.00	Benzo(g,h,i)perylene	200.00
Pyrene	100.00	Indeno(1,2,3-cd)pyrene	100.00

- 8.3 Calibration standards: Ultra Scientific (Dilute with CH₃CN)

Calib. Std.	Vol. of Intern. Std.	Final Vol.
Std. A	5.0 µl	1.0 ml
Std. B	10.0 µl	1.0 ml
Std. C	15.0 µl	1.0 ml

Std. D	20.0 µl	1.0 ml
Std. E	25.0 µl	1.0 ml

8.4 L.F.B. and SPIKE: Absolute Standards, Inc.

- a. Low LFB = Std B Spike 1,000 ml DI water w/ 10.0 µl of intermediate std
- b. LFB = Std C Spike 1,000 ml DI water w/ 20.0 µl of intermediate std
- c. Sample Spiking Spike 1,000 ml sample w/ 20.0 µl of intermediate std

9. ANALYSIS

Start-up conditions (2695):

- 9.1 Turn on Alliance 2695, Waters 474 fluorescence detector, Waters 996 PDA detector. Make certain the 2 detectors are connected correctly.
- 9.2 Set column temperature to 30 °C.
- 9.3 Turn Degasser "On".
- 9.4 Perform "Dry Prime", "Wet Prime" and "Purge Injector" functions.
- 9.5 Open the Millennium Software.
- 9.6 PDA detector settings preset within the Millennium data system make sure detector is on and calibrated. Fluorescence detector: Program mode should be already set-up with the detector. Make sure T=0 before injection.
- 9.7 Instrument Conditions

Degasser: on

Flow Rate: 1.5ml/min

<u>Step</u>	<u>Time</u>	<u>%A (H2O)</u>	<u>%C (ACN)</u>	<u>Curve</u>
1	--	50	50	--
2	5	50	50	5
3	28	8	92	5
4	29	50	50	2
5	34	50	50	2
6	44	50	50	2
7	50	20	80	2
8	53	20	80	2
9	75	20	80	2

Column Oven Temp: 30°C

Sample Injected Vol. 50ul, use limited volume inserts.

Detectors: PDA detector: start wavelength set at 210 nanometer,
end wavelength set at 400 nanometer.

Fluorescence settings are as follows (example): Program mode T = 0

Initial:	t=0	ex 224	em 330	
Step 1:	t=3	ex 224	em 330	
Step 2:	t=3	gain x10		
Step 3:	t=3	attn:32		
Step 4:	t=8.5	ex 260	em 320	ACNAPE
Step 5:	t=8.5	gain x10		
Step 6:	t=8.5	attn: 64		
Step 7:	t=10.6	ex 260	em 380	PHENAN
Step 8:	t=10.6	gain x100		
Step 9:	t=10.6	attn: 16		
Step 10:	t=12.9	ex 250	em 420	FLANTH
Step 11:	t=12.9	gain x100		
Step 12:	t=12.9	attn: 4		
Step 13:	t=15.0	ex 270	em 385	BaANTH
Step 14:	t=15.0	gain x10		
Step 15:	t=15.0	attn: 8		
Step 16:	t=18.0	ex 280	em 410	BbFLUR
Step 17:	t=18.0	gain x10		
Step 18:	t=18.0	attn: 8		
Step 19:	t=22.5	ex 270	em 466	DBahAN
Step 20:	t=22.5	gain x100		
Step 21:	t=22.5	attn: 8		
Step 22:	t=27.5	ex 300	em 466	No Peak
Step 23:	t=27.5	gain x100		
Step 24:	t=27.5	attn: 8		
Step 25:	t=0			

9.8 Data Station: Empower Software

10. QA/QC REQUIREMENTS

- 10.1 Method blanks (reagent blanks) in order to demonstrate that glassware and reagents are free of any interference, a reagent blank must be analyzed at the beginning of each analysis. If the blank produces any interference within any of the 16 constituents, determine the source of the interference and eliminate that interference before proceeding.
- 10.2 Laboratory Fortified Blank - Analyze at least one laboratory fortified blank, LFB, with every 20 samples or one per run, whichever is greater. The fortification concentration of each analyte should be 10 times the EDL or the MCL, whichever is less. If the recovery of any analyte falls outside the control limits, the source of the problem must be identified and corrected.
- 10.3 Verify calibration standards quarterly by analyzing a standard prepared from reference material obtained from an independent or second source. Results must be within +/- 30% of those used to routinely check calibration. Weekly, run a low level standard to check the reportable detection level, RDL.
- 10.4 Samples must be extracted within 7 days after collection. Duplicates are run on 10% of samples, or once during run, whichever is greater. Results should be within +/- 30%.
- 10.5 The laboratory must add a known concentration of spike solution, the same as used for LFB, at least 10% of samples or once per analytical run, whichever is greater. Recoveries should be within +/- 30%.

11. PREVENTIVE MAINTENANCE AND CORRECTIVE ACTIONS

- 11.1 Glassware - Glassware must be carefully cleaned. Clean all glassware as soon as practical after use by thoroughly rinsing with last solvent used in it. Follow by washing with hot water and 10% HCl and thoroughly rinsing with tap water followed by reagent water. After drying, heat in oven. Do not heat volumetric glassware above 220°C. Thorough rinsing with acetone may be substituted for heating.
- 11.2 Reagents - The use of high purity solvents and reagents will help to minimize contamination problems.
- 11.3 Carryover - Contamination carryover may occur when a sample containing a low concentration of analytes is analyzed immediately following a sample containing a high concentration. Use solvent rinses between samples to minimize carryover.
- 11.4 All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory method blanks.

12.0 NO OR POOR CHROMATOGRAPHY

- 12.1 Check all the standards. If calibration and/or QC lie outside the acceptable limits we must first re-run the standards to assure that the injection procedure is working properly. After the re-run if standards still lie outside the acceptable limits we must make a new standard or conduct instrument maintenance to assure that calibration and QC lie with acceptable parameters. For elevated spike recoveries make sure that the sample matrix is clean. If sample matrix is altering recoveries then a new matrix must be selected. If instrument performance is acceptable the samples must be re-extracted. If the sample lie outside allowable holding time they must be re-sampled.
- 12.2 Request a re-sample if it has unusual hits.

Record all corrective actions in the maintenance logbook. Include a complete description of the problem and what action(s) were taken to correct it.

SOP PROCEDURE CHANGE
For EPA Method 550.1

INITIALS

lvs--

ATL

METHOD 550.1 (REVISED 12/05/06)

Refrigerate D.I water overnight in 1L SPE bottles

•

Clear Check-Valves with plastic pick on each extractor

•

Purge each extractor unit using "**Purge method 550.9**"

Collect Purge Waste and store in Haz. Waste bottle

•

Transfer samples into a 1L amber bottle with 5mL of methanol

•

Add the spike(LFL: 10ul, LFB: 20ul, Spike: 20ul)

•

Assemble C-18 Empore disk

•

Load bottles onto each extractor.

On 4790 extractors, twist bottle 3/4 way to release air.

•

Load extractors with method "**Method 550.1**". **Start extractors.**

•

After the sample has been extracted,
transfer to a 125 ml erl-flask with 10g of Na_2SO_4
rinse the tube with 2ml of Acetonitrile twice

•

repeat the above rinse step with 2ml aliquot of CH_2CL_2 two more times

•

after 4th rinse, make sure extract is dry and Na_2SO_4 is free flowing.

Wait a minimum of 15 min

•

Transfer to small Zymark tube
rinse the erl-flask with two-2 ml of acetonitrile,
add the rise to the Zymark tube

•

repeat the above rinse with two 4ml aliquots of CH_2CL_2 twice

•

Zymark settings: Temp: 42, Pressure: 12 psi

•

concentrate the extract to 1ml and transfer to a amber gc autosampler vial

•

To clean extractors after use:

Rinse extractors w/ warm water by holding down purge & abort key simultaneously

ORANGE COUNTY WATER DISTRICT

EPA METHOD 550

Standard Preparation and Quality Control

Extraction Solvent ---- Acetonitrile

1. Calibration Standard: Ultra Scientific, Catalog # CUS-3327, stock concentration = 10 - 200 µg/ml

2. 2nd Source Standard: Absolute Standards, Catalog # 95109, stock concentration = 10 - 200 µg/ml

<u>Analytes</u>	<u>Concentration</u> <u>ug/ml</u>	<u>Analytes</u>	<u>Concentration</u> <u>ug/ml</u>
Naphthalene	100	Benzo(a)anthracene	10
Acenaphthylene	200	Chrysene	10
Acenaphthene	100	Benzo(b)fluoranthene	20
Fluorene	20	Benzo(k)fluoranthene	10
Phenanthrene	10	Benzo(a)pyrene	10
Anthracene	10	Dibenzo(a,h)anthracene	20
Fluoranthene	20	Benzo(g,h,i)perylene	20
Pyrene	10	Indeno(1,2,3-cd)pyrene	10

A. Calibration Standards, Ultra Scientific

<u>Standard</u>	<u>Volume of</u> <u>Standard</u>	<u>Final</u> <u>Volume</u>
STD A	5.0 µL	1 mL
STD B	10.0 µL	1 mL
STD C	15.0 µL	1 mL
STD D	20.0 µL	1 mL
STD E	25.0 µL	1 mL

B. Check Standard, Ultra Scientific

<u>Volume of</u> <u>Standard</u>	<u>Final</u> <u>Volume</u>
20.0 µL	1 mL

C. Low LFB, LFB, Spike, and Spike Duplicate, Ultra Scientific

<u>Standard</u>	<u>Volume of</u> <u>Standard</u>	<u>Final</u> <u>Volume</u>
Low LFB	10.0 µL	1 mL
LFB	20.0 µL	1 mL
Spike	20.0 µL	1 mL
Spike Dup	20.0 µL	1 mL

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

EPA Method 551.1

Revision 4.0

DETERMINATION OF CHLORINATION DISINFECTION BYPRODUCTS AND CHLORINATED SOLVENTS IN DRINKING WATER BY LIQUID-LIQUID EXTRACTION AND GAS CHROMATOGRAPHY WITH ELECTRON CAPTURE DETECTION

File Name: M:\SOP\ORGANIC\EPA_Method_SOPs\551.1_111009.doc Effective Date: 11/10/09
Revision: 4 Supersedes: 10/11/04

1. SCOPE & APPLICATION

1.1 Summary of method:

EPA Method 551.1 is used to analyze: Trihalomethanes (THMs), Halonitriles (HANs), other DBPs, Chlorinated Solvents, Pesticides, and Herbicides. OCWD uses this method to monitor for THMs, HANs, and the other DBPs. * The pesticides/herbicides targets, outlined within this method, are provided as an option within this SOP. A 50 ml aliquot of sample is extracted with 3 mls of Methyl-t-butyl ether (MTBE). 2 µL of the extracted sample is then injected into a GC equipped with an electron capture detector. Calibration standards and check standard are extracted in the same manner as the samples. Analysis time is approximately 60 minutes for the DBPs and CISOLVs. The option, for the analysis of specified pesticides and herbicides, would extend the run time to 90 minutes. Analytical results must be confirmed by a second column. When high enough concentrations of any analyte are detected, confirm by GC/MS. A separate vial with sodium sulfite must be collected for Chloral Hydrate – labeled as 551A on the sample vials. All other targets can be determined using the ammonium chloride – as the de-chlorinating reagent – labeled as 551.

1.2 Sample Collection

1.2.1 Samples for EPA method 551.1 are collected in 60 ml clear VOA vials. Four vials for each site are taken so that spikes and/or duplicates can be processed. Two sets of four are taken for each site – one set for Chloral Hydrate (551A) preserved with 0.6 grams sodium sulfite, and another set for all the other targets preserved with 0.6 grams of buffer - ammonium chloride.

1.2.2 When sampling, allow the tap to flush for several minutes so that the water temperature stabilizes. Adjust the flow to 500 ml/minute and collect the sample. When sampling, allow the tap to flush for several

minutes so that the water temperature stabilizes. Adjust the flow to 500 ml/minute and collect the sample. Fill the sample vials to just overflowing – but take care not to flush out the buffer/dechlorination reagents. Fill the bottles so there is no headspace and cap.

1.3 Sample Storage

- 1.3.1 All samples must be chilled and delivered to the laboratory at 4°C. Samples are stored at 4 °C and away from light until extraction. The method allows for a 14-day extraction holding time – however samples should be analyzed as soon as possible after collection (based on efficiency). Any samples extracted after 14 days are invalid and should not be analyzed. A request for resample form must be completed for those sites failing this requirement.
- 1.3.2 Once samples are extracted – the extracts should be stored at less than -10°C (stored within the organic explosion proof freezer). Samples must be analyzed within 14 days after extraction. Any samples failing the above sample storage requirements are invalid, and a request for resample form must be sent to the WQ department.
- 1.3.3 Sample extracts will expire after 24 hours under room temperature. Because most of the 551/A samples sets will exceed this time limit, it is necessary to load the latter half of the samples the next day. As such, the start time for the sample set needs to be planned accordingly.

2. **Reagents and Standards**

- 2.1 Reagent water - helium purged Millipore water
- 2.2 MTBE - (Methyl-tert-butyl ether) - extraction solvent - B & J GC² grade
- 2.3 Acetone/Methanol - standard solvent - B & J GC² grade.
- 2.4 EPA method 551 stock standard mix from Ultra Scientific. Surrogate standard – decafluorobiphenyl, Internal standard – bromofluorobenzene. Stock solution stored in freezer. Most stable for 4 months. Chloral hydrate standards have been shown to be stable for 2 months.
- 2.5 Sodium Chloride, NaCl - ACS Reagent grade -baked in muffle furnace @ 400°C for one hour - stored in amber glass bottle.
- 2.6 Sodium Sulfate, Na₂SO₄ – ACS Reagent grade –baked in muffle furnace @ 400°C for ½ hour – stored in amber glass bottle
- 2.7 Phosphate buffer mixture - 2.50% Sodium Phosphate, dibasic/ 97.5% Potassium

Phosphate, monobasic by weight. Add 0.6 grams of the mixture to each 60 ml sampling vial.

2.8 Ammonium Chloride - NH_4Cl - ACS reagent grade - used as the dechlorinating reagent for all targets except Chloral Hydrate - add 6mg of NH_4Cl to each 60ml sample vial.

2.9 Sodium Sulfite - Na_2SO_3 - ACS Reagent grade - used as a dechlorinating reagent for the analysis of chloral hydrate. Add 6mg of Na_2SO_3 to each 60 ml sample vial.

2.10 Reagent Blank Water

2.10.1 Reagent blank water is free of any organic contaminants. Boil a volume of 18 megohm water for approximately 15 minutes. While maintaining a temperature of 90°C , purge the water with UHP He for 1 hour.

2.11 Glassware Washing

2.11.1 Wash glassware with hot water, 10% HCl, hot tap water, and deionized water. Allow glassware to bake at 220°C overnight.

3. APPARATUS AND EQUIPMENT

3.1 Sample containers - 60 mL clear VOA vial, equipped with a PTFE septum.

3.2 Vials - autosampler screw cap with PTFE faced septa.

3.3 Micro syringes - 10 μL , 25 μL , and 50 μL gas-tight Hamilton Syringes.

3.4 GC column: primary column - DB-1 with 1.0 μm film thickness 0.32 mm X 30 meter long.

3.5 GC equipped with ECDs able to do temperature programming.

3.5.1 Activate the proper method. The linear velocity of the carrier gas is set at 25 cm/second.

3.5.2 The column temperature is programmed to hold at 35°C for 9 minutes and then increase to 40°C at $0.3^\circ\text{C}/\text{minute}$ - hold for 3 minutes. Increase to 120°C at $6^\circ\text{C}/\text{minute}$, and hold for 5 minutes. Increase to 150°C at $10^\circ\text{C}/\text{minute}$, and hold for 4.42 minutes. Increase to 235°C at $30^\circ\text{C}/\text{minute}$, and hold for 5 minutes.

3.5.3 Total run time approximately 50 minutes (50.17) - for DBPs and CLSOLVs. For analysis of pesticides and herbicides consult the EPA method 551.1 (rev. 1.0). Injector temperature: 200°C . Detector temperature: 290°C .

3.5.4 GC Parameters: VARIAN 3500s equipped with ECDs

DB-1 primary confirm by GC/MS or DB-624

Initial Column Temperature:

35°C

Initial Column Hold Time:

9.00 minutes

Program	Final Temp	Rate	Hold Time
1	40°C	0.3°C/minute	3.00 minutes
2	120°C	6.0°C/minute	5.00 minutes
3	150°C	10°C/minute	4.42 minutes
4	220°C	30.0°C/minute	2.00 minutes

Total Run Time - 53 minutes

Flow - primary column A (DB-1) in mL/minute:

1.8

Pressure - primary column A (DB-1) in PSI:

8.5

Column Velocity in cm/second:

25.0

Injector A & B Temperature:

200°C

Detector Temperature:

290°C

ECD Initial Attenuation:

16 range 10

(autozero on)

4. ANALYTES

<u>LIMS CODE</u>	<u>ANALYTE</u>	<u>OCWD RDL</u>
CHCl3	Chloroform	0.10 ug/L
111TCA	1,1,1-Trichloroethane	0.10 ug/L
CCl4	Carbon Tetrachloride	0.10 ug/L
TCAN	Trichloroacetonitrile	0.10 ug/L
DCAN	Dichloroacetonitrile	0.10 ug/L
CHBrCl	Bromodichloromethane	0.10 ug/L
TCE	Trichloroethylene	0.10 ug/L
ClHYDR **	Chloral Hydrate **	0.10 ug/L
11DC2P	1,1-Dichloro-2-Propanone	0.10 ug/L
ClPICR	Chloropicrin	0.10 ug/L
CHBr2Cl	Dibromochloromethane	0.10 ug/L
BCAN	Bromochloroacetonitrile	0.10 ug/L
EDB	1,2-Dibromoethane	0.10 ug/L
PCE	Tetrachloroethylene	0.10 ug/L
111TCP	1,1,1-Trichloropropanone	0.10 ug/L
CHBr3	Bromoform	0.10 ug/L
DBAN	Dibromoacetonitrile	0.10 ug/L
DBCP	1,2-Dibromo-3-chloropropane	0.10 ug/L
DFBP	Decafluorobiphenyl (surrogate std)	0.10 ug/L

** - Chloral Hydrate – samples must be taken in a separate vial – special arrangements must be made with the laboratory to have the vials prepared.

5. EXTRACTION

- 5.1 Let sample containers warm up to room temperature; use a transfer pipette to remove 10 mls of sample from each 60ml vial. Using this 10 ml aliquot, check the pH of the sample. It must be within a pH range of 4.5 and 5.5. If the pH is outside of this range, a new sample must be collected.
- 5.2 Replace the cap and weigh the vial to the nearest 0.1g – note the weight onto the extraction log sheet. For calibration and check standards, laboratory fortified samples, and reagent blanks use a 50ml-graduated cylinder to transfer the 50 ml sample volume into the vial.
- 5.3 Spike the surrogate and QA/QC samples according to the appropriate levels. Afterwards, add 3.0 ml of MTBE to each sample vial by using a dispenser – pre-measure to ensure that 3.0 mls of MTBE is delivered. Add 10 g of the muffle treated NaCl to each vial. Recap and shake vigorously for 4 minute.
- 5.4 Allow the water and MTBE phases to separate for at least 10 minutes. Draw off an aliquot (1.0 ml) of MTBE into the auto-sampler vial using a Pasteur pipette, making sure that none of the water is drawn into the vial. If possible, make-up a second vial in the same manner for back-up analysis.
- 5.5 Dispose of the vials contents properly and re-weigh the vial with the correct cap to attain the sample volume. Again, weigh to the nearest 0.1g and note this on the extraction log sheet. The net weight of the sample will be inputted into the chromatography software to adjust for the correct volume for each sample.
- 5.6 The extracts should be analyzed as soon as possible after extraction – same day if possible. The extracts should be stored in a freezer (<-10 C) until the analytical system is ready for analysis. Maximum storage time of sample extract is fourteen days.
- 5.7 Sample extracts will expire after 24 hours under room temperature. Because most of the 551/A samples sets will exceed this time limit, it is necessary to load the latter half of the samples the next day. As such, the start time for the sample set needs to be planned accordingly.

6. Calibration/Standardization

- 6.1 Prepare secondary dilution standards by preparing a 1/10 and a 1/100 dilution of the stock standard - both of which are prepared with acetone*. *When purchasing commercial stock standards avoid solutions made up in methanol (except Chloral Hydrate). Methanol can cause degradation of most of the haloacetonitriles. For these reasons, acetone should be used as the primary solvent for stock standard and primary dilution standard preparation. The two diluted standards will allow for further setup of calibration standards.

- 6.2 Prepare a calibration curve, which consists of 0.10 ppb, 0.20 ppb, 0.40 ppb, 0.80 ppb, and 1.0 ppb. This calibration curve has been found to be efficient for the analysis of groundwater samples before point of chlorination. It may be necessary to increase the concentrations of the calibration curve if the samples are coming after point of chlorination or from a known highly chlorinated system.
- 6.3 Follow extraction procedures for extracting these standards. Each analysis run contains this 5-point curve and the 0.40 ppb standard is run as the back standard. Follow the standard preparation outline. If the ratio of response to concentration (response factor) is constant over the working range ($< 10\%$ RSD), linearity through the origin can be assumed. A high (0.40 ppb) LFB (laboratory fortified blank) and a low (0.1 ppb) LFB are analyzed with each extraction run.
- 6.4 Spikes/Duplicates
 - 6.4.1 The spiking concentration is 0.4 ppb. All values from the spike sample are documented; and, after enough data are acquired, a control chart with upper and lower warning and control limits is graphed.
 - 6.4.2 Report all spike data on Control Chart Sheet. Once a month, the values from these sheets will be entered into a computer for control chart generation.

7. QA/QC REQUIREMENTS

- 7.1 Before the analysis of samples can begin, a laboratory performance check (LPC) sample must be analyzed to demonstrate instrument performance. This must be demonstrated before each daily run. The LPC sample checks for instrument sensitivity (Lindane), chromatographic performance (hexachloropentadiene), column performance (bromodichloromethane/trichloroethylene, bromacil/alachlor), and analyte breakdown (endrin). All criteria must pass before samples can be processed
 - 7.1.1 LPC Criteria:
 - Instrument sensitivity – signal to noise >3
 - Chromatographic Performance – PGF between 0.80 and 1.15
 - Column Performance – resolution > 0.50 for both analyte sets
- 7.2 A Continuous Calibration Check (CCC) sample is analyzed with each analytical run for validation of the calibration curve. The CCC sample is analyzed at 0.40 ppb. Recoveries must fall between 75% and 125% for all the targets. The recoveries of at least 90% of the analytes determined must fall between 80% and 120%.
- 7.3 A low level standard (LFB-low) must be run at 0.10 ppb to ensure that the system is capable of low level analysis - RDL is confirmed. The LFB-high is analyzed at 0.40 ppb with each sample run. A set of low and high LFBs are analyzed with each run,

the recoveries must be within +/- 25%.

- 7.4 Spikes & Duplicates must be analyzed at a frequency of at least 10% of the sample load. Spikes are analyzed at 0.40 ppb and recoveries must fall between 75% and 125%. Duplicates must not reflect a relative percent difference (RPD) greater than 25% for any one analyte. The RPD for 90% of the analytes being determined must be less than 20%.
- 7.5 Surrogate recovery must be monitored for every sample and QA/QC run. Recoveries must fall within the range of 80% to 120%. Samples that fail must be either re-analyzed or re-extracted if within holding time limits. Re-calibration of the system may be required to meet this requirement.
- 7.6 A reagent water blank must be analyzed with each analytical run to demonstrate that interferences from the system are under control.
- 7.7 To establish the ability to achieve low detection levels and to generate accurate and precise chromatography, the analyst must analyze seven low level replicates (0.10 ppb). The MDL is then calculated based on the precision and accuracy of these results. The acceptance criterion calls for a recovery of +/- 20% and an MDL that does not exceed 0.05 ppb. Anytime major maintenance to the system occurs a new MDL determination must be performed. Compliance to this shows that system performance is acceptable and sample analysis can begin. The low level LFB can be utilized to generate on-going MDL studies.

8. REPORTING REQUIREMENTS

- 8.1 For any positive hit, a confirmation must be performed. If in high enough concentrations a GC/MS analysis can be performed. If not, the sample must be re-analyzed using the confirmation column.
- 8.2 If any result is within 75% of an action level, the water quality (WQ) department must be notified immediately. Sample results will be carried through the LIMS system. The raw data will be written-up in a standard data package - with QA/QC recoveries of spikes and standards noted. This data package will then be reviewed by either the senior chemist or the supervising chemist for completeness. Once approved the final form will be generated and approved by the laboratory director. Any abnormalities will be noted on the data package sign-off sheet (corrective actions will also be noted).

9. CORRECTIVE ACTIONS

- 9.1 Calibration Standards/QC standards fail-
 - 9.1.1 Check all the standards. If calibration and/or QC lie outside the acceptable limits we must first re-run the standards to assure that the injection procedure is working properly.
 - 9.1.2 If standards are still outside the acceptable limits, re-extract a new standard or conduct instrument maintenance.

- 9.1.3 For elevated spike recoveries make sure that the sample matrix is clean. If sample matrix is altering recoveries, then a new matrix should be selected. If LFB's fail, check standard. Conduct a method review in both cases. The method review would include verification of standard lots and actual process steps. All staff members involved in the processing of samples would be included in this process.
 - 9.1.4 If instrument performance is acceptable, the samples must be re-extracted. If the samples are outside allowable holding time they must be re-sampled.
 - 9.1.5 If LPC fails, check the standard or replace with new standard, and re-analyze. If LPC fails again, stop the instrument and perform necessary preventive maintenance on the analytical system.
 - 9.1.6 If front check standard fails, check standards, make up new dilutions and re-analyze.
 - 9.1.7 Request a re-sample if necessary
- 9.2 All corrective actions will be noted in the maintenance logbook and the data review sign-off sheet. Scheduled preventive maintenance functions for the replacement of gas filters, septa, injection port inserts, and column replacement should be conducted regularly. Solvent blanks must be analyzed with each run in order to monitor any interferences. Reagent water must also be monitored, on every analytical run, for contamination.

10. PREVENTIVE MAINTENANCE

- 10.1 Changing of septum and gas cylinders requires that the column oven, detectors and injectors be cooled down. Additionally, detectors need to be turned off after cooling. When injector and column ovens have been brought up to temperature and allowed to equilibrate, turn on detector and activate the method. Check the syringe to ensure that it is being inserted properly and solvent/sample is unrestricted. Retention times must be monitored to ensure they remain consistent throughout the run. If retention times do vary, check septum and column for leaks and deterioration. The wash reservoir must contain the proper solvent (MTBE / 3/4 full).

<u>CHANGE</u>	<u>REASON</u>	<u>DATE</u>	<u>INITIALS</u>
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Changed surrogate curve to 0.2, 0.5, 1.0, 1.5, 2.0 PPB	Cal E (3.0 PPB) was oversaturated	09/23/09	PP
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ORANGE COUNTY WATER DISTRICT

EPA METHOD 551 - Rev. 2 (07/28/09)

Use purged bottled drinking water for all QCs

PREPARATION OF STANDARDS AND QUALITY CONTROLS

1. **CALIBRATION STANDARD:** Ultra Scientific - Stock Mix = 2000ug/ml
1st Dilution: Dilute 100ul of stock to 1ml Acetone - conc. = 200ug/ml - Std #1
2nd Dilution: Dilute 5ul of Std #1 to 1 ml Acetone - conc. = 1.0 ug/ml - Std #2- Working std.
2. **2ND SOURCE STANDARD:** Restek - Stock Mix = 2000ug/ml
1st Dilution: Dilute 100ul of stock to 1ml Acetone - conc. = 200ug/ml - Std #1
2nd Dilution: Dilute 5ul of Std #1 to 1 ml Acetone - conc. = 1.0 ug/ml - Std #2- Working std.

<u>Analyze</u>	<u>Analyze</u>
Bromochloroacetonitrile	Dibromochloromethane
Bromodichloromethane	1,2-Dibromo-3-chloropropane
Bromoform	1,2-Dibromoethane
Carbon Tetrachloride	Dichloroacetonitrile
Chloroform	Trichloroacetonitrile
Chloropicrin	Tetrachloroethene
Dibromoacetonitrile	1,1,1-trichloroethane
1,1-Dichloroacetone	Trichloroethene
1,1,1-Trichloroacetone	

Surrogate: Ultra Sci (Decafluorobiphenyl) IST-152 Stock 1000 ug/mL

Dilute 10 ul of Stock into 1mL of Acetone, **Working Surrogate** - conc = 10ug/mL

Spike 1, 2.5, 5, 7.5, 10 uL of 10ug/mL **Working Surrogate** into calibration vials A-E, respectively

Spike 5ul of 10ug/ml **Working Surrogate** into each sample

A. LAB PERFORMANCE CHECK STANDARD: Ultra Sci. - Custom Mix Std: **CUS-3964**
Spike 1ml 551 MTBE with 1ul of CUS-3964 and 30ul of std #2 (Working std) Chloral Hydrate (1ug/ml)

B. CALIBRATION STANDARD: Ultra Scientific

<u>Calib. Std. Conc.</u>	<u>Volume of Std.</u>
Std A - 0.10 ug /L	5.0ul of Std #2
Std B - 0.20 ug/L	10.0ul of Std #2
Std C - 0.40 ug/L	20.0ul of Std #2
Std D - 0.80 ug/L	40.0ul of Std #2
Std E - 1.00 ug/L	50.0ul of Std #2

C. LFB & SPIKE: Protocol

LOW LFB = 0.10 ug/L Spike 50ml purged bottled drinking water with 5ul of Std #2

LFB = 0.40 ug/L Spike 50 ml purged bottled drinking water water with 20 ul of Std #2

SAMPLE SPK = 0.40 ug/L Spike sample with 20 ul of Std #2

ORANGE COUNTY WATER DISTRICT

EPA METHOD 551A – Chloral Hydrate

Use purged bottled drinking water for all QCs

PREPARATION OF STANDARDS AND QUALITY CONTROLS

1. **CALIBRATION STANDARD:** Ultra Scientific - Stock Mix = 1000ug/ml
1st Dilution: Dilute 100ul of stock to 1ml Methanol - conc. = 100ug/ml - #1
2nd Dilution: Dilute 10ul std #1 to 1 ml Methanol - conc. = 1.0 ug/ml - #2-Working std.
2. **2ND SOURCE STANDARD:** Accustandard - Stock Mix = 1000ug/ml
1st Dilution: Dilute 100ul of stock to 1ml Methanol - conc. = 100ug/ml - #1
2nd Dilution: Dilute 10 ul of std #1 to 1 ml Methanol - conc. = 1.0 ug/ml - #2- Working std.

Analyze

Chloral Hydrate

Surrogate: Ultra Sci (Decafluorobiphenyl) IST-152 Stock 1000 ug/mL

Dilute 10 ul of Stock into 1mL of Acetone, **Working Surrogate** - conc = 10ug/mL

Spike 1, 2.5, 5, 7.5, 10 uL of 10ug/mL **Working Surrogate** into calibration vials A-E, respectively

Spike 5ul of 10ug/ml **Working Surrogate** into each sample

A. CALIBRATION STANDARD: Ultra Scientific

<u>Calib. Std. Conc.</u>	<u>Volume of Std.</u>
Std A - 0.10 ug /L	5.0ul of Std #2
Std B - 0.20 ug/L	10.0ul of Std #2
Std C - 0.40 ug/L	20.0ul of Std #2
Std D - 0.80 ug/L	40.0ul of Std #2
Std E - 1.00 ug/L	50.0ul of Std #2

B. LFB & SPIKE: Accustandard

LOW LFB = 0.10 ug/L Spike 50 ml purged bottled drinking water water with 5 ul of Std #2.

LFB = 0.40 ug/L Spike 50 ml purged bottled drinking water water with 20 ul Std #2

SAMPLE SPK = 0.40 ug/L Spike sample with 20 ul of Std #2

METHOD 551A - Chloral Hydrate

Rev. 3 - 09/23/09

let samples reach room temperature

use graduated cylinder to measure 50 mL **DI water** for QA/QC samples
Add preservative (0.6 g sodium sulfite/phosphate buffer mix)

pipette out 10 mls from each sample vial, check pH.
If pH is not within 4.5 - 5.5, then a new sample must be collected.

cap and weigh each vial - note the weight on extraction log
measure to nearest 0.1g

add the spike: LFBL:5ul, LFBH: 20ul, SPK:20ul; conc.=1.0ug/ml
inject the standards: 5ul, 10ul, 20ul, 40ul, 50ul: conc.=1.0ug/ml
add surrogate: 1, 2.5, 5, 7.5, 10 ul - conc.= 10ug/ml

For samples, add 5ul surrogate (conc = 10ug/ml)

add 10.0 g of muffle furnace treated NaCl to each vial

add 3 mL of 551 MTBE

Cap vial and shake vigorously for 4 min.

wait at least 10 min. for the layers to separate
transfer the MTBE extract into 2 autosampler vials (one with insert and one without insert)

discard remaining contents of the vial - air dry the vials
re-weigh the cap and vial to determine sample volume
note the weight onto the extraction log

store extracts in freezer at -10°C

METHOD 551.1

Rev. 4.0 - 09/23/09

let samples reach room temperature

use graduated cylinder to measure 50 mL **DI water** for QA/QC samples
Add preservative (0.6 g ammonium chloride/phosphate buffer mix)

pipette out 10 mls from each sample vial, check pH.
If pH is not within 4.5 - 5.5, then a new sample must be collected.

cap and weigh each vial - note the weight on extraction log
measure to nearest 0.1g

add the spike: LFBH: **5ul**, LFBH: **20ul**, SPK: **20ul**; conc.=1.0ug/ml
inject the standards: **5ul**, **10ul**, **20ul**, **40ul**, **50ul**; conc.=1.0ug/ml
add the surrogate: **1, 2.5, 5, 7.5, 10 ul** --conc = 10ug/ml

For samples, add **5ul** surrogate (conc = 10ug/ml)

add 10.0 g of muffle furnace treated NaCl to each vial

add 3 mL of 551 MTBE

Cap vial and shake vigorously for 4 min.

wait at least 10 min. for the layers to separate
transfer the MTBE extract into 2 autosampler vials (one with insert and one without insert)

discard remaining contents of the vial - air dry the vials
re-weigh the cap and vial to determine sample volume
note the weight onto the extraction log

store extracts in **freezer at -10°C**

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

EPA Method 552.2

Rev. 4.0

DETERMINATION OF HALOACETIC ACIDS IN DRINKING WATER BY LIQUID-LIQUID EXTRACTION, DERIVATIZATION, AND GAS CHROMATOGRAPHY WITH ELECTRON CAPTURE DETECTION

File Name: M:\Sop\Organic\epa method sop\552_2_010709.doc Effective Date: 12/17/2008
Revision: 4 Supersedes: 09/13/2007

1. SCOPE & APPLICATION

1.1 Summary of method

EPA Method 552.2 is used to analyze the haloacetic acids: monochloroacetic acid, monobromoacetic acid, dibromoacetic acid, dichloroacetic acid, bromochloroacetic acid, bromodichloroacetic acid, chlorodibromoacetic acid, tribromoacetic acid, trichloroacetic acid. EPA Method 552.2 can also be used to analyze Dalapon. The internal standard used is 1,2,3 trichloropropane and the surrogate is 2,3 dibromopropanoic acid. A 40 ml volume of sample is pH adjusted and then extracted with 4.0 mls of MTBE. The extract is dried and the haloacetic acids are then converted to their methyl esters by the addition of acidic methanol followed by slight heating. The sample extract is analyzed on a Varian CP 3800 gas chromatograph equipped with an Electron Capture Detector (ECD). The acidic extract is neutralized by a back extraction with a saturated solution of sodium bicarbonate. Calibration standards are extracted in the same manner as the samples.

1.2 Sample Collection

Samples for EPA method 552.2 are collected in 250 mL glass bottles. One bottle is collected for each site, spikes and duplicates are taken from extra bottles. Ammonium chloride is added to each bottle to produce a concentration of 100 mg/L. When sampling, allow the tap to flush for several minutes so that the water temperature stabilizes. Adjust the flow to 500 ml/minute and collect the sample. Do not rinse the sample bottle with the sample – fill the bottle so there is no headspace and cap. Seal the bottle and agitate by hand for 1 minute to completely dissolve the ammonium chloride.

1.3 Sample Storage

All samples must be chilled and delivered to the laboratory at 4°C. Samples are stored at 4°C and away from light until extraction. The method allows for a 14-day extraction holding time – however samples should be analyzed as soon as possible after collection (based on efficiency). Any samples extracted after 14 days are invalid and should not be analyzed. A request for resample form must be completed for those sites failing this requirement.

1.4 Extract Storage

Store extracts within organic explosion proof freezer (<-10°C). Extracts are to be stored at -10°C away from light and must be analyzed within 14 days. (If stored at 4°C, extracts must be analyzed within 7 days – *not* the preferred storage procedure).

2. **Reagents and Standards**

- 2.1 MTBE: methyl-t-butyl ether - Burdick & Jackson - capillary GC² solvent.
- 2.2 Sodium Sulfate: Anhydrous granular. Heat at 400°C, for up to 4 hrs to remove phthalates and other interfering organic substances.
- 2.3 Copper II Sulfate Pentahydrate, CuSO₄·3H₂O, ACS grade
- 2.4 Sulfuric Acid: Reagent grade.
- 2.5 Sodium Bicarbonate, NaHCO₃, ACS reagent grade.
- 2.6 Ammonium Chloride, NH₄Cl, ACS reagent grade.
- 2.7 Sulfuric Acid, concentrated, ACS reagent grade.
- 2.8 Saturated Sodium Bicarbonate solution: Add sodium bicarbonate to a volume of water, mixing periodically until the solution has reached saturation.
- 2.9 1,2,3 trichloropropane: 99+%.
- 2.10 2,3-dibromopropionic acid: 99%.
- 2.11 Methanol: GC² grade.
- 2.12 Deionized water: Produced by Milli-Q system.

3. **APPARATUS AND EQUIPMENT**

- 3.1 60-ml test tubes fitted with Teflon-lined screw caps

- 3.2 50ml amber TFE lined glass bottles
- 3.3 15-ml graduated conical tubes with Teflon lined screw caps
- 3.4 Analytical balance - capable of weighing to 0.0001 gram.
- 3.5 Water bath
- 3.6 pH meter
- 3.7 CP 3800 Varian gas chromatograph with electron capture detector
- 3.8 DB-1 and DB-5 capillary gas chromatograph columns
- 3.9 10 μ L, 25 μ L 100 μ L, 250 μ L and 500 μ L syringes
- 3.10 Disposable pipets
- 3.11 GC autosampler vials

4. ANALYTES

This is a gas chromatographic method used to analyze for disinfection by-products (DBPs) in finished drinking water. The following compounds can be determined using this method:

<u>LIMS code</u>	<u>Analyte</u>
MCAA	Monochloroacetic Acid
DCAA	Dichloroacetic Acid
TCAA	Trichloroacetic Acid
MBAA	Monobromoacetic Acid
DBAA	Dibromoacetic Acid
24DCPH	2,4-Dichlorophenol
246TCP	2,4,6-Trichlorophenol
BCAA	Bromochloroacetic Acid
BDCAA	Bromodichloroacetic Acid
CDBAA	Chlorodibromoacetic Acid
DALAPON	Dalapon
TBAA	Tribromoacetic Acid

5. EXTRACTION

- 5.1. Remove samples from storage and allow them to equilibrate to room temperature. Transfer 40 mls of sample into a long 60ml glass vial tube with a teflon-lined

screw cap. Add the surrogate, calibration, and spike standards. Adjust the pH to less than 0.5 by adding at least 2ml of concentrated sulfuric acid.

- 5.2. Cap, shake and then check the pH with a pH paper or meter. Note this on the extraction log. Quickly add 2 grams of copper II sulfate pentahydrate.
- 5.3. Quickly add 16 grams of muffled sodium sulfate and shake until dissolved. Wait 5 minutes. Add 4.0 ml of MTBE and shake by hand vigorously for approximately 2 minutes.
- 5.4. Allow the phases to separate for approximately 5 minutes. Transfer approximately 3 mL of the MTBE extract to a clean graduated receiving tube.
- 5.5. Add 1 ml of 10% sulfuric acid in methanol to each tube. Cap the tubes and place in a water bath/heating block at 50°C and maintain for 2 hours. Place an ice bath on top of the tubes to help reduce any loss of MTBE.
- 5.6. Allow the tubes to cool before carefully adding 4 ml of saturated sodium bicarbonate solution to each centrifuge tube in 1ml increments.
- 5.7. Shake the tubes for 2 minutes with repeated venting to allow for the neutralization reaction to be complete. Transfer to 2 mL to a conical vial and add the internal standard. Run the analysis as soon as possible

6. Instrument Conditions: GC Method 1 for 552 Compounds.

6.1. Column Temp:	Initial column temperature:	35°C		
	Hold time:	0.5 min		
	Final temperatures:	100°C	160°C	260°C
	Rate °C / min:	5	20	100
	Hold times:	0 min	1 min	2 min
	Total Run Time	13.5	17.5	20.5 min
6.2. Flow:	Helium linear velocity:	25 cm/sec		
	Flow	1.5 ml/min		
	Initial column pressure:	7.0 psi		
	Hold time:	25 min		
	Final pressure:	16.0 psi		
	Rate psi/min:	2		
	Hold time:	1 min		
	Total Run Time	30.5		
6.3 Injection:	Injector Program:	Initial	On	10
		0	Off	0
		0.6	On	50
		1.5	On	100
		5	On	20
	Injector temperature:	250°C hold 1 min.		

6.4. Detector Temp: 280°C

7. PREPARATION OF ACIDIC METHANOL SOLUTION

7.1 10% sulfuric acid in methanol solution - add 5ml of sulfuric acid drop wise to 20-30 ml of methanol contained in a 50.0 ml volumetric flask that has been placed in an ice bath. Let cool to about room temperature and dilute to mark with additional methanol.

8. QA/QC REQUIREMENTS

8.1 Spikes must be analyzed at a frequency of at least 10% of the sample load. Spikes are analyzed at 10.0 ppb.

8.2 On a daily run, a Laboratory Performance Check sample must be analyzed before the analysis of samples to demonstrate instrument performance.

8.3 A low level standard must be run at 1.0 ppb to ensure that the system is capable of low level analysis - RDL is confirmed. A Continuous Calibration Check sample should also be included on each analytical run for validation of the calibration curve – recoveries for all targets must fall between 70% and 130%.

8.4 Each day, reagent water blank must be analyzed to demonstrate that interferences from the analytical system are under control. To establish the ability to achieve low detection levels and to generate accurate and precise chromatography, the analyst must analyze seven low level replicates (1.0 ppb). The MDL is then calculated based on the precision and accuracy of these results. The acceptance criterion calls for a recovery of +/- 20%. Anytime major maintenance to the system occurs a new MDL determination must be performed. Compliance to this shows that system performance is acceptable and sample analysis can begin.

8.5 The surrogate standard (2,3-dibromopropionic acid) recovery is monitored for all standards, QA/QC samples, and samples processed by the method.

8.5.1 The recovery of the surrogate must be within +/- 30%.

8.5.2 If the recovery is not within specification, potential system and method performance problems must be verified to be in control. The sample extract is then reanalyzed. If the results are within surrogate recovery limits, report only this data.

8.5.3 If the data fails the recovery specifications for the surrogate, recheck the continuous calibration check standard (CCC). If the CCC fails, recalibrate the system after a thorough review of system and method parameters. If the CCC is within specifications, re-extract the sample and re-analyze. The sample must

still meet holding time requirements for the method. If the sample is outside the extraction holding time – report the sample as “NA” – not analyzed. Within the comments section of the LIMS report, explain how the sample failed QA/QC. Fill out a request for resample form and send to the WQ department as soon as possible.

8.6 The internal standard (1,2,3-trichloropropane) must be monitored by area count for each QA/QC sample and site sample.

8.6.1 A mean IS response is calculated from the current 5-point calibration curve. The IS response in area count should not deviate from this mean IS by more than 30% for any run. It is also acceptable if the IS response is within 15% of the daily CCC standard.

8.6.2 If the deviation is outside of the 30% specifications, check instrument parameters and re-inject the extract. If this meets specifications report the results for this injection for the sample. If the 30% specification continues to fail – recheck the CCC standard to verify the calibration. Recalibrate the system if the CCC fails. If the original extract continues to fail this specification re-extract the sample. The sample must still meet holding time requirements for the method. If the sample is outside the extraction holding time – report the sample as “NA” – not analyzed. Within the comments section of the LIMS report, explain how the sample failed QA/QC. Fill out a request for resample form and send to the WQ department as soon as possible.

8.7 The calibration is extracted by the same procedure as the samples described in Section 5 using reagent water that contains the same concentration of preservative as the sample. Calibration must contain at least five points. All analytes must fall within +/- 30%. If this recovery cannot be achieved a new calibration curve must be made.

9. REPORTING REQUIREMENTS

9.1 For any positive hit, a confirmation must be performed. If in high enough concentrations a GC/MS analysis can be performed. If not, the sample must be re-analyzed using the confirmation column. If any result is within 75% of an action level, the water quality (WQ) department must be notified immediately. Sample results will be carried through the LIMS system. The raw data will be written-up in a standard data package - with QA/QC recoveries of spikes and standards noted. This data package will then be reviewed by either the senior chemist or the supervising chemist for completeness. Once approved the final form will be generated and approved by the laboratory director. Any abnormalities will be noted on the data package sign-off sheet (corrective actions will also be noted).

10. PREVENTIVE MAINTENANCE AND CORRECTIVE ACTIONS

10.1 Glassware - Glassware must be carefully cleaned. Clean all glassware as soon as

practical after use by thoroughly rinse. Follow by washing with hot water and 10%HCl and thoroughly rinsing with tap water followed by reagent water. After drying, heat in oven. Do not heat volumetric glassware. Thorough rinsing with acetone may be substituted for heating.

- 10.2 Reagents - The use of high purity solvents and reagents will help to minimize contamination problems.
- 10.3 Carryover - Contamination carryover may occur when a sample containing a low concentration of analytes is analyzed immediately following a sample containing a high concentration. Use solvent rinses between samples to minimize carryover.
- 10.4 Minimize contact of the samples, reagents, or solvents with any plastics. This will help reduce contamination.
- 10.5 Record all corrective actions in the maintenance logbook. Include a complete description of the problem and what actions were taken to correct it. Solvent blanks must be analyzed with each run in order to monitor any interferences. Reagent water must also be monitored, on every analytical run, for contamination.
- 10.6 Calibration Standards/QC standards fail-
 - 10.6.1 Check all the standards. If calibration or QC lies outside the acceptable limits, re-run the standards to assure that the injection procedure is working properly.
 - 10.6.2 If standards are still outside the acceptable limits, re-extract a new standard or conduct instrument maintenance.
 - 10.6.3 For elevated spike recoveries make sure that the sample matrix is clean. If sample matrix is altering recoveries then a new matrix must be selected. If LFB's fail, check the standard. Conduct a method review in both cases. The method review would include verification of standard lots and actual process steps. All staff members involved in the processing of samples would be included in this process.
 - 10.6.4 If instrument performance is acceptable the samples must be re-extracted. If the sample lie outside allowable holding time they must be re-sampled.
 - 10.6.5 If LPC fails, check the standard or replace with new standard, and re-analyze. If LPC fails again, stop the instrument and perform necessary preventive maintenance of the analytical system.
 - 10.6.6 If front check standard fails, check standards, make up new dilutions and re-analyzed.
 - 10.6.7 Request re-sample if it's necessary.
 - 10.6.8 If there are hits, confirm with second Column.
 - 10.6.9 Changing of septum and gas cylinders requires that the column oven, detectors and injectors be cooled down. Additionally, detectors need to be turned off after cooling. When injector and column ovens have been brought up to temperature and allowed to equilibrate, turn on detector and activate the method. Check the syringe to ensure that it is being inserted properly and solvent/sample is unrestricted. Retention times must be monitored to ensure

they remain consistent throughout the run. If retention times do vary, check septum and column for leaks and deterioration. The wash reservoir must contain the proper solvent (MTBE / 3/4 full).

[illegible]

METHOD 552.2, Rev. 1.0 (micro-extraction)

Let sample container warm up to room temperature, turn on water bath

add 40 ml of sample to the tube
use a graduated cylinder

add surrogate (1,2,5,10,15, 20 for cal. std, 5 ul for samples, conc= 100 ug/mL))
calibration (1,2,5,10,15, 20) and spike (5, 10) standards, conc=40ug/L
calibration check (10 of second source, 5 ul of surrogate)

adjust the pH to < 0.5 by adding at least 2 ml of concentrated sulfuric acid

Quickly add 2 g of copper II sulfate pentahydrate and shake until it's dissolved

Quickly add 16 g of baked sodium sulfate and shake until it's dissolved
wait 5 minutes

Add 4 ml of MTBE and shake for 2 min.

Allow the phases to separate for approximately 5 min.
transfer approximately 3 ml of the MTBE extract into a new tube

Add 1 ml of 10% sulfuric acid in Methanol to each tube
Cap the tubes and place in water bath at 50 C for 2 hrs
Place an ice bath on top of the tubes to help reduce any loss of MTBE

Allow the tubes to cool

Add 4 ml of saturated sodium bicarbonate solution to each tube in 1 ml increment

Shake the tubes for 2 min with repeated venting

Transfer 2.0 ml to a conical vial
Add internal standard 4.0 uL

Transfer content to 2 amber gc vials (1 with limited volume insert, 1 without)
Store extracts in freezer

ORANGE COUNTY WATER DISTRICT

EPA METHOD 552

Standard Preparation and Quality Control

Calibration standards, QC's and samples are extracted using the same procedure.

Extraction Solvent ---- MTBE

Standards:

1. Calibration Standard: Ultra Scientific, Catalog # CUS-3372, Stock Concentration = 100 ug/mL

Working Standard: Dilute 400 uL of stock to 1.0 mL with MTBE Concentration = 40.0 ug/mL

2. 2nd Source Standard: Accustandard, Catalog # S-3662-0.1X, Stock Concentration = 100 ug/mL

Working Standard: Dilute 400 uL of stock to 1.0 mL with MTBE Concentration = 40.0 ug/mL

Analytes

Bromoacetic Acid
Bromochloroacetic Acid
Bromodichloroacetic Acid
Chloroacetic Acid
Chlorodibromoacetic Acid

Analytes

Dalapon
Dibromoacetic Acid
Dichloroacetic Acid
Tribromoacetic Acid
Trichloroacetic Acid

3. Surrogate: Accustandard (2,3-Dibromopropionic Acid), Catalog # M-552.2-SS,

Stock Concentration = 1000 ug/mL

Working Standard: Dilute 100 uL of stock to 1.0 mL with MTBE Concentration = 100 ug/mL

4. Internal Standard: Accustandard (1,2,3-Trichloropropane), Catalog # M-552.2-IS

Stock Concentration = 1000 ug/mL

Preparation:

A. Calibration Standard Preparation, Ultra Scientific

Standard Concentration	Volume of Working Std	Volume of Working Surrogate	Surrogate Concentration	Volume of Internal Std	Final Volume
STD A - 1.0 ug/L	1.0 uL	1.0 uL	2.5 ug/L	4.0 uL	2.0 mL
STD B - 2.0 ug/L	2.0 uL	2.0 uL	5.0 ug/L	4.0 uL	2.0 mL
STD C - 5.0 ug/L	5.0 uL	5.0 uL	12.5 ug/L	4.0 uL	2.0 mL
STD D - 10.0 ug/L	10.0 uL	10.0 uL	25.0 ug/L	4.0 uL	2.0 mL
STD E - 15.0 ug/L	15.0 uL	15.0 uL	37.5 ug/L	4.0 uL	2.0 mL
STD F - 20.0 ug/L	20.0 uL	20.0 uL	50.0 ug/L	4.0 uL	2.0 mL

B. Check Standard Preparation, Accustandard

Standard Concentration	Volume of Working Std	Volume of Working Surrogate	Surrogate Concentration	Volume of Internal Std	Final Volume
10.0 ug/L	10.0 uL	5.0 uL	12.5 ug/L	4.0 uL	2.0 mL

C. Low LFB, LFB, Spike, and Spike Duplicate Preparation, Accustandard

Standard Concentration	Volume of Working Std	Volume of Working Surrogate	Surrogate Concentration	Volume of Internal Std	Final Volume
Low LFB - 5.0 ug/L	5.0 uL	5.0 uL	12.5 ug/L	4.0 uL	2.0 mL
LFB - 10.0 ug/L	10.0 uL	5.0 uL	12.5 ug/L	4.0 uL	2.0 mL
Spike - 10.0 ug/L	10.0 uL	5.0 uL	12.5 ug/L	4.0 uL	2.0 mL
Spike Dup - 10.0 ug/L	10.0 uL	5.0 uL	12.5 ug/L	4.0 uL	2.0 mL

Revised: 03/24/09 mah

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

N-NITROSODIMETHYLAMINE (NDMA) LOW LEVEL

DETERMINATION OF N-NITROSODIMETHYLAMINE (NDMA) IN WATER BY GAS CHROMATOGRAPHY WITH AN ION TRAP MS DETECTOR USING METHANOL CHEMICAL IONIZATION

File Name: M:\SOP\Organic\epa method sop\
NDMA-lowlevel_0101.doc
Revision: 3

Effective Date: 10/25/2004
Supersedes: 2 (03/01/2001)

1. SUMMARY OF METHOD

This method is used to determine N-nitrosodimethylamine (NDMA) in groundwater. The method employs an isotopic dilution technique for the calculation of results - using NDMA-d6 as the surrogate. A one-liter volume of sample is extracted with methylene chloride in a separatory funnel. The methylene chloride extracts are concentrated to a volume of 1ml. Analysis is performed by a GC/MS system equipped with an ion trap detector using methanol chemical ionization.

2. ANALYTES

This is a gas chromatographic (GC) method applicable to the determination of N-nitrosodimethylamine in groundwater and finished drinking water. The following compounds are determined using this method:

LIMS code	analyte
NDMA-d6	NDMA-d6
NDMA	N-nitrosodimethylamine

3. APPARATUS AND EQUIPMENT

- 3.1 Sample Bottles - Two-liter amber glass bottles fitted with a screw cap lined with Teflon.
- 3.2 Autosampler vials - equipped with Teflon-lined septum.
- 3.3 Analytical Balance: Capable of weighing accurately to nearest 0.0001 g.

File Name: M:\SOP\Organic\EPA_Method_SOPs\NDMA-
lowlevel_0101.doc
Revision: 3 (03/01/2001)

- 3.4 Volumetric flasks.
- 3.5 3800 Varian gas chromatograph with DB-VRX column – ion trap detector. Equipped with an 8200 autosampler for injecting samples into the GC.
- 3.6 Column: Fused Silica Capillary column, DB-VRX 60 meters long x 0.32 mm I.D. with a 1.8 micron film thickness.
- 3.7 Disposable Pasteur Pipettes and graduated cylinders (1000ml, 100ml, and 10ml).

4. REAGENTS AND CONSUMABLE MATERIALS

- 4.1 Reagent Water - Millipore Milli-Q System or equivalent.
- 4.2 Methylene Chloride: Burdick & Jackson - capillary GC² solvent.
- 4.3 Methanol: Burdick & Jackson - capillary GC² solvent.
- 4.4 Sodium Sulfate: (ACS) Granular, anhydrous. Heat sodium sulfate at 400°C four hours, and store sodium sulfate at 130°C.
- 4.5 Acetone: Burdick & Jackson - capillary GC solvent.
- 4.6 Sodium chloride, crystal, ACS grade - Heat treat in a shallow tray at 400°C for a minimum of 4 hours to remove interfering organic substances.

5. SAMPLE COLLECTION, PRESERVATION AND HANDLING

- 5.1 Samples are collected in amber two-liter bottles, fitted with a screw cap lined with Teflon. Store samples at 4°C. All samples must be extracted immediately or before 14 day after collection. The extract should be analyzed within 14 days after extraction. All samples are preserved with sodium sulfite – 40-50 mg/L.

6. EXTRACTION

- 6.1 Transfer one liter of the sample to the two-liter separatory funnel. Spike all samples (QA/QC and sites) with 5 ul of the NDMA-d6 surrogate standard (5ug/ml), to produce a 25ppt surrogate/internal standard mix. Add 100 g of NaCl to the sample, seal, and shake to dissolve the salt. Add 60 ml of methylene chloride to the separatory funnel and extract the sample by vigorously shaking the funnel for 2 minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The

optimum technique depends upon the sample, but may include stirring, filtration through glass wool, centrifugation, or other physical methods. Collect the methylene chloride extract in a 250-ml Erlenmeyer flask containing approximately 7 grams of anhydrous sodium sulfate.

- 6.2 Add a second 60-ml volume of methylene chloride to the separatory funnel and repeat the extraction procedure a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner. Swirl flask to dry extract; allow flask to sit for 15 minutes.
- 6.3 Transfer combined extract into concentrate tube and rinse the flask with 2-25 ml of methylene chloride. Concentrate the extract to 1 ml final volume with Zymark turbo-Vap using nitrogen gas, less than 10.0 psi pressure at 35°C water bath temperature.
- 6.4 Transfer the extract into the appropriate autosampler vial- TFE-fluorocarbon-sealed, screw-cap vial and store. Refrigerate at 4°C until analysis by GC/MS –keep in the dark..

7. ANALYSIS

- 7.1 The low level analysis of NDMA is an isotopic dilution method using the NDMA-d6 as the surrogate to compensate for extraction efficiencies. Thus a critical part of this analysis is the use and injection of this QA/QC standard. Because of the low level (ppt) ranges, any type of contamination or interference can cause analytical problems. Thus, reagent blanks must be monitored for every extraction run - monitoring of reagent blanks is essential to the success of this method. If results for reagent blanks rise above 0.5 ppt level - corrective actions must be performed. Analysis will utilize at least a 10-point calibration curve – more points should be added if looking at sites within the plant to cover the expected NDMA levels. Verify the calibration by measurement of two calibration check standards, one at the beginning and one at the end of analytical run. The calibration check standard is spiked to a 25 ppt level and is used to verify the calibration curve – should be within +/- 20% of the expected value. LFBs (laboratory fortified blanks - (low: 2.0ppt and high: 25.0 ppt)) are analyzed at the start and end of every analysis – they should be within +/- 30% of the true value. Standards used for these QA/QC samples must be ordered from a second source whenever possible – the surrogate/internal NDMA-d6 is order through Cambridge. For extended runs, check standards should be interspersed with samples at regular intervals. If the response of NDMA for the LFBs varies from the predicted response by more than +/- 30%, test must be repeated using fresh calibration standards or corrective actions must be initiated. The ten-point calibration should be 2.0, 5.0, 7.0, 10.0, 25.0, 50.0, 100.0, 150.0, 200.0 and 250.0 ppt. Any results above 200 ppt must be confirmed with a standard that is within +/- 20% of the actual result or the calibration curve should be extended. The %RSD for the calibration curve should be less than 20%. All extracts and standards are in methylene chloride.

Instrument Conditions:

1. Initial column temperature: 35°C
2. Hold time: 4 minutes
3. Final temperature: 200°C
4. Temperature rate: 20°C/minute to 140°C
140°C to 200°C @ 50°C/minute

5. Hold time: 4.55 minutes @ 200°C
6. Helium linear velocity: 1.2 ml/min
7. Injection - 8ul with 45 second delay
8. Injector temperature: 37 °C initial and programmed to 200 °C.
Hold - @ 37 °C for 0.80 min - ramp to 200 °C @ 100° C/minute hold for 12.57 min.

9. Detector : Ion trap
10. Transferline 215 °C.
11. Mass Range 40 to 85 m/z
12. Trap temperature 135 °C.
13. Background Mass 40 m/z
14. Ionization time 200(sec)
15. CI max ioniz. time 2000 (usec)
16. CI max reaction time 80 (msec)
17. CI ioniz. storage time 5.0 m/z
18. Reagent ion eject 12.5 V
19. CI reaction storage 15.0 m/z
20. CI background mass 40 m/z

8. QA/QC REQUIREMENTS

- 8.1 Laboratory Reagent Blank - Run before samples. Use to demonstrate that all glassware and reagent interference's are under control. If any contamination peaks are produced, determine source of contamination and eliminate interference.

- 8.2 Laboratory Fortified Blank - Must analyze at least two laboratory fortified blank (LFB) one after every calibration and one at the end of the analysis. The fortification concentration of each analyte should confirm the ability to detect at the reportable level. If the recovery of any analyte falls outside the control limits, the source of the problem must be identified and corrected.

- 8.3 Standards - Verify calibration standards quarterly by analyzing a standard prepared from reference material obtained from an independent or second source. EPA performance evaluations are an excellent process to determine the validity of the method. Results must be within +/- 20% of those used to routinely check calibration. Daily, run a low-level standard to check the reportable detection level, RDL.

- 8.4 Samples - Samples must be extracted within 14 days after collection. Samples must be stored at 4°C until ready for extraction. Duplicates are run on 10% of samples, or once during run, whichever is greater. Results should be within +/- 30%. Extracts should be analyzed within 14 days after extraction.
- 8.5 Spike Recoveries - The laboratory must add a known concentration of spike solution @ 25 ppt, the same as used for LFB, to at least 10% of samples or once per analytical run, whichever is greater. Recoveries should be within the acceptable range +/- 30%. Wherever possible, run a second source standard for spikes.
- 8.6 QC Requirements - Analyze EPA QC check sample with known values if available. The results for each analyte must be within the EPA acceptance criteria. Semi-annually, generate a series of MDLs – at least seven replicates at a 2.0 ppt level. The MDL should be 3X lower than the RDL of the method.

9. PREVENTIVE MAINTENANCE AND CORRECTIVE ACTIONS

- 9.1 Glassware - Glassware must be carefully cleaned. Clean all glassware as soon as practical after use by thoroughly rinsing with last solvent used in it. Follow by washing with hot water and detergent and thoroughly rinsing with tap water followed by reagent water. After drying, heat in oven. Do not heat volumetric glassware above 220°C. Thorough rinsing with acetone may be substituted for heating.
- 9.2 Reagents - The use of high purity solvents and reagents will help to minimize contamination problems.
- 9.3 Carryover - Contamination carryover may occur when a sample containing a low concentration of analytes is analyzed immediately following a sample containing a high concentration. Use solvent rinses between samples to minimize carryover.
- 9.4 All reagents and apparatus must be routinely demonstrated to be free from interference under the conditions of the analysis by running laboratory method blanks.
- 9.5 Record all corrective actions in the maintenance logbook. Include a complete description of the problem and what actions were taken to correct it.

[illegible]

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

N-NITROSODIMETHYLAMINE (NDMA) LOW LEVEL

DETERMINATION OF N-NITROSODIMETHYLAMINE (NDMA) IN WATER BY GAS CHROMATOGRAPHY WITH AN ION TRAP MS DETECTOR USING METHANOL CHEMICAL IONIZATION

File Name: M:\SOP\Organic\epa method sop\
NDMA-lowlevel_0101.doc

Effective Date: 10/25/2004

Revision: 3

Supersedes: 2 (03/01/2001)

1. SUMMARY OF METHOD

This method is used to determine N-nitrosodimethylamine (NDMA) in groundwater. The method employs an isotopic dilution technique for the calculation of results - using NDMA-d6 as the surrogate. A one-liter volume of sample is extracted with methylene chloride in a separatory funnel. The methylene chloride extracts are concentrated to a volume of 1ml. Analysis is performed by a GC/MS system equipped with an ion trap detector using methanol chemical ionization.

2. ANALYTES

This is a gas chromatographic (GC) method applicable to the determination of N-nitrosodimethylamine in groundwater and finished drinking water. The following compounds are determined using this method:

LIMS code	analyte
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NDMA-d6	NDMA-d6
---------	---------

NDMA	N-nitrosodimethylamine
------	------------------------

3. APPARATUS AND EQUIPMENT

3.1 Sample Bottles - Two-liter amber glass bottles fitted with a screw cap lined with Teflon.

3.2 Autosampler vials - equipped with Teflon-lined septum.

3.3 Analytical Balance: Capable of weighing accurately to nearest 0.0001 g.

File Name: M:\SOP\Organic\EPA_Method_SOPs\NDMA-
lowlevel_0101.doc

Page 1 of 6

Revision: 3 (03/01/2001)

- 3.4 Volumetric flasks.
- 3.5 3800 Varian gas chromatograph with DB-VRX column – ion trap detector. Equipped with an 8200 autosampler for injecting samples into the GC.
- 3.6 Column: Fused Silica Capillary column, DB-VRX 60 meters long x 0.32 mm I.D. with a 1.8 micron film thickness.
- 3.7 Disposable Pasteur Pipettes and graduated cylinders (1000ml, 100ml, and 10ml).

4. REAGENTS AND CONSUMABLE MATERIALS

- 4.1 Reagent Water - Millipore Milli-Q System or equivalent.
- 4.2 Methylene Chloride: Burdick & Jackson - capillary GC² solvent.
- 4.3 Methanol: Burdick & Jackson - capillary GC² solvent.
- 4.4 Sodium Sulfate: (ACS) Granular, anhydrous. Heat sodium sulfate at 400°C four hours, and store sodium sulfate at 130°C.
- 4.5 Acetone: Burdick & Jackson - capillary GC solvent.
- 4.6 Sodium chloride, crystal, ACS grade - Heat treat in a shallow tray at 400°C for a minimum of 4 hours to remove interfering organic substances.

5. SAMPLE COLLECTION, PRESERVATION AND HANDLING

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6. EXTRACTION

- 6.1 Transfer one liter of the sample to the two-liter separatory funnel. Spike all samples (QA/QC and sites) with 5 ul of the NDMA-d6 surrogate standard (5ug/ml), to produce a 25ppt surrogate/internal standard mix. Add 100 g of NaCl to the sample, seal, and shake to dissolve the salt. Add 60 ml of methylene chloride to the separatory funnel and extract the sample by vigorously shaking the funnel for 2 minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The

optimum technique depends upon the sample, but may include stirring, filtration through glass wool, centrifugation, or other physical methods. Collect the methylene chloride extract in a 250-ml Erlenmeyer flask containing approximately 7 grams of anhydrous sodium sulfate.

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- 6.3 Transfer combined extract into concentrate tube and rinse the flask with 2-25 ml of methylene chloride. Concentrate the extract to 1 ml final volume with Zymark turbo-Vap using nitrogen gas, less than 10.0 psi pressure at 35°C water bath temperature.
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7. ANALYSIS

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2. Hold time: 4 minutes
3. Final temperature: 200°C
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140°C to 200°C @ 50°C/minute

5. Hold time: 4.55 minutes @ 200°C
6. Helium linear velocity: 1.2 ml/min
7. Injection - 8ul with 45 second delay
8. Injector temperature: 37°C initial and programmed to 200°C.
Hold - @ 37°C for 0.80 min - ramp to 200°C @ 100°C/minute hold for 12.57 min.

9. Detector : Ion trap
10. Transferline 215°C.
11. Mass Range 40 to 85 m/z
12. Trap temperature 135°C.
13. Background Mass 40 m/z
14. Ionization time 200(sec)
15. CI max ioniz. time 2000 (usec)
16. CI max reaction time 80 (msec)
17. CI ioniz. storage time 5.0 m/z
18. Reagent ion eject 12.5 V
19. CI reaction storage 15.0 m/z
20. CI background mass 40 m/z

8. QA/QC REQUIREMENTS

- 8.1 Laboratory Reagent Blank - Run before samples. Use to demonstrate that all glassware and reagent interference's are under control. If any contamination peaks are produced, determine source of contamination and eliminate interference.

- 8.2 Laboratory Fortified Blank - Must analyze at least two laboratory fortified blank (LFB) one after every calibration and one at the end of the analysis. The fortification concentration of each analyte should confirm the ability to detect at the reportable level. If the recovery of any analyte falls outside the control limits, the source of the problem must be identified and corrected.

- 8.3 Standards - Verify calibration standards quarterly by analyzing a standard prepared from reference material obtained from an independent or second source. EPA performance evaluations are an excellent process to determine the validity of the method. Results must be within +/- 20% of those used to routinely check calibration. Daily, run a low-level standard to check the reportable detection level, RDL.

- 8.4 Samples - Samples must be extracted within 14 days after collection. Samples must be stored at 4°C until ready for extraction. Duplicates are run on 10% of samples, or once during run, whichever is greater. Results should be within +/- 30%. Extracts should be analyzed within 14 days after extraction.
- 8.5 Spike Recoveries - The laboratory must add a known concentration of spike solution @ 25 ppt, the same as used for LFB, to at least 10% of samples or once per analytical run, whichever is greater. Recoveries should be within the acceptable range +/- 30%. Wherever possible, run a second source standard for spikes.
- 8.6 QC Requirements - Analyze EPA QC check sample with known values if available. The results for each analyte must be within the EPA acceptance criteria. Semi-annually, generate a series of MDLs – at least seven replicates at a 2.0 ppt level. The MDL should be 3X lower than the RDL of the method.

9. PREVENTIVE MAINTENANCE AND CORRECTIVE ACTIONS

- 9.1 Glassware - Glassware must be carefully cleaned. Clean all glassware as soon as practical after use by thoroughly rinsing with last solvent used in it. Follow by washing with hot water and detergent and thoroughly rinsing with tap water followed by reagent water. After drying, heat in oven. Do not heat volumetric glassware above 220°C. Thorough rinsing with acetone may be substituted for heating.
- 9.2 Reagents - The use of high purity solvents and reagents will help to minimize contamination problems.
- 9.3 Carryover - Contamination carryover may occur when a sample containing a low concentration of analytes is analyzed immediately following a sample containing a high concentration. Use solvent rinses between samples to minimize carryover.
- 9.4 All reagents and apparatus must be routinely demonstrated to be free from interference under the conditions of the analysis by running laboratory method blanks.
- 9.5 Record all corrective actions in the maintenance logbook. Include a complete description of the problem and what actions were taken to correct it.

[illegible]

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

1,4-Dioxane Test

DETERMINATION OF 1,4-DIOXANE IN WATER BY PURGE AND TRAP AND CAPILLARY COLUMN GAS CHROMATOGRAPHY/ MASS SPECTROMETRY

File Name: M:\SOP\Organic\epa method sop\1,4-Dioxane.doc Effective Date: 10/25/2004
Revision: 0 Supersedes: 10/28/2004

1. SUMMARY OF METHOD

1,4-dioxane is commonly found in treated wastewater effluent and landfill leachate due to the extensive use of, 1,4-dioxane as a stabilizer for chlorinated solvents such as 1,1,1-trichloroethane (TCA) and a contaminant in some surfactant compounds used in herbicides. Also a variety of personal care products; shampoo, liquid soaps, sunscreens, moisturizing lotions, baby lotions, and hair lotions contained 1,4-dioxane with levels ranging from 3 to 100 parts per million. As more occurrences and distributions of 1,4-dioxane were reported in several states including California, the interest in a reliable and fast analytical method to detect sub ppb levels 1,4-dioxane has increased.

Recently, 1,4-dioxane, which the US EPA classifies as a B2 probable human carcinogen, has been detected in the specific ground and surface waters. The findings of 1,4-dioxane in the water systems prompted the need of extensive monitoring of the compound in the drinking water. But the currently available methods have high detection limits of 10 to 50 µg/L. The high reportable detection limits are the results of poor extraction efficiency and volatile nature of the compound.

Due to the poor purging efficiency and infinite water solubility of 1,4-dioxane, the detection limit of purge-trap techniques is 100 times higher than the other purge able compounds with EPA method 524.2. The OCWD lab has initiated a series of modifications for the purge-trap extraction and instrumentation of EPA method 524.2 to improve the sensitivity and reproducibility for the determination of 1,4-dioxane in water. To improve purging efficiency, the purge time has been increased to 20 minutes from 11 minutes instead of increasing the purge flow of 40 ml/minute to prevent foaming of the heavy matrix samples. Also a different type of trap, which contains more carbopackTM, could improve the response and the shape of 1,4-dioxane peak. For the instrumentation, the GC/MS/MS has been applied to retrieve the ions from GC/MS, which removed the background ions to help the identification of low ppb levels of 1,4-dioxane from wastewater samples.

The improved method generated the method detection limit of 0.2 ppb and very reproducible data without manual labor and using only 25 ml of sample compared to the

1000 ml-sample extraction of the isotope dilution method. The split test between purge-trap and isotope dilution method showed the excellent correlation for drinking water and wastewater samples. The improved purge-trap and GC/MS/MS techniques will be very resourceful in saving labor and 100% solvent less extraction with fast turn-around time, high precision, and comparable sensitivity to the isotope dilution method.

2. ANALYTES

- 2.1 This is a gas chromatographic mass spectrometry (GC/MS/MS) method, applicable to the determination of 1,4-dioxane.

<u>LIMS code</u>	<u>Analyte</u>
14DIOX	1,4-dioxane

3. APPARATUS AND EQUIPMENT

- 3.1 Sample Bottles - 250 ml amber glass bottles & 40 ml amber vials - fitted with an open top screw cap lined with Teflon.
- 3.2 Purge and Trap unit – tekmar Aqua Tek 50/2050autosampler and Tekmar 3000 urge and Trap. Use a Vocarb 4000 trap – “T” trap from Supelco.
- 3.3 Varian Model 3800 gas chromatograph with a (Varian) Saturn 2000 GC/MS system.
- 3.4 Column: Fused Silica Capillary column, 60 meter x 0.32 mm ID DB-VRX with 1.8 micron film thickness.
- 3.5 Volumetric flasks (200ml, 100ml, and 50ml), and Hamilton micro syringes - 10ul to 250ul.

4. REAGENTS AND CONSUMABLE MATERIALS

- 4.1 Reagent Water - Millipore Milli-Q System or equivalent.
- 4.2 Standard stock for 1,4-dioxane – 100 ug/ml in methanol, were obtained from Ultra Scientific; 250 Smith Street, North Kingstown, RI and Accustandard; 125 Market Street, New Haven, CT. The stock solutions from Accu Standard were diluted with methanol to serve as a reference standard for recovery studies.
- 4.3 Internal standard – Fluorobenzene; 2000 ug/ml in methanol was purchased from Ultra Scientific. 1,4-dioxane-d8; 1000 ug/ml in methanol, were acquired from Cambridge Isotope Laboratories; 50 Frontage Road, Andover, MA.

- 4.4 GC² Methanol - Burdick and Jackson.

- 4.5 Ascorbic acid - ACS grade – if source water is chlorinated.
- 4.6 UHP grade Helium carrier gas.
- 4.7 Hydrochloric acid (1+1) - carefully add a measured volume of concentrated HCl to an equal volume of reagent water. Only if needed for project requirements.

5. SAMPLE COLLECTION, PRESERVATION AND HANDLING

- 5.1 All samples must be collected in four amber 40ml vials while spikes are collected in 250 mL amber bottles. The 40ml vials and the 250ml amber glass bottles should be filled just to overflowing. All samples must be chilled to 4°C at the time of collection, and they must be maintained at that temperature until ready for analysis. Samples must be stored away from all contaminating organic solvent vapors. Total hold time from sample collection to analysis is 14 days. If the samples are not analyzed by this period, they must be discarded. The Water Quality Department must be informed in order to resample the site.

6. ANALYSIS

6.1 Tuning with BFB:

Sauturn 2000 must meet the BFB criteria before analyses are performed. Inject 1.0 ul of a 25ng/ul Bromofluorobenzene (BFB) standard directly into the column. Obtain a background corrected mass spectrum of BFB peak and confirm that all the key M/Z criteria in Table A are achieved. If the tune does not pass, adjust the tune parameters and rerun BFB. Hardcopy the BFB report.

6.2 Calibration Curve:

A six point standard calibration curve must be run depending upon the concentration range desired. Examples of concentrations used in the curve are: 1.0, 2.0, 5.0, 10.0, 20.0, and 30.0 ppb. You may increase the range of the calibration curve, however a 1.0 ppb standard must be run to confirm the RDL of the analyte. A concentration of 2.0 ppb of the internal is injected into every sample and standard via the Tekmar purge/trap unit. The recoveries and area counts are tracked to insure a properly running instrument. The standard calibration curve for 1,4-dioxane must be within a +/- 20% relative standard deviation. Once the calibration curve has been established, it must be verified on each working day by analyzing the continuous calibration check standard. Typically, a standard calibration curve can last approximately 1 month.

6.3 Standard Preparation:

Calibration standards are prepared from stock solutions with helium purged DI.

Working standards are valid for 14 days from the day they are made. Stock standards are good for one month or when QA/QC data shows they need to be replaced.

Preparing internal and surrogate standards for the AquaTek 50:

The internal standard mix from Ultra Scientific – Fluorobenzene - is at 2000 ug/mL. Add 250uL of this to a 100 ml volumetric flask containing GC grade methanol. Bring to a final volume of 100mls of GC² methanol. We have found that this internal is stable and does not interfere with 1,4-dioxane.

Baking the column:

Bake the column whenever any changes are made to the system that introduces air into the system such as cutting the ends of the column or installing a new column, or any work done on the Tekmar autosampler or purge and trap. The system – both purge & trap and the GC/MS/MS, should be periodically baked to remove water vapor and organic interferences.

Notes:

1. Scan numbers of 1,4-dioxane and the internal standard should be documented so as to monitor the life of the column. The column should be replaced when resolution has dropped below an acceptable level
2. Data is collected for each run under specific file names within the software system. Mass spectral data are obtained with electron impact (EI) ionization at 70 eV electron energy. For samples that have ion abundance over the system's working range, a dilution with reagent water is necessary. Tentatively identified samples by comparison of mass spectrum (after background subtraction) to a reference spectrum in a user library. Ions above 10% relative abundance in the mass spectrum of the standard must be present in the spectrum of the component and should agree within absolute 10%. The GC retention time of the sample component must be within 10 scans of the time observed for that same compound when a calibration solution was analyzed.
3. Samples should be analyzed as soon as possible after collection –but have a 14-day holding time. Communication between the lab and the water quality department is important to understand each sample. There may be specific conditions or problems associated with each sample – an example would be frothing or very high levels. The more a chemist knows about the sample, the better he or she can provide quality assurance and processes, which can produce reliable results. If data shows that the value of the result is outside the calibration range – the sample must be diluted or additional standards analyzed to bracket the value within +/-20% of the value.

TABLE A

BFB KEY M/Z ABUNDANCE CRITERIA

MASS	CRITERIA
50	15 to 40% of mass 95
75	30 to 60% of mass 95
95	base peak, 100% relative abundance
96	5 to 9% of mass 95
173	<2% of mass 174
174	>50% of mass 95
175	5 to 9% of mass 174
176	>95% but <101% of mass 174
177	5 to 9% of mass 176

MS and GC Conditions:

1. Initial column temperature: 35°C
2. Hold time: 2 minutes
3. Final temperature: 220°C
4. Temperature rate: 3.1°C /minute to 100°C
100°C to 220°C @ 30°C /minute
5. Hold time: 1 minutes @ 220°C
6. Helium linear velocity: 1 ml/min
7. Total run time: 27.97 minutes
8. injector temperature: 150°C
9. Detector: Ion trap MS/MS
10. Transfer line 220°C.
11. Mass Range 40 to 100 m/z
12. Trap temperature 135°C.
13. Background Mass 40 m/z
14. EI current: 80 μ amps
15. Scan time: 0.36 seconds
16. Filament/Multiply delay: 14 minutes
17. Reagent ion ejection: 20 V
18. Ion storage level: 35.0 m/z
19. Ejection amplitude: 20 volts
20. High edge amplitude: 15 volts
21. Wave form: resonance

Tekmar ALS/3000/3100 Purge and Trap Conditions:

- | | | |
|----|----------------------|---------------|
| 1. | Purge Time: | 20.00 minutes |
| | Sparger Jacket Temp: | 50°C |
| 2. | Bake Time: | 10.00 minutes |
| 3. | Pre-Heat: | 245°C |
| 4. | Desorb: | 250°C |
| 5. | Bake: | 260°C |

Archon autosampler Conditions:

- | | | |
|----|--------------------------|---------------|
| 1. | Settle: | 0.3 minutes |
| 2. | Prepurge: | 30 seconds |
| 3. | Sample Pressurize: | 40-60 seconds |
| 4. | Sample Transfer: | 75 seconds |
| 5. | Internal Standard Fill: | 5 seconds |
| 6. | Internal Standard Trans: | 50 seconds |
| 7. | Backflush: | Off |
| 8. | Desorb Time: | 4 minutes |
| 9. | Transfer Line Rinse: | Off |

7. QA/QC REQUIREMENTS

- 7.1 Laboratory Reagent Blank - Run before samples. Use to demonstrate that all glassware and reagent interference are under control. If any contamination peaks are produced, determine source of contamination and eliminate interference.
- 7.2 Laboratory Fortified Blank - The fortification concentration of each analyte should confirm the ability to detect at the reportable level. If the recovery of any analyte falls outside the control limits, +/-30%, the source of the problem must be identified and corrected.
- 7.3 Standards - Verify calibration standards quarterly by analyzing a standard prepared from reference material obtained from an independent or second source calibration. Daily, run a low level standard to check the reportable detection level, 1ppb for RDL.
- 7.4 Samples - Samples must be analyzed within 14 days after collection. Samples must be stored at 4°C or below until ready for analysis. Duplicates are run on 10% of samples, or once during run, whichever is greater. Results should be within +/- 20%.
- 7.5 Spike Recoveries - The laboratory must add a known concentration of spike solution, the same as used for LFB, to at least 10% of samples or once per analytical run, whichever is greater. Recoveries should be within the acceptable range, +/- 30%. Wherever possible, run a second source standard for spikes.

- 7.7 Continuous Calibration Check Standard: Daily analyze a 5 ppb continuous calibration check standard. Also, confirm the RDL. The concentration measured using the calibration curve must be within +/-30% of the true value of the concentration in the calibration solution. If this condition is not met, recalibration may be required.

8. PREVENTIVE MAINTENANCE AND CORRECTIVE ACTIONS

- 8.1 Glassware - Glassware must be carefully cleaned. Do not heat volumetric glassware above 220°C.
- 8.2 Reagents - The use of high purity solvents and reagents will help to minimize contamination problems.
- 8.3 Carryover - Contamination carryover may occur when a sample containing a low concentration of analytes is analyzed immediately following a sample containing a high concentration. Use volatile free reagent water rinses between samples to minimize carryover.
- 8.4 All reagents and apparatus must be routinely demonstrated to be free from interference under the conditions of the analysis by running laboratory method blanks. Minimize contact of the samples and reagents with solvent vapors (methylene chloride). This will help reduce contamination.
- 8.5 A refrigerator blank should be run at least once a month. This blank, volatile free reagent water, is sealed in a 40 ml vial and placed in the VOC storage refrigerator for one month. Analyzed each month, it should be free of any organic contamination. Freons are the most likely interference to be picked up by this blank.
- 8.6 Record all corrective actions in the maintenance log book. Include a complete description of the problem and what actions were taken to correct it.

SOP PROCEDURE CHANGE

For 1,4-Dioxane test

Increase purging temp. to 50 °C from 40 °C

Increase the purging efficiency

10/25/04

1y

This image shows a single sheet of white paper with horizontal ruling lines. The lines are evenly spaced and run across the width of the page. There are no margins, text, or other markings on the paper.

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

1,2,3-TCP Test

DETERMINATION OF 1,2,3-TRICHLOROPROPANE IN WATER BY PURGE AND TRAP AND CAPILLARY COLUMN GAS CHROMATOGRAPHY/ MASS SPECTROMETRY

File Name: M:\SOP\Organic\epa method sop\1,2,3-TCP.doc
Revision: 0

Effective Date: 10/25/2004
Supersedes: 11/27/2002

1. SUMMARY OF METHOD

Due to the poor purging efficiency of 1,2,3-TCP, the detection limit of purge-trap techniques is 100 times higher than the other purge able compounds with EPA method 524.2. The OCWD lab has initiated a series of modifications for the purge-trap extraction and instrumentation of EPA method 524.2 to improve the sensitivity and reproducibility for the determination of 1,2,3-TCP in water. To improve purging efficiency, the purge time has been increased to 20 minutes from 11 minutes instead of increasing the purge flow of 40 ml/minute to prevent foaming of the heavy matrix samples and sparging temperature was increased to 60 °C. For the instrumentation, the GC/MS/MS has been applied to retrieve the ions from GC/MS, which removed the background ions to help the identification of low ppt levels of 1,2,3-TCP from water samples.

2. ANALYTES

- 2.1 This is a gas chromatographic mass spectrometry (GC/MS/MS) method, applicable to the determination of 1,2,3-TCP.

Lims code Analyte

123TCP **1,2,3-Trichloropropane**

3. APPARATUS AND EQUIPMENT

- 3.1 Sample Bottles - 250 ml amber glass bottles & 40 ml amber vials - fitted with an open top screw cap lined with Teflon.

- 3.2 Purge and Trap unit – Varian Archon autosampler and Tekmar 3100 urge and Trap.

Use a Vocab 4000 trap – “T” trap from Supelco.

- 3.3 Varian Model 3800 gas chromatograph with a (Varian) Saturn 2000 GC/MS system.
- 3.4 Column: Fused Silica Capillary column, 60 meter x 0.32 mm ID DB-VRX with 1.8 micron film thickness.
- 3.5 Volumetric flasks (200ml, 100ml, and 50ml), and Hamilton micro syringes - 10ul to 250ul.

4. REAGENTS AND CONSUMABLE MATERIALS

- 4.1 Reagent Water - Millipore Milli-Q System or equivalent.
- 4.2 Standard stock for 1,2,3-trichloropropane – 1000 ug/ml in methanol, were obtained from Ultra Scientific; 250 Smith Street, North Kingstown, RI and Accustandard; 125 Market Street, New Haven, CT. The stock solutions from Accu Standard were diluted with methanol to serve as a reference standard for recovery studies.
- 4.3 Internal standard – 1,2,3-trichloropropane-d5; 1000 ug/ml in methanol was purchased from Cambridge Isotope Laboratories; 50 Frontage Road, Andover, MA.
- 4.4 GC² Methanol - Burdick and Jackson.
- 4.5 Ascorbic acid - ACS grade – if source water is chlorinated.
- 4.6 UHP grade Helium carrier gas.

5. SAMPLE COLLECTION, PRESERVATION AND HANDLING

- 5.1 All samples must be collected in four amber 40ml vials while spikes are collected in 250 mL amber bottles. The 40ml vials and the 250ml amber glass bottles should be filled just to overflowing. If the concentration of the Trihalomethanes is important and the sample is known to be from a chlorinated source, ascorbic acid (25mg/40ml vial) must be added to the sample prior to collection. Do not flush out the rapidly dissolving ascorbic acid. All samples must be chilled to 4°C at the time of collection, and they must be maintained at that temperature until ready for analysis. Samples must be stored away from all contaminating organic solvent vapors. Total hold time from sample collection to analysis is 14 days. If the samples are not analyzed by this period, they must be discarded. The Water Quality Department must be informed in order to resample the site.

6. ANALYSIS

6.1 Tuning with BFB:

Sauturn 2000 must meet the BFB criteria before analyses are performed. Inject 1.0 ul of a 25ng/ul Bromofluorobenzene (BFB) standard directly into the column. Obtain a background corrected mass spectrum of BFB peak and confirm that all the key M/Z criteria in Table A are achieved. If the tune does not pass, adjust the tune parameters and rerun BFB. Hardcopy the BFB report.

6.2 Calibration Curve:

A six point standard calibration curve must be run depending upon the concentration range desired. Examples of concentrations used in the curve are: 5.0, 10, 25, 50, 100, and 150 ppt. You may increase the range of the calibration curve, however a 5.0 ppt standard must be run to confirm the RDL of analyte. A concentration of 40 ppt of the internal is injected into every sample and standard via the Archon autosampler unit. The recoveries and area counts are tracked to insure a properly running instrument. The standard calibration curve for 1,2,3-trichloropropane must be within a +/- 20% relative standard deviation. Once the calibration curve has been established, it must be verified on each working day by analyzing the continuous calibration check standard. Typically, a standard calibration curve can last approximately 1-2 month.

6.3 Standard Preparation:

Calibration standards are prepared from stock solutions with helium purged DI. Working standards are valid for 14 days from the day they are made. Stock standards are good for six month or when QA/QC data shows they need to be replaced.

Preparing internal and surrogate standards for the Archon autosampler:

The stock internal standard mix from Cambridge Isotopes – 1,2,3-Trichloropropane-d₅ - is at 100mg/mL. Add 5 ul of the stock internal standard into 1 ml volumetric flask to make 500 ug /ml intermediate internal standard. Add 4uL of this to a 5 ml volumetric flask containing GC grade methanol. Bring to a final volume of 5mls of GC² methanol. We have found that this internal is stable and does not interfere with 1,2,3-Trichloropropane.

Baking the column:

Bake the column whenever any changes are made to the system that introduces air into the system such as cutting the ends of the column or installing a new column, or any work done on the autosampler or purge and trap. The system – both purge & trap and the GC/MS/MS, should be periodically baked to remove water vapor and organic interferences.

Notes:

1. Scan numbers of 1,2,3-trichloropropane and the internal standard should be documented so as to monitor the life of the column. The column should be replaced when resolution has dropped below an acceptable level
2. Data is collected for each run under specific file names within the software system. Mass spectral data are obtained with electron impact (EI) ionization at 70 eV electron energy. For samples that have ion abundance over the system's working range, a dilution with reagent water is necessary. Tentatively identified samples by comparison of mass spectrum (after background subtraction) to a reference spectrum in a user library. Ions above 10% relative abundance in the mass spectrum of the standard must be present in the spectrum of the component and should agree within absolute 10%. The GC retention time of the sample component must be within 10 scans of the time observed for that same compound when a calibration solution was analyzed.
3. Samples should be analyzed as soon as possible after collection –but have a 14 day holding time. Communication between the lab and the water quality department is important to understand each sample. There may be specific conditions or problems associated with each sample – an example would be frothing or very high levels. The more a chemists knows about the sample, the better he or she can provide quality assurance and processes, which can produce reliable results. If data shows that the value of the result is outside the calibration range – the sample must be diluted or additional standards analyzed to bracket the value within +/-20% of the value.

TABLE A

BFB KEY M/Z ABUNDANCE CRITERIA

MASS	CRITERIA
50	15 to 40% of mass 95
75	30 to 60% of mass 95
95	base peak, 100% relative abundance
96	5 to 9% of mass 95
173	<2% of mass 174
174	>50% of mass 95
175	5 to 9% of mass 174
176	>95% but <101% of mass 174
177	5 to 9% of mass 176

MS and GC Conditions:

1. Initial column temperature: 35°C
2. Hold time: 2 minutes
3. Final temperature: 220°C
4. Temperature rate: 3.1°C /minute to 100°C
100°C to 220°C @ 30°C /minute
5. Hold time: 1 minutes @ 220°C
6. Helium linear velocity: 1 ml/min
7. Total run time: 27.97 minutes
8. injector temperature: 150°C
9. Detector: Ion trap MS/MS
10. Transfer line 220°C.
11. Mass Range 40 to 100 m/z
12. Trap temperature 135°C.
13. Background Mass 40 m/z
14. EI current: 80 μ amps
15. Scan time: 0.36 seconds
16. Filament/Multiply delay: 14 minutes
17. Reagent ion ejection: 20 V
18. Ion storage level: 35.0 m/z
19. Ejection amplitude: 20 volts
20. High edge amplitude: 15 volts
21. Wave form: resonance

Tekmar ALS 3100 Purge and Trap Conditions:

- | | | |
|----|-------------|---------------|
| 1. | Purge Time: | 20.00 minutes |
| 2. | Bake Time: | 10.00 minutes |
| 3. | Pre-Heat: | 245°C |
| 4. | Desorb: | 250°C |
| 5. | Bake: | 260°C |
| 6. | Purge Temp: | 60°C |

Archon autosampler Conditions:

- | | | |
|----|--------------------------|---------------|
| 1. | Settle: | 0.3 minutes |
| 2. | Prepurge: | 30 seconds |
| 3. | Sample Pressurize: | 40-60 seconds |
| 4. | Sample Transfer: | 75 seconds |
| 5. | Internal Standard Fill: | 5 seconds |
| 6. | Internal Standard Trans: | 50 seconds |
| 7. | Backflush: | Off |
| 8. | Desorb Time: | 4 minutes |
| 9. | Transfer Line Rinse: | Off |

7. QA/QC REQUIREMENTS

- 7.1 Laboratory Reagent Blank - Run before samples. Use to demonstrate that all glassware and reagent interference are under control. If any contamination peaks are produced, determine source of contamination and eliminate interference.
- 7.2 Laboratory Fortified Blank - The fortification concentration of each analyte should confirm the ability to detect at the reportable level. If the recovery of any analyte falls outside the control limits, +/-30%, the source of the problem must be identified and corrected.
- 7.3 Standards - Verify calibration standards quarterly by analyzing a standard prepared from reference material obtained from an independent or second source calibration. Daily, run a low level standard to check the reportable detection level, 5ppt for RDL.
- 7.4 Samples - Samples must be analyzed within 14 days after collection. Samples must be stored at 4°C or below until ready for analysis. Duplicates are run on 10% of samples, or once during run, whichever is greater. Results should be within +/- 20%.
- 7.5 Spike Recoveries - The laboratory must add a known concentration of spike solution, the same as used for LFB, to at least 10% of samples or once per analytical run, whichever is greater. Recoveries should be within the acceptable range, +/- 30%. Wherever possible, run a second source standard for spikes.

- 7.7 Continuous Calibration Check Standard: Daily analyze a 10 ppt continuous calibration check standard. Also, confirm the RDL. The concentration measured using the calibration curve must be within $\pm 30\%$ of the true value of the concentration in the calibration solution. If this condition is not met, recalibration may be required.

8. PREVENTIVE MAINTENANCE AND CORRECTIVE ACTIONS

- 8.1 Glassware - Glassware must be carefully cleaned. Do not heat volumetric glassware above 220°C.
- 8.2 Reagents - The use of high purity solvents and reagents will help to minimize contamination problems.
- 8.3 Carryover - Contamination carryover may occur when a sample containing a low concentration of analytes is analyzed immediately following a sample containing a high concentration. Use volatile free reagent water rinses between samples to minimize carryover.
- 8.4 All reagents and apparatus must be routinely demonstrated to be free from interference under the conditions of the analysis by running laboratory method blanks. Minimize contact of the samples and reagents with solvent vapors (methylene chloride). This will help reduce contamination.
- 8.5 A refrigerator blank should be run at least once a month. This blank, volatile free reagent water, is sealed in a 40 ml vial and placed in the VOC storage refrigerator for one month. Analyzed each month, it should be free of any organic contamination. Freons are the most likely interference to be picked up by this blank.
- 8.6 Record all corrective actions in the maintenance log book. Include a complete description of the problem and what action were taken to correct it.

SOP PROCEDURE CHANGE

For 1,2,3-trichloropropane test

CHANGE

REASON

DATE

INITIALS

Increase purging temp to 60 °C from 50 °C

To improve the purging efficiency

10/25/04

ly

[illegible]

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

ENDOCRINE DISRUPTING CHEMICALS - EDCs

DETERMINATION OF ENDOCRINE DISRUPTING CHEMICALS IN WATER BY LIQUID-SOLID EXTRACTION AND LIQUID CHROMATOGRAPHY/ MASS SPECTROMETRY

File Name: M:\SOP\Organic\epa method sop\Hormone_100609.doc Effective Date: 10/06/09
Revision: 5 Supersedes: 10/06/09

1. SUMMARY OF METHOD

A measured volume of sample of approximately 1 liter is extracted using a C18 disk. Manual SPE is used. The Endocrine Disrupting Chemicals (Hormones) are eluted from the disk with small quantities of Methylene Chloride. The extract is concentrated to 1.0 ml using the Zymark concentrator - using gentle heat and UHP nitrogen gas. The final extract is injected into the Waters/Micromass ZQ - LC/MS system for separation, identification, and quantitation. Both an internal and surrogate standard are added to every sample. Sample quantification is conducted using an external calibration method.

2. ANALYTES

This is a Liquid Chromatographic Mass Spectrometry (LC/MS) method, applicable to the determination hormone compounds. The following compounds can be determined using this method. The LC/MS system is run in both positive and negative electrospray mode, depending on target compound.

Targets	MW	CAS #	LIMS ID	RDL
Estrone	270	53-16-7	ESTRON	10 ng/L
Epitestosterone (cis-Testosterone)	288	481-30-1	EPITES	10 ng/L
Testosterone (trans-)	288	58-22-0	TESTOR	10 ng/L
Estriol	288	50-27-1	ESTRIO	10 ng/L
17-alpha-Estradiol	272	57-91-0	aESTRA	10 ng/L
17-beta-Estradiol	272	50-28-2	bESTRA	10 ng/L
17-alpha-Ethynylestradiol	296	57-63-6	aETEST	10 ng/L
Progesterone	314.5	57-83-0	PRGSTR	10 ng/L
Diethylstilbestrol	268	56-53-1	DESTBL	10 ng/L

QA/QC Targets

Targets are separated based on Calibration standards – Restek

Surrogate Standards

2,3,5,6-Tetrafluoro-4-(pentafluorophenyl) phenol

Internal Standards

Bisphenol-A – d6

3. APPARATUS AND EQUIPMENT

- 3.1 Sample Bottles – 2.5 liter amber glass bottles fitted with a screw cap lined with teflon.
- 3.2 Autosampler vials - 2 mL amber glass, screw cap with Teflon septa. (Waters Vials)
- 3.3 Concentrator Tube - Zymark 250 mL tubes used with the Zymark Turbo-Vap.
- 3.4 Analytical Balance: Capable of weighing accurately to nearest 0.0001 g.
- 3.5 Zymark Turbo-Vap - used to concentrate extracts.
- 3.6 Waters / Micromass ZQ - Liquid Chromatograph/Mass Spectrometer -Quaternary pump, autosampler, column oven and Empower Data workstation.
- 3.7 Column: Phenomenex – Gemini C18 (2.0 x 150 mm, 5 um).
- 3.8 Disposable pasteur pipets, graduated cylinders (1000ml), 1ml volumetric flasks, and Hamilton micro syringes - 10 ul to 100 ul.

4. REAGENTS AND CONSUMABLE MATERIALS

- 4.1 Reagent Water - Millipore Milli-Q System or equivalent.
- 4.2 Methylene Chloride – GC² quality
- 4.3 Acetonitrile - HPLC quality.
- 4.4 Methanol - GC² and HPLC quality

4.5 Empore disks (C-18): 0.5 grams of 8 um octadecyl bonded silica in 47 mm x 0.5 mm disk.

4.6 Nitrogen Generator -- Domnick Hunter

5. SAMPLE COLLECTION, PRESERVATION AND HANDLING

5.1 Samples are collected in amber 2.5 liter bottles, fitted with a screw cap lined with teflon. Keep samples sealed from collection time until analysis. Sampling equipment must be free of plastic tubing, gaskets, and other parts that may leach interfering analytes into the water sample.

5.2 All samples should be iced or refrigerated at 4 °C and kept in the dark from the time of collection until extraction.

5.3 Sample extraction hold time is 14 days, extract analysis hold time is 28 days.

6. EXTRACTION

(See the attached printout for Manual Extraction flow chart)

Liquid - Solid phase extraction (SPE):

Assemble C-18 Empore disk and filtration apparatus. Add 10 mL of CH₃CN, allow it to soak into the disk for 3 minutes then apply vacuum to remove the solvent completely. Apply 10 mL of methanol and allow it to soak into the disk for 3 minutes. Do not allow the disk to run dry until the end of the extraction step. Add 10 ml of DI water to keep the disk wet. Then add 1 liter of sample into the filtration reservoir, add the surrogate, LFBs, spikes and apply vacuum. Adjust the pressure to 25 ml/min flow rate (<10 in-hg). After the sample is processed, pull air through disk for 10 minutes to remove residual water from the disk. Remove the filter base and place a small Zymark or a clear 40-ml vial into the receiver. Add 5 ml of acetonitrile to the reservoir (rinsing the sides of the reservoir) and allow to soak for 3 minutes before applying vacuum. Repeat with a second and third with 5 ml acetonitrile. Repeat the eluting step once more with one 5 ml aliquot of methylene chloride. Transfer to Zymark tube, if it is not already in one, rinse the clear vial with two 2ml portions of acetonitrile, adding the rinse to the Zymark tube. Evaporate the eluant with a Turbo-Vap concentration workstation to 0.1mL, add **Methanol :H₂O (60:40)** to bring the volume to 1 ml. Rinse the low 25% of the Zymark tube, bring up the final volume to 1mL. Add 5uL of 10ug/ml Internal Standard, mix well and transfer to 1 LC/MS (Waters vials) brown autosampler vial. Store in the refrigerator at 4 °C

7. ANALYSIS

7.1 Initial Calibration: A five point calibration curve is required for this method; using standards STD-A (5ppt), B (10ppt), C (20ppt), D (50ppt) and E (100ppt)). For each analyte and surrogate, calculate the mean RF and the relative standard deviation (RSD). The %RSD of any analyte or surrogate within the calibration range must be less than 30%. If not -- inject other standards or perform system

maintenance and begin the system analysis and performance check over.

7.2 Continuous Calibration check:

- Reagent blank must be monitored for every extraction run - monitoring of reagent blank is essential to the success of this method. Verify the calibration by measurement of two mid-point calibration check standards, one at the beginning and one at the end of the run. The check standard area counts must be within +/- 30% of the standard curve. LFBs (laboratory fortified blanks) should also be analyzed with each extraction run. Standards used for these QA/QC samples must be ordered from a second source whenever possible. For extended runs, check standards should be interspersed with samples at regular intervals. If the response of any analyte varies from the predicted response by more than +/- 30%, test must be repeated using fresh calibration standards. Data is collected and processed by Empower software. Any results above the highest calibration standard must be confirmed with a standard that is within +/- 20% of the actual result or the sample must be diluted to within the calibration range.

7.3 LC/MS Operating Conditions:

LC Conditions: Column Temperature: 35°C
Gradient elution:

<u>Time</u>	<u>Flow</u>	<u>Solvent A</u> <u>H2O</u>	<u>Solvent B</u> <u>ACN</u>	<u>Solvent C</u> <u>0.1%NH4OH</u>	<u>Curve</u>
0	0.30ml/min	0	25	75	--
0.5	0.30	0	25	75	6
15	0.30	0	95	5	6
16	0.30	0	25	75	1
30	0.30	0	25	75	1

MS Conditions:

Scan: SIR (Single Ion Monitoring)
Acquire time: 0 to 16 Min. for both ES- and ES +

	<u>Mass (m/z)</u>	<u>Dwell (secs)</u>	<u>Cone Volts</u>
ES-	267.29	0.30	35.0
	269.30	0.30	30.0
	271.31	0.40	25.0
	287.39	0.40	25.0
	295.33	0.30	30.0
	331.10	0.40	25.0
	241	0.30	35.0
ES+	289.32	0.30	25.0

8. QA/QC REQUIREMENTS

- 8.1 Laboratory Reagent Blank - Run before samples. Use to demonstrate that all glassware and reagent interferences are under control. If any contamination peaks are produced, which inhibits quantitative of target compounds, determine source of contamination and eliminate interference.
- 8.2 Laboratory Fortified Blank - Must analyze at least two laboratory fortified blanks (LFBs) with each set of extractions. Run a LFB-low and an LFB-high with each analytical run. The fortification concentration of each analyte should confirm the ability to detect at the reportable level. If the recovery of any analyte falls outside the control limits, the source of the problem must be identified and corrected.
- 8.3 Standards - Verify calibration standards quarterly by analyzing a standard prepared from reference material obtained from an independent or second source. EPA performance evaluations are an excellent process to determine the validity of the method. Results must be within +/- 30% of those used to routinely check calibration. For every batch of sample, run a low level standard to check the reportable detection level, RDL.
- 8.4 Samples - Samples must be extracted within 14 days after collection – unless specific targets need to be determined. Samples must be stored at 4°C until ready for extraction. Duplicates are run on 10% of samples, or once during run, whichever is greater. Results should be within +/- 20%. Extracts should be analyzed within 28 days after sample extraction.
- 8.5 Spike Recoveries - The laboratory must add a known concentration of spike solution, the same as used for LFB, to at least 10% of samples or once per analytical run, whichever is greater. Recoveries should be within the acceptable range. Wherever possible, run a second source standard for spikes.
- 8.6 QC Requirements - Analyze EPA QC check sample with known values *if available*. The results for each analyte must be within the EPA acceptance criteria. Annually, analyze EPA Performance Evaluation samples. Analyze these check samples whenever major maintenance to the system occurs to ensure the validity of the method.
- 8.7 When running the daily midpoint standard, ensure that the absolute areas of the quantitation ions of the internal and surrogate standards have not decreased by more than 30% of the continuing calibration check or more than 50% of the initial calibration curve.

9. PREVENTIVE MAINTENANCE AND CORRECTIVE ACTIONS

- 9.1 Glassware - Glassware must be carefully cleaned. Clean all glassware as soon as practical after use by thoroughly rinsing with last solvent used in it. Follow by washing with hot water and detergent and thoroughly rinsing with tap water followed by reagent water. After drying, heat in oven. Do not heat volumetric glassware above 220°C. Thorough rinsing with acetone may be substituted for heating.
- 9.2 Reagents - The use of high purity solvents and reagents will help to minimize contamination problems.
- 9.3 Carryover - Contamination carryover may occur when a sample containing a low concentration of analytes is analyzed immediately following a sample containing a high concentration. Use solvent rinses between samples to minimize carryover.
- 9.4 All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory method blanks. Minimize contact of the samples, reagents, or solvents with any plastics. This will help reduce contamination. Interferences by phthalate esters can pose a major problem in pesticide analysis.
- 9.5 Record all corrective actions in the maintenance log book. Include a complete description of the problem and actions were taken to correct it.

EDCs - Hormones Method – Daily Instrument Performance Checks

Sample Description	Frequency	Acceptance Criteria	Remedial Action
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Low Level CCC – “SFA”	Each Analysis Run	70 – 130% target recovery	Instrument Maintenance & Check Standards
Mid Level CCC – “SFC”	Each Analysis Run	70 -130% target recovery	Instrument Maintenance & Check Standards
“RB” – reagent blank	Each Extraction Set	All targets must be less than 1/3 of the RDL	Isolate Source of Contamination and Re-Extract
Low LFB – “LFL” – reportable level	Each Analysis Run	50 – 150% target recovery	Check SPE Disk Lots – Verify Extraction Procedures – Re-Extract
LFB – “LFB” - mid level	Each Analysis Run	70 – 130% target recovery	Check SPE Disk Lots – Verify Extraction Procedures – Re-Extract
Sample Spikes – “S” & “K” “S” – spike “K” – spike dup	Each Analysis Run – 10% minimum of total sample load	70 – 130% recovery	Investigate Matrix Issues – Check Standards and Re-Extract
Field Sample	Run Analysis	Check Surrogate Recovery 50 – 150%	Investigate Matrix Issues – Check Standards and Re-Extract
Back Standards – “SBs”	Each Analysis Run – Every 10 samples must be bracketed with a CCC std	70 – 130% target recovery	Instrument Maintenance & Check Standards
Initial Calibration - “STs”	Started Before Each Analysis Run	Must use 5-pt calibration Lowest Standard must be at or below reportable detection level (RDL) Calib. Curve - Less than 20% RSD & R ² >0.970	Check Standard Lots & QC – Re-shoot or Open New Standards Instrument Maintenance
Sample Duplicate – “D”	Each Analysis Run – 10% minimum of total sample load	50% RPD	Results Reported – Re-Extract if possible
MDLs	Each New SPE Lot or Major Instrument Maintenance (at <i>least</i> twice per year)	Calculated MDL must be no more than 1/3 the RDL	Instrument Maintenance, Extraction Procedures, & Check Standards
IDC – Initial Demonstration of Competency	Change in instrument, extraction technique, or chemistry of the method.	70 – 130% target recovery and <20% RSD	Instrument Maintenance, Extraction Procedures, & Check Standards – Re-Run

For HOMONES Method

[illegible]

Endocrine Disrupting Chemicals Extraction - Hormones

add 10-ml of CH₃CN to the assembled SPE apparatus (with C-18 disk)

turn the vacuum pump on and off quickly,
allowing a small amount of solvent to drip through.
let the disk soak for 3 minutes

aspirate the remaining solvent, drying the disk completely

add 10-ml of methanol to the disk

aspirate a small amount of the solvent, leaving some on top of the disk
let the solvent soak the disk for 3 min, then apply vacuum
LEAVE A LAYER OF SOLVENT ON TOP OF THE DISK, KEEP DISK WET!

DO NOT LET THE DISK GO DRY!!!

add 10-ml of D.I. to disk, aspirate a small amount of water
Apply vacuum, aspirate remaining water, but always keep the disk wet.

add 1000-ml of sample to the reservoir

add the spike (LOW: **10ul of 1ug/ml mix**)

(LFB & Spike: **5ul of 10 ug/ml mix**)

10 ul of Surrogate #1 (2ug/ml stock)

turn on vacuum, adjust the pressure to 50 ml/min flow rate (<10 in-hg)

after the sample has been processed, pull air through disk for 10 min

remove the filter base, insert a small Zymark or a clear 40-ml tube into the receiver

add 5 ml of CH₃CN to disk, **rinsing the sides of the reservoir**

let a small amount drip through, and allow it to soak for 3 minutes
before applying vacuum and aspirate the remaining solvent

repeat the above eluting step with 2 more 5-ml aliquots of CH₃CN

repeat the eluting step once more with one 5-ml aliquot of CH₂CL₂

transfer to zymark tube, if it is not already in one
rinse the clear tube with two 2-ml portions of CH₃CN, adding the rinse to the Z. tube

Zymark settings: Temp: 40, Pressure: 9 psi

concentrate the extract to almost **dryness** and **remove the Z. tube ASAP**

add 60:40 methanol:water to bring the volume to 1.0 ml, add 5ul of 10ug/ml Internal Std

rinse around the bottom of the flask few times

transfer the extract to 2 amber autosampler vials (1 with limited-volume insert)

ORANGE COUNTY WATER DISTRICT

LC/MS METHOD - ENDOCRINE DISRUPTING CHEMICALS - EDCs

1. Calibration Standard: **Restek** - Custom Mix Stock = 1,000 ug/ml

Working Std #1: dilute 10 ul of stock to 1ml Methanol - Conc. 10 ug/ml

Working Std #2: dilute 100 ul of working std #1 to 1ml Methanol - Conc. 1 ug/ml

<u>Analyze</u>	<u>True MW</u>	<u>LC/MS - MW</u>	<u>LIMS ID</u>	<u>CAS #</u>
Estrone	270-	269.32	ESTRON	53-16-7
Epitestosterone (cis-Testosterone)	288+	289.30	EPITES	481-30-1
Testosterone (trans-)	288+	289.30	TESTOR	58-22-0
Estriol	288-	287.29	ESTRIO	50-27-1
17a-Estradiol	272-	271.29	aESTRA	57-91-0
17b-Estradiol	272-	271.29	bESTRA	50-28-2
17a-Ethynylestradiol	296-	295.32	aETEST	57-63-6
Progesterone	314+	315.54	PRGSTR	57-83-0
Diethylstilbestrol	268-	267.28	DESTBL	56-53-1
2,3,5,6-tetrafluoro-4-(pentafluorophenyl) phenol - Surrogate (TFPFPP)	333-	331	S1	
Bisphenol A-d ₁₆	244	241	I1	

A. CALIBRATION STANDARD: Restek - dilute with 60:40 Methanol:H₂O

(Base on 1 ml final ext. vol)

Calib. Std. (ng/L)	Std Mix & Caffeine	Vol. of 2 ug/ml Surrogate #1	Vol. of 10 ug/ml Internal	Final Dil. Vol.
Std A - 5 ng/L	5 µl of 1ug/ml	2.5 ul	5 ul	1.0 ml
Std B - 10 ng/L	10 µl of 1ug/ml	5ul	5 ul	1.0 ml
Std C - 20 ng/L	20 µl of 1ug/ml	10 ul	5 ul	1.0 ml
Std D - 50 ng/L	5 µl of 10ug/ml	15 ul	5 ul	1.0 ml
Std E - 100 ng/L	10 µl of 10ug/ml	25 ul	5 ul	1.0 ml

B. CALIBRATION CHECK:

Low Cali Check = 5 ng/L Spike 1ml MeOH with 5 µl of 1 ug/ml Std

High Cali Check = 20 ng/L Spike 1ml MeOH with 20 µl of 1 ug/ml Std

C. LFB & SPIKE:

Low LFB = 10 ng/L Spike 1,000ml DI water with 10 µl of 1 ug/ml Std

LFB = 50 ng/L Spike 1,000ml DI water with 5 µl of 10 ug/ml Std

Spk/Spk Dup = 50 ng/L Spike 1,000ml sample with 5 µl of 10 µg/ml Std

D. SURROGATE STANDARD SOLUTION:

Dissolve 0.001 g of 2,3,5,6-tetrafluoro-4-(pentafluorophenyl)phenol in 1 ml Methanol

Stock Concentration = 1000µg/ml

Working Standard 1: dilute 100 ul of stock to 1ml Methanol - Conc. 100 ug/ml

Working Standard 2: dilute 20 ul of std # 1 to 1ml Methanol - Conc. 2 ug/ml

***Spike standards and samples with 10µl of 2 ug/ml Std to give conc. of 20 ng/L

E. INTERNAL STANDARD SOLUTION:

Dissolve 0.001 g of Bisphenol A-d₁₆ in 10 ml Methanol

Stock Concentration = 100µg/ml

Working Standard: dilute 100 ul of stock to 1ml Methanol - Conc. 10 ug/ml

***Spike standards and samples with 5µl of 10 ug/ml Std to give conc. of 50 ng/L

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

EPA METHOD 521

DETERMINATION OF NITROSAMINES IN DRINKING WATER BY SOLID PHASE EXTRACTION AND CAPILLARY COLUMN GAS CHROMATOGRAPHY WITH LARGE VOLUME INJECTION AND CHEMICAL IONIZATION TANDEM MASS SPECTROMETRY (MS/MS)

File Name: EPA 521
Revision:

Effective Date: 11/06/2008
Supersedes:

1. SUMMARY OF METHOD

This method is used to determine 8 nitrosoamines in drinking water by solid phase extraction and gas chromatography with large volume injection and chemical ionization tandem mass spectrometry (MS/MS). The method employs a solid phase extraction method using 2 gm, 80 to 120 mesh size, coconut carbon cartridge. Analytes and a surrogate analyte (NDMA-d6) are extracted by passing a 500 ml volume of sample through a coconut carbon cartridge. The organic compounds are eluted from the solid phase with a small quantity of methylene chloride. The methylene chloride extracts are concentrated to a volume of 1ml and an internal standard (NDPA-d14) is added. Analysis is performed by a GC/MS/MS system equipped with 60 m gas chromatographic column and an ion trap mass spectrometry detector using positive chemical ionization with methanol liquid.

2. ANALYTES

This is a gas chromatographic (GC) method applicable to the determination of N-Nitrosoamines in groundwater and finished drinking water. The following compounds are determined using this method:

Nitrosamine	Abbrev.	Formula	MW	CAS #	Internal Std.
<i>N</i> -Nitrosodimethylamine	NDMA	$C_2H_6N_2O$	74	62-75-9	d ₁₄ -NDPA
<i>N</i> -Nitrosomethylethylamine	NMEA	$C_3H_8N_2O$	88	10595-95-6	d ₁₄ -NDPA
<i>N</i> -Nitrosodiethylamine	NDEA	$C_4H_{10}N_2O$	102	55-18-5	d ₁₄ -NDPA
<i>N</i> -Nitrosomorpholine	NMOR	$C_4H_8N_2O_2$	116	59-89-2	d ₁₄ -NDPA
<i>N</i> -Nitrosodi-n-propylamine	NDPA	$C_6H_{14}N_2O$	130	621-64-7	d ₁₄ -NDPA
<i>N</i> -Nitrosopyrrolidine	NPYR	$C_4H_8N_2O$	100	930-55-2	d ₁₄ -NDPA
<i>N</i> -Nitrosopiperidine	NPIP	$C_5H_{10}N_2O$	114	100-75-4	d ₁₄ -NDPA
<i>N</i> -Nitrosodi-n-butylamine	NDBA	$C_8H_{18}N_2O$	158	924-16-3	d ₁₄ -NDPA

3. APPARATUS AND EQUIPMENT

- 3.1 Sample Bottles - Two-liter amber glass bottles fitted with a screw cap lined with Teflon.
- 3.2 Autosampler vials - equipped with Teflon-lined septum.
- 3.3 Analytical Balance: Capable of weighing accurately to nearest 0.0001 g.
- 3.4 Volumetric flasks.
- 3.5 3800 Varian gas chromatograph with DB-VRX column – Varian 4000 MS/MS ion trap detector. Equipped with a Combi PAL autosampler
- 3.6 Column: Fused Silica Capillary column, DB-VRX 60 meters long x 0.32 mm I.D. with a 1.8 micron film thickness.
- 3.7 Auto trace – Caliper Science, Hopkinton, MA
- 3.8 Disposable Pasteur Pipettes and graduated cylinders (1000ml, 100ml, and 10ml).

4. REAGENTS AND CONSUMABLE MATERIALS

- 4.1 Reagent Water - Millipore Milli-Q System or equivalent.
- 4.2 Methylene Chloride: Burdick & Jackson - capillary GC² solvent.
- 4.3 Methanol: Brudick & Jackson - capillary GC² solvent.
- 4.4 Sodium Sulfate: (ACS) Granular, anhydrous. Heat sodium sulfate at 400°C four hours, and store sodium sulfate at 130°C.
- 4.5 Acetone: Burdick & Jackson - capillary GC solvent.
- 4.6 Sodium chloride, crystal, ACS grade - Heat treat in a shallow tray at 400°C for a minimum of 4 hours to remove interfering organic substances.
- 4.7 Sodium thiosulfate: (ACS) Granular, anhydrous.
- 4.8 Activated carbon cartridges, 6 ml size, 2,000 mg from United Chemical Technology Part number: EU52112M6.

5. SAMPLE COLLECTION, PRESERVATION AND HANDLING

- 5.1 Samples are collected in amber two-liter bottles, fitted with a screw cap lined with Teflon. Store samples at 4°C. All samples must be extracted immediately or before 14 day after collection. The extract should be analyzed within 14 days after extraction. All samples are preserved with sodium thiosulfate – 80-100 mg/L.

6. EXTRACTION

- 6.1 Transfer 500 ml of sample to 1-liter amber bottles. All QA/QC samples must be added sodium sulfite. Add 5ul of NDMA-d6 (surrogate standard, 2.5ug/ml) into every sample. Place 500 ml samples in Auto trace SPE system.
- 6.2 Cartridge Clean Up and Conditioning: Rinse each cartridge with two, 3mL methylene chloride. Let the cartridge drain dry after each flush. Then rinse the cartridge with two, 3mL methanol, Do not allow the methanol to elute below the top of the cartridge packing. From this point, do not allow the cartridge packing to go dry. The system is set up for sample flow rate, 8 ml/min (70 min for a 500 ml sample) After all of the sample has passed through the SPE cartridge, draw air or nitrogen through the cartridge for 15min using nitrogen gas at 20 psi. The cartridge should appear dry (clear black color) before continuing with the elution steps.
- 6.3 Soak and Collect 5 ml and 3 ml fractions using methylene chloride into a sample tube. Collect additional 3 ml fraction into the sample tube using methylene chloride. Pass the eluate through the drying column, which is packed with approximately 5-7 g of anhydrous sodium sulfate, and collect in a clean collection tube. Wash the sodium sulfate with at least 2 mL methylene chloride and collect in the same tube.
- 6.4 Concentrate the sample to approximately 0.9 ml – do not concentrate the extract to less than 0.5 ml. Make any volume adjustments with methylene chloride – to the 1.0 ml marked level. Transfer the 1.0 ml extract into a autosampler vial and store the sample extract in the refrigerator at 0 °C or below.

7. ANALYSIS

- 7.1 The low level analysis of nitrosoamines is a international standard method using the NDPA-d14. Because of the low level (ppt) ranges, any type of contamination or interference can cause analytical problems. Thus, reagent blanks must be monitored for every extraction run - monitoring of reagent blanks is essential to the success of this method. If results for reagent blanks rise above 0.5 ppt level - corrective actions must be performed. Analysis will utilize at least a 5-point calibration curve – more points should be added if looking at sites within the plant to cover the expected Nitrosamines levels. Verify the calibration by measurement of two calibration check standards, one at the beginning and one at the end of analytical run. The calibration check standard is spiked to a 25 ppt level and is used to verify the calibration curve – should be within +/- 30% of the expected value. LFBs (laboratory fortified blanks - (low: 2.0ppt and high:

25.0 ppt)) are analyzed at the start of every analysis – they should be within +/- 50% for low spike and +/- 30% for high spike of the true value.

7.2 Standards used for these QA/QC samples must be ordered from a second source whenever possible – the surrogate/internal NDMA-d6/NDPA-d14 ordered through Absolute Standard and Cambridge isotopes. For extended runs, check standards should be interspersed with samples at regular intervals. If the response of nitrosoamines for the LFBs varies from the predicted response by more than +/- 30%, test must be repeated using fresh calibration standards or corrective actions must be initiated. The 6-point calibration should be 2.0, 5.0, 10, 25.0, 40.0, 50.0 ppt. Any results above 50 ppt must be confirmed with a standard that is within +/- 20% of the actual result or the calibration curve should be extended. The %RSD for the calibration curve should be less than 30%. All extracts and standards are in methylene chloride.

7.3 Instrument conditions for Saturn 4000 GC/MS/MS/CI

MRM (custom resonant)

1.	Ionization storage level	35 m/z
2.	Ejection amplitude	20 Volts
3.	Low edge offset	4 steps
4.	High edge offset	4 steps
5.	High edge amplitude	15.0 Volts
6.	Isolation time	10 msec
7.	Excitation time	20 msec
8.	Modulation range	2 steps
9.	Modulation rate	3000 μ sec/step
10.	Number of frequencies	1
11.	CID frequency offset	0 Hertz

Gas chromatograph conditions for nitrosamines analyses

1079 Injector Program			
Temp (°C)	Rate (°C/min)	Hold (min)	Total (min)
30	0	0.34	0.34
250	200	30	31.44

Time (min)	Split State	Split Ratio	Injection volume	8 μ L
Initial	On	10	Plunger Inject speed	0.5 μ L/sec

0.34	Off	Off	Post injection delay	99 sec
1.50	On	100		
30.0	On	20		

Column Temperature Program: DB-VRX, 60 m .32 mm ID, 1.8 um film thickness

Temp.(°C)	Rate (°C/min.)	Hold time (min.)	Total time (min.)
35	0	4	4.0
100	20	2	9.25
160	5	2	23.25
180	2	1	34.25
220	65	1.13	36

CI/MS/MS parameters

CI gas: methanol	Eject.amp: 15.0 m/z
CI storage level: 19.0 m/z	Backgroundd mass: 40 m/z
Max. ion time: 2000 microsec	Max. reaction.time: 120 millisec
Target TIC 3000 counts	Prescan time: 200 microsec

Segment	Description	Start time	End time	Low mass	High Mass	Ioniz. Mode	Ion Prep
1	Fil/Mul delay	0	12.5	40		CI auto	
2	NDMA	12.5	14.9	40	82	CI auto	MRM
3	NMEA	14.9	17.4	55	91	CI auto	MS/MS
4	NDEA	17.4	21.4	70	104	CI auto	MRM
5	NDPA	21.4	25.9	53	146	CI auto	MRM
5	NMOR	21.4	25.9	53	146	CI auto	MRM
5	NPYR	21.4	25.9	53	106	CI auto	MS/MS
6	NPIP	25.9	29.4	40	116	CI auto	MS/MS
7	NDBA	29.4	35.5	40	160	CI auto	MS/MS

Segment	Channel		Precursor mass	Isolation window	Quan ion (Product ion)	Waveform type	Excit stor level	Excit ampl
2	NDMA	1	75	2	47	Resonant	35	0.41
	NDMA-d6	2	81	2	50	Resonant	35	0.40
3	NMEA	1	89	1	61	Resonant	35	0.36

4	NDEA	1	103	1	75	Resonant	40	0.39
5	NMOR	1	117	1.5	86	Resonant	40	0.39
5	NDPA	2	131	1.5	89	Resonant	50	0.33
5	NDPA-d14	3	145	1.5	97	Resonant	50	0.37
5	NPYR	4	101	1.5	55	Resonant	40	0.39
6	NPIP	1	115	1.5	69	Resonant	50	0.39
7	NDBA	1	159	1.5	57	Resonant	50	0.40

8. QA/QC REQUIREMENTS

- 8.1 Laboratory Reagent Blank - Run before samples. Use to demonstrate that all glassware and reagent interference's are under control. If any contamination peaks are produced, determine source of contamination and eliminate interference.
- 8.2 Laboratory Fortified Blank - Must analyze at least two laboratory fortified blank (LFB) one after every calibration and one at the end of the analysis. The fortification concentration of each analyte should confirm the ability to detect at the reportable level. If the recovery of any analyte falls outside the control limits, the source of the problem must be identified and corrected.
- 8.3 Standards - Verify calibration standards quarterly by analyzing a standard prepared from reference material obtained from an independent or second source. EPA performance evaluations are an excellent process to determine the validity of the method. Results must be within +/- 30% of those used to routinely check calibration. Daily, run a low-level standard to check the reportable detection level, RDL.
- 8.4 Samples - Samples must be extracted within 14 days after collection. Samples must be stored at 4 °C until ready for extraction. Duplicates are run on 10% of samples, or once during run, whichever is greater. Results should be within +/- 30% for high spike (25 ppt) and +/- 50 % for low spike (2-5 ppt) Extracts should be analyzed within 14 days after extraction.
- 8.5 Spike Recoveries - The laboratory must add a known concentration of spike solution @ 25 ppt, the same as used for LFB, to at least 10% of samples or once per analytical run, whichever is greater. Recoveries should be within the acceptable range +/- 30%. Wherever possible, run a second source standard for spikes.
- 8.6 QC Requirements - Analyze EPA QC check sample with known values if available. The results for each analyte must be within the EPA acceptance criteria. Semi-annually, generate a series of MDLs – at least seven replicates at a 2.0 ppt level. The MDL should be 3 times lower than the RDL of the method.

9. PREVENTIVE MAINTENANCE AND CORRECTIVE ACTIONS

- 9.1 Glassware - Glassware must be carefully cleaned. Clean all glassware as soon as practical after use by thoroughly rinsing with last solvent used in it. Follow by

washing with hot water and detergent and thoroughly rinsing with tap water followed by reagent water. After drying, heat in oven. Do not heat volumetric glassware above 220°C. Thorough rinsing with acetone may be substituted for heating.

- 9.2 Reagents - The use of high purity solvents and reagents will help to minimize contamination problems.
- 9.3 Carryover - Contamination carryover may occur when a sample containing a low concentration of analytes is analyzed immediately following a sample containing a high concentration. Use solvent rinses between samples to minimize carryover.
- 9.4 All reagents and apparatus must be routinely demonstrated to be free from interference under the conditions of the analysis by running laboratory method blanks.
- 9.5 Record all corrective actions in the maintenance logbook. Include a complete description of the problem and what actions were taken to correct it.

[illegible]

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

EPA Method 527 Revision 1, April 2005

DETERMINATION OF SELECTED PESTICIDES AND FLAME RETARDANTS IN DRINKING WATER BY SOLID PHASE EXTRACTION AND CAPILLARY COLUMN GAS CHROMATOGRAPHY/ MASS SPECTROMETRY (GC/MS)

File Name: M:\SOP\Organic\epa method sop\527_123008.doc Effective Date: 12/30/2008
Revision: 1.2 Supersedes: 1.1

1. SUMMARY OF METHOD

A 1-liter water sample is extracted using a SDVB (polystyrene divinyl benzene) disk. Target analytes and surrogate compounds are extracted by passing the measured volume of sample thru the solid phase extraction (SPE) with SDVB disk. The organic compounds are eluted from the disk with small quantities of ethyl acetate (EtAC) and methylene chloride (CH₂Cl₂). The extract is dried with anhydrous sodium sulfate and concentrated to 1 ml using the Zymark concentrator - using gentle heat and UHP nitrogen gas. Internal standards are added to every sample after concentration. 2uL of the final extract is injected into a GC/MS system for separation, identification and quantitation. The concentration of each analyte is determined by using the internal standard technique. Surrogate analytes are added to all Field and Quality Control Samples to monitor the extraction efficiency of the target analytes.

2. ANALYTES

This is a gas chromatographic mass spectrometry (GC/MS) method, applicable to the determination of selected semivolatile organic compounds in finished drinking water. The following compounds can be determined using this method.

Target	MW	Quan Mass	CAS #	LIMS ID
Atrazine		200	1912-24-9	ATRAZ
Bifenthrin		181	82657-04-3	BIFNTH
Bromacil		205	314-40-9	BROMAC
Chlorpyrifos		197	2921-88-2	CIPYRI
Dimethoate		87, 125	60-51-5	DMTH
Esbiol		123	28434-00-6	ESBIOL
Esfenvalerate		167	66230-04-4	ESFENV
Fenvalerate		167	51630-58-1	FENVLR
Hexabromobiphenyl		308	59080-40-9	245HBB
2,2',4,4',5,5'-Hexabromodiphenyl ether		643.5	68631-49-2	BDE153
Hexazinone		171	51235-04-2	HEXZON
Kepone		272	143-50-0	KEPONE
Malathion		173	121-75-5	MALATH
Mirex		272	2385-85-5	MIREX

Target	MW	Quan Mass	CAS #	LIMS ID
Norflurazon		145	27314-13-2	NORFLR
Nitrofen		283	1836-75-5	NITROF
Oxychlordane		185	27304-13-8	OXYCHL
Parathion		291	56-38-2	PARA
2,2',4,4',5-Pentabromodiphenyl ether		403.8	60348-60-9	BDE99
2,2',4,4',6-Pentabromodiphenyl ether		403.8	189084-64-8	BDE100
Prometryne		241	7287-19-6	PROMET
Propazine		229	139-40-2	PROPAZ
Terbufos-Sulfone		153	56070-16-7	TERSUL
2,2',4,4'-Tetrabromodiphenyl ether		326	5436-43-1	BDE47
Thiobencarb		100	28249-77-6	THIO
Vinclozolin		212	50471-44-8	VINCZL

Target - Internal Standards	MW	Quan Mass	CAS #	LIMS ID
Acenaphthene-d ₁₀		164	15067-26-2	I1
Phenanthrene-d ₁₀		188	1517-22-2	I2
Chrysene-d ₁₂		240	1719-03-5	I3

Target - Surrogate Standards	MW	Quan Mass	CAS #	LIMS ID
1,3-Dimethyl-2-Nitrobenzene		134	81-20-9	S1
Triphenylphosphate		326	115-86-6	S2
Perylene-d ₁₂		264	1520-96-3	S3

Internal Standards (@ 500 ug/ml in Acetone)

	Cas #
Acenaphthene-d ₁₀ (I1)	15067-26-2
Phenanthrene-d ₁₀ (I2)	1517-22-2
Chrysene-d ₁₂ (I3)	1719-03-5

Surrogate Standards (@500 ug/ml in Acetone)

1,3-Dimethyl-2-Nitrobenzene (S1)	81-20-9
Triphenylphosphate (S2)	115-86-6
Perylene- d ₁₂ (S3)	1520-96-3

3. APPARATUS AND EQUIPMENT

- 3.1 Sample Bottles - Two liter amber glass bottles fitted with polytetrafluoroethylene (PTFE) lined screw caps.
- 3.2 Autosampler vials – amber vials equipped with PTFE-lined septa.
- 3.3 Volumetric Flasks – Class A, sizes 1, 5 and 10 mL
- 3.4 Graduated Cylinders - 10, 100 & 1000 mL

- 3.5 Hamilton Micro Syringes – Size include 10, 25, 50, 100, 250 and 500 ul
- 3.6 Collection Tube – 45 ml clear vials
- 3.7 Concentrator Tube - Zymark 150 mL tubes used with the Zymark Turbo-Vap.
- 3.8 Zymark Turbo-Vap - used to concentrate extracts
- 3.9 Analytical Balance: Capable of weighing accurately to nearest 0.0001 g.
- 3.10 SPE disk extraction: vacuum extraction manifold.
- 3.11 CP-3800 Varian gas chromatograph with a Saturn 2200 Ion Trap Mass Spectrometer and a data workstation. Equipped with a CP-8200 autosampler for injecting samples into the GC.
- 3.12 Column: Fused Silica Capillary column, Varian Factor Four with 5m guard column 30meters long x 0.25 mm I.D. with a 0.5 micron film thickness.
- 3.13 Disposable pasteur pipets

4. REAGENTS AND CONSUMABLE MATERIALS

- 4.1 Reagent Water - Millipore Milli-Q System or equivalent.
- 4.2 Methylene Chloride, Methanol, Acetone, Ethyl Acetate: Burdick & Jackson - capillary GC² High Purity solvent.
- 4.3 Sodium Sulfate: (ACS) Granular, anhydrous. Heat sodium sulfate at 400°C four hours.
- 4.4 SPE disk: 47mm diameter and 0.5 mm thick, manufactured with a polystyrene divinylbenzene (SDVB) sorbent phase.
- 4.5 Helium carrier gas: UHP grade. Nitrogen gas: UHP grade.

5. SAMPLE COLLECTION, PRESERVATION AND HANDLING

- 5.1 Samples are collected in 2.5L amber bottles, fitted with a screw cap lined with polypropylene. Keep samples sealed from collection time until analysis. Sampling equipment, including automatic samplers, must be free of plastic tubing, gaskets, and other parts that may leach interfering analytes into the water sample.
- 5.2 Preservation reagents are added to each sample bottle prior to shipment to the filed.

Compound	Amount	Purpose
L-Ascorbic Acid	0.10 g/L	Dechlorination
Ethylenediaminetetraacetic acid trisodium salt	0.35 g/L	Inhibit metal-catalyzed hydrolysis of targets
Potassium Dihydrogen Citrate	9.40 g/L	pH 3.8 buffer mixture, inhibit microbial growth and Analyte degradation

- 5.3 All samples should be iced or refrigerated at 10 °C or below and kept in the dark from the time of collection until extraction. Residual chlorine must be reduced at the time of sample collection with 100 mg of ascorbic acid per liter. Sodium sulfite and sodium thiosulfate cannot be used because they were found to degrade target analytes. After addition to the sample, seal the bottle and shake until contents dissolve. Store all samples at 6°C or below until extraction. All samples must be extracted within 14 days after collection. The extract should be stored at 0°C or less and analyzed within 28 days after sample extraction.

6. EXTRACTION:

- 6.1 Manual Liquid - Solid Phase Extraction (SPE):

See the attach SPE Flow Chart.

7. ANALYSIS

- 7.1 **MS Tune Check** -- The first step for the instrumental analysis of this method is the mass calibration of the system. Using GC/MS DFTPP method, inject 1ul aliquot of the 5ng/ul (ug/ml) solution of DFTPP into the GC/MS system. Acquire a mass spectrum that includes data for m/z 45-450. A single spectrum of the DFTPP peak, an average spectrum of the three highest points of the peak, or an average spectrum across the entire peak to evaluate the performance of the system. This criterion must be met for each day's analytical run. If the system repeatedly fails the criteria – retune the system until it passes. The DFTPP mass spectrum must meet all the criteria in Table 1.

EnviroPro software is used to determine whether the ion abundance criteria is met. For DFTPP check – Click on “Custom Report” icon. Choose “527.swt”. Go to “Tune Report Setup”. Select file. Change Retention time if needed. Choose “Tune Report”. Use print icon to print.

- 7.2 **Initial Calibration:** A six point calibration curve is required for this method; using standards STD-A (0.1ppb), B (0.5ppb), C (1.0ppb), D (2.0ppb), E (5.0ppb), F (10.0ppb). At least five calibration curve points must be utilized for each target compound. For each analyte and surrogate, calculate the mean RF and the relative standard deviation (RSD). The %RSD of any analyte or surrogate within the

calibration range must be less than 30%. If not – inject other standards or perform system maintenance and begin the system analysis and performance check over. Use the GC/MS data system software to generate a **linear regression** or **quadratic** calibration curve. Forcing the calibration curve through the origin is not recommended.

7.3 **Continuous Calibration Check (CCC):** The CCC verifies the initial calibration at the beginning and end of each group of analyses, and after every tenth sample during analyses. The beginning CCC for each analysis batch must be at or below the MDL. Subsequent CCCs should alternate between a medium and high concentration.

- a. The absolute areas of the quantitation ions of the internal standards must be +/-30% from the average area measured during initial calibration. If any IS area has changed by more this amount, the following remedial action must be taken. Major maintenance such as cleaning the ion source, the mass analyzer, replacing filament assemblies, replacing or shortening GC column, etc., require returning to the initial calibration step (Sect 7.2)
- b. The calculated amount for each analyte for medium and high level CCCs must be +/-30% of the true value. The calculated amount for each analyte for the lowest CCC level must be +/-50% of the true value. If these criteria are not met, then all data for the problem analyte must be considered invalid, and remedial action (Sect. 7.3.c) should be taken which may require recalibration.
- c. **REMEDIAL ACTION:** Major maintenance such as cleaning the ion source, cleaning the mass analyzer, replacing filament assemblies, replacing or shortening GC column, etc., require returning to the initial calibration step (Sect 7.2)

Exception: If the continuing calibration fails because the calculated concentration is greater than 130% (150% for the low-level CCC) for a particular target compound, and Field Sample extracts show no detection for that target compound, non-detects may be reported without re-analysis.

Data is collected and processed by Saturn GC/MS software. A calibration file must be created by analyzing the six point calibration curve and entering these values into the cali file. An unknown is identified by comparing its mass spectra with the NIST98 library. Its purity must exceed 800 for positive identification. Any results above the highest calibration standard must be confirmed with a standard that is within +/- 20% of the actual result or the sample must be diluted to within the calibration range.

GC Conditions:

1.	Seg	Temp	Rate	Hold Time	Total
	1	35	0	0.0	0.0
	2	200	25	2.0	8.6
	3	230	4	1.0	17.1
	4	310	5	1.2	34.3

2. Helium linear velocity: 30 cm/sec
Constant Column Flow: 1.4 ml/min

3. Split injection program:

Time	Split Rate	Split Ratio
Initial	On	20
0.1	Off	Off
3.50	On	100
5.0	On	20

4. Injector temperature :

Temp	Rate	Hold	Total
40°C	----	0	0
300°C	200	20	21.3

5. Transfer Line Temp: 280 °C
Trap Temp: 225 °C
Manifold: 80 °C

6. 2 ul sample is injected

MS Conditions :

1. Mass range : 45 to 450 amu
2. Seconds/scan : 0.80
3. Acquire time : 35.3 minutes
4. Fil/Mul delay : 5.75 minutes

	<u>Start</u>	<u>End</u>	<u>Low Mass</u>	<u>High Mass</u>
Seg #1:	5.7min	24.12 min	45	450
Seg #2:	24.12 min	34.30 min	45	645

5. Peak threshold : 1 count
6. Mass defect : 0 mmn/100 amu
7. Background mass : 45 amu
8. Ionization mode : EI
9. Auto ion control : ON
10. Cal gas : OFF

8. QA/QC REQUIREMENTS

- 8.1 Laboratory Reagent Blank - Run before samples. Use to demonstrate that all glassware and reagent interferences are under control. If any contamination peaks are produced, determine source of contamination and eliminate interference.

- 8.2 Laboratory Fortified Blank - Must analyze at least two laboratory fortified blanks (LFBs) with each set of extractions. Run a LFB-low and an LFB-high with each analytical run. The fortification concentration of each analyte should confirm the ability to detect at the reportable level. If the recovery of any analyte falls outside the control limits, the source of the problem must be identified and corrected.
- 8.3 Standards - Verify calibration standards quarterly by analyzing a standard prepared from reference material obtained from an independent or second source. EPA performance evaluations are an excellent process to determine the validity of the method. Results must be within +/- 20% of those used to routinely check calibration. Daily, run a low level standard to check the reportable detection level, RDL.
- 8.4 Samples - Samples must be extracted within 14 days after collection – unless specific targets need to be determined. Samples must be stored at <6°C until extraction. Duplicates are run on 10% of samples, or once during run, whichever is greater. Extracts should be stored at <= 0°C and analyzed within 28 days after sample extraction.
- 8.5 Spike Recoveries - The laboratory must add a known concentration of spike and spike dup solution, the same standard used for LFBs, to at least 10% of samples or once per analytical run, whichever is greater. Recoveries should be within the acceptable range.
- 8.6 QC Requirements - Analyze EPA QC check sample with known values if available. The results for each analyte must be within the EPA acceptance criteria. Annually, analyze EPA Performance Evaluation samples. Analyze these check samples whenever major maintenance to the system occurs to ensure the validity of the method.
- 8.7 When running the daily midpoint standard, ensure that the absolute areas of the quantitation ions of the internal and surrogate standards have not decreased by more than 30% of the continuing calibration check or more than 50% of the initial calibration curve.

9. PREVENTIVE MAINTENANCE AND CORRECTIVE ACTIONS

- 9.1 Glassware - Glassware must be carefully cleaned. Clean all glassware as soon as practical after use by thoroughly rinsing with last solvent used in it. Follow by washing with hot water and 10% HCl and thoroughly rinsing with tap water followed by reagent water. After drying, heat in oven. Do not heat volumetric glassware above 220°C. Thorough rinsing with acetone may be substituted for heating.
- 9.2 Reagents - The use of high purity solvents and reagents will help to minimize contamination problems.

- 9.3 Carryover - Contamination carryover may occur when a sample containing a low concentration of analytes is analyzed immediately following a sample containing a high concentration. Use solvent rinses between samples to minimize carryover.
- 9.4 All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory method blanks. Minimize contact of the samples, reagents, or solvents with any plastics. This will help reduce contamination. Interferences by phthalate esters can pose a major problem in pesticide analysis.
- 9.5 Record all corrective actions in the maintenance log book. Include a complete description of the problem and what action were taken to correct it.

Table 1. Ion Abundance Criteria For Bis(Perfluorophenyl)Phenyl Phosphine, (Decafluorotriphenyl Phosphine, DFTPP)

<u>Mass</u>	<u>Relative Abundance Criteria</u>	<u>Purpose of Checkpoint</u>
51	10-85% of the base peak	Low-mass sensitivity
68	<2% of mass 69	Low-mass resolution
70	<2% of mass 69	Low-mass resolution
127	10-80% of the base peak	Low- to mid-mass resolution
197	<2% of mass 198	Mid-mass resolution
198	Base peak or >50% of 442	Mid-mass resolution & sensitivity
199	5-9% of mass 198	Mid-mass resolution & isotope ratio
275	10-60% of base peak	Mid- to high-mass sensitivity
365	>0.5% of base peak	Baseline threshold
441	Present and <150% mass 443	High-mass resolution
442	Base peak or >30% of mass 198	High-mass resolution & sensitivity
443	15-24% of mass 442	High-mass resolution & isotope ratio

SOP PROCEDURE CHANGE
For EPA Method 527

<u>CHANGE</u>	<u>REASON</u>	<u>DATE</u>	<u>INITIALS</u>
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Added Internal & Surrogate Quan Mass tables	Future Reference	12/30/08	LVS-----
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ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

EPA Method 529, Rev. 1.0

DETERMINATION OF EXPLOSIVES AND RELATED COMPOUNDS IN DRINKING WATER BY SOLID PHASE EXTRACTION AND CAPILLARY COLUMN GAS CHROMATOGRAPHY/MASS SPECTROMETRY (GC/MS)

File Name: M:\SOP\Organic\epa method sop\529_0906.doc
OCWD Revision: 2

Effective Date: 09/28/2006
Supersedes: 1

1. SUMMARY OF METHOD

A measured volume of sample of approximately 1 liter is extracted using a solid phase extraction (SPE) cartridge, containing 500 mg of a divinylbenzene/vinylpyrrolidone copolymer. Manual SPE is used. The organic compounds are eluted from the cartridge with small quantities of ethyl acetate. The extract is dried with sodium sulfate and concentrated to 1 ml using the Zymark concentrator - using gentle heat and UHP nitrogen gas. The final extract is injected into a GC/MS system for separation, identification, and quantitation. Internal and surrogate standards are added to every sample. The method is applicable to explosives and related compounds in drinking water.

2. ANALYTES

2.1 This is a gas chromatographic mass spectrometry (GC/MS) method, applicable to the determination of a wide range of organic compounds. The following compounds can be determined using this method. They are separated based on the Calibration Standards used for this method – AccuStandard:

Explosives –AccuStandard Full Scan MS Calibration Set - M-529-MS-SET

Target	MW	Quan Mass	CAS#	LIMS ID
2-Amino-4,6-dinitrotoluene	197	180 or 78	35572-78-2	2ADNT
4-Amino-2,6-dinitrotoluene	197	180	19406-51-0	4ADNT
3,5-Dinitroaniline	183	183 or 64	618-87-1	35DNA
1,3-Dinitrobenzene	168	80 or 75	99-53-4	13DNT
2,4-Dinitrotoluene	182	165	121-14-2	24DNT
2,6-Dinitrotoluene	182	165	606-20-2	26DNT
Hexahydro-1,3,5-trinitro-1,3,5-Triazine (RDX)	222	210	121-82-1	RDX
Nitrobenzene	123	51 or 77	98-95-3	NBENZ
2-Nitrotoluene	137	65	88-72-2	2NTOLU
3-Nitrotoluene	137	91	99-08-1	3NTOLU
4-Nitrotoluene	137	65 or 91	99-99-0	4NTOLU
1,3,5-Trinitrobenzene	213	75 or 213	99-35-4	135TNB
2,4,6-Trinitrophenylmethylnitramine (Tetryl)	287	194 or 77	479-45-8	TETRYL
2,4,6-Trinitrotoluene (TNT)	227	210	118-96-1	246TNT

<u>Target</u>	<u>MW</u>	<u>Quan Mass</u>	<u>CAS#</u>	<u>LIMS ID</u>
3,4-Dinitrotoluene (IS)	182	63 or 182	610-39-9	529IS1
Nitrobenzene-d5 (SUR)	128	82	4165-60-0	529S1
1,3,5-trimethyl-2nitrobenzene (SUR)	165	148 or 91	603-71-4	529S2
1,2,4-trimethyl-5-nitrobenzene (SUR)	165	148 or 91	610-91-3	529S3

QA/QC Targets

Targets are separated based on Calibration standards – Full Scan MS Calibration Set - M-529-MS-SET - AccuStandard

GC/MS Tune Standard – 4-8083 1 Analytes (@ 2000 ug/ml in Methanol) – Supelco

Bromofluorobenzene (BFB) (CAS#460-00-4) in Methanol – injection of 5 ng (1 ul of a 5 ug/ml working std)

Internal Standard – M-529-IS 1 Analytes (@ 2.0 mg/ml in Ethyl Acetate) – AccuStandard

Surrogate Standards – M529-SAFS 3 Analytes (@ 100 ug/ml in Methanol) – AccuStandard

Spike Fortification Solution – M-529- ISFS 14 Analytes (@ 200 ug/ml in Ethyl Acetate) – AccuStandard

3. APPARATUS AND EQUIPMENT

- 3.1 Sample Bottles – Two-liter amber glass bottles fitted with a screw cap lined with Teflon.
- 3.2 Autosampler vials - equipped with Teflon-lined septum.
- 3.3 Concentrator Tube - Zymark 250 mL tubes used with the Zymark Turbo-Vap.
- 3.4 Analytical Balance: Capable of weighing accurately to nearest 0.0001 g.
- 3.5 Zymark Turbo-Vap - used to concentrate extracts.
- 3.6 3800 Varian gas chromatograph with a Saturn Ion Trap Mass Spectrometer and a data workstation. Equipped with an 8200 autosampler for injecting samples into the GC.
- 3.7 Column: Fused Silica Capillary column, J & W Scientific – DB-5MS: 15 meters long x 0.25 mm I.D. with a 0.25 micron film thickness.
- 3.8 Disposable pasteur pipettes, graduated cylinders (1000ml, 100ml, and 10ml), and Hamilton micro syringes - 10 ul to 100 ul.

4. REAGENTS AND CONSUMABLE MATERIALS

- 4.1 Reagent Water - Millipore Milli-Q System or equivalent.
- 4.2 Methanol, Ethyl Acetate: Burdick & Jackson - capillary GC² solvent.
- 4.3 Copper Sulfate – ACS grade. Used as a sample preservative for inhibiting microbial activity.
- 4.4 Trizma Pre-Set Crystals (pH 7.0 Buffer). Used as a binding agent for free chlorine, and to keep the copper in solution. Alternatively, a two component buffer of tris(hydroxymethyl)aminomethane [CAS# 77-86-11] and tris(hydroxymethyl)aminomethane hydrochloride [CAS# 1185-53-1] may be used.
- 4.5 Sodium Sulfate: (ACS) Granular, anhydrous. Heat sodium sulfate at 400°C four hours, and store sodium sulfate at 130°C.
- 4.6 SPE cartridges: 0.5 grams of divinylbenzene/vinylpyrrolidone copolymer. Waters Porapak RDX (WAT 047220).
- 4.7 Helium carrier gas: UHP grade. Liquid carbon dioxide: technical grade. Nitrogen gas: UHP grade.

5. SAMPLE COLLECTION, PRESERVATION AND HANDLING

- 5.1 Samples are collected in amber 2-liter amber bottles, fitted with a screw cap lined with Teflon. Allow the bottle to have headspace, however as much as possible fill the bottle to 3 cm of the top. Samples must be sealed from collection time until analysis. All sampling equipment must be free of materials, which would leach interfering analytes into the water sample. These materials would include plastic tubing, gaskets, and other parts. Any new sampling materials should be noted within the chain of custody, or within the sampler's log notes.
- 5.2 All samples should be iced or refrigerated at or below 6 °C and kept in the dark from the time of collection until extraction. Samples also must be dechlorinated and preserved with a microbial inhibitor at the time of collection. Trizma Pre-Set Crystals pH 7 buffer is added to the samples at a concentration of 5.0 g/L. Copper Sulfate is added as a microbial inhibitor at a concentration of 0.5 g/L. The copper sulfate and Trizma should be added to the sample bottles as dry salts prior to transporting the samples bottles to the site. After addition of the sample, seal the bottle and shake for one minute. All samples are shipped and stored at or below 6°C until ready for extraction. All samples must be extracted within 14 days after collection. The extract should be analyzed within 30 days after sample extraction. The extract should be stored in amber glass autosampler vials, and kept at 0°C until ready for analysis.

6. EXTRACTION

- 6.1 Check pH of samples. Verify pH is between 5 and 7, and free of residual chlorine.
- 6.2 Assemble SPE cartridge and filtration apparatus. Add sample preservation to LFB and LRB and any other QA/QC samples. Spike the LFB or LFM as required. Add the surrogate fortification solutions to each sample and mix immediately until homogeneous. Analyze an LFB and LFM for every 20 samples.
- 6.3 Cartridge clean-up and conditioning: Rinse each cartridge with 3x 5 ml aliquots of ethyl acetate, let the cartridge drain dry after each flush. Repeat with three 5 ml portions of GC2 methanol but do not allow the cartridge to go dry. Rinse with 2X 10 ml portions of reagent water. Stop the flow with 4 cm of reagent water in the column – ready to begin adding sample to the cartridge to process.
- 6.4 Adjust vacuum/ flow rate to process samples at 10 to 15 ml/min. After sample has passed through the cartridge, draw air through the cartridge for 10 – 15 minutes at a high vacuum (10 – 15 in. Hg). Do not dry the cartridge for more than 10 to 15 minutes.
- 6.5 Under each extraction cartridge, place a glass collection vial. Rinse the inside of each sample bottle with 5 ml of ethyl acetate and pass through the cartridge and into the receiver. Repeat with another 5 ml aliquot and pass the solvent through the cartridge under low vacuum and flow rate, dropwise rate.
- 6.6 Dry the final extract by passing it through an anhydrous sodium sulfate drying tube – prewetted with a small volume of ethyl acetate. Rinse with 3 ml of ethyl acetate. Concentrate the final dry extract to 0.9 ml using the Zymark concentrator – at 40° C water bath and nitrogen flow. Spike each extract with the internal standard and bring volume up to 1.0 ml with ethyl acetate.

7. ANALYSIS

- 7.1 The first step for the instrumental analysis of this method is the mass calibration of the system. Inject into the GC/MS system a 1ul aliquot of the 5 ng/ul solution of BFB – Bromofluorobenzene. The resulting spectra should meet the ion abundance criteria for BFB, referenced in the table below. Acquire a mass spectrum that includes data for m/z 45-250. A single spectrum at the apex of the peak, or an average of the three spectra at the apex of the peak, or an average spectrum across the entire GC peak may be used to evaluate the performance of the system. This criterion must be met for each day's analytical run. If the system repeatedly fails the criteria – retune or clean the system until it passes.

Mass (m/z)	Relative Abundance Criteria	Purpose of Checkpoint
50	15-40% of mass 95	Low-mass sensitivity
75	30-80% of mass 95	Low-mass sensitivity
95	Base peak, 100% relative abundance	Low-mass resolution and sensitivity
96	5-9% of mass 95	Low-mass resolution and isotope ratio
173	<2% of mass 174	Mid-mass resolution
174	>50% of mass 95	Mid-mass resolution and sensitivity
175	5-9% of mass 174	Mid-mass resolution
176	>95% but <101% of mass 174	Mid-mass resolution and isotope ratio

177	5-9% of mass 176	Mid-mass resolution
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- 7.2 Once the system's daily performance checks have passed, inject a midlevel standard STD-D (2.0 ppb). For full scan mass analysis and analyte calibration, inject 2 ul of the calibration curve standards. Acquire the data from m/z45-250 with a total cycle time of 0.7 sec or less. Examine the stored GC/MS data with the data system software. The GC/MS/DS peak identification software should be able to recognize a GC peak in the appropriate retention time window for each of the compounds in the calibration solution, and make correct identifications.
- 7.3 If all performance criteria are met, inject 2 ul of each of the calibration solutions using the same GC/MS conditions.
- 7.4 Initial Calibration: A six point calibration curve is required for this method; using standards STD-A (0.1ppb), B (0.5ppb), C (1.0ppb), D (2.0ppb), E (5.0ppb), F (10.0ppb). At least five calibration curve points must be utilized for each target compound. For each analyte and surrogate, calculate the mean RF and the relative standard deviation (RSD). The %RSD of any analyte or surrogate within the calibration range must be less than 30%. If not – inject other standards or perform system maintenance and begin the system analysis and performance. It is acceptable to calibrate using 5 points – dropping the lowest or the highest concentration if it is not linear. Points in the middle of the calibration range may not be dropped.
- 7.5 Continuous Calibration check: Verify the initial calibration at the beginning and end of each group of analysis, and after every tenth field sample during analyses. The beginning CCC each day should be at or near the MRL in order to verify instrument sensitivity prior to any analyses. Subsequent CCCs can alternate between a medium and high concentration standard. Determine the absolute areas of the quantitation ions of the internal standard have not changed by more than 30% from the areas measured in the most recent continuing calibration check, or by more than 50% from the areas measured during initial calibration. If this area has changed by more than these amounts, adjustments must be made to restore system sensitivity. For each analyte and surrogate, the calculated amount for each analyte for medium and high level CCCs must be within 70-130% of the true value. The calculated amount for the lowest calibration point for each analyte must be within 50-150% of the true value. **If the CCC in the middle or at the end of an analysis batch fails because the calculated concentration is >130% of the true value, and field sample extracts showed no detection of method analytes, non-detects may be reported without re-analysis.**
- 7.6 EPA method 529 is used to monitor for low-level organics in ground water. Because of these low levels, any type of contamination or interference can cause analytical problems. Thus, reagent blanks must be monitored for every extraction run - monitoring of reagent blanks is essential to the success of this method. Background from method analytes and interferences should be $\leq 1/3$ the MRL.

GC Conditions:

1.	Seg	Temp	Rate	Hold Time	Total
	1	45	0	3.3	3.3

2	100	9	0.5	9.91
3	145	12	0.5	14.16
4	210	20	0.5	17.91
5	290	60	0.5	19.74

2. Helium linear velocity: 30 cm/sec
3. Splitless injection with 3.5 minute delay
4. Injector temperature (1079): Initial 60°C, then 250°C @ 200°C/minute hold for 12.00 minute
5. Transfer Line Temp: 280 °C
6. 2 ul sample is injected at 10 ul/sec - sandwich technique is used with a 0.2 ul solvent plug

MS Conditions:

1. Mass range : 45 to 250 amu
2. Seconds/scan : 0.39
3. Acquire time : 19.74 minutes
4. Fil/Mul delay : 5.5 minutes
5. Peak threshold : 1 count
6. Mass defect : 0 mmn/100 amu
7. Background mass : 43 amu
8. Ionization mode : EI
9. Auto ion control : ON
10. Cal gas : OFF

8. QA/QC REQUIREMENTS

- 8.1 Laboratory Reagent Blank - Run before samples. Use to demonstrate that all glassware and reagent interferences are under control. If any contamination peaks are produced, determine source of contamination and eliminate interference.
- 8.2 Laboratory Fortified Blank - Must analyze at least two laboratory fortified blanks (LFBs) with each set of extractions. Run a LFB-low and an LFB-high with each analytical run. The fortification concentration of each analyte should confirm the ability to detect at the reportable level. If the recovery of any analyte falls outside the control limits, the source of the problem must be identified and corrected.
- 8.3 Standards - Verify calibration standards quarterly by analyzing a standard prepared from reference material obtained from an independent or second source. EPA performance evaluations are an excellent process to determine the validity of the method. Results must be within +/- 30% of those used to routinely check calibration. Daily, run a low level standard to check the reportable detection level, RDL. Results must be within +/- 50% for the lowest standard.

- 8.4 Samples - Samples must be extracted within 14 days after collection. Samples must be stored at 4°C until ready for extraction. Duplicates are run on 10% of samples, or once during run, whichever is greater. Results should be within +/- 20%. Extracts should be stored at 0°C or less and analyzed within 30 days after sample extraction.
- 8.5 Spike Recoveries - The laboratory must add a known concentration of spike solution, the same as used for LFB, to each extraction batch (a set of up to 20 field samples). A laboratory fortified sample matrix duplicate should also be fortified, extracted and analyzed identically to the sample spike. Recoveries should be within the acceptable range (+/- 30%). Relative percent differences for spike and spike duplicate should fall in the range of $\pm 30\%$. Wherever possible, run a second source standard for spikes.
- 8.6 QC Requirements - Analyze EPA QC check sample with known values if available. The results for each analyte must be within the EPA acceptance criteria. Annually, analyze EPA Performance Evaluation samples if available. Analyze these check samples whenever major maintenance to the system occurs to ensure the validity of the method.
- 8.7 When running, the analyst must monitor the peak area of the IS in all injections during each analysis day. The analyst must ensure that the absolute areas of the quantitation ions of the internal standard have not deviated from the response in the most recent CCC by more than 30%, and must not deviate by more than 50% from the area measured during initial analyte calibration. Surrogate recovery criteria are 70-130% for the fortified amount for all method surrogates.
- 8.8 Request a resample if sample failed QA/QC, or has unusual hits.

9. PREVENTIVE MAINTENANCE AND CORRECTIVE ACTIONS

- 9.1 Glassware - Glassware must be carefully cleaned. Clean all glassware as soon as practical after use by thoroughly rinsing with last solvent used in it. Follow by washing with hot water and 10% HCl and thoroughly rinsing with tap water followed by reagent water. After drying, heat in oven. Do not heat volumetric glassware above 220°C. Thorough rinsing with acetone may be substituted for heating.
- 9.2 Reagents - The use of high purity solvents and reagents will help to minimize contamination problems.
- 9.3 Carryover - Contamination carryover may occur when a sample containing a low concentration of analytes is analyzed immediately following a sample containing a high concentration. Use solvent rinses between samples to minimize carryover.
- 9.4 All reagents and apparatus must be routinely demonstrated to be free from

interferences under the conditions of the analysis by running laboratory method blanks. Minimize contact of the samples, reagents, or solvents with any plastics. This will help reduce contamination. Interferences by phthalate esters can pose a major problem in pesticide analysis.

- 9.5 Record all corrective actions in the maintenance log book. Include a complete description of the problem and what actions were taken to correct it.

[illegible]

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

EPA Method 535

Version 1.1

MEASUREMENT OF CHLOROACETANILIDE AND OTHER ACETAMIDE HERBICIDES DEGRADATES IN DRINKING WATER BY SOLID PHASE EXTRACTION AND LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY (LC/MS/MS)

File Name: M:\SOP\Organic\EPA_Method_SOP\535_123008.doc Effective Date: 12/30/2008
Revision: 1.1 (12/30/08 lvs) Supersedes: 1.0

1. SUMMARY OF METHOD

A measured volume of sample (250-mLs) is extracted using a solid phase extraction (SPE) cartridge containing 0.5 grams of nonporous graphitized carbon. Manual SPE is used with the Varian Vac-Elut 20 system. The targets of interest are the chloroacetanilide and acetamide herbicide degradates; ethanesulfonic acids (ESA) and oxanilic acids (OA). The targets are retained upon the carbon and eluted with a small amount of methanol containing 10 mM ammonium acetate. The methanol extract is concentrated to dryness using the Zymark concentrator - using gentle heat and UHP nitrogen gas. The extract is reconstituted with 1 ml of water containing 5 mM ammonium acetate. The final extract is injected into the LC/MS/MS system for separation, identification, and quantitation. Internal standard procedure is used for quantification, and surrogate standards are added to every sample to monitor for extraction efficiencies.

2. ANALYTES

This is a Liquid Chromatographic/Tandem Mass Spectrometry (LC/MS/MS) method, applicable to the determination herbicide degradates. The following compounds can be determined using this method.

Targets	MW	CAS #	LIMS ID
Acetochlor ESA		187022-11-3	ACTESA
Acetochlor OA		184992-44-4	ACTOA
Alachlor ESA		142363-53-9	ALAESA
Alachlor OA		171262-17-2	ALAOA
Dimethenamid ESA		205939-58-8	DMAESA
Dimethenamid OA		-	DMAOA
Flufenacet ESA		-	FLFESA
Flufenacet OA		-	FLFOA
Metolachlor ESA		171118-09-5	MTAESA
Metolachlor OA		152019-73-3	MTAOA
Propachlor ESA		-	PRPESA
Propachlor OA		-	PRPOA

QA/QC Targets

Internal Standard:

Butachlor ESA

IS Stock Solution: 1,000 mg/ml in methanol – stable for six months when stored at 4 °C or less.

IS Primary Dilution Standard Solution:
100 ug/ml in methanol – stable for six months when stored at 4 °C or less

Surrogate Standards:

Dimethachlor ESA

Surrogate Stock Solution:
1,000 mg/ml in methanol – stable for six months when stored at 4 °C or less.

Surrogate Primary Dilution Standard Solution:
25 ug/ml in methanol – stable for 6 months when stored at 4 °C or less.

Analyte Standards:

12 Target Compounds

Analyte Stock Solution:
1,000 mg/ml in methanol.

Analyte Primary Dilution Standard Solution:
25 ug/ml in methanol – stable for 6 months when stored at 4 °C or less.

3. APPARATUS AND EQUIPMENT

- 3.1 Sample Bottles – 1 liter amber glass bottles fitted with a screw cap lined with PTFE (polytetrafluoroethylene) lined screw caps.
- 3.2 Autosampler vials - 2 mL amber glass, screw cap with PTFE septa.
- 3.3 Volumetric Flasks - Class A, various sizes used for preparation of standards.
- 3.4 Graduated Cylinders - Various sizes.
- 3.5 Micro Syringes - Various sizes - Hamilton micro syringes - 10 ul to 100 ul.
- 3.6 Analytical Balance: Capable of weighing accurately to 0.0001 g - Sartorius

- 3.7 Disposable pasteur pipets - 1mL
- 3.8 Zymark Turbo-Vap - used to concentrate extracts. Concentrator Tubes - Zymark 250 mL tubes used with the Zymark Turbo-Vap.
- 3.9 Vacuum Extraction Manifold – Vac-Elut 20 with vacuum pump.
- 3.10 Applied BioSystems 4000 Q Trap LC/MS/MS System. – Agilent 1200 Liquid Chromatograph, LEAP CTC autosampler, column oven and data workstation.
- 3.11 Column: Thermo HyPURITY C18 (100 x 2.1 mm, 5 um). Part #: 22105-102130

4. REAGENTS AND CONSUMABLE MATERIALS

- 4.1 Reagent Water - Millipore Milli-Q System or equivalent.
- 4.2 Ammonium Chloride – sample dechlorinating agent
- 4.3 Methanol – High purity
- 4.4 SPE Cartridges - (graphitized nonporous carbon): 0.5 grams in 6ml cartridge.
- 4.5 Nitrogen Generator - Parker LC/MS Gas Generator Source 5000 – Tri-Gas
- 4.6 Ammonium Acetate – Sigma-Aldrich ACS grade
- 4.7 10 mM Ammonium Acetate/Methanol – 0.7708 g ammonium acetate to 1 L of methanol. Stable for 18 days.
- 4.8 5 mM Ammonium Acetate/Reagent Water – 0.3854 g ammonium acetate to 1 L of reagent water (pH ~6.5). Should be made up fresh with each run.

5. SAMPLE COLLECTION, PRESERVATION AND HANDLING

- 5.1 Samples are collected in 1-Liter amber glass bottles, fitted with a screw cap lined with PTFE. Each bottle contains 100 mg of ammonium chloride, used as a de-chlorinating agent. Do not rinse or overfill the bottle during sample collection. Keep samples sealed from collection time until analysis. Sampling equipment must be free of plastic tubing, gaskets, and other parts that may leach interfering analytes into the water sample.
- 5.2 After collection, cap the bottle, and agitate by hand for ~1 minute to dissolve the ammonium chloride. Samples must not exceed 10 °C during transport, ice or chemical freeze packs should be used during shipment and temperature verified upon delivery into the lab. All samples should be iced or refrigerated at or below 6 °C and kept in the dark from the time of collection until extraction. Samples must be extracted within 14 days of collection. Sample extracts are stored at 4 °C or less and must be analyzed within 28 days after sample extraction.

6. EXTRACTION

Liquid - Solid phase extraction (SPE):

- Assemble the Varian Vac-Elut 20 cartridge vacuum filtration apparatus.
- Add ammonium chloride to all QA/QC checks (LFBs low & high and RB) to provide the same level as within field samples 100mg/L. Use 250ml sample bottles to measure out samples to be processed.
- Add to each sample and QA/QC checks – the surrogate standard to produce a final concentration of 0.48 ug/L.
- Spike all LFBs low & high, matrix spike & matrix spike dup with appropriate standard mix (see table X below).
- Condition cartridges, making sure that cartridge packing material does not go dry during any part of the conditioning procedure. If the cartridge goes dry during the conditioning phase, the conditioning must be started over.
 - Rinse each cartridge with 20 ml of 10 mM ammonium acetate/methanol.
 - Rinse each cartridge with 30 ml of reagent water – without allowing the water to drop below the top edge of the packing material.
 - Add 3 ml of reagent water to the cartridge, attach a 140 ml Varian reservoir tube, turn on the vacuum and begin adding the sample to the cartridge.
- Adjust the flow rate to ~ 10-15 ml/min using the vacuum and process the sample. Rinse the cartridge with 5 ml of reagent water. Once processed through the cartridge, draw air through the cartridge for 3 minutes at a high vacuum.
- Once all samples have been processed on the Vac-Elut 20 - open the top and insert collection tubes into the extraction tank. Elute the targets from the cartridge with 15 mls of the 10mM ammonium acetate/methanol at a low vacuum (~5 ml/min) at a drop-wise rate.
- Concentrate the extracts to dryness using the Zymark Turbo-Vap II concentrators, which uses a water bath at 60 °C and a gentle stream of nitrogen.
- Add 2mL of 5 mM ammonium acetate/reagent water to the Zymark, rinse the walls of the Zymark, transfer to 5 ml volume metric flask. Rinse the Zymark again with 2 mL of mM ammonium acetate/reagent water, add this to 5 mL volume metric flask. Bring the final volume to 5 mL, add 10uL of Internal standard, mix well and transfer the extract into 2 amber autosampler vials.

7. ANALYSIS

- 7.1 Initial Calibration: A six-point calibration curve is used for this method; using standards STD-A (0.20ppb), B (0.50ppb), C (1.00ppb), D (2.00ppb), E (3.00ppb), and F (4.00ppb). For each analyte the recovery must be within 70-130% of the true value for all but the lowest level of calibration. The lowest calibration level CCC must be within 50-150% of the true value. Surrogate recovery must be within 70-130% of the true value. If not – inject new calibration standards or perform system maintenance until the criteria passes.

7.2 Continuous Calibration check:

Reagent blank must be monitored for every extraction run - monitoring of reagent blank is essential to the success of this method. The reagent blank must demonstrate that all target analytes are below 1/3 the MRL. Verify the calibration by measurement of two mid-point calibration check standards, one at the beginning and one at the end of the run. The check standard area counts must be within +/- 30% of the standard curve. LFBs (laboratory fortified blanks) should also be analyzed with each extraction run. Standards used for these QA/QC samples must be ordered from a second source whenever possible. For extended runs, check standards should be interspersed with samples at regular intervals. No more than ten samples analyzed before a calibration check is analyzed. If the response of any analyte varies from the predicted response by more than +/- 30%, test must be repeated using fresh calibration standards. Data is collected and processed by Analyst 1.4.2 software. Any results above the highest calibration standard must be confirmed with a standard that is within +/- 20% of the actual result or the sample must be diluted to within the calibration range.

LC Conditions: Column Temperature: 65°C

Gradient elution:		Solvent A	Solvent B
<u>Total Time</u>	<u>Flow</u>	<u>%5mM Ammonium Acetate</u>	<u>%Methanol</u>
0	0.25ml/min	70	30
6	0.25	70	30
7	0.25	50	50
16	0.25	20	80
17	0.25	70	30
20	0.25	70	30

MS/MS Conditions:

	(ES-)	Retention <u>Time (min)</u>	<u>MW</u>	Precursor <u>Ion (m/z)</u>	Product <u>Ion (m/z)</u>	Collision <u>Energy(ev)</u>
Acetochlor ESA	ACTESA	18.10	315.1	314	162	-32
Acetochlor OA	ACTOA	17.20	265.3	264	146	-16
Alachlor ESA	ALAESA	17.90	315.1	314	160	-34
Alachlor OA	ALAOA	17.00	265.3	264	160	-16
Dimethenamid ESA	DMAESA	15.90	----	320	80	-58
Dimethenamid OA	DMAOA	15.20	----	270	198	-16
Flufenacet ESA	FLFESA	13.70	----	274	80	-50
Flufenacet OA	FLFOA	12.50	----	224	152	-14
Metolachlor ESA	MTAESA	18.50	329.12	328	80	-60
Metolachlor OA	MTAOA	18.20	279.33	278	206	-16
Propachlor ESA	PRPESA	12.90	----	256	80	-40
Propachlor OA	PRPOA	11.20	----	206	134	-14
Dimethachlor ESA	S1 (SUR)	13.80	301.34	300	80	-54
Butachlor ESA	I1 (IS)	23.00	357.15	356	80	-66

8. QA/QC REQUIREMENTS

- 8.1 Laboratory Reagent Blank - Run before samples. Use to demonstrate that all glassware and reagent interferences are under control. If any contamination peaks are produced, which inhibits quantitative of target compounds, determine source of contamination and eliminate interference.
- 8.2 Laboratory Fortified Blank - Must analyze at least two laboratory fortified blanks (LFBs) with each set of extractions; analyze a LFB-low (MRL) and an LFB-high with each analytical run. The fortification concentration of each analyte should confirm the ability to detect at the reportable level. If the recovery of any analyte falls outside the control limits, the source of the problem must be identified and corrected.
- 8.3 Standards - Verify calibration standards quarterly by analyzing a standard prepared from reference material obtained from an independent or second source. ERA carries known performance evaluations (PE) samples for the 535 UCMR2 targets – and should frequently verified on analytical runs as an additional QA/QC check. This helps determine the validity of the method and the system and provide additional support to our QA/QC of this method. Results must be within +/- 30% of those used to routinely check calibration. For every batch of sample, run a low level standard to check the minimum reportable level, MRL.
- 8.4 Samples must be extracted within 14 days after collection. Samples must be stored at 6°C (or less) until ready for extraction. Duplicates are run on 10% of samples, or once during run, whichever is greater. Relative percent difference (RPD) should be within 0-30% for all targets. Extracts should be analyzed within 28 days after sample extraction, all extracts must be stored in amber glass vials at 4°C or less in the dark.
- 8.5 Spike Recoveries - The laboratory must add a known concentration of spike solution, the same as used for LFB, to at least 10% of samples or once per analytical run, whichever is greater. A spike and spike duplicate is analyzed within each batch. Relative percent difference (RPD) should be within 0-30% for all targets. Recoveries should be within the acceptable range 60-140% (50-150% at the MRL). Wherever possible, run a second source standard for spikes if available.
- 8.6 QC Requirements - Analyze ERA QC check sample with known values if available. The results for each analyte must be within the acceptance criteria. Analyze these check samples whenever major maintenance of the system occurs, to ensure the validity of the method. This sample should be run at least monthly during the UCMR2 program to insure system verification. Analyze EPA Performance Evaluation samples, if available, during our certification process.
- 8.7.1 When running the daily midpoint standard, ensure that the absolute areas of the quantitation ions of the internal and surrogate standards have not decreased by more than 30% of the continuing calibration check or more than 50% of the initial calibration curve.

QA/QC & Initial Demonstration Requirements (Summary)

<i>EPA Method Reference</i>	<i>Requirement</i>	<i>Specification & Frequency</i>	<i>Acceptance Criteria</i>
Section 9.2.1	Initial Demonstration of Low System Background	Analyze a Reagent Blank prior to any other IDC steps, or anytime a new lot of SPE materials or reagents are used.	Demonstrate that all target analytes are $\leq 1/3$ the MRL and that possible interferences from reagents, extraction media and glassware do not prevent the identification and quantitation of target analytes.
Section 9.2.2	Initial Demonstration of Precision (IDP)	Analyze 4-7 replicate LFBs/CCCs fortified at midrange concentration.	%RSD must be $\leq 20\%$
Section 9.2.3	Initial Demonstration of Accuracy (IDA)	Calculate average recovery for replicates used in IDP.	Mean recovery + 30% of true value.
Section 9.2.4	Minimum Reporting Level (MRL) Confirmation	Fortify, extract and analyze 7 replicate LFBs at the proposed MRL. Use the equation provided to verify the MRL. Repeat after major instrument or operational changes.	See Section 9.2.4 for confirmation criteria.
Section 8.1 to 8.4	Sample Collection Preservation and Holding Time	14 days, protected from light, with addition of ammonium chloride	Iced or refrigerated at 10°C or less for up to 48 hours to allow time for shipping; refrigerated at 6°C or less after arrival at the laboratory.
Section 8.4	Extract Holding	28 days	Stored at 4°C or less in amber vials.
Section 9.3	Laboratory Reagent Blank (RB)	Include a RB with each extraction batch (up to 20 samples). Analyze prior to analyzing samples and determine to be free from interferences.	Demonstrate that all target analytes are below 1/3 the MRL, and that possible interferences from reagents and glassware do not prevent the identification and quantitation of target analytes.
Section 9.4 and 10.3	Continuing Calibration Check (CCC)	Verify initial calibration by analyzing a low level (at the MRL or below) CCC prior to analyzing samples. CCCs are then injected after every 10 samples and after the last sample, rotating concentrations to cover the calibrated range of the instrument.	Recovery for each analyte must be within 70-130% of the true value for all but the lowest level of calibration. The lowest calibration level CCC must be within 50-150% of the true value.
Section 9.5	Laboratory Fortified Blank (LFB)	Analyze at least one LFB daily or one for each extraction batch of up to 20 field samples. Rotate the fortified concentration between low, medium and high amounts.	Results of LFB analyses must be 70-130% of the true value for each analyte and surrogate for all fortified concentrations greater than the lowest CAL point. Results of LFBs corresponding to the lowest CAL point must be 50-150% of the true value.

Section 9.6	Internal Standard	Butachlor ESA is added to all standards and extracts.	Peak area counts of the IS in LFBs, RBs and sample extracts must be within 50-150% of the average peak area in the initial calibration.
Section 9.7	Surrogate Standards (SUR)	The surrogate, Dimethachlor ESA, is added to all calibration standards, samples, LFBs, Matrix Spikes & Dups, RBs, and duplicates.	Surrogate recovery must be within 70-130% of the true value. Samples that fail criteria must be reported as suspect due to surrogate recovery or potential matrix effect.
Section 9.8 & 9.9	Matrix Spikes & Spike Duplicates – and field duplicates.	Analyze a matrix spike and matrix spike duplicate (and field duplicate) with each extraction batch (20 samples or less) fortified with method analytes at a concentration \geq the native concentration.	Recoveries not within 60-140% (50-150% at the MRL) of the fortified amount may indicate a matrix effect. RPD – relative percent difference – should be within 0-30%.
Section 9.10	Resolution Check	Monitor once for every 24-hour period.	Alachlor ESA and acetochlor ESA that are not baseline resolved must have a resolution of 1.0 or greater using the equation in Section 9.10.
Section 9.11	Quality Control Sample (if available)	Analyze at least quarterly or when preparing new standards, as well as during the IDC.	If analyzed as a calibration sample, CCC criteria apply. If analyzed as an LFB, those criteria apply.
Section 10.2	Initial Calibration	Use internal standard calibration technique to generate an average RF, or first or second order calibration curve for each analyte. Our lab uses a 6-point Cal standards.	When each calibration standard is calculated using the calibration curve, the results should be 70-130% of the true value for all but the lowest standard. The lowest standard should be 50-150% of the true value.

9. PREVENTIVE MAINTENANCE AND CORRECTIVE ACTIONS

- 9.1 Glassware - Glassware must be carefully cleaned. Clean all glassware as soon as practical after use by thoroughly rinsing with last solvent used in it. Follow by washing with hot water and detergent and thoroughly rinsing with tap water followed by reagent water. After drying, heat in oven. Do not heat volumetric glassware above 220°C. Thorough rinsing with acetone may be substituted for heating.
- 9.2 Reagents - The use of high purity solvents and reagents will help to minimize contamination problems.
- 9.3 Carryover - Contamination carryover may occur when a sample containing a low concentration of analytes is analyzed immediately following a sample containing a high concentration. Use solvent rinses between samples to minimize carryover.
- 9.4 All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory method

blanks. Minimize contact of the samples, reagents, or solvents with any plastics. This will help reduce contamination. Interferences by phthalate esters can pose a major problem in pesticide analysis.

- 9.5 Record all corrective actions in the maintenance logbook. Include a complete description of the problem and what actions were taken to correct it.

SOP PROCEDURE CHANGE
For EPA 535 Method

<u>CHANGE</u>	<u>REASON</u>	<u>DATE</u>	<u>INITIALS</u>
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Update the LC condition		2/8/07	LVS
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Entered the retention time for the Rest of the compounds		12/30/08	LVS
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ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

Phenols by LC/MS

DETERMINATION OF ENDOCRINE DISRUPTING CHEMICALS IN WATER BY LIQUID-SOLID EXTRACTION AND LIQUID CHROMATOGRAPHY/ MASS SPECTROMETRY

File Name: M:\SOP\Organic\epa method sop\Phenols_0109.doc
Revision: 1

Effective Date: 01/14/09
Supersedes:

1. SUMMARY OF METHOD

A measured volume of sample of approximately 1 liter is extracted using a solid phase extraction (SPE) cartridge containing 1 g of a modified polystyrene divinyl benzene copolymer Manual SPE is used. The organic compounds are eluted from the cartridge with small quantities of Methylene Chloride. The extract is dried and concentrated to 1.0 ml using the Zymark concentrator - using gentle heat and UHP nitrogen gas. The final extract is injected into the Waters/Micromass ZQ - LC/MS system for separation, identification, and quantitation. Both an internal and surrogate standard are added to every sample. Sample quantification is conducted using an external calibration method.

2. ANALYTES

This is a Liquid Chromatographic Mass Spectrometry (LC/MS) method, applicable to the determination phenolic compounds. The following compounds can be determined using this method. The LC/MS system is run in both positive and negative electrospray mode, depending on target compound.

Targets	MW	LC/MS MW	CAS #	LIMS ID	RDL
4-Nonylphenol	220.3	219.36	104-40-5	NONYPH	1 ug/L
4-n-Octylphenol	206	205.2	1806-26-4	4nOCPH	1 ug/L
4-tert-Octylphenol	206	205.2	140-66-9	4tOCPH	1 ug/L
Bisphenol A	228.3	227.3	80-05-7	BisPHA	1 ug/L
Pentachlorophenol	264	263.3	87-86-5	PCP	1 ug/L
2,4,6-Trichlorophenol	196.1	195.1	88-06-2	246TCP	1 ug/L
4-Phenylphenol (4-Hydroxybiphenyl)	170	169.2	92-69-3	PHNYPH	1 ug/L
Tetrabromobisphenol A	543.9	542.9	79-94-7	TBBISA	1 ug/L
Nonylphenol diethoxylate	308	309	20427-84-3	NONYDI	10 ug/L
Nonylphenol monoethoxylate	264.4	265.4	104-35-8	NONYMO	10 ug/L

QA/QC Targets

Targets are separated based on Calibration standards – Restek

Surrogate Standards

4-(4-bromophenyl) phenol

Internal Standards

Bisphenol-A – d16

3. APPARATUS AND EQUIPMENT

- 3.1 Sample Bottles – 2.5 liter amber glass bottles fitted with a screw cap lined with teflon.
- 3.2 Autosampler vials - 2 mL amber glass, screw cap with Teflon septa. (Waters Vials)
- 3.3 Concentrator Tube - Zymark 250 mL tubes used with the Zymark Turbo-Vap.
- 3.4 Analytical Balance: Capable of weighing accurately to nearest 0.0001 g.
- 3.5 Zymark Turbo-Vap - used to concentrate extracts.
- 3.6 Waters / Micromass ZQ - Liquid Chromatograph/Mass Spectrometer -Quaternary pump, autosampler, column oven and Empower Data workstation.
- 3.7 Column: Phenomenex – Gemini C6 phenyl (2.0 x 150 mm, 5 um).
- 3.8 Disposable pasteur pipets, graduated cylinders (1000ml), 1ml volumetric flasks, and Hamilton micro syringes - 10 ul to 100 ul.

4. REAGENTS AND CONSUMABLE MATERIALS

- 4.1 Reagent Water - Millipore Milli-Q System or equivalent.
- 4.2 Methylene Chloride – GC² quality
- 4.3 Acetonitrile - HPLC quality.
- 4.4 Methanol - GC² and HPLC quality
- 4.5 Extraction cartridge: Varian Bond Elute PPL SPE cartridge, 1 grams of highly cross-linked, and chemically modified styrene divinyl benzene copolymer. Varian part #: 12255002.

4.6 Nitrogen Generator -- Dornick Hunter

5. SAMPLE COLLECTION, PRESERVATION AND HANDLING

- 5.1 Samples are collected in amber 2.5 liter bottles, fitted with a screw cap lined with teflon. Keep samples sealed from collection time until analysis. Sampling equipment must be free of plastic tubing, gaskets, and other parts that may leach interfering analytes into the water sample.
- 5.2 All samples should be iced or refrigerated at 4 °C and kept in the dark from the time of collection until extraction.
- 5.3 Sample extraction hold time is 14 days, extract analysis hold time is 14 days.

6. EXTRACTION

- 6.1 Transfer one liter of sample to 1-liter bottles. All samples will be at a pH of less than 2. All QA/QC samples must be added sodium sulfite and adjusted to this pH using 1:1 HCl:H₂O solution. Add 10ul of the surrogate (SS) compound fortification solution. Results in a 20 ug/L concentration of the SS target in water.
- 6.2 Cartridge Clean Up and Conditioning: Rinse each cartridge with four, 3mL methylene chloride. Let the cartridge drain dry after each flush. Then rinse the cartridge with three, 3mL methanol. **DO NOT** allow the methanol to elute below the top of the cartridge packing. **From this point, do not allow the cartridge packing to go dry.** Rinse with three, 3mL 0.05N HCl, turn off the vacuum before the dilute acid level drops below the top edge of the packing. Add 3mL additional 0.05N HCl to the cartridge, attach the transfer tube, and turn on the vacuum, and begin adding sample to the cartridge.

Adjust the vacuum so that the approximate flow rate is 20mL/min (50min for a 1L sample). After all of the sample has passed through the SPE cartridge, draw air or nitrogen through the cartridge for 15 min at high vacuum (10-15 in Hg). The cartridge should appear dry (light tan color) before continuing with the elution steps.

Rinse the inside of each sample bottle with 6 mL methylene chloride and use vacuum to pull the solvent through the transfer tube and through the cartridge, collecting the solvent in a collection tube. Remove the transfer tubing from the top of the cartridge. Add 2 mL methylene chloride to the top of the cartridge with a disposable pipette. Pull this solvent through the cartridge at low vacuum, such that the solvent exits the cartridge in a drop-wise fashion. Repeat this step once. Pass the eluate through the drying column, which is packed with approximately 5-7 g of anhydrous sodium sulfate, and collect in a clean collection tube. Wash the sodium sulfate with at least 2 mL methylene chloride and collect in the same tube.

- 6.3 Concentrate the sample to approximately 0.1 ml. Add 60:40 Methanol: H₂O to bring the volume to 1 ml. Rinse the low 25% of the Zymark tube, bring up the final volume to 1mL Add 10 ul of the internal standard. Make any volume adjustments with 60:40 Methanol: H₂O – to the 1.0 ml marked level. Transfer the 1.0 ml extract into 2 Waters autosampler vial (1 with limited-volume insert) and store the sample extract in the refrigerator at 0°C or below.

7. ANALYSIS

- 7.1 Initial Calibration: A five point calibration curve is required for this method; using standards STD-A (1ppb), B (2 ppb), C (5 ppb), D (10 ppb) and E (20 ppb)). For each analyte and surrogate, calculate the mean RF and the relative standard deviation (RSD). The %RSD of any analyte or surrogate within the calibration range must be less than 30%. If not – inject other standards or perform system maintenance and begin the system analysis and performance check over.
- 7.2 Continuous Calibration check:
- Reagent blank must be monitored for every extraction run - monitoring of reagent blank is essential to the success of this method. Verify the calibration by measurement of two mid-point calibration check standards, one at the beginning and one at the end of the run. The check standard area counts must be within +/- 30% of the standard curve. LFBs (laboratory fortified blanks) should also be analyzed with each extraction run. Standards used for these QA/QC samples must be ordered from a second source whenever possible. For extended runs, check standards should be interspersed with samples at regular intervals. If the response of any analyte varies from the predicted response by more than +/- 30%, test must be repeated using fresh calibration standards. Data is collected and processed by Empower software. Any results above the highest calibration standard must be confirmed with a standard that is within +/- 20% of the actual result or the sample must be diluted to within the calibration range.

7.3 LC/MS Operating Conditions:

LC Conditions: Column Temperature: 40°C
Gradient elution:

<u>Time</u>	<u>Flow</u>	<u>Solvent A</u>	<u>Solvent B</u>	<u>Solvent C</u>	<u>Curve</u>
		<u>H₂O</u>	<u>ACN</u>	<u>5mM Ammonium Acetate</u>	
0	0.30ml/min	0	50	50	--
0.5	0.30	0	50	50	5
13	0.30	0	100	0	5
14	0.30	0	50	50	1
40	0.30	0	50	50	1

MS Conditions:

Scan: SIR (Single Ion Monitoring)
Acquire time: 0 to 15 Min. for both ES- and ES +

	<u>Mass (m/z)</u>	<u>Dwell (secs)</u>	<u>Cone Volts</u>
ES-	169.2	0.30	45
	195.1	0.30	50
	205.2	0.30	50
	219.36	0.30	45
	227.3	0.30	45
	241.6	0.30	50
	247.11	0.30	45
	263.00	0.30	45
	542.9	0.30	55
ES+	265.4	0.30	50
	309.00	0.30	50

8. QA/QC REQUIREMENTS

- 8.1 Laboratory Reagent Blank - Run before samples. Use to demonstrate that all glassware and reagent interferences are under control. If any contamination peaks are produced, which inhibits quantitative of target compounds, determine source of contamination and eliminate interference.
- 8.2 Laboratory Fortified Blank - Must analyze at least two laboratory fortified blanks (LFBs) with each set of extractions. Run a LFB-low and an LFB-high with each analytical run. The fortification concentration of each analyte should confirm the ability to detect at the reportable level. If the recovery of any analyte falls outside the control limits, the source of the problem must be identified and corrected.
- 8.3 Standards - Verify calibration standards quarterly by analyzing a standard prepared from reference material obtained from an independent or second source. EPA performance evaluations are an excellent process to determine the validity of the method. Results must be within +/- 30% of those used to routinely check calibration. For every batch of sample, run a low level standard to check the reportable detection level, RDL.
- 8.4 Samples - Samples must be extracted within 14 days after collection – unless specific targets need to be determined. Samples must be stored at 4°C until ready for extraction. Duplicates are run on 10% of samples, or once during run, whichever is greater. Results should be within +/- 20%. Extracts should be analyzed within 14 days after sample extraction.
- 8.5 Spike Recoveries - The laboratory must add a known concentration of spike solution, the same as used for LFB, to at least 10% of samples or once per

analytical run, whichever is greater. Recoveries should be within the acceptable range. Wherever possible, run a second source standard for spikes.

- 8.6 QC Requirements - Analyze EPA QC check sample with known values *if available*. The results for each analyte must be within the EPA acceptance criteria. Annually, analyze EPA Performance Evaluation samples. Analyze these check samples whenever major maintenance to the system occurs to ensure the validity of the method.
- 8.7 When running the daily midpoint standard, ensure that the absolute areas of the quantitation ions of the internal and surrogate standards have not decreased by more than 30% of the continuing calibration check or more than 50% of the initial calibration curve.

9. PREVENTIVE MAINTENANCE AND CORRECTIVE ACTIONS

- 9.1 Glassware - Glassware must be carefully cleaned. Clean all glassware as soon as practical after use by thoroughly rinsing with last solvent used in it. Follow by washing with hot water and detergent and thoroughly rinsing with tap water followed by reagent water. After drying, heat in oven. Do not heat volumetric glassware above 220°C. Thorough rinsing with acetone may be substituted for heating.
- 9.2 Reagents - The use of high purity solvents and reagents will help to minimize contamination problems.
- 9.3 Carryover - Contamination carryover may occur when a sample containing a low concentration of analytes is analyzed immediately following a sample containing a high concentration. Use solvent rinses between samples to minimize carryover.
- 9.4 All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory method blanks. Minimize contact of the samples, reagents, or solvents with any plastics. This will help reduce contamination. Interferences by phthalate esters can pose a major problem in pesticide analysis.
- 9.5 Record all corrective actions in the maintenance log book. Include a complete description of the problem and actions were taken to correct it.

Phenols Method – Daily Instrument Performance Checks

Sample Description	Frequency	Acceptance Criteria	Remedial Action
Low Level CCC – “SFA”	Each Analysis Run	70 – 130% target recovery	Instrument Maintenance & Check Standards
Mid Level CCC – “SFC”	Each Analysis Run	70 -130% target recovery	Instrument Maintenance & Check Standards
“RB” – reagent blank	Each Extraction Set	All targets must be less than 1/3 of the RDL	Isolate Source of Contamination and Re-Extract
Low LFB – “LFL” – reportable level	Each Analysis Run	50 – 150% target recovery	Check SPE Disk Lots – Verify Extraction Procedures – Re-Extract
LFB – “LFB” - mid level	Each Analysis Run	70 – 130% target recovery	Check SPE Disk Lots – Verify Extraction Procedures – Re-Extract
Sample Spikes – “S” & “K” “S” – spike “K” – spike dup	Each Analysis Run – 10% minimum of total sample load	70 – 130% recovery	Investigate Matrix Issues – Check Standards and Re-Extract
Field Sample	Run Analysis	Check Surrogate Recovery 50 – 150%	Investigate Matrix Issues – Check Standards and Re-Extract
Back Standards – “SBs”	Each Analysis Run – Every 10 samples must be bracketed with a CCC std	70 – 130% target recovery	Instrument Maintenance & Check Standards
Initial Calibration - “STs”	Started Before Each Analysis Run	Must use 5-pt calibration Lowest Standard must be at or below reportable detection level (RDL) Calib. Curve - Less than 20% RSD & R2>0.970	Check Standard Lots & QC – Re-shoot or Open New Standards Instrument Maintenance
Sample Duplicate – “D”	Each Analysis Run – 10% minimum of total sample load	50% RPD	Results Reported – Re-Extract if possible
MDLs	Each New SPE Lot or Major Instrument Maintenance (at least twice per year)	Calculated MDL must be no more than 1/3 the RDL	Instrument Maintenance, Extraction Procedures, & Check Standards
IDC – Initial Demonstration of Competency	Change in instrument, extraction technique, or chemistry of the method.	70 – 130% target recovery and <20% RSD	Instrument Maintenance, Extraction Procedures, & Check Standards – Re-Run

SOP PROCEDURE CHANGE
For HOMONES Method

CHANGE

REASON

DATE

INITIALS

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

EPA Method 556

Last updated: 11/09/2009

DETERMINATION OF CARBONYL COMPOUNDS IN DRINKING WATER BY PENTAFLUOROBENZYLHYDROXYLAMINE DERIVATIZATION AND CAPILLARY GAS CHROMATOGRAPHY WITH ELECTRON CAPTURE DETECTION

1. SUMMARY OF METHOD

EPA method 556 is used in the District's monitoring of carbonyl compounds in drinking water. A 20 ml volume of water sample is adjusted to pH = 4 with potassium hydrogen phthalate (KHP) and the analytes are derivatized at 35°C for 2 hrs with 15 mg of o-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine (PFBHA) reagent. The oxime derivatives are extracted from the water with 4 ml hexane containing internal standard. The extract is processed through two acid wash steps, and then analyzed by GC-ECD.

2. ANALYTES

This is a gas chromatographic method optimized for the determination for selected carbonyl compounds in finished drinking water and raw source water. The analytes applicable to this method are derivatized to their corresponding pentafluorobenzyl oximes. The oxime derivatives are then extracted from the water with hexane. The hexane extracts are analyzed by capillary gas chromatography with electron capture detection (GC-ECD) and quantitated using procedural standard calibration. Below is a list of the target analytes:

Formaldehyde
Acetaldehyde
Propanal
Butanal
Pentanal
Hexanal
Heptanal
Octanal
Nonanal
Decanal
Cyclohexanone
Crotonaldehyde

Benzaldehyde
Glyoxal (ethanedial)
Methyl glyoxal

Internal Standards

1,2-Dibromopropane - (400 µg/L in hexane)

Add 40 µL of internal standard stock solution (10,000µg/mL) directly to 1 L of hexane in a volumetric flask. Cap flask and invert three times to ensure thorough mixing.

Surrogate Standards

2',4',5' -Trifluoroacetophenone (20 µg/ml in acetonitrile)

3. APPARATUS AND EQUIPMENT

- 3.1 Sample Bottles – 40 ml amber glass, screw cap vials and caps equipped with Teflon-faced silicone septa.
- 3.2 Autosampler vials – amber vials equipped with PTFE-lined septum.
- 3.3 Analytical balance: Capable of weighing accurately to nearest 0.0001 g.
- 3.4 Water bath - Capable of maintaining 35±2 °C
- 3.5 Volumetric Flasks
- 3.6 Gas Chromatography—Capillary Gas Chromatograph equipped with a split/splitless injector, or other injector suitable for trace analysis, and an electron capture detector
- 3.7 Primary Column- 30 m x 0.25mm Varian Factor Four VF-5ms, 0.25 µm film thickness
- 3.8 Confirmation Column- 30 m x0.25 mm J&W DB-1, 0.25µm film thickness

4. REAGENTS AND CONSUMABLE MATERIALS

- 4.1 Reagent Water – Millipore Milli-Q system or equivalent.

- 4.2 ACETONITRILE, HEXANE -- Burdick & Jackson - capillary GC² solvent.
- 4.3 POTASSIUM HYDROGEN PHTHALATE (KHP)--ACS grade or better.
- 4.4 AMMONIUM CHLORIDE-- ACS grade or better.
- 4.5 SULFURIC ACID—concentrated and 0.2N (add 5 mL of concentrated sulfuric acid to 900 ml reagent water).
- 4.6 COPPER SULFATE PENTAHYDRATE –ACS grade or better.
- 4.7 O-(2, 3, 4, 5, 6- PENTAFLUOROBENZYL)-HYDROXYLAMINE HYDROCHLORIDE (PFBHA) – Prepare a fresh 15mg/ml solution in reagent water at the same day when the samples are processed.

5. SAMPLE COLLECTION, PRESERVATION AND HANDLING

- 5.1 Samples are collected in 40 ml amber container, fitted with a polypropylene screw cap and PTFE-faced silicon septa. The collected sample should be headspace-free. Keep samples sealed from collection time until analysis. Sampling equipment must be free of plastic tubing, gaskets, and other parts that may leach interfering analytes into the sample.
- 5.2 Preservation reagents (15mg of copper sulfate pentahydrate) are added to each sample bottle prior to shipment to the field. If water may contain free chlorine, 15mg of ammonium chloride must also be added.
- 5.3 All samples should be iced or refrigerated at 4 °C and kept in the dark from the time of collection until extraction. After addition to the sample, seal the bottle and shake until contents dissolve. Store all samples at 4°C until ready for extraction. All samples must be extracted within 7 days after collection. The extract should be stored at 4°C or less and analyzed within 14 days after sample extraction.
- 5.4 The processing of a field blank (travel blank - TB) is recommended along with each sample set. A sample bottle for the TB is filled at the laboratory, with the same preservation reagents added, and shipped to the site(s) along with the other sample bottles. The TB is processed in the same manner as the samples.
- 5.5 Prior to and during sample processing, the exposure of sample to the ambient air (which may contain formaldehyde) should be minimized.

6. EXTRACTION

- 6.1 Remove the samples from storage and allow them to equilibrate to room temperature.
- 6.2 Remove 20 mL of sample and discard. Mark the level of the remaining sample volume on the outside of the bottle for later sample volume determination.
- 6.3 Add 200 mg KHP to the sample for pH=4.
- 6.4 Add 20 μ L surrogate solution.
- 6.5 Add 1 mL of freshly prepared PFBHA reagent. Cap and swirl gently to mix.
- 6.6 Place all samples in a water bath at 35 °C for 2 hrs for derivatization.
- 6.7 After derivatization, remove vials from water bath and cool to room temperature for 10 min.
- 6.8 To each vial add 0.05 ml (approximately 2 to 4 drops) of concentrated sulfuric acid.
- 6.9 Add 4 mL of hexane that contains the internal standard.
- 6.10 Shake at 720 rpm for 3 min. Let stand for approximately 5 min to permit phases to separate.
- 6.11 Draw off hexane layer into a 40 mL amber vial containing 3 mL 0.2 N sulfuric acid. Shake manually for 30 sec and let stand for 5 min for phase separation.
- 6.12 Draw off top hexane in two autosampler vials.

7. ANALYSIS

- 7.1 Identify the method analytes in the sample chromatogram by comparing the retention time of the suspect peak to the retention time of an analyte peak (or isomer peaks) in a calibration standard or the laboratory fortified blank.
- 7.2 Two chromatographic peaks (E and Z isomers) will be observed for many of the target compounds. Formaldehyde will have only one peak, and double peaks for Octanal, Nonanal, and decanal usually co-elute. Glyoxal and methyl glyoxal may have more than one peaks. These double peaks are indicative of the method performance. When possible, both peaks should be used in calibration and quantitation.
- 7.3 Initial Calibration: A six point calibration curve is required for this method; using standards STD-A (10 ppb), B (20 ppb), C (40 ppb), D (60 ppb), E (80 ppb), F (100 ppb). At least five calibration curve points must be utilized for each target compound. For each analyte and surrogate, calculate the mean RF and the relative standard deviation (RSD). The %RSD of any analyte or surrogate within the

calibration range must be less than 30%. If not – inject other standards or perform system maintenance and begin the system analysis and performance check over.

GC Conditions:

- | 1. | Seg | Temp | Rate | Hold | Total |
|----|-----|------|----------|------|-------|
| | 1 | 50 | 0 | 1.0 | 1.0 |
| | 2 | 220 | 4°C/min | 1.0 | 44.50 |
| | 3 | 250 | 20°C/min | 8.0 | 54.00 |
2. Carrier Gas: Helium
3. Column: Varian Factor Four VF-5ms, 0.25 mm x30 m, film thickness 0.25um
4. Injection: 2 uL Splitless, 220°C

8. QA/QC REQUIREMENTS

- 8.1 Laboratory Reagent Blank - Run before samples. LRB is to demonstrate that all glassware and reagent interferences are under control. If any contamination peaks are produced, determine source of contamination and eliminate interference.
- 8.2 Laboratory Fortified Blank - Must analyze at least two laboratory fortified blanks (LFBs) with each set of extractions. Run a LFB-low and an LFB-high with each analytical run. The fortification concentration of each analyte should confirm the ability to detect at the reportable level. If the recovery of any analyte falls outside the control limits (70-130%), the source of the problem must be identified and corrected.
- 8.3 Standards - Verify calibration standards quarterly by analyzing a standard prepared from reference material obtained from an independent or second source. EPA performance evaluations are an excellent process to determine the validity of the method. Results must be within +/- 20% of those used to routinely check calibration. On a daily basis, run a low level standard to check RDL.
- 8.4 Samples - Samples must be extracted within 7 days after collection – unless specific targets need to be determined. Samples must be stored at 4°C until extraction. Duplicates are run on 10% of samples, or once during a single run, whichever is greater. Results should be within +/- 20%. Extracts should be analyzed within 14 days after sample extraction.

- 8.5 Spike Recoveries - The laboratory must add a known concentration of spike solution, the same as used for LFB, to at least 10% of samples or once per analytical run, whichever is greater. Recoveries should be within the acceptable range. Wherever possible, run a second source standard for spikes.
- 8.6 QC Requirements - Analyze EPA QC check sample with known values if available. The results for each analyte must be within the EPA acceptance criteria. Semi-annually, analyze EPA Performance Evaluation samples. Analyze these check samples whenever major maintenance to the system occurs to ensure the validity of the method.

9. PREVENTIVE MAINTENANCE AND CORRECTIVE ACTIONS

- 9.1 Glassware - Glassware must be carefully cleaned. After drying, heat in oven at 130 °C for several hours before use. Do not heat volumetric glassware. After cleaning, glassware should be stored in a clean environment to prevent any accumulation of dust or other contaminants.
- 9.2 Reagents - The use of high purity solvents and reagents will help minimize contamination problems. The most likely interferences are formaldehyde and acetaldehyde in the reagent water. The use of high-purity water with lowest possible formaldehyde concentration is important for standard preparation.
- 9.3 Carryover - Contamination carryover may occur when a sample containing a low concentration of analytes is analyzed immediately following a sample containing a high concentration. Use solvent rinses between samples to minimize carryover.
- 9.4 Minimize contact of the samples, reagents, or solvents with any plastics. This will help reduce contamination. Care should be taken to minimize exposure of reagents and sample water with laboratory air, which may contain trace amount of formaldehyde.
- 9.5 Record all corrective actions in the maintenance log book. Include a complete description of the problem and what actions were taken to correct it.
- 9.6 In case of poor chromatography, the following corrective actions need to be taken.
- 9.6.1 Check all the standards. If calibration and/or QC lie outside the acceptable limits we must first re-run the standards to assure that the injection procedure is working properly. After the re-run if standards still lie outside the acceptable limits we must make a new standard or conduct instrument maintenance to assure that calibration and QC lie with acceptable parameters. For elevated spike recoveries make sure that the sample matrix is clean. If sample matrix is altering recoveries then a new matrix must be selected. If instrument performance is acceptable the samples must be re-

extracted. If the sample lies outside allowable holding time they must be re-sampled.

9.6.2 Request re-sampling if necessary.

9.7 If there are hits, confirm by spiking sample. Analytical hit will be increased by the amount spiked in.

DATE INITIALS

This image shows a single sheet of white paper with horizontal ruling lines. The lines are evenly spaced and run across the width of the page. There are no margins or other markings visible on the paper.

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURES

EMPOWER PRO SOFTWARE FOR HPLC METHODS

Empower Pro Software Procedures

File Name: M:\Sop\Organic\waters_millenium\Empower Pro_111809.doc

Effective Date: 11/18/09

Revision: 1

Supersedes:

1. SUMMARY OF METHOD

This SOP describes the procedures used to acquire, process, review, reprocess, print, export and backup data within the Empower Pro Software. This SOP also describes a method for a library search of an unknown peak.

2. ACQUIRING DATA

- 2.1 The Empower Pro program is booted from the hard drive. The system will default to loading Empower.
- 2.2 Log-in as "Analyst", there is no password. Administrator log-in requires password.
- 2.3 You will be in the "Empower Pro" window. There are 6 icons with labeled "Run Sample"; "Browse Project"; "Configure System"; "Process Data"; "Review Data" and "Print Data"
- 2.4 Click on "Run Samples" icon. You will see 2 windows titled "Project" and "Chromatographic System". Highlight to select your project.
- 2.5 Choose the Detector:
For EPA_531; 547 select HPLC_2475_ONLY
For EPA_549; 532 select HPLC_2998_ONLY
For EPA_550 select HPLC_2475_2998
Click OK

Note: Confirm that the pump, auto-sampler and detectors are on. Make sure the mobile phases, solvents and needle rinse solvent are full. Also, check your waste bottle.

- 2.6 In Run Sample window, click on "Samples" tab at the bottom of Sample Set Method.
Select: File/New Sample Set Method/Based on Existing Sample Set Method - load the most recent sample set.

Note: Follow the Daily Setup Tips before continue to next step.

- 2.7 The first line in the Sample Set should have a Clear Calibration command in the column titled 'Function.' Click on the box and choose this option from the pick list. Go to the next column titled 'Method Set Report' and click on it. Choose a method set from the pick list. You will not be able to run samples if a method set is not specified. The Clear Calibration command will clear all calibration curves in memory. If this is not done, Empower may simply append today's calibration curve onto the calibration curve that it has in its memory.
- 2.8 Once the sample set is loaded, input the sample names to reflect current standards and lab numbers. Click on the Setup Instruments icon which is located next to the Monitor icon at the bottom right side of the window. Then click the Run icon which is the icon that has a green line across the icon. Empower will ask you to name the sample set. Name the sample set by Method_Date (Ex. 549_111809). Your run will begin once you type in the name of the sample set and then hit enter.

3. REVIEWING / PROCESSING DATA

- 3.1 Once your run is complete, close the Run Sample window. (optional - because samples can be processed without closing the run sample window)
- 3.2 Go to Empower Pro Main Window.
Click on Browse Project icon/ select Project/OK
- 3.3 Batch processing may not give you the desired baseline integration for correct quantification of unknowns, QAQC samples, or nonlinear calibration curves. To correct for these errors process sample set/injection manually. Follow these steps:
1. Go to Empower Main Window. Click on Browse Project → select Project → OK
 2. Click on the **Sample Set** tab within **Project** window.
 3. Highlight the sample set name to be processed manually. Double click to open the sample set.
 4. Highlight calibration standards click Process. You should now be in the Background Processing and Reporting window. Check Clear Calibration box and select Calibration Only in the "How" window → **OK**
 5. Click on Results tab in the Project Window to view calibration results
 6. Highlight the calibration results → right click and select Review. In this Review window, samples can be manually integrated. Click on **Calibrate/Save** after each injection is manually integrated. Saving results will also save new calibration curves.
 7. Once the calibration curves are established, process the QCs and samples in the same manner. Follow step 3 to open sample set.

8. Highlight QCs and samples click Process → select Quantitate Only in the “How” window → **OK**. Follow step 5 to open results window.
9. Follow step 6 but click on **Integrate/Save** after each injection is manually integrated.

To view calibration curve(s) click on the **Curves** tab in the **Project** window. Click on **Update** if you do not see the most recent processed curve(s). Highlight the desired curve, **Right** click and choose **Compare**.

4. REPROCESSING DATA

- 4.1 To redraw baselines on a sample, click on the peak start or peak end arrow that is located underneath the chromatogram. Drag this arrow and release to establish the proper peak start/end then click Quantitate, Save (to save the results).

Note: You may not see this result file in the Review Window. You need to exit the Review window, go back to the Project window -- click on result tab to reenter the Result View Table. Your reprocessed result file should now be at the top of the table and can be identified by the processing time.

- 4.2 To redraw baselines on a standard, follow step one but click Calibrate instead of Quantitate. Then reprocess your samples using the reprocessed calibration curve. Do this by highlighting the samples you want to reprocess using the ‘new’ calibration curve (the one that has the standard with the redrawn baseline), then Process. The reprocessed results will only be seen in the project window under Result Tab. To view and print the reprocessed results, highlight the standards and samples of interested.
- 4.3 If changes to the method result in changes in retention times, the retention times may be updated as follows. First, select a Result, then Review. Click on Process Method button to open the method. Click on 'Components' tab located in the middle of the window. Enter the retention time then Save Method, and reprocess the Sample Set.

5. PRINTING / EXPORTING DATA

Printing and Exporting data can be done simultaneous or one at a time depends on the Analyst. The following steps can be use for both.

To print a batch processed samples, in the Results tab highlight the desired samples, Right click and choose Print. The Background Processing & Reporting window will be activated. In the Reporting portion of this window Method is preset with “use acquisition Method Set” -- do not change. Click OK.

Note: Exported files will be exported to **C:\Empower\Export\EPAXXX.ars** extension. No chromatograms will be exported, only results.

6. ADDITIONAL PROCESSING GUIDELINES

- 6.1 Alter sample after the analysis is done. Go to Project window select Sample Set tab, highlight a sample set, right click and select Alter Sample. While in the Alter Sample window, you may change the sample type, function, etc.. You may change the calibration curve from a three point to a single point and the standard amounts from a 10 ppb standard to a 5 ppb standard. To change the standard concentration, click on Edit/Amount then enter the values. Close this window to go back to Alter Sample Set window. Save the sample set. Reprocess the Sample Set.
- 6.2 To draw a perpendicular to separate overlapping peaks, depress the control button, then click on the chromatogram where you want the perpendicular drawn. To remove a perpendicular, depress the control button, then click on the diamond beneath the baseline.
- 6.3 To review a chromatogram during a run, open the project window. Click Sample Set, double click on the Sample Set, then highlight the sample in the Injections window, right click on process and select "quantitate only" click OK

7. BACKING UP PROJECTS

- 7.1 Data is archived by backing up projects. First create a directory on the M:\Archive\ drive for each method. This is where you want to store the project. Go to Configure System → select project → right click and select Backup Project Type comments for the project to be backup → click Next → browse the M:\ for each project → click Next to start backup project.
- 7.2 After the backup project is completed → check to make sure there are two files with extension .INF and .EXP in the back up folder. If not, re-do the backup project.

8. RESTORING PROJECTS

- 8.1 To restore a project, go to Configure System → select project → right click and select Restore Project, Browse M:\ and select the project to be restored → Next → Enter the project name → Next to start Restore; then the DOS screen appears and ".. importing table" statements should be scrolling down the screen.

9. CREATING/CLONING NEW PROJECTS

- 9.1 After you have backed up a project, a new project needs to be created. Clone a new project before delete the project that you have backed up.

10. LIBRARY SEARCH OF AN UNKNOWN PEAK

- 10.1 From the 532_Project window, select Result tab, highlight the sample and double click to open Review window.

- 10.2 In the Review window, select the unknown peak and right click at the apex of the peak select Extract Spectrum @...
- 10.3 In the Spectrum Review window, click Library, Open Library, choose DIURON. Then click off peaks that you do not want to match against the library by clicking on the spectrum displayed on bottom the right side of the window. Click on the spectrum and it will turn gray. The spectrum that you wish to compare with the library will still be in color. Then go to Library and click on Match against DIURON. If the unknown peak is confirmed as diuron, Empower will display the match angle results. If the unknown peak is not confirmed as diuron, Empower will say that a match was not found.

SOP PROCEDURE CHANGE
For MILLENNIUM SOFTWARE FOR HPLC METHODS

<u>CHANGE</u>	<u>REASON</u>	<u>DATE</u>	<u>INITIALS</u>
SOP	New Software- Empower Pro		LVS

2690 Daily Setup Tips

Introduction:

Most of the problems that people experience with the 2690, involve the solvent management system. Improper preparation of solvents, or running the system dry, can cause bubbles or clogs to form in the pumping system. The purpose of this document, is to help the user understand how to properly run the system, and to provide a guide in correcting problems caused by poorly prepared solvents, improper priming, and bubbles that may become trapped in the system.

Daily Operation:

1. Solvent Filtration:

- A. All solvents should be filtered to .45 micron before use.
- B. Buffers should be filtered as the last step before putting them on the system. Complete all pH adjustments etc. before filtering your buffer.
- C. Rinse all containers with filtered solvent before filling them.
- D. Solvents that are used directly out of the bottles that they are shipped in generally do not need to be filtered to remove particulates, however, filtering them is a good way to degass the solvent.
- E. Cover all solvent reservoirs with aluminum foil to keep dust out of your filtered solvents. Parafilm has a nasty tendency to extract organics that will show up in your gradient runs.

2. Degassing:

- A. Vacuum filtration is the quickest and most thorough way of degassing solvents.
- B. Solvents that were degassed yesterday are not degassed today. Over time, atmospheric gasses dissolve into your solvents. Most solvents will absorb considerable quantities of gas in 12-24 hours, and will need to be degassed again.
- C. The degassers in the 2690 are meant to be used as "polish" degassers to remove gasses that have been absorbed by your solvents since you degassed them. This allows you to run the instrument for a considerable period of time before the solvents need to be degassed again. They were never intended to be used to degas solvents that were saturated with dissolved gas (such as organic solvents packed under nitrogen).
- D. A quick degassing method that works best with organic solvents is sonication. Placing the entire solvent bottle in an ultrasonic bath for 10-15 min. will remove most of the gas.

General Note on the Vacuum Degasser: The vacuum degasser works by passing the solvent through a bundle of thin walled semi permeable tubes. The outside of the tubes is subjected to a vacuum. Gas in the solvent diffuses out of the solvent, through the wall of the tubes and into the vacuum chamber. This process takes time. If you set a flow rate through the degasser chamber faster than 3 ml/min, the amount of time the solvent spends in the chamber is too short for the gas to be removed from the solvent. This can happen if you use a syringe to pull solvents through the pumping system, or if you set a flow rate during the priming procedure that is too high.

3. Priming the system

- A. If you are going to put a buffer on the system, flush the line with 100% degassed water first. Perform a "dry prime" for 5 min to flush out any organic solvent that the previous user may have left in the line. This will avoid precipitation problem when you prime with your buffer.
- B. In general, start off by priming the individual lines that you will be using. Use the "dry prime" routine and prime each line for 5 min. During the prime, watch the system pressure. If you see pressure fluctuations, open the drawoff valve, pull with the syringe for 3-5 seconds, and close the valve. This should pull out any bubbles that are present in the pump heads.
 Note: At 3 ml/min during the dry prime, it takes 3-4 min before the new solvent reaches the pump heads from the solvent reservoir.
- C. After all of the lines have been individually primed, flush the degasser chambers by doing the following:
 - 1) Set the composition to flush all of the lines that you are going to use with equal volumes of solvent.
 - 2) Press the "Direct Function" Key
 - 3) Select "Wet Prime"
 - 4) Set a flow rate equal to 2.5 times the number of lines you are going to prime. Example: for 3 lines, set a flow rate of 7.5 ml/min
 - 5) Set a time of 20 min.
 - 6) Press "Start"

Note: This will flush each of the degasser chambers that you are using with 5 volumes of solvent. The flow rate is kept at 2.5 ml/min/chamber so that the degasser can operate properly. \

- 4. Purge the injector by doing the following:
 - A. Press the "Function" key
 - B. Select "Purge Injector"
 - C. Enter "6" column volumes
 - D. Check the "compression check" box
 - E. Press Start.
- 5. Start normal flow for the initial conditions for your analysis. Try to keep from raising the flow rate faster than 0.5 ml/min/min to avoid column damage.

Trouble Shooting

Pressure Fluctuations.

- 1. Are the solvents miscible?
 - a. Some solvents are not miscible with each other at all proportions. Check to make sure that the solvents you are using are miscible.
- 2. Buffer salts and ion pairing reagents can precipitate at high organic modifier concentrations.
 - a. Precipitated buffer salts will clog the check valves and tubing in the pump. If this happens, try flushing the pump with 100% water by using the "dry prime" function.
 - b. In general, Methanol will cause salts to precipitate at a lower concentration than Acetonitrile.
 - c. The 2690 has smaller tubing, and check valves in the pumping system than previous Waters systems. The smaller tubing is needed to reduce system volume for microbore operations, but it is somewhat more prone to clogging by precipitated buffer and particulates.

- 3 Bubbles may become lodged in the degasser or in the pump heads.
- a. Particularly with high water mobile phases, surface tension may cause bubbles to stick to surfaces in the degasser, or in the pump heads. Methanol is used to remove bubbles from the system because of its low surface tension and good wetting properties.
 - b. Clearing bubbles from the degasser chambers and pump heads is a 4 step process.
 - 1) Flush buffers from the lines using 100 % degassed water
 - 2) Flush the lines with 100 % degassed methanol.
 - 3) Flush the methanol out of the buffer lines with 100% degassed water.
 - 4) Flush the water out with your buffer.

**8. STANDARD
OPERATING PROCEDURES
INORGANIC**

CHAPTER 8

STANDARD OPERATING PROCEDURES INORGANIC AND MICROBIOLOGY

INORGANIC METHODS

Standard Operating Procedures for inorganic methods included in Chapter 8 are as follows:

TAB NAME – SOP NAME

- Alk. Auto – Alkalinity by Autotritator
- Alk. Manu – Alkalinity, Total and Phenolphthalein (Manual Methods)
- Ammonia Dist – Ammonia (as Nitrogen) When Distillation Is Required
- Boron Curcu. – Boron (Curcumin Method as a back-up)
- CN by FIA – Cyanide by FIA
- COD – Chemical Oxygen Demand
- COD Reactor Digestion – COD by Block Digestor Method
- Color
- Dis/Tot S²⁻ – Dissolved Sulfide/Total Sulfide Methylene Blue Colorimetric Method (Photometric Method)
- EC – Electrical Conductivity
- Fluoride – Fluoride by Probe
- GFAA – Graphite Furnace Atomic Absorption
- Hg-Cold Vapor – Mercury by Cold Vapor Atomic Absorption
- Hydrogen Peroxide (H₂O₂)
- ICP-OES Perkin Elmer – Trace Elements by Inductively Coupled Plasma-Optical Emission Spectroscopy
- ICP/MS – Inductively Coupled Plasma / Mass Spectrometry
- Inorganic DBP – Inorganic Disinfection Byproducts (Dionex DX500 IC)
- Iodine # - Determination of the Iodine Number of Activated Carbon
- Ion Chrom. – Ion Chromatography
- Lime CaO – Analysis of Lime Samples

- MBAS – Methylene Blue Active Substances (MBAS) [Surfactants]
- NH_3 by FIA – Ammonia (as nitrogen) by FIA
- NO_3/NO_2 by FIA – Nitrate/Nitrite (as nitrogen) by FIA
- Org-N/TKN by FIA – Organic Nitrogen and Total Kjeldahl Nitrogen by FIA
- Perchlorate – Perchlorate (Dionex DX500 IC)
- pH
- Residual CL_2 - Residual Chlorine
- Set. Solid – Settleable Solids (Volumetric Method)
- Silica – Silica (Molybdosilicate Method)
- Suitability Test
- Sulfide
- Sus. Sol – Suspended Solids
- TDS – Total Dissolved Solids (TDS) also known as Total Filterable Residue
- TOC-High Level – Total and Dissolved Organic Carbon O.I. Model 1010 High-Level Analysis
- TOC/DOC-Low Level – Total and Dissolved Organic Carbon O.I. Model 1010 Low-Level Analysis
- TON – Threshold Odor Number
- Total Hardness – Hardness, Total (mg/l as CaCO_3)
- Trace Metals Prep
- Trace Metals Sample Handling
- Turbidity
- UVAB – U.V. Absorbance or Percent Transmittance (CARY)
- Dissolved Cr VI – Dissolved Hexavalent Chromium

MICROBIOLOGY

- Coli MPN – Coliforms by Multiple Tube Fermentation Technique
- F. Coli. – Fecal Coliform Analysis (Membrane Filter Method)
- HPC – Heterotrophic Plate Count (A. Pour Plate Method, B. Spread Plate Method)
- Quanti-Tray – Total and E. Coli Analysis by Quanti Tray Method
- Total Coli. – Total Coliform Analysis (Membrane Filter Method)

**Please See the Standard Operating
Procedures Binder**

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

ALKALINITY AUTOTITRATOR

File Name: M:\SOP\INORGNIC\ALKALINITY AUTO.doc
Revision: 10

Effective Date: 6/19/2008
Supersedes: 9 (3/7/07)

1. REFERENCES

- 1.1 *Standard Methods* 20th Ed. #2320 B
- 1.2 *EPA Method for Analysis of Water and Waste*, #310.1 (adapted for use with autotitrator). This method complies with EPA-approved methodology for analysis of drinking water and wastewater.

2. HOLDING TIME

- 2.1 **Total Alkalinity:** 14 days @ 4° C. **pH and Phenolphthalein Alkalinity:** no holding time; analyze immediately.

3. INTERFERENCES

- 3.1 Soaps, oily matter, suspended solids and precipitates may coat the glass electrode and cause sluggish response. Clean electrode occasionally.

Note: EPA Performance Evaluation instructions specify titration of deionized water blank for subtraction from sample alkalinity concentration. Because of the limitation of the instrument method when titrating ultra pure water this method leads to over correction and subsequent low results. Do not use a blank correction with the auto-titration method, and check results with manual method.

4. APPARATUS

- 4.1 Brinkmann model 716 DMS Titrino autotitrator
- 4.2 Sample changer model 748 DH with combination pH/reference electrode.
- 4.3 Plastic sample beakers, 250 mL; **now used as disposable sample containers.**

5. REAGENTS

- 5.1 Buffer Solutions: pH 5, 7, 8, and 10, NIST traceable
- 5.2 Titrant: 0.020N H₂SO₄

- 5.3 Soaking Solution, 3M KCl : Dissolve 223.665g KCl in 1000 ml water.
- 5.4 Stock Standard, 1000 mg/L: Dissolve 1.060g Na₂CO₃, dried at 140°C for 4 hours, in 1000 mL of CO₂-free (boiled) water. Prepare fresh monthly.
- 5.5 Working Standard, 100 mg/L: Dilute 100 ml of Stock Standard in 1000 mL of CO₂-free (boiled) water. Prepare fresh weekly.

6. PROCEDURE

Note: Greg DeMattia of Brinkmann Instruments can often provide valuable assistance. Voicemail: 1-800-645-3050 X2472; Cellphone: 1-949-678-4347.

Note: Use a 50 mL sample size for analysis. Do not filter, dilute, concentrate or alter sample.

Note: Before analysis, make sure the bottles are filled with their respective contents; namely, 0.020N Sulfuric acid and DI water. **Empty waste container when it is more than ½ full.** Fill the electrode with 3M KCl soaking solution. **Check that the attached drying tube is filled with Ascarite™, and that it has been changed within the last month.**

6.1 Startup Procedure

6.1.1 Turn 748 DH sample changer and 716 DMS Titrino Power on **at power strip.**

6.1.2 Turn on the computer and the printer

6.1.3 If necessary, convert the hardware configuration from Chlorine to pH/Alkalinity (**TAKE CARE NOT TO BREAK FRAGILE PARTS WHEN FOLLOWING THESE STEPS**):

6.1.3.1 Grasp the Chlorine titrant bottle holder by the upright plastic cylinder at the **front** of the unit, pull toward yourself to disengage from the mechanism, and remove from the top of the Titrino unit.

6.1.3.2 Carefully set the Chlorine unit on the bench top, and put the Alkalinity unit in its place atop the Titrino unit, sliding it back along the top of the Titrino unit until it locks into place and the motorized lever in front of the unit automatically rotates from one side to the other. Be careful not to drop either unit, spill the titrant(s), or tangle the titration tubing or cables of the two units.

6.1.3.3 Remove the platinum wire Cl₂ electrode from the electrode holder (it should have been in the 2:00 position, looking down from above). Place the electrode in its holder on the right side of the autotitrator unit (**store this electrode in DI water; it must be kept wet**).

6.1.3.4 Tuck the Cl₂ titration tube into the tubing clamp. Remove the pH combination electrode from the KCl solution container, and place the electrode in the electrode holder at the 4:00 position.

6.1.4 Place pH buffers into the 748 sample changer as follows:

Cup 46: pH 10 Buffer

Cup 47: pH 8 Buffer (or other buffer value meeting *Standard Methods* requirement of ± 2 pH units from sample value(s))

Cup 48: pH 7 Buffer

6.1.5 Place DI water, CC standard, 2nd source and samples to the 748 sample changer as follows:

Cup 1 and 2: DI water

Cup 3: CC standard

Cup 4: MDL standard (5 mg/L as CaCO₃)

Cup 5: 2nd Source (pH5 buffer or other as appropriate)

Cup 6 - 45: Samples

NOTE: for ROP and UVP samples, insert 3 samples of each for pH only to act as “rinses” for the pH electrode before the “real” pH/Alkalinity sample of each, respectively.

6.2 Sample Analysis

6.2.1 Double click on **Titrimo** icon. Type your initials in user text box. Click **OK** to log on. **TWC Main Menu** window appears.

6.2.2 From the **View** menu, choose **worklists**. **TWC Worklist Manager (1)** window appears

6.2.3 To display the list of Macros, click the down arrow next to the **No Macro** box. Click **CalphAlk2** from the list. You might have to scroll through the list to find it.

6.2.4 From the **Sample** menu, choose **Add** to create Worklist. Click on **Add** as many as the number of samples to be run. From the **Sample** menu, choose **Line Edit Mode**. Type sample ID number in Id#1 column and sample reference number in Id#2 column (example: 00060123-01, 00060123 is ID number and 01 is reference number). Select a Sub Macro for each sample. To display the list of Sub Macro, click on each cell in Sub Macro column. Choose **DETALK** from the list by scrolling through the list. **DETALK** is for both pH and alkalinity analysis; **if you wish to measure pH only (necessary with the 2nd Source pH 5 buffer), select “pH.”** Print out the Worklist.

*** If you choose to use the presaved worklist template instead type a new worklist, you must save the worklist before you start to run.**

Note: Type DI H₂O (twice), CC STD, MDL, and 2nd Source in line 1, 2, 3, 4 and 5 respectively.

Note: Samples can be added anytime during the analysis. Just repeat 6.2.4.

- 6.2.5 Click **“green arrow icon”** to start the analysis. The electrode will move to cup #48(pH 7) and the calibration will start. If the electrode doesn't go to cup #48, stop the analysis and reset. To reset, Click **“Changer”** icon from TWC main menu window. Go to **Command** and choose **Reset**. Restart the analysis by clicking **“green arrow”** icon again.

Note: Each sample Id # will be removed from the list, when the analysis is completed.

6.3 Creating Results Table (not currently used)

- 6.3.1 After the analysis is completed, create results table. From **View** menu, choose **Results**. TWC Result Manager (1) appears. Select the samples you want to report by clicking sample ID one by one while you're holding left control key. Alternatively, hold down the **Shift** key and press the **Down Arrow** key to highlight all the samples in your run.
- 6.3.2 Go to **Report**. Select **Microsoft Excel**. Execute Excel Template appears. Select **Sample of Excel Report #2- Multiple Samples.xlt**. Click **Open** to create results table. List Builder Dialog appears. Select table column header by clicking on it and click **“>>”**. Recommended headers are **Sample ID, User Id, Run Date, Run Time, Result1-ID, Result1 Value, Result2-ID, Result2 Value, Result3-ID, Result3 Value**. Click **OK**. Excel will create result summary table automatically. Format the table if necessary by **highlighting columns and using Text Align buttons to line up labels and results (Left Align Column A; Center Align all other columns)**. Print your result summary table.

6.4 Reporting results

It is very important that results be reported consistently as follows. The four possible endpoints include two inflection point endpoints: **“phenol”** and **“Total,”** and two fixed pH endpoints: **“FEP83”** and **“FEP45.”** If the pH is less than or equal to 8.3, do not report phenolphthalein alkalinity (ALKPHE). If the pH is greater than 8.3, report the **lower** of the **“phenol”** or **“FEP83”** values (only report inflection point value if it represents a pH value higher than or equal to 8.3). Similarly, report **“Total”** as the total alkalinity value if it is **less** than or equal to the **“FEP45”** value and the titration curve is

smooth and contains a typical inflection point. Otherwise, report the "FEP45" value for total alkalinity (TOTALK).

6.5 Shutdown Procedure

6.5.1 Close TWC Result Manager (1) window. Go to File from TWC Worklist Manager (1) window. Choose EXIT. Exit Titrimo Workcell by clicking "Opened Door" icon. **Shut down computer.**

6.5.2 Turn off 716 DMS Autotitrator, 748 DH Sample changer and computer. This completes the proper shutdown procedure.

Note: Fill the electrode with 3M KCl if necessary and make sure that the electrode storage beaker has enough 3M KCl soaking solution. (Change it every week.)

7. QUALITY CONTROL

7.1 **At start of sample run, include a pH 5 buffer solution (or buffer value < 10 and 3 pH units less than calibration standard in Cup 47 above). Result must be within ± 0.1 pH unit of theoretical (4.9 – 5.1, or other values as appropriate); if not, shut down analysis and correct problem before proceeding.** Run one duplicate for every ten samples or one per run for less than ten samples. Values should agree within 10%. Analyze two control chart check standards per run; results should be within control limits. EPA Performance Evaluation samples are run for external validation. Results should be within EPA-specified acceptance limits. Analyze sodium carbonate solution with second source titrant monthly (manual titration) and compare with reading obtained with primary titrant in autotitrator. Results should agree within 10%.

Holding time for pH = No holding time; analyze immediately (we normally analyze as soon as possible the same day received)

Holding time for Alkalinity = 14 days. While this is not specified as "Total Alkalinity only," we have found that analysis of Phenolphthalein Alkalinity (and subsequent calculations of OH, CO₃, and sometimes HCO₃ Alkalinity may be unreliable under the following circumstances, due to the significant number of samples we receive with pH close to the phenolphthalein endpoint:

1. Sample as collected has pH of < 8.3, and pH rises to ≥ 8.3 before being analyzed for phenolphthalein alkalinity. Phenolphthalein alkalinity will be **overestimated**.
2. Sample as collected has pH of ≥ 8.3 , and pH drops to < 8.3 before being analyzed for phenolphthalein alkalinity. Phenolphthalein alkalinity will be **underestimated**.

The simpler case is if the pH of sample as collected is < 8.3 ; if the alkalinity autotitrator later detects phenolphthalein alkalinity, the analyst can just enter "0" instead of the inaccurate result in the worksheet. In the reverse case, there **should** be Phenolphthalein, OH, and CO₃ Alkalinity, but none will be detected. "NA" should be entered for these on the worksheet, **as well as for** HCO₃, since the calculation cannot be performed properly. If the pH stays "on the same side" of the 8.3 endpoint, it is OK to report alkalinity, with supervisor approval (who will evaluate the likely accuracy of the result).

8. TROUBLESHOOTING

8.1 Make sure the electrode is clean, filled, and properly calibrated. Quality control standards (see above) should be no more than 1 week old. Check titrant regularly for changes in concentration or contamination. Before entering alkalinity values, check pH from autotitrator against laboratory manual pH readings obtained on the day sample was received, if any. If titration pH is greater than 8.3 and manual pH is less than or equal to 8.3, do not enter phenolphthalein alkalinity value. Enter 0 instead.

8.2 Check the numeric results and the graphic of the titration to verify that the inflection point was detected at the proper point, and that it is (normally) close to the theoretical pH of 4.5 (on the y-axis). See "Reporting Results" above.

SOP PROCEDURE CHANGE
FOR ALKALINITY AUTOTITRATOR

CHANGE	DATE	INITIAL
Revised Procedure #1 and Quality Control per ELAP	02/02/01	JMD
auditor to comply with <i>Standard Methods</i>		
Various changes to holding time, buffer solutions, procedure and results reporting	8/24/04	JMD
Changed name of default macro to TcalphAlk3m	9/21/04	JMD
Various changes to results reporting	10/12/04	SW
Changed <i>Standard Methods</i> edition; added Greg DeMattia's Phone #s; added module switching instructions for converting Amperometric Chlorine to Alkalinity	1/10/05	JMD
Amended with a worklist template option	07/18/05	LTL
Added rinse cups for IWF-RO-B and IWF-QUV-B; other minor changes	4/10/06	JMD
Clarified when to use fixed and inflection endpoints for titration results reporting	3/7/07	JMD
Added note on waste container, references to MDL; updated sample IDs; other minor changes.	6/19/08	JMD

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

AMMONIA (AS NITROGEN) WHEN DISTILLATION IS REQUIRED

File Name: M:\SOP\INORGNIC\AMMONIADIST.doc
Revision: 1

Effective Date: 06/27/2001
Supersedes: N/A

1. VAPODEST CONNECTIONS

1.1 Make all necessary tubing connections to **Vapodest** w/proper tubing as specified in the manual

1.1.1 H_3BO_4 (2-4% boric acid)

1.1.1.1 Prime pump by pressing the H_3BO_4 button

1.1.2 H_2O (DI water) for sample dilution

1.1.2.1 Prime pump by pressing the H_2O button

1.1.3 H_2O (DI water) for steam generator

1.1.4 Cooling water (tap water) for condenser; *minimum 1.3 bar!*

1.1.5 Water Outlet to drain for condenser

1.1.6 Sample suction to drain for sample tube waste

1.1.7 NaOH (32% w/w sodium hydroxide)

1.1.7.1 Prime pump by pressing the NaOH button

1.2 Connect the TL-Easy (titrator)

1.2.1 Connect the RS232 cable from the Vapodest to the TL-Easy, RS232-1 socket

1.2.2 Connect the electrode cable to the Vapodest and electrode to the cable and insert in vessel

1.2.3 Insert burette tip into distillation receiver/titration vessel

2. TL-EASY

2.1 Priming burette

- 2.1.1 Fill titrant reservoir with proper titrating reagent (0.02N H₂SO₄)
- 2.1.2 Prime burette according to the instructions in the TL-Easy manual to remove all air in burette and tubing
- 2.2 Calibrate pH electrode
 - 2.2.1 Calibrate daily according to the instructions in the TL-Easy manual
 - 2.2.1.1 Press the [CAL] button on the front of the TL-Easy.
 - 2.2.1.2 Insert electrode into buffer 7.00 and press [Start]
 - 2.2.1.3 Wait until "Buffer 1" is completed. The TL-Easy will prompt to place buffer 2 on.
 - 2.2.1.4 Rinse electrode with DI water
 - 2.2.1.5 Place Buffer 4.00 on and insert electrode, press [Start]
 - 2.2.1.6 After successful calibration, press "Mode" key until Titrator reads "EQ"
- 2.3 Entering the calculation
 - 2.3.1 Hold down "SET" key
 - 2.3.1.1 Select Calculation
 - 2.3.1.2 Select F1
 - 2.3.1.3 Enter a value of 280 for F1 by using arrow keys
 - 2.3.1.4 Enter a value of the blank for B by using the arrow keys
 - 2.3.1.5 Enter a value of sample size, for Q, by using arrow keys

3. VAPODEST PROGRAMMING

- 3.1 Turn on tap water for condenser
- 3.2 Make sure all reservoirs are full, especially the DI water for the steam generator. *An error message will appear if there is no tap water or DI water!*
- 3.3 You will receive the following message, when the unit is first turned on "Low water, press Enter". Press [Enter] and the steam generator will fill. The display will read "Filling steam generator"

- 3.4 The Vapodest is ready when the display reads "**Distillation = 1, Programming = 2**"
- 3.5 Entering the Programming mode = 2 will display the following parameters:
- 3.5.1 **Program #**; Enter 0 – 9 for 10 memory locations
- 3.5.2 **Add H₃BO₄**; Enter time that will dispense the volume necessary,
~10mL/sec
- 3.5.2.1 **Add H₃BO₄** = ~5 for both Nitrogen Ammonia & TKN (50mLs)
- 3.5.3 **Add H₂O**; Enter time that will dispense the volume necessary, *~10mL/sec.*
- 3.5.3.1 **Add H₂O** = 0 for Nitrogen Ammonia, = ~6 for TKN (60mLs) to insure a 6 fold dilution of the residual acid.
- 3.5.4 **Add NaOH**; Enter time that will dispense the volume necessary,
~10mL/sec.
- 3.5.4.1 **Add NaOH** = 0 for Nitrogen Ammonia & = ~6-7 for TKN (basic; 11pH)
- 3.5.5 **Reac. time**; Enter time before distillation begins.
- 3.5.5.1 **Reac. Time** = 0 for both Nitrogen Ammonia & TKN
- 3.5.6 **Dist. time**; Enter time to collect the necessary distillate volume.
Normally, 30mL/min is the distillation rate at 100%.
- 3.5.7 **Steam capac.**; Enter 100% for ammonia or TKN applications. *This is the power of the steam generator! 150watts/10%*
- 3.5.8 **Suction time**; Enter the time necessary to aspirate out the residue in the distillation tube. *This is very much dependent on the total volume in the distillation tube. Approximately 10mL/sec.*
- 3.5.9 Once the program is set, you need to only recall the appropriate method.
Note that all times are approximate and have to be determined experimentally!

4. RUNNING SAMPLES

4.1 Nitrogen Ammonia

4.1.1 Perform a blank

- 4.1.1.1 The volume titrated will be placed in the TL-Easy under section **2.3.1.4**, for the blank "B"
- 4.1.2 Sample preparation
 - 4.1.2.1 Add 50mLs borate buffer to sample
 - 4.1.2.2 Adjust pH of sample to 9.5pH with 6N NaOH
Note; if sample was preserved, adjust pH with NaOH before adding buffer!
 - 4.1.2.3. Transfer sample to distillation tube and place on Vapodest
- 4.1.3 Distillation
 - 4.1.3.1 The Vapodest is ready when the display reads "**Distillation = 1, Programming = 2**"
 - 4.1.3.2 Enter "1" for distillation (if program has been written and stored as is Section 3)
 - 4.1.3.3 Enter the program # for the application (i.e. Nitrogen Ammonia = 1 & TKN=2)
 - 4.1.3.4 Enter to start distillation
- 4.1.4 Titration of ammonia
 - 4.1.4.1 The TL-Easy will start titrating after the distillation is completed.
Make sure the TL-Easy is primed and calibrated according to section 2
- 4.2 Organic Nitrogen & TKN (Total Kjeldahl Nitrogen)
 - 4.2.1 Perform a blank
 - 4.2.1.1 The volume titrated will be placed in the TL-Easy under section **2.3.1.4**, for the blank "B"
 - 4.2.2 Sample preparation
 - 4.2.2.1 Sample is digested under acidic conditions with an appropriate catalyst
 - 4.2.2.2 After digestion and cooling of sample, transfer digestion tube to the Vapodest
 - 4.2.3 Distillation

4.2.3.1 The Vapodest is ready when the display reads “**Distillation = 1, Programming = 2**”

4.2.3.2 Enter “1” for distillation (if program has been written and stored as is Section 3)

4.2.3.3 Enter the program # for the application (i.e. Nitrogen Ammonia = 1 & TKN=2)

4.2.3.4 Enter to start distillation

4.2.4 Titration of ammonia

4.2.4.1 The TL-Easy will start titrating after the distillation is completed.
Make sure the TL-Easy is primed and calibrated according to section 2

5. Daily Maintenance

5.1. Make sure all reservoirs are full

5.2. Make sure tap water is turned on while running; turn off at the end of the day

5.3. Run DI water as a sample to “Steam-out” and clean the unit at the beginning and end of each day

5.4. Remove the viton cone and rinse with water to remove any NaOH crystals

6. Monthly Maintenance

6.1. Remove the NaOH reservoir and place dip tube in a hot water reservoir

6.2. Press the “NaOH” button to push the hot water through the NaOH pump; run 250mL twice

6.3. Press the “Empty” button to remove the waste from the tube

6.4. Place the dip tube back in the NaOH reservoir and press the “NaOH” button to prime the NaOH

CHANGE	REASON	DATE	INITIALS
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Page 6 of 6

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

CYANIDE BY FIA (Do NOT use FIA_CN.doc SOP)

File Name: M:\SOP\INORGNIC\FIA_CN.doc
Revision: 12

Effective Date: 8/31/2009
Supersedes: 11 (5/18/2009)

1. REFERENCES

- 1.1 *Standard Methods* 20th Ed. #4500-CN, pp. 4-32 to 4-53.
- 1.2 *EPA Methods for Analysis of Water and Waste*, #335.2 and 335.3.
- 1.3 Lachat Instruments QuikChem Method 10-204-00-1-A. This method complies with EPA-approved methodology for analysis of drinking water and wastewater.

2. SAMPLE HANDLING AND PRESERVATION

- 2.1 Prepare sample collection bottles in advance. Add 1.7 g NaOH pellets to a clean 2 liter brown plastic bottle. Add sodium arsenite solution (**see below**) to remove interference from chlorine or other oxidizing agents (use 1 mL per mg/L chlorine residual expected per L of sample). **Get chlorine residual values for GWRS samples from the DPD residual chlorine method analyst (and have him/her also analyze any CN samples not normally run for chlorine, e.g. Q1).** After sample collection and preservation, verify the pH. It must be at least 12. **Completeness of dechlorination may be checked using KI/Starch test paper. See Standard Methods for more detail.** Samples can be held for up to 14 days at 4°C.

3. APPARATUS

- 3.1 Reflux distillation apparatus with gas absorber.
- 3.2 LACHAT QuikChem 8000 Flow Injection Analyzer.

4. REAGENTS

- 4.1 **Sodium arsenite solution. Dissolve 0.930 g NaAsO₂ in DI water and dilute to 500 mL. (CAUTION: Toxic—take care to avoid ingestion.) Prepare fresh weekly. This is the dechlorinating agent.**
- 4.2 Sodium Hydroxide Solution, 1.25N. Dissolve 50 g sodium hydroxide (NaOH) in

DI water and dilute to 1000 mL or 200g NaOH in DI water dilute to 4L.

- 4.3 Sodium Hydroxide Solution, 0.25N. Dissolve 40 g sodium hydroxide in DI water and dilute to 4 L.
- 4.4 Sulfuric Acid, concentrated.
- 4.5 Magnesium Chloride Solution. Dissolve 510 g magnesium chloride hexahydrate ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$) in DI water and dilute to 1000 mL.
- 4.6 Carrier, 0.25M Sodium Hydroxide. In a 1 L volumetric flask dissolve 10.0g sodium hydroxide (NaOH) in DI water and dilute to mark. DEGAS WITH HELIUM PURGE BEFORE USING. Record in logbook.
- 4.7 Phosphate Buffer, 0.71M. In a 1 L volumetric flask, dissolve 97 g anhydrous potassium dihydrogen phosphate (potassium phosphate, monobasic, anhydrous, KH_2PO_4) in DI water and dilute to mark. Prepare fresh monthly. DEGAS WITH HELIUM BEFORE USING. Record in Prep Logbook.
- 4.8 Chloramine-T Hydrate. To a 500 mL volumetric flask add about 250 mL DI water, then add 2.0 g chloramine-T [$\text{CH}_3\text{C}_6\text{H}_4\text{SO}_2\text{N}(\text{Cl})\text{Na} \cdot \text{H}_2\text{O}$]. **Make sure this is hydrate and NOT trihydrate.** Dilute to mark. Prepare fresh daily. DEGAS BEFORE USING. Record in Prep Logbook.
- 4.9 Pyridine-Barbituric Acid Reagent. This reagent is purchased pre-made from a suitable vendor like Ricca. **IMPORTANT NOTE!!!** The pre-made reagent needs to be diluted before use on the FIA. Dilute 250mL of concentrated stock reagent (based on the 6% w/v barbituric acid; 30% v/v pyridine; and 6% v/v HCl recipe that Ricca uses) to 1 L with DI water. If a precipitate has formed, filter reagent through a 0.45 μm membrane filter. Record in Prep Logbook.
- 4.10 Cyanide Standard, 1000 mg/L. In a 1 L volumetric flask dissolve 2.0 g potassium hydroxide (KOH) in 500 mL DI water. Add 2.51 g potassium cyanide (KCN). Dissolve, then dilute to mark with DI water. Prepare fresh weekly or restandardize weekly following the procedure in Standard Methods. CAUTION! KCN IS HIGHLY TOXIC! AVOID CONTACT WITH SOLID OR SOLUTION! **Note: A prepared standard may be purchased from a suitable vendor.**
- 4.11 Intermediate Cyanide Standard, 20 $\mu\text{g/mL}$. Dilute 10.0 mL stock standard to 500 mL with 0.25N NaOH.
- 4.12 Working Cyanide Standard, 1 $\mu\text{g/mL}$. Dilute 10.0 mL intermediate standard to 200 mL with 0.25N NaOH.
- 4.13 Prepare calibration standards as per directions in the Standards/Reagents Preparation Logbook.

- 4.13.1 Standard A, 100 µg/L CN. Dilute 50 mL of working standard to 250 mL with 0.25N NaOH.
- 4.13.2 Standard B, 50 µg/L CN. Dilute 25 mL of working standard to 250 mL.
- 4.13.3 Standard C, 40 µg/L CN. Dilute 20 mL of working standard to 250 mL.
- 4.13.4 Standard D, 20 µg/L CN. Dilute 10 mL of working standard to 250 mL.
- 4.13.5 Standard E, 10 µg/L CN. Dilute 10 mL of Std A to 100 mL.
- 4.13.6 Standard F, 5 µg/L CN. Dilute 5 mL of Std A to 100 mL.
- 4.13.7 Standard G, 2 µg/L CN. Dilute 2 mL of Std A to 100 mL.
- 4.13.8 Standard H, Blank. Use 0.25N NaOH.
- 4.13.9 Control Chart Standard, 40 µg/L CN. Same as Standard C.
- 4.13.10 Method Detection Limit Sample (MDL), 2 µg/L CN. Same as Standard G.
- 4.13.11 Reportable Detection Limit Sample (RDL), 5 µg/L CN. Same as Std. F.
- 4.13.12 Second Source Standard, 40 µg/L CN. Follow procedures for preparing Intermediate and Working Standards using material from a second source other than the Calibration Stock. Then follow as per Standard C.
- 4.13.13 Spiked Sample Preparation. See Section 7.6.

NOTE: HAVE THE PERSON DISTILLING YOUR SAMPLES PREPARE A BLANK, CCSTD #1, CCSTD #2, AND 2ND SOURCE TO VERIFY THE RECOVERY FROM THE DISTILLATION PROCESS.

5. SAMPLE PREPARATION

- 5.1 Place 500 mL of sample in boiling flask containing several boiling stones. **Measure** 50 mL 1.25N sodium hydroxide solution into the absorber scrubber. Connect the condenser, absorber and trap. **Turn ON cooling water.**
- 5.2 Start a slow stream of air through the sample by applying vacuum to the trap. Adjust the vacuum so that **a 0.5-1.0 cm layer of bubbles is maintained at the top of the liquid in the absorber scrubber.**
- 5.3 Slowly add 25 mL concentrated sulfuric acid through the inlet tube. Rinse the inlet tube with DI water. Allow 3 minutes for mixing.

- 5.4 Add 20 mL magnesium chloride solution through the inlet tube and wash with DI water.
- 5.5 Heat to boiling. Reflux for one hour.
- 5.6 Turn off heat and continue the airflow for at least 15 minutes.
- 5.7 Drain the absorber solution into a 250 mL **plastic** volumetric flask. Wash the scrubber with DI water and add to the flask. Dilute to the mark with DI water.

6. SYSTEM PROCEDURE

- 6.1 Power up the FIA and computer by pressing the RED switch on the power strip at the back of the instrument. Then press the ON button on the computer.
- 6.2 Press **Exit** on the monitor to remove the Resolution Notifier or wait till it disappears automatically.
- 6.3 Press **Ctrl-Alt-Delete** simultaneously. At log on screen, log on using the same log on procedure as you would use for all the other computers (i.e. Username: your initials and enter your password). Then click **OK** or just press **Enter**.
- 6.4 Click on the **Omnion 3.0** icon.
- 6.5 Click on **Configuration** at top of screen. Then **Autosamplers**. A new screen will open. Click on box **Initialize Autosampler**. The autosampler will initialize, then go to the wash station. Now close the autosampler window.
- 6.6 Click on **Open** icon. A daughter window opens on the screen to the last method that was run. If the currently displayed method is not the one you want, press the yellow folder located at the top left of the window and you will move up one directory to the **Methods** directory. To open the method that you wish to use, click **Method**, choose the method you want, then open a recent run file. (Doing this insures that the most up to date timing values will apply to your run.)
- 6.7 The **Run Window** opens. The autosampler **Run Worksheet** window should open, but if it doesn't, click on the sample list icon at the bottom far left of the screen. Once open begin typing your worksheet. Highlight the first available sample ID box. Type in the sample LIMS# and press **ENTER**. The cursor should now move to the next sample row below the one you just entered your data in. Sample type is **Unknown**. (**NOTE:** You **can not** use the arrow keys to move around or to go and down, the data will not be entered.)
- 6.8 The undistilled CC Standard is in location S15 on the autosampler. It is highlighted green in the run worksheet. The sample type is **Check Standard**. Highlight the row to verify the correct pass/fail criteria. In the **Run Properties** window, check the DQM Limits. The **Known Concentration** should be **40 µg/L**.

The >+ **Conc.Limit** should be **44 µg/L** and the <-**Conc.Limit** should be **36 µg/L**. The DQM Action messages should be PASS, FAIL, Continue, and Continue for both the upper and lower limits. The protocol for naming the CC Standard is **QACCxyymmddC**, where x is the number of the CC Standard in the run, and yymmdd is year, month, day.

- 6.9 The MDL is in location S14 on the autosampler. The sample type is **Unknown**. Do not use Method Detection Limit for the sample type or the sample will be analyzed seven replicates. The MDL sample is named **MDL01yymmddM**.
- 6.10 To enter a **Spike and Spike Duplicate** in a new location, first change sample type from Unknown to **Spike and Spike Duplicate**. When you do this, three RED rows appear, one for the unspiked sample, one for the spiked sample and one for the spike duplicate. Move the cursor to the **Sample No.** column and left click to highlight the spike duplicate row. Next, click on the icon located in the lower left, second from left, that looks like a file card. A daughter window appears. Under **Properties** enter the known spike concentration, **10µg/L**. Next, for DQM test limits, check box next to + **%Recovery Limit** and enter **120**. Move down to bottom and enter messages for DQM Actions: PASS, FAIL, Continue and Continue. Then check box next to <- **%Recovery Limit** and enter **80**. Again, enter DQM Action messages. Use the LIMS designations of S and K for spike and spike duplicate respectively. Finally, click lower left icon again to return to sample page.
- 6.11 To enter a **Duplicate**, change sample type from Unknown to **Duplicate**. This will add two orange-colored rows, the first for the unknown sample and the second for the sample duplicate. There will be **NO** DQM actions for this. Use the LIMS designation of Q for the sample duplicate.
- 6.12 To **Insert** or **Delete** any sample rows, first click on the **Sample No.** column. Right click. A dropdown menu will appear. Use **Delete** to remove any rows. Use **Insert** to add one row or **Insert Many** to add more than one row. The row or rows will be added immediately above the row you initially highlighted. **When adding rows, make sure to change the number of replicates to 2.**
- 6.13 Make sure all the **Trigger OFF** and **Weight 1.0000g** boxes are checked off.
- 6.14 To renumber the sample cups, click on the first sample below the blue standards area. Click in the **Sample No.** column and click and drag the cursor to the last row. Release and the entire sample tray should turn blue. Then, without moving the mouse, right click and a dropdown menu should appear. Click on the last item **Columns->**, then another menu appears. Click on the first item **AutoNumber Cups**.
- 6.15 When you have the sample tray the way you want it, click on **Run** in the upper left of the screen. Then **Save as Default New Run**. Click OK. To print the sample tray worksheet, click **Run**, then **Export Worksheet Data**. It will

automatically be printed at the shared printer.

- 6.16 Check the condition of all pump tubing and replace if necessary. **This is a high back-pressure method and requires the use of Tygon pump tubes. DO NOT use Duraprene pump tubing for this method. DO NOT mix Tygon and Duraprene.** Place the reagent lines in a container of DI water. Correctly set the tension on the pump tubes. The pump speed is **35**. Position the pyridine-barbituric acid reagent under the small fume hood next to the FIA. Connect the long length of tygon tubing to the reagent waste nipple and place the end in the hood so that it empties into the cyanide reagent waste container. Turn the fume hoods ON. Check for any leaks or flow problems.

PROCEDURE FOR INSTALLATING PUMP TUBING

- 6.16.1 Place correct pump tubing onto pump cartridges.
- 6.16.2 Move tension levers to the right-most position.
- 6.16.3 Turn the pump ON by pressing the red button to start the pump rollers rotating. Install pump cartridge by engaging one end of cartridge into cartridge holder, then clamp down other end until it snaps into place. The tension levers must always be on the left side of the pump. An arrow indented on the top of the cartridge shows the flow direction.
- 6.16.4 Move the tension lever to the left until it clicks once. The tension levers should be approximately in the 12 o'clock position.
- 6.16.5 After the analysis, release tension on the pump tubing by moving the tension levers back to the right, then releasing one side of the pump cartridge from the pump cartridge holder.

After pumping DI water through the manifold, and checking to see that the heater has reached the required temperature of **60°C**, place the reagent lines in their corresponding reagent bottles. Check for any leaks or obstructions or pulsating pump tubing.

- 6.16 Load the sample tray with samples. The MDL sample goes in position S14, the CC Standard in position S15, and the Second Source sample in position S16. Place the standards in the standards rack.
- 6.17 Click on the **Start** button located at the top, fourth from the left, with the green arrow.
- 6.18 Verify the correct channel (Channel 1) at the bottom of the screen. There will be an icon with the channel number on it. To view the diagram, click on the channel icon. The **Channel** screen will come up. The baseline will begin at the top left of the screen. When the first standard comes out, the peak will appear, pushing the

baseline down. You can view the entire run by ensuring the two boxes at lower left, **Track Data** and **Auto Scale** are checked.

- 6.19 To view the calibration data, click on the icon located on the left side of the screen that looks like a calibration curve.
- 6.20 As the first peaks appear on the diagram (or channel) window, you will notice a blue box will automatically be drawn around each peak. This is the **Peak Expectation Window**. There will also be a red peak height and baseline drawn. If this information doesn't look right, click on the run icon (now a red dot) and stop the run. Move the cursor to any white part of the screen and right click. This opens a dropdown menu. Click on **Adjust Peak Expectation Window**. A black box now appears around the peak, surrounding the blue box. Left click and the black box changes to red and the shape of the cursor changes to a left-right and up-down arrow shape. Move the cursor to the left vertical side of the box and the cursor shape now changes to a left-right arrow shape. Left click and drag the left side of the red box to where you want the peak detection to begin. Do the same to the right side. Then move cursor outside of box into the white background area and right click. A dropdown menu will appear. Select **Rerun Peak Detection**. The box will change back to blue and be repositioned to reflect the changes you made. The red baseline will also be redrawn. If the peak detection box looks OK, click on the **Start** icon to restart the analysis. Monitor the first few standard peaks to ensure that the changes were correct. If not, repeat the above process. When the analysis is finished, save the method with the updated information. (**NOTE: Saving the method is only necessary if changes were made to the peak detection or timing parameters. Normally, changes are not required.**)
- 6.21 The minimum correlation coefficient is 0.9950, however 0.9990 or better can be easily attained.
- 6.22 When the run is finished, the run icon will switch from the red dot to the green arrow. A message will pop up on the screen: "Export File Path: Access permission to export file is denied. The file may be open with another application." Click OK
- 6.23 Place all pump tubes in DI water to flush the manifold. Continue flushing for **at least five minutes**, then remove the lines from the water and continue pumping until dry. Release tension on the pump tubes. Properly dispose of the reagent waste.
- 6.24 To print the run report, click on **Tools** at the top of the screen. Click on **Custom Report**, then the printer icon. When finished, click **Report** at the top left, then **Close**.
- 6.25 The run is automatically saved and assigned a file name in the following format: OM_M_DD_YYYY_HH_MM_SSAM(orPM).OMN. For housekeeping, click and drag the run file to the corresponding monthly folder.

- 6.26 To transfer the run data to a memory stick, insert the memory stick into a USB port. Minimize the "Removable Disk (F:)" window. Go to the **Run Properties** window, click the **Run** tab, then click the **Export Data to File** tab.
- 6.27. To exit from Omnion, click on **Run** at the upper left, then **Exit**. You will be prompted "Exit Omnion?", then OK.

7. QUALITY CONTROL

- 7.1 Method Detection Limit (MDL). A MDL sample (the lowest concentration standard) must be analyzed with each batch of samples to be run. Since cyanide is not run as frequently, it is a good idea to run seven replicates of the MDL sample with each run to insure sufficient data for the MDL calculation. Calculate the MDL as follows:

$$\text{MDL} = (t) \times (S)$$

where,

t = Student's t value of 3.14 for seven replicates.

S = standard deviation of the seven replicates.

- 7.2 Laboratory Reagent Blank (LRB). The laboratory must analyze at least one LRB with each batch of samples. Values that exceed the MDL indicate laboratory or reagent contamination and corrective actions must be taken before continuing the analysis.
- 7.3 Control Chart Standard (CC STD). The laboratory must analyze a control chart standard immediately following the calibration, after every tenth sample, and at the end of the sample run. **Digest two separate solutions of 20 mL of 1 mg/L CN solution diluted to 500 mL and treat the same as samples.** Analysis of the control chart standard following calibration must verify that the instrument is within $\pm 10\%$ of calibration. Subsequent analyses of the control chart standard must verify the calibration is STILL within $\pm 10\%$. If the control chart standard is outside the limits, the sample analysis must be aborted, the cause for failure determined and/or the instrument recalibrated. All samples following the last acceptable control chart standard must be reanalyzed.
- 7.4 Reportable Detection Limit (RDL). A RDL sample must be analyzed with each batch of samples to be run. This is to verify that the instrument can detect this value. The naming protocol is **QARDLyymmddW**.
- 7.5 Duplicate Samples. As a minimum, run one duplicate for every ten samples in a batch, or one duplicate for a batch of less than ten samples. The relative percent difference (RPD) should be $\leq 10\%$.

- 7.6 Laboratory Fortified Sample Matrix (LFM). The laboratory must analyze a **minimum of 10% of routine samples** with the addition of a known amount of analyte added for a **spike and a spike duplicate**. For a **10µg/L spike**, add 5 mL of a 1 mg/L CN solution to 500 mL of sample **before digestion**. **It is the responsibility of the cyanide digestion person to make up the working standard and spike the sample(s)**. Calculate the percent recovery as follows:

$$R = [(C_s - C) / s] \times 100\%$$

where,

R = percent recovery

C_s = spiked sample concentration

C = unspiked sample concentration

s = concentration equivalent of analyte added to sample

The percent recovery range should be 80 - 120%. If the recovery of the analyte falls outside the acceptable range and the control chart standard is shown to be in control, the problem is judged to be either matrix- or solution- related, not system-related.

- 7.7 Second Source. A second source sample from a manufacturer other than that from which the calibration standards were obtained must be analyzed with each batch run, preferably immediately following the calibration, to provide additional performance data.
- 7.8 Performance Evaluation Sample. Analyzed on an annual basis as required by ELAP.

8. TROUBLESHOOTING

- 8.1 Refer to the instrument manual for troubleshooting hints. Record any unusual events or routine maintenance in the Maintenance Logbook.

9. DATA TRANSFER TO LIMS

- 9.1 First, make sure the correct run file is open.
- 9.2 Insert the memory stick into the USB port.
- 9.3 In the **Run Properties** window, click on **Export Data to File** tab.
- 9.4 Continue to Aspen. Use **CN File (FIA)** to import data.

SOP PROCEDURE CHANGE
FOR CYANIDE BY FIA

<u>CHANGE</u>	<u>DATE</u>	<u>INITIALS</u>
Revised	3/6/00	CAN
Revised Secs. 6.16 to include degas and fume control details, 6.17 to reflect current hardware, 6.23 for calibration curve printing, "Quality Control" to add directions for making spikes and control chart standards.	12/11/03	JMD
Directions for using Pyridine/Barbituric Acid Reagent from Ricca and Chloramine-T Hydrate and NOT Trihydrate	07/08/04	JAB
Switched from 1 liter bottle to 2 liter brown plastic bottle	02/04/05	JAB
Minor changes to sodium arsenite recipe and sample prep	2/7/05	JMD
Revised software method operating procedures to reflect new Omnion 3.0 use differences.	05/25/05	PH
Section 4.1 1.25M NaOH modified to reflect authorization to 4 times volume of reagents.	09/07/2005	FC
Described use of new Omnion 3.0 software in more detail.	9/22/2005	JAB
Clarified that the digestion person is responsible for spiking	2/13/06	JMD
Naming protocol for CC STD.	8/7/06	JAB
Section 6.18 Added comment about NOT mixing Tygon and Duraprene pump tubing. Increased NaOH in 2.1 from 1.6 to 1.7 grams to insure ph 12 or above. Removed reference to WF-21 sample points in 2.1. Added reminder to turn on cooling water in section 5.1.	6/5/08	JAB and JMD
Added Sections 6.18.1 thru 5 concerning recommended procedure for installing pump tubing.	4/7/2009	JAB

SOP PROCEDURE CHANGE
FOR CYANIDE BY FIA

<u>CHANGE</u>	<u>DATE</u>	<u>INITIALS</u>
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Added to Section 6.16 about correct installation of pump tubes. Added RDL requirements. Changed Calibration Standard F from 4ppb to 5ppb for the RDL.	5/18/09	JAB
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Clarified dechlorination requirements for GWRS samples by revising Sec. 2.1, moving sodium arsenite prep instructions to top of reagent list.	8/31/09	JMD/PH
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ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

CYANIDE BY MICRO DIST FIA (Do NOT use FIA_CN.doc SOP)

File Name: M:\SOP\INORGNIC\FIA_CN.doc
Revision: 13

Effective Date: 10/15/2009
Supersedes: 12 (8/31/2009)

1. REFERENCES

- 1.1 *Standard Methods* 20th Ed. #4500-CN, pp. 4-32 to 4-53.
- 1.2 *EPA Methods for Analysis of Water and Waste*, #335.2 and 335.3.
- 1.3 Lachat Instruments QuikChem Method 10-204-00-1-X (MICRO DIST Method).

2. SAMPLE HANDLING AND PRESERVATION

- 2.1 Prepare sample collection bottles in advance. Add 1.8 g NaOH pellets to a clean 2 liter brown plastic bottle. Add sodium arsenite solution (see below) to remove interference from chlorine or other oxidizing agents (use 1 mL per mg/L chlorine residual expected per L of sample). Get chlorine residual values for GWRS samples from the DPD residual chlorine method analyst (and have him/her also analyze any CN samples not normally run for chlorine, *e.g.* Q1). After sample collection and preservation, verify the pH. It must be at least 12. Completeness of dechlorination may be checked using KI/Starch test paper. See *Standard Methods* for more detail. Samples can be held for up to 14 days at 4°C.

3. APPARATUS

- 3.1 MICRO DIST repipettor
- 3.2 MICRO DIST tube press
- 3.3 MICRO DIST block (Lachat Part No. A17100)
- 3.4 LACHAT QuikChem 8000 Flow Injection Analyzer.

4. REAGENTS

- 4.1 Sodium arsenite solution. Dissolve 0.930 g NaAsO₂ in DI water and dilute to 500 mL. (CAUTION: Toxic—take care to avoid ingestion.) Prepare fresh

- weekly. This is the dechlorinating agent. Record in logbook.
- 4.2 Sodium Hydroxide Solution, 0.95N. Dissolve 38 g sodium hydroxide (NaOH) in DI water and dilute to 1000 mL. Record in logbook.
 - 4.3 Sodium Hydroxide Solution, 0.25N. Dissolve 40 g sodium hydroxide in DI water and dilute to 4 L.
 - 4.4 Sulfuric Acid, concentrated.
 - 4.5 7.11M H₂SO₄ / 0.79M MgCl₂ Solution: In the hood, dissolve 161 g MgCl₂ · 6H₂O in 400 to 500 ml DI water in a 1.5L or 2L beaker. **SLOWLY** add 380 ml concentrated H₂SO₄ (**CAUTION—SOLUTION WILL BECOME VERY HOT!**), cool down to room temperature, and dilute to 1000mL.
 - 4.6 Carrier, 0.25M Sodium Hydroxide. In a 1 L volumetric flask dissolve 10.0 g sodium hydroxide (NaOH) in DI water and dilute to mark. **DEGAS WITH HELIUM PURGE BEFORE USING.** Record in logbook.
 - 4.7 Phosphate Buffer, 0.71M. In a 1 L volumetric flask, dissolve 97 g anhydrous potassium dihydrogen phosphate (potassium phosphate monobasic, anhydrous, KH₂PO₄) in DI water and dilute to mark. Prepare fresh monthly. **DEGAS WITH HELIUM BEFORE USING.** Record in Prep Logbook.
 - 4.8 Chloramine-T Hydrate. To a 500 mL volumetric flask add about 250 mL DI water, then add 2.0 g chloramine-T [CH₃C₆H₄SO₂N(Cl)Na · H₂O]. **Make sure this is hydrate and NOT trihydrate.** Dilute to mark. Prepare fresh daily. **DEGAS BEFORE USING.** Record in Prep Logbook.
 - 4.9 Pyridine-Barbituric Acid Reagent. This reagent is purchased pre-made from a suitable vendor like Ricca. **IMPORTANT NOTE!!!** The pre-made reagent needs to be diluted before use on the FIA. Dilute 250mL of concentrated stock reagent (based on the 6% w/v barbituric acid; 30% v/v pyridine; and 6% v/v HCl recipe that Ricca uses) to 1 L with DI water. If a precipitate has formed, filter reagent through a 0.45µm membrane filter. Record in Prep Logbook.
 - 4.10 Cyanide Standard, 1000 mg/L. In a 1 L volumetric flask dissolve 2.0 g potassium hydroxide (KOH) in 500 mL DI water. Add 2.51 g potassium cyanide (KCN). Dissolve, then dilute to mark with DI water. Prepare fresh weekly or restandardize weekly following the procedure in Standard Methods. **CAUTION! KCN IS HIGHLY TOXIC! AVOID CONTACT WITH SOLID OR SOLUTION! Note: A prepared standard may be purchased from a suitable vendor.**
 - 4.11 Intermediate Cyanide Standard, 20 µg/mL. Dilute 10.0 mL stock standard to 500 mL with 0.25N NaOH.
 - 4.12 Working Cyanide Standard, 1 µg/mL. Dilute 10.0 mL intermediate standard to

200 mL with 0.25N NaOH.

- 4.13 Prepare calibration standards as per directions in the Standards/Reagents Preparation Logbook.
- 4.13.1 Standard A, 100 µg/L CN. Dilute 25 mL of working standard to 250 mL with 0.25N NaOH.
- 4.13.2 Standard B, 50 µg/L CN. Dilute 10 mL of working standard to 200 mL.
- 4.13.3 Standard C, 40 µg/L CN. Dilute 10 mL of working standard to 250 mL.
- 4.13.4 Standard D, 20 µg/L CN. Dilute 5 mL of working standard to 250 mL.
- 4.13.5 Standard E, 10 µg/L CN. Dilute 10 mL of Std A to 100 mL.
- 4.13.6 Standard F, 5 µg/L CN. Dilute 5 mL of Std A to 100 mL.
- 4.13.7 Standard G, 2 µg/L CN. Dilute 2 mL of Std A to 100 mL.
- 4.13.8 Standard H, Blank. Use 0.25N NaOH.
- 4.13.9 Control Chart Standard, 40 µg/L CN. Same as Standard C.
- 4.13.10 Method Detection Limit Sample (MDL), 2 µg/L CN. Same as Standard G.
- 4.13.11 Reportable Detection Limit Sample (RDL), 5 µg/L CN. Same as Std. F.
- 4.13.12 Second Source Standard, 40 µg/L CN. Follow procedures for preparing Intermediate and Working Standards using material from a second source other than the Calibration Stock. Then follow as per Standard C.
- 4.13.13 Spiked Sample Preparation. See Section 7.6.

NOTE: HAVE THE PERSON DISTILLING YOUR SAMPLES PREPARE A BLANK, CCSTD #1, CCSTD #2, AND 2ND SOURCE TO VERIFY THE RECOVERY FROM THE DISTILLATION PROCESS.

5. SAMPLE PREPARATION using MICRO DIST

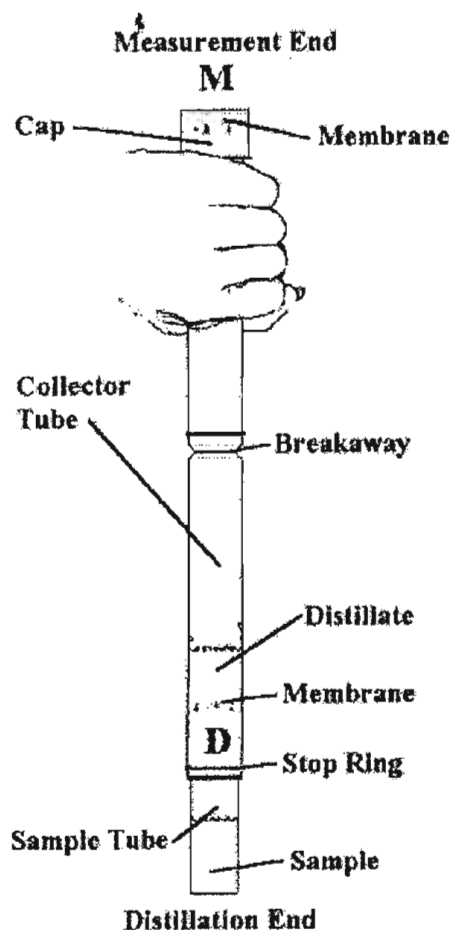


Figure 1. Collector Tube

- 5.1 Turn on the heater block. It will take about 40 minutes to warm up to 120° C.
- 5.2 Prepare User-Fill collector tubes: with the M end up, use automatic pipette to add 1.59 ml of 0.95N NaOH to each collector tube, place a membrane filter on the top and cap it before using (Figure 1).
- 5.3 Put the sample tubes into the sample rack, up to 21 for the block. Add one boiling chip to each tube. Pipette 6.0ml of calibration standard, sample, spike, blank, CC standard or 2nd source standard into each sample tube (Figure 2).

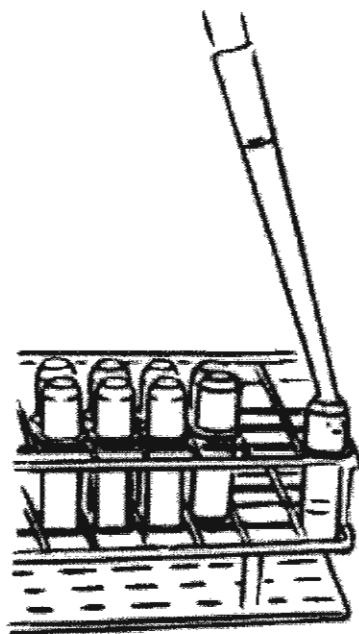


Figure 2. Placing Sample in Tubes

- 5.4 Add 0.1 ml (2 drops) conc. H_2SO_4 (may use nitrogen preservation acid dispenser), then add 0.75 ml of 7.11M H_2SO_4 /0.79M MgCl_2 solution to the sample tube using repipettor. **IMMEDIATELY** push the D end of the collector tube over the open end to start the seal.



Figure 3. Adding 7.11M H_2SO_4 / 0.79M MgCl_2 Solution

- 5.5 Place the assembly in the press, putting the sample tube through the hole in the white base. Grip the collector tube firmly, press down on the handle until the stop ring on the sample tube hits the D end of the collector tube. **Do not force the handle down with excessive pressure. IMPORTANT—after applying proper pressure to tube assembly, examine the sample tube and collector tube stop ring to make sure they are flush or close to it, and that they are aligned on the same straight line axis (otherwise tubes will not insert into the block to the proper depth). If not properly aligned, re-align by hand, or re-insert into press and gently apply more pressure.**

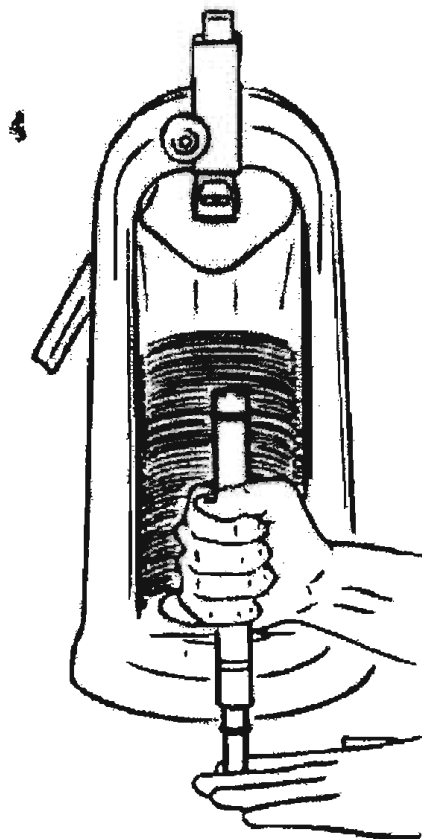


Figure 4. Starting the Seal

- 5.6 Put on the heat-resistant gloves. Push the sample tube and D end of each tube all the way into the preheated block so that collector tube stop ring touches the block. Set the timer for 30 minutes.

- 5.7 When the 30 minutes is up, put on the heat-resistant gloves. Remove the first tube from the block and immediately pull off its sample tube using a downward, twisting motion to a waste bucket within 4 seconds.

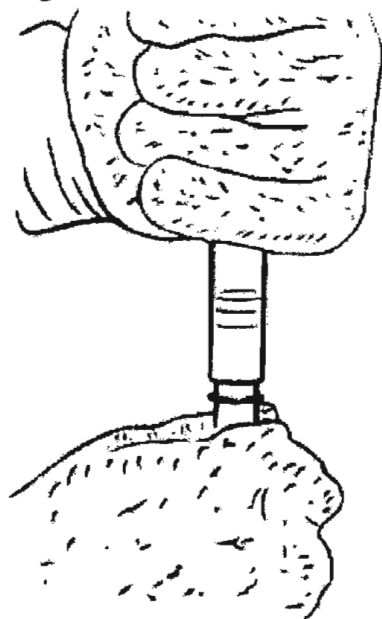


Figure 5. Removing the Sample Tube

- 5.8 Invert each collector tube and place it into the collector tube rack with the D end up. It should take less than 2 minutes to pull out 21 tubes.
- 5.9 Allow tubes to cool for at least 10 minutes. With the D end still up, break the collector tube in half at the score line. Rinse the D end with DI water into the M end, and discard the D end.

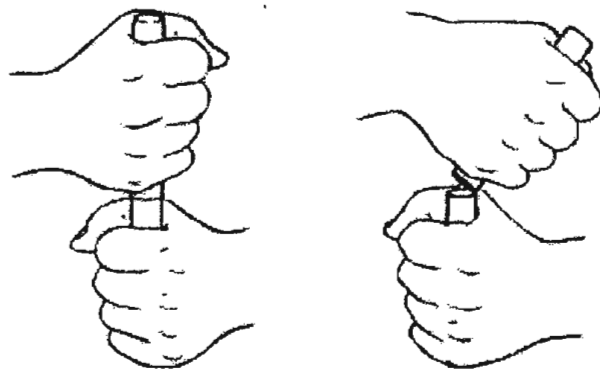


Figure 6. Breaking the D End of the Collector Tube

- 5.10 Dilute the remaining M end to the 6.0 ml mark with DI water. Seal both ends with Parafilm and shake it to homogenize. Remove Parafilm and transfer sample to glass FIA sample tube. Sample is now ready for analysis by FIA.

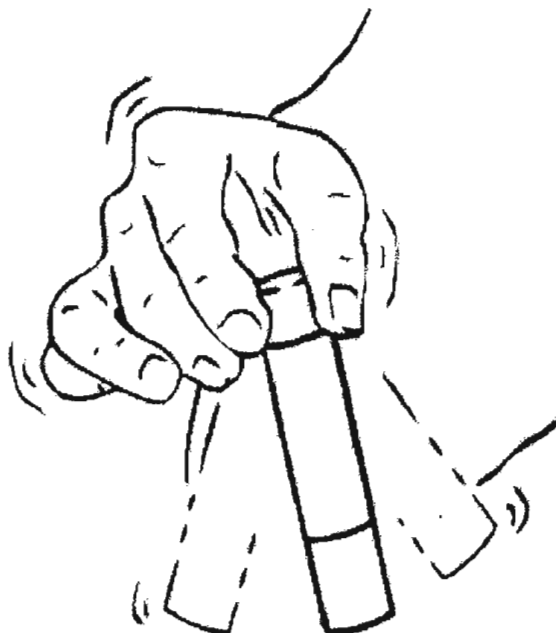


Figure 7 shaking the tube

6. SYSTEM PROCEDURE

- 6.1 Power up the FIA and computer by pressing the RED switch on the power strip at the back of the instrument. Then press the ON button on the computer.
- 6.2 Press **Exit** on the monitor to remove the Resolution Notifier or wait till it disappears automatically.
- 6.3 Press **Ctrl-Alt-Delete** simultaneously. At log on screen, log on using the same log on procedure as you would use for all the other computers (i.e. Username: your initials and enter your password). Then click **OK** or just press **Enter**.
- 6.4 Click on the **Omnion 3.0** icon.
- 6.5 Click on **Configuration** at top of screen, then **Autosamplers**. A new screen will open. Click on **Initialize Autosampler** button. The autosampler will initialize, then go to the wash station. Close the autosampler window.
- 6.6 Click on **Open** icon. A window opens to the last method that was run. If it is not the one you want, click the yellow folder with the up arrow at the top of the window to open the **Methods** directory. Choose the method you wish to use and then click to open the most recent run. (Doing this insures that the most recent timing updates will apply to your run).
- 6.7 The **Run Window** opens. The autosampler **Run Worksheet** window should open, but if it doesn't, click on the sample list icon at the bottom far left of the screen.

Once open begin typing your worksheet. Highlight the first available sample ID box. Type in the sample LIMS# and press **ENTER**. The cursor should now move to the next sample row below the one you just entered your data in. Sample type is **Unknown**. (**NOTE:** You **can not** use the arrow keys to move around or to go and down, the data will not be entered.)

- 6.8 The undistilled CC Standard is in location S15 on the autosampler. It is highlighted green in the run worksheet. The sample type is **Check Standard**. Highlight the row to verify the correct pass/fail criteria. In the **Run Properties** window, check the DQM Limits. The **Known Concentration** should be **40 µg/L**. The **>+ Conc.Limit** should be **44 µg/L** and the **<-Conc.Limit** should be **36 µg/L**. The DQM Action messages should be PASS, FAIL, Continue, and Continue for both the upper and lower limits. The protocol for naming the CC Standard is **QACCxyymmddC**, where x is the number of the CC Standard in the run, and yymmdd is year, month, day.
- 6.9 The MDL is in location S14 on the autosampler. The sample type is **Unknown**. Do not use Method Detection Limit for the sample type or the sample will be analyzed seven replicates. The MDL sample is named **MDL01yymmddM**.
- 6.10 To enter a **Spike and Spike Duplicate** in a new location, first change sample type from Unknown to **Spike and Spike Duplicate**. When you do this, three RED rows appear, one for the unspiked sample, one for the spiked sample and one for the spike duplicate. Move the cursor to the **Sample No.** column and left click to highlight the spike duplicate row. Next, click on the icon located in the lower left, second from left, that looks like a file card. A daughter window appears. Under **Properties** enter the known spike concentration, **10µg/L**. Next, for DQM test limits, check box next to **+ %Recovery Limit** and enter **120**. Move down to bottom and enter messages for DQM Actions: PASS, FAIL, Continue and Continue. Then check box next to **<- %Recovery Limit** and enter **80**. Again, enter DQM Action messages. Use the LIMS designations of S and K for spike and spike duplicate respectively. Finally, click lower left icon again to return to sample page.
- 6.11 To enter a **Duplicate**, change sample type from Unknown to **Duplicate**. This will add two orange-colored rows, the first for the unknown sample and the second for the sample duplicate. There will be **NO** DQM actions for this. Use the LIMS designation of Q for the sample duplicate.
- 6.12 To **Insert** or **Delete** any sample rows, first click on the **Sample No.** column. Right click. A dropdown menu will appear. Use **Delete** to remove any rows. Use **Insert** to add one row or **Insert Many** to add more than one row. The row or rows will be added immediately above the row you initially highlighted. **When adding rows, make sure to change the number of replicates to 2.**
- 6.13 Make sure all the **Trigger OFF** and **Weight 1.0000g** boxes are checked off.

- 6.14 To renumber the sample cups, click on the first sample below the blue standards area. Click in the **Sample No.** column and click and drag the cursor to the last row. Release and the entire sample tray should turn blue. Then, without moving the mouse, right click and a dropdown menu should appear. Click on the last item **Columns->**, then another menu appears. Click on the first item **AutoNumber Cups**.
- 6.15 When you have the sample tray the way you want it, click on **Run** in the upper left of the screen. Then **Save as Default New Run**. Click OK. To print the sample tray worksheet, click **Run**, then **Export Worksheet Data**. It will automatically be printed at the shared printer.
- 6.16 Check the condition of all pump tubing and replace if necessary. **This is a high back-pressure method and requires the use of Tygon pump tubes. DO NOT use Duraprene pump tubing for this method. DO NOT mix Tygon and Duraprene.** Place the reagent lines in a container of DI water. Correctly set the tension on the pump tubes. The pump speed is **35**. Position the pyridine-barbituric acid reagent under the small fume hood next to the FIA. Connect the long length of Tygon tubing to the reagent waste nipple and place the end in the cyanide reagent waste container directly under the hood. Check for any leaks or flow problems.

PROCEDURE FOR INSTALLING PUMP TUBING

- 6.16.1 Place correct pump tubing onto pump cartridges.
- 6.16.2 Move tension levers to the right-most position.
- 6.16.3 Turn the pump ON by pressing the red button to start the pump rollers rotating. Install pump cartridge by engaging one end of cartridge into cartridge holder, then clamp down other end until it snaps into place. The tension levers must always be on the left side of the pump. An arrow indented on the top of the cartridge shows the flow direction.
- 6.16.4 Move the tension lever to the left until it clicks once. The tension levers should be approximately in the 12 o'clock position.
- 6.16.5 After the analysis, release tension on the pump tubing by moving the tension levers back to the right, then releasing one side of the pump cartridge from the pump cartridge holder.

After pumping DI water through the manifold, and checking to see that the heater has reached the required temperature of **60°C**, place the reagent lines in their corresponding reagent bottles. Check for any leaks or obstructions or pulsating pump tubing.

- 6.17 Load the sample tray with samples. The MDL sample goes in position S14, the

CC Standard in position S15, and the Second Source sample in position S16.
Place the standards in the standards rack.

- 6.18 Click on the **Start** button located at the top, fourth from the left, with the green arrow.
- 6.19 Verify the correct channel (Channel 1) at the bottom of the screen. There will be an icon with the channel number on it. To view the diagram, click on the channel icon. The **Channel** screen will come up. The baseline will begin at the top left of the screen. When the first standard comes out, the peak will appear, pushing the baseline down. You can view the entire run by ensuring the two boxes at lower left, **Track Data** and **Auto Scale** are checked.
- 6.20 To view the calibration data, click on the icon located on the left side of the screen that looks like a calibration curve.
- 6.21 As the first peaks appear on the diagram (or channel) window, you will notice a blue box will automatically be drawn around each peak. This is the **Peak Expectation Window**. There will also be a red peak height and baseline drawn. If this information doesn't look right, click on the run icon (now a red dot) and stop the run. Move the cursor to any white part of the screen and right click. This opens a dropdown menu. Click on **Adjust Peak Expectation Window**. A black box now appears around the peak, surrounding the blue box. Left click and the black box changes to red and the shape of the cursor changes to a left-right and up-down arrow shape. Move the cursor to the left vertical side of the box and the cursor shape now changes to a left-right arrow shape. Left click and drag the left side of the red box to where you want the peak detection to begin. Do the same to the right side. Then move cursor outside of box into the white background area and right click. A dropdown menu will appear. Select **Rerun Peak Detection**. The box will change back to blue and be repositioned to reflect the changes you made. The red baseline will also be redrawn. If the peak detection box looks OK, click on the **Start** icon to restart the analysis. Monitor the first few standard peaks to ensure that the changes were correct. If not, repeat the above process. When the analysis is finished, save the method with the updated information.
- (NOTE: Saving the method is only necessary if changes were made to the peak detection or timing parameters. Normally, changes are not required.)**
- 6.22 The minimum correlation coefficient is 0.9950, however 0.9990 or better can be easily attained.
- 6.23 When the run is finished, the run icon will switch from the red dot to the green arrow. A message will pop up on the screen: "Export File Path: Access permission to export file is denied. The file may be open with another application." Click OK
- 6.24 Place all pump tubes in DI water to flush the manifold. Continue flushing for **at least five minutes**, then remove the lines from the water and continue pumping until dry. Release tension on the pump tubes. Properly dispose of the reagent waste.

- 6.25 To print the run report, click on **Tools** at the top of the screen. Click on **Custom Report**, then the printer icon. When finished, click **Report** at the top left, then **Close**.
- 6.26 The run is automatically saved and assigned a file name in the following format: OM_M_DD_YYYY_HH_MM_SSAM(orPM).OMN. For housekeeping, click and drag the run file to the corresponding monthly folder.
- 6.27 To transfer the run data to a memory stick, insert the memory stick into a USB port. Minimize the "Removable Disk (F:)" window. Go to the **Run Properties** window, click the **Run** tab, then click the **Export Data to File** tab.
- 6.28 To exit from Omnion, click on **Run** at the upper left, then **Exit**. You will be prompted "Exit Omnion?", then OK.

7. QUALITY CONTROL

- 7.1 Method Detection Limit (MDL). A MDL sample (the lowest concentration standard) must be analyzed with each batch of samples to be run. Since cyanide is not run as frequently, it is a good idea to run seven replicates of the MDL sample with each run to insure sufficient data for the MDL calculation. Calculate the MDL as follows:

$$\text{MDL} = (t) \times (S)$$

where,

t = Student's t value of 3.14 for seven replicates.

S = standard deviation of the seven replicates.

- 7.2 Laboratory Reagent Blank (LRB). The laboratory must analyze at least one LRB with each batch of samples. Values that exceed the MDL indicate laboratory or reagent contamination and corrective actions must be taken before continuing the analysis.
- 7.3 Control Chart Standard (CC STD). The laboratory must analyze a control chart standard immediately following the calibration, after every tenth sample, and at the end of the sample run. **Distill two separate solutions of 40 ppb CN std treat the same as samples.** Analysis of the control chart standard following calibration must verify that the instrument is within $\pm 10\%$ of calibration. Subsequent analyses of the control chart standard must verify the calibration is STILL within $\pm 10\%$. If the control chart standard is outside the limits, the sample analysis must be aborted, the cause for failure determined and/or the instrument recalibrated. All samples following the last acceptable control chart standard must be reanalyzed.

- 7.4 Reportable Detection Limit (RDL). A RDL sample must be analyzed with each batch of samples to be run. This is to verify that the instrument can detect this value. The naming protocol is **QARDLyymmddW**.
- 7.5 Duplicate Samples. As a minimum, run one duplicate for every ten samples in a batch, or one duplicate for a batch of less than ten samples. The relative percent difference (RPD) should be $\leq 10\%$.
- 7.6 Laboratory Fortified Sample Matrix (LFM). The laboratory must analyze a **minimum of 10% of routine samples** with the addition of a known amount of analyte added for a **spike and a spike duplicate**. For a **10 μ g/L spike**, add 1 mL of a 1 mg/L CN solution to 100 mL of sample **before distillation**. **It is the responsibility of the cyanide digestion person to make up the working standard and spike the sample(s)**. Calculate the percent recovery as follows:

$$R = [(C_s - C) / s] \times 100\%$$

where,

R = percent recovery

C_s = spiked sample concentration

C = unspiked sample concentration

s = concentration equivalent of analyte added to sample

The percent recovery range should be 80 - 120%. If the recovery of the analyte falls outside the acceptable range and the control chart standard is shown to be in control, the problem is judged to be either matrix- or solution- related, not system-related.

- 7.7 Second Source. A second source sample from a manufacturer other than that from which the calibration standards were obtained must be analyzed with each batch run, preferably immediately following the calibration, to provide additional performance data.
- 7.8 Performance Evaluation Sample. Analyzed on an annual basis as required by ELAP.

8. TROUBLESHOOTING

- 8.1 Refer to the instrument manual for troubleshooting hints. Record any unusual events or routine maintenance in the Maintenance Logbook.

9. DATA TRANSFER TO LIMS

- 9.1 First, make sure the correct run file is open.
- 9.2 Insert the memory stick into the USB port.
- 9.3 In the **Run Properties** window, click on **Export Data to File** tab.
- 9.4 Continue to Aspen. Use **CN File (FIA)** to import data.

SOP PROCEDURE CHANGE
FOR CYANIDE BY FIA

<u>CHANGE</u>	<u>DATE</u>	<u>INITIALS</u>
Revised	3/6/00	CAN
Revised Secs. 6.16 to include degas and fume control details, 6.17 to reflect current hardware, 6.23 for calibration curve printing, "Quality Control" to add directions for making spikes and control chart standards.	12/11/03	JMD
Directions for using Pyridine/Barbituric Acid Reagent from Ricca and Chloramine-T Hydrate and NOT Trihydrate	07/08/04	JAB
Switched from 1 liter bottle to 2 liter brown plastic bottle	02/04/05	JAB
Minor changes to sodium arsenite recipe and sample prep	2/7/05	JMD
Revised software method operating procedures to reflect new Omnion 3.0 use differences.	05/25/05	PH
Section 4.1 1.25M NaOH modified to reflect authorization to 4 times volume of reagents.	09/07/2005	FC
Described use of new Omnion 3.0 software in more detail.	9/22/2005	JAB
Clarified that the digestion person is responsible for spiking	2/13/06	JMD
Naming protocol for CC STD.	8/7/06	JAB
Section 6.18 Added comment about NOT mixing Tygon and Duraprene pump tubing. Increased NaOH in 2.1 from 1.6 to 1.7 grams to insure ph 12 or above. Removed reference to WF-21 sample points in 2.1. Added reminder to turn on cooling water in section 5.1.	6/5/08	JAB and JMD
Added Sections 6.18.1 thru 5 concerning recommended procedure for installing pump tubing.	4/7/2009	JAB

<u>CHANGE</u>	<u>DATE</u>	<u>INITIALS</u>
Added to Section 6.16 about correct installation of pump tubes. Added RDL requirements. Changed Calibration Standard F from 4ppb to 5ppb for the RDL.	5/18/09	JAB
Clarified dechlorination requirements for GWRS samples by revising Sec. 2.1, moving sodium arsenite prep instructions to top of reagent list.	8/31/09	JMD/PH

[illegible]

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

CHEMICAL OXYGEN DEMAND

File Name: M:\SOP\INORGNIC\COD.doc
Revision: 2

Effective Date: 5/23/2000
Supersedes: 1 (12/18/1997)

1. REFERENCES

- 1.1 *Standard Methods* 18th, 19th, and 20th Eds., #5220 B
- 1.2 *EPA Methods for Analysis of Water and Waste*, # 410.2.

2. HOLDING TIME

- 2.1 Acidify sample to pH <2 with H₂SO₄, cool to 4°C, hold for up to 28 days; best if analyzed without delay.

3. INTERFERENCES

- 3.1 Straight-chain aliphatic compounds are effectively oxidized with the addition of silver sulfate as a catalyst. Mercuric sulfate is added to complex chloride, which acts as a positive interference. For samples exceeding 2mg/L NO₂-N, add 10mg sulfamic acid for each mg NO₂-N to eliminate interference.

4. APPARATUS

- 4.1 Reflux apparatus - Consisting of 250mL round-bottom flasks with ground-glass 24/40 necks and air condensers with 24/40 ground-glass joints, and heating mantles.

5. REAGENTS

- 5.1 Potassium dichromate stock solution, 0.25N - Dissolve 12.259g potassium dichromate, K₂Cr₂O₇, primary standard grade, previously dried at 103°C for 2 hours, in DI water and dilute to 1000mL.
- 5.2 Potassium dichromate standard solution, 0.025N - Dilute 100.0mL stock to 1000mL with DI water.

- 5.3 Sulfuric acid reagent - Add 24.7g silver sulfate, Ag_2SO_4 , to 2.5L bottle of conc. H_2SO_4 . Let stand 1 to 2 days to dissolve Ag_2SO_4 .
- 5.4 Mercuric sulfate solution, 0.4g in 5mL - Measure 160g mercuric sulfate, HgSO_4 , into 4L erlenmeyer flask. Add 1600mL DI water and stir. Carefully add 400mL conc. H_2SO_4 . Continue stirring until completely dissolved.
- 5.5 Ferrous ammonium sulfate stock solution, 0.25N - Dissolve 49g FAS, $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$, in DI water. Add 10mL conc. H_2SO_4 , cool, and dilute to 500mL.
- 5.6 Ferrous ammonium sulfate standard solution, 0.025N - Dilute 200.0mL stock to 2000mL with DI water. Standardize daily. Add 10.0mL 0.025N $\text{K}_2\text{Cr}_2\text{O}_7$ to 100 mL DI water. Add 30mL conc. sulfuric acid reagent and cool. Titrate with FAS using 3 drops ferroin indicator.
- $$N(\text{FAS}) = 0.25/\text{mL FAS used}$$
- 5.7 Ferroin indicator solution - Dissolve 1.485 g 1,10-phenanthroline monohydrate and 695 mg $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ in DI water and dilute to 100 mL.
- 5.8 Potassium hydrogen phthalate (KHP) stock - Dry potassium hydrogen phthalate, $\text{HOOC}_6\text{H}_4\text{COOK}$, to constant weight at 120C. Dissolve 425 mg in DI water and dilute to 1000mL. This solution has a theoretical COD of 500mg/L O_2 . Solution stable for 3 months when refrigerated.
- 5.9 KHP standard, 10mg/L - Dilute 10.0mL stock to 500mL with DI water.

6. PROCEDURE

- 6.1 Pipet 20.0mL of well-mixed sample into 250mL round-bottom flask. Include two DI water blanks and two FAS standardization flasks.
- 6.2 Add to each flask (except for FAS stand. flasks) the following:
- 6.2.1 several boiling chips
 - 6.2.2 5.0mL mercuric sulfate solution
 - 6.2.3 10.0mL 0.025N $\text{K}_2\text{Cr}_2\text{O}_7$
 - 6.2.4 30mL sulfuric acid reagent
- 6.3 Rinse down neck of flask with water, place air condenser on flask and swirl to mix contents.
- 6.4 Place flask on heating mantle, reflux 2 hours, cool, rinse down condenser with 100mL cold DI water.

6.5 Cool flask to room temperature and titrate excess dichromate with 0.025N FAS, using 3 drops ferroin indicator. Titrate to first sharp color change from blue-green to reddish brown. Note: blue-green color may reappear.

6.6 After finishing titration, COD waste is discarded into waste container.

7. CALCULATION

7.1 $\text{mg/L COD} = (A - B) \times N \times 8000/\text{mL sample}$

7.2 A = mL FAS used for blank

7.3 B = mL FAS used for sample

7.4 N = Normality of FAS

8. QUALITY CONTROL

8.1 Run one duplicate for every ten samples, or one duplicate per run for less than ten samples; values should agree within 10%. Check standard should be within 2 sigma control limits or +/- 20%, whichever is less. EPA Performance Evaluation samples are used for external validation; results should be within EPA-specified acceptance limits. Second source standards are analyzed once per month and should be within 10% of expected reading.

9. TROUBLESHOOTING

9.1 Sample should be diluted if, after adding reagents, sample color turns green. This indicates that sample has oxidized all available dichromate and has a higher than expected COD.

SOP PROCEDURE CHANGE
FOR COD

CHANGE

DATE

INITIALS

Updated *Standard Methods* reference

5/23/00

JMD

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

CHEMICAL OXYGEN DEMAND Colorimetric Measurement, 0-150 mg/L COD

File Name: M:\SOP\INORGNIC\COD-reactor digestion.doc
Revision: 3

Effective Date: 5/28/2008
Supersedes: 2 (11/19/2007)

1. REFERENCES

- 1.1 *Standard Methods* 18th, 19th, and 20th Eds., #5220 B
- 1.2 *EPA Methods for Analysis of Water and Waste*, # 410.2.
- 1.3 HACH'S Dichromate Reactor Digestion Method; Method 8000

2. HOLDING TIME

- 2.1 Acidify sample to pH <2 with H₂SO₄, cool to 4°C, hold for up to 28 days; best if analyzed without delay.

3. INTERFERENCES

- 3.1 Straight-chain aliphatic compounds are effectively oxidized with the addition of silver sulfate as a catalyst. Mercuric sulfate is added to complex chloride, which acts as a positive interference. For samples exceeding 2 mg/L NO₂-N, add 10mg sulfamic acid for each mg NO₂-N to eliminate interference.

4. APPARATUS

- 4.1 HACH COD Reactor Model 45600, Cat. No. 45600-18
- 4.2 HACH Portable Datalogging Spectrometer

5. REAGENTS

- 5.1 Vials containing premeasured reagents, including catalysts and chloride compensator. Cat. No. 21259-25, 25/pkg.
- 5.2 **Potassium hydrogen phthalate (KHP) stock standard, 500 mg/L: Lightly crush and then dry a sufficient amount of primary standard solid KHP (HOCC₆H₄COOK) to constant weight at 110°C. Dissolve 425 mg in DI water and dilute to 1000 mL. Good for 3 months.**
- 5.3 **KHP Check Standard solution, 100 mg/L: dilute 100 mL of stock solution to 500 mL. Make fresh each time.**

6. PROCEDURE

- 6.1 Mix thoroughly 100 mL or a representative amount of sample in a 250mL beaker. Prepare aliquots if necessary.
- 6.2 Turn on the COD Reactor. Preheat to 150°C. Verify that the TIMER switch is set to Infinity. Place the plastic shield in front of the reactor. Verify temperature of the block by placing a dial thermometer supplied with the instrument in the temperature well provided in the block. **Note:** When the HEATING indicator begins to cycle on & off, the block temperature is stable.
- 6.3 Under the fume hood, remove the cap of the COD Digestion Reagent Vial. Hold the vial at a 45-degree angle and pipet 2.0 mL of sample into the vial. For greater accuracy, three replicates should be analyzed and the results averaged.
- 6.4 Replace the vial cap tightly. Rinse the outside of the COD vial with DI water and wipe clean with paper towel. **Note :** Spilled reagent will affect test accuracy. Do not run test with vials that has been spilled.
- 6.5 Hold the vial by the cap and invert gently several times to mix the contents. **Caution:** The vial will become very hot during mixing.
- 6.6 Prepare a blank. One blank must be run with each set of samples. Run samples and blanks with the same lot of vials.
- 6.7 Place the prepared vials in the preheated COD Reactor. Temperature will drop when loaded with the vials. Wait for a few minutes for the temperature to stabilize at 150°C.
- 6.8 Heat the vials for 2 hours. Set the TIMER switch to the TIMER position to automatically turn off the power of the digester at end of digestion. Set dial to 120 minutes.
- 6.9 Wait about 20 minutes for the vials to cool down to 120° C or less.
- 6.10 Invert each vial several times while still warm. Place the vials into a rack. Wait until they have cooled to room temperature.

7. COLORIMETRIC MEASUREMENT

- 7.1 Enter the stored program number, which is **430**. The display will show: **Dial nm to 420**
- 7.2 The next display will show: **Zero Sample**; then **mg/L COD LR**
- 7.3 Place the COD Vial adapter into the cell holder with marker to the right.

- 7.4 Clean the outside of the blank with a Kimwipe® to remove any fingerprints or other marks.
- 7.5 Place the blank into the adapter with the HACH logo facing the front of the instrument. Place the cover on the adapter.
- 7.6 Press **ZERO**. The display will show : **Zeroing..** then **0. mg/L COD LR**. Remove cover and blank vial.
- 7.7 Proceed with the samples. Clean the outside of the vials with Kimwipes® before placing them in the adapter. Replace cover.
- 7.8 Press: **READ**. The display will show: **Reading..** then the results in **mg/L COD** will be displayed. Transfer the COD reading to the COD data sheet.

8. QUALITY CONTROL

- 8.1 Run one duplicate for every ten samples, or one duplicate per run for less than ten samples; values should agree within 10%. 100 mg/L check standard should be within 2 sigma control limits or +/- 20%, whichever is less. PT samples are used for external validation; results should be within specified acceptance limits. Second source standards (**may use ERA QC and older PT standards**) are analyzed once per month and should be within 10% of expected reading.
- 8.2 EDL for this program is 2 mg/L COD. Protect unused digestion vials from light.

9. TROUBLESHOOTING

- 9.1 Sample should be diluted if, after adding reagents, sample color turns green. This indicates that sample has oxidized all available dichromate and has a higher than expected COD.

SOP PROCEDURE CHANGE
FOR COD

CHANGE	DATE	INITIALS
Updated <i>Standard Methods</i> reference	5/23/00	JMD

Changed references to EPA PE to PT samples	11/19/07	JMD
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Added secs. 5.2 and 5.3 (preparation of check std. solution), other minor changes.	5/28/08	JMD
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ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

COLOR

File Name: M:\SOP\INORGNIC\COLOR.doc
Revision: 3

Effective Date: 11/19/2007
Supersedes: 2 (3/06/2000)

1. DISCUSSION

- 1.1 Color in water may result from the presence of natural metallic ions (Fe & Mn), humus and peat materials, plankton, weeds, and industrial wastes. Color is removed to make water suitable for general and industrial consumptions
- 1.2 The term "COLOR" refers to mean true color, that is, the color of water from which turbidity is removed. The term "APPARENT COLOR" includes color due to substances in solution and also due to suspended matter. The latter is determined on the original sample without filtration or centrifugation.

2. REFERENCES

- 2.1 *Standard Methods*, 18th Ed., #2120B (pp. 2-1 - 2-3)
- 2.2 *EPA Methods for Analysis of Water and Wastes* (1979, rev. 1983), 110.2 (wastewater only). This method complies with EPA-approved methodology for analysis of drinking water and wastewater.

3. HOLDING TIME

- 3.1 48 hours @ 4°C; glass or plastic container.

4. INTERFERENCES

- 4.1 Turbidity will cause the apparent color to be noticeably higher than the true color.
- 4.2 Method is pH dependent.

5. APPARATUS

- 5.1 Nessler tubes, matched, 50-mL tall form.

6. REAGENTS

- 6.1 Stock Color Solution: Dissolve 0.1246 g potassium chloroplatinate (K_2PtCl_6) and 0.1 g crystallized cobaltous chloride ($CoCl_2 \cdot 6H_2O$) in DI water containing 10 mL concentrated HCl. (Alternately use commercial prepared stock, ie. Ricca Color

Standard – 500 units). Dilute to 100 mL in a volumetric flask. This stock standard has a color of 500 units. Prepare permanent standards having colors of 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60 and 70 by diluting 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0 and 7.0 mL stock color standard with DI water to 50 mL in Nessler tubes. Protect these standards from evaporation and contamination by covering them with polyethylene stoppers (hollow type).

7. PROCEDURE

- 7.1 Remove suspended matter from sample by centrifuging. If sample still contains turbidity, remove by filtration as described in *Standard Methods* #2120 C. Also use filtration for true color (TRCOLR) determination. If sample is not filtered, the result is apparent color (APCOLR).
- 7.2 Determine sample color by filling a matched Nessler tube to the 50-mL mark with sample and visually comparing it to the standards. Look vertically downward through the tubes toward a white or specular surface placed at such an angle that light is reflected upward through the columns of liquid. If the color exceeds 70 units, dilute sample with DI water in known proportions until the color is within the range of the standards.

Calculation if sample is diluted:

$$\text{Color units} = \frac{A \times 50}{V}$$

Where:

A = Observed color of diluted sample

V = mL sample taken for dilution

Record color results in whole numbers and record as follows:

<u>Color Units</u>	<u>Record to Nearest</u>
1-50	1
51-100	5
101-250	10
251-500	20

8. QUALITY CONTROL

- 8.1 Run one duplicate for every ten samples, or one duplicate per run for sets of less than ten samples; values should agree within 10%. PT samples are used for external validation; results should be within specified acceptance limits. Analyze one second source standard (must be within 10% of theoretical value) and two control chart check standards (10 color units) per run; plot the analyzed values of

the control chart standards and insure that they are within the control limits. If not, correct the problem and reanalyze all samples analyzed since the last "in control" control chart results.

9. TROUBLESHOOTING

- 9.1 Since biological activity may change the color characteristics of a sample, the analysis should be performed as soon as possible; see "Holding Time" above.
- 9.2 The color value of water is extremely pH dependent and invariably increases as the pH of the water is raised. When reporting a color value, specify the pH at which the color is determined.

SOP PROCEDURE CHANGE
FOR COLOR

**CHANGE
INITIALS**

REASON

DATE

JMD

Changed EPA PE to PT samples

11/19/2007

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

DISSOLVED SULFIDE/TOTAL SULFIDE¹ METHYLENE BLUE COLORIMETRIC METHOD (PHOTOMETRIC METHOD)

File Name: M:\SOP\INORGNIC\SULFIDE1.doc
Revision: 1

Effective Date: 12/01/1994
Supersedes: N/A

Sulfide reacts with dimethyl-p-phenylenediamine in the presence of FeCl_3 to produce methylene blue, which is measured at 664 nm through a 1-cm cell.

1. REFERENCES

- 1.1 EPA *Methods for Analysis of Water and Waste* # 376.2.
- 1.2 *Standard Methods*, 18th, 19th, and 20th Eds. #4500-S²⁻ D; as adapted for OCWD lab use.

2. HOLDING TIME

- 2.1 (See flowchart, p. 6). Best if analyzed immediately. **Preservation:** a. For total sulfide, or dissolved if there is no suspended matter present in the sample, add 12 drops $\text{Zn}(\text{OAc})_2$ solution/300 mL sample + 6N NaOH solution (dropwise to raise pH above 9 [use pH paper to verify]; start with 6 drops and increase if necessary), stopper with no air bubbles under stopper, mix by rotating back and forth vigorously about a transverse axis for 1 minute or longer, and hold at 4°C for up to 7 days. b. For dissolved sulfide, **first** request that the sample be collected in duplicate, **next** add 12 drops NaOH to each sample bottle, **then finally** add 12 drops of AlCl_3 solution to each sample bottle. Rotate back and forth vigorously about a transverse axis for 1 minute or longer to flocculate contents. Let settle until reasonably clear supernatant can be drawn off (about 5-15 min.; do not wait longer than necessary). Combine supernatant from the duplicate bottles into a clean "BOD" bottle, filling to the top with no air bubble under the stopper. If the sample was not collected in duplicate, pour the supernatant into a clean BOD bottle and fill with DI H_2O , being careful not to aerate the sample (see below). The sample may now be analyzed directly or preserved for later analysis as in "a." above.

3. INTERFERENCES

- 3.1 Dissolved Oxygen (D.O.) – be careful not to aerate samples **or** reagents (also, DI used for preparation of standards should not contain excess D.O.).
- 3.2 Color: each sample is analyzed against its own sample blank in the spectrophotometer to correct for this interference.

¹*If no suspended solids present, can assume dissolved sulfide equals total sulfide.

- 3.3 Turbidity, suspended solids: The OCWD lab's sulfide samples usually do not contain suspended solids. If present, and if analysis of **dissolved** sulfide is desired, flocculate and settle out the suspended matter as under "Preservation" above (using 12 drops of 6N NaOH and 12 drops of AlCl_3 solution) and analyze the clear **liquor** (supernatant).
- 3.4 Sulfite, thiosulfate, iodide and many other soluble substances can be eliminated by precipitating sulfide as ZnS, removing the supernatant containing the interfering species, and analyzing the ZnS after replacing the supernatant with DI. This procedure is also used for concentrating sulfide. If sample is suspected of containing these interferences, do not directly analyze an unpreserved sample without first adding $\text{Zn}(\text{OAc})_2$ and NaOH. See "Preservation" above and "PROCEDURE" below.

4. APPARATUS

- 4.1 UV/VIS Spectrophotometer @ 664 nm; 1-cm cell.

5. REAGENTS

- 5.1 Zinc acetate, 2N: dissolve 220 g $\text{Zn}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 2\text{H}_2\text{O}$ in 870 ml DI. Makes 1 L of solution.
- 5.2 NaOH, 6N.
- 5.3 Amine-sulfuric acid stock solution: dissolve 27 g N,N-dimethyl-*p*-phenylenediamine oxalate (Eastman cat. # 5672 or J.T. Baker are suitable—purchase fresh and discard if dark) in a cold mixture of 50 ml conc. H_2SO_4 and 20 ml DI in a 100-ml volumetric flask. Cool and dilute to mark. (Discard if dark). Store in dark glass bottle.
- 5.4 Amine-sulfuric acid reagent: dissolve 25 ml amino-sulfuric acid stock (above) with 975 ml 1+1 H_2SO_4 . Store in dark bottle. Solution should be clear.
- 5.5 Ferric chloride solution: dissolve 100 g $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in 40 ml DI.
- 5.6 Diammonium hydrogen phosphate solution: dissolve 400 g $(\text{NH}_4)_2\text{HPO}_4$ in 800 ml DI.
- 5.7 Sodium sulfide crystals: $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ (for preparation of standard curve).
- 5.8 6N Aluminum chloride $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ —dissolve contents of a previously unopened 100-g bottle in 144 ml DI.

6. PROCEDURE

- 6.1 If sample has not been previously preserved and concentration of sample or removal of interferences is desired, add 12 drops $\text{Zn}(\text{OAc})_2$ solution and 6N NaOH dropwise to pH 9 to sample. Stopper with no air bubbles under stopper, and mix by rotating back and forth vigorously about a transverse axis for 1 minute or longer. Let precipitate settle for 30 min and decant as much supernatant as possible without loss of precipitate. Refill bottle with DI water, resuspend the precipitate, and withdraw a sample. If concentration of the sample is desired, refill only partially with DI water, measure the total volume of DI water + precipitate, and withdraw a sample. When calculating the sulfide concentration, multiply the result by the ratio of final to initial sample volume.
- 6.2 Transfer 7.5 ml of sample to each of two test tubes (we use tubes of approx. 125 mm x 15 mm), labeling them A and B (B is the blank).
 - 6.2.1 To A add 0.5 ml amine-sulfuric acid reagent and 0.15 ml (3 drops) FeCl_3 . Mix immediately by inverting slowly, only once.
 - 6.2.2 To B (the blank) add 0.5 ml 1+1 H_2SO_4 and 0.15 ml FeCl_3 and mix.
- 6.3 Wait 3-5 minutes and add 1.6 ml $(\text{NH}_4)_2 \text{HPO}_4$ to each tube. Dissolve precipitate on vortex mixer.

Note: Blue color will develop in Tube A in about a minute if sulfide is present (allow additional time if initial pink color hasn't faded).
- 6.4 Wait 3-15 minutes (if zinc acetate was used, wait at least 10 minutes) and read absorbencies at 664 nm with 1-cm cell.
 - 6.4.1 Zero spectrophotometer with blank (Tube B)
 - 6.4.2 Record absorbance in Tube A.
 - 6.4.3 Determine concentration from calibration curve.

7. STANDARDIZATION

7.1 Preparing Standards:

There is no exact gravimetric method for making sulfide standards of pre-determined strength, so sulfide solutions of approximate concentrations are made up and their strengths then determined exactly using the Iodometric method. At the same time, the Methylene Blue method is used to determine absorbance of these standards so that a calibration curve of absorbance vs. concentration can be constructed.

Note: Standard Methods discusses comparing the error of the methylene blue visual method with the Iodometric method: this is strictly for determining the

concentration (standardization) of the methylene blue dye used in the visual comparison method and has nothing to do with the photometric method we are using here.

7.2 $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ solution:

Put several grams of clean, washed crystals of $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ into small beaker. Don't quite cover with water. Stir occasionally for a few minutes. Pour into another container. Solution is good for a few hours.

Add one drop of this solution to 1 L DI and mix. Immediately determine: 1) sulfide concentration by the iodometric method and 2) absorbance by the methylene blue method.

Repeat determinations by varying drops of Na_2S (or volume of water) till at least 5 standards have been run, with a range of 0.1-2.0 mg/L sulfide.

OR:

Can attempt approximation of standards ranging from 0.1 mg/L to 2.0 mg/L as follows:

7.3 1000 mg/L:

Weigh out 7.491 g $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ into a small beaker.² Don't quite cover with water. Stir occasionally for a few minutes. Pour into 1L volumetric flask and dilute to mark.

To cover the analytical range of interest usually encountered:

7.4 2 mg/L:

Dilute 2 ml 1000 mg/L to 1000 ml.

Make up standards by diluting 2 mg/L in test tubes as follows:

0.1 mg/L: 1.0 ml to 20 ml
0.5 mg/L: 5.0 ml to 20 ml
1.0 mg/L: 10 ml to 20 ml
1.5 mg/L: 15 ml to 20 ml
2.0 mg/L: no dilution

Determine absorbencies and construct a calibration curve.

8. QUALITY CONTROL

8.1 Run 10% duplicate samples (at least one duplicate per run). Readings should agree within 10%.

²** $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ = 240.176 g = 7.491
S²⁻ 32.06 g

- 8.2 Include one detection limit standard of 0.1 mg/L (most of our samples are at or below the detection limit).

CHANGE	DATE	INITIALS
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³*If no suspended solids present, can assume dissolved sulfide equals total sulfide.

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

ELECTRICAL CONDUCTIVITY (EC) ($\mu\text{S}/\text{cm}$) (also known as Specific Conductance; see *Standard Methods*, sec. 2510A)

File Name: M:\SOP\INORGNIC\EC.doc
Revision: 6

Effective Date: 11/19/2007
Supersedes: 5 (10/15/2007)

1. REFERENCES

- 1.1 *Standard Methods* 20th Ed. #2510 B, pp. 2-46 to 2-47.
- 1.2 *EPA Methods for Analysis of Water and Waste*, #120.1. This method complies with EPA - approved methodology for analysis of drinking water and wastewater.

2. HOLDING TIME

- 2.1 28 days at 4°C.

3. INTERFERENCES

- 3.1 Contaminated measurement cell; clean and replatinize (Pt cell only) if necessary following manufacturer's instructions.
- 3.2 While the conductivity meter has temperature compensation algorithms for both KCl standards and natural waters, sample temperature different from calibration solution or significantly different from the standard measurement temperature of 25°C can affect results. The temperature coefficient of most waters is only approximately the same as that of standard KCl solution; the more the temperature of measurement deviates from 25.0°C, the greater the uncertainty in applying any temperature correction. Always be sure that the sample to be analyzed and the calibration solution are at the same temperature, preferably as close to 25.0°C as possible.

4. APPARATUS

- 4.1 Self-contained conductivity meter capable of measuring conductivity with an error not exceeding 1% or 1 $\mu\text{S}/\text{cm}$, whichever is greater; currently using Orion Model 162A.
- 4.2 Conductivity cell: platinum reference or graphite monitoring. If non-platinum (graphite) cell is used for measurements, calibrate monthly by comparing reading from non-platinum cell with results obtained with platinum cell. Use appropriate correction factor, if any, to correct all measurements and document calibrations in a permanent log book kept with the instrument.

4.3 Thermistor temperature sensor, integral with measurement cell.

5. REAGENTS

5.1 Calibration solutions (all three needed):

5.1.1 Thermo Orion #011008 (100 $\mu\text{S}/\text{cm}$)

5.1.2 Thermo Orion #011007 (1413 $\mu\text{S}/\text{cm}$)

5.1.3 Thermo Orion #011006 (12.9 mS/cm)

5.2 Second Source Standards (may use either or both):

5.2.1 Potassium Chloride (KCl). 0.010 M: 1413 $\mu\text{S}/\text{cm}$ @ 25°C (VWR cat. #62344-936 or equivalent).

5.2.2 Potassium Chloride 1000 $\mu\text{S}/\text{cm}$ (Ricca #5888.01-32)

6. **PROCEDURE: (NOTE: Thermo Orion has confirmed that there are significant errors in the Model 162A instruction manual. This SOP has been confirmed with Thermo Orion Technical Service; if there is an inconsistency between the SOP and the instruction manual, follow the SOP).**

6.1 Plug instrument power cord into wall outlet. Instrument will go through a brief self-test. Holding in "yes" key will not change or enhance this process (despite what is written in instruction manual).

6.2 Press "setup" button on instrument. Instrument defaults should have the following settings visible on readout; if so, press "yes" to advance to next parameter. If not, use up and down arrow keys to scroll to correct setting, then press "yes" to accept:

TC (Temperature Compensation)	2.1%
REF (Reference Temperature)	25°C
TDS FCTR	0.66
CAL SEL (Calibration Select)	MUL (Multiple Point Calibration)
RDY PRNT	OFF
SET DATE	Current year, month, day
SET TIME	Current hour, minute
SET BAUD	1200

After scrolling through the above settings, making changes as necessary, press "mode" button to return to measurement mode.

6.3 Set up two 600 mL beakers containing DI water for rinsing. The measurement cell should always be rinsed in the beakers in the same sequence; that way, as the first beaker accumulates a slight conductivity from drops of samples carried over, the second beaker will remain relatively pure. The cell should have been stored dry in a beaker padded with paper towels. If not, rinse well in fresh DI solution. Always blot cell dry with a Kimwipe® after rinsing to avoid diluting standard solutions. Place cell into snap-cap beaker of Orion 100 $\mu\text{S}/\text{cm}$ calibration solution, moving it slowly

through solution and making sure cell surface is free of air bubbles.

- 6.4 Press “cal” button; readout should read “MUL”, then “SET P-1?”. Press “yes”.
- 6.5 The display will now indicate the current temperature in the top field and the standard value of 199.9 mS, with the first digit flashing. Press “cal” until the readout reads 199 μ S (Note: The display scrolls through ranges 199.9 mS, 19.99 mS, 1999 μ S, and 199.9 μ S in that order. If you miss the correct range, keep pressing “cal” until it comes up again).
- 6.6 Refer to the temperature vs. uncorrected EC chart taped to the side of the centrifuge for the Orion standards. Using the up and down arrow keys, enter the uncorrected EC reading for the Orion 100 μ S standard into the readout. For example, if the temperature of the standard is 22.0°C, enter “94.0” into the readout, not the nominal value of 100.0 μ S. The corrected values are only valid if the solution temperature is 25.0°C. When readout is correct, press the “yes” key to accept.
- 6.7 The meter will now display “SET P-2?”. Rinse the cell well in DI water, blot dry, and place cell in a snap-cap beaker of Orion 1413 μ S/cm calibration solution as above (NOTE: always calibrate the meter by placing the cell into the conductivity standards starting from the lowest concentration and working up to the higher concentrations in order to reduce any effect from carryover during calibration). Press the “yes” key, then proceed as in 6.5 and 6.6, but this time using the 1999 μ S range and the uncorrected value for the Orion 1413 μ S standard at ambient temperature.
- 6.8 The meter will now display “SET P-3?”. Proceed as above, this time using the Orion 12.9 mS/cm standard. When “SET P-4?” appears on the display, press “mode” to return to measurement mode. The instrument is now calibrated. “MODE”, “SET CELL”, and “PRINT” will appear on the display in succession, followed by the normal temperature and conductivity readouts. You may verify calibration compensated for ambient temperature by analyzing the calibration standards in order as samples—results should very nearly equal theoretical values (**normally within 2%**).
- 6.9 Analyze second source standard; again, readout should very nearly equal theoretical value as specified at 25.0°C (**also, within 2% should be attainable**).
- 6.10 Before analyzing samples, press “setup” again, and scroll TC value down using down arrow keys from 2.1% (used for calibration and QC standards) to “nLFn” (below 0.0). This is a temperature calibration algorithm used for natural waters. Press “yes” to accept, then “mode” to return to measurement mode. Change back to 2.1% when analyzing standard solutions.
- 6.11 Follow the same procedure as above for samples, making sure that the samples are **as close as practical** to the same temperature as the calibration solution. **Write down the EC reading when “READY” appears on the display. Be careful to**

distinguish between values displayed as $\mu\text{S}/\text{cm}$ and mS/cm . A good practice is to report all results as $\mu\text{S}/\text{cm}$, as this is the input the Aspen LIMS requires.

- 6.12 Besides the “nLFn” temperature compensation for natural waters, the EC meter has an “nLFU” setting for ultrapure waters accessible through the “setup” key. Use this setting when analyzing DI water for bacterial suitability. Be sure to return to the correct setting after DI analysis is completed.

7. CALCULATION

None necessary unless graphite cell readout differs from platinum cell, then apply a correction factor.-Instrument readout will automatically switch to mS/cm (millisiemens per centimeter) when the measured conductivity exceeds $1999 \mu\text{S}/\text{cm}$. Be sure to report any such high values correctly.

8. QUALITY CONTROL

- 8.1 Run one duplicate for every 10 samples or one duplicate per run for sets less than 10 samples. Values should agree within 10%, **however, < 1% should be easily attainable.** Analyze 2 Control Chart standards per run and insure that analyzed values are within control limits. 2nd source standard: EM Science cat. # PX1413: should agree with calibration standard within $\pm 10\%$. EPA Performance Evaluation samples are used for external validation. Results should be within EPA specified acceptance limits.

- 8.2 **There are 3 quality control reports that print out if necessary when the Inorganic Data Review Report is printed:**

8.2.1 Field / Lab EC Report: If lab and field EC differ enough for this report to be printed, repeat EC analysis, note reanalysis result on report, and notify supervisor, who will evaluate the need for notification of Water Quality.

8.2.2 TDS / EC Balance Report: Calls out samples where TDS / EC ratio is not between 0.55 and 0.7. Give to supervisor for evaluation of need for repeat analyses.

8.2.3 Exception Report: Treat the same as with any other method—repeat all appropriate results if still within holding time and note on report. Submit to supervisor for approval.

9. TROUBLESHOOTING

- 9.1 See "Interferences," "Procedure" #3 above.

SOP PROCEDURE CHANGE
FOR EC

[illegible]

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

FLUORIDE BY PROBE

File Name: M:\SOP\INORGNIC\FLRDPRBE.doc
Revision: 5

Effective Date: 3/14/2008
Supersedes: 4 (6/13/2007)

1. REFERENCES

- 1.1 Orion Instruction Manual: Fluoride Electrodes, Model 96-0900 (1983).
- 1.2 *Standard Methods* (20th Ed.) #4500-F⁻-C; *EPA Methods for Analysis of Water and Waste* #340.2

2. HOLDING TIME

- 2.1 28 days; no special requirements for preservation

3. APPARATUS

- 3.1 pH/specific ion meter (we use an Orion #920).
- 3.2 Orion specific electrode for Fluoride (Model #96-0900). **Do not store dry; store in small storage bottle w/ lower F⁻ calibration standard (0.1 mg/L – 100 mg/L).**
- 3.3 Magnetic stirrer, with layer of insulation to prevent gradual heating of solution

4. REAGENTS

- 4.1 100 mg/L fluoride standard. **Currently using VWR Cat. # VW3407-2.**
- 4.2 **Second source standard. Currently using Ricca Cat. # 3171-16, 100 mg/L F.**
- 4.3 TISAB III (a pH 5 buffer containing a chelating agent, CDTA).

5. INTERFERENCES

- 5.1 TISAB buffer eliminates the following interferences: pH out of 5-9 range; polyvalent cations such as Si⁺², Fe⁺³, and most commonly, Al⁺³.

6. PROCEDURE

- 6.1 Standardizing the Instrument:

- 6.1.1 Be sure to remove rubber cap from fluoride electrode and uncover filling hole. "Pump" the electrode to insure free flow of filling solution, and refill with KCl/AgCl filling solution.
- 6.1.2 Be sure fluoride electrode and reference pin are plugged into **system 1**.
- 6.1.3 Using **plastic** 200-mL volumetric flasks and volumetric pipets, prepare 2 standards which bracket the expected sample range and differ in concentration by a factor of 10 (i.e., a 0.5 and 5.0 mg/L standard or a 1.0 and 10 mg/L std.). **Measure** 50 mL of each standard into separate 150-mL plastic beakers and add **5** mL of TISAB III and a magnetic stir bar to each.
- 6.1.4 Press MODE key on the model 920 analyzer until "CONC" appears on the display.
- 6.1.5 Press FUNCTION key until display reads STD(1).
- 6.1.6 Place beaker containing the lower in concentration of the two standards on a stir plate and immerse the fluoride electrode in the solution. Use the magnetic stirrer to stir at a moderate rate, taking care to avoid any contact between the stir bar and the electrode, and eliminating any air bubbles from the fluoride electrode crystal surface.
- 6.1.7 When "READY" appears on display after reading has stabilized, press \wedge or \vee until correct standard concentration appears, then press "ENTER".
- 6.1.8 Remove electrode from 1st standard, rinse with DI water, and place in beaker containing 2nd (higher) standard. When "READY" appears on display after reading has stabilized, press \wedge or \vee until correct standard concentration appears, then press "ENTER".
- 6.1.9 The meter is now calibrated. Rinse electrode with DI water. Press FUNCTION key until "SLOPE" appears on the display. Record the information in the logbook.

6.2 Samples

- 6.2.1 **Measure** 40 ml sample + 4 ml TISAB III and use 50 ml Dispo beakers for samples.
- 6.2.2 Read concentration in mg/L directly from readout, allowing approximately 30 seconds to 2 minutes for stabilization. Report to 3 significant figures except: when < 1.00 mg/L, use 2 sig. figs.. Report values less than 0.10 mg/L as "<0.10 mg/L".
- 6.2.3 Between readings, rinse electrode and blot with Kimwipe□.
- 6.2.4 Check calibration periodically or at end of run.

6.2.5 When finished, put meter in STDBY; turn off stirrer. Store electrode (see Sec. 3.2).

Notes: Samples and standards should be at same temperature, preferably room temperature. Highly acidic or highly basic samples should be adjusted to pH 5-6 before addition of TISAB. Re-calibrate at least once in two hours.

6.3 To Check DI Water for Fluoride Concentration

Reagent: Prepare 10 mg/L fluoride standard.

6.3.1 Wash electrode by dipping Kimwipe® in 1-3% Ivory liquid solution and carefully wash off electrode. Rinse well.

6.3.2 Add 99 ml DI and 1 ml TISAB III into plastic beaker.

6.3.3 Wait 6-7 minutes for equilibrium.

6.3.4 Meantime, set Mode to KA/1 and STD VALUE to 10.0; set Slope to established value for instrument, or about -59.

6.3.5 When equilibrium is reached, press SET CONC. Display will read 10.0, then begin to drop.

6.3.6 Add 1.0 ml of 10 mg/L standard. Display will drop rapidly and show the fluoride conc in about 15 seconds.

--Detection limit is 0.006 mg/L.

7. ELECTRODE CARE

7.1 Fluoride Electrode — (black probe) - essentially maintenance free, except for keeping electrode filled with KCl/AgCl filling solution. To store, blot dry, re-cover filling hole, and replace **in storage solution (see Sec. 3.2)**. Do not store in water.

8. QUALITY CONTROL

8.1 Run at least one duplicate sample for every ten samples; every run should contain at least 1 duplicate. Duplicates should agree within 10%.

8.2 Include a pair of quality control standards (0.50 mg/L) with each run. Our quality control procedure specifies standards must be within 2 sigma control limits on quality control chart or $\pm 20\%$, whichever is less.

8.3 Include a second source standard with each run. Compare with published acceptance range.

- 8.4 Include a detection limit standard if sample results are "less than detection limit".
- 8.5 Check calibration periodically or at end of run.
- 8.6 In every run analyze a known addition (spiked sample) to check the results of a direct measurement. Should agree within $\pm 4\%$ if the proper conditions for known addition exist. See Orion instruction booklet for procedure.

9. TROUBLESHOOTING

- 9.1 If QC checks indicate precision or accuracy deficiencies, review procedure above, including section on electrode care. Refer to Orion manual "trouble-shooting checklist". Make sure wires are plugged securely into back of meter. Repeat measurements, making up fresh standard if necessary.

[illegible]

Hydrogen Peroxide

**ORANGE COUNTY WATER DISTRICT
STANDARD OPERATING PROCEDURE**

**POTASSIUM TITANIUM OXALATE DETERMINATION OF HYDROGEN
PEROXIDE CONCENTRATION**

File Name: M:\SOP\INORGNIC\HYDROGEN
PEROXIDE TITANIUM ANALYSIS
METHOD.DOC

Effective Date: 4/3/08

Revision: 3

Supersedes: Rev 2
(2/14/06)

SCOPE:

This method is suitable for the determination of hydrogen peroxide in aqueous effluents and raw sewage in the range 0.1 mg/L to 50 mg/L as H₂O₂.

PRINCIPLE:

The sample is clarified by pretreatment with aluminum chloride and sodium hydroxide solutions, and interfering substances are flocculated. The hydrogen peroxide present in the sample and standards reacts with potassium titanium oxalate. In an acidified solution, this reaction forms a yellow pertitanic acid complex. The colored complex is measured spectrophotometrically against an established concentration curve, at a 400 nm wavelength.

REAGENTS:

All: Reagents should be of analytical reagent grade, unless otherwise stated. Do not increase volumes of reagents, as only small volumes are used in the analysis, and the risk of contamination increases the longer a particular reagent is used.

1. Potassium permanganate 0.1N solution: Dissolve 3.2 g KMnO₄ in DI water and dilute to the mark in a 1 L volumetric flask.
2. Aluminum Chloride (484 g/L): Dissolve 121 g of AlCl₃·6H₂O in 150 ml of DI water and dilute to the mark in a 250 mL volumetric flask.
3. Potassium titanium oxalate solution (same as **Potassium bis(oxalato)oxotitanate (IV)**): Dissolve 25 g of Potassium titanium oxalate in 400 mL of DI water, warming if necessary, and dilute to the mark in a 500 mL volumetric flask.

Note 1: Potassium titanium oxalate is toxic and should be handled with care.

Note 2: **Store reagent in the incubator to prevent the solute from dropping out of solution.**

Note 3: Reorder this reagent as Potassium bis(oxalato)oxotitanate (IV) from Alfa Aesar. Stock # 89620.

4. Sodium Hydroxide solution: dissolve 60 g of NaOH in 150 mL DI water, cool, and dilute to the mark in a plastic 250 mL volumetric flask.

File Name: M:\SOP\INORGNIC\HYDROGEN PEROXIDE TITANIUM ANALYSIS
METHOD.doc
Revision: 3 (4/3/08)

5. Sulfuric Acid (1 + 9) solution: Slowly add 50 ml conc. H_2SO_4 (d 1.84) to 450 ml of DI water in a 1 L beaker with stirring. Cool. Do not dilute further—reagent is ready to use.
6. Sulfuric Acid (1 + 17) solution: Slowly add 20 ml of conc. H_2SO_4 (d 1.84) to 340 ml of DI water in 1 L beaker with stirring. Cool. Do not dilute further—reagent is ready to use.
7. Hydrogen Peroxide Stock Solution ACS grade, 29 – 30 % conc. (purchased).

Calibration of Standard Solution:

1. Pipet 7.5 mL of the stock Hydrogen Peroxide solution into a 2 L volumetric flask and dilute to the mark with DI water.
2. Using a graduated cylinder, add 50 ml DI water and 10 ml of (1 + 9) H_2SO_4 , to a 250 mL Erlenmeyer flask. Add 0.1 N Potassium Permanganate (KMnO_4) dropwise, until the appearance of a faint permanent pink color (approximately 1 drop).
3. Pipette 50.0 ml from the 2 L Hydrogen Peroxide solution above, into the Erlenmeyer flask.
4. Titrate the contents of the flask with 0.1 N Potassium Permanganate (KMnO_4) solution, until the same pink color is obtained. The initial pink color will fade upon initial titrating and reappear at the end point.
5. Calculate the concentration of Hydrogen Peroxide solution in the 2 L volumetric flask as follows:

$$\begin{aligned}\text{H}_2\text{O}_2 \text{ mg/L} &= T \times N \times 340 \text{ mg/L} \\ T &= \text{ml of } \text{KMnO}_4 \text{ titrant used} \\ N &= \text{Normality of } \text{KMnO}_4 \text{ solution (0.1)}\end{aligned}$$

Calibration Curve:

1. Dilute 5 ml of the standard calibration solution above, which is contained in the 2 L volumetric flask, to 250 mL with DI water. This is your working standard solution, used to make up your calibration standards.
2. Prepare approximate calibration standards of 0, 1 ppm, 2 ppm, 3 ppm, 4 ppm, 5 ppm and 6 ppm by pipetting 0, 1 mL, 2 mL, 3 mL, 4 mL, 5 mL, and 6 mL respectively of the Working Standard into seven separate 25 mL volumetric flasks, then adding DI water to each sufficient to make a total volume of 20 mL. The actual concentrations will be dependent upon the concentration of the working standard solution in (1) above. For example, if the Stock 2 L solution concentration were determined to be 1292 ppm by titration, diluting 5 ml of the 1292 ppm solution to 250 ml with DI water would yield a 25.84 ppm H_2O_2 solution. Using 1 ml of this solution, diluted to 25 ml with DI water, **after** addition of developing reagents, would yield an actual H_2O_2 concentration of 1.03 ppm. These values should be entered into the standards list, of the spectrophotometer program used. (Refer to Procedure For Analysis Section below).
3. Read all standards and samples at a wavelength of 400 nm.

Pretreatment of samples:

1. Fill a 300 mL BOD bottle to the top with the sample to be analyzed.
2. Pipette 1 mL of aluminum chloride solution and 1 mL of sodium hydroxide solution into each sample. Pipette the liquids below the surface of the sample liquid. Stopper bottle, mix well, and allow to sit for at least 30 minutes. Use the supernatant (clear part of sample) for the analysis.

Procedure for Analysis:

1. To a 25 ml volumetric flask, add 20 ml of sample supernatant or required amount of working standard solution diluted to 20 mL for desired standard concentration.
2. Add 2.5 ml of (1 + 17) Sulfuric Acid.
3. Add 2.0 ml of Potassium Titanium Oxalate solution.
4. Dilute to the 25 ml mark with DI water, and mix.
5. Prepare a blank correction for each sample by adding 2.5 ml of (1 + 17) Sulfuric Acid to 20 mL of sample supernatant, and diluting with DI water to the 25 ml mark. Subtract this concentration H_2O_2 value from its corresponding sample concentration H_2O_2 value, to arrive at the actual value of H_2O_2 for the sample.
6. Read standards and samples at 400 nm wavelength on the spectrophotometer.
7. Calculate correct sample concentration for dilution of 20 to 25 mL (multiply by 1.25).

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ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

TRACE ELEMENTS BY INDUCTIVELY COUPLED PLASMA-OPTICAL EMISSION SPECTROSCOPY

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REFERENCES:

EPA Methods for the Determination of Metals in Environmental Samples (Supplement I, 1994), 200.7, 200.15

Code of Federal Regulations 40 (July 1, 2006), Part 136, Appendix C

Standard Methods, 20th Ed., #3120 (pp. 3-34 to 3-40).

Perkin Elmer ICP-OES Optima 4300 DV WinLab32 Software User's Guide, Version 2.2
Cetac Technologies Inc., U-5000AT Ultrasonic Manual

1. SCOPE AND APPLICATION

- 1.1 This method may be used for the determination of dissolved, suspended, total, or total recoverable elements in drinking water, surface water, domestic and industrial wastewaters.
- 1.2 Dissolved elements are determined in filtered and acidified samples. Appropriate steps must be taken in all analyses to ensure that potential interferences are taken into account. This is especially true when dissolved solids exceed 1500 mg/L. (See 5.)
- 1.3 Total elements are determined after appropriate digestion procedures are performed. Since digestion techniques increase the dissolved solids content of the samples, appropriate steps must be taken to correct for potential interference effects. (See 5.)

2. SUMMARY OF METHOD

- 2.1 The method describes a technique for the simultaneous multi-element determination of trace elements in solution. The basis of the method is the measurement of atomic emission by an optical spectroscopic technique. Samples are nebulized and the aerosol that is produced is transported to the plasma torch where excitation occurs. Characteristic atomic-line emission spectra are produced by a radio-frequency inductively coupled plasma (ICP). The spectra are dispersed by a Echelle polychromator and the intensities of the lines are monitored by a segmented-array, Charge-coupled-device Detector. It is a solid state imaging device that provides simultaneous detection with simultaneous background measurement and is controlled by a computer system. A background correction technique is used to compensate

for variable background contribution to the determination of trace elements but seems to be unnecessary at concentration levels found in our samples. The position selected for the background intensity measurement, on either or both sides of the analytical line are determined by the complexity of the spectrum adjacent to the analyte line. The position used is free of spectral interference and reflects the same change in background intensity as occurs at the analyte wavelength measured. See discussion of additional interferences named in 5.1 (and tests for their presence as described in 5.2).

3. DEFINITIONS

- 3.1 Dissolved--Those elements that will pass through a 0.45 μm membrane filter.
- 3.2 Suspended--Those elements that are retained by a 0.45 μm membrane filter.
- 3.3 Total--The concentration determined on an unfiltered sample following vigorous digestion (9.3)
- 3.4 Total recoverable--The concentration determined on an unfiltered sample following treatment with hot, dilute mineral acid (9.4).
- 3.5 Instrumental detection limit-- The concentration equivalent to a signal, due to the analyte, which is equal to three times the standard deviation of a series of ten replicate measurements of a reagent blank signal at the same wavelength.
- 3.6 Sensitivity--The slope of the analytical curve, i.e. functional relationship between emission intensity and concentration.
- 3.7 Instrument check standard--multi-element standard of known concentrations prepared by the analyst to monitor and verify instrument performance on a daily basis. (See 7.6.1)
- 3.8 Interference check sample--A solution containing both interfering and analyte elements of known concentration that can be used to verify background and inter-element correction factors. (See 7.6.2)
- 3.9 Laboratory Fortified Blank (LFB) -- An aliquot of LRB to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control and whether the laboratory is capable of making accurate and precise measurements.
- 3.10 Laboratory Fortified Sample Matrix (LFM) - An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.
- 3.11 Quality control sample--A solution obtained from an outside source having known concentration values to be used to verify the calibration standards.

- 3.12 Calibration standards--a series of known standard solutions used by the analyst for calibration of the instrument (i.e., preparation of the analytical curve).
- 3.13 Linear dynamic range--The concentration range over which the analytical curve remains linear.
- 3.14 Reagent blank--A volume of deionized, distilled water containing the same acid matrix as the calibration standards carried through the entire analytical scheme.
- 3.15 Calibration blank--A volume of deionized, distilled water acidified with HNO₃ and HCl.
- 3.16 Method of standard addition-- The standard addition technique involves the use of the unknown and the unknown plus a known amount of standard. See 10.6.1)

4. SAFETY

- 4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets is available to all personnel involved in chemical analyses.

5. INTERFERENCES

- 5.1 Several types of interference effects may contribute to inaccuracies in the determination of trace elements. They can be summarized as follows:
- 5.2 Spectral interferences can be categorized as 1) overlap of a spectral line from another element; 2) unresolved overlap of molecular band spectra; 3) background contribution from continuous or recombination phenomena; and 4) background contribution from stray light from the line emission of high concentration elements. The first of these effects is compensated by utilizing a computer correction of the raw data, requiring the monitoring and measurement of the interfering element. The second effect may require selection of an alternate wavelength. The third and fourth effects can usually be compensated by a background correction adjacent to the analyte line. In addition, users of simultaneous multi-element instrumentation must assume the responsibility of verifying the absence of spectral interference from an element that could occur in a sample but for which there is no channel in the instrument array. Listed in Table 2 are some interference effects for the recommended wavelengths given in Table 1. The data in Table 2 are intended for use only as a rudimentary guide for the indication of potential spectral interferences. For this purpose, linear relations between concentration and intensity for the analytes and the interferents can be assumed.
- 5.3 Physical interferences are generally considered to be effects associated with the sample nebulization and transport processes. Such properties as change in viscosity and surface tension can cause significant inaccuracies especially in samples that may contain high dissolved solids and/or acid concentrations. A peristaltic pump is used to lessen these

interferences. If these types of interferences are still operative, they must be reduced by dilution of the sample and/or utilization of standard addition techniques.

- 5.4 Chemical Interferences are characterized by molecular compound formation, ionization effects and solute vaporization effects. Normally these effects are not pronounced with the ICP technique, however, if observed they can be minimized by careful selection of operating conditions that is, incident power, observation position, and so forth), by buffering of the sample, by matrix matching, and by standard addition procedures. These types of interferences can be highly dependent on matrix type and the specific analyte element.
- 5.5 It is recommended that whenever a new or unusual sample matrix is encountered, a series of tests be performed prior to reporting concentration data for analyte elements. These tests, as outlined in 5.2.1 through 5.2.4, will ensure the analyst that neither positive nor negative interference effects are operative on any of the analyte elements thereby distorting the accuracy of the reported values.
- 5.6 Serial dilution--If the analyte concentration is sufficiently high (minimally a factor of 10 above the instrumental detection limit after dilution), an analysis of a dilution should agree within 5 % of the original determination (or within some acceptable control limit (14.3) that has been established for that matrix). If not, a chemical or physical interference effect should be suspected.
- 5.7 Spike addition--The recovery of a spike addition added at a minimum level of 10X the instrumental detection limit (maximum 100X) to the original determination should be recovered to within 90 to 110 percent or within the established control limit for that matrix. If not, a matrix effect should be suspected. The use of a standard addition analysis procedure can usually compensate for this effect. Caution: The standard addition technique does not detect coincident spectral overlap. If suspected, use of computerized compensation, an alternate wavelength, or comparison with an alternate method is recommended.
- 5.8 Comparison with alternate method of analysis--When investigating a new sample matrix, comparison tests may be performed with other analytical techniques such as inductively coupled plasma/mass spectrometry, atomic absorption spectrometry, or other approved methodology.
- 5.9 Wavelength scanning of analyte line region to detect potential spectral interferences—This is performed once at initial instrument setup.

6. APPARATUS

- 6.1 Inductively Coupled Plasma-Optical Emission Spectrometer (Perkin Elmer Corp), Ultrasonic Nebulizer (CETAC U-5000RT), Autosampler 93 (Perkin Elmer).
- 6.2 Computer controlled atomic emission spectrometer with background correction.
- 6.3 Radiofrequency generator.
- 6.4 Argon gas supply, cryogenic liquid, high-purity grade (99.996% or better).

7. OPERATING CONDITIONS

STARTUP PROCEDURE:

- 7.1 Before starting, clear out the water from both water traps located on the wall behind the instrument. Press the release valve located beneath each trap. Hold it open till all the water and vapor is released. Check the dessicant in black container next to the traps, to insure that the dessicant is still good. There is a glass viewing area at the bottom of the container to check the dessicant quality. Blue indicates that the dessicant is still good and pink indicates that it is spent and needs to be replaced. Notify maintenance to replace the dessicant and order new replacement dessicant from Grainger. **Turn ON** computer and monitor. Note that there are two analysis methods. Follow only the instructions that pertain to the method chosen for the analysis. For the analysis of Ca, B, K, Mg and Na use Method 200.7 Cyclonic#1. (Verify the correct conditions for this method. Plasma = 15; Auxiliary = 0.2; Nebulizer = 0.54; RF Power = 1450). This uses the cyclonic spray chamber. If using this method for analysis, disregard any references to the CETAC ultrasonic nebulizer. If analyzing for Cr, Fe and V, use Method 200.7 Trace. (Verify the correct conditions for this method. Plasma = 16; Aux. = 0.2; Neb. = 0.54; RF Power = 1450). This method utilizes the ultrasonic nebulizer. Please follow any directions pertaining to this below and disregard any mention of the cyclonic spray chamber.
- 7.2 If using the CETAC unit, **Press Power** switch located on rear of CETAC nebulizer. Unit takes approximately 15 minutes to reach operating parameters (140 degrees C +/- 2 degrees C on heater and 3 degrees C +/- 1 degree Celsius on cooler).
- 7.3 **Open** liquid argon gas located outside.
- 7.4 **Turn On** ICP-OES chiller, located outside.
- 7.5 When computer is finished booting up, press **ALT-CTL-DELETE**. At login screen press **ENTER**.
- 7.6 Double Click on **WinLab32** icon. The computer automatically boots up to the **Diagnostics** window. Look at and check the following icons:
 - 7.6.1 **Spectrophotometer:** this will show you how long the instrument has till it has warmed up and is ready to analyze samples.
 - 7.6.2 **Plasma:** informs user if the communication to the generator is OK.
 - 7.6.3 **System:** A window showing blue lines connecting the computer, auto sampler and ICP instrument should be active and viewable. All the blue lines should turn green, indicating that communication between each unit has been established. If not, refer to the appropriate tab in the active window, to see what the problem is.
 - 7.6.4 Connect the autosampler to either the ultrasonic or cyclonic spray chamber instrument pump lines, depending on which method you are going to perform.
- 7.7 When the instrument is ready, click in **FILE**, click on **OPEN**, select **WORKSPACE**. Click

on **VIEW ALIGN.WSP**. Click on **File**, **Open**, click on **Method** and click on either Trace or Cyclonic1, then **OK**. **NOTE:** Before step 7.8, insure that the pump tubing is in good condition. Pump tubing **at a minimum** should be changed after every 8 hours of use.

- 7.8 Click on the **PLASMA CONTROL** Window. ******IMPORTANT NOTE: Before turning plasma ON, remove moisture from air line moisture trap located on wall behind instrument. Failure to do this thoroughly may result in plasma shutting off prematurely during run.**
- 7.9 Click the plasma ON/OFF button to **ON** and allow the plasma to equilibrate for about 10 minutes. You do not need to have the cyclonic nebulizer functioning while the plasma is on. The plasma can run dry on this instrument. Once the plasma is ignited, **Open Method** you plan to use for the analysis. If you are running with the cyclonic spray chamber, you need to turn on the heater for the nebulizer box. Press the "Heat" button on the Plasma Control window. Click on the **Method** Icon to activate the method window. Click on **Sampler** in the method window. Compare the conditions shown in the method with those activated in the **Plasma Control** window. If within a minute of initial plasma ignition these conditions are not shown or do not match, then manually enter and click **Apply**. **DO NOT PROCEED TO STEP 10 UNTIL THESE VALUES ARE PRESENT OR CHANGED TO THE METHOD SELECTED VALUES OR THE PLASMA WILL NOT BE OPTIMIZED FOR YOUR RUN.**
- 7.10 In **VIEW ALIGN.WSP** of the **Spectrometer Control Window** click on **Hg Realign** to profile the instrument.
- 7.11 When the Hg realignment is finished, click on **Radial** under Manual Settings, Plasma. Aspirate a 10 ppm Manganese solution and allow to equilibrate in the plasma. When solution is equilibrated, click on **Align View**, **OK**. When finished, click on **Apply**. Do not remove the probe from the solution yet.
- 7.12 Click on **Axial**, under Manual Settings, Plasma. Click on **Align View**, **OK**. When finished, click on **Apply**. The realignment and Hg profile values are printed out. File the printout with your data packet. Remove the probe from the 1 ppm Mn solution and place it in the wash solution.
- 7.13 Click on **FILE**. Click on **OPEN**. Click on **WORKSPACE**.
- 7.14 Double click on **AUTO.WSP**.
- 7.15 Click on **SAMPLE INFO** icon.
- 7.16 Click on **FILE**. Click on **OPEN**. Click on **SAMPLE INFO FILE**. Click on **TEMPLATE Cyclonic.SIF** or **Trace Template.sif**. Type in the sample LIMS number. Type in sample **Aliquot** (normally 97 ml unless sample is diluted. Standard are 100 ml). Under the **Diluted to Volume** type in 100 ml for all types of samples and standards. If a samples is to be designated as a Spike Recovery or Duplicate sample, then proceed as follows: **the original sample must be before the sample you indicate as a duplicate or if it is to be designated as a spike, the spiked sample must come after the unspiked sample**. Double click on the **Matrix Check Sample** cell, of the sample so designated. The Matrix Check Sample window appears. Click on either **Duplicate** or **Recovery (Spike)**, whichever the sample is to be designated. Do not worry about the set numbers for duplicates. As long as you followed the

above placement of samples, the imputed numbers showing will be correct. For spiked samples you must also select the **Set Number**. This number indicates the proper concentrations of the spike. Please check the method you are using for spike sets and corresponding concentrations. To add samples to the Sample Information File while running, recall the desired sample information file. Add your samples to the list. Click on **Append to Analysis List** located on upper right of the sample info file page. Type in then **Sample** starting number, followed by a hyphen and then the last **sample** number. **DO NOT USE THE AUTOSAMPLER NUMBER**. Press **OK**. The analyzing samples page on the auto analysis window should show the updated sample file names. Please note that updates to the sample info file can only be done in the Winlab32 continuous window of the sample info file. You can not use Winlab32 Offline windows sample info file.

7.17 When finished typing in the sample table information. click on **FILE**. Click on **SAVE AS**. Click on **SAMPLE INFO FILE**. Under file name in Year-Month subdirectory, type in the usual Year-Month-Day lab convention for naming of files (i.e. 070216 for February 16, 2007). Then click on **SAVE**. To print a copy of the **Sample Information File**, proceed as follows:

7.17.1 Activate the **Sample Information File** by clicking on the sample info window (indicated by the gray top bar turning blue).

7.17.2 Click on **FILE**. Click on **PRINT**.

7.17.3 Click on **Active Window**. Click **OK**.

7.18 **To begin the analysis:**

7.18.1 Click on **Analysis** at top of window. Click on **Clear Spectra Display**.

7.18.2 Click on **Analysis** at top of window. Click on **Clear Results Display**.

7.18.2.1 Click on **Analysis** at top of window. Click on **Clear Reagent Blank**.

7.18.2.2 Click on **Analysis** at top of window. Click on **Clear Calibration Blank**.

7.18.2.3 Click on **Analysis** at top of window. Click on **New Calibration**.

7.18.2.4 Activate the **Automated Analysis Control** window, by clicking on it if visible, or clicking on the icon labeled **Auto** at the top of the window.

7.18.2.5 Click on **Setup** Tab to activate this window. Under "**Method and Sample Location**" double click on **Method** and highlight the method to be used. Then double click. (Use Method 200.7 Cyclonic#1 or Method 200.7 Trace). Under **Sample Information File**, click on **OPEN**. Select the **Sample Info File** that contains your sample list to be analyzed. Under **Result Data Set Name**, click on **OPEN**. Type in the same file name as the sample info file and press **OK**. Click in box "**Print Log During Analyses**".

7.18.2.6 Click on **Analyze** Tab. After placing your standards and samples in the appropriate tray area, you may begin the analysis in one of two ways. Standard positions in tray are as follows: 1 = Blank; 2 = Standard 1; 3 =

Standard 2; 4 = Standard 3; 5 = Standard 4; 6 = Standard 5; 7 = Standard 6; and 8 = C.C. Std. Click on the **Analyze All**. Selecting this will analyze the standards and then the samples. **This is not recommended**. This does not allow you to check and refine your calibration before analysis. The recommended procedure is to first click on **Calibrate**. This will perform a calibration then stop and allow you to ensure that all elements have a correlation coefficient of 0.995 or better and if not, to correct the problem before proceeding. Print out the calibration curves as follows. Click **Calibration Icon**, click **FILE**, then click **PRINT**. Click **ACTIVE WINDOW**. If and when the calibration is OK, click on **ANALYZE SAMPLES**. ****NOTE: It is highly recommended that you check the peak wavelengths before proceeding with the sample analysis. To do this, click on the **Examine** icon to enter the **Examine Spectra** window. Click on **Data** to open the current calibration data set. Choose the Blank and Standards you wish to examine. Check the spectra for each analyte. Usually the peak wavelength is ok. If not, move the cursor so it is aligned with the center of the peak. If the peak is outside the window, click and drag the **P** so the peak is within the window. When everything looks ok, click on **Method**, then **Update Method Parameters**, then **Update and Save Method** button. You need to do this for each element you make adjustments to. Then proceed to **ANALYZE SAMPLES**.

7.18.2.7 Samples will now be analyzed according to your sample info file and the method.

7.19 The method of standard additions is not normally required on samples analyzed by the OCWD lab. If necessary, refer to this section in the complete EPA Method 200.7.

REAGENTS AND STANDARDS

- 8.1 Acids used in the preparation of standards and for sample processing must be ultra-high purity grade (Baker-Mallinckrodt ULTREX) or equivalent. Redistilled acids are acceptable.
- 8.2 Nitric acid, conc. (sp gr 1.41).
Hydrochloric Acid, conc. (sp gr 1.19).
- 8.3 Nitric acid (1+1): Add 500 mL conc. HNO_3 (sp. gr 1.41) to 400 ml Milli Q water and dilute to 1 liter.
Hydrochloric acid (1+1): Add 500 ml conc. HCl (sp gr 1.19) to 400 ml Milli Q Water and dilute to 1 liter.
- 8.4 Milli Q water —produced by Milli-Q Gradient A-10 System (Millipore) fed by whole-lab deionization system. Use Milli-Q water for the preparation of all reagents, calibration standards and as dilution water.
- 8.5 Calibration standard stock solutions are purchased pre-prepared (SPEX Corp., Metuchen, NJ). Observe expiration date printed by manufacturer on bottle label and order fresh mixed standards in time to replace expired solution. Calibration standards must be initially verified

using a quality control sample and monitored weekly for stability.

8.6 Stock solutions are as follows:

8.6.1 10,000 mg/L Stock solutions for: Ca, K, Mg, and Na.

8.6.2 1,000 mg/L Stock solutions for: B, Cr, and Fe.

8.6.3 Blanks -- Four types of blanks are required for the analysis. The calibration blank is used in establishing the analytical curve, the laboratory reagent blank is used to assess possible contamination from the sample preparation procedure, the laboratory fortified blank is used to assess routine laboratory performance and a rinse blank is used to flush the instrument uptake system and nebulizer standards, check solutions, and samples to reduce interferences between standards, check solutions, and samples to reduce memory interferences.

8.7 The calibration blank for aqueous samples and extracts is prepared by acidifying reagent water to the same concentrations of the acids as used for the standards. The calibration blank should be stored in a FEP bottle.

8.8 The laboratory reagent blank (LRB) must contain all the reagents in the same volumes as used in the processing of the samples. The LRB must be carried through the same entire preparation scheme as the samples including sample digestion, when applicable.

8.9 The laboratory fortified blank (LFB) is prepared by fortifying an aliquot of the laboratory reagent blank with all analytes to a suitable concentration using the following recommended criteria, Ca, Mg, Na = 50 mg/L, K = 5.0 mg/L and all other analytes 0.2 mg/L or a concentration approximately 100 times their respective MDL, whichever is greater. The LFB must be carried through the same entire preparation scheme as the samples including sample digestion, when applicable.

8.10 Rinse blank is prepared by acidifying reagent water to the same concentrations of acids as used in the calibration blank and stored in a convenient manner.

8.11 In addition to the calibration standards, an instrument check standard, an interference check sample and a quality control sample are also required for the analyses.

8.12 The instrument check standard is prepared by the analyst by combining compatible elements at a concentration equivalent to the midpoint of their respective calibration curves.

8.12.1 The interference check sample is prepared by the analyst in the following manner. Select a representative sample that contains minimal concentrations of the analytes of interest by known concentration of interfering elements that will provide an adequate test of the correction factors. Spike the sample with the elements of interest at the approximate concentration of either 100 ug/L or 5 times the estimated detection limits given in Table 1. (For effluent samples of expected high concentrations, spike at an appropriate level.) If the types of samples analyzed are varied, a synthetically prepared sample may be used if the above criteria and intent are met.

8.12.2 The quality control sample should be prepared in the same acid matrix as the

9. SAMPLE HANDLING AND PRESERVATION

- 9.1 For the determination of trace elements, contamination and loss are of prime concern. Dust in the laboratory environment, impurities in reagents and impurities on laboratory apparatus that the sample contacts are all sources of potential contamination. Sample containers can introduce either positive or negative errors in the measurement of trace elements by (a) contributing contaminants through leaching or surface desorption or (b) by depleting concentrations through adsorption. Thus the collection and treatment of the sample prior to analysis requires particular attention. Laboratory glassware including the sample bottle (whether polyethylene, polypropylene or FEP-fluorocarbon) should be thoroughly washed with detergent and tap water; rinsed with (1+1) nitric acid, tap water, (1+1) hydrochloric acid, tap and finally deionized distilled water in that order (if disposable, pre-cleaned bottles as mentioned in 9.3 are not used). NOTE 2: Use caution if considering using chromic acid to remove organic deposits from glassware; (normally unnecessary for OCWD laboratory samples). A commercial product, NOCHROMIX, available from Godax Laboratories, 6 Varick St., New York, NY 10013, may be used in place of chromic acid. Chromic acid should not be used with plastic bottles. NOTE 3: If it can be documented through an active analytical quality control program using spiked samples and reagent blanks, that certain steps in the cleaning procedure are not required for routine samples, those steps may be eliminated from the procedure.
- 9.2 Before collection of the sample a decision must be made as to the type of data desired, that is dissolved, suspended, total recoverable or total, so that the appropriate preservation and pretreatment steps may be accomplished. Filtration, acid preservation, etc., are to be performed at the time the sample is collected or as soon as possible, thereafter.
- 9.3 Samples for metals analysis must always be collected in a separate, metal-free container provided by the laboratory to sampling personnel. The container will typically be a disposable, certified, pre-cleaned plastic bottle supplied by I-Chem, Eagle-Picher, or other vendor, and will either contain nitric acid suitable for preserving the sample, or not, as appropriate (see below).
- 9.3.1 For the determination of dissolved elements the **unacidified** sample must be filtered through a 0.45 µm membrane filter as soon as practical after collection **in a pre-cleaned bottle not containing acid**. Single-use plastic filtering apparatus are recommended to avoid possible contamination (**Corning 431155 or eq.**). **First, rinse the filter and apparatus with 50-100 mL DI water**. Then use the first 50-100 mL of sample to rinse the filter and filter flask. Discard this portion and collect the required volume of filtrate. Acidify the filtrate with (1+1) HNO₃ to a pH of 2 or less. Normally, 3 mL of (1+1) acid per liter should be sufficient to preserve the sample. **Store in the bottom half of the filter apparatus using the screw-cap closure. Do not use a filter for more than one sample.**
- 9.3.2 Suspended elements are rarely specified for analysis by the OCWD laboratory. Refer to full EPA Method 200.7 for details if necessary.

- 9.3.3 For determination of total recoverable elements in aqueous samples, the samples must be acid preserved prior to aliquoting for either sample processing or determination by direct spectrochemical analysis. For proper preservation samples are not filtered, but acidified with (1+1) nitric acid to pH < 2. Preservation is to be done at the time of sample collection for total and total recoverable elements. **The Water Quality Department will collect these samples in disposable, certified, pre-cleaned plastic bottles supplied by I-Chem, Eagle-Picher, or other vendor, already containing a measured amount of nitric acid.** If for some reason field preservation is impossible, it is recommended that the samples be returned to the laboratory as soon as possible after collection and acid preserved upon receipt in the laboratory. **If there is doubt whether field preservation is appropriate, Water Quality will normally collect the sample in an un-acidified bottle.** In either case, following acidification, the sample should be mixed and held for sixteen hours. (Normally, 3 mL of (1+1) nitric acid per liter of sample is sufficient for most ambient and drinking water samples). The pH of all aqueous samples must be tested immediately prior to withdrawing an aliquot for processing to ensure the sample has been properly preserved. If for some reason such as high alkalinity the sample pH is verified to be > 2, more acid must be added and the sample held for sixteen hours, and the acidification and holding process repeated until the sample is verified to be pH < 2. If properly acid preserved, the sample can be held up to 6 months before analysis. NOTE: When the nature of the sample is either unknown or is known to be hazardous, acidification should be done in a fume hood.

10. SAMPLE PREPARATION

- 10.1 For the determinations of dissolved elements, the filtered, preserved sample may often be analyzed as received. The acid matrix and concentration of the samples and calibration standards must be the same. (See Note 6.) If a precipitate formed upon acidification of the sample or during transit or storage, it must be redissolved before the analysis by adding additional acid and/or by heat as described in 9.3. With the exception of silver, where this method is approved for the determination of certain metal and metalloid contaminants in drinking water, samples may be analyzed directly by pneumatic nebulization without acid digestion if the sample has been properly preserved with acid and has turbidity of < 1 NTU at the time of analysis. This total recoverable determination procedure is referred to as "direct analysis". Check all drinking water samples for turbidity before analysis, and submit all those having an NTU value of 1 or greater to the Total Recoverable or Total element procedures.
- 10.2 Suspended elements are rarely specified for analysis by the OCWD laboratory. Refer to full EPA Method 200.7 for details if necessary. NOTE 4: In place of filtering, the sample after diluting and mixing may be centrifuged or allowed to settle by gravity overnight to remove insoluble material.
- 10.3 Total elements are rarely specified for analysis by the OCWD laboratory. Refer to full EPA Method 200.7 for details if necessary. NOTE 5: When determining boron in aqueous samples, only plastic, PTFE or quartz labware should be used from time of sample collection to completion of analysis. When possible, borosilicate glass should be avoided to prevent contamination of these analytes. NOTE 6: If the sample analysis solution has a different acid concentration from that given in 9.4, but does not introduce a physical interference or affect the analytical result, the same calibration standards may be used.

- 10.4 For the determination of total recoverable elements, choose a measured volume of a well mixed, acid preserved sample appropriate for the expected level of elements and digest in open vessel digestion block (Environmental Express) according to manufacturer's instructions. Adjust the volume to 50 mL and mix. The sample is now ready for analysis. Concentrations so determined shall be reported as "total."

11. CALCULATION

11.1 Reagent blanks (7.5.2) should be subtracted from all samples. This is particularly important for digested samples requiring large quantities of acids to complete the digestion.

11.2 If dilutions were performed, the appropriate factor must be applied to sample values.

11.3 Data should be rounded to the tenth or units place, as appropriate, and all results should be reported in µg/L up to three significant figures.

12. SHUTDOWN PROCEDURE

12.1 Aspirate deionized water for at least 3 minutes. If using the CETAC Ultrasonic Nebulizer, rinse entire transducer faceplate and its surrounding area by introducing water through the auxiliary rinse port of the aerosol chamber, using the attached spray bottle. This prevents accumulation of corrosive liquid samples on the transducer faceplate and the glassware.

12.2 Turn OFF sample peristaltic pump under plasma control window. If using the Ultrasonic nebulizer, turn of the Operate button and let ultrasonic nebulizer run dry for 15 seconds.

12.3 Press the green FAST PUMP switch on the nebulizer to allow the pump to drain all the liquid from the system.

12.4 Turn OFF the green FAST PUMP switch followed by the POWER switch located on the back of the ultrasonic nebulizer.

12.5 Turn off the plasma and the gas supplies accordingly.

12.6 Turn OFF cooling system.

13. QUALITY CONTROL (INSTRUMENTAL)

13.1 Check the instrument standardization by analyzing appropriate quality control check standards as follows:

13.2 Analyze an appropriate instrument check standard (7.6.1) containing the elements of interest at a frequency of 10%. This check standard is used to determine instrument drift. If agreement is not within 5% of the expected values or within the established control limits, whichever is lower, the analysis is out of control. The analysis should be terminated, the problem corrected, and the instrument recalibrated. Analyze the calibration blank at a frequency of 10%. The result should be within the established control limits of two standard deviations of the mean value. If not, repeat the analysis two more times and average the three results. If the

average is not within the control limit, terminate the analysis, correct the problem and recalibrate the instrument.

13.2.1 To verify inter-element and background correction factors analyze the interference check sample at the beginning, end, and at periodic intervals throughout the sample run. Results should fall within the established control limits of 1.5 times the standard deviation of the mean value. If not, terminate the analysis, correct the problem and recalibrate the instrument.

13.2.2 A quality control sample obtained from an outside source must first be used for the initial verification of the calibration standards. A fresh dilution of this sample shall be analyzed every week thereafter to monitor their stability. If the results are not within 5% of the true value listed for the control sample, prepare a new calibration standard and recalibrate the instrument. If this does not correct the problem, prepare a new stock standard and a new calibration standard and repeat the calibration.

13.2.3 Samples with results within 90% or more of the upper limit of the linear dynamic range are diluted and re-analyzed.

For Sections 12 and 13, see the complete EPA Method 200.7 (Appendix C to *Code of Federal Regulations*, Part 136. The following are additional mandatory quality control checks required by EPA Method 200.7, Revision 4.4, May 1994 (numbering sequence follows referenced revision and is different from the numbering of the Appendix C version used elsewhere in this Standard Operating Procedure):

14. ASSESSING LABORATORY PERFORMANCE (MANDATORY)

14.1 Laboratory reagent blank (LRB) -- The laboratory must analyze at least one LRB (Sect. 7.10.2) with each batch of 20 or fewer samples of the same matrix. LRB data are used to assess contamination from the laboratory environment. LRB values that exceed the MDL indicate laboratory or reagent contamination should be suspected. When LRB values constitute 10% or more of the analyte level determined for a sample or is 2.2 times the analyte MDL whichever is greater, fresh aliquots of the samples must be prepared and analyzed again for the affected analytes after the source of contamination has been corrected and acceptable LRB values have been obtained.

14.2 Laboratory fortified blank (LFB) -- The laboratory must analyze at least one LFB (Sect. 7.10.3) with each batch of samples. Calculate accuracy as percent recovery using the following equation:

$$R = \frac{\text{LFB} - \text{LRB}}{s} \times 100$$

where: **R -- percent recovery.**
 LFB = laboratory fortified blank.
 LRB = laboratory reagent blank.
 s -- concentration equivalent of analyte added to fortify the LBR solution.

If the recovery of any analyte falls outside the required control limits of 85--115%, that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.

- 14.3 The laboratory must use LFB analyses data to assess laboratory performance against the required control limits of 85--115%. When sufficient internal performance data become available (usually a minimum of twenty to thirty analyses), optional control limits can be developed from the mean percent recovery (\bar{x}) and the standard deviation (S) of the mean percent recovery. These data can be used to establish the upper and lower control limits as follows:

$$\begin{aligned}\text{UPPER CONTROL LIMIT} &= \bar{x} + 3s \\ \text{LOWER CONTROL LIMIT} &= \bar{x} - 3s\end{aligned}$$

The optional control limits must be equal to or better than the required control limits of 85--115%. After each five to ten new recovery measurements, new control limits can be calculated using only the most recent twenty to thirty data points. Also, the standard deviation (S) data should be used to establish an ongoing precision statement for the level of concentrations included in the LFB. These data must be kept on file and be available for review.

14.3.1 Assessing Analyte Recovery and Data Quality

- 14.3.1.1 Sample homogeneity and the chemical nature of the sample matrix can affect analyte recovery and the quality of the data. Taking separate aliquots from the sample for replicate and fortified analyses can in some cases assess the effect. Unless otherwise specified by the data user, laboratory or program, the following laboratory fortified matrix (LFM) procedure (Sect 9.4.2) is required.
- 14.3.1.2 The laboratory must add a known amount of each analyte to a minimum of 10% of the routine samples. In each case the LFM aliquot must be a duplicate of the aliquot used for sample analysis and for total recoverable determinations added prior to sample preparation. For water samples, the added analyte concentration must be the same as that used in the laboratory fortified blank (Sect. 7.10.3). (For notes on Ag, Ba, and Sn see Sects. 1.7 & 1.8.) Over time, samples from all routine sample sources should be fortified. NOTE: The concentration of calcium, magnesium, sodium and strontium in environmental waters, along with iron and aluminum in solids can vary greatly and are not necessarily predictable. Fortifying these analytes in routine samples at the same concentration used for the LFB may prove to be of little use in assessing data quality for these analytes. For these analytes sample dilution and reanalysis using the criteria given in Section 9.5.2 is recommended. Also, if specified by the data user, laboratory or program, samples can be fortified at higher concentrations, but even major constituents should be limited to < 25 mg/L so as not to alter the sample matrix and affect the analysis.
- 14.3.1.3 Calculate the percent recovery for each analyte, corrected for background concentrations measured in the unfortified sample, and compare these

values to the designated LFM recovery range of 70-130% or a 3 sigma recovery range calculated from the regression equations given in Table 9. Recovery calculations are not required if the concentration added is less than 30% of the sample background concentration. Percent recovery may be calculated in units appropriate to the matrix, using the following equation:

$$R = \frac{C_s - C}{s} \times 100$$

where: R = percent recovery.

C_s = fortified sample concentration.

C = sample background concentration.

s = concentration equivalent of analyte added to fortify the sample.

- 14.4 If the recovery of any analyte falls outside the designated LFM recovery range, and the laboratory performance for that analyte is shown to be in control (Sect. 9.3), the recovery problem encountered with the fortified sample is judged to be matrix related, not system related. The data user should be informed that the result for that analyte in the unfortified sample is suspect due to either the heterogeneous nature of the sample or matrix effects and analysis by method of standard addition or the use of an internal standard(s) (Sect. 11.5) should be considered.

15. QUALITY CONTROL

- 15.1 Complete the ICP Data Review form. See Exhibit F on page 14.
- 15.2 Run calibration check standards at start and end of run. See Table F.1 for acceptance limits.
- 15.3 Run seven (7) Detection Limit Standards with each run. This should be labeled as follows: MDLxxyyymmddM, where xx is the number of the MDL in the run, yy is year, mm is month, and dd is day. If you do this, the MDL data will be easier to retrieve in Aspen. See Table F.2 for acceptance limits.
- 15.4 Run a Laboratory Reagent Blank every 10 analysis. For example, run a method blank at the 10th, 20th 30th, etc., position. See Table F.3 for acceptance limits.
- 15.5 Run a Digestion Blank if samples were digested. See Table F.3 for acceptance limits.
- 15.6 Run at least one routine spike sample to measure recovery for every 10 samples analyzed. See Table F.4 for acceptance limits.
- 15.7 A Second Source standard is analyzed with each run. See Table F.5 for an example of the concentrations of a specific second source standard and acceptance limits. Note that this is an example only, other second source standards can be used. Include a list of the concentrations of the second source standards with the data review.
- 15.8 Run one duplicate for every ten samples, or one duplicate per run for sets of less than ten

samples. Values should agree within 10 %.

- 15.9 Participation in performance evaluation studies is recommended using USEPA external standards.
- 15.10 Run a **Background Interference Check Standard** with each run. See Table F.6 for acceptance limits.
- 15.11 Dilute and rerun any sample where the Fe result is greater than 10 mg/L.
- 15.12 Rerun samples that precede a failed control chart standard.

Table F.1 ICP QAQC Calibration Check Standard Acceptance Limits

Element	OCWD RDL	CC STD Concentration	CC STD Acceptance Range (%)	CC STD Acceptance Range (Units)	CC STD Low Limit	CC STD High Limit
Na	0.1 mg/L	50 mg/L	+/-10%	+/- 5 mg/L	45 mg/L	55 mg/L
K	0.1 mg/L	5 mg/L	+/- 10%	+/- 0.5 mg/L	4.5 mg/L	5.5 mg/L
Mg	0.1 mg/L	50 mg/L	+/-10%	+/- 5 mg/L	45 mg/L	50 mg/L
Ca	0.1 mg/L	50 mg/L	+/-10%	+/- 5 mg/L	45 mg/L	55 mg/L
B	0.1 mg/L	0.5 mg/L	+/- 20%	+/- 0.1 mg/L	0.4 mg/L	0.6 mg/L
Cr	1.0 ug/L	100 ug/L	+/- 10%	+/- 10 ug/L	90 ug/L	110 ug/L
Fe	1.0 ug/L	500 ug/L	+/-10%	+/- 50 ug/L	450 ug/L	550 ug/L

Table F.2 ICP QAQC Detection Limit Standard Acceptance Limits

Element	OCWD RDL	Detection Limit STD Concentration	Detection Limit STD Acceptance Range (%)	Detection Limit STD Acceptance Range (Units)	Detection Limit STD Low Limit	Detection Limit STD High Limit
Na	0.5 mg/L	0.5 mg/L	+/- 50%	+/- 0.25 mg/L	0.25 mg/L	0.75 mg/L
K	0.5 mg/L	0.5 mg/L	+/- 50%	+/- 0.25 mg/L	0.25 mg/L	0.75 mg/L
Mg	0.5 mg/L	0.125 mg/L	+/- 50%	+/- 0.0625 mg/L	0.0625 mg/L	0.1875 mg/L
Ca	0.5 mg/L	0.5 mg/L	+/- 50%	+/- 0.25 mg/L	0.25 mg/L	0.75 mg/L
B	0.1 mg/L	0.25 mg/L	+/- 50%	+/- 0.125 ug/L	0.125 ug/L	0.375 ug/L
Cr	1.0 ug/L	1.0 ug/L	+/- 50%	+/- 0.5 ug/L	0.5 ug/L	1.5 ug/L
Fe	1.0 ug/L	1.0 ug/L	+/- 50%	+/- 0.5 ug/L	0.5 ug/L	1.5 ug/L

Table F.3 ICP QAQC Laboratory Reagent Blank and Digestion Blank Acceptance Limits

Element	OCWD RDL	Method Blank Acceptance Limit
Na	0.1 mg/L	0.05 mg/L
K	0.1 mg/L	0.05 mg/L
Mg	0.1 mg/L	0.05 mg/L
Ca	0.1 mg/L	0.05 mg/L

B	0.1 mg/L	0.05 mg/L
Cr	0.1 ug/L	0.05 ug/L
Fe	1.0 ug/L	0.5 ug/L

Table F.4 ICP QAQC Spiked Sample Acceptance Limits				Spike Conc. 3		
Element	OCWD RDL	Spiked Sample Concentration	Spiked Sample Acceptance Range (%)	Spiked Sample Acceptance Range (Units)	Spiked Sample Low Limit	Spiked Sample High Limit
Na	0.1 mg/L	100 mg/L	+/- 30%	+/- 30 mg/L	70 mg/L	130 mg/L
K	0.1 mg/L	2 mg/L	+/- 30%	+/- 0.6 mg/L	1.4 mg/L	2.6 mg/L
Mg	0.1 mg/L	100 mg/L	+/- 30%	+/- 30 ug/L	70 ug/L	130 ug/L
Ca	0.1 mg/L	100 mg/L	+/- 30%	+/- 30 mg/L	70 mg/L	130 mg/L
B	0.1 mg/L	1 mg/L	+/- 30%	+/- 0.3 ug/L	0.7 ug/L	1.3 ug/L
Cr	1.0 ug/L	100 ug/L	+/- 30%	+/- 30 ug/L	70 ug/L	130 ug/L
Fe	1.0 ug/L	100 ug/L	+/- 30%	+/- 30 ug/L	70 ug/L	130 ug/L

16. AUTOMATIC DATA TRANSFER PROCEDURE

- 16.1 Place a floppy disc in drive A of the ICP-OES computer station.
- 16.2 **Double Click** on **Data Manager** icon, located on the Desktop area.
- 16.3 Highlight the **Results** you wish to transfer.
- 16.4 **Click** on **Export** icon.
- 16.5 **Click** on **Use Existing Design**.
- 16.6 **Click** on **Browse**. If transferring trace metals from an ultrasonic run, **Click** on **Aspen Trace Metal File.Xpt**. If transferring metals done by the cyclonic spray chamber, **Click** on **Aspen Cyclonic Export File.xpt**. After making your selection, **Click** on **Open**.
- 16.7 **Click** on **Finish**.
- 16.8 **Click** on **Export Data**. When finished exporting data, remove the floppy disc and move to any LIMS station computer and proceed with the data transfer to LIMS described below, in paragraph H.

17. DATA TRANSFER TO LIMS

- 17.1 Place Floppy disc containing exported data in drive A.
- 17.2 Log on to Aspen LIMS.

- 17.3 **Click on Import Data icon.**
- 17.4 **Click on Select File Type** and select either **ICP Trace file** or **ICP (Cyclonic file)**, depending on run to be imported.
- 17.5 **Select File Location** by Clicking on **Browse** and highlight the file to be imported and **Click on Open.**
- 17.6 **Click on Import File.**
- 17.7 **Click on Start Import.**
- 17.8 **Enter your Initials** and **Click OK.**
- 17.9 **Click on Continue.**
- 17.10 A window pops up and asks if you are importing dissolved metals. Check your backlog answer appropriately. If you do have dissolved metals to report, then click on the appropriate LIMS number so that the Yes/No shows Yes. When finished **Click on Continue.**
- 17.11 **Click on Send to LIMS.**
- 17.12 If no errors in the samples occur, proceed to step 13. Otherwise, note the erred LIMS # sample and **Click Return to Data Import** to correct the problem. When the problem is corrected, again **Click on Send to LIMS.**
- 17.13 **Click on Create New WS.**
- 17.14 Click OK at **Append Exceptions to Test and QC File** window. **Click on Cancel.**
- 17.15 You should now be at the main menu for Aspen LIMS. **Click on Enter Sample Results, Review Existing Worksheet** and find your newly created worksheet. Proceed from this point as in Normal login procedure (i.e. analysis date, your initials, print copy, send ALN, Etc).

ORANGE COUNTY WATER DISTRICT MAIN LABORATORY

ICP Data Review

Analytical Chemist: _____

Analysis Date: _____

Reviewing Chemist: _____

Review Date: _____

Supervising Chemist: _____

Approval Date: _____

Data Review Checklist

	Analytical Chemist (Y or N)	Reviewing Chemist (Y or N)
1. Calibration Check Standards (1 per 10 samples and at the end of run)	_____	_____
2. Calibration Check Standards (Within Limits – see Table F.1)	_____	_____
3. MDL Standards (1 set of 7 standards per run)	_____	_____
4. MDL Standards (Within Limits – see Table F.2)	_____	_____
5. RDL Standard (1 per run)	_____	_____
6. RDL Standard (Within Limits)	_____	_____
7. Laboratory Reagent Blank (Every 10 th position)	_____	_____
8. Laboratory Reagent Blank (Within Limits - see Table F.3)	_____	_____
9. Digestion Blank (1 per digestion batch. If not applicable, enter N/A)	_____	_____
10. Digestion Blank (Within Limits – see Table F.3. If not applicable, enter N/A)	_____	_____
11. Digested LFB (QC-1) (1 per digestion batch)	_____	_____
12. Digested LFB (Within Limits)	_____	_____
13. Spike (Minimum 1 per every 10 samples run or part of 10 samples.)	_____	_____
14. Spike (Within Limits – see Table F.4)	_____	_____
15. Second Source Standard (1 per run)	_____	_____
16. Second Source Standard (Within Limits – see Table F.5)	_____	_____
17. Duplicate Samples (1 every 10 samples)	_____	_____
18. Duplicate Samples (Results within 10%)	_____	_____
19. Background Interference Check Standard (With each run. If not applicable, enter N/A)	_____	_____
20. Background Interference Check Standard (Within limits – see Table F.6)	_____	_____

Checklist #	Chemist (Initial)	Data Review Comments for all above “N” entries	Supervisor (Initial)
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____

SOP PROCEDURE CHANGE
FOR TRACE ELEMENTS BY INDUCTIVELY
COUPLED PLASMA-OPTICAL EMISSION SPECTROSCOPY

CHANGE	DATE	INITIALS
Revised Section F	11/1/99	MY, JMD, PH, JAB, FC
Added Tables F.1 to F.5	11/5/99	MY, JMD, PH, JAB, FC
Changed Note in Section A, ignition instructions in Section B per Ben Chalfont of BAIRD/TJA	4/10/00	JMD, PH, JAB, FC
Referenced and incorporated portions of <i>Code of Federal Regulations 40</i> , Part 136, Appendix C	1/25/01	JMD
Section 6, 7, 8, shutdown procedure, G and H amended To conform to new instrumentation (CETAC Unit, 4300DV, and Aspen LIMS automatic data transfer).	12/1/02	PH
Refresh	08/13/03	JAB
SOP Updated to correct spelling errors. Added clarification To SOP for better clarity and continuity.	08/27/04	PH
Added comments about removal of moisture before starting plasma and checking calibration in the Examine Window	10/25/04	JAB
Updated SOP to reflect correct spiking requirement, of 1 for every 10 samples or part of 10 samples analyzed. Also, updated the attached ICP Data Review sheet to reflect The same information	8/25/05	PH
Added information on the correct conditions to use, (7.1) depending on the method. Also added a comment (15.3) about how to label the MDL so the data can be retrieved in Aspen.	12/07/2005	JAB
Revised dissolved metals procedure in Section 9	2/16/2007	JMD
Revised Section 7.1 to reflect procedure to clear water traps daily. Formally in section 7.6.1	10/14/2008	PH
Revised ICP Data Review sheet. Add lines 17 and 18 requiring That a Reportable Detection Limit Standard be run with each run	5/11/2009	PH

SOP PROCEDURE CHANGE
FOR TRACE ELEMENTS BY INDUCTIVELY
COUPLED PLASMA-OPTICAL EMISSION SPECTROSCOPY

CHANGE	DATE	INITIALS
Revised ICP/ICP-MS Data Review sheet. Moved RDL up to top below MDL and added requirement for <u>digested LFB</u>	7/6/2009	JAB
Added Section 13.2.3 Requires dilution of samples > 90% or more of linear dynamic range.	11/12/2009	PH

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

TRACE ELEMENTS BY INDUCTIVELY COUPLED PLASMA / MASS SPECTROSCOPY (ICP/MS)

File Name: M:\SOP\INORGANIC\ICP_MS SOP.doc
Revision: 1

Effective Date: 11/18/2009
Supersedes:

1. REFERENCES

- 1.1 EPA Method 200.8 Determination of Trace Elements in Waters and Wastes By Inductively Coupled Plasma – Mass Spectrometry, Revision 5.4, EMMC Version, May 1994.
- 1.2 Standard Methods, 20th Ed., #3125 (pp. 3-44 to 3-52).
- 1.3 Perkin Elmer Sciex Elan ICP-MS DRC II, with Software, Version 3.4 (Build 3.4.57.1002) 1994-2009.
- 1.4 ESI SC Autosampler with software Version 2.2.0.8, Elemental Scientific, Inc. 2009.

2. SAMPLE HANDLING AND PRESERVATION

Prior to the collection of an aqueous sample, consideration should be given to that type of data required, (i.e., dissolved or total recoverable), so that appropriate preservation and pretreatment steps can be taken. The pH of all aqueous samples **must** be tested immediately prior to aliquoting for processing or “direct analysis” to ensure the sample has been properly preserved. If properly acid preserved, the sample can be held up to 6 months before analysis.

2.1 Dissolved

For determination of dissolved elements, the **unacidified** sample must be filtered through a 0.45-µm pore diameter membrane filter at the time of collection (**in a pre-cleaned bottle not containing acid**) or as soon thereafter as practically possible. Single-use plastic filtering apparatus are recommended to avoid contamination (**Corning 431155 or eq.**). **First, rinse the filter and apparatus with 50-100 mL DI water.** Then use the first 50-100 mL of sample to rinse the filter and filter flask. Discard this portion and collect the required volume of filtrate. Acidify the filtrate with (1+1) HNO₃ to a pH of 2 or less. Normally, 3 mL of (1+1) acid per liter should be sufficient to preserve the sample. **Store in the bottom half of the filter apparatus using the screw-cap closure. Do not use a filter for more than one sample.**

2.2 Total Recoverable

For the determination of total recoverable elements in aqueous samples, samples are **not** filtered, but acidified with (1+1) nitric acid to pH < 2 (normally, 3ml of (1+1) acid per liter of sample is sufficient for most ambient and drinking water samples).

Preservation may be done at the time of collection. **The Water Quality Department will collect these samples in disposable, certified, pre-cleaned plastic bottles supplied by I-Chem, Eagle-Picher, or other vendor, already containing a measured amount of nitric acid.** If for some reason field preservation is impossible, it is recommended that the samples be returned to the laboratory as soon as possible after collection and acid preserved upon receipt in the laboratory. **If there is doubt whether field preservation is appropriate, Water Quality will normally collect the sample in an un-acidified bottle.** Following acidification, the sample should be mixed, held for sixteen hours, and then verified to be pH < 2 just prior to withdrawing an aliquot for processing or "direct analysis". If for some reason such as high alkalinity the sample pH is verified to be > 2, more acid must be added and the sample held for sixteen hours until verified to be pH < 2.

3. HOLDING TIME

3.1 6 months (28 days for Hg).

4. INTERFERENCES

4.1 Isobaric elemental interferences, polyatomic ion interferences, abundance sensitivity, physical and memory interferences. Please refer to EPA Method 200.8 for further explanations of terms and solutions to these types of problems.

5. APPARATUS

5.1 Perkin-Elmer Sciex 6000 Inductively Coupled Plasma – Mass Spectrometer, with an AS90 Autosampler.

6. QUALITY CONTROL

6.1 Run one duplicate for every ten samples, or one duplicate per run for sets of less than ten samples; values should agree within 10%. A Control Chart Standard must be analyzed at the beginning, before the first sample, then after every ten samples, and finally at the end of the run. A blank must be analyzed after each CC standard. Control chart check standards should be within 2 sigma control limits or +/- 20%, whichever is less. The Control Standard satisfies the requirement in section 9.3.4 of the EPA Method 200.8 requirement, for calibration monitoring and recalibration of the instrument. EPA Performance Evaluation samples are used for external validation; result should be within EPA-specified acceptance limits. Second source standards are analyzed once per run

and must be within 10% of expected reading or within the certified fact sheet range for that second source. Run one spike / spike duplicate sample set for every 10 samples, and/or one spike / spike duplicate sample set for sets less than 10 samples. Spike % Recovery should be between 70%-130% for all analytes per EPA Method 200.8, however, in actuality, a range of 90% to 110% is easily attainable. If run has digested samples, then run a digested spike. **Note:** Add the internal standard to all digested samples after the digestion. Each operator is required to run one MDL study on the instrument with each new assignment to it (See section 9.2.4 of EPA Method 200.8). Please refer to actual method for total references. This is easily accomplished by analyzing several MDL samples each time you run. Label MDL as XXYYDDM, where xx is the number of the MDL, yymmdd is year, month, and day. The last M indicates that it is an MDL standard. By doing this, the data will be easier to retrieve in Aspen.

- 6.2 Prepare and analyze a Reportable Detection Limit sample with each run to verify the ability to meet the laboratory RDL reporting limits. Label as QARDLyymmddW.

7. TROUBLESHOOTING

- 7.1 Use process of elimination to determine the problem. Refer to the flow chart. If any quality control check shows process is "out of control", repeat test, rerunning any sample measurements made since last "in control" calibration.

8. PROCEDURE

- 8.1 Make the Calibration Standards, C.C. Standard, Second Source Standard, RDL Standard and MDL Standard prior to beginning.
- 8.2 Turn on computer.
- 8.3 Turn on monitor.
- 8.4 The instrument vacuum and the chiller should remain on at all times. Once a week, check the chiller liquid level and fill as required. See the instrument hardware manual for proper level and fluid to use.
- 8.5 Turn on the ESI SC autosampler power, by toggling the I/O switch to the I position (O position is the off). The switch is located on the right side panel of the autosampler when facing the unit.
- 8.6 Turn on the PC3 Fast sample introduction system, which is attached to the torch outside area, next to the peristaltic pump. The toggle switch is located to the rear of the PC3 Fast unit, and has two (2) positions. The upper one depicts 3 rain drop

symbols and the lower depicts a snowflake. Press the switch to the rain drops symbol, as you are facing the front of the ICP-MS DRC II unit.

- 8.7 Double click on the **ESI SC Icon**.
- 8.8 Click on **“Initialize Autosampler”**.
- 8.9 Click on **“Rinse/Wash”**, to fill the wash station reservoirs.
- 8.10 Click on **“Initialize Autosampler”** again.
- 8.11 Click on the minus (-) sign to minimize the ESI SC autosampler control window. Note: The ESI SC autosampler window needs to remain open during the analysis, in order for it to control and synchronize the autosampler with the ICP_MS DRC II and its' software.
- 8.12 Double click on the **“Elan”** icon and it will automatically open to the optimization workspace.
- 8.13 Properly connect all peristaltic pump tubing and replace after every 8 hours of operation, or as warranted.
- 8.14 Click on the **“Device”** icon at the top of the monitor screen.
- 8.15 Click on the **“direction”** arrow which points counter-clockwise. The pump speed will register 48 rpm. Run this for approximately 2 minutes or until the carrier and internal standards lines are filled with their corresponding solutions. Then manual change to 6.0 rpm, by highlighting the 48.0 rpm and typing in 6.0 rpm.
- 8.16 Click on the **“Instrument”** icon at the top of the monitor screen.
- 8.17 In the **“Instrument”** window under **Plasma**, click on **{Start}**. Allow the instrument to warm up before beginning any analysis (approximately 15 minutes).
- 8.18 Click on the **“Smart Tune”** icon at top of monitor screen. The **“Run in the manual mode”** should be checked already, but if not, click on it to place a check mark there. Take the carrier line out of the carrier solution and place it in a 1 ppb tuning solution. Allow sufficient uptake time to allow for the tuning solution to equilibrate in the plasma. Then click on **“Optimize”**. A Mass Tuning and Daily Performance will be performed, and if it passes both, then save the optimization to the default file upon exiting. The printout will tell you if the Mass Tuning and Daily Performance have passed the set criteria. There is no need to check the individual results. If the Mass Tuning and Daily performance do not pass, it will automatically do a Nebulizer, and Autolens calibration and again perform the mass tuning and daily Performance. In the event that it again fails to pass, reconfigure the selections to run in Smart Tune to include a Pulse and Analog

Stage, followed by a Dual detector Calibration, followed by a Daily Performance check.

Daily Performance Requirements 1 PPB Tuning Solution

Mg Sensitivity:	>6,000 cps/1 ppb	(Net Intensity Mean)
In Sensitivity :	>30,000 cps/1 ppb	
U Sensitivity:	>30,000 cps/1 ppb	
Ba⁺⁺/Ba⁺:	≤0.03	
CeO/Ce:	≤0.03	
Background:	<2 cps @ Mass 220	

- 8.19 Upon successful completion of both the Mass Tuning and Daily Performance checks, save the Mass Tuning Calibration to the Default Optimization file, upon exiting Smart Tune. Then place the peristaltic pump Carrier line into some rinse solution to clean out the line of the Tuning Solution and then place it back into the Carrier bottle.
- 8.20 Click on 'File' and then click on "**Open Workspace**". Click on "**EPA 200.8.wrk**".
- 8.21 Click on the Sample window to activate it. Click on "**File**", click on "**Open**" and click on "**EPA 200.8 Template.sam**". The tray sample positions are numbered by the Tray# first and then the position in that tray. There are 21 positions in each of the four (4) trays). For example, the first sample in tray one (1) would be designated as 101. Tray 1 sample 21 would be 221, and so on.
- 8.22 If necessary, type in the number 101 as the first A/S Loc number and insure that "**Run Blank, Stds. and Sample**" is in the row of the column labeled "**Measurement Action**". After this initial labeling, all subsequent rows in this column should have only "**Run Sample**". Insure that in the methods column that **C:\elandata\Method\FAST_EPA200.8_shortv1.0.mth** is entered for each sample. Under the "**Sample Type**" column, highlight the field for your spikes and duplicate samples and **right** click. Select the appropriate designation (i.e. QC Spike or QC Duplicate). For spikes, upon selecting the **QC Spike** designation, a notification of the spike and spike table being used appears. Example: If Sample 23 is the unspiked reference sample, then your selection should show **Spike – 1 of 23**, indicating that spike table 1, the normal spike table utilized, is being used and the reference sample is 23. IF you are using a different Spike Table, then change the 1 to the appropriate table you are using. For the **Spike Duplicate**, select the next sample as a **QC Spike** and when the pop up window opens showing **Spike – 1 of 24**, change the **24** to **23**, which is the actual reference sample. Also, by

numbering the spikes with the same autosampler number location, there is no need to have a separate spike sample container, as the spike will be sampled twice from the same autosampler location. The corresponding spike concentrations for each spike table are listed in the method Q.C. Tab area. **NOTE: Both spike sample and duplicate samples must have the exact same name. DO NOT USE THE “S” AND “D” DESIGNATIONS USED IN OTHER INSTRUMENTATION METHODS. It is recommended that you analyze the undiluted sample LAST, AFTER the 2X and spike samples. This way, the undiluted sample values will be transferred to LIMS.**

- 8.23 When finished with the Sample table, click “**File**”, then click “**Save As**”, and at the pop up window, save the sample table file with the normal lab naming system of YYMMDD, under the appropriate sub folder. To obtain a printed copy of your samples, click on “**File**”, and then click “**Print**”.
- 8.24 Click on the green “**R**” icon to the left of the monitor screen.
- 8.25 Load the **Dataset** file you wish to store your data in, by clicking on “**Load**”, then click on “**EPA 200.8**”, then click on the **year/month** sub folder that you are saving the data to.
- 8.26 Load the **Sample** file by clicking on “**Load**”, and then in the pop up window, click on the sample file you wish to run, and click “**OK**”.
- 8.27 Close the green “**R**” window.
- 8.28 Click on the Sample window to activate it and then click on the square located in the upper right of the window to enlarge the Sample window.
- 8.29 Left click on the “**Batch Index #1**” to highlight it and with the mouse, drag it down to highlight your entire batch file that you are going to run.
- 8.30 Click on the “**Analyze Batch**” to begin the analysis.
- 8.31 Select **YES** to clear the previously acquired QC data.
- 8.32 Select **YES** to clear the blank and current calibration. Note: if you click on the green R icon, the calibration selected should now read <no file loaded>. Until the sample probe enters the first sample, the computer will not accept any commands.
- 8.33 Minimize the autosampler window and click on the calibration window to activate it. This will allow you to see and review your calibration data.
- 8.34 When the run is completed, Place the carrier and Internal Standard lines in MQ water to flush them out. After a few minutes, remove the lines from the MQ water and allow the pump tubing to pump dry.

9. SHUTDOWN

9.1 Daily shutdown procedure.

9.1.1 Click on the **Device Control** icon). Click on **Stop**.

9.1.2 Click on the **instrument** icon).

9.1.3 Click on **Stop** under plasma.

9.1.4 Loosen the peristaltic pump tubing.

9.1.5 Turn off autosampler power.

9.1.5 Exit the Elan program and close the ESI SC autosampler.

9.1.6. Turn off the power to the PC3 nebulizer.

9.1.7. Turn of the computer and monitor.

9.2 Extended shutdown procedure. Perform for weekends and if instrument not to be used for more than a week.

9.2.1 Leave the vacuum on.

9.2.2 Turn the Chiller off. It only needs to be on when the instrument is running.

10. STANDARD PREPARATION PROCEDURE

NOTE: ALL CALIBRATION STANDARDS, SECOND SOURCE STANARDS, CHECK STANDARDS AND SPIKES ARE TO BE MADE FRESH EACH RUN. RECORD IN ICP-MS STANDARDS PREP LOGBOOK.

10.1 Intermediate 1 mg/L mercury solution. Weigh out 2.5 g of 10 mg/L mercury stock solution and dilute to 25 g with milli-Q water.

10.2 Standards Preparation

10.2.1 Calibration Blank

Weigh out 48.5 g of milli-Q water and add 1 ml of 1+1 Nitric Acid and 0.5 ml 1+1 Hydrochloric Acid. Add 100 µl Internal Standards Stock also containing 50 µg/L Gold Solution.

10.2.2 Calibration Standard 1:

Weigh out 0.05 g of Calibration Stock 1, 0.05 g of Calibration Stock 2, 0.05 g of Mercury intermediate 1 mg/L stock, and 0.05 g of Calibration stock 4. Dilute to 48.5 g with milli-Q water, add 1 ml of 1+1 Nitric Acid and 0.5 ml 1+1 Hydrochloric Acid. Add 100 µl Internal Standards Stock Solution also containing 50 ug/L Gold Solution.

10.2.3 Calibration Standard 2:

Pipette 0.1 g of Calibration Stock 1, 0.1 g of Calibration Stock 2, 0.1 g of Mercury intermediate 1 mg/L stock, and 0.5 g of Calibration Stock 4. Dilute to 49 g with milli-Q water, add 1 ml of 1+1 Nitric Acid and 0.5 ml 1+1 Hydrochloric Acid. Add 100 µl Internal Standards Stock Solution, which also contains the Gold solution.

10.2.4 Calibration Standard 3:

Pipette 0.5 g of Calibration Stock 1, 0.5 g Calibration Stock 2, 0.25 g of Mercury intermediate 1 mg/L stock, and 5 g of Calibration Stock 4. Dilute to 48.5 g with milli-Q water, add 1 ml of 1+1 Nitric Acid and 0.5 ml 1+1 Hydrochloric Acid. Add 100 µl Internal Standards Stock Solution, which also contains the Gold Solution.

10.3 Check Standards Preparation:

10.3.1 QC Standard 3

Add 0.125 g of 1 mg/L Mercury Intermediate Stock Solution.
Add 0.250 g of Calibration Stock 1 and 2.
Add 2.5 g of Calibration Stock 4.
Dilute to 48.5 g with milli-Q water
Add 1 ml of 1+1 Nitric Acid
Add 0.5 ml of 1+1 Hydrochloric Acid.
Add 100 µl of Internal Standards Stock Solution, which also contains the Gold solution.

10.3.2 QC Standard 4:

Same sample and position as the Calibration Blank.

10.3.3 Second Source CPI International Standard

Add 0.125 g of CPI 1 mg/L Intermediate Stock Solution.
Add 0.250 g of CPI Stock Solutions 1 and 2.
Add 2.5 g of CPI Stock Solution 4.
Dilute to 48.5 g with milli-Q water.
Add 1 ml 1+1 Nitric Acid.
Add 0.5 ml Hydrochloric Acid.
Add 100 µl Of the Internal Standards Stock Solution, which also contains the Gold solution.

10.4 MDL and RDL:

10.4.1 MDL

Prepare 200 ppb Intermediate Standard.
Add 1.0 g each of Calibration Stock #1 and Calibration Stock #2.
Add 1.0 g of Mercury Stock
Dilute to 50 g with Milli-Q DI water.
Then, to a clean tube,
Add 0.25 g of Intermediate MDL Standard and 0.5 g of Stock #4.
Dilute to 48.5 g with Milli-Q water.
Add 1.0 mL of 1+1 Nitric Acid
Add 0.5 mL of 1+1 Hydrochloric Acid
Add 100 µL of Internal Standard, which also contains gold.

10.4.2 RDL

Prepare Intermediate RDL Standard containing 200 ppb of all elements EXCEPT Be, Sb, Tl at 100 ppb and Hg at 20 ppb.
Add 1.0 g of RDL Standard Stock and 1.0 g of Calibration Stock #2, plus 0.1 g of Mercury Stock.
Dilute to 50 g with Milli-Q water.
Then, to a clean tube,
Add 0.25 g of Intermediate RDL Standard.
Dilute to 48.5 g with Milli-Q water.
Add 1.0 mL of 1+1 Nitric Acid
Add 0.5 mL of 1+1 Hydrochloric Acid
Add 100 µL of Internal Standard, which also contains gold.

11. SPIKE PREPARATION

11.1 Spike Table 1:

0.125 g of 10 ppm Calibration Stock solution 1 and 2.
0.05 g of 1 ppm Hg intermediate solution.*
1.25 g of 100 ppm Calibration Stock solution 4.
Add 25 g of sample.
Dilute to 48.5 g with Milli-Q water
Add 1 ml 1+1 Nitric Acid.
Add 0.5 ml 1+1 Hydrochloric Acid
Add 100 µl of Internal Standard Stock, which also contains the Gold solution.
* To make the 1 ppm Hg Intermediate solution, weigh out 2.5 g of 10 ppm Hg Stock solution and dilute to 25 g.

Note: This is a 2X dilution of the sample. Make the unspiked sample a 2X dilution also for matrix matching purposes.

11.2 Spike Table 3:

0.5 g of 10 ppm Calibration Stock solution 1 and 2.

0.025 g of 10 ppm Hg Stock solution.
2.5 g of 100 ppm Calibration Stock solution 4.
Add 25 g of sample.
Dilute to 48.5 g with Milli-Q water
Add 1 ml of 1+1 Nitric Acid.
Add 0.5 ml 1+1 Hydrochloric Acid
Add 100 µl of Internal Standard Stock, which also contains the Gold solution.

Note: This is a 2X dilution of the sample. Make the unspiked sample a 2X dilution also for matrix matching purposes.

12. SAMPLE PREPARATION

12.1 Weigh samples during preparation to insure that the total solids is < 0.5%.

Prepare unknown matrix samples as follows:

48.5 g of sample.
Add 1 ml of 1+1 Nitric Acid.
Add 0.5 ml 1+1 Hydrochloric Acid.
Add 100 µl of Internal Standard Stock, which also contains the Gold solution.

12.2 Prepare Brine samples as follows (i.e. Arlington Desalter Brine.)

1 g of sample.
47.5 g Milli-Q water.
Add 1 ml of 1+1 Nitric Acid.
Add 0.5 ml 1+1 Hydrochloric Acid.
Add 100 µl of Internal Standard Stock, which also contains the Gold solution.

% RECOVERY RANGES (Per EPA Method 200.8)

C.C. STDS 85 – 115 %

Spikes 70 – 130 % (At least 10% of samples must be spiked.)

Internal Stds 60 – 125%

[illegible]

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

TRACE ELEMENTS BY INDUCTIVELY COUPLED PLASMA / MASS SPECTROSCOPY (ICP/MS)

File Name: M:\SOP\INORGNIC\ICP_MS SOP.doc
Revision: 6

Effective Date: 7/13/2009
Supersedes: 5 (3/27/2009)

1. REFERENCES

- 1.1 EPA *Method 200.8 Determination of Trace Elements in Waters and Wastes By Inductively Coupled Plasma – Mass Spectrometry, Revision 5.4, EMMC Version, May 1994.*
- 1.2 *Standard Methods*, 20th Ed., #3125 (pp. 3-44 to 3-52).
Perkin Elmer Sciex Elan 6000/6100 Software, Version 2.3.1, Nov. 1999

2. SAMPLE HANDLING AND PRESERVATION

Prior to the collection of an aqueous sample, consideration should be given to that type of data required, (i.e., dissolved or total recoverable), so that appropriate preservation and pretreatment steps can be taken. The pH of all aqueous samples **must** be tested immediately prior to aliquoting for processing or “direct analysis” to ensure the sample has been properly preserved. If properly acid preserved, the sample can be held up to 6 months before analysis.

2.1 Dissolved

For determination of dissolved elements, the **unacidified** sample must be filtered through a 0.45- μ m pore diameter membrane filter at the time of collection (**in a pre-cleaned bottle not containing acid**) or as soon thereafter as practically possible. Single-use plastic filtering apparatus are recommended to avoid contamination (**Corning 431155 or eq.**). **First, rinse the filter and apparatus with 50-100 mL DI water.** Then use the first 50-100 mL of sample to rinse the filter and filter flask. Discard this portion and collect the required volume of filtrate. Acidify the filtrate with (1+1) HNO₃ to a pH of 2 or less. Normally, 3 mL of (1+1) acid per liter should be sufficient to preserve the sample. **Store in the bottom half of the filter apparatus using the screw-cap closure. Do not use a filter for more than one sample.**

2.2 Total Recoverable

For the determination of total recoverable elements in aqueous samples, samples are **not** filtered, but acidified with (1+1) nitric acid to pH < 2 (normally, 3ml of (1+1) acid per liter of sample is sufficient for most ambient and drinking water samples).

Preservation may be done at the time of collection. **The Water Quality Department will collect these samples in disposable, certified, pre-cleaned plastic bottles supplied by I-Chem, Eagle-Picher, or other vendor, already containing a measured amount of nitric acid.** If for some reason field preservation is impossible, it is recommended that the samples be returned to the laboratory as soon as possible after collection and acid preserved upon receipt in the laboratory. **If there is doubt whether field preservation is appropriate, Water Quality will normally collect the sample in an un-acidified bottle.** Following acidification, the sample should be mixed, held for sixteen hours, and then verified to be $\text{pH} < 2$ just prior to withdrawing an aliquot for processing or "direct analysis". If for some reason such as high alkalinity the sample pH is verified to be > 2 , more acid must be added and the sample held for sixteen hours until verified to be $\text{pH} < 2$.

3. HOLDING TIME

3.1 6 months (28 days for Hg).

4. INTERFERENCES

4.1 Isobaric elemental interferences, polyatomic ion interferences, abundance sensitivity, physical and memory interferences. Please refer to EPA Method 200.8 for further explanations of terms and solutions to these types of problems.

5. APPARATUS

5.1 Perkin-Elmer Sciex 6000 Inductively Coupled Plasma – Mass Spectrometer, with an AS90 Autosampler.

6. QUALITY CONTROL

6.1 Run one duplicate for every ten samples, or one duplicate per run for sets of less than ten samples; values should agree within 10%. A Control Chart Standard must be analyzed at the beginning, before the first sample, then after every ten samples, and finally at the end of the run. A blank must be analyzed after each CC standard. Control chart check standards should be within 2 sigma control limits or $\pm 20\%$, whichever is less. The Control Standard satisfies the requirement in section 9.3.4 of the EPA Method 200.8 requirement, for calibration monitoring and recalibration of the instrument. EPA Performance Evaluation samples are used for external validation; result should be within EPA-specified acceptance limits. Second source standards are analyzed once per run and must be within 10% of expected reading or within the certified fact sheet range for that second source. Run one spike / spike duplicate sample set for every 10 samples, and/or one spike / spike duplicate sample set for sets less than 10 samples. Spike % Recovery should be between 70%-130% for all analytes per EPA Method 200.8, however, in actuality, a range of 90% to 110% is easily attainable. If run has digested samples, then run a digested spike. **Note:** Add the internal standard to all digested samples after the digestion. Each operator is

required to run one MDL study on the instrument with each new assignment to it (See section 9.2.4 of EPA Method 200.8). Please refer to actual method for total references. This is easily accomplished by analyzing several MDL samples each time you run. Label as MDLxxymmddM, where xx is the number of the MDL, yymmdd is year, month, and day. By doing this, the data will be easier to retrieve in Aspen.

- 6.2 Prepare and analyze a Reportable Detection Limit sample with each run to verify the ability to meet the laboratory RDL reporting limits. Label as QARDLyymmddW.

7. TROUBLESHOOTING

- 7.1 Use process of elimination to determine the problem. Refer to the flow chart. If any quality control check shows process is "out of control", repeat test, rerunning any sample measurements made since last "in control" calibration.

8. PROCEDURE

- 8.1 Turn on liquid argon gas cylinder. (Regulator pressure should be between 70 – 120 psi.)
- 8.2 Check chiller coolant level and turn on chiller, if coolant at proper level..
- 8.3 Turn on computer.
- 8.4 Turn on monitor.
- 8.5 Turn on autosampler.
- 8.6 Press **Ctrl + Alt + Del** to log onto the computer after it boots up. Log on password is **admin**, and is case sensitive. Press { **ENTER** } at the log on screen.
- 8.7 Double click on the **elan** icon.
- 8.8 Click on the vacuum { **Start** } and wait for the instrument to reach the proper vacuum (typically this is 2.20e – 005 Torr or less). When the instrument reaches the proper vacuum, the area on the picture of the instrument turns from yellow to green. **Note:** It is a good idea to leave the instrument shutdown with the vacuum, chiller, and argon gas still on, Monday thru Thursday and shutdown completely for weekend.
- 8.9 Connect the peristaltic pump tubing and place tube in wash solution. **It is best if you change to new pump tubing.**
- 8.10 Activate the “**Device control window**”. If not visible, click on the “ **Open the devices window**” icon, which is the 12th icon from the left.

- 8.11 Click on { **Connect** }.
- 8.12 Click on the “**direction**” arrow which points counter-clockwise. Wait for the wash station reservoir to fill before proceeding to next step.
- 8.13 Click on **File**. Click on **Open Workspace**. Click on **200.wrk**. Click on **Open**.
- 8.14 In the “**Instrument**” window under **Plasma**, click on { **Start** }.
- 8.15 When the blue “**ignition sequence**” is in the middle of the box, activate the method window if showing or left click on the first icon on the left, labeled {**Cu**}. Left click on **Sampling**, then left click on **probe**. Left click on { **Go To Rinse** }. Then click **OK**. Allow the instrument to equilibrate (about 45 minutes).
- 8.16 While waiting for the instrument to warm up, make the standards, c.c. standards, spikes and prepare your samples for the autosampler run.
- 8.17 Open the “**Sample window**” by clicking on the second icon from the left (it has a picture of flasks on it), and maximize this window. Click **Batch** tab if necessary, then **File, Open, Template**.
- 8.18 If necessary, type in the number eight (8) the first A/S Loc number and insure that “**Run Blank, Stds. and Sample**” is in the row of the column labeled “**Measurement Action**”. After this initial labeling, all subsequent rows in this column should have only “**Run Sample**”. Insure that in the methods column that **C:\elandata\Method\epa200.mth** is entered for each sample. Under the “**Sample Type**” column, highlight the field for your spikes and duplicate samples and **right** click. Select the appropriate designation (i. e. spike or duplicate). For spikes, upon selecting the **QC Spike** designation, a request for the **Spike Table** to use appears. Enter the appropriate spike table number (normally 1). The corresponding spike concentrations for each spike table are listed in the method Q.C. Tab area. **NOTE: Both spike sample and duplicate samples must have the exact same name. DO NOT USE THE “S” AND “D” DESIGNATIONS USED IN OTHER INSTRUMENTATION METHODS. It is recommended that you analyze the undiluted sample LAST, AFTER the 2X and spike samples. This way, the undiluted sample values will be transferred to LIMS.**
- 8.19 Click on **File**. Click on **Save As**. Click on the **Year-month** sample directory and type in the autosampler file name to save it as. Use the normal lab convention in naming the file (i.e. year-month-day).
- 8.20 Click on **File**. Click on **Print**. This will print a copy of your autosampler tray.
- 8.21 Click on **File**. Click on **Open Workspace** and select “**Tuning.wrk**”, and click to open.

- 8.22 Place autosampler probe in a 10 ppb solution containing Mg, Rh, Pb and Ba, in 2% nitric acid. Wait for the standard to equilibrate in the plasma, then click on **“Tune Mass Spec”**. When the tuning is completed, check to see that the values fall within the following ranges:

Measured Mass Ranges

He	2.966 to 3.066
Mg	23.935 to 24.035
Rh	102.855 to 102.955
Ce	139.855 to 139.955
Pb	207.927 to 208.027
U	238.000 to 238.100

These values are ± 0.05 amu from the actual mass. The **“Measured Peak Width”** values must be **0.7 ± 0.05 amu** before analysis can begin. This is required by EPA Method 200.8, Sections 6.1.1 and 10.2.1. If any of the values are outside of the required limits, try repeating the tuning step several times until the proper values are obtained. If you still are unable to obtain good values, try to make changes manually. To **increase** a measured peak width, **decrease** the **Resolution DAC** value. Conversely, to **decrease** a measured peak width, **increase** the **Resolution DAC** value. Changing the Resolution DAC value by 30 units results in about a 0.1 amu change in the peak width. To manually adjust the measured mass, go to the **Interactive** window by clicking on the fifth icon from the left of the icons located along the top of the screen. You will see mass peak windows for the tuning elements. Enlarge the top of the peak for the element of interest by clicking and dragging. There will be a diamond indicating the measured mass. Move it to the left or right to adjust the mass and bring it into the allowable range. **If you are not sure about how to do this, please ask someone who knows.**

NOTE: Do not remove the sample probe from the 10 ppb solution at this point, if all of the above criteria are met. You will need it for the next step.

- 8.23 After the tuning has been completed, click on **File**, then **Save As**, and select **Default** to save the tuning file. To print the tuning file, click **File**, **Print**, verify that **Tuning.rop** is selected, then click the **Print** button at the bottom of the screen.
- 8.24 The autosampler probe should already be in the 10 ppb solution containing Mg, Rh, Pb and Ba. Click on **File**, **Open Workspace**, and select **“Daily Performance.wrk”**.

- 8.25 Click on **“Analyze Sample”**. When the reading is finished, review the following parameters and insure that the results are within the following limits

Daily Performance Requirements

Mg Sensitivity:	>20,000 cps (Net Intensity Mean)
Rh Sensitivity:	>150,000 cps
Pb Sensitivity:	>100,000 cps
Ba⁺⁺/Ba⁺:	≤0.03
CeO/Ce:	≤0.03
Background:	<30 cps @ Mass 220

A copy of the Daily Performance file is printed automatically. If all parameters are satisfactory, place the autosampler probe in the wash station and allow the system to rinse, then continue to the next step. If not, refer to the **Optimization Procedures Flowchart** in the ELAN 6000 manual. The flowchart is a step-by-step procedure for optimizing the parameters to meet the daily performance requirements. Repeat the tuning if any optimization changes were made.

- 8.26 Click on **File**. Click on **Open Workspace**. Click on **200.wrk**. Click on **Open**.
- 8.27 Click on the **Method** icon (the first icon on the left with the symbol Cu) and insure that the method selected is **EPA200.mth**. If not, click on **File**, Click on **Open**, and find and select this method, then click on **Open**.
- 8.28 Open the sample window (click on the icon second from the left with flasks), and maximize the window. With the sample window active, click on **File**, and click on **Open**. Double click on the **year-month** folder your sample tray is in. Highlight your tray and click **Open**.
- 8.29 Click on the **green “R”** icon. Insure that the proper information is in each area.
- 8.29.1 **Method:** should be **EPA200.mth**
- 8.29.2 **Dataset:** This usually needs to be changed and should be the year-month file you are currently using. Example, for July 2000, it should be **\\EPA200.8\0007**. If the dataset is incorrect, click on **Load**, highlight the correct dataset, then click on **OK**.
- 8.29.3 **Sample:** This should have your year-month file and then your autosampler file name. Example: **007\000725.sam**. If not, click on **Load** and find your correct files and load them.
- 8.29.4 **Report Template:** No need to change. Should be **200 qc report.rop**.
- 8.29.5 **Tuning:** **default.tun**. No need to change

8.29.6 **Optimization: default.dac.** No need to change.

8.29.7 **Calibration:** Currently has **000615.cal**. This is OK. You do not need to make any changes here. When you start the analysis, ignore the current calibration (explained below) file selected. When the analysis starts, the name will change to untitled until the calibration is complete and you save the current calibration. All results on the current sample analysis run will refer to the new calibration for results.

8.29.8 **Polyatomic: elan.ply.** No need to make changes.

When all of the fields are checked and correct, click on **DONE**.

- 8.30 With the sample window still active and maximized, move the cursor to the **number 1** next to the autosampler Sample 8 position. Hold down Shift, then use Down Arrow to highlight samples until the entire listing of your samples is blacked out. Essentially, for a normal run, your entire screen will appear blacked out. When done, click on **Analyze Batch**.
- 8.30 Select **YES** to clear the previously acquired QC data.
- 8.31 Select **YES** to clear the blank and current calibration. Note: if you click on the green R icon, the calibration selected should now read <no file loaded>. Until the sample probe enters the first sample, the computer will not accept any commands.
- 8.32 Minimize the autosampler window and click on the 6th icon from the left to activate the calibration window. This will allow you to see and review your calibration data. This icon has a magnifying glass and a straight line in it.
- 8.33 When the run is completed, allow the system to flush for a while and then remove the wash station line from the reservoir bottle and place it in the glass container. Allow all of the wash solution to be drained from the wash line and station area. Then place the sample probe in Milli-Q water and allow the system to again flush out the acidified wash solution residual. When finished, place the probe back into the holder at the wash station.

9. SHUTDOWN

- 9.1 Daily shutdown procedure.
- 9.1.1 Click on the **Device Control** icon (12th icon from the left). Click on **Stop**.
- 9.1.2 Click on the **instrument** icon (the 11th icon from the left).
- 9.1.3 Click on **Stop** under plasma.
- 9.1.4 Loosen the peristaltic pump tubing.

- 9.1.5 Turn off autosampler power.
- 9.1.5 Exit the Elan program and shutdown the computer.
- 9.2 Extended shutdown procedure (Perform for weekends and if instrument not to be used for more than 3 or 4 days).
 - 9.2.1 Click on the **Device Control** icon (12th icon from the left). Click on **Stop**.
 - 9.2.2 Click on the **instrument** icon (the 11th icon from the left).
 - 9.2.3 Click on **Stop** under plasma.
 - 9.2.4 Click on **Stop** under vacuum.
 - 9.2.5 Loosen the peristaltic tubing.
 - 9.2.6 Turn off autosampler power.
 - 9.2.7 Exit the Elan program and shutdown the computer.
 - 9.2.8 Turn off the chiller.
 - 9.2.9 Turn off the argon gas supply.

10. STANDARD PREPARATION PROCEDURE

NOTE: ALL CALIBRATION STANDARDS, SECOND SOURCE STANDARDS, CHECK STANDARDS AND SPIKES, ARE TO BE MADE FRESH EACH RUN. RECORD IN ICP-MS STANDARDS PREP LOGBOOK.

- 10.1 Intermediate 1 mg/L mercury solution. Weigh out 2.5 g of 10 mg/L mercury stock solution and dilute to 25 g with milli-Q water.
- 10.2 Standards Preparation
 - 10.2.1 Calibration Blank
Weigh out 48.5 g of milli-Q water and add 1 ml of 1+1 Nitric Acid and 0.5 ml 1+1 Hydrochloric Acid. Add 100 µl Internal Standards Stock also containing 50 µg/L Gold Solution.
 - 10.2.2 Calibration Standard 1:
Weigh out 0.05 g of Calibration Stock 1, 0.05 g of Calibration Stock 2, 0.05 g of Mercury intermediate 1 mg/L stock, and 0.05 g of Calibration stock 4. Dilute to 48.5 g with milli-Q water, add 1 ml of 1+1 Nitric Acid

and 0.5 ml 1+1 Hydrochloric Acid. Add 100 µl Internal Standards Stock Solution also containing 50 ug/L Gold Solution.

10.2.3 Calibration Standard 2:

Pipette 0.1 g of Calibration Stock 1, 0.1 g of Calibration Stock 2, 0.1 g of Mercury intermediate 1 mg/L stock, and 0.5 g of Calibration Stock 4. Dilute to 49 g with milli-Q water, add 1 ml of 1+1 Nitric Acid and 0.5 ml 1+1 Hydrochloric Acid. Add 100 µl Internal Standards Stock Solution, which also contains the Gold solution.

10.2.4 Calibration Standard 3:

Pipette 0.5 g of Calibration Stock 1, 0.5 g Calibration Stock 2, 0.25 g of Mercury intermediate 1 mg/L stock, and 5 g of Calibration Stock 4. Dilute to 48.5 g with milli-Q water, add 1 ml of 1+1 Nitric Acid and 0.5 ml 1+1 Hydrochloric Acid. Add 100 µl Internal Standards Stock Solution, which also contains the Gold Solution.

10.3 Check Standards Preparation:

10.3.1 QC Standard 1 and 3 (Have the same concentration.)

Add 0.125 g of 1 mg/L Mercury Intermediate Stock Solution.

Add 0.250 g of Calibration Stock 1 and 2.

Add 2.5 g of Calibration Stock 4.

Dilute to 48.5 g with milli-Q water

Add 1 ml of 1+1 Nitric Acid

Add 0.5 ml of 1+1 Hydrochloric Acid.

Add 100 µl of Internal Standards Stock Solution, which also contains the Gold solution.

10.3.2 QC Standard 4:

Same sample and position as the Calibration Blank.

10.3.3 Second Source CPI International Standard

Add 0.125 g of CPI 1 mg/L Intermediate Stock Solution.

Add 0.250 g of CPI Stock Solutions 1 and 2.

Add 2.5 g of CPI Stock Solution 4.

Dilute to 48.5 g with milli-Q water.

Add 1 ml 1+1 Nitric Acid.

Add 0.5 ml Hydrochloric Acid.

Add 100 µl Of the Internal Standards Stock Solution, which also contains the Gold solution.

10.4 MDL and RDL:

10.4.1 MDL

Prepare 200 ppb Intermediate Standard.
Add 1.0 g each of Calibration Stock #1 and Calibration Stock #2.
Add 1.0 g of Mercury Stock
Dilute to 50 g with Milli-Q DI water.
Then, to a clean tube,
Add 0.25 g of Intermediate MDL Standard and 0.5 g of Stock #4.
Dilute to 48.5 g with Milli-Q water.
Add 1.0 mL of 1+1 Nitric Acid
Add 0.5 mL of 1+1 Hydrochloric Acid
Add 100 µL of Internal Standard, which also contains gold.

10.4.2 RDL

Prepare Intermediate RDL Standard containing 200 ppb of all elements EXCEPT Be, Sb, Tl at 100 ppb and Hg at 20 ppb.
Add 1.0 g of RDL Standard Stock and 1.0 g of Calibration Stock #2, plus 0.1 g of Mercury Stock.
Dilute to 50 g with Milli-Q water.
Then, to a clean tube,
Add 0.25 g of Intermediate RDL Standard.
Dilute to 48.5 g with Milli-Q water.
Add 1.0 mL of 1+1 Nitric Acid
Add 0.5 mL of 1+1 Hydrochloric Acid
Add 100 µL of Internal Standard, which also contains gold.

11. SPIKE PREPARATION

11.1 Spike Table 1:

0.125 g of 10 ppm Calibration Stock solution 1 and 2.
0.05 g of 1 ppm Hg intermediate solution.*
1.25 g of 100 ppm Calibration Stock solution 4.
Add 25 g of sample.
Dilute to 48.5 g with Milli-Q water
Add 1 ml 1+1 Nitric Acid.
Add 0.5 ml 1+1 Hydrochloric Acid
Add 100 µl of Internal Standard Stock, which also contains the Gold solution.
* To make the 1 ppm Hg Intermediate solution, weigh out 2.5 g of 10 ppm Hg Stock solution and dilute to 25 g.

Note: This is a 2X dilution of the sample. Make the unspiked sample a 2X dilution also for matrix matching purposes.

11.2 Spike Table 3:

0.5 g of 10 ppm Calibration Stock solution 1 and 2.
0.025 g of 10 ppm Hg Stock solution.

2.5 g of 100 ppm Calibration Stock solution 4.
Add 25 g of sample.
Dilute to 48.5 g with Milli-Q water .
Add 1 ml of 1+1 Nitric Acid.
Add 0.5 ml 1+1 Hydrochloric Acid
Add 100 µl of Internal Standard Stock, which also contains the Gold solution.

Note: This is a 2X dilution of the sample. Make the unspiked sample a 2X dilution also for matrix matching purposes.

12. SAMPLE PREPARATION

- 12.1 Weigh samples during preparation to insure that the total solids is < 0.5%.
Prepare unknown matrix samples as follows:
48.5 g of sample.
Add 1 ml of 1+1 Nitric Acid.
Add 0.5 ml 1+1 Hydrochloric Acid.
Add 100 µl of Internal Standard Stock, which also contains the Gold solution.
- 12.2 Prepare Brine samples as follows (i.e. Arlington Desalter Brine.)
1 g of sample.
47.5 g Milli-Q water.
Add 1 ml of 1+1 Nitric Acid.
Add 0.5 ml 1+1 Hydrochloric Acid.
Add 100 µl of Internal Standard Stock, which also contains the Gold solution.

% RECOVERY RANGES (Per EPA Method 200.8)

C.C. STDS	85 – 115 %
Spikes	70 – 130 % (At least 10% of samples must be spiked.)
Internal Stds	60 – 125%

SOP PROCEDURE CHANGE

FOR TRACE ELEMENTS BY INDUCTIVELY COUPLED PLASMA /

MASS SPECTROSCOPY (ICP/MS)

[illegible]

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

INORGANIC DISINFECTION BYPRODUCTS by IC EPA METHOD 300.1

File Name: M:\SOP\INORGNIC\IC_IDBP.doc

Revision: 7

Effective Date: 6/29/2009

Supersedes: 5/4/2009

1. REFERENCES

- 1.1 EPA *Methods for the Determination of Inorganic Substances in Environmental Samples* #300.1 (1997)
- 1.2 ICS-3000 *Ion Chromatography Operator's Manual*, Dionex.
- 1.3 *Chromeleon* Software Manual, Dionex.

2. SAMPLE COLLECTION, PRESERVATION, AND HOLDING TIME

- 2.1 Ethylenediamine (EDA) preservation solution, as described below, must be added to each sample to be collected for bromate, chlorite and chlorate. Add 250 µl of 100 mg/mL EDA solution to a 500 mL sample bottle. To prepare 100 mg/mL EDA solution: Dilute 2.8 ml of EDA (99+%) (CASRN 107-15-3) to 25 ml with Milli-Q DI water. **Prepare fresh monthly.**

Samples must be collected in 500mL brown plastic, wide-mouth bottles containing EDA preservative.

<u>Analyte</u>	<u>Preservation</u>	<u>Holding Time</u>
Bromate	50 mg/L EDA	28 Days
Bromide	None Required	28 Days
Chlorate	50 mg/L EDA	28 Days
Chlorite	50 mg/L EDA, Cool to 4°C	14 Days

3. APPARATUS

- 3.1 Ion Chromatography System, Dionex ICS-3000, dual channel, equipped with CD dual conductivity detectors, DP dual gradient pumps, EG reagent-free (RFIC) eluent generator system, AS autosampler, *Chromeleon* software, computer and printer.
- 3.2 Dionex AS-19 separator column, AG-19 guard column, ASRS-300 self-regenerating suppressor column.

- 3.3 Waters Sep-Pak C-18 cartridge for removal of organics.
- 3.4 Gelman Acrodisc syringe filter, 0.45 μm pore size (Part Number 4438 or equivalent) and 10cc B-D sterile syringe.
- 3.5 Helium gas (high purity).

4. REAGENTS

- 4.1 **Milli-Q DI water.** There are two 2L reservoirs labeled System 2 IDBP.

5. STANDARD STOCK SOLUTIONS

Note: Dry all chemicals used to make the stock solutions at 105°C for 30 minutes. Use Milli-Q DI water to dilute the stock solutions to the required volume. **(Commercially prepared stocks also listed for use.)**

- 5.1 Bromide (1000 mg/L): RICCA: Cat. # 1180-4 or
Dissolve 1.2876 g of Sodium Bromide (NaBr) to 1L. The solution is stable for 1 month.
- 5.2 Bromate (1000 mg/l): SPEX Cat. # AS-BRO39-2X or
Dissolve 1.1798 g of Sodium Bromate (NaBrO_3) to 1L. The solution is stable for 1 month.
- 5.3 Chlorate (1000 mg/L): SPEX Cat. # AS-CLO39-2X or
Dissolve 1.2753 g of Sodium Chlorate (NaClO_3) to 1L. The solution is stable for 1 month.
- 5.4 Chlorite (1000 mg/L): SPEX Cat. # AS-CLO29-2X or
Dissolve 1.6760 g of Sodium Chlorite (NaClO_2) to 1L. The solution is stable for 2 weeks. **NOTE:** Commercially available Sodium Chlorite is only 80% Chlorite. Therefore the normal number of grams per liter of 1.3410, needs to be corrected for by multiplying 100/80 or 1.25, in order to ensure solution is based on 100 % Sodium Chlorite.
- 5.5 Surrogate Solution: 0.50 mg/mL dichloroacetate (DCA) prepared by dissolving 0.065 g dichloroacetic acid, potassium salt ($\text{Cl}_2\text{CHCO}_2\text{K}$) in DI water and dilute to 100 mL in a volumetric flask. **Prepare fresh every 3 months.**

6. CALIBRATION STANDARDS

Prepare fresh weekly. EDA must be added to calibration standards at 50 mg/L.

- 6.1 Intermediate 10 mg/L Working Solution:
Pipette 10 ml of 1000 mg/l Bromide, 10 ml of 1000 mg/L Bromate, 10 ml of 1000 mg/L Chlorate, and 10 ml 1000 mg/L Chlorite solutions into a 1L volumetric flask. Dilute to the mark with Milli-Q DI water.
- 6.2 Calibration Standards
- 6.2.1 **STD5** (500 ppb)
Pipette 50 ml of 10 mg/L working solution, add 0.5 mL of EDA preservative and dilute to 1L with Milli-Q DI water.
NOTE: Prepare a second 500 ppb solution WITHOUT EDA and use to prepare the remaining working standards.
- 6.2.2 **STD4** (250 ppb)
Pipette 100 ml of STD5 (500 ppb), add 100 uL of EDA and dilute to 200 ml with Milli-Q water.
- 6.2.3 **STD3** (125 ppb) LFB.
Pipette 50 ml of STD5 (500 ppb), add 100 uL of EDA and dilute to 200 ml with Milli-Q water.
- 6.2.4 **STD2** (50 ppb) QACC.
Pipette 20 ml of STD5 (500 ppb), add 100 uL of EDA and dilute to 200 ml with Milli-Q water.
- 6.2.5 **STD1** (25 ppb) MDL.
Pipette 10 ml of AUTOCAL6R (500 ppb), add 100 uL of EDA and dilute to 200 ml with Milli-Q water.
- 6.2.6 **BLANK**
Add 0.5 mL of EDA to 1 L of Milli-Q water.
- 6.2.7 **RDL** (10µg/L ClO₂, ClO₃, Br, 5µg/L BrO₃)
Prepare intermediate standard solution by pipetting 1mL each of stock ClO₂, ClO₃, and Br, and 0.5mL of stock BrO₃ into a 1 L volumetric flask and dilute to 1L with Milli-Q DI water. Mix well. Then pipet 10mL intermediate standard to a 1 L volumetric flask. Add 0.5mL EDA, then dilute to 1L with Milli-Q water.
- 6.2.8 Prepare blank, standards and QC samples for analysis as follows:
Pipet 20mL aliquot of blank or standard solution to a disposable 50mL plastic beaker. Add 40µL of surrogate. Swirl to mix. Use a plastic disposable pipet to transfer sample to autosampler vial. The standard is now ready for analysis.

7. PROCEDURE

7.1 START-UP PROCEDURE:

- 7.1.1 Turn ON computer and log in as if you were logging on to the district network. Double-click on the *Chromeleon* icon. After doing this, you should notice the green "Connected" lights come ON for the DC module, DP module and EG module.
- 7.1.2 The control panel window should open. You should see two control panels, one for Anions on the left and one for IDBP on the right. You can maximize or minimize each control panel by using the arrow buttons next to the control panel title for each method. You can toggle to the Browser window by using Control/Tab. The browser is where you will create the sequences, start batches, review chromatograms and store data files.
- 7.1.3 From the control panel window, click the "Gradient Pump" tab. Under "Flow Control" click the "Motor" switch from red to green to turn ON the gradient pump. You should notice a green light on the DP module for Pump 2 Flow. Then click the "Home" tab, followed by the **Start Up** button at the top of the control panel. This will turn ON the CR-TC, and the eluent generator. Verify these are on by observing the green lights on the EG module for EGC 2 and CR-TC 2. The Cell Heater and Column switches should already be ON as visible on the screen. It will also turn ON the suppressor. On the DC module, the green light should be on for Suppressor 2. Also, the Upper and lower oven lights should be on. And Valve 2 should indicate the Inject position. Click on the "Detector Compartment" tab. At "Suppressor 1 Settings", verify that Type is ASRS_4mm, Mode is ON, and the Current setting is 112 mA. Click the "Home" tab and confirm that the current reading is **112 mA**.
- 7.1.4 If the instrument is used on a routine basis, there is really no need to prime the gradient pump. If you need to, click on "Gradient Pump" tab. The motor should be ON and the flow rate at 1.0 mL/min. Click the "Prime Control" switch. A "Check Results" screen will appear, with the message:
"Pump 2. Purging will deliver a high flow to your system. Ensure that the purge valve is open to protect your columns and fluidic system."
Open the door to the DP module and open the purge valve for Pump 2 about a quarter to half turn. Click OK, and the priming will start. To stop at any time before the 300 second default time, click the "Prime Control" switch to OFF and **remember to close the purge valve**. Priming automatically shuts off the suppressor, so you need to **remember to turn the suppressor ON after priming**.

7.1.5 The instrument makes its own hydroxide eluent. All you need to do is add water. Make sure the reservoirs are filled with Milli-Q DI water. Water can be added at any time before or during the analysis. **If the analysis will be running overnight, it is a good idea to add water just before you leave in the evening.**

7.2 CREATING A SEQUENCE:

7.2.1 Go to the browser to create a sequence of samples to be analyzed. Hit **Ctrl / Tab** to toggle between the browser and control panel screens.

7.2.2 Open the IDBP Template. If the template is locked, right-click, properties, properties of sequence "IDBP Template", General tab, Lock Status, Locked or Unlocked. Click to unlock the template. **After creating your new sequence and saving it, please go back and lock the IDBP Template for future use.**

7.2.3 The **column titles** of the sequence are as follows:

No. is Sequence Number

Name is Sample ID

Type is **Standard** or **Unknown**

Pos. is Autosampler Vial Position (1 – 100)

Inj. Vol. is **250 µL**

Program is **AS19PRG.prg**

Method is **Default**

Status is **Single**. (After the sequence batch has been started, the status will change from Single to Running. After a sample has been completed, the status will change to Finished. If a sample was stopped for any reason in the middle of the analysis, the status will change to Interrupted. The status will have to be changed back to Single and the sequence resaved to rerun.)

Dil. Factor is **1** unless the sample is diluted, then use the proper factor.

- 7.2.4 For STD1 thru STD5, use **Standard** as the type of sample. All other samples including Blank, CC Standards, LFB, MDL, RDL, 2nd Source, Spikes, Duplicates and Samples, use **Unknown** as the sample type. For diluted samples, don't forget to enter the correct dilution factor. You can place the standards and samples anywhere on the autosampler tray. For instance, if you need to run IDBP and Anions simultaneously, you could place the IDBP samples in A/S positions 1 thru 50 and the Anion samples in positions 51 thru 100. If you have more than 50 samples for IDBP, place the 51st sample in A/S position number 1 and continue. Same for Anions. **Just remember to put the correct number for the sample in the Pos. column of the sequence.** To add more lines at the end of the sequence, highlight the last row by clicking on the gray **No.** box. Then Down Arrow. You will be asked, "*Append New Line?*" Click "Yes", then continue the Down Arrow to add more rows.
- 7.2.5 When the sequence is ready to save, use the following protocol: File, Save As, IDBP DATA 2008, 0809 Folder, 080915 (YYMMDD). Note: You can add to the sequence at any time during the analysis, **just remember to Save As.** To print the sequence, go to **File, Print Sequence**, then **OK.**
- 7.2.6 If you want to add a stop program at the end of the sequence, go to the **Program** column and click in the box. Choose the **STOP SYS 2** program for IDBP. Change the **Sample Type** to **Blank.**
- 7.2.7 Use the 1.5mL glass vial with the slit septa cap for analysis (Dionex P/N 055427). Use a disposable plastic transfer pipet to add sample to the vial up to the 1.5mL mark. **DO NOT fill to the top. The autosampler needle vent must not be immersed in liquid.** Place samples in autosampler tray. Do not remove tray from the autosampler. The door can be opened at any time during analysis to add samples. **Be careful to add samples only when the autosampler needle is idle.**

Sample Prep: Run all samples 1X. Filter all samples **first** through 0.45µm Acrodisc and Sep-Pak (See IC_Anions.doc for Sep-Pak pretreatment requirements). Then, to 20.0mL filtered sample, add 40µL DCA surrogate. Mix well, then transfer to autosampler vial. To prepare a **matrix spike**, add 10.0mL filtered sample plus 10.0mL STD2 (50ppb) plus 40µL surrogate, mix and transfer to autosampler vial*. If sample needs dilution (sometimes needed for samples with high chlorate or chloride), prepare proper dilution, add 40µL surrogate, and don't forget to put correct dilution factor in sequence.

* Note that a spike is a 2X dilution.

7.3 STARTING ANALYSIS:

- 7.3.1 Before starting, check the system conductivity. Click the “Conductivity Detector” tab and check the Total Signal. For IDBP, the Total Signal should be close to 1.0 μ S to begin analysis (usually around 1.2 μ S, depending on the quality of the Milli-Q water). It may take several hours to reach equilibrium, especially if the instrument has not been run recently.
- 7.3.2 To check the baseline before starting analysis, click on the 13th icon from left, Blue Dot. Data Acquisition – IDBP. Click OK to start baseline acquisition. Click Blue Dot a second time to turn off acquisition. **Note: You must turn OFF acquisition before starting the analysis.**
- 7.3.3 Prime the autosampler syringe before starting analysis. Go to “Autosampler” tab on Control Panel. Click on “Syringe Prime” button. The syringe will move down and up twice for a total flush of 500 μ L. If you notice bubbles in the line from the DI water reservoir to the valve, gently tap the line when the syringe has moved about 1/3 of the way on the downward cycle to try to get the bubbles into the syringe. Hopefully, the bubbles will be pumped out to waste on the upward cycle. When adding water to the reservoir, make sure to remove any bubbles or air gaps **BEFORE** priming. You will probably have to remove the tubing at the valve end and try to remove any air bubbles, then reattach the line to the valve. If you have any trouble, please ask for help from someone who has experience in doing this procedure.
- 7.3.4 When ready to start the analysis, click Batch, Start. You will see the message *“Start Batch on IDBP? Remove any sequence batch lists you do not want to run.”* Make sure just the batch list of the sequence you want to run is shown and highlight it. An example is 0809/080915 JAB. Click **Start**. The autosampler needle will start moving and the first row of the sequence will be highlighted green.
- 7.3.5 Run time is about 33 minutes per sample. When each sample is done, the status will change from “Running” to “Finished”.

7.4 CHROMATOGRAM REVIEW:

- 7.4.1 You can review the chromatogram of a standard or sample, and make changes to it at any time. If changes involve updating the method for that particular run, the changes take effect immediately and will apply to all samples waiting to be analyzed.
- 7.4.2 Double-click on a sample. This will take you to the “Integration” window. You will see the chromatogram to the left, the calibration curve to the right, and the results table at the bottom. You can see peak names, area

counts, retention times and amounts for the individual analytes. There are five tabs at the bottom of the window: **Integration; Calibration; Peak Analysis; Summary; and Audit Trail**. You can check the correlation coefficients by clicking the “Calibration” tab.

- 7.4.3 Double-click on a peak name and you will see the integration points for that analyte and the peak start and stop points.
- 7.4.4 To identify a peak, put the cursor on a peak, then double-click. A “Peak Properties” box will appear. If the peak is misidentified, select the correct peak, then click the “Thumb-Tack” symbol at right, and the peak will assume the new identity.
- 7.4.5 After making any changes to a chromatogram, **File, Close**. You will be prompted to save the changes. Click **Yes**. Notice a message at the upper left of the chromatogram. It will say “Modified By ???,” and if a peak ID was changed, “Peak ID Manually Assigned.”
- 7.4.6 Other changes can be made using “QNT Editor” (23rd icon from left). There are five tabs at the bottom: **General; Detection; Peak Table; Amount Table; and Calibration**. Go to the “Calibration” tab to disable or enable a standard curve point. You should be careful using QNT Editor. If you are not sure how to do something, please ask for help.
- 7.4.7 Helpful icons located across the top of the browser screen include: **Acquisition; Integration; QNT Editor; Printer Layout; Show Calibration Curve; Previous Sample; Next Sample; Automatic Tool; Full Size; Autoscale**.
- 7.4.8 When the run is finished and any and all changes have been made, it is time to print the results. First, highlight all standards and samples you want to print. If a stop program was used, don’t include this sample in the batch to print. **File, Batch Report**. The “Batch Report” screen will appear. At “Print Options”, make sure “Printout” box is checked, then **OK**. A second “Batch Report” screen appears. Click **OK**.

7.5 SHUTDOWN:

- 7.5.1 If the stop program was used (and if it worked), the pump, EGC, CR-TC, and suppressor should be OFF. Check the control panel and the modules to verify. **Again, as explained in Section 7.2.6, for the shutdown sample, change the sample type to BLANK and the program to STOP SYS 2**. If you didn’t use the stop program, go to the control panel and click the **SHUT DOWN** button at the top. This will shut off the pump, EGC, CR-TC and suppressor. Verify by looking at the modules. The column heater should be left ON.

7.6 DATA TRANSFER TO LIMS:

- 7.6.1 Insert a memory stick into USB port.
- 7.6.2 Go to browser and open the sequence batch to be transferred. Double-click on the first standard, STD1.
- 7.6.3 Click on **Printer Layout** icon at top (next to yellow **QNT Editor** icon).
- 7.6.4 Click on **Summary** at bottom of page.
- 7.6.5 Go to **File**, click **Close**, and you should be back at the browser page with your selected sequence showing.
- 7.6.6 Highlight **only** the first standard in the sequence, STD1.
- 7.6.7 Click on **File**, then **Batch Report**.
- 7.6.8 Unclick **Printout** check mark.
- 7.6.9 Put a check mark in the **Export Options** box.
- 7.6.10 Put a check mark in the **ASCII Text Format** box. Then click on **Next**.
- 7.6.11 Under **Export Results**, change **SST** to **Summary**.
- 7.6.12 Click on **Finish**, then **OK**. Finally, click on **OK** when shown in the **Batch Report** window. The data will be transferred to the memory stick in the following format:
E:\IDBP\IDBPDATA2009\0906\090629.seq\0001.TXT
- 7.6.13 Open *Aspen LIMS*. At "Import Data" use **IDBP File – ICS3000** for IDBP. Open the E-drive and double-click past the IDBP Folder, past the IDBPDATAyear folder, past the Month folder, past the sequence folder, to 0001 text data file. This will contain the data to be imported.

8. QUALITY CONTROL

- 8.1 Surrogate recoveries should fall between 90 – 115% for proper instrument performance and analyst technique to be verified.
- 8.2 An initial demonstration of performance should be made. This includes a curve, second source standard and MDL study. Also, whenever a new operator takes over, the MDL study should be done.

A mid-range control chart standard (CCSTD) and a calibration blank must be analyzed immediately following calibration, after every ten samples and at the end of the run. The CCSTD must be within $\pm 10\%$. If it fails, analysis must be stopped and the problem corrected and if necessary recalibrate the instrument.

- 8.3 One laboratory reagent blank (LRB) must be analyzed with each batch of samples and it must not exceed the MDL. The LRB is treated exactly like a sample.
- 8.4 One laboratory fortified blank (LFB) must be analyzed with each batch of samples and must be within $\pm 10\%$. We use STD3 as the LFB.
- 8.5 One Second Source Standard must be analyzed with each batch of samples. Values must be within $\pm 10\%$ of the expected reading.
- 8.6 One Reportable Detection Limit (RDL) standard must be analyzed with each batch of samples. Values must validate the RDL set by the OCWD Lab.
- 8.7 Spike and spike duplicate samples must be run with each batch. For batches with ten or fewer samples, run one spike and spike duplicate. For batches with more than ten samples, at least ten percent of samples shall have a spike and spike duplicate. The spike concentration should be at least four times the MDL. Spike recovery acceptance limits should be $\pm 10\%$. See Section 7.2 on spike preparation.
- 8.8 Sample duplicates must be run on at least ten percent of samples in the batch.

9. MAINTENANCE

- 9.1 Monitor the eluent generation cartridge. From the Control Panel, click the "Eluent Generator" tab. Under EGC_2 you will see the serial number, expiration date, and **Remaining Ion Count**. When this value gets down to below 10% remaining, it is time to order a replacement cartridge. The Dionex Part Number for the EGC-II KOH EluGen Cartridge is 058900. The Part Number for the CR-ATC Anion Continuously Regenerated Trap Column is 060477. Follow Section 9.12 of the *ICS-3000* manual for replacement instructions or call Dionex for service.

10. TROUBLESHOOTING

Please refer to the Dionex *ICS3000 Ion Chromatography Operator's Manual*. Possible causes for poor results include:

- 10.1 Columns need cleaning or replacement.
- 10.2 Incorrect dilution of sample.

10.3 Leak in system.

11. LABORATORY NUMBERS AND QC CODES

11.1 In order to facilitate automatic sample and QC data transfer, please use the nomenclature designated in this section. Lab numbers in Aspen are structured using the following protocol. For example, lab number 08090006-01 indicates the sixth sample received in September of 2008 (08 is year, 09 is month and 0006-01 is sample ID). QC codes are required for various reports and charts.

<u>QC Code</u>	<u>Definition</u>	<u>Example</u>
S	Spike	08090006-01S
K	Spike Duplicate	08090006-01K
Q	Sample Duplicate	08090006-01Q
R	Recheck (No transfer)	08090006-01R
C	Control Chart Standard	QACCXYMMDDC
		X is number of CC STD in batch.
		YYMMDD is year, month, day.

<u>QC Code</u>	<u>Definition</u>	<u>Example</u>
M	MDL	MDL01YYMMDDM
W	RDL	QARDLYYYMMDDW

SOP PROCEDURE CHANGE
INORGANIC DISINFECTION BYPRODUCTS by IC

CHANGE	DATE	INITIALS
Added reference to EPA Method 300.1	11/18/09	JMD
Deleted reference to EPA Method 300.0	10/11/00	JMD
Added preservative information	11/29/00	PH
Clarified preservative information in Section 2.1	6/7/04	JMD
Clarification of the sample prep procedure for the analysis of bromate.	10/12/04	PH
Added cat. # of stock standard solutions to 4.1; 4.2; 4.3 and 4.4.	2/10/06	FC
Chromeleon Software	11/21/2006	JAB
Sample dilution and spike surrogate aliquot adjusted to 20 uL instead of 40 uL, to reflect diluted sample volume.	4/25/2007	PH
As per discussion with SF, PH and JMD, changed surrogate Volume back to 40 uL for spike. Also added section 7.8 about RDL sample.	11/27/2007	JAB
Completely revised SOP based on ICS3000	09/15/2008	JAB
Changed sample prep procedure. Filter BEFORE adding surrogate.	01/22/2009	JAB
Minor changes as per JMD	3/5/2009	JAB
Changes to Sec. 11.1 Added Automatic LIMS transfer codes for MDL and RDL samples.	5/4/2009	PH
Revised Section 7.6 Data Transfer as per PH.	6/29/2009	JAB

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

ION CHROMATOGRAPHY EPA METHOD 300.0

File Name: M:\SOP\INORGANIC\IC_ANIONS.doc
Revision: 12

Effective Date: 6/29/2009
Supersedes: 11 (5/4/2009)

1. REFERENCES

- 1.1 EPA *Methods for the Determination of Inorganic Substances in Environmental Samples* (1993) #300.0 (rev. 2.1)
- 1.2 *Standard Methods* 20th Edition, #4110B.
- 1.3 EPA *Method 300.0 Determination of Inorganic Anions by Ion Chromatography* (revision 2.2)
- 1.4 *ICS-3000 Ion Chromatography Operator's Manual*, Dionex.
- 1.5 *Chromeleon Software Manual*, Dionex.

2. HOLDING TIME

<u>Analyte</u>	<u>Preservation</u>	<u>Holding Time</u>
Chloride	None required	28 days
Fluoride	None required	28 days
Bromide	None required	28 days
Nitrate-N	Cool to 4°C	48 hours
Nitrite-N	Cool to 4°C	48 hours
o-Phosphate-P	Cool to 4°C	48 hours
Sulfate	Cool to 4°C	28 days

NOTE: It is **vital** to observe the preservation and holding time requirements for nitrate and nitrite-N, especially in the case of Title 22 drinking water samples, in case an Action Level Notification needs to be sent (see SOP for Action Level Notifications). Results over the Action Level for nitrite-N (0.75 mg/L), nitrate-N (7.5 mg/L), or nitrate as nitrate (33.75 mg/L) on Title 22 or other drinking water samples **must be dealt with promptly!**

3. INTERFERENCES

- 3.1 Organic acids, high concentration of any one ion, ions with similar retention time, ions eluting near water dip, contaminants on the exchange resin, contamination of reagents, glassware, or apparatus, and the presence of air bubbles in the column, tubing or conductivity detector are all possible. In practice, interferences are rare in the normal range of OCWD lab samples.

4. APPARATUS

- 4.1 Analytical balance.
- 4.2 Ion Chromatograph, Dionex ICS-3000, dual channel, equipped with CD dual conductivity detectors, DP dual gradient pumps, EG reagent-free (RFIC) eluent generator system, AS autosampler, *Chromeleon* software, computer and printer.
- 4.3 Dionex AS-18 Anion separator column, AG-18 guard column, ASRS-300 self-regenerating suppressor column.
- 4.4 Waters Sep-Pak C-18 cartridge for removal of organics, Gelman Acrodisc syringe filter, 0.45 μm pore size (Part Number 4438 or equivalent) and 10cc B-D sterile syringe.
- 4.5 Helium gas (high purity).

5. REAGENTS

- 5.1 **Milli-Q water.** There are two 2L reservoirs labeled System 1 Anions.

6. STANDARDS PREPARATION

NOTE: At present, laboratory is using custom-prepared stock standard solutions from CPI to prepare the calibration standards.

CPI P/N 4400-130735 contains 5000 mg/L Cl, 4000 mg/L SO₄, and 500 mg/L NO₃-N.

CPI P/N 4400-130736 contains 100 mg/L F, NO₂-N, Br, and PO₄-P.

<u>Anion</u>	<u>Salt</u>	<u>Amount g/L</u>
F ⁻	NaF	2.2101
Cl ⁻	NaCl	1.6485
NO ₂ -N	NaNO ₂	4.9259*
Br ⁻	KBr	1.4893
NO ₃ -N	KNO ₃	7.2182
PO ₄ -P	Na ₂ HPO ₄	4.5832
SO ₄	NaSO ₄	1.4780

****Note: All salts listed above except NaNO₂ need to be dried to a constant weight at 105 °C. NaNO₂ should be dried to a constant weight in a desiccator.***

- 6.1 Calibration Standards:
Dilute working standards should be prepared weekly.

6.1.1 STD5: Dilute 20 mL of EACH CPI stock to 1000 mL with milli-Q.

<u>Anions</u>	<u>mg/L</u>	<u>ml of 1000 ppm/L</u>
F	2	2
Cl	100	100
NO ₂ -N	2	2
Br	2	2
SO ₄	80	80
NO ₃ -N	10	10
PO ₄ -P	2	2

6.1.2 STD4: 100 ml of STD5 to 200 ml with milli-Q water.

<u>Anions</u>	<u>mg/L</u>
F	1
Cl	50
NO ₂ -N	1
Br	1
SO ₄	40
NO ₃ -N	5
PO ₄ -P	1

6.1.3 STD3 (LFB): 50 ml of STD5 to 200 ml with milli-Q water.

<u>Anions</u>	<u>mg/L</u>
F	0.50
Cl	25
NO ₂ -N	0.50
Br	0.50
SO ₄	20
NO ₃ -N	2.5
PO ₄ -P	0.50

6.1.4 STD2: 20 ml of STD5 to 200 ml with milli-Q water.

<u>Anions</u>	<u>mg/L</u>
F	0.20
Cl	10
NO ₂ -N	0.20
Br	0.20
SO ₄	8
NO ₃ -N	1
PO ₄ -P	0.20

6.1.5 STD1 (MDL): 10 mL of STD5 to 200 mL with milli-Q water.

<u>Anions</u>	<u>mg/L</u>
F	0.1
Cl	5
NO ₂ -N	0.1
Br	0.1
SO ₄	4
NO ₃ -N	0.5
PO ₄ -P	0.1

6.2 **Second Source Standard** is prepared using stock standards from Ricca Chemical Company or equivalent source (diluted to 1 L):

NOTE: Make sure to verify the stock concentrations before following the recipe below.

<u>Anions</u>	<u>mg/L</u>	<u>ml of std./L milli-Q water</u>
F	1.0	(1.00 mL of 1000 mg/L stock)
Cl	50	(50.0 mL of 1000 ")
NO ₂ -N	1.25	(5.00 mL of 250 ")
Br	0.5	(0.50 mL of 1000 ")
SO ₄	50	(50.0 mL of 1000 ")
NO ₃ -N	5.0	(50.0 mL of 100 ")
PO ₄ -P	1.0	(1.00 mL of 1000 ")

6.3 **Control Chart Standard** is prepared by diluting 150 ml of STD5 to 200ml with milli-Q water:

<u>Anions</u>	<u>mg/L</u>
F	1.5
Cl	75
NO ₂ -N	1.5
Br	1.5
SO ₄	60
NO ₃ -N	7.5
PO ₄ -P	1.5

- 6.4 **Reportable Detection Limit Sample** is prepared by first making an intermediate standard (10mL Stock #2 and 1mL Stock #1, diluted to 500mL), then diluting 25mL of the intermediate to 500mL with milli-Q water:

<u>Anions</u>	<u>mg/L</u>
F	0.1
Cl	0.5
NO ₂ -N	0.1
Br	0.1
NO ₃ -N	0.1
PO ₄ -P	0.1
SO ₄	0.4

7. SAMPLE PREPARATION

- 7.1 Samples must be filtered through Sep-Pak and Acrodisc filter. Assemble the syringe with Acrodisc filter first, then Sep-Pak, with the “long tube” of Sep-Pak attached to outlet of Acrodisc filter, and the “short tube” at the end. Samples should be aqueous solutions only, not acid-preserved, and within the analytical concentration range of the elements of interest (diluted if necessary—usually a 1X, 2X, and 4X dilution). Rinse syringe with sample and elute sample with pressure on syringe plunger through Sep-Pak and Acrodisc filter into a clean disposable beaker. Then, with a clean plastic transfer pipet, transfer sample into labeled autosampler vial

Note: To prepare Sep-Pak C-18 cartridge, first rinse with 5 mL methanol to activate the stationary phase, then rinse with 10 mL Milli-Q DI water. Store prepped Sep-Pak cartridges in a specimen container filled with DI water and a secure top to keep the cartridges moist.

Note: See Section 12 on how to prepare a spike sample.

8. PROCEDURE

8.1 Start-Up Procedure:

- 8.1.1 Turn ON computer and log in as if you were logging on to the District network. Double-click on the *Chromeleon* icon. After doing this, you should notice the “Connected” green light come ON for the DC module, DP module and EG module.

- 8.1.2 The control panel window should open. You should see two control panels, one for Anions on the left and one for IDBP on the right. You can maximize or minimize each control panel by using the arrow buttons next to the control panel title for each method. You can toggle to the Browser window by using Control/Tab. The browser is where you will create the sequences, start batches, review chromatograms and store data files.
- 8.1.3 From the control panel window, click the “Gradient Pump” tab. Under “Flow Control” click the “Motor” switch from red to green to turn ON the gradient pump. You should notice a green light on the DP module for Pump 1 Flow. Then click the “Home” tab, followed by the **Start Up** button at the top of the control panel. This will turn ON the CR-TC and eluent generator. Verify these are on by observing the green lights on the EG module for EGC 1 and CR-TC 1. The Cell Heater and Column switches should already be ON as visible on the screen. It will also turn ON the suppressor. On the DC module, the green light should be on for Suppressor 1. Also, the Upper and Lower oven lights should be ON. And Valve 1 should indicate the Inject position. Click on the “Detector Compartment” tab. At “Suppressor 1 Settings”, verify that Type is ASRS_4mm, Mode is ON, and the Current setting is 107 mA. Click the “Home” tab and confirm that the current reading is **107 mA**.
- 8.1.4 If the instrument is used on a routine basis, there is really no need to prime the gradient pump. If you need to, click on the “Gradient Pump” tab. The motor should be ON and the flow rate at 1.0 mL/min. Click the “Prime Control” switch. A “Check Results” screen will appear, with the message:
“Pump 1. Purging will deliver a high flow to your system. Ensure that the purge valve is open to protect your columns and fluidic system.”
Open the door to the DP module and open the purge valve for Pump 1 about a quarter to a half turn. Click OK, and the priming will start. To stop at any time before the 300 second default time, click the “Prime Control” switch to OFF and **remember to close the purge valve**. Priming automatically shuts off the suppressor, so you need to **remember to turn the suppressor ON after priming**.
- 8.1.5 The instrument makes its own hydroxide eluent. All you need to do is add water. Make sure that the reservoirs are filled with milli-Q DI water. Water can be added at any time before or during the analysis. **If the analysis will be running overnight, it is a good idea to add water just before you leave in the evening.**

8.2 Creating a Sequence:

- 8.2.1 Go to the browser to create a sequence of samples to be analyzed. Hit **Ctrl / Tab** to toggle back and forth between the browser and the control panel.

- 8.2.2 Open the Anion Template. If the template is locked, right-click, properties, properties of sequence "Anion Template", General tab, Lock Status, Locked or Unlocked. Click to unlock the template. **After creating your new sequence and saving it, don't forget to lock the template for future use.**
- 8.2.3 The **column titles** of the sequence are as follows:
 No. is Sequence Number
 Name is Sample ID
 Type is **Standard** or **Unknown**
 Pos. is Autosampler Vial Position (1 – 100)
 Inj. Vol. is **25.0 µL**
 Program is **Anion Gradient NEW 10-43.pgm**
 Method is **ANIONS METHOD**
 Status is **Single**. (After the sequence batch has been started, the status will change from Single to Running. After a sample has been completed, the status will change to Finished. If a sample was stopped in the middle of the analysis, the status will change to Interrupted. The status will have to be changed back to Single and the sequence resaved to rerun).
 Dil. Factor is 1 unless the sample is diluted, then use proper factor.
- 8.2.4 For STD1 thru STD5, use **Standard** as the type of sample. All other samples including CC Standards, LFB, MDL, RDL, 2nd Source, Spikes, Duplicates and Samples, use **Unknown** as the type of sample. For diluted samples, don't forget to enter the correct dilution factor. You can place the standards and samples anywhere on the autosampler tray. For instance, if you need to run Anions and IDBP simultaneously, you could place the Anion samples in A/S position numbers 1 thru 50 and the IDBP samples in position numbers 51 thru 100. If you have more than 50 samples for Anions, place the 51st sample in A/S position number 1 and continue. Same for IDBP. Place the 51st sample in position 51 and continue. **Just remember to put the correct number for the sample in the Autosampler column.** To add more lines at the end of a sequence, highlight the last row by clicking the gray **No.** box. Then Down Arrow. You will be asked, "*Append New Line?*" Click "Yes", then continue the Down Arrow to add rows.
- 8.2.5 When the sequence is ready to save, use the following protocol:
File, Save As, Anion DATA 2008, 0809 Folder, 080910 (YYMMDD).
Note: You can add to the sequence at any time during the analysis, **just remember to Save afterwards.** To print, go to **File, Print Sequence**, then **OK.**

- 8.2.6 If you want to add a stop program at the end of the sequence, go to the **Program** column and click in the box. Choose the **STOP PRG** for Anions. Change the **Sample Type** to **Blank**. **Place a vial containing DI water in the STOP position.**
- 8.2.7 Use the 1.5 mL glass vial with the slit septa cap for analysis (Dionex P/N 055427, on HTE). Use a disposable plastic transfer pipet to add sample to the vial up to the 1.5mL mark. **DO NOT over-fill. The needle vent must not be immersed in liquid.** Place samples in autosampler tray. Do not remove tray from the autosampler. The door can be open at any time during analysis to add samples. **Add samples only when the needle is idle.**
- 8.3 Start Analysis:
- 8.3.1 Before starting, check the system conductivity. Click the “Conductivity Detector” tab and check the Total Signal. For Anions, the Total Signal must be below 1 μ S to begin the analysis. It usually is around 0.3 μ S, depending on the quality of the milli-Q DI water. This level is usually reached shortly after start-up, but it may take longer if the instrument has not been run recently.
- 8.3.2 To check the baseline before starting analysis, click on the 13th icon from the left, a BLUE DOT. Data Acquisition – Anions. Click OK to start baseline acquisition. Click the BLUE DOT a second time to turn off acquisition. **Note: You must turn OFF acquisition before starting the analysis. This can also be done by using a button on the Status page.**
- 8.3.3 Prime the autosampler syringe before starting the analysis. Go to the “Autosampler” tab on the Control Panel. Click on “Syringe Prime” button. The syringe will move down and up 4 times for a total of 1 mL. If you notice any bubbles in the line from the DI water reservoir to the valve, gently tap the line when the syringe has moved about 1/3 of the way on the downward cycle to try to get the bubbles into the syringe. Hopefully, the bubbles will be pumped out of the syringe on the upward cycle. When adding water to the reservoir, make sure to remove any bubbles or air gaps **BEFORE** priming. You will probably have to remove the tubing at the valve end and try to remove any air, then reattach the line to the valve. If you have any trouble, please ask someone who has experience in doing this procedure.

- 8.3.4 When ready to start the sequence, click Batch, Start. *“Start Batch on Anions? Remove any sequence batch lists you do not want to run. Make sure just the batch list of the sequence you want to run is shown and highlight it. An example is 0809/080910 JAB. If it is the one you want to run, then click **Start**. The autosampler needle will start moving to the rinse position and the current row of the sequence will be highlighted green. (NOTE: Column temperature is heated to a default of 30°C. When you click Start, the column begins cooling. When it drops below 23°C, the autosampler will move to the first sample vial. The delay can be significant, especially if the room temperature is warm).*
- 8.3.5 Run time is about 23 minutes per sample. When a sample is done, the status will change from “Running” to “Finished”.
- 8.4 Chromatogram Review:
- 8.4.1 You can review a standard or sample chromatogram and make changes to it at any time after that standard or sample has finished being analyzed. If changes involve updating the method for that particular run, the changes take effect immediately and will apply to all finished samples as well as those waiting to be analyzed.
- 8.4.2 Double click on the sample ID. This will take you to the “Integration” window. You will see the chromatogram on the left, the calibration curve on the right and the results table at the bottom. You can see peak names, area counts, retention times and amounts for the individual analytes. There are five tabs at the bottom of the window: **Integration; Calibration; Peak Analysis; Summary; and Audit Trail**. You can check the correlation coefficients by clicking the “Calibration” tab.
- 8.4.3 Double-click on a peak name and you will see the integration points for that analyte and the peak start and stop points.
- 8.4.4 To identify a peak, put the cursor on a peak, then double-click. A “Peak Properties” box will appear. If the peak is misidentified, select the correct peak, then click the “Thumb-Tack” symbol at right, and the peak will assume the new identity.
- 8.4.5 After making any changes to a chromatogram, **File, Close**. You will be prompted to save the changes. Click **Yes**. Notice a message at the upper left of the chromatogram, after the Sequence File Number. It will say “Modified By XXX,” and if a peak ID was changed, “Peak ID Manually Assigned.”

- 8.4.6 Other changes can be made in the 'QNT Editor' (23rd icon from the left). There are five tabs at the bottom: **General; Detection; Peak Table; Amount Table; and Calibration.** Go to the "Calibration" tab to disable or enable a standard curve point. You should know what you are doing if working in the QNT Editor. If you are not sure how to do something, please ask for help.
- 8.4.7 Helpful icons located across the top of the browser screen include **Acquisition; Integration; QNT Editor; Printer Layout; Show Calibration Curve; Previous Sample; Next Sample; Automatic Tool; Full Size; Autoscale.**
- 8.4.8 When the run is finished and any and all changes have been made, it is time to print your results. First, highlight all standards and samples you want to print. If you used the STOP PROGRAM, don't include this sample in the batch. Then **File, Batch Report.** A "Batch Report" screen will appear. At "Print Options", make sure "Printout" box is checked, then **OK.** A second "Batch Report" screen appears. Click **OK** (will appear after a short delay for processing). After printing, double click on STD5 of the sequence, then click on the "Printer Layout" icon (24th from the left at the top of the page). At the bottom of the page, click on **Calibration Batch 2** tab, then **Print, OK.** This will print the second page of the calibration curves.
- 8.5 Shutdown:
- 8.5.1 If the STOP PROGRAM was used (and if it worked), the pump, EGC, CR-TC, and suppressor should be OFF. Check the Control Panel and the individual modules to verify. **Again, as explained in Section 8.2.6, for the shutdown sample, change the sample type to BLANK and the program to STOP PRG.** If you didn't use the stop program, go to the control panel ("Home" tab) and click the **SHUT DOWN** button at the top. This will shut off the pump, EGC, CR-TC, and suppressor. Verify by looking at the individual modules. The column heater should be left ON.
- 8.6 Data Transfer to LIMS:
- 8.6.1 Insert memory stick into USB port.
- 8.6.2 Go to browser and open sequence batch you want to transfer. Double-click on the first standard, STD1.
- 8.6.3 Click on **Printer Layout** icon at top (next to yellow **QNT Editor** icon).

- 8.6.4 Click on **Summary** at bottom of page.
- 8.6.5 Go to **File**, click **Close**, and you should be back at the browser page with your selected sequence showing.
- 8.6.6 Highlight **only** the first standard in the sequence, STD1.
- 8.6.7 Click on **File**, then **Batch Report**.
- 8.6.8 Unclick **Printout** check mark.
- 8.6.9 Put a check mark in the **Export Options** box.
- 8.6.10 Put a check mark in the **ASCII Text Format** box. Then click on **Next**.
- 8.6.11 Under **Export Results**, change SST to **Summary**.
- 8.6.12 Click on **Finish**, then **OK**. Finally, click on **OK** when shown in the **Batch Report** window. The data will be transferred to the memory stick in the following format:
E:\Anions\AnionDATA2009\0906\090629.seq\0001.TXT
- 8.6.13 Open *Aspen LIMS*. At "Import Data" use **IC File – ICS3000** for Anions. Open the E-drive and double-click past the Anions Folder, past the AnionDATAyear folder, past the Month folder, past the sequence folder, to 0001 text data file. This will contain the data to be imported.

9. QUALITY CONTROL

- 9.1 An initial demonstration of performance should be made. This includes a curve, second source standard (QCS) and a MDL study. Also whenever a new operator takes over the test a MDL study should be done.

A mid-range control chart standard (CCSTD) and a calibration blank must be analyzed immediately following calibration, after every ten samples and at the end of the run. The CCSTD must be within +/- 10%, if it is not you can analyze another CCSTD, if it also fails, the analysis must be stopped and the problem corrected and if necessary recalibrate the instrument.
Note: If you wish you can run CCSTDs more frequently to check the performance of the instrument.
- 9.2 One laboratory reagent blank (LRB) must be analyzed with each batch of samples, it must not exceed the MDL. A LRB is treated exactly like as a sample would be treated.
- 9.3 One laboratory fortified blank (LFB) must be analyzed with each batch of samples, this must be within +/- 10%. We use STD3 as the LFB.

- 9.4 One Second Source Standard is analyzed with each run. Values must be within +/- 10% of the expected reading.
- 9.5 One Reportable Detection Limit (RDL) sample is analyzed with each batch. Values must validate the Reportable Detection Limits set by the OCWD lab.
- 9.6 Spike and spike duplicate samples must be run with each batch. For batches with ten or fewer samples, run one spike and spike duplicate. For batches with more than ten samples, at least ten percent of samples shall have a spike and spike duplicate analysis. The spike concentration should be at least four times the MDL. Spike recovery acceptance limits should be 90% to 110%. See Section 10 for instructions on spike preparation.
- 9.7 Sample duplicates must be run on at least ten percent of samples in the batch.

10. SPIKE PREPARATION

- 10.1 Pipet 5mL of sample to be spiked into a disposable beaker.
- 10.2 Pipet 5mL of **STD5** into the same beaker.
- 10.3 Transfer 10mL Milli-Q DI water to the same beaker and mix thoroughly using a swirling motion.

NOTE: This produces a 4X dilution of the sample. Make sure a 4 is entered in the Dilution Factor column of the sequence. Be sure to run a 4X dilution of the same sample adjacent to the spiked sample in the autosampler, omitting the spiking solution and replacing it with 5mL of Milli-Q DI water.

- 10.4 Formula for determining % recovery is:

$$P = 100 \times \frac{\text{Spiked sample conc.} - \text{unspiked sample conc.}}{\text{Spike conc.}}$$

Use the Spike Recovery spreadsheet for calculations when preparing the Data Package.

11. MAINTENANCE

- 11.1 Monitor the eluent generation cartridge. From the Control Panel, click the "Eluent Generator" tab. Under EGC_1 you will see the serial number, expiration date, and **Remaining Ion Count**. When this value gets down to below 10% remaining, it is time to order a replacement cartridge. The Dionex Part Number for EGC-II KOH EluGen Cartridge is 058900. The Part Number for CR-ATC Anion Continuously Regenerated Trap Column is 060477. Follow Section 9.12 of the *ICS-3000* manual for replacement instructions or call Dionex for service.

12. TROUBLESHOOTING

Please refer to the *Dionex ICS3000 Ion Chromatography Operator's Manual*. Possible causes of poor results include:

12.1 Columns need cleaning or replacement.

12.2 Incorrect dilution of sample.

12.3 Leak in system.

13. LABORATORY NUMBERS AND QC CODES

13.1 In order to facilitate automatic sample and QC data transfer, please use the nomenclature designated in this section. Lab numbers in Aspen are structured using the following protocol. For example, lab number 08090006-01 indicates the sixth sample received in September of 2008 (08 is the year, 09 is the month, and 0006-01 is the sample ID). QC codes are required for various reports and charts.

<u>QC Code</u>	<u>Definition</u>	<u>Example</u>
S	Spike	08090006-01S
K	Spike Dup.	08090006-01K
Q	Sample Dup.	08090006-01Q
R	Recheck (No Transfer)	08090006-01R
C	Control Chart Standard	QACCXYMMDDC Where X is the number of the CC Std in the batch sequence.
M	MDL	MDL01YYMMDDM
W	RDL	QARDLYYMMDDW

SOP PROCEDURE CHANGE
FOR ION CHROMATOGRAPHY

CHANGE	REASON	DATE	INITIALS
Modified for Excel File Transfer		9/4/98	MY
Added Section C		9/29/98	MY
Added Section C.2, error msg when generating IC file		2/10/99	MY
Modify Section C for PeakNet .csv file creation		9/18/2000	MY/PH
Updated SOP to reflect changes made by moving Anions method from 4000i to DX-500.		4/6/2001	JAB
Updated Section 5 replace Conifer with Aspen		1/29/2002	MY
Updated Sec. 5 modifying eluent preparation instructions Clarified Sec. 8.1.6 and spiking instructions in Sec. 12 Added "R" for "Recheck" to QC codes. In data transfer section, replaced references to Conifer with Aspen, revised Instrument File Generation Secs. 3.1 and 3.2. Replaced "Excel File Generation" section with Aspen Data Import And revised Data Review and Peer Review Procedures.		6/12/03	JMD
Updated 6.1 to include frequency of standard preparation and updated section 10 to comply with EPA 300.0		12/8/03	JJR
Inorganic laboratory automatic data transfer number and code designations Update, for Aspen automatic reporting of MDLs and CC Standards reports.		03/08/05	PH
New Chromeleon Software		11/22/2006	JAB
Added 6.4 Reportable Detection Limit Sample		11/27/2007	JAB
Completely revised because of new ICS3000 IC system		09/10/2008	JAB
Minor revisions as per JMD		3/5/2009	JAB
Changes to Sec. 11.1 Added Automatic LIMS transfer codes for MDL and RDL samples.		5/4/2009	PH

SOP PROCEDURE CHANGE
FOR ION CHROMATOGRAPHY

CHANGE	REASON	DATE	INITIALS
Revised Section 8.6 Data Transfer as per PH		6/29/2009	JAB

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

METHYLENE BLUE ACTIVE SUBSTANCES (MBAS) [SURFACTANTS]

File Name: M:\SOP\INORGNIC\MBAS.doc
Revision: 9

Effective Date: 7/28/2009
Supersedes: 8 (11/24/2008)

1. REFERENCES

- 1.1 *Standard Methods*, 20th Ed. #5540 C
- 1.2 *EPA Methods for Analysis of Water and Waste*, # 425.1.

2. HOLDING TIME

- 2.1 48 hours at 4°C

3. INTERFERENCES

- 3.1 Positive interferences from all other MBAS species present. Organic sulfonates, sulfates, carboxylates and phenols, and inorganic thiocyanates, cyanates, nitrates and chlorides also may transfer more or less methylene blue into chloroform layer. Interferences from chloride and nitrate largely eliminated by backwash step. Negative interferences from presence of cationic surfactants and other cationic materials such as amines. Sulfides may react with methylene blue to form a colorless reduction product. Eliminate this interference by oxidation with hydrogen peroxide.

4. APPARATUS

- 4.1 Spectrophotometer – Varian Cary 50 or equivalent, for use at 652 nm, providing light path of 10 cm or longer.
- 4.2 Separatory funnels – 250, 500 or 1000 mL, with TFE stopcocks and stoppers.

5. REAGENTS

- 5.1 Stock Linear Alkylbenzene Sulfonate (LAS) solution - weigh an amount of reference LAS (from EPA) equal to 1.000g LAS on a 100% active basis. For example, if LAS is stated as 5.3% active, $100/5.3 = 18.868$ g to be weighed out and diluted to 1000 mL with DI water. This will yield a concentration of 1000 mg/L LAS.
- 5.2 Standard LAS solution - Dilute 10.0 mL LAS to 1000 mL with DI water. This will yield a 10 mg/L solution. Prepare daily.

- 5.3 Phenolphthalein indicator - Dissolve 5g phenolphthalein in 500mL 95% ethyl or isopropyl alcohol and add 500mL DI water.
- 5.4 Sodium hydroxide, 1N - Dissolve 40g NaOH in DI water and dilute to 1000mL.
- 5.5 Sulfuric acid, 1N - Add 28mL conc. H_2SO_4 to 500mL DI water and dilute to 1000mL.
- 5.6 Sulfuric acid, 6N - Add 167mL conc. H_2SO_4 to 800mL DI water and dilute to 1000mL.
- 5.7 Chloroform – **min. 99.8%, ACS spectrophotometric grade, Sigma-Aldrich #154733-2L or equivalent.** CAUTION: CHCl_3 vapors are toxic. Take appropriate safety precautions when handling.
- 5.8 Methylene blue reagent - Dissolve 200mg methylene blue in 200mL DI water. Transfer 60mL to a 2000mL volumetric flask. Add 1000mL water, 82mL 6N H_2SO_4 and 100g sodium phosphate, monobasic, monohydrate, $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$. Dissolve completely, then dilute to 2000mL.
- 5.9 Wash solution - Add 82mL 6N H_2SO_4 to 1000mL water in a 2000mL flask. Add 100g $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ and dissolve. Dilute to 2000mL.
- 5.10 Glass wool - Pre-extract with CHCl_3 .

6. PROCEDURE

6.1 STANDARDS

- 6.1.1 Prepare a series of 6 **250 mL** separatory funnels with 0, 0.2, 0.5, 1.00, 3.00 and 5.00 mL standard LAS solution (0, 0.02, 0.05, 0.1, 0.3 and 0.5 mg/L), **added to sufficient water** to make total volume 100mL in each funnel.
- 6.1.2 Adjust pH by adding 1 drop phenolphthalein indicator **to solution in funnel, then make alkaline** by dropwise addition of 1N NaOH. Discharge pink color by dropwise addition of 1N H_2SO_4 .
- 6.1.3 Add 25 mL methylene blue reagent and 10 mL CHCl_3 . **Stopper securely and rock** funnel vigorously for 30 seconds. Let phases separate. Draw off CHCl_3 layer into second funnel.
- 6.1.4 Repeat extraction two additional times, using 10 mL CHCl_3 each time.

6.2 SAMPLES

- 6.2.1 Sample size: Select sample volume on the basis of expected MBAS concentration:

Expected MBAS concentration (mg/L)	Sample Taken (mL)	Sep. funnel size (mL)
<0.025	500	1000
0.025-0.080	400	1000
0.080-0.40	250	500
>0.40	100	250

6.2.2 **Do not increase volume of methylene blue solution or chloroform.** If expected MBAS concentration is above 0.5 mg/l, dilute sample before extraction. For Title 22 and RO product samples, use 500ml sample volume. **Other wastewater** samples tend to form an emulsion, hence use 100 ml sample volume.

6.2.3 **Treat samples the same as standards per 6.1 above. Proportionally increase the amount of phenolphthalein added to sample sizes larger than 100 mL. Also, the buffering effect of most samples will necessitate adding a larger volume of NaOH to adjust pH.**

6.3 Combine all CHCl_3 extracts in second separatory funnel. Add 50mL wash solution and rock vigorously for 30 seconds. Let layers separate, then draw off CHCl_3 layer through funnel containing plug of pre-extracted glass wool into a 50-mL (changed from past use of 100-mL) volumetric flask. Extract wash solution twice more with 10mL CHCl_3 each time, and add extracts to volumetric flask. Rinse glass wool and funnel with CHCl_3 . Collect washings in flask, dilute to mark and mix.

6.4 Measure absorbance at 652nm using a 10-cm (changed from past use of 5 cm) cell path. **Correct sample readings based on the 100 mL volume used for standards, e.g., for 500 mL of sample, divide concentration reading from spectrophotometer by 5; for 200 mL sample, divide by 2, etc. Likewise, if you must use a sample size less than 100 mL in the case of high MBAS or heavy emulsions, multiply by 2 for 50 mL, 4 for 25 mL, etc. Also see sec. 7.1 below.** Note: all the modifications in this paragraph are necessary to obtain a detection limit of 0.02mg/L.

Note: Discard all waste CHCl_3 from sep funnels and flasks into container labeled "Recovered CHCl_3 " (not "waste CHCl_3 ") and store in cabinet until full. Then transfer container to hazardous materials storage room.

7. QUALITY CONTROL

7.1 Run one duplicate for every ten samples, or one duplicate per run **of less than ten samples**; values should agree within 10%. Check standard should be within 2 sigma control limits or 20%, whichever is less. Second source standards are analyzed once per month and should be within 10% of expected reading. Also, analyze an MDL (0.02mg/L) standard once a month. Run one spiked sample each time, using a sample and separatory funnel volume based on the expected sample concentration. The spike recovery should be 75% - 125% (when spiking 500 mL sample, **divide unspiked concentration by 5, but do not divide spike value by 5**).

8. TROUBLESHOOTING

- 8.1 Extracts should be clear (not turbid or showing evidence of emulsion). Make sure pH is adjusted for standards and samples.

SOP PROCEDURE CHANGE
FOR METHYLENE BLUE ACTIVE SUBSTANCES (MBAS) [SURFACTANTS]

[illegible]

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

AMMONIA (as nitrogen) by FIA

File Name: M:\SOP\INORGNIC\FIA_NH3.doc
Revision: 12

Effective Date: 4/27/2009
Supersedes: 11 (4/7/2009)

1. REFERENCES

- 1.1 *Standard Methods* 20th Ed. #4500NH₃-H.
- 1.2 *EPA Methods for Analysis of Water and Waste*, #350.1.
- 1.3 Lachat Instruments QuikChem Method 10-107-06-1-K. This method complies with EPA-approved methodology for the analysis of drinking water and wastewater.

2. HOLDING TIME

- 2.1 Acidify sample to pH <2 with H₂SO₄ (approx. 2 mL conc. H₂SO₄ per liter), cool to 4°C, hold for up to 28 days; best if analyzed without delay.

3. INTERFERENCES

- 3.1 Calcium and magnesium ions may precipitate if present in sufficient concentration. Tartrate or EDTA is added to the sample in-line in order to prevent this problem.
- 3.2 Color, turbidity and certain organic species may interfere. Turbidity is removed by manual filtration. Sample color may be corrected for by running the samples through the manifold without adding the color formation reagents. Proceed as follows:
 - 3.2.1 Calibrate the system in the normal manner.
 - 3.2.2 Analyze the samples.
 - 3.2.3 Place reagent and carrier lines in DI water and allow the baseline to stabilize.
 - 3.2.4 Disable the DQM features and inject samples again without recalibrating.
 - 3.2.5 Subtract the background concentration from the original concentration to give the color-corrected concentration.

- 3.3 Samples should be adjusted to room temperature prior to analysis.
- 3.4 **This method has been tested and found to have acceptable recovery without initial sample distillation with standard OCWD sample streams (GWRS, Green Acres Project, Deep Wells, Monitoring Wells, Drinking Water Wells, Prados, OCRs, PDP/MDPs, and R&Ds). Any new waste discharger's samples or other new source of samples, or, any increase in the concentration of ammonia in the above waste streams above previous levels requires distillation of the samples before automated FIA analysis by this method. More conveniently, submit samples from waste streams that require pre-distillation to analysis by the automated Kjeldahl distillation/titration/digestion unit (equivalent to *Standard Methods* 4500-NH₃ B and E).**

4. APPARATUS

- 4.1 LACHAT QuikChem 8500 Flow Injection Analyzer.

5. REAGENTS

- 5.1 Sodium Phenolate. In a 1-L volumetric flask, add 88 mL of 88% liquified phenol to approximately 600 mL DI water. While stirring, slowly add 32 g sodium hydroxide (NaOH). Cool and dilute to the mark. Store in amber glass bottle. **Prepare fresh weekly. Discard if reagent turns dark. DO NOT DEGAS THIS REAGENT.** Record in Reagent Prep Logbook.

NOTE: Until further notice, use only *Guaranteed Reagent (GR)* grade liquefied phenol, min. 88%, from EMD. VWR Catalog #EM-PX0511-1.

- 5.2 Sodium Hypochlorite. In a 500 mL volumetric flask, dilute 250 mL regular Clorox bleach [5.25% sodium hypochlorite (NaOCl)] to the mark with DI water. **Prepare fresh prior to use. DO NOT DEGAS THIS REAGENT.** Record in Reagent Prep Logbook.
- 5.3 Buffer. In a 2 L volumetric flask, dissolve 100.0 g disodium ethylenediamine tetraacetic acid (Na₂EDTA) and 11.0 g sodium hydroxide (NaOH) in about 1800 mL DI water, then dilute to the mark. Prepare fresh monthly. **THIS REAGENT MUST BE DEGASSED PRIOR TO USE.** Record in Reagent Prep Logbook.
- 5.4 Sodium Nitroprusside. In a 1-L volumetric flask, dissolve 3.50 g sodium nitroprusside, Na₂Fe(CN)₅NO·2H₂O, also known as sodium nitroferricyanide dihydrate, in DI water and dilute to the mark. Store in amber glass bottle. Prepare fresh every two weeks. **DO NOT DEGAS THIS REAGENT.** Record in Reagent Prep Logbook.
- 5.5 Carrier. Milli-Q DI water. **DEGAS PRIOR TO USE.**

- 5.6 To degas the buffer and carrier, bubble helium gas through the solutions vigorously for several minutes.
- 5.7 Ammonia Standards and QA/QC Samples. (Record in Standards Prep Logbook).
- 5.7.1 Stock Ammonia Standard, 1,000 mg/L $\text{NH}_3\text{-N}$. Commercially prepared from Ricca or equivalent.
- 5.7.2 Standard A, 20.0 mg/L $\text{NH}_3\text{-N}$. Dilute 4 mL of Stock to 200 mL with Milli-Q DI water.
- 5.7.3 Standard B, 8.0 mg/L $\text{NH}_3\text{-N}$. Dilute 2 mL of Stock to 250 mL with Milli-Q DI water.
- 5.7.4 Standard C, 2.0 mg/L $\text{NH}_3\text{-N}$. Dilute 10 mL of Std A to 100 mL with Milli-Q DI water.
- 5.7.5 Standard D, 1.0 mg/L $\text{NH}_3\text{-N}$. Dilute 5 mL of Std A to 100 mL with Milli-Q DI water.
- 5.7.6 Standard E, 0.4 mg/L $\text{NH}_3\text{-N}$. Dilute 5 mL of Std B to 100 mL with Milli-Q DI water.
- 5.7.7 Standard F, 0.2 mg/L $\text{NH}_3\text{-N}$. Dilute 10 mL of Std C to 100 mL with Milli-Q DI water.
- 5.7.8 Standard G, 0.1 mg/L $\text{NH}_3\text{-N}$. Dilute 5 mL of Std C to 100 mL with Milli-Q DI water.
- 5.7.9 Standard H, Blank. This is Milli-Q DI water.
- 5.7.10 Control Chart Standard, 8.0 mg/L $\text{NH}_3\text{-N}$. Same as Std B.
- 5.7.11 Method Detection Limit Sample (MDL), 0.2 mg/L $\text{NH}_3\text{-N}$. Same as Std F.
- 5.7.12 Reportable Detection Limit Sample (RDL), 0.1 mg/L $\text{NH}_3\text{-N}$. Same as Std. G.
- 5.7.13 Second Source Standard Stock, 100 mg/L $\text{NH}_3\text{-N}$. Commercially prepared from Thermo or equivalent.
- 5.7.14 Second Source Standard, 8 mg/L $\text{NH}_3\text{-N}$. Dilute 20 mL of Second Source Stock to 250 mL with Milli-Q DI water.

5.7.15 **Spiked Sample Preparation.** Add 1 mL of 100 mg/L Stock to a 50 mL volumetric flask and dilute to the mark with sample. This is a 2 mg/L spike.

6. PROCEDURE

- 6.1 Turn ON the computer FIRST. Power up the FIA by pressing the RED switch on the power strip at the right side of the instrument.
- 6.2 Double click on the **Omnion 3.0** icon.
- 6.3 Click on **Configuration** at top of screen, then **Autosamplers**. A new window will open. Click on box **Initialize Autosampler**. The autosampler will initialize, then go to the wash station position. Close the autosampler window.
- 6.4 Click on **Open** icon. A window opens to the last method that was run. If it is not the one you want, click the yellow folder with the up arrow at the top of the window to open the Methods directory. Choose the method you want to run, then click to open the most recent run (doing this insures that the most recent timing updates will apply to your run).
- 6.5 The screen is divided into three windows. **Run Worksheet** is at the upper left, **Run Properties** is at the upper right, and **Channel** is along the bottom. You will be prompted, “**Do you want to change the setpoints of the relevant heaters?**” Answer YES, then notice the heater Temp and Setpoint indications along the lower left side of the Channel window, in this case **60°C**.
- 6.6 Enter sample information in the **Run Worksheet** window. Highlight the sample ID box. Type in the sample LIMS number and press ENTER. The cursor will move to the next sample row. Sample type is **UNKNOWN**.
- 6.7 The CC Standard is in location S9 on the autosampler. It is highlighted green in the run worksheet. The sample type is **CHECK STANDARD**. Highlight the row to verify the correct pass/fail criteria. In the **Run Properties** area of the screen, check the DQM Limits. The **KNOWN CONCENTRATION** should be **8.00 mg/L**. The **>+ CONC. LIMIT** should be **8.80 mg/L** and the **<- CONC. LIMIT** should be **7.20 mg/L**. The DQM Action messages should be PASS, FAIL, Continue and Continue. The protocol for naming the CC Standard is **QACCxyymmddC**, where x is the number of the CC Standard in the run, and yymmdd is year, month, day.
- 6.8 The MDL sample is in location S6 on the autosampler. The sample type is **UNKNOWN**. Do not use Method Detection Limit for the sample type or the sample will be analyzed seven replicates. The MDL sample is named **MDL01yymmddM**.

- 6.9 To enter a spike and spike duplicate, change the sample type to **SPIKE AND SPIKE DUPLICATE**. Three rows colored red will appear, one for the unspiked sample, one for the spiked sample and one for the spiked duplicate sample. Move the cursor to the spike duplicate row and click to highlight. In the **Run Properties** area, enter the known spike concentration of **2.0 mg/L**. Check the **>+ %Recovery Limit** box and enter the upper limit of **115%**. Enter the DQM Action messages of **PASS, FAIL, Continue, and Continue**. Check the **<- %Recovery Limit** box and enter the lower limit of **85%**. Again, enter the DQM Action messages. Use the LIMS designations of **S** and **K** for spike and spike duplicate respectively. Then continue entering samples in the **Run Worksheet**.
- 6.10 To enter a duplicate, change the sample type to **DUPLICATE**. Two rows colored orange will appear, the first for the unknown sample, and the second for the sample duplicate. There is no DQM.
- 6.11 To insert or delete a sample row, first highlight a sample row, then right-click. A dropdown menu will appear. Use **Insert** or **Insert Many** to add rows and **Delete** to remove rows. **When adding rows, make sure to change the number of replicates to 2.**
- 6.12 Make sure all **Trigger OFF** and **Weight 1.0000g** boxes are checked.
- 6.13 To renumber the sample cups, click on the first sample row below the blue standards area. Click in the Sample No. column and drag the cursor down to the last sample row. Release and the entire sample area should turn blue. Then, without moving the cursor, right-click and the dropdown menu will appear. Click on the last item, **Columns ->**, and a second dropdown menu will appear. Click on the first item, **AutoNumber Cups**.
- 6.14 To save the tray, click on the **Run** button in the upper left of the screen, then **Save As Default New Run**, then **OK**. To print the sample tray worksheet, click **Run**, then **Export Worksheet Data**.
- 6.15 Check the condition of all pump tubing and replace if necessary. **This is a high backpressure method and requires the use of Tygon pump tubes. DO NOT use Duraprene pump tubes for this method. DO NOT mix Tygon and Duraprene.** Place the reagent lines in a container of DI water. Correctly set the tension on the pump tubes. The pump speed is **35**. **Make sure the waste lines are at the top of the waste container and not hanging down in liquid. This can cause a backpressure flow problem.**

PROCEDURE FOR INSTALLING PUMP TUBING

6.15.1 Place correct pump tubing onto pump cartridges.

6.15.2 Move tension levers to the right-most position.

6.15.3 Turn the pump ON by pressing the blue Manual Run / Stop button to start the pump rollers rotating. Install pump cartridge by engaging one end of the cartridge into cartridge holder, then clamp down other end until it snaps into place. The tension levers must always be on the left side of the pump. An arrow indented on the top of the cartridge shows the flow direction.

6.15.4 Move the tension lever to the left until it clicks once. The tension levers should be approximately in the 12 o'clock position.

6.15.5 After the analysis, release tension on the pump tubing by moving the tension levers back to the right, then releasing one side of the pump cartridge from the pump cartridge holder.

After pumping DI water through the manifold, place the reagent lines in their corresponding reagent bottles. Check for any leaks or obstructions or pulsating pump tubing.

6.16 Load the sample tray with samples. The MDL sample goes in position S6, the CC Standard in position S9, and the Second Source sample in position S10. Place the standards in the standards rack.

6.17 Click on the **Start** button located at the top.

6.18 View the diagram in the **Channel** window. As the first peaks appear, you will notice a box outlined in blue will automatically be drawn around each peak. This is the **Peak Expectation Window**. There will also be a peak height and baseline drawn in red. **IF THIS INFORMATION DOESN'T APPEAR TO BE CORRECT**, stop the run by clicking the **Stop** button. Move the cursor to any part of the background and right-click. A dropdown menu will appear. Click on **Adjust Peak Expectation Window**. A box outlined in black will now appear around the peak, surrounding the blue box. Left-click and the black box changes to red and the shape of the cursor changes to a left-right and up-down arrow shape. Move the cursor to the left vertical side of the box and the cursor shape changes to a left-right arrow. Left-click and drag the left side of the red box to where you want the peak detection to begin. Do the same for the right side, changing where you want the peak detection to end. Then move the cursor outside the box, into the background area, and right-click. The dropdown menu will appear. Select **Rerun Peak Detection**. The box will change back to blue and be repositioned to reflect the changes you made. The red baseline will also be redrawn. If the peak detection box looks OK, click on the **Start** button to restart the analysis. Monitor the first few standards to verify that the changes were correct. If not, repeat the above process. When the analysis is finished, save the method with the updated information. **(Note: Saving the method is only necessary if changes were made to the peak detection or timing parameters. Normally, changes are not required and you will not have to do this step.)**

- 6.19 The minimum correlation coefficient is 0.9950, however 0.9990 or better can be easily attained.
- 6.20 When the run has finished, a message will pop up on the screen: "Export File Path: Access permission to export file is denied. The file may be opened with another application." Click OK. Also note that the **Start** button is highlighted.
- 6.21 Flush all reagent lines and manifold tubing by placing the reagent lines in a container of DI water. Continue flushing for **at least five minutes**, then remove all lines from the water and continue pumping until all reagent lines, manifold tubing and waste lines are dry. Release tension on the pump tubes. Properly dispose of waste.
- 6.22 Omnion automatically saves the run and assigns a file name using the following protocol: OM_MM_DD_YYYY_HH_MM_SSAM (or PM) .OMN. Make sure the run file is in the correct monthly folder.
- 6.23 To print the run report, go to **Tools** at the top of the screen, then **Custom Report**, then click the printer icon. When finished, click **Report** at the top left, then **Close**.
- 6.24 Transfer the run data to a memory stick. Insert the memory stick into a USB port. Minimize the "Removable Disk (F:) window. Click the **Run** tab in the **Run Properties** window, then click the **Export Data to File** tab.
- 6.25 To exit from Omnion, click on **Run** at the upper left, then **Exit**. You will be prompted "Exit Omnion?", then OK. Don't forget to turn OFF power to the instrument.

7. QUALITY CONTROL

- 7.1 Method Detection Limit (MDL). A MDL sample (in this case the second lowest concentration standard) must be analyzed with each batch of samples to be run. When data for seven replicates has been acquired, calculate the MDL as follows:

$$\text{MDL} = (t) \times (S)$$

where

t = Student's t value 3.14 for seven replicates.

S = standard deviation of the replicate analyses.

Run MDL sample at beginning of each run.

- 7.2 Laboratory Reagent Blank (LRB). The laboratory must analyze at least one LRB with each batch of samples. Values that exceed the MDL indicate laboratory or reagent contamination and corrective actions must be taken before continuing the analysis.

- 7.3 Control Chart Standard (CC STD). The laboratory must analyze a Control Chart Standard immediately following daily calibration, after every tenth sample, and at the end of the sample run. Analysis of the Control Chart Standard following calibration must verify that the instrument is within $\pm 10\%$ of calibration. Subsequent analyses of the Control Chart Standard must verify the calibration is STILL within $\pm 10\%$. If the Control Chart Standard is outside the limits, the sample analysis must be aborted, the cause for failure determined and/or the instrument recalibrated. All samples following the last acceptable Control Chart Standard must be reanalyzed.
- 7.4 Reportable Detection Limit (RDL). A RDL sample (The lowest concentration standard) must be analyzed with each batch of samples to be run. The naming protocol is **QARDLyymmddW**.
- 7.5 Duplicates. As a minimum, run one duplicate analysis for every ten samples, and/or one for each sample set of less than ten samples. The relative percent difference (RPD) should be $\leq 10\%$.
- 7.6 Laboratory Fortified Sample Matrix (LFM). The laboratory must analyze a routine sample with a known amount of analyte added as a spike and spike duplicate, **at least once** per run, or **10%** if more than 10 samples. Calculate the percent recovery as follows:

$$R = [(C_s - C) / s] \times 100\%$$

where,

R = percent recovery

C_s = spiked sample concentration

C = unspiked sample concentration

s = concentration equivalent of analyte added to sample.

The percent recovery range should be **85 – 115%**. If the recovery of the analyte falls outside the acceptable range and the Control Chart Standard is shown to be in control, the problem is judged to be either matrix or solution related, not system related.

- 7.7 Second Source. A second source sample from a manufacturer other than that from which the calibration standards were obtained must be analyzed with each batch run, preferably immediately following daily calibration, to provide additional performance data.
- 7.8 Performance Evaluation Sample. Analyzed on an annual basis as required by ELAP.

8. TROUBLESHOOTING

- 8.1 Refer to the instrument manual for troubleshooting hints. **MANDATORY:** Note any unusual events or routine maintenance in the Maintenance Logbook.

9. DATA TRANSFER TO LIMS

- 9.1 First, make sure the correct run file is open.
- 9.2 Insert the memory stick into a USB port.
- 9.3 In **Run Properties** click on **Export Data to File** tab.
- 9.4 Continue to Aspen. Use **NH3-N File (FIA)** to import data.

SOP PROCEDURE CHANGE
FOR FIA NH3

<u>CHANGE</u>	<u>DATE</u>	<u>INITIALS</u>
Updated <i>Standard Methods</i> reference	5/23/00	JMD
Added #4 to "Procedure" requiring pre-distillation for new sample sources.	1/22/01	JMD
Made minor correction to wording in 3.2, added "prepare fresh daily" to hypochlorite reagent prep instructions, corrected pump instructions, added spike dup. requirements.	2/18/04	JMD
Revised software method operating procedures to reflect new Omnion 3.0 use differences.	06/07/05	PH
Updated method to "Low Flow" Lachat 10-107-06-1-K	7/13/05	JAB
Section 5.3 Buffer modified to reflect authorization to double volume of reagents.	09/07/2005	FC
Added more detailed information about use of new Omnion 3.0 software.	9/27/2005	JAB
Naming protocol for CC STD.	8/7/2006	JAB
In Section 6.10, added comment about only using Tygon tubing for this method.	6/6/2008	JAB
Minor changes as per JMD	3/9/2009	JAB
Added Sections 6.15.1 thru 5 about proper procedure for installing pump tubing.	4/7/09	JAB
Revised SOP to better reflect change from QuikChem 8000 to 8500. Added requirements for RDL. Added 0.1 ppm standard to calibration to meet RDL requirement.	4/27/09	JAB

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

FIA INSTRUMENT INTERFACE (LACHAT)

File Name: M:\SOP\INORGNIC\FIATransfer.doc
Revision: 2

Effective Date: 12/05/05
Supersedes: 3 (12/08/2003)

1. SUMMARY

- 1.1 After analysis is run, data will be reformatted using WinLab software. It will then be manipulated by a macro and saved to a file for transfer into Aspen. Sample data will be saved to a worksheet, while QC information will be saved to the appropriate control chart or QC worksheet; i.e. spikes and CC standards will be saved to control charts, while mdl's will be saved to mdl worksheets. No manual data entry will be necessary, and QC data will always be current and printable at any time.

2. PRE-ANALYSIS PREPARATION IN SAMPLE TRAY FILE

- 2.1 Sample ID's must be complete (11 characters); i.e. 03040545-14, not 545-14 or 03040545, etc.
- 2.2 Sample ID's for sample duplicates must end in "Q"; i.e. 03040545-14Q; **for spikes, use S; for spike duplicates, use K, for MDL standards use M, for rechecks (not to be transferred to Aspen), use R.**
- 2.3 Any samples or standards that do not need to be saved in Aspen must have an ID less than 12 characters long. This rule mainly applies to ERA standards, calibration standards and blanks.
- 2.4 Do not change the calibration, or MDL (as entered in template) and QC standard names, as they are already appropriately entered in the method.

3. CREATING INSTRUMENT FILE

- 3.1 When a run is complete, insert a disk into the disk drive of the computer.
- 3.2 From the menu at the top of the screen, select "Open" and select file to be transferred. Click on "Open". Click on the "Run Properties Icon", located at the bottom left of the monitor screen. Click on the "Run" tab and then click on "Export Data to File".

4. TRANSFERRING DATA TO ASPEN

- 4.1 Insert floppy disk into disk drive of computer. Log onto Aspen and choose "Import Data" from the main menu; the import window will appear.

- 4.2 Select the appropriate FIA File Type (FIA-NO3, FIA-NO2, FIA-NH3, FIA-ORGN, FIA-CN) to open the corresponding FIA Interface file.
- 4.3 Select "File Location," "Browse." Double click on desired file.
- 4.4 Select "Start Import", and the imported data will then appear. Select "Import File," "Continue."
- 4.5 Select "Send to Lims".
- 4.6 When the Forms dialog box appears, select "Create New WS".
- 4.7 All sample data is then sent to a new worksheet, while QC data remains on the screen. Disregard warning message ("Warning! Not all records...").
- 4.8 Select "OK" on dialog box .
- 4.9 The QC data and all other data will now be stored in the appropriate place in Aspen.
- 4.10 Return to the main menu by selecting the "Cancel" button.
- 4.11 Select "Enter Sample Results", then "Review Existing Worksheet"; open the worksheet containing the data just transferred. (It should be the last worksheet in the list.)
- 4.12 Check the data, then select "Calculate Results".
- 4.13 After calculations are complete, select "Print Inorganic Data Review".
- 5.1 Exit Aspen. When exiting the worksheet, a dialog box appears stating, "This worksheet has not been approved. Approve Now?" Select "No".
- 5.2 After the data has been reviewed by another chemist, log onto Aspen and reopen the worksheet containing that data.
- 5.3 Select "Mark Status", then mark tests approved (analysis date and analysts initials are entered automatically with the transfer to Aspen).

Exit Aspen

SOP PROCEDURE CHANGE
FOR FIA INSTRUMENT INTERFACE (LACHAT)

**CHANGE
INITIALS**

REASON

DATE _____

Revised QC Codes, Sec. 2, data transfer particulars to conform to current practice.

11/18/03

JMD

Minor changes to Sec. 4 to reflect current practice

12/08/03

JMD

Revision to Section 3, reflecting new Omnion 3 exporting of data to file.

12/05/05

PH

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

NITRATE/NITRITE (as nitrogen) by FIA INCLUDES NO₃+NO₂-N; NO₃-N; NO₃; and NO₂-N

File Name: M:\SOP\INORGNIC\FIA_NO₃+NO₂.doc
Revision: 18

Effective Date: 5/19/2009
Supersedes: 17 (4/7/2009)

1. REFERENCES

- 1.1 *Standard Methods* 20th Ed. #4500NO₃-F.
- 1.2 *EPA Methods for the Determination of Inorganic Substances in Environmental Samples* (1993), #353.2.
- 1.3 Lachat Instruments QuikChem Method 10-107-04-1-J for NO₃+NO₂-N, and Method 10-107-04-1-B for NO₂-N. This method complies with EPA-approved methodology for analysis of drinking water and wastewater.

2. HOLDING TIME

- 2.1 Best if analyzed immediately. See below for preservation methods.
- 2.2 Drinking Water: (Nitrate, non-chlorinated sample): Acidify sample to pH <2 with H₂SO₄ (approx. 2 mL conc. H₂SO₄ per liter), cool to 4°C, hold for up to 14 days. (Nitrate, chlorinated sample): Cool to 4°C, hold for up to 28 days. (Nitrite): Cool to 4°C, hold for up to 48 hours.
- 2.3 Wastewater: (Nitrate): Cool to 4°C, hold for up to 48 hours. (Nitrate + nitrite): Acidify sample to pH <2 with H₂SO₄ (approx. 2 mL conc. H₂SO₄ per liter), cool to 4°C, hold for up to 28 days. (Nitrite): Cool to 4°C, hold for up to 48 hours.

3. INTERFERENCES

- 3.1 Residual chlorine can interfere by oxidizing the cadmium column.
- 3.2 Sample turbidity may interfere. Turbidity can be removed by filtration through a 0.45µm pore diameter membrane filter prior to analysis.
- 3.3 Samples should be adjusted to room temperature prior to analysis.

4. APPARATUS

- 4.1 LACHAT QuikChem 8000 Flow Injection Analyzer.
- 4.2 Cadmium-Copper Reduction Column (Lachat Part #50237A).

5. REAGENTS

- 5.1 15N Sodium hydroxide. In a sufficiently-sized container, add 150 g NaOH very slowly to **250 mL** water. **CAUTION:** The solution will get very hot! Stir until dissolved. Then cool and store in a plastic bottle. Record in Standards/Reagents Preparation Logbook.
- 5.2 Ammonium chloride buffer, pH 8.5. In a 2-L volumetric flask, dissolve 170.0g ammonium chloride (NH_4Cl) and 2.0 g disodium ethylenediamine tetraacetic acid dihydrate ($\text{Na}_2\text{EDTA}\cdot 2\text{H}_2\text{O}$) in 1,600 mL DI water. Dilute to the mark and invert to mix. **Filter through 0.45 μm membrane filter.** Adjust the pH to 8.5 with 15N sodium hydroxide. Record in Standards/Reagents Preparation Logbook. .
- 5.3 Sulfanilamide color reagent. In a 1-L volumetric flask, add 800 mL Milli-Q water. Then add 100 mL of 85% phosphoric acid (H_3PO_4), 40.0 g sulfanilamide and 1.0 g N-(1-naphthyl)ethylenediamine dihydrochloride (NED). Stir to dissolve for 30 minutes. Dilute to the mark and mix. **Filter through 0.45 μm membrane filter.** Store in a dark bottle. This solution is stable for one month. Record in Standards/Reagents Preparation Logbook. This recipe may be doubled by adding 1600mL Milli-Q water to a 2L flask, then adding 200mL H_3PO_4 , 80.0g sulfanilamide and 2.0g NED. Stir to dissolve, then dilute to mark and mix. Filter and store up to one month.
- 5.4 Carrier. **Milli-Q** water. May need to degas.
- 5.5 Stock **nitrate** standard, 1,000 mg/L $\text{NO}_3\text{-N}$. Purchase commercially prepared, usually from SPEX..
 - 5.5.1 2nd Source: 100 mg/L $\text{NO}_3\text{-N}$. Purchase commercially prepared from a manufacturer other than that of the primary standard source, usually from Ricca.
- 5.6 Stock **nitrite** standard, 1,000 mg/L $\text{NO}_2\text{-N}$. Purchase commercially prepared, usually from SPEX..
 - 5.6.1 2nd Source: 250 mg/L $\text{NO}_2\text{-N}$. Purchase commercially prepared, usually from Ricca.

5.7 **Nitrate Nitrogen:** Prepare working standards containing 10.0; 5.0; 2.0; 1.0; 0.25; 0.1; and 0 mg/L NO₃-N as per directions in the Standards/Reagents Preparation Logbook.

5.7.1 Intermediate Standard, 50 mg/L NO₃-N. Dilute 10 mL of Stock to 200 mL with Milli-Q DI water.

5.7.2 Standard A, 10 mg/L NO₃-N. Dilute 50 mL of Intermediate Standard to 250 mL with Milli-Q DI water.

5.7.3 Standard B, 5 mg/L NO₃-N. Dilute 25 mL of Intermediate Standard to 250 mL with Milli-Q DI water.

5.7.4 Standard C, 2 mg/L NO₃-N. Dilute 10 mL of Intermediate Standard to 250 mL with Milli-Q DI water.

5.7.5 Standard D, 1 mg/L NO₃-N. Dilute 10 mL of Std A to 100 mL with Milli-Q DI water.

5.7.6 Standard E, 0.25 mg/L NO₃-N. Dilute 25 mL of Std D to 100 mL with Milli-Q DI water.

5.7.7 Standard F, 0.10 mg/L NO₃-N. Dilute 10 mL of Std D to 100 mL with Milli-Q DI water.

5.7.8 Standard G, Blank. Milli-Q DI water.

5.7.9 Control Chart Standard, 5 mg/L NO₃-N. Same as Standard B.

5.7.10 Method Detection Limit Sample (MDL), 0.1 mg/L NO₃-N. Same as Std F.

5.7.11 Reportable Detection Limit Sample (RDL), 0.1 mg/L NO₃-N. Same as F.

5.7.12 Second Source Standard, 5 mg/L NO₃-N. Dilute 5 mL of Second Source stock to 100 mL with Milli-Q DI water.

5.7.13 Spiked Sample Preparation. Add 1 mL of 100 mg/L Second Source stock to a 50 mL volumetric flask and dilute to the mark with sample. This is a 2 mg/L spike.

5.8 **Nitrite Nitrogen:** Prepare working standards containing 0.500; 0.250; 0.100; 0.050; 0.025; 0.010; 0.005; 0.002; and 0 mg/L as NO₂-N as per directions in the Standards/Reagents Preparation Logbook. **NOTE: NO₂-N working and calibration standards are only good for 24 hours (as per DHS requirements). They may be made up and used in the afternoon, and then re-used the next day providing <24 hours have elapsed.**

5.8.1 Intermediate Standard, 1.0 mg/L NO₂-N. Dilute 1.0 mL of Stock to 1,000

mL with Milli-Q DI water.

- 5.8.2 Standard A, 0.500 mg/L NO₂-N. Dilute 50 mL of Intermediate to 100 mL with Milli-Q DI water.
- 5.8.3 Standard B, 0.250 mg/L NO₂-N. Dilute 25 mL of Intermediate to 100 mL with Milli-Q DI water.
- 5.8.4 Standard C, 0.100 mg/L NO₂-N. Dilute 10 mL of Intermediate to 100 mL with Milli-Q DI water.
- 5.8.5 Standard D, 0.050 mg/L NO₂-N. Dilute 5 mL of Intermediate to 100 mL with Milli-Q DI water.
- 5.8.6 Standard E, 0.025 mg/L NO₂-N. Dilute 10 mL of Standard B to 100 mL with Milli-Q DI water.
- 5.8.7 Standard F, 0.010 mg/L NO₂-N. Dilute 10 mL of Standard C to 100 mL with Milli-Q DI water.
- 5.8.8 Standard G, 0.005 mg/L NO₂-N. Dilute 5 mL of Standard C to 100 mL with Milli-Q DI water.
- 5.8.9 Standard H, 0.002 mg/L NO₂-N. Dilute 2 mL of Standard C to 100 mL with Milli-Q DI water.
- 5.8.10 Standard I, Blank. Milli-Q DI water.
- 5.8.11 Control Chart Standard, 0.025 mg/L NO₂-N. Same as Standard E.
- 5.8.12 Method Detection Limit Sample (MDL), 0.002 mg/L NO₂-N. Same as H.
- 5.8.13 Reportable Detection Limit Sample (RDL), 0.002 mg/L NO₂-N. Same as H.
- 5.8.14 Second Source Standard, 0.025 mg/L NO₂-N. First, prepare a 2.5 mg/L Intermediate by diluting 5 mL of Second Source Stock to 500 mL. Then dilute 5 mL of Intermediate to 500 mL with Milli-Q DI water. NOTE: Save some of the 2.5 mg/L Intermediate to prepare spikes.
- 5.8.15 Spiked Sample Preparation. Add 1 mL of 2.5 mg/L Intermediate to a 50 mL volumetric flask and dilute to the mark with sample. This is a 0.05 mg/L spike.

5.9 For the **Nitrate** analysis, include a column reduction recovery check standard at the beginning of the tray, before the samples, consisting of a 5.0 mg/L Nitrite nitrogen (NO₂-N) sample. To check that the cadmium reduction column is at least 75% effective, ensure that the analyzed result of this sample is between 4.5 and 6.25 mg/L as nitrate-nitrogen. If the result is greater than 6.25, stop the analysis and replace the cadmium column.

5.9.1 Remove cadmium from exhausted columns and store cadmium waste in hazardous waste storage area.

6. **PROCEDURE: (NITRATE + NITRITE AS NITROGEN)**

- 6.1 Power up the FIA by flipping the **RED** switch on the power strip at the back of the instrument. Then press the **ON** button on the computer
- 6.2 Press **Exit** on the monitor to remove the Resolution Notifier or wait until it disappears automatically.
- 6.3 Press **Ctrl-Alt-Delete** simultaneously. At log on screen, log on using the same log on procedure as you would use for all the other computers (i.e. Username: your initials and enter your password). Then click **OK** or just press **Enter**.
- 6.4 Double Click on the **Omnion 3.0** icon.
- 6.5 Click on **Configuration** at top of screen. Then **Autosamplers**. A new screen will open. Click on **Initialize Autosampler** button. The autosampler will initialize, then go to the wash station. Close the autosampler window..
- 6.6 Click on **Open** icon. A daughter window opens on the screen to the last method that was run. If the currently displayed method is not the one you want, then press the yellow folder with the "up arrow" on it, located at the top right of the window, and you will move up one directory, to the **Methods** directory. To open the method that you wish to use, click **Method**, choose the method you wish to use, and then a recent run file. (Doing this insures that the most up to date timing values will apply to your run.)
- 6.7 The **Run Window** opens. The autosampler **Run Worksheet** window should open, but if it doesn't, click on the sample list icon at the bottom far left of the screen. Once open, begin typing your worksheet. Highlight the first available sample ID box. Type in the sample LIMS# and press **ENTER**. The cursor should now move to the next sample row below the one you just entered your data in. Sample type is **Unknown**. (**NOTE: You can not** use the arrow keys to move around or go up and down, the data will not be entered.)

- 6.8 The CC Standard is in location S15 on the autosampler. It is highlighted green in the run worksheet. The sample type is **Check Standard**. Highlight the row to verify the correct pass/fail criteria. In the **Run Properties** window, check the DQM Limits. The **Known Concentration** value should be **5.0 for NO₃-N**, or **0.025 for NO₂-N**. Next, for DQM test limits, check the box next to **>+ Conc. Limit** and enter **5.5 for NO₃-N** or **0.0275 for NO₂-N**. Move down to bottom of screen and enter messages for DQM Actions: PASS, FAIL, Continue, and Continue. Then, check the box next to **<- Conc. Limit** and enter **4.5 for NO₃-N** or **0.0225 for NO₂-N**. Again, move down to bottom of screen and enter DQM Action messages. Finally, click the lower left icon again to return to sample page. The protocol for naming the CC Std. is: **QACCxyymmddC**, where **x** denotes the CC Standard number in the run, and **yymmdd** stands for year, month and day.
- 6.9 The MDL is in location S14 on the autosampler. The sample type is **Unknown**. The MDL sample is named **MDL01yymmddM**.
- 6.10 To enter a **Spike and Spike Duplicate** in a new location, first change **Sample Type** from Unknown to **Spike and Spike Duplicate**. When you do this, three red rows appear, one for the unspiked sample, one for the spiked sample and one for the spike duplicate. Move the cursor to the **Sample No.** column and left click to highlight the spike duplicate row. Next, click on the icon located in lower left, second from left, that looks like a file card. A daughter window appears. Under **Properties** enter the known spike concentration, in this case **2.0 for NO₃-N** or **0.050 for NO₂-N**. Next, for DQM test limits, check box next to **>+ %Recovery Limit** and enter **115**. Move down to bottom and enter messages for DQM Actions: PASS, FAIL, Continue, and Continue. Then check box next to **<- %Recovery Limit** and enter **85**. Again, enter DQM Action messages. Finally, click lower left icon again to return to sample page. **NOTE:** Use "S" following the LIMS number, to indicate the sample is a spike and use "K" to indicate the sample is a spike duplicate.
- 6.11 To enter a **Duplicate**, change **Sample Type** from Unknown to **Duplicate**. This will add two orange-colored rows, the first for the unknown sample and the second for the sample duplicate. There will be **NO** DQM actions for this. Use the LIMS designation of **Q** for the sample duplicate.
- 6.12 To **Insert** or **Delete** any sample rows, first click on the **Sample No.** column. Right click. A dropdown menu will appear. Use **Delete** to remove any rows. Use **Insert** to add one row or **Insert Many** to add more than one row. The row or rows will be added immediately above the row you initially highlighted. **When adding rows, make sure to change the number of replicates to 2.**
- 6.13 Make sure all of the **Trigger OFF** and **Weight 1.0000g** boxes are checked off.

- 6.14 To renumber the sample cups, click on the first sample below the blue standards area. Click in the **Sample No.** column and click and drag the cursor to the last row. Release and the entire sample tray should turn blue. Then, without moving the mouse, right click and a dropdown menu should appear. Click on the last item **Columns ->**, then another menu appears. Click on the first item, **AutoNumber Cups**.
- 6.15 When you have the sample tray the way you want it, click on **Run** in the upper left of the screen. Then **Save as Default New Run**. Click OK. To print the sample tray worksheet, click **Run**, then **Export Worksheet Data**. It will automatically be printed at the shared printer.
- 6.16 Check the condition of all pump tubing and replace if necessary. **This method requires the use of Tygon pump tubes. DO NOT mix Tygon and Duraprene pump tubes.** Correctly set the tension on the pump tubes. The pump speed is 35. Check for any leaks or flow problems.

PROCEDURE FOR INSTALLING PUMP TUBING

- 6.16.1 Place correct pump tubing onto pump cartridges.
- 6.16.2 Move tension levers to the right-most position.
- 6.16.3 Turn the pump ON by pressing the red button to start the pump rollers rotating. Install pump cartridge by engaging one end of cartridge into cartridge holder, then clamp down other end until it snaps into place. The tension levers must always be on the left side of the pump. An arrow indented on the top of the cartridge shows the flow direction.
- 6.16.4 Move the tension lever to the left until it clicks once. The tension levers should be approximately in the 12 o'clock position.
- 6.16.5 After the analysis, release tension on the pump tubing by moving the tension levers back to the right, then releasing one side of the pump cartridge from the pump cartridge holder.

Place the reagent lines in their corresponding reagent bottles. Check for any leaks or obstructions or pulsating pump tubing.

When running NO₃+NO₂-N, make sure all reagents have reached the flow cell, and NO bubbles are present. THEN turn the blue switching valve located on the manifold to place the cadmium reduction column IN-LINE. DO NOT PUMP AIR BUBBLES OR WATER THROUGH THE COLUMN!

- 6.17 Load the sample tray with samples. The MDL sample goes in position S14, the CC Standard in position S15, and the Second Source Standard in position S16. Place the standards in the standards rack.

- 6.18 Click on the **Start** icon located at the top, fourth from left, with the green arrow.
- 6.19 Verify the correct channel at bottom of screen (Channel 2 for NO₂-N or Channel 3 for NO₃+NO₂-N). There will be an icon with the channel number on it. To view the fiagram, click on the channel icon at the bottom. The **Channel** screen will come up. The baseline will begin at the top left of the screen. When the first standard comes out, the peak will appear, pushing the baseline down. You can view the entire run by ensuring the two boxes at lower left, **Track Data** and **Auto Scale** are checked.
- 6.20 To view the calibration data, click on the icon located on the left side of the screen that looks like a calibration curve.
- 6.21 As the first peaks appear on the fiagram (or channel) window, you will notice a blue box will automatically be drawn around each peak. This is the **Peak Expectation Window**. There will also be a red peak height and baseline drawn. If this information doesn't look right, click on the run icon (now a red circle) and stop the run. Move the cursor to any white part of the screen and right click. This opens a dropdown menu. Click on **Adjust Peak Expectation Window**. A black box now appears around the peak, surrounding the blue box. Left click and the black box changes to red and the shape of the cursor changes to a left-right and up-down arrow shape. Move the cursor to the left vertical side of the box and the cursor shape changes to left-right arrow shape. Left click and drag the left side of the red box to where you want the peak detection to begin. Do the same to the right side. Then move cursor outside of box into white background area and right click. A dropdown menu will appear. Select **Rerun Peak Detection**. The box will change back to blue and be repositioned to reflect the changes you made. The red baseline will also be redrawn. If the peak detection box looks OK, click on the **Start** icon to restart the analysis. Monitor the first few standard peaks to ensure that the changes were correct. If not, repeat the above process. When the analysis is finished, save the method with the updated information. **(NOTE: Saving the method is only necessary if changes were made to the peak detection or timing parameters. Normally, changes are not required.)**
- 6.22 The minimum correlation coefficient is 0.9950, however 0.9990 or better can be easily attained.
- 6.23 When the run is finished, the run icon will switch from the red circle to the green arrow. Take the cadmium column off-line **FIRST** (when running NO₃+NO₂-N), **then** place reagent lines in DI water to flush manifold. Continue flushing for **at least five minutes**, then remove lines from water and continue pumping until dry. Release tension on pump tubes. Properly dispose of waste.
- 6.24 After the run is finished, a message will pop up on the screen: "Export File Path: Access permission to export file is denied. The file may be opened with another application." Click OK.
- 6.25 Omnion automatically saves the run and assigns a file name using the protocol

OM_M_DD_YYYY_HH_MM_SSAM(or PM).OMN. For housekeeping of the run files, click and drag the run file to the correct monthly folder for each method.

6.26 To print the run report, click on **Tools** at top in run window. Click on **Custom Report**, then the printer icon. When finished, click **Report** at the top left, then **Close**.

6.27 To transfer the run data to a memory stick, insert the memory stick into a USB port. Minimize the "Removable Disk (F:)" window. Go to the **Run Properties** window, click the **Run** tab, then click the **Export Data to File** tab.

6.28 To exit from Omnion, click on **Run** at the upper left, then **Exit**. You will be prompted "Exit Omnion?", then OK.

7. PROCEDURE: NITRITE AS NITROGEN

7.1 The nitrite procedure is identical to the nitrate + nitrite procedure except for the following:

7.1.1 Replace "nitrate" with "nitrite".

7.1.2 Nitrite is run on channel 2. There is no cadmium column for this method.

8. QUALITY CONTROL

8.1 Method Detection Limit (MDL). A MDL sample (the lowest concentration standard) must be analyzed with each batch of samples to be run. To determine MDL values, take seven replicate aliquots of reagent water spiked at a concentration of two to three times the estimated instrument detection limit, in this case the lowest standard, and process through the entire analytical method. Calculate the MDL as follows:

$$\text{MDL} = (t) \times (S)$$

where,

t = Student's t value 3.14 for seven replicates.

S = standard deviation of the replicate analyses.

- 8.2 Laboratory Reagent Blank (LRB). The laboratory must analyze at least one LRB with each batch of samples. Values that exceed the MDL indicate laboratory or reagent contamination and corrective actions must be taken before continuing the analysis.
- 8.3 Control Chart Standard. The laboratory must analyze a Control Chart Standard immediately following the daily calibration, after every tenth sample, and at the end of the sample run. Analysis of the Control Chart Standard following calibration must verify that the instrument is within "10% of calibration. Subsequent analyses of the Control Chart Standard must verify the calibration is STILL within "10%. If the Control Chart Standard is outside the limits, the sample analysis must be aborted, the cause for failure determined and/or the instrument recalibrated. All samples following the last acceptable Control Chart Standard must be reanalyzed.
- 8.4 Reportable Detection Limit (RDL). A RDL sample (lowest concentration standard) must be analyzed with each batch of samples to be run. This is to verify that the instrument can detect this value. The naming protocol for the RDL is **QARDLyymmddW**.
- 8.5 Duplicate Sample Analysis. As a minimum, run one duplicate for every ten samples in a batch, or one duplicate for a batch of less than ten samples. The relative percent difference (RPD) should be $\leq 10\%$.
- 8.6 Laboratory Fortified Sample Matrix (LFM). The laboratory must analyze a **minimum of 10% of routine samples, or one per batch of less than ten samples**, with the addition of a known amount of analyte added for a **spike and a spike duplicate**. Calculate the percent recovery as follows:

$$R = [(C_s - C) / s] \times 100\%$$

where,

R = percent recovery

C_s = spiked sample concentration

C = unspiked sample concentration

s = concentration equivalent of analyte added to sample.

The percent recovery range should be **85-115%**. If the recovery of the analyte falls outside the acceptable range and the Control Chart Standard is shown to be in control, the problem is judged to be either matrix- or solution-related, not system-related.

- 8.7 Second Source. A second source sample from a manufacturer other than that from which the primary calibration standards were obtained, must be analyzed with each batch run, preferably immediately following the calibration, to provide additional performance data.
- 8.8 For the NO₃+NO₂-N analysis, run a known nitrite-nitrogen standard (5 mg/L NO₂-N) to verify cadmium reduction column efficiency. See Section 5.9.

- 8.9 Performance Evaluation Samples. Analyzed on an annual basis as required by ELAP.

9. TROUBLESHOOTING

- 9.1 Refer to the instrument manual for troubleshooting hints. MANDATORY, record any unusual events or routine maintenance in the Maintenance Logbook.

10. REPORTING REQUIREMENTS FOR TITLE 22 SAMPLES

- 10.1 This is the procedure for reporting nitrate and nitrite results for Title 22 samples when the results exceed levels requiring an ALN. In these situations, an ALN is sent to the personal group ALNWQINO. Due to the importance of the nitrate and nitrite results to the producers, the following ALN reporting procedure was developed to meet their needs.
- 10.2 Samples that are Title 22 samples are listed in your backlog report.
- 10.3 Enter your data in **Aspen**. Once you've entered your data, click on the 'Calculate Results.' This is very important. If you do not click on this button you may not receive the following messages.
- 10.4 If a nitrate and/or nitrite result exceeds an ALN level, the following messages will appear when you enter your data. **Aspen** will send you a message box stating "Result exceeded ALN" followed by a message box stating "Lab # 98010000-00 is a Title22 Sample that requires an ALN for nitrate or nitrite. Please see your supervisor before sending this ALN."
- 10.5 Do not send an ALN until the supervisor reviews the data. If the supervisor is not available, a senior chemist can review this data.
- 10.6 Fill out a Data Quality Assurance Review report.
- 10.7 In the header section, check ALN and 24 Hr RUSH.
- 10.8 In the Abnormal data section, enter the Lab#, the Sample ID/Date, and the result.
- 10.9 Have your supervisor initial/approve this data. If the supervisor is not available, then a senior chemist can initial/approve the data.
- 10.10 Send the ALN as soon as possible.

The above steps must be completed within 24 hours after the last sample of a run is printed. For example, if you set up a run which ends at midnight, you are expected to complete the above steps before the end of the following working day. If you set up a run which ends during the working day, you are expected to complete the above steps on that day or the end of

the next working day at the latest. Weekends and holidays do not count as working days. However, if you are on sick leave or vacation, it will be your responsibility to communicate the status of your samples to the backup chemist. It will then be the responsibility of the backup chemist to complete the above steps.

11. DATA TRANSFER TO LIMS

- 11.1 First, make sure the correct run file is open.
- 11.2 Insert the memory stick into the USB port.
- 11.3 In the **Run Properties** window, click on **Export Data to File** tab.
- 11.4 Continue to Aspen. Use **NO3 File (FIA)** or **NO2-N File (FIA)**, depending on which method you are importing.

SOP PROCEDURE CHANGE
FOR FLA NO₃+NO₂ or NO₂-N

<u>CHANGE</u>	<u>DATE</u>	<u>INITIALS</u>
Added Section J - Reporting Title 22 Samples	2/4/98	MY
Added Note to Procedure, #22, p. 5	8/27/98	JAB/JMD
Added Nitrite Standard to the Procedure	7/9/99	LTL
Revised	3/6/00	CAN
Updated <i>Standard Methods</i> reference	5/23/00	JMD
Added #20 to "Procedure:" include NO ₂ -N reduction check standard	1/22/01	JMD
Added caution that NO ₂ -N working & calibration standards good for ≤ 24 hours.	10/9/03	JMD
Changed carrier from DI to Milli-Q water; added 2 nd source concs.; Various changes to Sections 6, 7, 8;	11/18/03	JMD
Revised to reflect changes to NO ₂ +NO ₃ -N method. Now using Lachat method 10-107-04-1-J. Calibration range 0 – 10 mg/L	6/23/04	JAB
Revised software method operating procedures to reflect new Omnion 3.0 use differences.	5/25/05	PH
Section 5.1 NH ₄ Cl buffer and 5.3. Color reagent modified to reflect authorization to double volume of reagents.	9/7/2005	FC
Described use of new Omnion 3.0 software in more detail.	9/21/2005	JAB
Naming protocol for CC STD.	8/7/2006	JAB
Corrected Error in number of spikes to be run, to conform to lab Protocol. 1 Spike per 10 samples not 20 samples, and any part of 10 samples.	8/23/07	PH

SOP PROCEDURE CHANGE
FOR FLA NO3+NO2 or NO2-N

<u>CHANGE</u>	<u>DATE</u>	<u>INITIALS</u>
Section 6.11. Added note about NOT mixing Tygon and Duraprene tubing.	6/6/2008	JAB

Added Section 8.4 to clarify duplicate analysis requirement.	10/15/2008	JAB

Added Sections 6.11.1 thru 5 concerning proper procedure for installing pump tubing.	4/7/2009	JAB

Added Section 6.16 about correct installation of pump tubes. Added RDL requirements.	5/19/2009	JAB

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

FIA INSTRUMENT INTERFACE (LACHAT)

File Name: M:\SOP\INORGNIC\FIATransfer.doc
Revision: 2

Effective Date: 12/05/05
Supersedes: 3 (12/08/2003)

1. SUMMARY

- 1.1 After analysis is run, data will be reformatted using WinLab software. It will then be manipulated by a macro and saved to a file for transfer into Aspen. Sample data will be saved to a worksheet, while QC information will be saved to the appropriate control chart or QC worksheet; i.e. spikes and CC standards will be saved to control charts, while mdl's will be saved to mdl worksheets. No manual data entry will be necessary, and QC data will always be current and printable at any time.

2. PRE-ANALYSIS PREPARATION IN SAMPLE TRAY FILE

- 2.1 Sample ID's must be complete (11 characters); i.e. 03040545-14, not 545-14 or 03040545, etc.
- 2.2 Sample ID's for sample duplicates must end in "Q"; i.e. 03040545-14Q; **for spikes, use S; for spike duplicates, use K, for MDL standards use M, for rechecks (not to be transferred to Aspen), use R.**
- 2.3 Any samples or standards that do not need to be saved in Aspen must have an ID less than 12 characters long. This rule mainly applies to ERA standards, calibration standards and blanks.
- 2.4 Do not change the calibration, or MDL (as entered in template) and QC standard names, as they are already appropriately entered in the method.

3. CREATING INSTRUMENT FILE

- 3.1 When a run is complete, insert a disk into the disk drive of the computer.
- 3.2 From the menu at the top of the screen, select "Open" and select file to be transferred. Click on "Open". Click on the "Run Properties Icon", located at the bottom left of the monitor screen. Click on the "Run" tab and then click on "Export Data to File".

4. TRANSFERRING DATA TO ASPEN

- 4.1 Insert floppy disk into disk drive of computer. Log onto Aspen and choose "Import Data" from the main menu; the import window will appear.

- 4.2 Select the appropriate FIA File Type (FIA-NO3, FIA-NO2, FIA-NH3, FIA-ORGN, FIA-CN) to open the corresponding FIA Interface file.
- 4.3 Select "File Location," "Browse." Double click on desired file.
- 4.4 Select "Start Import", and the imported data will then appear. Select "Import File," "Continue."
- 4.5 Select "Send to Lims".
- 4.6 When the Forms dialog box appears, select "Create New WS".
- 4.7 All sample data is then sent to a new worksheet, while QC data remains on the screen. Disregard warning message ("Warning! Not all records...").
- 4.8 Select "OK" on dialog box .
- 4.9 The QC data and all other data will now be stored in the appropriate place in Aspen.
- 4.10 Return to the main menu by selecting the "Cancel" button.
- 4.11 Select "Enter Sample Results", then "Review Existing Worksheet"; open the worksheet containing the data just transferred. (It should be the last worksheet in the list.)
- 4.12 Check the data, then select "Calculate Results".
- 4.13 After calculations are complete, select "Print Inorganic Data Review".
- 5.1 Exit Aspen. When exiting the worksheet, a dialog box appears stating, "This worksheet has not been approved. Approve Now?" Select "No".
- 5.2 After the data has been reviewed by another chemist, log onto Aspen and reopen the worksheet containing that data.
- 5.3 Select "Mark Status", then mark tests approved (analysis date and analysts initials are entered automatically with the transfer to Aspen).

Exit Aspen

**CHANGE
INITIALS**

REASON

DATE _____

11/18/03

JMD

12/08/03

JMD

12/05/05

PH

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

ORGANIC NITROGEN and TKN by FIA

File Name: M:\SOP\INORGNIC\FIA_Organic-N.doc
Revision: 15

Effective Date: 11/5/2009
Supersedes: 14 (10/8/2009)

1. REFERENCES

- 1.1 *Standard Methods* 20th Ed., #4500-N_{ORG} D.
- 1.2 EPA Method 351.2 *Determination of Total Kjeldahl Nitrogen by Semi-Automated Colorimetry*, Revision 2.0, August 1993.
- 1.3 Lachat Instruments QuikChem Method 10-107-06-2-H, *Determination of Total Kjeldahl Nitrogen by Flow Injection Analysis Colorimetry, (Copper Catalyst / Block Digestor Method)*, Revision Date, May 13, 2008. This method complies with EPA-approved methodology for analysis of drinking water and wastewater,

2. SAMPLE COLLECTION, PRESERVATION AND STORAGE

- 2.1 Samples may be collected in plastic or glass bottles. Acidify sample to pH <2 with H₂SO₄ (approx. 2 mL conc. H₂SO₄ per liter), cool to 4°C, and analyze as soon as possible after collection. If storage is required, samples are maintained at 4°C and may be held for up to 28 days.

3. INTERFERENCES

- 3.1 Samples must not consume more than 10% of the sulfuric acid during the digestion. The buffer will accommodate a range of 4.5 to 5.0% (v/v) H₂SO₄ in the diluted digestion sample with no change in signal intensity.
- 3.2 High nitrate concentrations (10x or more than the TKN level) result in low TKN values. If interference is suspected, sample should be diluted and reanalyzed.
- 3.3 Digests must be free of turbidity. Some boiling stones have been shown to crumble upon vigorous vortexing. It is recommended that all digests be centrifuged before analysis.

4. APPARATUS

- 4.1 LACHAT QuikChem 8500 Flow Injection Analyzer.
- 4.2 Block digester.

- 4.3 75 mL digestion tubes. Use non-volumetric straight tubes, available from SEAL Analytical or Environmental Express. Also use teardrop stoppers.
- 4.4 Boiling granules, Hengar Non-Selenized.
- 4.5 50 mL plastic centrifuge tubes with caps.
- 4.6 Centrifuge.

5. REAGENTS

- 5.1 Reagent water. ASTM Type II or equivalent deionized water.
- 5.2 Digestion Solution. In a 4 L beaker, dissolve 268 g of potassium sulfate (K_2SO_4) and 14.6 g copper sulfate ($CuSO_4$) in 1,600 mL DI water and stir to mix. Carefully add 268 mL of concentrated sulfuric acid (H_2SO_4). **Caution. This solution will get hot.** Allow to cool, then transfer to a 2 L volumetric flask and dilute to the mark with DI water.
Store in a warm place like incubator. If crystallization occurs, gently heat with stirring to redissolve. Record in Reagent Prep Logbook.
- 5.3 Thymolphthalein Indicator. Dissolve 0.4 g of thymol blue in 50 mL of ethanol, then add 50 mL of DI water. Record in Reagent Prep Logbook.
- 5.4 Borate Buffer. In a 1 L volumetric flask, dissolve 9.5 g of sodium tetraborate ($Na_2B_4O_7 \cdot 10H_2O$) in approximately 500 mL of DI water, then add 88 mL of 0.1N sodium hydroxide solution and dilute to mark with DI water. Record in Reagent Prep Logbook.
- 5.5 6N Sodium Hydroxide. In a 250 mL volumetric flask, dissolve 60 g of sodium hydroxide in DI water and dilute to mark. Record in Reagent Prep Logbook.
- 5.6 TKN Buffer. To a 2 L volumetric flask add 1,800 mL of DI water. Completely dissolve 70.0 g of sodium phosphate dibasic heptahydrate ($Na_2HPO_4 \cdot 7H_2O$). Next, add 40.0 g of disodium EDTA ($C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$). The EDTA will not dissolve until the sodium hydroxide has been added. Finally, add 100 g of sodium hydroxide (NaOH). Stir until dissolved. **Store in a warm place like incubator. This reagent is saturated. If a precipitate forms, the reagent must be discarded. Do not use if there are crystals in the bottom of the reagent bottle.** Record in the Reagent Prep Logbook. Prepare fresh monthly.
NOTE: THIS REAGENT MUST BE DEGASSED PRIOR TO USE.

- 5.7 Salicylate-Nitroprusside Reagent. In a 1 L volumetric flask, dissolve 150 g of sodium salicylate [salicylic acid sodium salt, $C_6H_4(OH)COONa$], and 1.0 g of sodium nitroprusside [sodium nitroferricyanide dihydrate, $Na_2Fe(CN)_5NO \cdot 2H_2O$] in about 800 mL of DI water. Dilute to mark. Store in a dark bottle and prepare fresh monthly. Record in Reagent Prep Logbook.
NOTE: DO NOT DEGAS THIS REAGENT.
- 5.9 Hypochlorite Solution. In a 500 mL volumetric flask, dilute 30 mL of regular Clorox bleach (5.25% sodium hypochlorite) to the mark with DI water. Prepare fresh prior to use. Record in Reagent Prep Logbook.
NOTE: DO NOT DEGAS THIS REAGENT.
- 5.10 0.8M Sodium Hydroxide. In a 2 L volumetric flask, dissolve 64 g of sodium hydroxide in DI water and dilute to mark. Record in Reagent Prep Logbook.
NOTE: THIS REAGENT MUST BE DEGASSED PRIOR TO USE.
- 5.11 Carrier. In a 2 L volumetric flask, dissolve 800 mL of digestion solution (see section 5.2) in about 500 mL of DI water. Dilute to the mark with DI water. The carrier is designed to match the digestion matrix. **Store in a warm place like incubator.** Record in Reagent Prep Logbook.
NOTE: THIS REAGENT MUST BE DEGASSED PRIOR TO USE.
- 5.12 Organic Nitrogen Standards and QA/QC Samples
- 5.12.1 Stock Nitrogen Standard, 250 mg/L N. In a 1 L volumetric flask, dissolve 0.9540 g of ammonium chloride (NH_4Cl) that has been dried for two hours at $110^\circ C$ in about 800 mL of DI water. Dilute to the mark and mix. **Record all standards preps in the Standards Prep Logbook.**
NOTE: IT IS ADVISABLE THAT A FRESH SET OF STANDARDS BE PREPARED AND INCLUDED WITH EACH BATCH OF SAMPLES TO BE DIGESTED.
- 5.12.2 Standard A, 5.0 mg/L N. Dilute 5 mL of the Stock Standard to 250 mL with DI water.
- 5.12.3 Standard B, 2.0 mg/L N. Dilute 2 mL of the Stock Standard to 250 mL with DI water.
- 5.12.4 Standard C, 1.0 mg/L N. Dilute 20 mL of Standard A to 100 mL with DI water.
- 5.12.5 Standard D, 0.5 mg/L N. Dilute 10 mL of Standard A to 100 mL with DI water.
- 5.12.6 Standard E, 0.25 mg/L N. Dilute 5 mL of Standard A to 100 mL with DI water.

- 5.12.7 Standard F, 0.1 mg/L N. Dilute 10 mL of Standard C to 100 mL with DI water.
- 5.12.8 Standard G, Blank. This is DI water.
- 5.12.9 Control Chart Standard, 1.0 mg/L N. Same as Standard C.
- 5.12.10 Method Detection Limit Sample (MDL), 0.1 mg/L N. Same as Standard F.
- 5.12.11 Reportable Detection Limit Sample (RDL), 0.1 mg/L N. Same as Standard F.
- 5.12.12 Second Source Standard, 1.0 mg/L N. Follow the procedures for preparing the Stock Standard and Intermediate Standards using material from a second source. Then follow as per Standard C.
- 5.12.13 Spiked Sample Preparation. Add 200 μ L of Stock Standard to a 50 mL tube and dilute to 50 mL with sample. This will make a 1 mg/L spike.

6. DIGESTION PROCEDURE - STANDARDS

- 6.1 To a clean 75 mL digestion tube, add 1 to 2 Hengar boiling granules.
IMPORTANT NOTE: CHECK TUBES FOR ANY CRACKS AND DISCARD BROKEN ONES. CRACKED TUBES EXPOSED TO HIGH HEAT CAN BE A SAFETY ISSUE.
- 6.2 Pipet 25.0 mL of standard to the digestion tube and add 10 mL of digestion solution. Place a teardrop stopper on the top of the tube. Place tubes in the tube rack.
- 6.3 Instructions for operation of the block digester are as follows:
- 6.3.1 Turn ON the block digester by pressing the ON/OFF button at the upper right corner of the controller. The home screen will be displayed, showing the current block temperature, controller model BDs, controller firmware version, and the Prgrm, Setup, and Stdby keys.
- 6.3.2 Press the RUN button. The Select Program screen displays a sketch of the temperature program for Program 1.
- 6.3.3 Press the \leftarrow or \rightarrow buttons on the numeric keypad to select the correct program, in this case, **Program 2**. Then press the $\sqrt{}$ button. The controller will beep and a red light on the block will come on, indicating the block is ON and in the heating mode.

- 6.3.4 At any time while the program is running, the operator can press the STOP button to stop the program and return to the home screen display. The program can also be paused by pressing the PAUSE button. The block will maintain the temperature at the time of pausing. Press the CONT button to continue the program.
- 6.3.5 Program 2 is for the digestion procedure. The block will be heated to 200 °C and will remain at the set temperature for one hour. Then the temperature will ramp at 4 °C per minute to 380 °C where it will be maintained for 30 minutes.
- 6.4 Place the tubes in the preheated block and start **Program 2.**
- 6.5 When Program 2 is finished, carefully lift the tube rack from the block digester and place it to the side of the block for cooling. Allow sufficient time for the tubes to cool, no more than 10 minutes, then add 25.0 mL of DI water to each tube. **Stop and wait longer if the water addition causes steaming, bubbling, or frothing reaction.**
- 6.6 Thoroughly vortex mix the standards and carefully transfer to plastic 50 mL centrifuge tubes. Cover tubes with caps to prevent ammonia contamination. Store in refrigerator until ready for analysis.
- 6.7 Before analysis, centrifuge standards for 10 minutes at **2,000 rpm (Sorvall Program #1)**. Decant supernatant to FIA autosampler tube for analysis.

7. DIGESTION PROCEDURE – SAMPLES

- 7.1 To a clean 75 mL digestion tube, add 1 to 2 Hengar boiling granules. **CHECK FOR CRACKED TUBES.**
- 7.2 Pipet 25.0 mL of sample to the digestion tube. Add 1 mL of borate buffer and two drops of thymolphthalein indicator. Then add 6N sodium hydroxide dropwise until the sample turns blue. Place tubes in the tube rack.
- 7.3 See Sections 6.3.1 thru 6.3.5 on how to operate the block digester. The exception is to use **Program 1.** Program 1 is for ammonia removal. The block is heated to 180 °C and held at that temperature for one hour, at which time the tube rack is removed to the cooling position.
- 7.4 Place the tubes in the preheated block and start **Program 1.** **NO TEARDROP STOPPERS DURING THIS STEP.**

- 7.5 When Program 1 is finished (one hour), or until sample volume is reduced by one half, carefully lift the tube rack and place it in the cooling position above the block. Allow 5 to 10 minutes for the tubes to cool, then add 10 mL of digestion solution and the teardrop stoppers and proceed with **Program 2** and Sections 6.5 thru 6.7.
- 7.6 Standards may be added to a rack containing samples at the beginning of **Program 2**. NOTE: IT IS ADVISABLE TO PREPARE A FRESH SET OF STANDARDS WITH EACH BATCH OF SAMPLES TO BE DIGESTED.

8. SYSTEM PROCEDURE

- 8.1 Turn ON computer FIRST. Then power up the FIA by pressing the red switch on the power strip at the right side of the instrument.
- 8.2 Double click on the **Omnion 3.0** icon.
- 8.3 Click on **Configuration** at top of screen, then **Autosamplers**. A new window will open. Click on box **Initialize Autosampler**. The autosampler will initialize, then go to the wash station position. Close the autosampler window.
- 8.4 Click on **Open** icon. A window opens to the last method that was run. If it is not the one you want, click the yellow folder with the up arrow at the top of the window to open the Methods directory. Choose the method you want to run, then click to open the most recent run. (Doing this insures that the most recent timing updates will apply to your run).
- 8.5 The screen is divided into three windows. **Run Worksheet** at the upper left, **Run Properties** at the upper right, and **Channel** along the bottom. You will be prompted, "**Do you want to change the setpoints of the relevant heaters?**" Answer YES, and notice the heater Temp and Setpoint indications along the lower left side of the Channel window, in this case 60°C.
- 8.6 Enter sample information in the **Run Worksheet** window. Highlight the sample ID box. Type in the sample LIMS number and press ENTER. The cursor will move to the next sample row. Sample type is **UNKNOWN**.

- 8.7 The CC Standard is in location S9 on the autosampler. It is highlighted green in the run worksheet. The sample type is **CHECK STANDARD**. Highlight the row to verify the correct pass/fail criteria. In the **Run Properties** area of the screen, check the DQM Limits. The **KNOWN CONCENTRATION** should be **1.00 mg/L**. The **>+ CONC. LIMIT** should be **1.10 mg/L** and the **<- CONC. LIMIT** should be **0.90 mg/L**. The DQM Action messages should be **PASS**, **FAIL**, **Continue**, and **Continue**. The protocol for naming the CC Standard is **10CCxyymmddC**, where x is the number of the CC Standard in the run, and yymmdd is year, month, day.
- 8.8 The MDL and RDL are in location S6 on the autosampler (**Standard F**). The sample type is **UNKNOWN**. Do not use Method Detection Limit for the sample type or the sample will be analyzed seven replicates. The MDL sample is named **MDL01yymmddM**, and the RDL sample is named **QARDLyymmddW**.
- 8.9 To enter a spike and spike duplicate, change the sample type to **SPIKE AND SPIKE DUPLICATE**. Three rows colored red will appear, one for the unspiked sample, one for the spiked sample and one for the spike duplicate. Move the cursor to the spike duplicate row and click to highlight. In the **Run Properties** area, enter the known spike concentration of **1.0 mg/L**. Check the **>+ %Recovery Limit** box and enter the upper limit of **120%**. Enter the DQM Action messages of **PASS**, **FAIL**, **Continue**, and **Continue**. Check the **<- %Recovery Limit** box and enter the lower limit of **80%**. Again, enter the DQM Action messages. Use the LIMS designations of **S** and **K** for spike and spike duplicate respectively. Then continue entering samples in the **Run Worksheet**.
- 8.10 To enter a duplicate, change the sample type to **DUPLICATE**. Two rows colored orange will appear, the first for the unknown sample, and the second for the sample duplicate. There will be no DQM.
- 8.11 To insert or delete a sample row, first highlight a sample row, then right click. A dropdown menu will appear. Use **Insert** or **Insert Many** to add rows and **Delete** to remove rows. **When adding rows, make sure to change the number of replicates to 2.**
- 8.12 Make sure all **Trigger OFF** and **Weight 1.0000g** boxes are checked.
- 8.13 To renumber the sample cups, click on the first sample row below the blue standards area. Click in the Sample No. column and drag the cursor down to the last sample row. Release and the entire sample area should turn blue. Then, without moving the cursor, right click and the dropdown menu will appear. Click on the last item, **Columns ->**, and a second dropdown menu will appear. Click on the first item, **AutoNumber Cups**.

- 8.14 To save the tray, click on **Run** in the upper left of the screen, then **Save as Default New Run**, then **OK**. To print the sample tray worksheet, click **Run**, then **Export Worksheet Data**.
- 8.15 Check the condition of all pump tubing and replace if necessary. **This is a high back-pressure method and requires the use of Tygon pump tubes. DO NOT use Duraprene pump tubes for this method. DO NOT mix Tygon and Duraprene.** Place the reagent lines in a container of DI water. Correctly set the tension on the pump tubes. The pump speed is 35. **Make sure the waste lines are at the top of the waste container and not hanging down in liquid. This can cause a backpressure flow problem.**

PROCEDURE FOR INSTALLING PUMP TUBING

- 8.15.1 Place correct pump tubing onto pump cartridges.
- 8.15.2 Move tension levers to the right-most position.
- 8.15.3 Turn the pump ON by pressing the blue Manual Run / Stop button to start the pump rollers rotating. Install pump cartridge by engaging one end of cartridge into cartridge holder, then clamp down other end until it snaps into place. The tension levers must always be on the left side of the pump. An arrow indented on the top of the cartridge shows the flow direction.
- 8.15.4 Move the tension lever to the left until it clicks once. The tension levers should be approximately in the 12 o'clock position.
- 8.15.5 After the analysis, release tension on the pump tubing by moving the tension levers back to the right, then releasing one side of the pump cartridge from the pump cartridge holder.

After pumping DI water through the manifold, place the reagent lines in their corresponding reagent bottles. **VERY IMPORTANT! MAKE SURE TO PUMP THE BUFFER FIRST! AFTER THE BUFFER REACHES THE VALVE, ADD THE OTHER REAGENTS, WITH THE SALICYLATE NITROPRUSSIDE REAGENT LAST! FAILURE TO DO THIS MAY RESULT IN A PRECIPITATE FORMING IN THE MANIFOLD TUBING!** Check for any leaks or obstructions or pulsating pump tubing.

- 8.16 Load the sample tray with samples. The MDL/RDL sample (**Standard F**) goes in position S6, the CC Standard in position S9, and the Second Source sample in position S10. Place the standards in the standards rack.
- 8.17 Click on the **Start** button located along the top of the screen.

- 8.18 View the diagram in the **Channel** window along the bottom of the screen. As the first peaks appear on the diagram, you will notice a box outlined in blue will automatically be drawn around each peak. This is the **Peak Expectation Window**. There will also be a peak height and baseline drawn in red. **IF THIS INFORMATION DOESN'T APPEAR TO BE CORRECT**, stop the run by clicking on the **Stop** button. Move the cursor to any part of the background of the screen and right-click. A dropdown menu will appear. Click on **Adjust Peak Expectation Window**. A box outlined in black will now appear around the peak, surrounding the blue outlined box. Left-click and the black box changes to red and the shape of the cursor changes to a left-right and up-down arrow shape. Move the cursor to the left vertical side of the box and the cursor shape changes to a left-right arrow. Left-click and drag the left side of the red box to where you want the peak detection to begin. Do the same for the right side, changing where you want the peak detection to end. Then move the cursor outside the box you were working on, into the background area, and right-click. The dropdown menu will appear. Select **Rerun Peak Detection**. The box will change back to blue and be repositioned to reflect the changes you made. The red baseline will also be redrawn. If the peak detection box looks OK, click on the **Start** button to restart the analysis. Monitor the first few standards to verify that the changes were correct. If not, repeat the above process. When the analysis is finished, save the method with the updated information. **(NOTE: Saving the method is only necessary if changes were made to the peak detection or timing parameters. Normally, changes are not required and you not have to do this step).**
- 8.19 The minimum correlation coefficient is 0.9950, however 0.9990 or better can be easily attained.
- 8.20 When the run has finished, a message will pop up on the screen: "Export File Path: Access permission to export file is denied. The file may be open with another application." Click OK. Also note that the **Start** button is highlighted.
- 8.21 Flush all reagent lines and manifold tubing by placing the **salicylate nitroprusside** reagent line in a container of DI water **FIRST** and the **buffer** reagent line in the water **LAST**. Continue flushing all reagent lines for **at least five minutes**, then remove all lines from the water and continue pumping until all reagent lines, manifold tubing and waste lines are dry. Release tension on the pump tubes. Properly dispose of waste.
- 8.22 Omnion automatically saves the run and assigns a file name using the protocol OM_MM_DD_YYY_HH_MM_SSAM (or PM).OMN. For housekeeping of the run files, make sure the run file is in the correct monthly folder.
- 8.23 To print the run report, go to **Tools** at the top of the screen, then **Custom Report**, then click the printer icon. When finished, click **Report** at the top left, then **Close**.

- 8.24 To transfer the run data to a memory stick, insert the memory stick in USB port. Minimize the “Removable Disk (F:)” window. Click the **Run** tab in the **Run Properties** window, then click the **Export Data to File** tab.
- 8.25 To exit from Omnion, click on **Run** at the upper left, then **Exit**. You will be prompted “Exit Omnion?”, then OK. Don’t forget to turn OFF power to the instrument.

9. QUALITY CONTROL

- 9.1 Method Detection Limit (MDL). A MDL sample (the lowest concentration standard) must be analyzed with each batch of samples to be run. When data from seven replicates has been acquired, calculate the MDL as follows:

$$\text{MDL} = (t) \times (S)$$

where,

t = Student’s t value (3.14 for seven replicates)

S = standard deviation

Run MDL sample at beginning of each run.

- 9.2 Laboratory Reagent Blank (LRB). The laboratory must analyze at least one blank with each batch of samples. Values that exceed the MDL indicate laboratory or reagent contamination and corrective actions must be taken before continuing the analysis.
- 9.3 Control Chart Standard (CC STD). The laboratory must analyze a Control Chart Standard immediately following calibration, after every tenth sample, and at the end of the sample run. Analysis of the Control Chart Standard following calibration must verify that the instrument is within $\pm 10\%$ of calibration. Subsequent analyses during the run must verify the calibration is STILL within $\pm 10\%$. If the Control Chart Standard is outside the limits, the sample analysis must be stopped, the cause for failure determined and/or the instrument recalibrated. All samples following the last acceptable Control Chart Standard must be reanalyzed.
- 9.4 Reportable Detection Limit (RDL). A RDL sample (the lowest concentration standard) must be analyzed with each batch of samples to be run. **The RDL is named QARDLyymmddW.**
- 9.5 Duplicate Samples. As a minimum, run one duplicate for every ten samples in a batch, or one duplicate for a batch of less than ten samples. The relative percent difference (RPD) should be $\leq 10\%$.

- 9.6 Laboratory Fortified Sample Matrix (LFM). The laboratory must add a known amount of analyte, for a spike and spike duplicate, to a minimum of 10% of the samples within a batch, or one for a batch of less than ten samples. Calculate the percent recovery as follows:

$$R = [(C_s - C) / s] \times 100\%$$

where,

R = percent recovery

C_s = spiked sample concentration

C = unspiked sample concentration

s = concentration equivalent of analyte added to sample

The percent recovery range should be **80% - 120%**. If the recovery of the analyte falls outside the acceptable range and the Control Chart Standard is shown to be in control, the problem is judged to be either matrix or solution related, not system related.

- 9.7 Second Source. A second source sample from a manufacturer other than that from which the calibration standards were obtained must be analyzed with each batch run, preferably immediately following the calibration, to provide additional performance data.
- 9.8 Performance Evaluation Sample. Analyzed on an annual basis as required by ELAP.

10. TROUBLESHOOTING

- 10.1 If the baseline drifts, peaks are too wide, or other problems with precision arise, clean the manifold tubing by the following procedure:
- 10.1.1 Place reagent lines in DI water a pump 2 – 5 minutes to clear any residual reagents.
 - 10.1.2 Place reagent lines in 1M hydrochloric acid (1 + 11) and pump for several minutes.
 - 10.1.3 Place reagent lines in DI water and pump for several minutes.
 - 10.1.4 Resume pumping reagents.
- 10.2 If the salicylate reagent has precipitated in the tubing, all Teflon manifold tubing **must be replaced**. Prevent this by priming system with buffer first before introducing remaining reagents to the manifold, adding the salicylate reagent line LAST.
- 10.3 Note any unusual events or routine maintenance in the Maintenance Logbook.

11. DATA TRANSFER TO LIMS

- 11.1 First, make sure the correct run file is open.
- 11.2 Insert the memory stick into the USB port.
- 11.3 In the **Run Properties** window click on **Export Data to File** tab.
- 11.4 Continue to Aspen. Use **ORG-N File (FIA)** to import data.
- 11.5 Edit analysis dates for TKN in the worksheet before reporting data to reflect the later of: a) the $\text{NH}_3\text{-N}$ analysis date, or b) the Organic Nitrogen analysis date.
- 11.6 If Total Nitrogen is to be reported, you will need to wait for the ammonia, nitrate, and nitrite values to be reported first.

SOP PROCEDURE CHANGE
FOR ORGANIC NITROGEN and TKN by FIA

<u>CHANGE</u>	<u>DATE</u>	<u>INITIALS</u>
Revised Buffer, Carrier formulas per Lachat method and added 0.8M NaOH	7/10/02	JAB
Added Spike Dup. in Sec. 9.4, TKN data handling in Sec. 11	11/18/03	JMD
Modified timing of water addition to digests, minor change to wording of 7.2, 8.2.	2/18/04	JMD
Revised software method operating procedures to reflect new Omnion 3.0 use differences.	06/07/05	PH
Section 5.2 Digestion solution; 5.4 Borate buffer and 5.6 TKN buffer modified to reflect authorization to double volume of reagents.	9/7/05	FC
Added more detailed information about use of new Omnion 3.0 software.	9/28/05	JAB
Revised carrier to match digestion matrix, per method. Use regular Hengar boiling chips. Data transfer to LIMS.	5/11/06	JAB
Naming protocol for CC STD.	8/7/06	JAB
Use of straight, non-volumetric digestion tubes and teardrop stoppers. Procedures for new block digester.	3/12/07	JAB
Added comment to Section 8.17 about using Tygon pump tubing. Also, several minor changes requested by JMD.	6/6/08	JAB
Added comments as per JMD	3/9/09	JAB
Added to Section 8.15 about correct installation of pump Tubes. Revised SOP to better reflect change from QuikChem 8000 to 8500. Added Section 9.4 about RDL	4/23/09	JAB
Removed reagent: 0.1N NaOH—not needed in method; changed centrifuge speed to 2000 rpm; corrected method opening instructions; corrected CC, MDL, 2 nd source, and RDL tray locations; other minor corrections.	10/8/09	JMD

SOP PROCEDURE CHANGE
FOR ORGANIC NITROGEN and TKN by FIA

<u>CHANGE</u>	<u>DATE</u>	<u>INITIALS</u>
Completely revised SOP based on change from Lachat Method 10-107-06-2-E to 10-107-06-2-H. This new method was designed with the use of copper as the catalyst. This new method also follows EPA 351.2 and is on the approved list in CFR40. The standards are made with ammonium chloride instead of glycine.	11/5/09	JAB

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

FIA INSTRUMENT INTERFACE (LACHAT)

File Name: M:\SOP\INORGNIC\FIATransfer.doc
Revision: 2

Effective Date: 12/05/05
Supersedes: 3 (12/08/2003)

1. SUMMARY

- 1.1 After analysis is run, data will be reformatted using WinLab software. It will then be manipulated by a macro and saved to a file for transfer into Aspen. Sample data will be saved to a worksheet, while QC information will be saved to the appropriate control chart or QC worksheet; i.e. spikes and CC standards will be saved to control charts, while mdl's will be saved to mdl worksheets. No manual data entry will be necessary, and QC data will always be current and printable at any time.

2. PRE-ANALYSIS PREPARATION IN SAMPLE TRAY FILE

- 2.1 Sample ID's must be complete (11 characters); i.e. 03040545-14, not 545-14 or 03040545, etc.
- 2.2 Sample ID's for sample duplicates must end in "Q"; i.e. 03040545-14Q; **for spikes, use S; for spike duplicates, use K, for MDL standards use M, for rechecks (not to be transferred to Aspen), use R.**
- 2.3 Any samples or standards that do not need to be saved in Aspen must have an ID less than 12 characters long. This rule mainly applies to ERA standards, calibration standards and blanks.
- 2.4 Do not change the calibration, or MDL (as entered in template) and QC standard names, as they are already appropriately entered in the method.

3. CREATING INSTRUMENT FILE

- 3.1 When a run is complete, insert a disk into the disk drive of the computer.
- 3.2 From the menu at the top of the screen, select "Open" and select file to be transferred. Click on "Open". Click on the "Run Properties Icon", located at the bottom left of the monitor screen. Click on the "Run" tab and then click on "Export Data to File".

4. TRANSFERRING DATA TO ASPEN

- 4.1 Insert floppy disk into disk drive of computer. Log onto Aspen and choose "Import Data" from the main menu; the import window will appear.

- 4.2 Select the appropriate FIA File Type (FIA-NO3, FIA-NO2, FIA-NH3, FIA-ORGN, FIA-CN) to open the corresponding FIA Interface file.
- 4.3 Select "File Location," "Browse." Double click on desired file.
- 4.4 Select "Start Import", and the imported data will then appear. Select "Import File," "Continue."
- 4.5 Select "Send to Lims".
- 4.6 When the Forms dialog box appears, select "Create New WS".
- 4.7 All sample data is then sent to a new worksheet, while QC data remains on the screen. Disregard warning message ("Warning! Not all records...").
- 4.8 Select "OK" on dialog box .
- 4.9 The QC data and all other data will now be stored in the appropriate place in Aspen.
- 4.10 Return to the main menu by selecting the "Cancel" button.
- 4.11 Select "Enter Sample Results", then "Review Existing Worksheet"; open the worksheet containing the data just transferred. (It should be the last worksheet in the list.)
- 4.12 Check the data, then select "Calculate Results".
- 4.13 After calculations are complete, select "Print Inorganic Data Review".
- 5.1 Exit Aspen. When exiting the worksheet, a dialog box appears stating, "This worksheet has not been approved. Approve Now?" Select "No".
- 5.2 After the data has been reviewed by another chemist, log onto Aspen and reopen the worksheet containing that data.
- 5.3 Select "Mark Status", then mark tests approved (analysis date and analysts initials are entered automatically with the transfer to Aspen).

Exit Aspen

**CHANGE
INITIALS**

DATE _____

11/18/03

12/08/03

12/05/05

PH

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

PERCHLORATE (DIONEX DX600 IC)

File Name: M:\SOP\INORGNIC\Perchlorate.doc
Revision: 7

Effective Date: 6/21/2007
Supersedes: 6 (6/21/2007)

1. REFERENCES

- 1.1 EPA *Determination of Perchlorate in Drinking Water Using Ion Chromatography* #314.0 (rev 1.0 [Nov. 1999])
- 1.2 *Methods for the Determination of Inorganic Substances in Environmental Samples* (1993) #300.0 (rev. 2.1)
- 1.3 Dionex DX600 Chromatography System Owner's Manuals
- 1.4 PeakNet Chromatography Software User's Guide. Reference CDHS letter to Dionex, dated April 10, 1997, H. Okamoto.
- 1.5 EPA UCMR (1999) List 1 and List 2, *Chemical Analytical Methods and Quality Control Manual*, December 2001.

2. SAMPLE COLLECTION, PRESERVATION, AND HOLDING TIME

- 2.1 Samples should be collected in plastic or glass bottles.

<u>Analyte</u>	<u>Preservation</u>	<u>Holding Time</u>
Perchlorate	None Required	28 Days

- 2.2 See Section 7 for sample pretreatment directions.
- 2.3 **If analyzing UCMR samples, total run time is limited to 30 hours and/or 20 samples per batch. Also, see Section 8 for QC requirements.**

3. REAGENTS

3.1 Regenerant: Milli- Q water.

3.2 Eluent: 120 mM NaOH

Weigh out 9.6 g Sodium Hydroxide (NaOH) and dilute to 2L with degassed milli-Q water.

DO NOT DEGAS THE ELUENT AFTER IT IS MADE.

4. STANDARD STOCK SOLUTIONS

Note: Dry all chemicals used to make the stock solutions at 105°C for 30 minutes.

Use Milli-Q water to dilute the stock solutions to the required volume.

Perchlorate (1000 mg/L): Dissolve 1.2312 g of Sodium Perchlorate (NaClO₄) to 1L. Alternately, use commercially prepared stock.

5. STANDARDS

Prepare fresh weekly.

5.1 Intermediate 500 ug/L Working Solution:

Prepare a 10 mg/L working standard solution. Pipette 10 ml of 1000 mg/l Perchlorate stock solutions into a 1L volumetric flask. Dilute to the mark with Milli-Q water. Prepare a 500 ug/L working solution. Pipette 25 ml of 10 mg/L standard solution into a 500 ml volumetric flask and dilute to the mark with milli-Q water. Do not degas the standard once it is made.

5.2 Calibration Standards - 5 Total

5.2.1 AUTOCAL5R / HIGH C.C. STD: (20 ppb)

Pipette 10 ml of 500 ug /L working solution and dilute to 250 ml with milli-Q water.

5.2.2 AUTOCAL4R (15 ppb)

Pipette 15 ml of 500 ug/L working solution (500 ppb) and dilute to 500 ml with milli-Q water.

5.2.3 AUTOCAL3R / MID C.C. STD: (10 ppb)

Pipette 10 ml of 500 ug/L working solution (500 ppb) and dilute to 500 ml with milli-Q water.

5.2.4 AUTOCAL2R / LOW C.C. STD: (4 ppb)

Pipette 8 ml of 500 ug/L working standard (500 ppb) and dilute to 1L with milli-Q water.

5.2.5 AUTOCAL1R: (2.5 ppb)

Pipette 25 ml of 20 ppb (AUTOCAL5R) and dilute to 200 ml with milli-Q water.

5.2.6 DETECTION LIMIT STANDARD: (2.0 ppb)

Pipette 20 mL of 20 ppb (AUTOCAL5R) and dilute to 200 mL with milli-Q water.

5.3 LABORATORY REAGENT BLANK (LRB): Milli-Q DI water.

5.4 LABORATORY FORTIFIED BLANK (LFB): Prepare a 4.0 ppb LFB using same stock solution (500 ppb Intermediate Std) as used to fortify Matrix Spike and Matrix Spike Duplicate. Pipette 0.4 mL of Intermediate Std (500 ppb) and dilute to 50 mL with Milli-Q DI water.

5.5 INSTRUMENT PERFORMANCE CHECK STD (IPC): Pipette 5 mL of CPI OCWD-LOW Anion Stock Standard (Contains 100 ppm F, NO₂-N, Br, PO₄-P) into a clean 500 mL volumetric flask. Add 50 mL of 1000 ppm Chloride Stock Standard. Add 50 mL of 1000 ppm Sulfate Stock Standard. Add 5 mL of 1000 ppm Nitrate-N Stock Standard. Add 20 mL of 500 ppb Intermediate ClO₄ Standard. Dilute to 500 mL with Milli-Q DI water. The final concentration should be as follows:

1 ppm	F
100 ppm	Cl
1 ppm	NO ₂ -N
1 ppm	Br
10 ppm	NO ₃ -N
1 ppm	PO ₄ -P
100 ppm	SO ₄
20 ppb	ClO ₄

Check the conductivity. It should be around 700 uS.

5.6 SECOND SOURCE STANDARD: (10 ppb)

Prepare a 10 mg/L working standard solution. Pipette 10 ml of 1000 mg/l Perchlorate stock solutions into a 1L volumetric flask. Dilute to the mark with Milli-Q water. Prepare a 500 ug/L working solution. Pipette 25 ml of 10 mg/L standard solution into a 500 ml volumetric flask and dilute to the mark with milli-Q water. Finally. Dilute 10ml of 500ppb working solution to 500ml with milli-Q water.

5.7 QC SAMPLE: Prepare and analyze a QC sample with each analysis. Order from ERA or other source. Follow manufacturers instructions for sample preparation. Concentration range is usually 10 – 20 ppb ClO₄.

6. PROCEDURE

6.1 Turn on the helium gas. (Regulator should read 100 psi at delivery end of regulator, not tank pressure part of gauge.)

6.2 Open reservoir gas valve to pressurize eluent container for perchlorate system.

- 6.3 Empty contents of Regenerate Bottles, then fill with **FRESH** milli-Q water. Pressurize the containers and adjust the regulator to deliver 10 mL/min regenerate flow, about 15 psi, for the SRS setting of 300 mA.
(**NOTE:** Recheck the pressure after the system is running, and adjust the pressure if necessary. Usually the pressure drops after startup.)
- 6.4 Turn ON DX-600 instrument modules, IP25 and CD20 for perchlorate. Then turn ON computer. (**NOTE:** If you want to run both perchlorate and chromeVI simultaneously, turn ON IP25 and AD25 for chromeVI method at the same time perchlorate modules are turned ON.)
- 6.5 The **PeakNet Main Menu** should now be on the monitor screen. Click on **Run**. Perchlorate and ChromeVI run windows appear on screen. Minimize the run window for ChromeVI.
- 6.6 Click on **File** and then click on **Load Method**. Alternatively you may also click on the **Method Icon**, located directly below the **File**. Double click on **Perchlorate** and click **OK**, or alternatively double click on **Perchlorate**.
- 6.7 Click on **Modes**. Under start, click on **Via Run Menu Command**. Then click **OK**. Program should now be load.)
- 6.8 If the pump needs priming, proceed as follows. Open the door to the IP25 isocratic pump module. On the pump control screen, press the **Left Arrow** button. The blinking cursor should be on **Remote**. Press the **Select** button once, then **Enter**. The pump should now be in **Local** mode. Open the pressure transducer waste valve (located at the top center of the pump) by turning the knob counterclockwise one to two turns. Press the **Prime** button. Allow the pump to prime until all air and previous eluent are purged and no air bubbles can be seen exiting the waste line. Press **Prime** again to return to normal flow rate and close pressure transducer waste valve. The pump is now ready for operation. **NOTE:** After priming, check the pressure reading on the **MAIN** menu screen. The reading from one stroke to the next should be within 3%. A variation of more than 3% indicates the pump is out of prime.
- 6.9 Reload the method to get the system back in the remote mode. Press **File**, **Load Method**, or press the Method Icon, highlight the method, and press **OK**. Click on **Modes**. Check to see if **Run via menu command** is selected, if not , select same and click **OK**.
- 6.10 To check for baseline stability, click on **Run**. Click on **Direct Control** (alternately you may use the icon). Click on **CD20**. Click on **Offset** and click **OK**. Click on the **Baseline icon** (3rd icon from the left). You should begin to see a baseline shortly. Check this baseline and the conductivity reading on the CD20 module, to ensure stability of the baseline (conductivity should be between 2.0 and 3.0 uS which equates with about 18 megaohms of water). The lower your baseline or conductivity, the lower your detection limit.

- 6.11 Click on **PeakNet Main Menu**, on the bottom of the monitor screen. Click on **Schedule**. Double click on Perchlorate, then TEMPLATE to open the template schedule for perchlorate. **PLEASE, use the template as it has all required QC already entered for you.** Enter samples to be analyzed, including required QC. The method is Perchlorate. In the Data File column enter date as YYMMDD. Save the schedule as YYMMDD.

Note: To change the monthly data storage subdirectory (example 0312 for December 2003) in which you must store all the data for a given month, proceed as follows:

- Click on **Start**, in lower left corner.
- Click on **PeakNet**.
- Click on **Data**.
- Click on **File**.
- Click on **New**.
- Click on **Folder**.
- Type new data storage file name.
- Press **Enter**.

- 6.12 Loading Sample Schedule to Start Run.

- Click on **File**.
- Click on **Load Schedule**.

Note: You may also click on the schedule icon, which is the second from the left, to load the schedule.

- Double click on Perchlorate, then YYMM, then choose schedule to be run.
- If **Starting line number** is on 1, for the sample number to start with (i.e. AUTOCAL1R), then go to the next step. If not, change the number to correspond to the sample you wish to start with.
- Click on **Store all data files in directory**.
- Click on **Browse**.
- Click on monthly data file you wish to store the data in (i.e. 0312 for December 2003 data storage).

Note: The path statement, for data storage, should show the following, using the above example: **c:\peaknet\data\perchlorate\yymm**

- Click on **Modes**.
- Under **Start successive runs** click on **Automatically**, then click **OK**. Status area at top of screen should now read, **Ready** and the sample ID to be run should be displayed. Go to the autosampler and press the **Run** button (first making sure that vial to be run is loaded properly). At the computer, press **Run**, then **Start** to start the perchlorate run. The instrument should now run all the samples listed in the sample schedule. You may add samples to the schedule at any time, however, you must

ensure that the schedule is closed and not open, when the analyzed sample starts to print. If the schedule is left open, the sample data for that sample will not be saved, or if it's a standard, the method calibration will not be updated.

6.13 SYSTEM NOTES:

6.13.1 Under **Perchlorate, Shutdown.met** is the shutdown method for Perchlorate. **This program only stops the pump and turns off the suppressor.** All the modular units are still powered up, as are the computer and monitor.

6.13.2 **WARNING.** Since the ASRS-II (suppressor) is operating in the external mode, the water regenerant reservoir will continue to pump water to the suppressor. **Operation of the suppressor without the eluent flowing will ruin the suppressor. As the reservoir container is pressurized, you must manually turn off the reservoir gas, by turning off the bottle's flow regulator. Also, loosen the bottle cap to ensure water doesn't back flow into the regulator.**

6.14 SYSTEM SHUTDOWN PROCEDURE:

You may include the shutdown method, **Perchlorate shutdown.met**, as the last step in the sample schedule. This will automatically shutdown perchlorate method, however, all the power is still on to the modular units (the 300 mAs to the suppressor is turned off only and the pump is turned off). You must turn off the gas supply to the regenerant bottle and release the pressure manually, to ensure that the regenerant flow is stopped. **(SEE WARNING ABOVE UNDER PARAGRAPH 6.13.2)** Shut off computer system first. Then you must manually press the ON/OFF switches to ALL modules for both systems to complete full shutdown. If the suppressor is not to be used for longer than one day, please refer to the proper attached shutdown procedure from the ASRS manual. Failure to follow these procedures will result in the baseline being extremely noisy and result in poor results.

6.15 Printing of the Calibration Curve Graph.

- Enter the **PeakNet Menu**.
- Click on **File**.
- Click on **Open Method**.
- Click on **Perchlorate** method.
- Make sure **IP25** icon is highlighted.
- Click on **Component Table** icon.
- Click on **Calibration** tab.
- Click on **Details** box.
- Click on **Print**.
- Click on **All Components**.
- Click on **OK**.

7. SAMPLE PRETREATMENT

- 7.1 Prior to analysis, EC of each sample should be measured. Refer to the chain of custody for Field EC value. Except PRADO and a few others, most samples do not require pretreatment. It is acceptable to initially analyze all samples, except those mentioned, without pretreatment, then make a determination which samples need to be reanalyzed with pretreatment. Samples containing a high concentration of sulfate (>200 ppm) will require pretreatment. Sulfate interferes with the determination of perchlorate. During analysis, if the sample conductivity is greater than 0.3 uS when the perchlorate retention time window is reached, the sample must be treated to remove sulfate interference.
- 7.2 Use Dionex *On Guard II* Barium cartridges for removal of sulfate. (Dionex Part Number 057094. Refer to Dionex *On Guard II* instruction manual for proper preparation and use of Barium cartridges). Use Dionex *On Guard Sample Prep* to prep more than one sample at a time. Initially flush the cartridge with 15 mL Milli-Q DI water and a maximum flow rate of 2 mL/minute. After flushing, add sample, discarding the first 6 mL. To determine the number of Ba cartridges required for a particular sample, use the following formula:

$$\frac{(\text{Sample EC}) \times (0.01) \times (48)}{192}$$

- 7.3 If any samples need pretreatment, QC protocol requires you to include a pretreated LRB Method Blank, pretreated 4 ppb LFB, and at least one pretreated Matrix Spike / Matrix Spike Duplicate set in your analysis batch. See Section 8.1.10.

8. QUALITY CONTROL

In order to ensure the proper precision and accuracy of reported data, the adherence to steps (1) through (10) below is mandatory. Any run not containing all of these required steps will be considered as unacceptable data, and will need to be repeated.

To verify the Matrix Conductivity Threshold (MCT) of the instrument, as part of each analysis batch you are required to prepare and analyze an Instrument Performance Check (IPC) standard. See Section 5.5 for directions on how to prepare the IPC. The IPC is to be analyzed prior to the analysis of ANY other sample, including LRB and C.C. STD. Recovery of perchlorate should be between 80 – 120% of the spike level, and the retention time should not shift more than 5%. If the IPC fails, the source of the problem must be determined and corrected before ANY sample analysis can begin.

Run LRB Method Blank.

Run 4 ppb Initial Low Level C.C. Standard. Value should be $\pm 20\%$.

Run 4 ppb LFB. Value should be $\pm 20\%$.

Run Second Source Standard.

Run a QC sample at least once a quarter.

Alternate continuing C.C. Standards as follows: Mid-Level, Low-Level, High-Level, Low-Level, Mid-Level, Low-Level, and so on. Values should be $\pm 20\%$.

Run on duplicate for every ten samples, or one duplicate per run for batches of less than ten samples. Values should agree within $\pm 10\%$.

Run a sample matrix spike and matrix spike duplicate (MS / MSD) once per 20 samples or analysis batch, whichever is more frequent, and alternate spike concentration between low-level (4 ppb) and mid-level (10 ppb). Recoveries should be between 80 – 120%. To prepare a 4 ppb spike, add 0.4 mL of 500 ppb Intermediate Standard to sample and dilute to 50 mL. To prepare a 10 ppb spike, add 1.0 mL of 500 ppb Intermediate Standard to sample and dilute to 50 mL.

If any sample matrix needs pretreatment, you are required to analyze a pretreated LRB Method Blank, a pretreated LFB (4 ppb), and at least one MS / MSD set. See Section 7.3.

Run Detection Limit Standard (2 ppb). Value should be $\pm 20\%$.

9. DATA TRANSFER TO LIMS

Laboratory Numbers and QC Codes. Use the following QC Codes when creating your sample schedule.

<u>QC Code</u>	<u>Definition</u>	<u>Example</u>
S	Spike	96080006-01S
K	Spike Duplicate	96080006-01K
Q	Sample Duplicate	96080006-01Q
M	MDL	MDCC1YYMMDDM (Example: MDLCC1050308M This would be the first MDL for March 8, 2005. For the second MDL, replace 1 with 2. For the third, replace 2 with 3, and so on.)
C	LOW 4 PPB CC	04CC1YYMMDDC
W	MID 10 PPB CC	10CC1YYMMDDW

T	HIGH 20 PPB CC	20CC1YYMMDDT
L	LFB	Doesn't transfer
F	LOW LFB	Doesn't transfer
B	Reagent Blank	RB000YYMMDDDB
R	Recheck	03060101-01R Doesn't transfer

Instrument File Generation. **(DO THIS BEFORE INSTRUMENT IS RUN AGAIN)**

To create the .csv file for use by the IC interface, go to the PeakNet main menu and click on the **BATCH** icon. From the top menu, select **File**, then **Open**. Highlight **Aspen.bch**, then click **Open**. From the top menu, select **Processing**, **Input**, **Select**, then find your schedule folder (for example 0601). Highlight your schedule, then click **Open**. (Disregard error message "Error Opening Data File...") Go to box titled **Process Injections** and subtract one from the total number of samples. (Don't transfer the shutdown sample.) Enter the correct number of samples to be transferred in the **Through** box. Click on **Export** and enter your file name (for example a:\030612.csv). Then **OK**.

Place formatted floppy disk in drive. Click on **Processing**, then **Start**. You will see your sample files being copied.

At the computer, open **Aspen**. Place disk in drive and click **Import Data**.

Select the File Type, in this case **CLO4** IC file.

Select Location – **Browse**. Highlight your data file, then click **Open**.

Start Import.

Continue. Enter analysis date and initials when prompted.

Once the import is complete, click **Send to LIMS** button and data will be sent to LIMS. Create a new worksheet when prompted.

Review the existing worksheet and check for errors.

ION CHROMATOGRAPHIC CONDITIONS

Columns: Guard Column – Dionex IonPac AG16
Separator Column – Dionex IonPac AS16

Eluent: 120 mM Sodium hydroxide

Run Time: 10 min

Flow Rate: 1.0 mL/min

Sample Volume: 750 uL

Retention Time: 9.0 min

Detection: Suppressed conductivity, ASRS-II, 300 mV, external water mode.

SOP PROCEDURE CHANGE
FOR PERCHLORATE

CHANGE	DATE	INITIALS
Moved Perchlorate to new DX600	3/1/01	JAB
Removed requirement to use degassed Milli-Q water to prepare standards and changed concentration of standards (Secs. 4&5); corrected shutdown program name and changed procedure for spiking sample in Sec. 6.	3/1/02	PH
Reemphasized requirements for UCMR samples, ie spike and spike duplicate and sample pretreatment.	11/14/03	JAB
Updated QC requirements for UCMR and sample pretreatment. Added requirement for IPC.	12/12/03	JAB
Updated SOP to reflect proper nomenclature for automated Transfer of quality control data into Aspen.	3/8/05	PH
Additional info for LIMS transfer.	01/19/06	JAB
Modified instruction for 2 nd Source standard preparation. (section 5.6)	06/21/07	SL

pH

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

pH

File Name: M:\SOP\INORGNIC\PH1.doc
Revision: 8

Effective Date: 4/3/2008
Supersedes: 7 (6/8/2007)

1. REFERENCES

- 1.1 *Standard Methods*, 20th Ed., #4500-H⁺B
- 1.2 ***Code of Federal Regulations*, Title 40, Part 136, Table II.**
- 1.3 *EPA Methods for Analysis of Water and Wastes* (1979, rev. 1983), 150.1. This method complies with EPA-approved methodology for analysis of drinking water and wastewater.

2. SUMMARY

- 2.1 Calibrate pH meter if necessary, correcting for temperature of buffer solution. Check calibration with additional buffer. Place probe in sample; allow to equilibrate; record pH reading.

3. HOLDING TIME

- 3.1 Analyze immediately (**15 minutes maximum per CFR Part 136**).

4. INTERFERENCES

- 4.1 Temperature of sample different from calibration buffer and/or significantly different from 25°C; organic matter, oily matter, soaps.

5. APPARATUS

- 5.1 pH meter: Orion EA 920 or other unit as appropriate.
- 5.2 Combination pH electrode: Epoxy body Thermo Orion Ross™ electrode.
- 5.3 Thermistor: Orion or equivalent.
- 5.4 Stir plate, magnetic stir bars, 50-mL disposable beakers.

6. PROCEDURE

- 6.1 Sample Preparation: Warm to room temperature (as close as possible to 25°C).
- 6.2 Calibration (**NOTE: Always use fresh pH buffer and discard after use.**):
- 6.2.1 **pH meter automatically detects a pH 7 buffer and will calibrate on correct temperature-compensated pH when "enter" key is pressed. Please follow the steps listed below exactly to comply with requirements of *Standard Methods*.**
 - 6.2.2 **If pH meter is on Standby (blank display), press "Function" key to activate.**
 - 6.2.2 Rinse combination electrode and thermistor with DI water **and shake off excess water or blot once gently with Kimwipe.** Move silicone rubber sleeve (if present) to uncover hole at the top of electrode. Fill electrode to level of hole with Ross electrode filling solution from squeeze bottle if necessary.
 - 6.2.3 Set disposable beaker containing pH 7 buffer and a small stir bar on stir plate. Turn on and adjust stirrer to a medium speed and lower electrode and thermistor into beaker. Ensure that stir bar does not contact electrode or thermistor.
 - 6.2.4 Press "function" key once. "STD(1)" should now appear on display. Wait for "READY" to appear on lower left of display after reading has stabilized (**should read close to pH 7**). Press "ENTER" key.
 - 6.2.5 Display should now read "STD(2)." *Standard Methods* requires calibration slope adjustment with a pH buffer "within 2 pH units of sample pH." Set disposable beaker containing pH 8 (or other pH to keep within 2 pH units of sample) buffer and a small stir bar on stir plate and lower electrode and thermistor into beaker. When "READY" appears on display, reading should have stabilized on pH 8.00. If not, use up or down arrow keys to make final adjustment to 8.00 (or other pH as appropriate). Press "ENTER" key.
 - 6.2.6 *Standard Methods* requires a check of pH meter response with a third buffer, below pH 10, approximately 3 pH units different from the second. Set disposable beaker of pH 5 buffer (or other buffer 3 pH units less than that used in 6.2.5) on stir plate and lower electrode and thermistor into beaker. **Display should now read "SAMPLE." If not, press "Function" key until it does.** The reading should be within 0.1 unit of theoretical (4.9-5.1). If it is not, see "Troubleshooting," below, particularly the portions related to electrode and potentiometer problems.
 - 6.2.7 The meter is now calibrated for the pH range of interest. Record all calibration information in pH calibration logbook. % Slope is found by pressing "function" key twice; must be within range 95-105%.

6.3 Sample pH determination:

- 6.3.1 Press "function" key until display once again reads "SAMPLE." Place disposable beaker containing sample on stir plate.
- 6.3.2 Read sample pH off display when "READY" mode appears.
- 6.3.3 Rinse the electrode and thermistor with DI water between samples as above.

6.4 Care of pH meter & electrode:

- 6.4.1 Cover combination electrode hole with silicone rubber sleeve after use.
- 6.4.2 Immerse electrode in storage solution (refer to mfr.'s instructions; usually pH 7 buffer + KCl). Mark original level of storage solution on disposable beaker and add DI water periodically to compensate for evaporation. Do not allow electrode to dry out.
- 6.4.3 When not in use, place meter in standby by holding down "standby" key until display goes blank.
- 6.4.4 **Note: Regular maintenance of electrode is very important! Refer to pH preventive maintenance logbook, mfr.'s instructions and "TROUBLESHOOTING" below.**

7. QUALITY CONTROL

- 7.1 Run one duplicate for every ten samples, or one duplicate per run for sets of less than ten samples; values should agree within 10%. ERA Performance Testing samples are used for external validation; result should be within specified acceptance limits. Analyze second source buffer weekly; must agree with theoretical within 10%. Record result in logbook.

8. TROUBLESHOOTING

The following is a list of possible causes of problems:

- 8.1 Low slope:
 - 8.1.1 Defective pH electrode
 - 8.1.2 Improper electrode conditioning
 - 8.1.3 Contamination of buffers, glassware, etc.
 - 8.1.4 Technique (not rinsing and/or stirring)
- 8.2 High slope:
 - 8.2.1 Measurement/calibration technique
 - 8.2.2 Buffer incorrect

- 8.2.3 Temperature variations
 - 8.2.4 Contamination
- 8.3 Drift:
 - 8.3.1 Membrane needs cleaning
 - 8.3.2 Reference potential unstable because: junction needs cleaning, filling solution is incorrect, or electrode is defective.
 - 8.3.3 Temperature variations
- 8.4 Erratic response:
 - 8.4.1 Irregular flow from reference junction (electrode needs cleaning)
 - 8.4.2 Electrical problems such as: improper grounding of stir plate, static, or main power supply surges.
 - 8.4.3 Defective sensing or reference electrode
- 8.5 Off-scale or overrange reading:
 - 8.5.1 Reference electrode not properly filled or junction blocked
 - 8.5.2 Electrodes not connected properly
 - 8.5.3 Defective electrode or meter
- 8.6 "Wrong answer"
 - 8.6.1 Incorrect buffer(s)
 - 8.6.2 Sample is incompatible with electrode

SOP PROCEDURE CHANGE
FOR pH

[illegible]

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

RESIDUAL CHLORINE

File Name: M:\SOP\INORGNIC\CL2.doc
Revision: 2

Effective Date: 6/2/2004
Supersedes: 1 (05/23/00)

1. REFERENCES

- 1.1 *Standard Methods*, 18th, 19th, and 20th Eds., #4500-Cl F
- 1.2 *EPA Methods for Analysis of Water and Wastes* (1979, rev. 1983), 330.4. This method complies with EPA-approved methodology for analysis of drinking water and wastewater.

2. HOLDING TIME

- 2.1 None; analyze immediately.

3. INTERFERENCES

- 3.1 **Peroxides and other oxidizing agents**, oxidized manganese, copper over approximately 10 mg/L (less than this is taken care of by EDTA in reagents); bromine, bromamine, iodine, turbidity and color.

4. APPARATUS

- 4.1 Brinkman digital buret, 50 mL. Calibrate quarterly by pumping 100 mL of titrant into a volumetric flask.

5. REAGENTS

- 5.1 DPD indicator solution: Dissolve 1.1g anhydrous DPD (N,N-Diethyl-p-phenylenediamine) sulfate in chlorine-free water containing 8ml 1+3 H₂SO₄, and 200 mg disodium EDTA. Make up to 1L and store in brown glass-stoppered bottle in the dark; discard when discolored. See *Standard Methods* for alternate formulas using DPD oxalate and DPD sulfate pentahydrate. **Also can use commercially prepared reagent. (Ricca #2655-32 or equivalent)**
- 5.2 DPD phosphate buffer: Dissolve 24 g anhydrous Na₂HPO₄ and 46 g anhydrous KH₂PO₄ in DI water. Combine with 100 ml DI water in which 800 mg EDTA have been dissolved. Make up to 1L and add 20mg HgCl₂ as preservative. **Also can use commercially prepared reagent. (Ricca #5805-32 or equivalent)**

- 5.3 Standard Ferrous Ammonium Sulfate (FAS) titrant: Dissolve 1.106 g $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ in DI water containing 1 ml 1+3 H_2SO_4 and make up to 1 L with freshly boiled and cooled DI water. Good for 1 month. Standardize FAS by combining 10 ml 1+5 H_2SO_4 , 5 ml conc H_3PO_4 , 2 ml 0.1% barium diphenylamine sulfonate indicator and 100 ml of FAS. Titrate with 0.025N potassium dichromate to a violet endpoint that persists for 30 s. The FAS titrant is equivalent to 100 μg Cl_2 / 1.00 ml. **Also can use commercially prepared reagent. Check titer on a monthly basis. (Ricca #3144-1 or equivalent)**
- 5.4 Potassium iodide, KI, crystals.
- 5.5 Chlorine-free water.

6. PROCEDURE

- 6.1 This procedure gives a direct reading (ml FAS = mg/L Cl_2) up to 5 mg/L; an aliquot should be diluted to 100ml if higher concentrations are present.
- 6.1.1 Mix 5ml buffer and 5ml DPD indicator solution in empty 250 ml erlenmeyer flask. Next add 100ml of sample. If red color develops **immediately** upon addition of sample, titrate **immediately** with FAS until color disappears (Reading A). This is free chlorine.
- 6.1.2 Add small scoop (about 1g) of KI to flask and mix to dissolve. Let stand for **exactly** 2 min and **immediately** continue titrating until red color disappears (Reading B). This is combined chlorine. **Begin titration promptly at the 2-minute mark after addition of KI, and titrate briskly to the endpoint. Do not add more titrant if color reappears after endpoint is reached.**

NOTE: High concentrations of combined chlorine can break through into the free chlorine fraction. *Standard Methods* states that the thioacetamide modification must be used when free chlorine is measured in the presence of >0.5 mg/L total chlorine. Alternatively, follow the following procedure for routine OCWD samples: 1. No free chlorine detected: follow procedure as above. 2. Free chlorine detected: repeat analysis with increasingly smaller aliquots of sample (50 mL, 25 mL, 10 mL) until no free chlorine detected, or until calculated free chlorine values of two consecutive dilutions are within $\pm 10\%$. 3. If no satisfactory result is found using #2, consult *Standard Methods* and use the thioacetamide modification.

6.2 Calculations

- 6.2.1 Total Chlorine =
(Reading A + Reading B) X Dilution Factor X Correction Factor

Note: (Correction Factor, if necessary, derived from standardization of FAS solution)

- 6.2.2 Dilution Factor =
100/number of mls of sample used. For example, if 75 mls of sample is

used instead of 100 mls, then the dilution factor is 100/75 or 1.3333.

7. QUALITY CONTROL

- 7.1 Run one duplicate for every ten samples, or one duplicate per run for sets of less than ten samples; values should agree within 10%. EPA Performance Evaluation samples are used for external validation; results should be within EPA-specified acceptance limits.
- 7.2 Run routine blanks to make sure water is chlorine-free.
- 7.3 If analyzing a drinking water sample for regulatory purposes, need to run a QC sample of known concentration to verify performance (i.e. ERA, HACH or SPEX).

8. TROUBLESHOOTING

- 8.1 If sample is added before buffer and indicator, test will not work.
- 8.2 Success also depends upon completion of procedural steps rapidly.
- 8.3 Proper pH of 6.2 to 6.5 is essential for accurate results.
- 8.4 Do not report any result obtained where the titration volume in "Procedure" #2 above is > 5 mL; both total and free chlorine results will be incorrect.

SOP PROCEDURE CHANGE
FOR RESIDUAL CHLORINE

[illegible]

**ORANGE COUNTY WATER DISTRICT
STANDARD OPERATING PROCEDURE**

RESIDUAL CHLORINE (AMPEROMETRIC AUTOTITRATOR METHOD)

File Name: M:\SOP\INORGNIC\AMPCL2 AUTO.doc
Revision: 4

Effective Date: 7/7/2008
Supersedes: 3 (11/19/2007)

1. REFERENCES

- 1.1 *Standard Methods* 20th Ed. #4500-Cl D
- 1.2 *EPA Method for Analysis of Water and Waste* (1979, rev. 1983), #330.1. This method complies with EPA-approved methodology for analysis of drinking water and wastewater.

2. HOLDING TIME

- 2.1 None; analyze immediately.

3. INTERFERENCES

- 3.1 Oxidized manganese, copper over approximately 10 mg/L, bromine, bromamine, and iodine. This method is less sensitive to interference by peroxides than the DPD titration methods, but interference from this or other oxidizing agents may occur.

4. APPARATUS

- 4.1 Brinkmann model 716 DMS Titrino autotitrator.
- 4.2 Sample changer model 748 DH with Cl₂ platinum wire electrode.
- 4.3 Plastic sample beakers, 250 mL (**use once only; do not wash**).

5. REAGENTS

- 5.1 Titrant: 0.00564N Phenylarsine Oxide (PAO), 1 mL = 0.2 mg Cl₂: Ricca Cat. # 5750-1.
- 5.2 pH 7 Phosphate Buffer (for Free Chlorine): LabChem Cat. # LC18520-1.
- 5.3 Potassium Iodide 5% w/v (for Total Chlorine): VWR Cat. # VW3458-2.
- 5.4 pH 4 Acetate Buffer (for Total Chlorine): VWR Cat. # VW3300-1.

6. PROCEDURE

Note: Greg DeMattia of Brinkmann Instruments can often provide valuable assistance.
Voicemail: 1-800-645-3050 X2472; Cellphone: 1-949-678-4347.

Note: Sample volume: Select a sample volume requiring no more than 2 ml phenylarsine oxide titrant. Thus, for chlorine concentrations of 2 mg/L or less, take a 200ml sample; for chlorine level in excess of 2 mg/L, use 100ml or proportionately less. Normally use a 200 mL sample size for free chlorine; **at present (July 2008), all GWRS samples have a consistent history of < 2 mg/L total chlorine, so you may use 200 mL sample for all.**

6.1 Startup Procedure

6.1.1 Turn 748 DH sample changer and 716 DMS Titrino Power on at power strip.

6.1.2 Turn on the computer and the printer

6.1.3 If necessary, convert the hardware configuration from pH/Alkalinity to Chlorine (**TAKE CARE NOT TO BREAK FRAGILE PARTS WHEN FOLLOWING THESE STEPS**):

6.1.3.1 Grasp the Alkalinity titrant bottle holder by the upright plastic cylinder at the **front** of the unit, pull toward yourself to disengage from the mechanism, and remove from the top of the Titrino unit.

6.1.3.2 Carefully set the Alkalinity unit on the bench top, and put the Chlorine unit in its place atop the Titrino unit, sliding it back along the top of the Titrino unit until it locks into place and the motorized lever in front of the unit automatically rotates from one side to the other. Be careful not to drop either unit, spill the titrant(s), or tangle the titration tubing or cables of the two units.

Note: Before analysis, make sure the titrant bottle is adequately filled with PAO solution.

6.1.3.3 Remove the pH combination electrode from the electrode holder (it should have been in the **4:00** position, looking down from above). Unscrew the cable connector from the electrode and place the electrode in a separate container of 3M KCl soaking solution.

6.1.3.4 Tuck the pH electrode lead into the tubing clamp, and unclamp the Cl₂ cable from the clamp, letting it hang free. Remove the platinum wire Cl₂ electrode from its holder on the **right** side of the Chlorine unit (it is OK to store this electrode dry), securely screw the cable connector into the top of the electrode, and place the electrode in the electrode holder at the **2:00** position.

6.1.4 Place DI water and samples onto the 748 sample

changer as follows:

Cups 1 and 2: DI water

Cups 3-48: Samples

Each sample needs to have two 250-mL beakers; one for free chlorine and a separate one for total chlorine. Duplicates (see “Quality Control” below) may be run on separate samples for free and total chlorine samples to avoid taking too much volume from a single sample bottle (however, all grabs for residual chlorine are now collected in gallon bottles to provide for adequate volume).

6.2 Sample Analysis

6.2.1 Logon to the computer with your initials, then press Enter.

6.2.2 Double click on **Titrimo** icon. Type your initial in user text box. Click **OK** to log on. **TWC Main Menu** window appears.

6.2.3 From the **View** menu, choose **worklists**. **TWC Worklist Manager (1)** window appears

6.2. To display the list of Macros, click the down arrow next to the **No Macro** box. Click **MAIN** from the list. You might have to scroll through the list to find it.

6.2.4 From the **Sample** menu, choose **NEW** to create the first line of the Worklist. Select a Sub Macro for the first sample line. To display the list of Sub Macros, click on the cell in the Sub Macro column. Choose **Met-Cl** from the list by scrolling through the list.

6.2.5 Click on **Add** as many as the number of samples to be run. From the **Sample** menu, choose **Line Edit Mode**. Enter “200” in “Sample Size” column (or other volume as appropriate). Type sequence numbers beginning with “1” (“1,2,3,4,” etc) in the ID#1 column. In the ID#2 column, type “DI” in the first two sample lines, then type sample ID number in Id#2 column for the remainder of the sample lines. Remember to allow two lines for each sample (and additional lines for the 10% duplicates). Type “Free” or “Total” as appropriate for each sample beaker in Id#3 column. Print out the Worklist.

Note: Samples can be added anytime during the analysis by repeating this step.

6.2.6 Dispense 1 mL of pH7 Phosphate Buffer into each beaker intended for free chlorine. Dispense **first** 1 mL of Potassium Iodide solution, **then** 1 mL of pH4 Acetate Buffer into each beaker intended for total chlorine. Remember that each sample needs **two** beakers: one for free chlorine and one for total chlorine; **this method will not** analyze both types of chlorine from the same sample beaker. The two DI water samples at the beginning of the run are

normally **Free** chlorine, **plus add one Total chlorine** DI water sample at the start of the **Total chlorine** samples.

- 6.2.5 **Before starting, reset autosampler by clicking “Changer” icon from TWC main menu window. Go to Command and choose Reset. Click Go To, then Soak. Then click “green arrow icon” to start the analysis. The electrode will move to cup #1. If the electrode doesn’t go to cup #1, stop the analysis and reset again.**

Note: Each sample Id # will be removed from the worklist when the analysis is completed.

6.3 Creating Results Table

- 6.3.1 After the analysis is completed, create results table. From **View** menu, choose **Results**. TWC Result Manager (1) appears. Select the samples you want to report by clicking sample ID one by one while you’re holding left control key. Alternatively, hold down the **Shift** key and press the **Down Arrow** key to highlight all the samples in your run.
- 6.3.2 Go to **Report**. Select **Microsoft Excel**. Execute Excel Template appears. Select **Sample of Excel Report #2- Multiple Samples.xlt**. List Builder Dialog appears. Select table column header by clicking on it and click “>>”. Recommended headers are **Sample ID, User Id, Run Date, Run Time, Units, Result1-ID, Result1 Value, Result2-ID, Result2 Value, Result3-ID, Result3 Value**. Click **OK**. Excel will create result summary table automatically. Format the table if necessary **by highlighting columns and using Text Align buttons to line up labels and results (Left Align Column 1; Center Align all other columns)**. Print your result summary table.
- 6.3.3 **Note:** Most of our samples have no free chlorine, and the result on the individual page printout will read, “missing fixed ep.” Total chlorine should be a number between 0.1 and 5.0 mg/L. Samples with total chlorine > 2.0 mg/L (these could have a yellow color when KI is added, and might read as “missing fixed ep”) **must** be diluted to less than 200 mL, with the correct volume entered into the “Sample Size” column, and reanalyzed as above.

6.4 Shutdown Procedure

- 6.4.1 Close TWC Result Manager (1) window. Go to File from TWC Worklist Manager (1) window. Choose EXIT. Exit Titrino Workcell by clicking “Opened Door” icon. **Shut down computer.**
- 6.4.2 Turn off 716 DMS Autotitrator, 748 DH Sample changer and computer. This completes the proper shutdown procedure.

7. QUALITY CONTROL

- 7.1 Run one duplicate for every ten samples or one per run for less than ten samples. If there is more than one sample, run duplicate free and total chlorine on separate samples. Values should agree within 10%. Run ERA Quality Control Performance Evaluation samples for free and total chlorine once per month for external validation. Results should be within ERA-specified acceptance limits. Analyze PT samples annually to maintain certification.

Holding time for residual chlorine: No holding time; analyze immediately.

8. TROUBLESHOOTING

- 8.1 Make sure the electrode is clean, with platinum wires in good condition. Check titrant regularly for changes in concentration or contamination.

SOP PROCEDURE CHANGE

FOR RESIDUAL CHLORINE (AMPEROMETRIC) AUTOTITRATOR

[illegible]

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

SETTLEABLE SOLIDS (Volumetric Method)

File Name: M:\SOP\INORGNIC\SETTLSL1.doc
Revision: 1

Effective Date: 12/01/1993
Supersedes: N/A

1. REFERENCES

- 1.1 *Standard Methods* 18th Ed. #2540 F, p.2-57
- 1.2 EPA *Methods for Chemical Analysis of Water and Wastes* (1979, rev. 1983) #160.5: Adapted for OCWD Lab use. This method complies with EPA-approved methodology for analysis of wastewater.

2. HOLDING TIME

- 2.1 EPA Methods state 48 hours at 4°C--however, the OCWD lab's preferred procedure is to test when sample arrives.

3. APPARATUS

- 3.1 Imhoff cone

4. PROCEDURE (volumetric)

- 4.1 Fill Imhoff cone to 1-L mark with well-mixed sample. Settle for 45 minutes, then gently stir sides of cone with a rod or by spinning. Allow to settle for another 15 minutes and record volume of settleable solids as milliliters per liter.

Note on reading volume:

If settled matter contains pockets of liquid, estimate their volume and subtract from volume of settled solids. In other words, try to estimate amount of "packed" solids. Don't count air spaces. Don't count floating material as settled matter.

Det. Limit: 0.1mL/L (though depends on sample composition)

Range of Imhoff Cone: 0.1mL/L to 40mL/L or 100mL/L. If sample goes beyond range, either dilute sample or estimate extended calibration of cone for an approximation of settleable matter. The formula for the volume of a cone is:

$$V = \frac{\pi r^2 h}{3}$$

$$\begin{aligned} \text{Circumference of section of cone} &= 2\pi r \\ \text{or } r &= C/2\pi \end{aligned}$$

CHANGE	DATE	INITIALS
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INITIALS

This image shows a single sheet of white paper with horizontal ruling lines. The lines are evenly spaced and run across the width of the page. There is no text or other markings on the paper.

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

SILICA (MOLYBDOSILICATE METHOD)

File Name: M:\SOP\INORGNIC\silica-cary.doc
Revision: 6

Effective Date: 7/13/2009
Supersedes: 5 (10/23/2008)

1. REFERENCES

- 1.1 EPA *Methods for Analysis of Water and Waste* #370.1
- 1.2 *Standard Methods*, 20th Edition #4500-SiO₂ C.

2. HOLDING TIME

- 2.1 Maximum 28 days @ 4°C in plastic container

3. INTERFERENCES

- 3.1 Color, turbidity of samples, large amount of iron and sulfide, contact with glass.

4. APPARATUS

- 4.1 Varian Cary 50-UV Spectrometer
- 4.2 Plastic 125mL Erlenmeyer flasks.
- 4.3 Plastic 1 L volumetric flasks

Note: Use plasticware rather than borosilicate glass where possible

5. REAGENTS

- 5.1 Hydrochloric Acid, HCl, 1+1.
- 5.2 Ammonium Molybdate Reagent:
Dissolve 10g of (NH₄)₆Mo₇O₂₄·4H₂O in a 100 mL volumetric flask stirring with low heat. Dilute to 100 mL with DI and filter if necessary. Then, adjust pH to 7 to 8 with silica-free NH₄OH or NaOH.
- 5.3 Oxalic Acid:
Dissolve 7.5 g of H₂C₂O₄ and dilute to 100 mL with DI.
- 5.4 Store all the silica reagents and standards in tightly stoppered plastic bottles.

6. STANDARD SOLUTIONS

- 6.1 1000mg/L silica stock standard: Ricca #6750.
- 6.2 50mg/L silica working standard: Transfer 50mL of 1000mg/L silica stock standard into a plastic 1000mL volumetric flask and dilute to the mark with recently boiled and cooled DI. Make a series of standards as follows and run a standard curve.

<u>mL of working standard solution (1 mL = 50 µg silica)</u>	<u>Concentrations of silica (mg/L) per 50 mL</u>
0.0	Blank
5.0	5.0
10.0	10.0
20.0	20.0
30.0	30.0
40.0	40.0
50.0	50.0

- 6.3 Second source standard – 1000 mg/L VWR 3461-2 (prepare working standard and dilute to one convenient working concentration as above).

7. PROCEDURE

- 7.1 Note: Make sure sipper line is taut by pushing in the lever and turning it clockwise.
- 7.1.1 Prepare two 125 mL Erlenmeyer flasks for each sample, and one for each standard in standard curve.
- 7.1.2 In one set of sample flasks, pipet 50 mL of sample and add 1.0 mL of 1+1 HCl and 2.0 mL oxalic acid and mix well. These are the sample blanks, and are not subject to the time limitations below.
- 7.1.3 Turn computer on. Type in **your normal network** User Name and Password to log in. Double click Cary WinUV icon. Double click on **Concentration**. Scroll up to Cary 50 and click OK. Go to **File**. Click on **Open Method**. Select **Silica**. Open. Click **Setup button**. Select **Samples** from menu. **Change number of samples** to numbers desired. Enter sample names. Verify wavelength = 410 nm. Click OK.
- 7.1.4 To 50.0 mL sample (or a smaller portion diluted to 50.0mL with DI) in the other set of flasks from 7.1.1, or standards, add in rapid succession 1.0 mL of 1+1 HCl and 2.0 mL ammonium molybdate reagent in a 125mL Erlenmeyer flask.
- 7.1.4 Mix well and let stand for 5 to 10 min.
- 7.1.5 Add 2.0 mL oxalic acid solution to the samples and mix well.

Click on **Zero** to run blank. Then click on **Start** to run analysis.

Read absorbance of solution at 410nm in the spectrophotometer after 2 min. but before 15 min. Each sample reading should be subtracted from its blank reading obtained from 7.1.2.

Place sipper line in standards and samples. Push on the Flush button, then push on the Start button. There is no need to push the Read button icon on the screen.

When done with analysis, you may edit results before printing. Go to **Edit**. Select **edit report** so you may be able to type in your name and enter the LIMs numbers after the readings (if not entered previously). Click on **Print** on screen (**Clear screen** would prompt you that information will not be saved). **Click OK**.

Flush out sipper line several times with DI water. Click on File to exit program. Shut down computer. Loosen sipper line by turning lever counterclockwise and then pulling it out.

7.2 For solid sample:

7.2.1 Determine the % solid of sample.

7.2.2 Weigh out about 0.7 to 1.0 gram of the dry sample into a 200mL volumetric flask.

7.2.3 Dissolve the solid in 1+1 HCl, make the volume up to 200mL.

7.2.4 Pipet 50mL into each blank (no molybdate) and sample flask. Proceed as steps 1 through 6 in Item 1 above.

7.2.5 Centrifuge each sample and blank.

7.2.6 Subtract the sample reading from its blank.

7.2.7 Calculate the % silica as follows:

$$\frac{\text{conc. obtained from Spec.}}{5(\text{weight of the dry sample})}$$

8. QUALITY CONTROL

8.1 Run one duplicate for every ten samples, or one duplicate per run for sets of less than ten samples; values should agree within 10%. Run two control chart standards of 10mg/L with run; check standards should be within 2 sigma control limits or 20%, whichever is less. Analyze second source standard at least once a month or whenever new working standard is made up. Run one spike sample (add 1.0mL of 1000ppm stock standard to 50mL of the sample, spiked concentration should be 20ppm.) each time; the spike recovery should be from 80%-120%

9. TROUBLESHOOTING

9.1 The following are the most likely sources of analytical problems:

File Name: M:\SOP\INORGANIC\silica-cary.doc
Revision: 6 (7/13/2009)

- 9.1.1 Color developing time.
- 9.1.2 Contamination from using glassware or improperly cleaned plasticware.
- 9.1.3 Appropriate concentrations of standards.

SOP PROCEDURE CHANGE
FOR SILICA (MOLYBDOSILICATE METHOD)

CHANGE	DATE	INITIALS
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Use the Varian Cary –50 spectrometer	1/20/2003	lgl
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Added run spike sample to section 8.1.	2/2/2005	FC
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Minor changes to emphasize use of plasticware and improve readability	12/9/2005	JMD
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Added spike instruction to section 8.1	3/23/2006	FC
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Changed <i>Standard Methods</i> reference	11/30/2006	JMD
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Various sequence, format, and editorial changes	10/23/2008	JMD
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Tightened spike recovery specification to 80-120%, minor editorial change to 7.1.2	7/13/2009	JMD
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ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

BACTERIOLOGICAL WATER SUITABILITY TEST

File Name: M:\SOP\INORGNIC\SUITABILITY.doc

Effective Date: 09/11/2002

Revision: 2

Supersedes: 01/01/1995

1. REFERENCES

- 1.1 Standard Methods For The Examination Of Water and Wastewater, 18th Edition, Section 9020B, 3-1, pages 9-5 to 9-7.
- 1.2 California Department of Health, Test of the Bacteriological Quality of Laboratory Water, Revised 1977

2. PROCEDURE

2.1 PREPARATION (IN OTHER WORDS – DO BEFORE STARTING DAY ONE)

2.1.1 Prepare at least five nutrient agar slants as follows:

2.1.1.1 In a 250 mL flask, rehydrate 4.6 grams of nutrient agar in 200 mL deionized water. In a double boiler, heat contents to boiling with constant stirring to completely dissolve. Sterilize agar in autoclave for 15 minutes at 121C.

2.1.1.2 Sterilize at least five 16 x 125 mm screw-cap tubes in autoclave for 30 minutes at 121C.

2.1.1.3 Aseptically dispense about 8 mL of tempered agar to each sterile tube and allow to cool in a slant position. The length of the agar slant surface should be 6.3 to 6.5 cm. This is very important. Store tubes in refrigerator after agar has solidified. Discard remaining liquid agar in trash. **DO NOT POUR DOWN SINK.**

2.1.2 Distill at least 10 liters of laboratory deionized water and store in borosilicate glass.

IMPORTANT NOTE: DO THIS SEVERAL DAYS BEFORE STARTING DAY ONE.

2.1.3 The day before starting Day One, inoculate an agar slant with *Enterobacter aerogenes** (stored in the freezer).

*This bacteria is non-pathogenic, but direct contact should be avoided since it may cause infections upon entering open wounds in the skin. Exercise caution.

2.1.3.1 Follow manufacturers instructions for preparing pure culture.

2.1.4 Thoroughly clean all glassware (refer to Equipment List, Glassware, 3.2). Rinse all glassware with distilled DI water (2.1.1). Dry sterilize in oven at 180C for 2 hours.

2.2 DAY ONE PROCEDURE

2.2.1 Inoculate a second nutrient agar slant from the revitalized stock culture. Incubate 18 to 24 hours at $35\text{C} \pm 0.5\text{C}$. This slant will have sufficient quality growth for the water quality test.

2.2.2 Using distilled deionized water, prepare phosphate buffered dilution water.

2.2.2.1 Prepare 150 mL stock phosphate buffer. Dissolve 5.1 g potassium dihydrogen phosphate, KH_2PO_4 , in 75 mL distilled deionized water. Adjust to pH 7.20 with 1 N NaOH and dilute to 150 mL. Use a sterile 250 mL screw-cap Erlenmeyer flask for this stock solution and boil for 2 minutes to kill vegetative cells. Refrigerate and store no longer than 4 weeks. Discard if solution becomes turbid.

2.2.2.2 Prepare 150 mL stock magnesium sulfate solution. Dissolve 7.5 g magnesium sulfate, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, in distilled deionized water and dilute to 150 mL. Use a sterile 250 mL Erlenmeyer flask for this solution and boil for 2 minutes. Refrigerate. Discard if solution becomes turbid.

2.2.2.3 Use 5.0 mL stock phosphate buffer and 20 mL magnesium sulfate solution diluted to 4 L with distilled deionized water. Fill 30 milk dilution bottles with 101 mL of buffered dilution water. Put screw caps on loosely and autoclave the bottles for 15 minutes at 15 psi. The final volume after cooling should be 99 mL. Fill 30 screw-cap tubes with 9.2 mL of buffered dilution water, then autoclave. The final volume should be 9.0 mL

2.2.3 Using distilled deionized water, prepare the following solutions:

2.2.3.1 Sodium Citrate Solution: Dissolve 0.087 g $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$ in 150 mL distilled deionized water.

2.2.3.2 Ammonium Sulfate Solution: Dissolve 0.180 g $(\text{NH}_4)_2\text{SO}_4$ in 150 mL distilled deionized water.

2.2.3.3 Salt mixture **excluding** iron sulfate: Dissolve 0.078 g magnesium sulfate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$); 0.075 g sodium chloride (NaCl); and 0.051 g calcium chloride ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) in 150 mL distilled deionized water.

The above three solutions are prepared in sterile 250 mL Erlenmeyer flasks with screw caps, then boiled 1 to 2 minutes to kill vegetative cells. Store in refrigerator.

2.3 DAY TWO PROCEDURE:

2.3.1 Prepare the following solutions **fresh**:

2.3.1.1 Iron sulfate solution: Dissolve 0.014 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ in 30 mL distilled deionized water.

2.3.1.2 Phosphate buffer solution, 1:25: Dilute 2.0 mL of the stock phosphate buffer with 48 mL distilled deionized water.

The above two solutions are made and boiled 1 to 2 minutes in 125 mL Erlenmeyer flasks. Cover with 50 mL beakers to prevent contamination. These solutions are not meant to be stored.

2.3.2 Boil 150 - 200 mL of the distilled deionized water (**REFEREE**) in a sterile 500 mL Erlenmeyer flask covered with a loose fitting screw cap for 1-2 minutes. Cool before using to prepare **FLASK A**.

2.3.3 Boil 150 - 200 mL of the laboratory DI water (**TEST**) in a sterile 500 mL Erlenmeyer flask covered with a loose fitting screw cap for 1-2 minutes. Cool before using to prepare **FLASK B**.

2.3.4 Aseptically prepare test solutions A and B in sterile 125 mL Erlenmeyer flasks according to the table below:

Note: Prepare 6 sets of A and 6 sets of B.

Media Reagents	FLASK A (mL) (Referee)	FLASK B (mL) (Test)
Sodium citrate solution	2.5	2.5
Ammonium sulfate solution	2.5	2.5
Salt mixture w/o FeSO_4	2.0	2.0
Ferrous sulfate solution	0.5	0.5
Phosphate buffer 1:25 (pH 7.3±0.1)	1.5	1.5
Referee water	21.0	NA
Laboratory Test water	NA	21.0
Total volume	30.0	30.0

2.3.5 Using distilled deionized water, prepare 500 mL of Plate Count Agar. In a 1 L Erlenmeyer flask, rehydrate 11.75 g of agar in 500 mL water. Heat in a

double boiler with constant mixing. Autoclave for 15 minutes at 121°C. Allow to cool to appropriate temperature in 44.5°C water bath before use.

- 2.3.6 Using the 18 to 24 hour culture of *Enterobacter aerogenes*, prepare dilutions as follows. Aseptically pipet 2 mL of sterile buffered dilution water from a 99 mL dilution bottle into the slant. Emulsify the bacteria by gently rubbing the bacterial growth with the pipet tip (**be careful not to scratch or tear the agar**). Pipet the suspension back into the 99 mL dilution bottle. Shake the stoppered dilution water bottle vigorously. Make serial dilutions according to the following:
- 2.3.6.1 Pipet 1 mL of the suspension into a second 99 mL dilution water bottle and shake vigorously. (100x)
- 2.3.6.2 Pipet 1 mL of the suspension into a third 99 mL dilution water bottle and shake vigorously. (10,000x)
- 2.3.6.3 Pipet 10 mL from the third bottle into a fourth 90 mL dilution water bottle and shake vigorously. (100,000x)
- 2.3.6.4 It may be necessary and probably a good idea to make one additional dilution. Pipet 1 mL of the suspension from the third bottle into a fifth 99 mL dilution water bottle and shake vigorously. (1,000,000x)
- 2.3.7 Pipet 0.5; 1 and 2 mL from dilution bottle four (100,000x) into three **FLASK As** and repeat for three **FLASK Bs**. Pipet 0.5; 1 and 2 mL from dilution bottle five (1,000,000x) into three **FLASK As** and repeat for three **FLASK Bs**.

Note: Do steps 2.3.6 and 2.3.7 while agar is being prepared.

- 2.3.8 Plate counts are made to verify the initial cell densities of **FLASK A** and **FLASK B**. Prepare pour plates for initial plate counts as follows: Pipet 1 mL from each **FLASK A** into triplicate sterile petri dishes labelled with the appropriate ID and dilution volume. Pipet 1 mL from each **FLASK B** into triplicate sterile petri dishes appropriately labelled. Within 20 minutes after pipeting, pour 10-15 mL of Plate Count Agar into the dishes. Carefully swirl, making a figure 8 motion, first in one direction, then in the reverse direction, for about 15 seconds, to evenly distribute the sample. Set aside and allow to cool.

Also, prepare sterility checks with 1 mL of each solution used (2.3.4). Sterility checks should be made on the media solutions, test and referee water, dilution water blank, air blank and plate count agar blank.

- 2.3.9 Invert the plates and incubate them for 24 ± 2 hrs at $35^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$.

- 2.3.10 Incubate **Flasks A** and **B** for 24 ± 2 hrs at $35^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$.

2.4 DAY THREE PROCEDURE:

- 2.4.1 Check the dilution water, media solutions, air blank, and control agar plates for contamination. **If contamination is suspected, STOP.** You will need to start testing from beginning.
- 2.4.2 Count the colonies on the initial plates using a Quebec colony counter or equivalent. Average the triplicate results for Flask A and for Flask B and record on the test form for future reference. The **acceptable** average should be 30-80 colonies per plate. Hopefully, one set of initial dilutions for **Flask A** and **Flask B** will yield a favorable count. If the count is excessively low or high, all the preceding steps should be reviewed and repeated up to this point. In the initial plate count, if there are less than 30 colonies, the test results will be erratic; and if there are greater than 80 colonies, the inhibitory effect will be lessened. A problem most typical of using plastic petri dishes is that condensation will form on the lids. Prevent this moisture from falling onto the agar surface by inverting the plates for incubation. If moisture gets onto the surface of the agar, confluent spreading growth will occur, thus rendering those plates useless.

IMPORTANT: The initial plate counts for **Flask A** and **Flask B** are important factors influencing the final test results. It is important that the initial counts for **A** and **B** be approximately equal to secure accurate data. If there is a wide difference in numbers (greater than 15 percent), the test should be repeated. Introducing a correction factor (ratio) could increase the test error dramatically. When the difference is less than 15 percent, a ratio may be used to correct for differences in the final counts:

$$\text{Corrected Final Count, B} = \frac{\text{Initial Count, A} \times \text{Final Count, B}}{\text{Initial Count, B}}$$

Past data have indicated that when the inoculum gives an initial plate count of 50 colonies per milliliter for **A** and **B**, the final plate count would be too numerous to count on the first three final dilutions (1.0, 0.1 and 0.001 mL). An initial count of 30 colonies per plate would probably exclude only the first two dilutions. An initial count of 80 would necessitate additional dilutions beyond 0.0001 mL.

- 2.4.3 Melt agar in a double boiler. Cool to 44-46°C.
- 2.4.4 Thoroughly mix the incubated **Flask A** and **Flask B** that give the acceptable number of colonies on the initial counts and prepare final plate counts. Dilutions of 1.0, 0.1, 0.01, 0.001, and 0.0001 mL are recommended by the California State Department of Health. As noted above, 5 dilutions should be used, the levels of which should vary according to the initial counts. **A range of 0.01 to 0.000001 mL may be more appropriate for all initial count densities between 30 and 80 colonies.** Make triplicate plates for each dilution of **Flask A** and **Flask B**. **Due to a 20 minute reproduction**

time for *Enterobacter aerogenes*, pour only 6 plates at a time. Pour an agar control plate, dilution water blank control plate, and air blank. Incubate all plates, inverted, for 24 ± 2 hrs. at $35^{\circ}\text{C} \pm 0.5$ C.

2.5 DAY 4 PROCEDURE:

2.5.1 Select plates from a dilution of **Flask A** and of **Flask B** that show 30 to 300 colonies in each of the three plates. Average the triplicate counts for each flask and apply the proper dilution factor to obtain the final count per milliliter. If necessary, compute the correction factor as discussed in 2.4.2.

2.5.2 Calculate the ratio as follows:

$$\frac{\text{Colony count / mL Flask B}}{\text{Colony count / mL Flask A}} = \text{Test Value}$$

2.5.3 Interpretation of results:

A ratio of from 0.8 to 1.2 (inclusive) indicates no toxic substances. A ratio of less than 0.8 indicates growth-inhibiting substances in the laboratory water sample. A ratio greater than 1.2 indicates growth-promoting substances in the laboratory water, but growth promoting substances may be ignored if the ratio is less than 3.0.

3. EQUIPMENT LIST

3.1 EQUIPMENT LIST

3.1.1 Dry Air Incubator, $35^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$.

3.1.2 Autoclave, 15 psi and 121°C .

3.1.3 Drying oven, 180°C .

3.1.4 Analytical balance.

3.1.5 Quebec Colony counter.

3.1.6 Hot plate with stirring mechanism.

3.1.7 Bunsen burner or equivalent.

3.1.8 All glass distillation apparatus.

3.1.9 Disposable inoculating loops.

3.1.10 Aluminum foil.

3.1.11 Permanent marker.

3.1.12 Plastic petri dishes.

3.2 GLASSWARE

3.2.1 Flask, Erlenmeyer, 2 L, quantity 5.

3.2.2 Flask, Erlenmeyer, 125 mL, quantity 20.

3.2.3 Beaker, 50 mL, quantity 20.

3.2.4 Flask, Screw cap, 250 mL, quantity 6.

3.2.5 Flask, Screw cap, 500 mL, quantity 4.

3.2.6 Bottle, Milk dilution, 99 mL, quantity 30.

3.2.7 Vial, Screw cap, 25 mL, (for 9 mL dilution blanks), quantity 30.

3.2.8 Vial, Screw cap, 16x125 mm, (for nutrient agar slants), quantity 10.

3.2.9 Pipet, serological, sterile, disposable, 1 mL, 5 mL, 10 mL.

3.3 CHEMICALS AND MEDIA

3.3.1 Glass distilled deionized water, at least 10 L.

3.3.2 Plate Count Agar.

3.3.3 Nutrient Agar.

3.3.4 ACS grade salts.

3.3.4.1 Potassium dihydrogen phosphate monobasic, KH_2PO_4 .

3.3.4.2 Magnesium sulfate, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$.

3.3.4.3 Sodium citrate, $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$.

3.3.4.4 Ammonium sulfate, $(\text{NH}_4)_2\text{SO}_4$.

3.3.4.5 Calcium chloride, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$.

3.3.4.6 Ferrous sulfate, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$.

3.3.4.7 Sodium chloride, NaCl .

3.3.4.8 Sodium hydroxide, NaOH , 1 N.

3.3.4.9 Pure culture of *Enterobacter aerogenes*.

SOP PROCEDURE CHANGE
FOR SUITABILITY TEST

CHANGE	DATE	INITIALS
Rewrote entire SOP based on California State Department of Health protocol.	09/11/02	JAB

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

DISSOLVED SULFIDE/TOTAL SULFIDE¹ METHYLENE BLUE COLORIMETRIC METHOD (PHOTOMETRIC METHOD)

File Name: M:\SOP\INORGANIC\SULFIDE1.doc
Revision: 1

Effective Date: 12/01/1994
Supersedes: N/A

Sulfide reacts with dimethyl-p-phenylenediamine in the presence of FeCl_3 to produce methylene blue, which is measured at 664 nm through a 1-cm cell.

1. REFERENCES

- 1.1 EPA *Methods for Analysis of Water and Waste* # 376.2.
- 1.2 *Standard Methods*, 18th, 19th, and 20th Eds. #4500-S²⁻ D; as adapted for OCWD lab use.

2. HOLDING TIME

- 2.1 (See flowchart, p. 6). Best if analyzed immediately. **Preservation:** a. For total sulfide, or dissolved if there is no suspended matter present in the sample, add 12 drops $\text{Zn}(\text{OAc})_2$ solution/300 mL sample + 6N NaOH solution (dropwise to raise pH above 9 [use pH paper to verify]; start with 6 drops and increase if necessary), stopper with no air bubbles under stopper, mix by rotating back and forth vigorously about a transverse axis for 1 minute or longer, and hold at 4°C for up to 7 days. b. For dissolved sulfide, **first** request that the sample be collected in duplicate, **next** add 12 drops NaOH to each sample bottle, **then finally** add 12 drops of AlCl_3 solution to each sample bottle. Rotate back and forth vigorously about a transverse axis for 1 minute or longer to flocculate contents. Let settle until reasonably clear supernatant can be drawn off (about 5-15 min.; do not wait longer than necessary). Combine supernatant from the duplicate bottles into a clean "BOD" bottle, filling to the top with no air bubble under the stopper. If the sample was not collected in duplicate, pour the supernatant into a clean BOD bottle and fill with DI H_2O , being careful not to aerate the sample (see below). The sample may now be analyzed directly or preserved for later analysis as in "a." above.

3. INTERFERENCES

- 3.1 Dissolved Oxygen (D.O.) -- be careful not to aerate samples **or** reagents (also, DI used for preparation of standards should not contain excess D.O.).
- 3.2 Color: each sample is analyzed against its own sample blank in the spectrophotometer to correct for this interference.

¹*If no suspended solids present, can assume dissolved sulfide equals total sulfide.

- 3.3 Turbidity, suspended solids: The OCWD lab's sulfide samples usually do not contain suspended solids. If present, and if analysis of **dissolved** sulfide is desired, flocculate and settle out the suspended matter as under "Preservation" above (using 12 drops of 6N NaOH and 12 drops of AlCl_3 solution) and analyze the clear **liquor** (supernatant).
- 3.4 Sulfite, thiosulfate, iodide and many other soluble substances can be eliminated by precipitating sulfide as ZnS, removing the supernatant containing the interfering species, and analyzing the ZnS after replacing the supernatant with DI. This procedure is also used for concentrating sulfide. If sample is suspected of containing these interferences, do not directly analyze an unpreserved sample without first adding $\text{Zn}(\text{OAc})_2$ and NaOH. See "Preservation" above and "PROCEDURE" below.

4. APPARATUS

- 4.1 UV/VIS Spectrophotometer @ 664 nm; 1-cm cell.

5. REAGENTS

- 5.1 Zinc acetate, 2N: dissolve 220 g $\text{Zn}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 2\text{H}_2\text{O}$ in 870 ml DI. Makes 1 L of solution.
- 5.2 NaOH, 6N.
- 5.3 Amine-sulfuric acid stock solution: dissolve 27 g N,N-dimethyl-*p*-phenylenediamine oxalate (Eastman cat. # 5672 or J.T. Baker are suitable—purchase fresh and discard if dark) in a cold mixture of 50 ml conc. H_2SO_4 and 20 ml DI in a 100-ml volumetric flask. Cool and dilute to mark. (Discard if dark). Store in dark glass bottle.
- 5.4 Amine-sulfuric acid reagent: dissolve 25 ml amino-sulfuric acid stock (above) with 975 ml 1+1 H_2SO_4 . Store in dark bottle. Solution should be clear.
- 5.5 Ferric chloride solution: dissolve 100 g $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in 40 ml DI.
- 5.6 Diammonium hydrogen phosphate solution: dissolve 400 g $(\text{NH}_4)_2\text{HPO}_4$ in 800 ml DI.
- 5.7 Sodium sulfide crystals: $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ (for preparation of standard curve).
- 5.8 6N Aluminum chloride $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ --dissolve contents of a previously unopened 100-g bottle in 144 ml DI.

6. PROCEDURE

- 6.1 If sample has not been previously preserved and concentration of sample or removal of interferences is desired, add 12 drops $\text{Zn}(\text{OAc})_2$ solution and 6N NaOH dropwise to pH 9 to sample. Stopper with no air bubbles under stopper, and mix by rotating back and forth vigorously about a transverse axis for 1 minute or longer. Let precipitate settle for 30 min and decant as much supernatant as possible without loss of precipitate. Refill bottle with DI water, resuspend the precipitate, and withdraw a sample. If concentration of the sample is desired, refill only partially with DI water, measure the total volume of DI water + precipitate, and withdraw a sample. When calculating the sulfide concentration, multiply the result by the ratio of final to initial sample volume.
- 6.2 Transfer 7.5 ml of sample to each of two test tubes (we use tubes of approx. 125 mm x 15 mm), labeling them A and B (B is the blank).
 - 6.2.1 To A add 0.5 ml amine-sulfuric acid reagent and 0.15 ml (3 drops) FeCl_3 . Mix immediately by inverting slowly, only once.
 - 6.2.2 To B (the blank) add 0.5 ml 1+1 H_2SO_4 and 0.15 ml FeCl_3 and mix.
- 6.3 Wait 3-5 minutes and add 1.6 ml $(\text{NH}_4)_2 \text{HPO}_4$ to each tube. Dissolve precipitate on vortex mixer.

Note: Blue color will develop in Tube A in about a minute if sulfide is present (allow additional time if initial pink color hasn't faded).
- 6.4 Wait 3-15 minutes (if zinc acetate was used, wait at least 10 minutes) and read absorbencies at 664 nm with 1-cm cell.
 - 6.4.1 Zero spectrophotometer with blank (Tube B)
 - 6.4.2 Record absorbance in Tube A.
 - 6.4.3 Determine concentration from calibration curve.

7. STANDARDIZATION

7.1 Preparing Standards:

There is no exact gravimetric method for making sulfide standards of pre-determined strength, so sulfide solutions of approximate concentrations are made up and their strengths then determined exactly using the Iodometric method. At the same time, the Methylene Blue method is used to determine absorbance of these standards so that a calibration curve of absorbance vs. concentration can be constructed.

Note: Standard Methods discusses comparing the error of the methylene blue visual method with the Iodometric method: this is strictly for determining the

concentration (standardization) of the methylene blue dye used in the visual comparison method and has nothing to do with the photometric method we are using here.

7.2 $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ solution:

Put several grams of clean, washed crystals of $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ into small beaker. Don't quite cover with water. Stir occasionally for a few minutes. Pour into another container. Solution is good for a few hours.

Add one drop of this solution to 1 L DI and mix. Immediately determine: 1) sulfide concentration by the iodometric method and 2) absorbance by the methylene blue method.

Repeat determinations by varying drops of Na_2S (or volume of water) till at least 5 standards have been run, with a range of 0.1-2.0 mg/L sulfide.

OR:

Can attempt approximation of standards ranging from 0.1 mg/L to 2.0 mg/L as follows:

7.3 1000 mg/L:

Weigh out 7.491 g $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ into a small beaker.² Don't quite cover with water. Stir occasionally for a few minutes. Pour into 1L volumetric flask and dilute to mark.

To cover the analytical range of interest usually encountered:

7.4 2 mg/L:

Dilute 2 ml 1000 mg/L to 1000 ml.

Make up standards by diluting 2 mg/L in test tubes as follows:

0.1 mg/L: 1.0 ml to 20 ml

0.5 mg/L: 5.0 ml to 20 ml

1.0 mg/L: 10 ml to 20 ml

1.5 mg/L: 15 ml to 20 ml

2.0 mg/L: no dilution

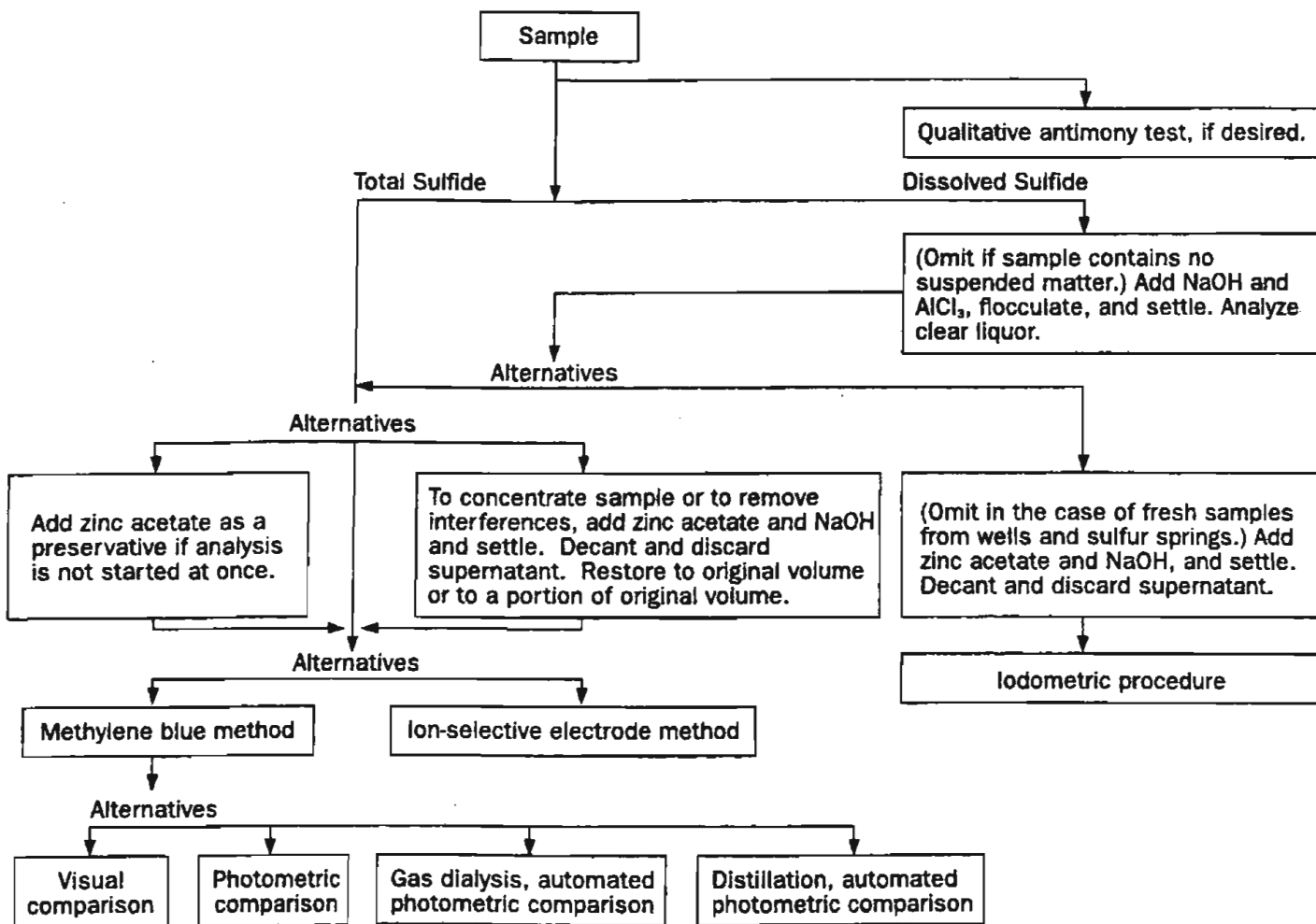
Determine absorbencies and construct a calibration curve.

8. QUALITY CONTROL

8.1 Run 10% duplicate samples (at least one duplicate per run). Readings should agree within 10%.

²** $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O} = \frac{240.176 \text{ g}}{32.06 \text{ g}} = 7.491$

- 8.2 Include one detection limit standard of 0.1 mg/L (most of our samples are at or below the detection limit).



INITIALS

[illegible]

Page 7 of 7

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

SUSPENDED SOLIDS (also known as Non-Filterable Residue)

File Name: M:\SOP\INORGNIC\SUSSOL.doc
Revision: 8

Effective Date: 6/6/2008
Supersedes: 7 (4/24/2008)

1. REFERENCES

- 1.1. *Standard Methods* 20th Ed. #2540 D and E.
- 1.2. *EPA Methods for Analysis of Water and Waste*, 160.2 and 160.4. This method complies with EPA-approved methodology for analysis of drinking water and wastewater.

2. HOLDING TIME

- 2.1. Cool to 4°C, good for 7 days.

3. INTERFERENCES

- 3.1. Filtration apparatus, filter material, pre-washing, and drying temperature are specified because these variables have been shown to affect the results.
- 3.2. Samples high in total dissolved solids (TDS, Filterable Residue), such as saline, brine, and waste waters may be subject to positive interference. Care must be taken in selecting the filtering apparatus so that washing of the filter and any dissolved solids in the filter minimizes this potential interference.
- 3.3. Non-representative particulates such as leaves, sticks, fish and lumps of fecal matter should be excluded from the sample if it is determined that their inclusion is not desired in the final result.
- 3.4. Preservation of the sample is not practical; analysis should begin as soon as possible. Refrigeration or icing to 4°C, to minimize microbiological decomposition of solids, is recommended.

4. APPARATUS

- 4.1. **Buchner funnel, 90 mm; PALL/Gelman 47 mm magnetic filter funnel; or Buchner funnel, 63mm diameter (alternate), as appropriate.**
- 4.2. **Glass microfibre filter (primary): Environmental Express "Double Weigh" 76 mm or 47 mm prewashed/preweighed (alternate: 5.5 cm diameter [Whatman 934-AH]).**
- 4.3. Suction flask, of sufficient capacity for sample size.
- 4.4. Drying oven, for operation at 103-105°C.

- 4.5. Aluminum weighing dishes (**included in primary for 4.2**).
- 4.6. Desiccator, provided with a desiccant containing a color indicator.
- 4.7. Muffle furnace, for operation at $550 \pm 50^{\circ}\text{C}$.
- 4.8. Graduated cylinder of suitable size for sample aliquot.

5. PROCEDURE

- 5.1. Preparation of glass-fiber filters:

If primary apparatus referenced above is used, skip this step; certified weight is pre-labeled on aluminum dish (just record in data notebook).

Insert filter with wrinkled side up in filtration apparatus. Apply vacuum and wash filter with three successive 20 ml portions of DI water. Continue suction to remove all traces of water and discard washings. Remove filter from filtration apparatus and transfer to an aluminum dish. Dry in an oven at 103 to 105°C for one hour (**set to 104°C and press Meas(ure) button to monitor actual oven temperature**). If volatile solids are to be measured, ignite at $550^{\circ}\text{C} (\pm 50^{\circ}\text{C})$ for 15 minutes in a muffle furnace. Cool in desiccator to balance temperature and weigh. Repeat cycle of drying or igniting, cooling, desiccating, and weighing until the weight difference is less than 4% of the previous weight or 0.5 mg, whichever is less. Store in desiccator until needed.

- 5.2. Selection of filter and sample size:

Choose sample volume to yield between 2.5 and 200 mg of dried residue, up to a maximum volume of 1 L. If more than 10 minutes are required to complete filtration, increase filter size or decrease sample volume but do not produce less than 2.5 mg residue except as noted above. For nonhomogeneous samples such as raw wastewater, use a large filter to permit filtering a representative sample. **For most samples, use 76 mm filters to allow use of maximum sample volume (up to 1 L). Use 47 mm filter only for 1-L aliquot samples with very low suspended solids (e.g., RO effluent).**

- 5.3. Assemble filtering apparatus and filter and begin suction. Wet filter with a small volume of DI water to seat it. Filter a measured volume of well-mixed sample through the glass fiber filter. Wash with three successive 10 ml volumes of DI water, allowing complete drainage between washings and continue to suction for about 3 minutes after filtration is complete. Carefully remove filter from filtration apparatus and transfer to an aluminum dish. Dry for at least one hour at 103 to 105°C in an oven (**set to 104°C and press Meas(ure) button to monitor actual oven temperature**), cool in a desiccator to balance temperature, and weigh. Repeat the cycle of drying, cooling, desiccating, and weighing of the residue until the weight difference is less than 4% of the previous weight or 0.5 mg, whichever is less.

Calculation:

$$\text{mg total suspended solids/L} = \frac{(A - B) \times 1,000}{\text{sample volume, ml}}$$

where: A = weight of filter plus dried residue, mg; and
B = weight of filter, mg.

6. QUALITY CONTROL

- 6.1. Run one duplicate for every 10 samples or one duplicate per run for sets less than 10 samples; values should agree within 5% of their average. EPA Performance Evaluation samples are used for external validation. Results should be within EPA specified acceptance limits.

7. TROUBLESHOOTING

- 7.1. If EPA-Performance Evaluation samples are not within acceptable limits, reevaluate techniques for possible errors. Also, if duplicates do not agree within 5% of their average, reevaluate technique for possible errors.

SOP PROCEDURE CHANGE
FOR SUSPENDED SOLIDS

CHANGE	DATE	INITIALS
Revised sections E.1 and 3 to reflect Standard Methods 18 th Ed., Section 2540D.3c.	01/10/2001	JAB
Updated <i>Standard Methods</i> reference; added references to alternate prewashed/preweighed glass fiber filters in Secs. 4 & 5; revised Sec. 5.3; corrected "Calculation" in Sec. 5 and removed Note.	3/11/2002	JMD
Revised sections 5.2; 6.1 and 7.1 to reflect Standard Methods 18 th Ed., Section 2540D.3b and 3c.	1/29/2003	FC
Clarified above and added maximum volume of 1 L	2/21/03	JMD
Removed reference to 18 th and 19 th Editions of Std. Methods; decreased minimum residue from 10 to 2.5 mg	1/19/06	JMD
Added reference to 76 mm Double Weigh filters and associated equipment	12/8/2006	JMD
Minor editorial changes	4/24/2008	JMD
Added reminder to press Meas button on oven to monitor actual oven temperature	6/6/2008	JMD

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

TOTAL DISSOLVED SOLIDS (TDS) (also known as Total Filterable Residue)

File Name: M:\SOP\INORGNIC\TDS.doc

Revision: 12

Effective Date: 10/19/2009

Supersedes: 11 (6/9/2009)

1. REFERENCES

- 1.1 *Standard Methods*, 20th Ed., #2540C
- 1.2 This method complies with EPA-approved methodology for analysis of drinking water and wastewater.

2. SUMMARY

- 2.1 Filter well-mixed sample through glass fiber filter. Dry measured volume of filtrate in weighed evaporating dish on water bath. Transfer to oven and dry to constant weight for at least 1 hour at 180 °C. Weigh cooled dish. Repeat cycle of drying, cooling, and weighing until weight change is less than 4% of previous weight or **less than 0.5 mg**, whichever is less. TDS = weight of residue divided by volume of sample (in mg/L).

3. HOLDING TIME

- 3.1 7 days @ 4 °C.

4. INTERFERENCES

- 4.1 **Balance not level (bubble not centered in level indicator) has been shown to affect results; always check that balance is level before every series of weighings.**
- 4.2 Too much residue in dish will entrap water during drying. Therefore, limit sample size to insure that no more than 200 mg residue is left after drying (e.g., if 50 mL sample, max concentration should be 4000 mg/L: use 25 mL sample for TDS expected to be from 4000 to 8000 mg/L, etc.

Example: Use 25 ml sample for **GWRS***-ROC** (historical average between 4000-8000 mg/L).
- 4.3 Too little residue in the dish can lead to imprecise results due to experimental variation. **If less than 2.5 mg of residue is left after drying, double the sample size and repeat. If more volume is needed than the evaporating dish can hold**

at one time, reserve the balance of filtered sample (up to a total of 1 L), and add to the dish in stages as the liquid evaporates. Use 200 mL for all GWRs*-FPW, GWRs***-UVP, and GWRs***-ROP samples.**

- 4.4 Highly mineralized water containing considerable Ca, Mg, Cl or SO₄ might be hygroscopic and it may need additional drying and rapid weighing.
- 4.5 Samples high in HCO₃ may need additional drying (so that all HCO₃ is converted to CO₃).

5. APPARATUS

- 5.1 Glass fiber filter discs: Whatman 934-AH **or equivalent** (5.5 cm size preferred).
- 5.2 Filtration apparatus.
- 5.3 Buchner funnel: OCWD Main Lab uses ~50ml size with above referenced discs.
- 5.4 Water bath, drying oven, and desiccator.
- 5.5 100-200 mL porcelain evaporating dishes.

6. PROCEDURE

- 6.1 Preparation:
 - 6.1.1 Store a supply of dishes (previously dried at 180 °C for 1 hour) in desiccator.
 - 6.1.2 **Check bubble level on balance before beginning any series of weighings, and adjust if bubble not centered in circle.**
 - 6.1.3 Weigh evaporating dishes and place on water bath.
 - 6.1.4 Wash filter (wrinkled side up) on filtration apparatus using three successive 20 mL portions of DI. Apply suction until dry. Discard washings.
- 6.2 Filter well-mixed sample through filter until sufficient sample plus 10-20ml excess is collected. Sample volume: usually 50 mL. **If necessary, adjust volume to keep within range of 2.5 to 200 mg dried residue in dish, up to a maximum of 1 L. If more than 10 min are required to complete filtration, increase filter size or decrease sample volume.**
- 6.3 Transfer measured volume of filtrate to weighed dish using Class "A" volumetric pipet and evaporate to dryness on boiling water bath.
- 6.4 Dry dish in oven for **at least one hour** at 180 ± 2 °C. **Per CDPH, it is OK to leave dishes in oven overnight.**

6.5 Cool in desiccator and weigh. Residue must be dried to a constant weight by repeated drying, cooling, desiccating and weighing of the residue until the weight difference is less than 4% of the previous weight or **less than 0.5 mg**, whichever is less.

6.6
$$\text{TDS (mg/L)} = \frac{\text{Final weight of dish (mg)} - \text{Initial weight of dish (mg)} \times 1,000}{\text{volume of filtrate used (mL)}}$$

7. QUALITY CONTROL

7.1 Run one duplicate for every **ten** samples, or one duplicate per run for sets of less than ten samples; **values should agree to within 5% of their average weight.**

7.2 Include a PT or other external standard once per month with sample run. If out of acceptable range (as given by EPA) or **if duplicates are off by more than 5% of their average weight**, review technique and rerun a standard. If still beyond acceptable range, review procedure and consider **all** potential sources of error (e.g., **balance not level**, constant weight of dried residue, cleanliness of dishes and/or glassware, condition of desiccator and desiccant, and/or sources of contamination that may be present in the laboratory such as dust).

SOP PROCEDURE CHANGE
FOR TOTAL DISSOLVED SOLIDS

CHANGE	DATE	INITIAL
Removed section on using graduated cylinder, increased time for drying sample residue in oven to ≥ 2 h. Clarified section on sample size under "Interferences."	8/11/98	JMD
Added note concerning GA well and DS-Arlington-Brine Samples in section D.1.	4/12/99	AYT
Changed section F.5 to reflect Standard Methods 18 th Ed. Section 2540C.2d.	1/10/01	JAB
Changed "Summary" to reflect above change	1/22/01	JMD
Corrected errors in calculation formula	5/6/02	JMD
Changed section 4.2, 6.2, 7.1 and 7.2 reflect Standard Methods 18 th Ed., section 2540C. 3c. and 3d.	2/10/03	FC
Clarified above and added maximum volume of 1 L	2/21/03	JMD
DHS allowed us to leave the sample in the oven overnight.	10/25/04	LY
Removed reference to 18 th and 19 th Editions of Std. Methods; decreased minimum residue from 10 to 2.5 mg	1/19/06	JMD
Added specific reference to IWF-QUV-B and IWF-QRO-B in 4.2	2/23/06	JMD
Changed above to GWRS nomenclature; changed 2-hour drying back to one hour; changed duplicate frequency back to every 10 samples; added reminder to check balance leveling; removed reference to EPA method 160.1 (Deleted as of 12 March 2007) other minor editorial changes.	11/9/07	JMD
Added reference to GWRS***-ROC to Sec. 4.2, removed obsolete sample references, other minor changes	6/9/09	JMD
Clarified that weight difference between weighings must be less than 0.5 mg	10/19/09	JMD

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

G.E. (SIEVERS) MODEL 900/5310C TOC

File Name: M:\SOP\INORGNIC\TOC_Sievers.doc
Revision: 7

Effective Date: 3/5/2009
Supersedes: 9/18/2008

1. REFERENCES

- 1.1 a. Sievers Model 900 *Operations and Maintenance Manual*.
b. Sievers Model 5310C *Operations and Maintenance Manual*.
- 1.2 *Standard Methods*, 20th ed. #5310C.
- 1.3 EPA *Methods for Analysis of Water and Wastes* #415.1. This method complies with EPA-approved methodology for analysis of wastewater.

2. SAMPLE COLLECTION, PRESERVATION AND HOLDING TIME

- 2.1 Collect sample in trace-clean 250mL amber glass bottle. If filtering for DOC, transfer filtrate to trace-clean 40mL amber glass vial. To minimize contamination, DO NOT handle septa with bare fingers. Use forceps or clean gloves. Pre-rinse sample bottles and vials before filling.
- 2.2 Acidify sample with H₃PO₄ (Aldrich high purity or equivalent) to pH <2. Refrigerate at 4°C for up to 28 days.
- 2.3 **TOC samples with apparent turbidity must be centrifuged prior to preservation.**

3. INTERFERENCES

- 3.1 Loss of volatile organic substances during sample mixing.
- 3.2 Inorganic Carbon not purged from sample during first part of instrument cycle – verify correct operation of Inorganic Carbon Remover (ICR) system.
- 3.3 **Positive interference from contaminated sample vials and bottles.**
- 3.4 Microbial contamination from high TOC samples can result in TOC measurements that deviate from expected values. **See Section 8.5 for maintenance procedure to minimize this problem.**

- 3.5 Chloride values above 600 mg/L can cause interference with the conductivity detector. Samples with a known chloride concentration above 600 mg/L must be diluted. A blank correction must be done to calculate the true value of the diluted sample, followed by the dilution factor.

4. APPARATUS

- 4.1 TOC Analyzer – Sievers Model 900 or 5310C TOC, Autosampler, and ICR, set-up and maintained in accordance with manufacturer's instructions.
- 4.2 Sample Vials – 40-mL, screw caps with Teflon septa used for autosampler. **Drinking water samples must be collected in amber glass vials or 250 mL bottles specially cleaned as for trace organics (see *Standard Methods* #5310B 1.d for details).** If using the septum-piercing feature of the autosampler, it is recommended that the **thinner** (0.065”) septa be used. **Use forceps or clean gloves when handling septa to minimize contamination.**
- 4.3 Filter apparatus for samples requiring Dissolved Organic Carbon (DOC) – *Nalgene* brand 0.45µm Nylon, 50mm diameter, 150mL capacity. Pre-rinse apparatus with at least 500mL TOC free Milli-Q DI water. (This is an OCWD-stocked item, #175-033-00014. Nalgene # 150-0045, VWR # 28199-349.)

5. REAGENTS

- 5.1 Reagent Water – Carbon-free, de-ionized, containing TOC <20 µg/L carbon. Produced as needed by Millipore "Milli-Q" unit (unit typically produces water with 4 µg/L C).
- 5.2 3% Hydrogen Peroxide – Prepare a 10X dilution from the 30% stock. This is used to prevent microbial contamination of the sample lines. See Section 8.5.
- 5.3 Ammonium Persulfate Oxidizer – This reagent comes pre-made in the form of a reagent cartridge from GE/Sievers. The part number is CAPF 90300-01. **(NOTE: Make sure you order #90300 instead of #90150. The volume is 300mL vs 150mL).** Good for up to three months. If stored in refrigerator before opening, shelf life is one year.
- 5.4 Phosphoric Acid – This reagent comes pre-made in the form of a reagent cartridge from GE/Sievers. The part number is CAPF 90310-01. If stored in refrigerator before opening, shelf life is one year.
- 5.5 Potassium Acid Phthalate Stock Solution, 1000 ppm carbon – Dissolve 2.12807 g potassium acid phthalate, KHP (previously dried at 110°C for at least two hours), in Milli-Q DI water and dilute to 1000 mL. Preserve with H₃PO₄ (Aldrich high-purity grade). Store in refrigerator at 4°C. Good for three months.

- 5.6 Calibration Standards – Acidify all standards to preserve and store in refrigerator @ 4°C. Record dates prepared in the Standards Prep Logbook.
- 5.6.1 25.0 mg/L carbon — Dilute 25 mL of 1000 mg/L stock standard to 1000 mL with Milli-Q water
- 5.6.2 10.0 mg/L carbon – Dilute 10 ml of 1000 mg/L stock standard to 1000 mL with Milli-Q water. **This is also the high level C. C. Standard.**
- 5.5.1 5.0 mg/L carbon – Dilute 5 mL of 1000 mg/L stock standard to 1000 mL with Milli-Q water.
- 5.6.3 2.0 mg/L carbon – Dilute 40 mL of 25 mg/L standard to 500 mL with Milli-Q water. **This is also the low level C. C. Standard.**
- 5.5.2 1.0 mg/L carbon – Dilute 20 mL of 25 mg/L standard to 500 mL with Milli-Q water.
- 5.6.4 0.5 mg/L carbon – Dilute 20 mL of 25 mg/L standard to 1000 mL with Milli-Q water.
- 5.6.5 0.05 mg/L carbon – Dilute 5 mL of 10 mg/L standard to 1000 mL with Milli-Q water. **This is the MDL standard.** The calculated MDL for the 7 MDL standards (one run each day over seven nonconsecutive days) should be < 0.3 mg/L, which is the California Health Departments DLR.
- 5.6.6 Blank – Use Milli-Q water. Rinse vial several times with Milli-Q before collecting blank.
- 5.7 Spike solution – Use 1000 mg/L stock standard. For a 2 ppm spike, dilute 200 µL of stock to 100 mL with sample.
- 5.8 Control chart standards, 2.0 mg/L and 10.0 mg/L – Refer to sections 5.5.2 and 5.5.4.
- 5.9 Second source standard. Use SPEX or ERA standard. Prepare as directed. Store in refrigerator at 4°C.

6. PROCEDURE

- 6.1 Turn ON computer. TOC analyzer, autosampler, and ICR should already be ON. Make sure vial door on analyzer is in closed position. Screen on analyzer will show “Main” mode in upper left.
- NOTE: If instrument has not been used in a while, turn off autosampler, then turn back on. This will re-home the position of the needle.

- 6.1.1 Press **Ctrl-Alt-Delete** simultaneously. At log on screen, log on using the same log on procedure as you would use for all the other computers (i.e. Username: your initials and enter your password). Then click **OK** or just press **Enter**
- 6.1.2 Double click on **DataPro900** or **DataPro5310C** icon. Program will automatically go through the “DataGuard StartUp Verification” checklist. After a pause of about 15 seconds, the main screen will come on, and “DataPro900 (COM 1)” will appear in the upper left. Screen on analyzer will now show “Autosampler Mode” in upper left.
- 6.2 Open new protocol. The first sample should be a blank. Choose “Blank” as the type of sample. The next two samples should be 0.5 mg/L standard and 2.0 mg/L standard. Choose “Standard” as the type of sample. Use “Standard” for C.C. Standard, MDL Standard and 2nd Source Standard. Use “Sample” for all samples.
- 6.2.1 The LIMS codes for C. C. standards are “02CCNYMMDDC” for the 2 mg/L standard and “10CCNYMMDDC” for the 10 mg/L standard, where N is the number of the C.C. standard in the run, and YYMMDD is year/month/day. Adjust this accordingly for a particular run.
- 6.2.2 Include samples, duplicates, spikes and spike duplicates. **Also include a clean-up vial containing 3% H₂O₂ at the end of the protocol. See Sec. 8.5.**
- 6.2.3 Plan to run all low TOC samples at the beginning of the run and the high TOC samples at the end. Include the 2.0 mg/L low level and 10 mg/L high level C. C. Standards. Run several DI rinse vials between the low TOC samples and high TOC samples to minimize any carryover. **Use “Sample” as the type for the rinse vials.**
- 6.2.4 **Add one drop or 0.1 mL of high purity H₃PO₄ to each sample vial to ensure complete removal of IC.**
- 6.3 To determine reagent flow rates for the samples to be run, you can use the Auto mode or you can manually set the flow rates. With Auto mode, the analyzer determines the optimum reagent flow rates. Note that using Auto mode for all samples will dramatically increase analysis time. It is recommended that you use Auto mode only for samples that have no historical data.
- 6.3.1 To use Auto mode, you don’t have to change anything, as this is the default setting for samples in the TEMPLATE protocol.
- 6.3.2 To manually set the flow rates, click once in the “Acid Oxid” column. A pop-up window “Reagent Settings” will appear. Click the “Manual” button, then put in the desired Acid and Oxidizer values. Then OK. You will need to do this for each sample you wish to set manually.

6.3.3 As a guide to determine manual flow rates, use the following criteria:

<u>TOC Concentration</u>	<u>Oxidizer Flow Rate</u>
25 – 50 ppm	2.8 – 13.5 µL/min
10 – 25 ppm	1.4 – 7.5 µL/min
5 – 10 ppm	0.7 – 2.8 µL/min
1 – 5 ppm	0.7 – 1.4 µL/min
<1 ppm	0.0 – 0.7 µL/min

<u>IC Concentration</u>	<u>Acid Flow Rate with ICR Inline</u>
50 – 100 ppm	4.0 µL/min
0 – 50 ppm	2.0 µL/min
DI water	not applicable

For samples with a TOC of less than 1 ppm, set the oxidizer rate to 0.1 and the acid rate to 0.5. For samples with a TOC of 1 ppm to 10 ppm, use 2.0 for the oxidizer and 2.0 of the acid. For samples with a TOC greater than 10 ppm, use AUTO.

- 6.4 All standards, QC, and samples will be run using four replicates and one reject.
- 6.5 Save protocol by selecting FILE, then SAVE PROTOCOL. DataPro will automatically assign a file name based on the current date. Preferably save as YYMMDD, then SAVE.
- 6.6 To run the protocol, first make sure the one you want to run is open. FILE, then OPEN, then OPEN PROTOCOL.
- 6.6.1 Confirm that the vials are in the correct positions on the autosample.
- 6.6.2 Click RUN button at the top of the protocol area.
- 6.6.3 The “Confirm Setup” dialog box displays to remind you to check that all vials are in their proper locations on the autosampler. If they are, click YES to continue.
- 6.6.4 If the instrument has not been run in over 24 hours, DataPro automatically prompts you to flush the reagent syringes. Generally you will see this message when you first run a protocol. Insert a 40 mL vial with Milli-Q DI water in the “Flush” position at the far upper right of the standards rack on the autosampler, then click YES. When the reagent flush is complete, the protocol will immediately begin running.
- 6.6.5 Note: When the instrument is not running, the FAULT light on the ICR will show red. This is normal. Once a run has started, the FAULT light will go out.

- 6.6.6 While a protocol is running, you can pause it and make changes to the portion of the protocol that has not yet been run. An example is adding more samples. Click on the PAUSE button. The system stops once the analysis of the current vial has finished. Make changes, the SAVE as a NEW protocol. Then click on the RESUME button.
- 6.6.7 When the run is finished, results will automatically be printed out.
- 6.6.8 Procedure for LIMS transfer of data is under construction.
- 6.6.9 When you are ready to exit DataPro, select FILE, then EXIT. A dialog box will appear, alerting you that control of the analyzer will transfer from the computer back to the display screen on the analyzer. "Exiting DataPro900. Analyzer will be placed in TOC mode." Click OK.
- 6.6.10 **Don't forget to empty waste container.**

7. QUALITY CONTROL

- 7.1 Run one duplicate for every ten samples, or one duplicate per run for batches of less than 10 samples. Values should agree within 10%.
- 7.2 A Blank and Control Chart sample are to be analyzed after the calibration standards, after every 10 samples, and at the end of the run. When running both low and high TOC samples in the same protocol, run the 2 mg/L low C.C. standard at the beginning and end of the batch of low TOC samples, followed by the 10 mg/L high C. C. standard at the beginning and end of the batch of high TOC samples. Values should be within 2 sigma control limits or $\pm 10\%$, whichever is less.
- 7.3 Make sure to run at least one **0.05 mg/L MDL standard** and a 0.5 mg/L standard during each protocol run. The calculated MDL for the 7 MDL standards (one run each day over seven nonconsecutive days) should be < 0.3 mg/L, which is the California Health Departments DLR.
- 7.4 A second source should be analyzed at the beginning of run, and should meet the acceptance criteria.
- 7.5 Analyze at least one matrix spike and spike duplicate per run. Recovery should be 90 – 110%.

8. ROUTINE MAINTENANCE

- 8.1 To ensure proper function of the analyzer, autosampler, and ICR, follow the maintenance guidelines and consumables replacement schedule spelled out in the *Operations and Maintenance Manual* that came with the instrument.

- 8.2 You can use DataPro900 to monitor the status of consumable items in the analyzer by selecting SETUP, then CONSUMABLES STATUS. This will cause the “TOC Consumables Status” dialog box to appear. It will show the usage and number of days of use remaining for UV lamp; Tubing; Resin Bed; Acid; and Oxidizer.
- 8.3 After replacing any consumable, a calibration verification should be performed. The verification occurs at a single concentration, in this case a 1 ppm standard concentration will be used because most of our samples are close to this range.
- 8.3.1 Make sure the analysis is stopped. Place the handle of the ICR into the BYPASS position.
- 8.3.2 Select FILE, then RUN SYSTEM PROTOCOLS, then VERIFICATION.
- 8.3.3 Select the concentration of the standard that you will be using. Then click OK to continue. You will be prompted to save the protocol you previously had open. Click YES to save it or NO to continue without saving it.
- 8.3.4 Click the CALIBRATE button. If the CONFIRM SETUP dialog box appears, double check the vial placement, then click YES to continue.
- 8.3.5 If the instrument has not been run in the past 8 hours, you must perform a reagent flush. Insert a 40 mL vial of low-TOC water in the Flush position at the far right on the autosampler standards rack. Click the YES button to continue. When the flush is complete, the calibration will automatically begin.
- 8.3.6 When the calibration verification is complete, the “Verification Values” window will be displayed. The calibration verification is successful if the following conditions are satisfied:
- 8.3.6.1 RSD is $\leq 3\%$.
- 8.3.6.2 TOC %Diff is $\pm 5\%$ or less.
- 8.3.6.3 IC %Diff is $\pm 5\%$ or less.
- 8.3.6.4 If these conditions are NOT satisfied and the verification FAILS, you may need to perform a calibration and then the verification procedure again. First consult the “Troubleshooting” section in the *Operation and Maintenance Manual* to determine if there is a problem with the analyzer.
- 8.3.6.5 Click OK to close the summary screen.
- 8.3.6.6 Return handle to the INLINE position on the ICR.

8.4 Check DI reservoir periodically, once every two weeks, to ensure sufficient supply of water. Make sure water level is up to blue line.

8.5 **Field Service Bulletin Number UPW 07-10A – “TOC, 900 and 5310C Biological Contamination”**

Analyzers that continually run high concentrations of TOC have the potential to build microbial contamination within the sample lines. This microbial contamination can result in TOC measurements that deviate from expected values.

Clean-up Procedure: Fill a 40mL vial with a **3% hydrogen peroxide** solution (prepare from the 30% concentrated H₂O₂ stock). Insert the vial at the end of the normal sample protocol. Set the number of reps to one. Set the acid flow rate to 0.3µL/min and the oxidizer flow rate to 0.1µL/min. **This procedure should be performed at the end of every sample protocol.**

9. TROUBLESHOOTING

9.1 Refer to the troubleshooting section in *Operations and Maintenance Manual*.

9.2 Periodically, it may be necessary to perform a TOC Autozero. This procedure will correct for minor differences in the response of the two CO₂ sensors. This adjustment is critical **ONLY** for the determination of LOW-LEVEL TOC concentrations. Refer to the *Operations and Maintenance Manual* for instructions on how to perform the autozero.

SOP PROCEDURE CHANGE
FOR TOTAL ORGANIC CARBON, SIEVERS MODEL 900

CHANGE	DATE	INITIALS
New SOP for Sievers TOC	4/8/2005	JAB
Changed part number reference for persulfate to reflect the 300mL size we will now be using.		
Changed references from <i>Ionics</i> to <i>GE</i> .	10/12/2005	JAB
Added that blank is to be run every 10 samples, just Like the check standard.	12/15/05	PH
Changed MDL standard concentration to 0.05mg/L (5.5.7)	01/23/06	SL
DOC filter 0.45µm nylon.	2/13/06	JAB
Changed log on in Procedure 6.1.1	10/25/06	PH
Password Changed	06/12/07	PH
Changed log on in Procedure 6.11	10/2/2007	FC
As per Field Service Bulletin UPW 07-10A, add Hydrogen Peroxide clean-up flush vial at end of every protocol run. This is to prevent microbial contamination of sample lines.	09/18/2008	JAB
Section 3.5, about interference from high chloride samples.	3/5/2009	JAB

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

THRESHOLD ODOR TESTING

File Name: M:\SOP\INORGNIC\ODOR1.DOC
Revision: 2

Effective Date: 4/22/2009
Supersedes: 1 (5/25/2000)

1. References

- 1.1 EPA Method 140.1; Standard Methods 20th Eds. #2150 B; ASTM Method D 1292-80

2. Holding Time

- 2.1 Must be completed as soon as possible after collection of the sample. If storage is necessary refrigerate for 24 hours, making sure no extraneous odors can be drawn into the sample as the water cools.

3. Interferences

- 3.1 Most tap waters and some waste are chlorinated. It is often desirable to determine the odor of the chlorinated sample as well as of the same sample after removal of chlorine. Dechlorination is achieved using sodium thiosulfate in exact stoichiometric quantity. It is important to check a blank to which a similar amount of dechlorinating agent has been added to determine if any odor has been imparted. Such odor usually disappears upon standing if excess reagent has not been added.

4. Apparatus

- 4.1 Odor-free glassware which are freshly cleaned shortly before use, with non-odorous soap and acid-cleaning solution followed by rinsing with odor-free water. The glassware used in odor testing should be reserved for that purpose only. Rubber, cork, and plastic stoppers must not be used.
- 4.2 Electric hot plate or tray with temperature control for performing odor test @ 60°C or wide-mouthed 500-ml Erlenmeyer flask with Petri dishes as cover plates.
- 4.3 Odor flasks: Glass-stoppered, 500-mL Erlenmeyer flasks, preferably opaque or dark-colored.
- 4.4 Sample bottles, glass stoppered or with TFE-lined closures.
- 4.5 Graduated cylinders: 200-,100-,50-, and 25-mL.
- 4.6 Thermometers: 0-100°C, chemical or metal-stem dial type.

4.7 Odor-free water generator: See Figure 1.

5. Reagents

- 5.1 Activated carbon- water purification grade. Carbon should be renewed after treating approximately 20 liters of water, or more often as necessary.
- 5.2 Odor-free water: must be prepared as needed by filtration through a bed of activated carbon. Distilled water is passed through the generator at a rate of 100 mL/min.. When the generator is first started, it should be flushed to remove carbon fines before the odor-free water is used.

Note: The quality of water obtained from the generator should be checked prior to use. The life of the carbon will vary with condition and amount of water filtered. Subtle odors of biological origin often are found if moist carbon filters stand idle between test periods. Detection of odor in the water coming through the carbon indicates that a change of carbon is needed.

6. Procedures

Precautions: Although extreme sensitivity is not required, exclude insensitive persons and concentrate on observers who have a sincere interest in the test. Avoid extraneous odor stimuli such as those contributed by scented soaps, perfumes, and shaving lotions. Insure that the tester is free from colds or allergies that affect odor response. A panel of 5 to 7 testers should be used, none of whom should know in advance which flask contains which dilution.

Threshold Measurement

- 6.1 Make at least four dilutions of each sample.
- 6.2 Add 200 mL, 100 mL, 50 mL, 25 mL, and 12.5 mL, of the sample into separate prepared, labeled Erlenmeyer flasks containing odor-free water to make a total volume of 200 mL.
- 6.3 Use a separate flask containing only odor-free water to serve as the reference for comparison. Use additional blanks if large number of samples are tested.
- 6.4 Cover each flask with foil and insert a thermometer into each one of them. Heat the dilutions and the reference to 61°C (EPA specifies 60+/-1°C; assumption is that sample will not cool below 59°C before reaching last panel member.
- 6.5 After the desired temperature is reached, shake flask containing the odor-free water first, remove stopper, and sniff vapors. Test the rest of the flasks containing the dilutions the same way. Sample dilutions should be presented to the panel in increasing order of concentration, with two or more blanks interspersed in the series near the expected threshold odor level.

- 6.6 Record the observations of each tester by indicating whether odor is noted (+ sign).

For example:

mL Sample

Diluted to

200 mL 12.5 0 25 0 50 100 200

Response - - + - + + +

7. Calculations

- 7.1 The threshold odor number (TON) is the dilution ratio at which odor is just detectable. In the example above, the first detectable odor occurred when 25 mL sample was diluted to 200 mL. Thus the threshold is 200 divided by 25, or 8.
- 7.2 The smallest TON that can be observed is 1, as in the case where the odor flask contains 200 mL. Undiluted sample. If no odor is detected at this concentration, report "No odor observed" instead of a threshold number. Anomalous responses sometimes occur; a low concentration may be called positive and a higher concentration may be called negative. In such a case, designate the threshold as that point after which no further anomalies occur.

For example:

Increasing concentration →

Response - - + - + + + +

Threshold X

where:

- signifies negative response , and
- + signifies positive response.

- 7.3 Use appropriate statistical methods to calculate the most probable average threshold from large number of panel results. Because a significant number of our samples exhibit individual T.O.N.'s of "no odor observed"(full strength not distinguishable from blank), the geometric mean often cannot be calculated. For this reason, T.O.N. is reported as a median and range of results. For example:

Sample Odor Series

mL of Odor-free water	mL of Sample	Observer Response				
		1	2	3	4	5
187.5	12.5	-	-	-	-	-
175	25	-	+	-	+	+
200	0	-	-	-	-	-
150	50	+	+	-	-	+
200	0	-	-	-	-	-
100	100	+	+	+	+	+
0	200	+	+	+	+	+

Circled plus equals threshold level.

Individual threshold odor numbers are obtained as follows:

Observer	T.O.N.
1	4
2	8
3	2
4	2
5	8
Range: 2-8	Median: 4

8. Precision and accuracy

- 8.1 There is no absolute T.O.N. The T.O.N. reflects the opinion of the tester at the time of testing. The value may vary for an individual with time of day or from day to day.

SOP PROCEDURE CHANGE
FOR THRESHOLD ODOR TESTING

[illegible]

Total Hardness

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

HARDNESS, Total (mg/l as CaCO₃)

File Name: M:\SOP\INORGNIC\HARDNESS.doc
Revision: 1

Effective Date: 5/23/2000
Supersedes: N/A

1. REFERENCES

- 1.1 *Standard Methods* 18th, 19th, and 20th Eds. #2340 B (preferred) & C.
- 1.2 *EPA Methods for Analysis of Water and Waste*, #130.2. This method complies with EPA - approved methodology for analysis of drinking water and wastewater.

2. HOLDING TIME

- 2.1 Six months when preserved with HNO₃ to pH <2.

3. INTERFERENCES (EDTA TITRIMETRIC METHOD)

- 3.1 Some metal ions interfere by causing fading or indistinct endpoints. Reduce this interference by adding an inhibitor, like MgCDTA, before titration, see below.
- 3.2 Suspended or colloidal organic matter. Eliminate by evaporating the sample to dryness on steam bath and heating in a muffle furnace @ 550°C until the organic matter is completely oxidized. Dissolve residue in 20 mL of 1N HCL, neutralize to pH 7 with 1N NaOH & make up to 50 mL with distilled water.

4. APPARATUS (EDTA TITRIMETRIC METHOD)

- 4.1 Standard laboratory titrimetric equipment.
- 4.2 125-mL Erlenmeyer flasks.

5. REAGENTS (EDTA TITRIMETRIC METHOD)

- 5.1 Buffer Solutions:
(Odorless buffers): Mix 55 mL conc. HCL with 400 mL DI water and then, slowly and with stirring, add 300 mL 2-aminoethanol (free of Al and heavier metals). Add 5.0 g magnesium salt EDTA and dilute to 1L with DI water.
- 5.2 Inhibitor: (For most waters, inhibitors are not necessary). If required, adjust acidic samples to pH 6 or higher with buffer or 0.1N NaOH. Add 250mg NaCN in powder form (caution: extremely toxic). Add buffer to adjust pH to 10.0 ± 0.1 and titrate.

Flush waste down drain with large quantities of water after insuring that no acid is present.

- 5.3 Indicator: Use a commercially available indicator such as Calgamite indicator or prepare in the lab as follows: Mix together 0.5 g Eriochrome Black -T and 100 g NaCl.
- 5.4 Standard EDTA titrant, 0.02N: Weigh out 3.723 g analytical grade disodium ethylene tetraacetate dihydrate, $\text{Na}_2\text{H}_2\text{C}_{10}\text{H}_{12}\text{O}_8 \cdot 2\text{H}_2\text{O}$, in a liter volumetric flask and dilute to the mark with DI water. Check with standard calcium solution by titration. Store in polyethylene.
- 5.5 Standard calcium solution 0.02N: Weigh out 1.000 g anhydrous calcium carbonate (primary standard low in metals) into a 500 mL flask. Add, a little at a time, 1+1 HCl until all of the CaCO_3 has dissolved. Add 200 mL DI water. Boil for a few minutes to expel CO_2 . Cool. Add a few drops of methyl red indicator and adjust to intermediate orange color by adding 3N NH_4OH or 1+1 HCl as required. Transfer into a 1L volumetric flask and dilute to the mark with DI water.
- 5.6 Hydrochloric acid solution, 1+1.
- 5.7 Methyl red indicator: Dissolve 0.10 g methyl red in DI water in a 100 mL volumetric flask and dilute to mark.
- 5.8 Ammonium hydroxide solution, 3N: Dilute 210 mL conc. NH_4OH to 1L with DI water.
- 5.9 Ammonium hydroxide solution, 1N: Dilute 70 L conc. NH_4OH to 1L with DI water.

6. PROCEDURE (ICP METHOD [PREFERRED METHOD])

- 6.1 Calculation:

$$\text{Total Hardness, mg/L CaCO}_3 = 2.497 [\text{Ca, mg/L}] + 4.118 [\text{Mg, mg/L}]$$

7. PROCEDURE (EDTA TITRIMETRIC METHOD)

- 7.1 Standardization of EDTA titrant: Pipet 10.0 mL standard calcium solution into 125 mL Erlenmeyer flask containing 50 mL DI water. Add 1 mL buffer solution. Add a small scoop of indicator. Titrate slowly with continuous stirring until the last reddish tinge disappears; add last few drops at 3-5 seconds. intervals to blue endpoint. Total titration time should be 5 minutes. Calculate normality of EDTA:

$$\text{N of EDTA} = 0.2 / \text{mL of EDTA}$$

- 7.2 Titration of sample — normal to high hardness:

- 7.2.1 Sample should require <15 mL EDTA titrant & titration should be completed within 5 min. of buffer addition.

- 7.2.2 Pipet 25 mL sample into flask, neutralize with 1N NH_4OH and dilute to about 50 mL.
- 7.2.3 Add 2 mL buffer solution.
- 7.2.4 If endpoint is not sharp, repeat adding inhibitor at this point.
- 7.2.5 Add a small scoop of indicator.
- 7.2.6 Titrate slowly with continuous stirring with standard EDTA titrant to blue endpoint, adding the last few drops at 3 to 5 second intervals.
- 7.3 Titration of sample — low hardness (<5 mg/L)
 - 7.3.1 Use a large sample (100 mL), without further dilution.
 - 7.3.2 Use proportionately larger amounts of buffer, inhibitor, and indicator.
 - 7.3.3 Use a microburet and run a blank using DI water.
 - 7.3.4 Calculations:

$$\text{Hardness (EDTA), mg CaCO}_3\text{/L} = A \times N \times 50,000/\text{mL sample}$$

Where A = mL titration for sample

- 7.4 Daylight or a daylight fluorescent lamp is highly recommended because ordinary incandescent lights tend to produce a reddish tinge in the blue at the endpoint.

8. QUALITY CONTROL

- 8.1 Run one duplicate for every 10 samples or one duplicate per run. Values should agree within 10%. Analyze 2 Control Chart standards per run. EPA Performance Evaluation samples are used for external validation. Results should be within EPA specified acceptance limits.

9. TROUBLESHOOTING

- 9.1 Check EDTA standardization periodically because of gradual deterioration especially if results are questionable.

CHANGE	REASON	DATE	INITIALS
Updated <i>Standard Methods</i> reference		5/23/00	JMD

5/23/00

JMD

[illegible]

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

TRACE METALS SAMPLE HANDLING OVERVIEW

File Name: M:\SOP\INORGNIC\TRACE METALS SAMPLE HANDLING OVERVIEW.doc Effective Date: 12/12/2001
Revision: 1 Supersedes: N/A

These procedures apply to trace metals only. Samples where only Na, K, Mg, Ca and B are requested may be analyzed by ICP-OES using Method 1 below regardless of turbidity and/or color.

Samples with gross or excessive settleable matter should be centrifuged and/or allowed to settle and the supernatant drawn or poured off before using procedures 2-6 below.

Samples not preserved in the field (e.g., those which will be filtered—see below) should be preserved as soon after receipt and/or filtration in the laboratory as possible by addition of HNO₃ to < pH 2 (3 mL 1+1 acid is a good starting point). After preservation in field or lab, mix sample and allow to stand for at least 16 hours. After 16 hours and again immediately before withdrawing an aliquot for processing or analysis, verify that pH is < 2. If not, add more acid and allow to stand another 16 hours. Repeat process as necessary.

1. Colorless samples w/ <1 NTU turbidity **ONLY** (includes Q-15s and most drinking water samples; does **not** include samples for Ag analysis, however—use Procedure #3 instead):
 - Add acid to sample (along with internal standards, modifiers, etc. if necessary) as specified in the appropriate SOP and analyze directly.
2. Westbay samples (station name contains “WB”) (only when analyzed for trace metals; “Level I’s” may be analyzed directly as in #1):

First pre-filter through glass fiber filter (watch sample usage—Westbay samples are usually limited volume). Then filter through a prewashed, ungridded 0.45 µm pore cellulose acetate or polycarbonate membrane filter in a plastic filter holder. After filtration, if the sample is a:

2.1 Colorless sample:

Add acid to sample (along with internal standards, modifiers, etc. if necessary) as specified in SOP and analyze directly.

2.2 All others:

Digest using microwave digester

3. Deep Wells, Green Acres Plant, R&D, GWRS, Water Factory 21 (except Q-10) and other wastewater samples:

Do not filter

Digest using microwave digester

4. Q-10 samples:

Do not filter

Digest using graphite/Teflon open vessel on hot plate

5. Surface water samples:

Filter **ALL** surface water samples unless specifically requested otherwise. First pre-filter through glass fiber filter (take from metals-free bottle, **not** general mineral bottle; store in new, clean metals-free bottle [do not return to original metals-free sample bottle]). Then filter through a prewashed, ungridded 0.45 µm pore cellulose acetate or polycarbonate membrane filter in a plastic filter holder. After filtration, if the sample is a:

- 5.1 Colorless sample with **NO** contribution from wastewater (rare)

Add acid to sample (along with internal standards, modifiers, etc. if necessary) as specified in SOP and analyze directly.

- 5.2 All other surface water samples (most OCWD SW samples):

Digest using microwave digester

6. All other aqueous samples submitted to OCWD Laboratory:

If requested, filter as in #5 above

Otherwise, treat as in #3 above

[illegible]

Turbidity

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

TURBIDITY

File Name: M:\SOP\INORGNIC\turbidity sop.doc
Revision: 7

Effective Date: 11/6/2009
Supersedes: 6 (4/24/2008)

1. REFERENCES

- 1.1 EPA *Methods for Analysis of Water and Waste*, #180.1.
- 1.2 *Standard Methods*, 20th Ed. #2130B, pp. 2-9 to 2-11.
- 1.3 Hach 2100AN Laboratory Turbidimeter Quick Reference Guide and Instrument Manual

2. HOLDING TIME

- 2.1 48 hours @ 4°C; best if analyzed immediately.

3. INTERFERENCES

- 3.1 Color, coarse suspended matter.

4. APPARATUS

Hach 2100AN Turbidimeter.

Ultrasonic water bath

Hach StablCal® stabilized Formazin standards. We use StablCal® ampuled Calibration Kit for 2100 AN turbidimeter, cat. #26595-05. Standards are good for 24 months from date of manufacture. Always keep standards in their original kit with the cover on. **Do not shake or invert <0.1 NTU standard!**

5. PROCEDURE

**Initial instrument settings: Range = AUTO, Ratio = OFF, Signal Avg. = ON.
Allow instrument to warm up for at least 60 minutes before using (instrument may be left on continuously).**

Currently (2008), the date/time memory battery is not operating and is not user-replaceable. Enter date and time each time instrument is powered up as follows:

- 1. Press SETUP to enter setup mode. The mode display will flash. Select the number 05 using the edit (up and down arrow) keys, then press ENTER.**

Set the hours and minutes (HH-MM) using the edit keys (24-hr format). Press ENTER to accept the new setting.

- 2. Select the number 06 using the up arrow key, then press ENTER. Set the month and day (MM-DD) using the edit keys. Press ENTER to accept the new setting.**
- 3. Select the number 07 using the up arrow key, then press ENTER. Set the year (YY) using the edit keys. Press ENTER to accept the new setting.**
- 4. Press SETUP to exit the setup mode. Pressing UNITS/EXIT at any time, prior to accepting the new value, exits the setup mode leaving original values intact.**

Hach recommends use of 20-, 200-, 1000-, and 4000-NTU Formazin standards for calibration of the Model 2100AN Turbidimeter (**a 7500-NTU standard is included in the StablCal® kit, but is not needed for samples in our turbidity range**). The procedure for calibration is as follows:

NOTE: Hach says that the instrument does not need to be calibrated with every use. Calibrate when your check standards or second source standards fall out of the $\pm 10\%$ range **specified in the Hach manual**, or every three months, whichever comes first. You can use different standards to calibrate although Hach doesn't recommend it. If you choose to do so, refer to the instrument manual sections 3.3.2 – 3.3.4.

- 5.1 Press CAL.** The CAL mode annunciator lights, and the small green LED digits in the mode display flashes 00. The NTU value of the blank from the previous calibration is displayed.
- 5.2 Remove the <0.1 NTU standard cell from the standards kit without shaking or inverting it.** Clean the cell by placing a drop of silicone oil on the cell and wiping with a Kimwipe. Place into the cell holder, with the triangular mark on the ampule aligned with the alignment mark on the cell holder. Close the cell cover and press ENTER. The instrument display counts down from 60 to 0, and then makes a measurement. This measurement is stored and used to calculate a correction factor for measurement of all NTU standards.
- 5.3 The instrument automatically increments to the next standard, displays the expected NTU value while the standard number (e.g., 01) is shown in the mode display. Remove the <0.1 NTU standard. For all remaining standards, invert the cell 10 times to mix and let sit 1-3 minutes before inserting into the cell holder. To save time, you may mix the next cell while the current cell is counting down its 60 seconds. Follow this procedure for all the remaining standards. When calibrating, RATIO mode will automatically switch on.**
- 5.4 When you are done entering all of the standards to be used for the calibration, press CAL/Zero. If you stop after the 4000 NTU standard, the displayed number will be 05; if the calibration is done including the 7500 NTU**

standard, it will be 00 and the previously measured value of the <0.1 NTU standard value is displayed. The instrument is now calibrated.

- 5.5 Press **CAL/Zero** again, and then press **PRINT**. Your calibration will now print out. Tear off and attach to your data. Press the **UNITS/EXIT** key to return to the operating mode without affecting your current calibration.
- 5.6 Make sure that the **RATIO** indicator light is off. Allow refrigerated samples to warm to room temperature. Perform the following steps as quickly as possible: mix sample thoroughly by **gently** inverting several times—in most cases, do not shake samples, as excessive suspension of bubbles will result. Pour the sample into the cell, wipe **the outside of the cell dry** with a Kimwipe, and wipe again with a Kimwipe moistened with a drop of silicone oil to make sure the outside is clean, dry, and transparent. Place the sample cell into the cell holder and close the cover, then press **ENTER** (this is necessary to clear the signal averaging buffer of the previous sample results). Read and record your results at the first stable reading (not a transient peak, and not at the end of a slow decline, indicating settling solids) of the continuously updating results. **Per Don Diepenbroek, Hach regional sales manager, do not wait more than 2 minutes have elapsed under most circumstances before recording the turbidity value.**

Note: do not normally use physical means to remove small bubbles which may interfere with measurement, however, if necessary in special cases, partially immerse filled cell in ultrasonic water bath to remove suspended bubbles. Turn the ultrasonic bath on for a minimum of 10 seconds, up to 60 seconds.

- 5.7 For all samples with turbidity higher than 40 NTU: **Dilute sample with appropriate volume of filtered sample to bring turbidity below 40 NTU. Dilution with DI water may result in incorrect results due to dissolution of some of the turbidity. Apply an appropriate factor to calculate the turbidity.**

6. QUALITY CONTROL

- 6.1 After calibrating the instrument measure a Formazin standard as a sample for your check standard and measure another Formazin standard from a different company as your second source; values should agree within 10%. **Hach Corp. does not recommend use of AMCO Clear® or other polymer bead-type turbidity standards, as the light scattering is not equivalent to Formazin standards.**
- 6.2 Run a duplicate every ten samples or one duplicate per run for sets with less than ten samples. Values should agree within 10%.

7 TROUBLESHOOTING

- 7.1 **Hach states that the displayed value with the sample chamber closed and no sample in place should be about 0.017 NTU. If the value is higher, carefully clean the windows in the sample chamber (and the EPA filter, if necessary) with a damp Kimwipe®.**

SOP PROCEDURE CHANGE **FOR TURBIDITY**

[illegible]

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

TRACE ELEMENTS AND METALS SAMPLE HANDLING AND PREPARATION

File Name: M:\SOP\INORGNIC\TraceMetalsPrep(NEW).doc Effective Date: 7/6/2009
Revision: 5 Supersedes: 4 (12/14/2001)

1. REFERENCES

- 1.1 EPA *Methods for the Determination of Metals in Environmental Samples* (June 1991), 200.1; Supplement I (May 1994), 200.2, 200.15 and 200.7
- 1.2 *Methods for Analysis of Water and Wastes* (1979, rev. 1983), 200.0.
- 1.3 *Code of Federal Regulations 40* (July 1, 2000), Part 136, Appendix C
- 1.4 *Standard Methods*, 18th, 19th, and 20th Eds., #3010-3030.

2. SCOPE AND APPLICATION

- 2.1 This method may be used for the sampling, sample handling and preparation of dissolved, suspended, total, or total recoverable elements in drinking water, surface water, domestic and industrial wastewaters. Analysis is to be performed by ICP/MS, ICP-OES, or other trace metal analysis technique. Hexavalent chromium (Cr^{+6}) analysis has its own unique requirements; see the Cr^{+6} SOP for details.

3. SUMMARY OF METHOD

- 3.1 The sample is collected and preserved in such a way as to maintain the concentrations of elements in solution to the levels in existence at the time of collection, and in a form compatible with recovery by the analytical technique used. An acidic environment is to be maintained at all times in the sample solution, and additional heat/acid treatment steps and acid matrix matching is specified, where appropriate.

4. DEFINITIONS

- 4.1 Dissolved--Those elements that will pass through a 0.45 μm membrane filter.
- 4.2 Suspended--Those elements that are retained by a 0.45 μm membrane filter.
- 4.3 Total--The concentration determined on an unfiltered sample following vigorous digestion (9.3)

- 4.4 Total recoverable--The concentration determined on an unfiltered sample following treatment with hot, dilute mineral acid (9.4).

5. SAFETY

- 5.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets is available to all personnel involved in chemical analyses.
- 5.2 At no time should there be any storage or use of flammable liquids/solvents within the metals digestion hoods. This area of the lab requires the use of specific inorganic acids, and therefore no organic solvents or flammable liquids should enter into this processing area. Any use of these materials in this area is strictly forbidden.

6. REAGENTS AND STANDARDS

- 6.1 Acids used in the preparation of standards and for sample processing must be ultra-high purity grade (Baker-Mallinckrodt ULTREX) or equivalent. Redistilled acids are acceptable.
- 6.1.1 Hydrochloric acid, conc. (sp gr 1.19).
- 6.1.2 Hydrochloric acid, (1+1): Add 500 mL conc. HCl (sp gr 1.19) to 400 mL deionized water and dilute to 1 liter.
- 6.1.3 Nitric acid, conc. (sp gr 1.41).
- 6.1.4 Nitric acid (1+1): Add 500 mL conc. HNO₃ (sp. gr 1.41) to 400 mL deionized water and dilute to 1 liter.
- 6.2 Deionized water—produced by Milli-Q Gradient A-10 System (Millipore) fed by whole-lab deionization system. Use Milli-Q water for the preparation of all reagents, calibration standards and as dilution water.
- 6.3 Hydrogen Peroxide solution, 30% H₂O₂, ACS Reagent Grade.

7. APPARATUS AND EQUIPMENT

- 7.1 Labware—since contamination is of prime consideration, work in a clean laboratory area designed for trace metal handling. All reusable labware (glass, plastic, Teflon) should be cleaned prior to use. After thorough cleaning with

detergent and water, soak labware for at least 4 hrs. in 1+1 nitric acid, followed by rinsing with water and oven drying.

- 7.2 Assorted class "A" pipets.
- 7.3 *Environmental Express* 50 mL metals-free centrifuge tubes.
- 7.4 *Environmental Express* heated block digestion system. See system instruction manual for details.
- 7.5 Membrane filtration apparatus.
- 7.6 Membrane filters, nylon, HPLC grade, 0.45 µm pore. Do not use "bacti"-type filters.

8. SAMPLE HANDLING AND PRESERVATION

- 8.1 For the determination of trace elements, contamination and loss are of prime concern. Dust in the laboratory environment, impurities in reagents and impurities on laboratory apparatus that the sample contacts are all sources of potential contamination. Sample containers can introduce either positive or negative errors in the measurement of trace elements by (a) contributing contaminants through leaching or surface desorption and (b) by depleting concentrations through adsorption. Thus the collection and treatment of the sample prior to analysis requires particular attention. Laboratory glassware including the sample bottle (whether polyethylene, polypropylene or FEP-fluorocarbon) should be thoroughly washed with detergent and tap water; rinsed with (1+1) nitric acid, tap water, (1+1) hydrochloric acid, tap and finally deionized distilled water in that order (See Notes 2 and 3). NOTE 2: Use caution if considering using chromic acid to remove organic deposits from glassware; (normally unnecessary for OCWD laboratory samples). A commercial product, NOCHROMIX, available from Godax Laboratories, 6 Varick St., New York, NY 10013, may be used in place of chromic acid. Chromic acid should not be used with plastic bottles. NOTE 3: If it can be documented through an active analytical quality control program using spiked samples and reagent blanks, that certain steps in the cleaning procedure are not required for routine samples, those steps may be eliminated from the procedure.
- 8.2 Before collection of the sample a decision must be made as to the type of data desired, that is dissolved, suspended, total recoverable or total, so that the appropriate preservation and pretreatment steps may be accomplished. Filtration, acid preservation, etc., are to be performed at the time the sample is collected or as soon as possible, thereafter.
 - 8.2.1 Samples for metals analysis must always be collected in a separate, metal-free container provided by the laboratory to sampling personnel. The container will typically be a disposable, certified, pre-cleaned plastic bottle supplied by I-Chem, Eagle-Picher, or other vendor, and will either

contain nitric acid suitable for preserving the sample, or not, as appropriate (see below).

- 8.2.2 For the determination of **dissolved elements** the sample must be filtered through a 0.45 μm membrane filter as soon as practical after collection. (Glass or plastic filtering apparatus are recommended to avoid possible contamination.) Use the first 50 - 100 mL to rinse the filter flask. Discard this portion and collect the required volume of filtrate. Acidify the filtrate with (1+1) HNO_3 to a pH of 2 or less. Normally, 3 mL of (1+1) acid per liter should be sufficient to preserve the sample. **NOTE: To date, the OCWD Water Quality Department has consistently requested that ALL surface water (Santa Ana River, Anaheim Lake, etc.) samples be filtered for dissolved trace metal analysis. In most cases, the unpreserved sample will have been prefiltered through a glass fiber filter prior to membrane filtration. No exceptions to this procedure will be made unless specifically requested otherwise.**
- 8.2.3 Suspended elements are rarely specified for analysis by the OCWD laboratory. Refer to full EPA Method 200.7 for details if necessary.
- 8.2.4 For determination of **total recoverable elements** in aqueous samples, the samples must be acid preserved prior to aliquoting for either sample processing or determination by direct spectrochemical analysis. For proper preservation samples are not filtered, but acidified with (1+1) nitric acid to $\text{pH} < 2$. Preservation is to be done at the time of sample collection for total and total recoverable elements. If for some reason field preservation is impossible, it is recommended that the samples be returned to the laboratory as soon as possible after collection and acid preserved upon receipt in the laboratory. In either case, following acidification, the sample should be mixed and held for sixteen hours. (Normally, 3 mL of (1+1) nitric acid per liter of sample is sufficient for most ambient and drinking water samples). The pH of all aqueous samples must be tested immediately prior to withdrawing an aliquot for processing to ensure the sample has been properly preserved. If for some reason such as high alkalinity the sample pH is verified to be > 2 , more acid must be added and the sample held for sixteen hours, and the acidification and holding process repeated until the sample is verified to be $\text{pH} < 2$. If properly acid preserved, the sample can be held up to 6 months before analysis. **NOTE:** When the nature of the sample is either unknown or is known to be hazardous, acidification should be done in a fume hood.

9. SAMPLE PREPARATION

NOTE: Sample prep person is responsible for preparing the digested blank, digested LFB (CC Std), sample duplicates, and digested spike/spike duplicates for each method. Follow the ICP-OES or ICP-MS SOPs for preparation procedures. This is required for QA/QC. Record date of prep on sample digestion tubes and in the Prep Logbook.

- 9.1 For the determinations of **dissolved elements**, the filtered, preserved sample may often be analyzed as received. The acid matrix and concentration of the samples and calibration standards must be the same. (See Note 6.) If a precipitate formed upon acidification of the sample or during transit or storage, it must be redissolved before the analysis by adding additional acid and/or by heat as described in 9.3. With the exception of silver, where this method is approved for the determination of certain metal and metalloid contaminants in drinking water, samples may be analyzed directly by pneumatic nebulization without acid digestion if the sample has been properly preserved with acid and has turbidity of < 1 NTU at the time of analysis. This total recoverable determination procedure is referred to as “direct analysis”. Check all drinking water samples for turbidity before analysis, and submit all those having an NTU value of 1 or greater to the Total Recoverable or Total Element procedures.
- 9.2 Suspended elements are rarely specified for analysis by the OCWD laboratory. Refer to full appropriate analytical method for details if necessary. NOTE 4: In place of filtering, the sample after diluting and mixing may be centrifuged or allowed to settle by gravity overnight to remove insoluble material.
- 9.3 Total elements are rarely specified for analysis by the OCWD laboratory. Refer to full appropriate analytical method for details if necessary. NOTE 5: When determining boron in aqueous samples, only plastic, PTFE or quartz labware should be used from time of sample collection to completion of analysis. When possible, borosilicate glass should be avoided to prevent contamination of these analytes. NOTE 6: If the sample analysis solution has a different acid concentration from that given in 9.4, but does not introduce a physical interference or affect the analytical result, the same calibration standards may be used.
- 9.4 For the determination of **total recoverable elements**, choose a measured volume of a well mixed, acid preserved sample appropriate for the expected level of elements and digest in *Environmental Express* block digestion system according to manufacturer’s instructions. Concentrations so determined shall be reported as “Total.” For details of other methods, see specific method.
- 9.5 Sample preparation for **ICP-OES analysis**:
- 9.5.1 For samples not needing digestion (i.e. Title 22, most groundwater, boron, Na, K, Ca, Mg), measure 97 mL sample into a clean 250-mL wide-mouth plastic bottle. Add 2 mL of (1+1) nitric acid and 1 mL of (1+1) hydrochloric acid.
- 9.5.2 For samples needing digestion (i.e. WF-21, Prado, surface water, deep wells), measure 50 mL of sample into an *Environmental Express* 50 mL metals-free centrifuge tube. Place tubes in *Environmental Express* digestion system and follow procedures spelled out in digestion block SOP. After the digestion procedure has finished and samples have cooled,

bring digested sample back up to the 50 mL initial volume with DI water. Samples are now ready for analysis.

- 9.5.3 For samples needing filtration prior to sample preparation, use nylon membrane filter that has been thoroughly rinsed with ASTM Type I water before filtering sample.
- 9.6 Sample Preparation for **ICP/MS Analysis:** see OCWD SOP for ICP/MS for special sample prep requirements.
- 9.7 All samples prepared for metals analysis must be recorded in a Prep Logbook. There are two, one for ICP-OES preps and one for ICP/MS preps. Use the following procedure to create a Prep Log.
 - 9.7.1 Enter ASPEN and click on the USER EXTENSION button. Under the ACTIVE/ARCHIVE REPORTS banner click on the INORGANIC DIGESTION LOG button.
 - 9.7.2 Choose the correct test method.
 - 9.7.2.1 X200.7 for ICP-OES.
 - 9.7.2.2 X200.7D for dissolved ICP-OES.
 - 9.7.2.3 X200.8 for ICP/MS.
 - 9.7.2.4 X200.8D for dissolved ICP/MS.
 - 9.7.3 Highlight sample of interest by clicking any point within that row. Doing so will change NO to YES in the SELECTED column. Continue this step until you have selected all pertinent samples.
 - 9.7.4 If the sample has a corresponding duplicate or spike that was prepped, click on the DUPLICATE and/or SPIKE buttons to the right of the sample list.
 - 9.7.5 When finished selecting samples, duplicates and spikes, click on the OPEN LOG button.

A preview of your prep log will appear. If you need to change the acid lot number, do so at this time. Verify the PREP BY and PREP DATE information. Click ALL buttons to fill columns.
 - 9.7.6 If the sample was digested using microwave or manually by open vessel, click the appropriate boxes.
 - 9.7.7 Then click the SAVE FORM button. Shortly, a message box will appear stating 'Form Successfully Updated'. Click OK.
 - 9.7.8 To print your Prep Log, click on PRINT FORM button at top.

[illegible]

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

ENVIRONMENTAL EXPRESS AUTOBLOCK

File Name: M:\SOP\INORGNIC\ENVIRONMENTAL
EXPRESS AUTOBLOCK.doc

Effective Date: 4/3/2008

Revision: 3

Supersedes: 2 (6/8/2007)

1. PROCEDURE

- 1.1 Plug black block digester power cord back into the fume hood power outlet if necessary.
- 1.2 Open the laptop cover.
- 1.3 Press the silver **ON/OFF** button near the screen to turn the laptop on.
- 1.4 Select "Profile 1" with Down Arrow key and press Enter.
- 1.5 At login screen, enter "admin" for password and press OK. At "Failed to Initialize the Network" message, press OK.
- 1.6 Using the touchpad and buttons, double click the **AutoBlock PC** menu choice and wait for the program to load.
- 1.7 Making sure vial carriers are in the "DOWN" position within the AutoBlock before adding vials (select Initialize if not), measure samples to be digested to the 50 mL mark on the digestion vial. Label vial and place the first vial in the first column to the left, as you face the digestion block and in the very front left position. Fill up the remaining positions in that column before proceeding to the second column. Fill column two completely before proceeding to the next column, etc. Set empty vials you have replaced with sample prepped vials aside and place them back in the holders at the end of the run. Leave all empty vials in place that are not replaced by a vial containing a sample. **(NOTE: This is especially important if a column is not completely filled with tubes containing samples. Empty tubes must complete the column so as to catch acid during the priming step.)** For each type of digestion (OES or MS), a blank, spike, and LFB (prepared by ICP analyst) must be digested for each run.
 - 1.7.1 Note 1: It is required that a vial, empty or full, be in every position to ensure proper heating and cooling during the digestion run.

- 1.7.2 Note 2: Vials both with and without samples in the last column used will have acid added; replace these empty vials with clean ones after each run.
- 1.8 Tap **Select Mode** until the **Method Mode** appears in the upper right hand corner.
- 1.9 Tap **Load**.
- 1.10 Tap the **Down Arrow** and tap method **ocwdicpms.ahb**. The “MS method” is used for both OES and MS preps.
- 1.11 Tap **Apply**.
- 1.12 Tap **Initialize**.
- 1.13 Tap **Start**.
- 1.14 Tap **OK** at Reagent Verification screen.
- 1.15 Tap **OK** at Temperature Verification of 95 °C.
- 1.16 Enter the number of columns to be digested. The default value is 1. To change, use the scroll bar to scroll down to the desired number selection of columns to be digested and tap it to select.
- 1.17 **TURN ON FUME HOOD SWITCH AND LEAVE ON THROUGHOUT DIGESTION.**
- 1.18 Tap **Apply**. Due to a minor bug, a number between 30,000 and 40,000 will appear with an “OK” button. Press it to continue. This may occur up to 5 times. The selected method should then start automatically. NOTE: There is a hesitation at this step. The reagent lines are flushed and primed for injection. Only vials in columns selected for digestion will have reagents added.
- 1.19 Print out digestion log.
- 1.20 When the digestion is finished and the samples have cooled (3-5 hours), add 100 µL of Internal Standard solution to each ICP_MS sample, then add Milli-Q water to each digested vial to bring volume up to the 50 mL mark. Cap and label with digestion log labels. On the AutoBlock screen, tap **Reset**, then **Initialize**. After the block has cooled enough that the exhaust fan has shut off (approx. 30 °C), shut off the fume hood, close the AutoBlock screen, shut down the laptop computer and close the lid.

SOP PROCEDURE CHANGE
FOR ENVIRONMENTAL EXPRESS AUTOBLOCK

CHANGE	DATE	INITIAL
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Clarified that metals prep person adds internal std. to MS	1/01/06	JMD
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Revised order of steps for greater accuracy	1/01/06	JMD
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Added Laboratory Fortified Blank, blank, and spike to digestion protocol	2/13/06	JMD
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Added header & footer, changed to reflect use of laptop computer, other minor editorial changes.	6/8/07	JMD
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Minor changes to shutdown procedures.	4/3/08	JMD
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ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

DEENA Automated Sample Preparation System

File Name:
Revision: 1

Effective Date: 10/30/2009
Supersedes:

1. PROCEDURE

1.1 Turn on the computer power, then press Ctrl-Alt-Delete to start.

User name: **Orange County** Password: press **Enter**

Log on to: **D32K5JC1** (this computer)

1.2 Turn on the power to the DEENA in the hood. The switch is located on the right side of the heating block, towards the back.

1.3 Double click on the **Launch ASD.exe** icon on the computer.



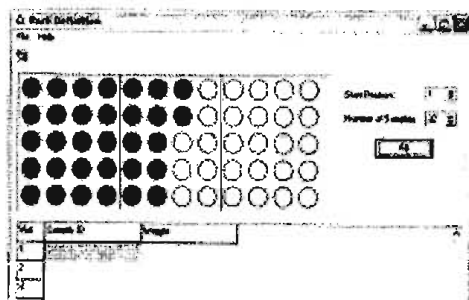
At this time DEENA will initialize.

1.4 Preheat the system. Click on **Set Temperature**  to **120°C**.

1.5 Prepare the samples to the digestion vials and load them on DEENA digest rack in the hood. (Note: Environmental Express vials are OK to use.)

1.6 At the computer, click on **file, open**, then open "**ocwd template .wsp**"

- 1.7 Click on **Rack Definition**  to set the number of samples.



Start Position: 1, then enter **Number of Samples** you will digest. Click on **Fill** button. At this point the sample positions will change to Blue. Confirm that the sample positions in NEENA and on the computer screen are the same. Check the reagents, especially Milli-Q water, and make sure there is enough for the run.

- 1.8 To start the run: Press **Go** 

- 1.9 When the run is finished, the PC screen will display as shown below:



- 1.10 Remove samples from DEENA, and check the sample level.
Cap the tubes, then take them to ICP room for analysis.

2. MAINTENANCE

- 2.1 See NEENA Operator's Manual.

SOP PROCEDURE CHANGE

FOR DEENA

CHANGE

DATE

INITIALS

New Method

10/30/09

FC

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

UV ABSORBANCE OR PERCENT TRANSMITTANCE

File Name: M:\SOP\INORGNIC\UVAB CARY.doc
Revision: 8

Effective Date: 6/6/2008
Supersedes: 7 (2/11/2008)

1. REFERENCES

- 1.1 *Standard Methods for the Examination of Water and Wastewater*, 19th Ed., Method 5910, pp. 5-60 to 5-62 and 20th Ed., Method 5910, pp. 5-65 to 5-68. This method complies with EPA-approved methodology for analysis of drinking water and wastewater.

2. HOLDING TIME

- 2.1 Analyze as soon as possible. **Maximum hold time 48 hours at 4 °C (analyze plant composites collected on Fridays no later than the following Monday).**

3. INTERFERENCES

- 3.1 Primary interferences include colloidal particles, UV-absorbing organics besides those of interest, and UV-absorbing inorganics, mainly ferrous iron, nitrate, nitrite and bromide.
- 3.2 Some oxidants and reducing agents (ozone, chlorate, chlorite, chloramines, thiosulfate) may also interfere.
- 3.3 Specific interferences can be corrected for; however, if corrections exceed 10% of total absorption, select another method or alternative wavelength.
- 3.4 Avoid reading samples with pH below 4 or above 10, as UV absorption may vary at these levels.

4. APPARATUS

- 4.1 Varian Cary 50 UV-visible spectrophotometer with computer and printer
- 4.2 Disposable plastic cups
- 4.3 Filters, glass fiber, pre-rinsed with DI water, Whatman 934-AH or equivalent.
- 4.4 Filter assembly, glass, TFE, porcelain (Buchner funnel) or stainless steel, capable of holding the selected filters.

5. PROCEDURE

- 5.1 Rinse filter and filter assembly with at least 50 mL of DI water. See *Standard Methods* for cases where sample pH needs adjustment or buffer addition. Rinse filtration apparatus with a small amount of sample, discard rinse solution, then filter 50 mL of sample. Prepare a DI water blank in the same manner.
- 5.2 Pour filtered sample into disposable beaker or other appropriate container, including a DI water blank and sample duplicate for every 10 samples (or one per run for runs of less than 10 samples), and allow to come to room temperature.
- 5.3 Turn on computer at power strip. Press Ctrl-Alt-Del. At "Log on to Windows" screen, log in with your initials and network password. Double click on Cary WinUV icon, then click on "OK." Double click on Simple Reads icon. Click on "Cary 50," then click on "OK." Click on CONNECT icon at top of screen if present.
- 5.4 Click on Setup. In read mode area make sure correct Read at Wavelength is chosen. Highlight default wavelength and select 254 or 228.
- 5.5 In Y mode area click on Abs, or %T for plant samples, then click on OK.
- 5.6 Confirm that correct cuvet (1 cm flow-through) is in light path, and make sure that sipper line is taut by pushing in the lever and turning clockwise.
- 5.7 Flush out the line with DI water by pressing FLUSH button on sipper 2-3 times. Press and release button; there is no need to hold it down. Click on the ZERO button on screen, **then click on Read to document the blank reading in Abs. or %.**
- 5.8 Place sipper line in **filtered** DI water and press the FLUSH button, then press the START button on the peristaltic pump to read blank. Again press and release; do not hold down. There is no need to click on the READ icon on the computer screen.
- 5.9 To read samples place sipper line in **filtered** sample and press the FLUSH button, then press the START button. There is no need to click on the READ icon on screen. **Repeat for each sample so that at least two readings are taken of each sample.**
- 5.10 **To enter lab numbers on the report after all readings are taken, click on Edit (on the menu bar), Edit Report; then type in sample numbers to the right of each reading.**
- 5.11 When done, click on PRINT on screen, then OK. Then click on CLEAR REPORT. The software will prompt you with a screen saying that unsaved data will be lost. Click on OK.

- 5.12 Flush out sipper line several times with **Trace-Clean® (if available)**, followed by DI water.
- 5.13 Click on FILE at top of screen and exit. Shut down computer. Loosen tension on the sipper peristaltic pump tubing by turning lever counterclockwise and pulling out. Switch off power at power strip.

6. UV %T SCANS

- 6.1 At the initial screen, click on SCAN icon.
- 6.2 Click on Setup button. Under CARY tab, start and end scan at desired wavelengths (usually starting at 310 nm and ending at 190 nm). At Y-Mode, indicate %T. At Y-Min, usual setting is 60 and Y-Max is 100. At Scan Controls, select Medium speed. Still on Setup, under Baseline tab, click on Baseline Correction. Under Reports tab, enter Operator Name and Sample ID under Comments. Also, at X-Y Pairs Table, click on box to include an X-Y Pairs Table with the report.
- 6.3 Using the Flush button on the sipper, flush and fill cell with DI water Blank. Click on Baseline button. The following message will appear: Scan Insert blank sample into sample compartment and press OK to collect 100%T baseline scan. Click OK.
- 6.4 After doing baseline scan, flush and fill cell with sample.
- 6.5 Click on 13th icon button from left (looks like graph). Click and drag cursor from bottom right to enlarge graph. Click on Graph at top of screen and go to Graph Preferences. For Grid Style, choose Solid. For Trace Width, choose 5 pixels, then OK. Then go to 4th icon button from left (Axes Scales). For X-Axis Scale, select 310 – 190. For Y-Axis Scale, choose 60-100.
- 6.6 Click on large START button. Save as month/year. Enter sample ID, then OK. Scan will now begin. After it has finished, click on Print.

7. QUALITY CONTROL

- 7.1 Run one blank every 10 samples (at least one per run). Run one duplicate for every ten samples or one duplicate per run for sets with less than ten samples; values should agree within 10%.
- 7.2 **Monthly, analyze the absorbance of a 0.5 mg/L KHP standard (may use TOC standard or dilution thereof); must be within 13% of the theoretical value of 0.009 cm⁻¹.**

[illegible]

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

HEXAVALENT CHROMIUM

File Name: M:\SOP\INORGNIC\CRVI.doc
Revision: 4

Effective Date: 10/10/2006
Supersedes: 3 (1/19/2006)

1. REFERENCES

- 1.1 Arar, E. J.; Long, S. E.; Pfaff, J. D. "Method 218.6 – Determination of Dissolved Hexavalent Chromium in Water, Groundwater, and Industrial Waste Water Effluents by Ion Chromatography", Revision 3.3, 1994, United States Environmental Protection Agency, Cincinnati, OH 45268.
- 1.2 *Standard Methods*, 19th Ed., #3500-Cr E.
- 1.3 Dionex Corporation Application Note 26, "Determination of Cr (VI) in Water, Waste Water, and Solid Waste Extracts".
- 1.4 Dionex Corporation Application Note 80, "Determination of Dissolved Hexavalent Chromium in Drinking Water, Groundwater and Industrial Waste Water Effluents by Ion Chromatography".

2. SUMMARY OF METHOD

- 2.1. An aqueous sample is filtered through a 0.45 um filter and the filtrate is adjusted to a pH of 9 to 9.5 with a concentrated buffer solution. A measured volume of the sample (500 uL) is introduced into the ion chromatograph. A guard column removes organics from the sample before the Cr (VI), as CrO_4^{2-} , is separated on a high capacity anion exchange separator column. Post-column derivatization of the Cr (VI) with diphenylcarbazide is followed by detection of the colored complex at 530 nm.

3. DEFINITIONS

- 3.1. Calibration Standard (CAL STD) – A solution prepared from the dilution of the stock standard solution. The CAL STD solutions are used to calibrate the instrument response with respect to analyte concentration.
- 3.2. Dissolved Analyte – The concentration of analyte in an aqueous sample that will pass through a 0.45 um membrane filter prior to pH adjustment.
- 3.3. Instrument Performance Check Solution (IPC) -- A solution of the method analyte, used to evaluate the performance of the instrument system with respect to a defined set of method criteria.

- 3.4. Laboratory Duplicates (LD1 and LD2) – Two aliquots of the same sample taken in the laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 indicates precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.
- 3.5. Laboratory Reagent Blank (LRB) – An aliquot of reagent water or other blank matrix that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents and internal standards that are used with other samples. The LRB is used to determine if the method analyte or other interferences are present in the laboratory environment, reagents or apparatus.
- 3.6. Laboratory Fortified Blank (LFB) – An aliquot of LRB to which a known quantity of the method analyte is added in the laboratory. The LFB is analyzed exactly like a sample and its purpose is to determine whether the methodology is in control and whether the laboratory is capable of making accurate and precise measurements.
- 3.7. Laboratory Fortified Matrix (LFM) – An aliquot of an environmental sample to which a known quantity of the method analyte is added in the laboratory. The LFM is analyzed exactly like a sample and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentration of the analyte in the sample matrix must be determined in a separate aliquot and the measured value in the LFM corrected for background concentration.
- 3.8. Linear Dynamic Range (LDR) – The concentration range over which the instrument response to an analyte is linear.
- 3.9. Method Detection Limit (MDL) – The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero.
- 3.10. Quality Control Sample (QCS) – A solution of the method analyte of known concentration which is used to fortify an aliquot of LRB or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check either laboratory or instrument performance.
- 3.11. Stock Standard Solution – A concentrated solution containing the method analyte prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

4. INTERFERENCES

- 4.1 Interferences which can affect the accurate determination of Cr (VI) may come from several sources.
 - 4.1.1 Contamination – A trace amount of Cr is sometimes found in reagent grade salts. Since a concentrated buffer solution is used in this method to

adjust the pH of samples, reagent blanks should be analyzed to assess for potential Cr (VI) contamination. Contamination can also come from improperly cleaned glassware or contact of caustic or acidic reagents or samples with stainless steel or pigmented material.

- 4.1.2 Reduction of Cr (VI) to Cr (III) can occur in the presence of reducing species in an acidic medium. At pH 6.5 or greater, however, CrO_4^{2-} which is less reactive than HCrO_4^- is the predominant species.
- 4.1.3 Overloading of the analytical column capacity with high concentrations of anionic species, especially chloride and sulfate, will cause a loss of Cr (VI). The column specified in this method can handle samples containing up to 5% sodium sulfate or 2% sodium chloride. Poor recoveries from fortified samples and tailing peaks are typical manifestations of column overload.

5. SAFETY

- 5.1 Hexavalent chromium is a toxic substance and a suspected carcinogen and should be handled with appropriate precautions. Extreme care should be exercised when weighing the salt for preparation of the stock standard. Material Safety Data Sheets should be made available to all personnel involved in the chemical analysis.

6. EQUIPMENT AND SUPPLIES

- 6.1 Dionex Model DX-600 Ion Chromatograph
 - 6.1.1 Instrument equipped with a pump capable of withstanding a minimum backpressure of 2000 psi and of delivering a constant flow of 1.5 mL/min and containing no metal parts in the sample, eluent or reagent flow path.
 - 6.1.1 Helium gas supply (High purity, 99.995%).
 - 6.1.2 Pressurized eluent container, plastic, 2 L size.
 - 6.1.3 Sample loop, 500 uL.
 - 6.1.4 Pressurized post-column reagent delivery module with a mixing tee and beaded mixing coil.
 - 6.1.5 Dionex IonPac NG1 guard column, placed before separator column and capable of removing strongly absorbing organics and particles that would otherwise damage the separator column.
 - 6.1.6 Dionex IonPac AS7 separator column, capable of separating CrO_4^{2-} from other sample constituents.

- 6.1.7 Dionex AD20 absorbance detector, a low-volume flow-through variable wavelength detector with a detection wavelength of 530 nm.
- 6.1.8 Computer with Dionex PeakNet software for receiving digital signals to record detector response in peak area as a function of time.
- 6.2 Labware – All reusable labware (glass, plastic, etc.), including sample containers, should be soaked overnight in laboratory grade detergent and water, rinsed with water, then soaked for 4 h in a mixture of dilute nitric acid and hydrochloric acid (1+2+9) followed by rinsing with tap water and ASTM Type I water.
 - 6.2.1 Plastic centrifuge tubes, 50 mL, metals-free.
 - 6.2.2 Pipettors, assorted fixed-volumes ranging from 25 uL to 2500 uL, with disposable metals-free tips.
 - 6.2.3 50 mL male luer-lock disposable syringes.
 - 6.2.4 0.45 um syringe filters, PALL Gelman Acro 50A.
- 6.3 Sample Processing Equipment
 - 6.3.1 Sample containers – High density polypropylene, 125 mL capacity.
 - 6.3.2 pH meter – pH range 0 – 14 with accuracy ± 0.03 pH units.
 - 6.3.3 0.45 um filter discs, 25 mm diameter (PALL Gelman Acro 50A, Mfr. P/N 4497 or equivalent).
 - 6.3.4 Plastic syringe, 10 mL or 60 mL capacity, disposable (BD 309604 or 309663 or equivalent).

7. REAGENTS AND STANDARDS

- 7.1 Reagents – All chemicals are ACS grade unless otherwise specified.
 - 7.1.1 Ammonium hydroxide, NH_4OH , (sp. gr. 0.902).
 - 7.1.2 Ammonium sulfate, $(\text{NH}_4)_2\text{SO}_4$.
 - 7.1.3 1,5-diphenylcarbazide.
 - 7.1.4 Methanol, HPLC grade.
 - 7.1.5 Sulfuric acid, concentrated, (sp. gr. 1.84).
- 7.2 Reagent Water – For all sample preparations and dilutions, ASTM Type I water is required. Millipore Milli-Q water is acceptable.

- 7.3 Cr (VI) Stock Standard Solution, 1000 mg/L – Dissolve 4.501 g of sodium chromate tetrahydrate, $\text{Na}_2\text{CrO}_4 \cdot 4\text{H}_2\text{O}$ in ASTM Type I water and dilute to 1 L. Transfer to a polypropylene storage container. As an alternative, use a commercially prepared and certified stock standard.
- 7.4 Laboratory Reagent Blank (LRB) – Prepare by adjusting pH of ASTM Type I water to 9 – 9.5 with buffer.
- 7.5 Laboratory Fortified Blank (LFB) – To an aliquot of LRB add an aliquot of stock standard to produce a final concentration of 1.0 ug/L of Cr (VI). The LFB must be carried through the entire sample preparation and analysis scheme.
- 7.6 Quality Control Sample (QCS) – A quality control sample must be obtained from a source outside the laboratory. Dilute according to instructions and analyze with samples.
- 7.7 Eluent, 250 mM $(\text{NH}_4)_2\text{SO}_4$ + 100 mM NH_4OH – Dissolve 33 g ammonium sulfate in 500 mL of ASTM Type I water, then add 6.5 mL ammonium hydroxide. Dilute to 1 L with ASTM Type I water. (Alternative reagent make-up: dissolve 66 g ammonium sulfate in 1000 ml of ASTM Type I water, then add 13 ml of ammonium hydroxide. Dilute to 2L with ASTM Type I water.) **Good for 7 days.**
- 7.8 Post-Column Reagent – Dissolve 0.5 g of 1,5-diphenylcarbazide in 100 mL of HPLC-grade methanol. Add to 500 mL of ASTM Type I water containing 28 mL of concentrated sulfuric acid (98%) while stirring. Dilute to 1 L with ASTM Type I water. **NOTE:** This reagent is stable for four to five days but should be prepared only as needed.
- 7.9 Buffer Solution – Dissolve 33 g of ammonium sulfate in ASTM Type I water and add 13 mL of ammonium hydroxide. Dilute to 100 mL with ASTM Type I water. **Prepare immediately before use.**

8. SAMPLE COLLECTION, PRESERVATION AND STORAGE

- 8.1 Filtration and pH adjustment should be performed as soon as possible after sample collection. For determination of dissolved Cr (VI), the sample is filtered through a 0.45 um syringe filter. Use a portion of the sample to rinse the filter, then collect the required volume of filtrate. Adjust the pH of all sample filtrates to 9 – 9.5 by slowly adding buffer and checking the pH with a pH meter, **before analyzing the sample. Do not analyze the sample until it has been filtered and pH adjusted.**
- 8.2 Ship and store the samples at 4°C. Bring samples to ambient temperature prior to analysis. Samples must be analyzed within 24 h of collection.

9. QUALITY CONTROL

- 9.1 This laboratory is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of

laboratory capability, and the analysis of laboratory reagent blanks (LRB), laboratory fortified blanks (LFB), and laboratory fortified sample matrix (LFM) as a continuing check on performance. This laboratory is required to maintain performance records that define the quality of the data thus generated.

9.2 Initial Demonstration of Performance (mandatory)

- 9.2.1 The initial demonstration of performance is used to characterize instrument performance (MDLs and linear dynamic range) and laboratory performance prior to sample analyses.
- 9.2.2 Method Detection Limit (MDL) – The MDL should be established using reagent water fortified at a concentration of two to five times the estimated detection limit. To determine the MDL value, take seven replicate aliquots of the fortified reagent water and process through the entire analytical method. Perform all calculations and report in the appropriate units. The MDL should be determined annually, when a new operator begins work or whenever there is a change in instrument analytical hardware or operating conditions.
- 9.2.3 Linear Dynamic Range (LDR) – The LDR should be determined by analyzing a minimum of seven calibration standards ranging in concentration from 0.2 ug/L to 100 ug/L. Samples having a concentration that is $\geq 90\%$ of the upper limit of the LDR must be diluted to fall within the bounds of the current calibration curve concentration range and reanalyzed.

9.3 Assessing Laboratory Performance (mandatory)

- 9.3.1 This laboratory must analyze at least one LRB (Section 7.4) with each set of samples. Reagent blank data are used to assess contamination from the laboratory environment. If the Cr (VI) value in the LRB exceeds the determined MDL, then laboratory or reagent contamination should be suspected. Any determined source of contamination should be corrected and the samples reanalyzed.
- 9.3.2 This laboratory must analyze at least one LFB (Section 7.5) with each set of samples. Calculate accuracy as percent recovery (Section 9.4.2). If the recovery of Cr (VI) falls outside the control limits (Section 9.3.3), then the procedure is judged out of control, and the source of the problem should be identified and resolved before continuing the analysis.
- 9.3.3 Until sufficient data become available (usually a minimum of 20 to 30 analyses), assess laboratory performance against recovery limits of 90 – 110%. When sufficient internal performance data becomes available, develop control limits from the percent mean recovery (\bar{x}) and the standard deviation (s) of the mean recovery. These data are used to establish upper and lower control limits as follows:

$$\text{UPPER CONTROL LIMIT} = \bar{x} + 3s$$

$$\text{LOWER CONTROL LIMIT} = \bar{x} - 3s$$

9.3.4 To verify that the instrument is properly calibrated on a continuing basis, run a LRB and an IPC (Section 3.3) after every ten analyses. If the measured concentration of the IPC (a midpoint calibration standard) deviates from the true concentration by more than $\pm 5\%$, perform another analysis of the IPC. If the discrepancy is still $\pm 5\%$ of the known concentration, the instrument must be recalibrated and the previous ten samples reanalyzed.

9.3.5 Quality Control Sample (QCS) – Each quarter, this laboratory should analyze one or more QCS. If the criteria provided with the QCS are not within $\pm 10\%$ of the stated value, corrective action must be taken and documented.

9.4 Assessing Analyte Recovery and Data Quality

9.4.1 This laboratory must spike a known amount of Cr (VI) to a minimum of 10% of samples. The concentration level can be the same as that of the LFB (Section 7.5). **Matrix spikes should be run in duplicate to check precision.**

9.4.2 Calculate the percent recovery for Cr (VI) corrected for background concentration measured in the unfortified sample and compare this value to the control limits established in Section 9.3.3 for the analysis of LFBs. Fortified recovery calculations are not required if the concentration of Cr (VI) added is less than 2X the sample background concentration. Percent recovery may be calculated using the following equation:

$$R = \frac{C_F - C}{F} \times 100$$

Where:

R = percent recovery

C_F = fortified sample concentration

C = sample background concentration

F = concentration equivalent of Cr (VI) added to sample

9.4.3 If the recovery of Cr (VI) falls outside control limits established in Section 9.3.3 and the recovery obtained for the LFB was shown to be in control (Section 9.3), the recovery problem encountered with the fortified sample is judged to be matrix related and not system related. The result for Cr (VI) in the unfortified sample must be labeled '**suspect matrix**'.

10. CALIBRATION AND STANDARDIZATION

- 10.1 Establish instrument operating conditions as indicated in Table 1. The flow rate of the eluent pump is set at 1.5 mL/min and the pressure of the post-column reagent delivery module adjusted so that the final flow rate of the post-column reagent from the detector is 2.0 mL/min. This requires manual adjustment and measurement of the final flow rate using a graduated cylinder and stop watch.
- 10.2 Before using the procedure (Section 11.0) to analyze samples, there must be data available documenting initial demonstration of performance. The required data and procedure is described in Section 9.2. This data must be generated using the same instrument operating conditions and calibration routine to be used for sample analysis. These documented data must be kept on file and be available for review by the data user.
- 10.3 The recommended calibration routine is given in Section 11.3.

11. PROCEDURE

- 11.1 Preserved samples should be brought to ambient temperature prior to analysis.
- 11.2 Initiate instrument operating configuration described in Section 10 and Table 1.
 - 11.2.1 Manually turn ON the two IP25 isocratic pumps, the CD20 conductivity detector and the AD25 absorbance detector. Then, turn ON the computer.
 - 11.2.2 Make sure there is enough eluent and post-column reagent available for the analysis.
 - 11.2.3 Turn the helium gas ON. Open valve on eluent module. Flip switch to ON on the PC10 pneumatic controller.
 - 11.2.4 Click on the RUN icon, highlight and maximize the ChromeVI method page.
 - 11.2.5 Load the ChromeVI method to start the pump and allow the system to equilibrate for at least 30 min.
 - 11.2.6 **Only if the instrument was not shut down properly as in 11.9 is it necessary to clean the absorbance cell before starting the analysis.** To do this, replace the post-column reagent bottle with the one marked "Methanol" and pressurize the chamber. Pump methanol through the cell for **no more than 5-10 minutes** or until the baseline stabilizes (excessive cleaning time with methanol is detrimental to the mixing coil). Next, replace the

methanol bottle with the one marked 'DI Water' and pump through cell until the baseline stabilizes. Finally, place post-column reagent bottle in chamber and allow the system to equilibrate. Otherwise, skip this step.

- 11.3 Calibration – Before samples are analyzed a calibration should be performed using a minimum of three calibration standards that bracket the anticipated concentration range of the samples. Calibration standards should be prepared from the stock standard (Section 7.3) by appropriate dilution with ASTM Type I water (Section 7.2). The standard solutions should be adjusted to pH 9 – 9.5 with the buffer solution (Section 7.9) prior to final dilution. Prepare the working calibration standards as per the following procedure. Record standards preparation in log book.
- 11.3.1 Intermediate Standard 1, 1000 ug/L – Place a clean metals-free centrifuge tube (Section 6.2.1) on balance and tare weight. Pipet 50 uL of 1000 mg/L Cr (VI) stock standard into the tube and add approximately 40 mL of Milli-Q water. Add buffer to adjust pH (about 100 uL is sufficient), then continue to add water to final weight of 50.00 g.
- 11.3.2 Intermediate Standard 2, 100 ug/L – Pipet 5000 uL of Intermediate Standard 1 into a clean, tared tube, adjust pH and bring to final weight of 50 g with Milli-Q water.
- 11.3.3 **CAL STD 1, 0.2 ug/L** – Dilute 100 uL of Intermediate Standard 2 to 50 g, adjusting pH before final dilution.
- 11.3.4 **CAL STD 2, 0.5 ug/L** – Dilute 250 uL of Intermediate Standard 2 to 50 g.
- 11.3.5 **CAL STD 3, 1.0 ug/L** – Dilute 500 uL of Intermediate Standard 2 to 50 g.
- 11.3.6 **CAL STD 4, 5.0 ug/L** – Dilute 250 uL of Intermediate Standard 1 to 50 g.
- 11.3.7 **CAL STD 5, 10.0 ug/L** – Dilute 500 uL of Intermediate Standard 1 to 50 g.
- 11.3.8 **CAL STD 6, 25.0 ug/L** – Dilute 1250 uL of Intermediate Standard 1 to 50 g.
- 11.4 Construct a calibration curve of analyte response (peak height or area) versus analyte concentration over a concentration range of one or two orders of magnitude. The coefficient of correlation (r) for the curve should be 0.999 or greater.

- 11.5 During the analysis of samples, the laboratory must comply with the required quality control described in Sections 9.3 and 9.4.
- 11.5.1 Laboratory Reagent Blank (LRB) – Filter Milli-Q water and adjust pH to 9 – 9.5 with buffer.
- 11.5.2 Laboratory Fortified Blank (LFB) – Dilute 500 uL of Intermediate Standard 2 (Section 11.3.2) with Milli-Q water, adjust pH and bring to final weight of 50 g with Milli-Q water. This yields a 1.0 ug/L concentration.
- 11.5.3 Instrument Performance Check (IPC) – Dilute 500 uL of Intermediate Standard 2 to 50 g. this yields a 1.0 ug/L concentration.
- 11.5.4 Laboratory Fortified Matrix (LFM) – Dilute 500 uL of Intermediate Standard 2 to 50 g with preserved sample. This yields a 1.0 ug/L spike concentration.
- 11.5.5 Quality Control Sample (QCS) – Follow manufacturer's specifications for preparation.
- 11.6 Sample concentrations that exceed the calibration range must be diluted and reanalyzed.
- 11.7 Create a sample schedule. Click on the SCHEDULE icon and open the Chrome (VI) schedule template. After entering the samples and required QC, save the schedule using the YYMMDD format. Then load the schedule to be run.
- 11.8 Load autosampler with samples to be analyzed and advance to first standard. Press HOLD/RUN key on autosampler to indicate RUN, then RUN and START on computer to start the analysis.
- 11.9 **When all samples are finished, flush cell with methanol for 5-10 minutes only (excessive cleaning time with methanol is detrimental to the mixing coil). To do this, simply replace post-column reagent reservoir with one containing methanol. After methanol, flush cell with DI water for 10 minutes.** Shut down computer first. Then turn OFF the pumps and detectors. Close the valve to the eluent and flip switch to VENT on PC10 pneumatic controller.

12. DATA ANALYSIS AND CALCULATIONS

- 12.1 The sample concentration can be calculated from the calibration curve. Report values in ug/L. Sample concentrations must be corrected for any Cr (VI) contamination found in the LRB.

- 12.2 The QC data obtained during sample analyses provide an indication of the quality of sample data and should be reported with sample results. This should be included in the data package for data review.

13. TROUBLESHOOTING

- 13.1 This method is simple and rugged but this troubleshooting guide has been included to minimize any down time. The guide lists symptoms of some of the common problems, their likely causes and remedies.

13.1.1 Symptom 1 – No peak observed.

- 13.1.1.1 Possible cause – No sample injected. Remedies include making sure the system gas pressure is ON and that the sample loop received the sample from the autosampler.
- 13.1.1.2 Possible cause – No post-column reagent flow. Check that the flow rate out of the cell is 2.0 mL/min.
- 13.1.1.3 Possible cause – Wrong detector wavelength. Check that the wavelength is set to 530 nm.

13.1.2 Symptom 2 – Noisy baseline.

- 13.1.2.1 Possible cause – Air bubble in cell. Flush cell with several mL of methanol.

13.1.3 Symptom 3 – Low column pressure.

- 13.1.3.1 Possible cause – Air in pump head. Prime pump with eluent.
- 13.1.3.2 Possible cause – Leak in system. Tighten or replace leaking fitting.

13.1.4 Symptom 4 – Excessive column pressure.

- 13.1.4.1 Possible cause – Improper flow rate. Check that pump flow rate is 1.5 mL/min.
- 13.1.4.2 Possible cause – Plugged fitting. With pump OFF, remove columns and reconnect eluent lines. Turn ON pump and check that the system pressure is less than 100 psi when valve is in either LOAD or INJECT position.
- 13.1.4.3 Possible cause – Plugged column bed support. Replace bed support.

13.1.5 Symptom 5 – Peak response too high or too low.

- 13.1.5.1 Possible cause – Incorrect sample loop size. Ensure sample loop is correct size.

13.1.5.2 Possible cause – Low post-column reagent flow rate.
Check that the flow rate from the detector waste line is 2.0 mL/min.

13.1.6 Symptom 6 – Poor peak shape and reasonable retention time.

13.1.6.1 Possible cause – Column is overloaded with a sample concentration that is too high. Dilute the sample and reanalyze.

13.1.7 Symptom 7 – Poor peak shape and incorrect retention time.

13.1.7.1 Possible cause – Eluent was prepared incorrectly.
Prepare new eluent.

13.1.7.2 Possible cause – Column is contaminated with strongly retained anions, metals, or organics. Pump acetonitrile through ONLY the NG1 guard column for 30 min, then rinse with Milli-Q water for 15 min. Pump 1 N HCl through all columns for one hour, rinse with water for 30 min, and reequilibrate with eluent for 30 min.

14. DATA TRANSFER TO LIMS

14.1 Laboratory Numbers and QC Codes. Use the following QC Codes when creating your sample schedule.

<u>QC Code</u>	<u>Definition</u>	<u>Example</u>
S	Spike	96080006-01S
K	Spike Duplicate	96080006-01K
Q	Sample Duplicate	96080006-01Q
M	MDL	MDL01YYMMDDM (Example: MDL01050308M)
C	1 PPB CC	01CC1YYMMDDC
L	LFB	Doesn't transfer
B	Reagent Blank	RB000YYMMDDB
R	Recheck	03060101-01R Doesn't transfer

14.2 Instrument File Generation. **(DO THIS BEFORE INSTRUMENT IS RUN AGAIN)**

- 14.2.1 To create the .csv file for use by the IC interface, go to the PeakNet main menu and click on the **BATCH** icon. From the top menu, select **File**, then Open. Highlight **Aspen.bch**, then click Open. From the top menu, select **Processing, Input, Select**, then find your schedule folder (for example 0601). Highlight schedule, then click Open. (Disregard error message "Error Opening Data File...") Go to box titled **Process Injections** and verify the correct number of samples to be transferred in the **Through** box. Click on **Export** and enter file name (for example a:\030612.csv). Then OK.
- 14.2.2 Place formatted floppy disk in drive. Click on **Processing**, then **Start**. You will see your sample files being copied.
- 14.2.3 At the computer, open Aspen. Place disk in drive and click **Import Data**. Select file type, in this case **CrVI** IC file. Select location – Browse. Highlight data file and Open. Start Import. Continue. Enter analysis date and initials when prompted. Once the import is complete, click **Send to LIMS** button and data will be sent to LIMS. Create new worksheet when prompted. Review worksheet and check for errors.

TABLE 1. ION CHROMATOGRAPHIC CONDITIONS

Columns:	Guard Column – Dionex IonPac NG1 Separator column – Dionex IonPac AS7
Eluent:	250 mM (NH ₄) ₂ SO ₄ 100 mM NH ₄ OH Flow rate = 1.5 mL/min
Post-Column Reagent:	2 mM 1,5-diphenylcarbazide 10% v/v CH ₃ OH 1 N H ₂ SO ₄ Flow rate = 0.5 mL/min
Detector:	Visible, 530 nm
Retention time:	3.3 min

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ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

COLIFORMS BY MULTIPLE TUBE FERMENTATION TECHNIQUE

File Name: M:\SOP\INORGNIC\MULTI_FM.TUB.doc
Revision: 5

Effective Date: 12/3/2008
Supersedes: 4 (11/10/2008)

NOTE: This SOP is for WASTEWATER and other NON-POTABLE water samples ONLY! Amendments have been added when applicable for DRINKING WATER samples that must conform to the TOTAL COLIFORM RULE.

1. REFERENCES

- 1.1 *Standard Methods for the Analysis of Water and Wastewater*, 18th, 19th, and 20th ed., APHA, AWWA, WEF., Sections 9221B and 9221E.
- 1.2 Manual for the Certification of Laboratories Analyzing Drinking Water, EPA 815-B-97-001, March 1997.
- 1.3 Code of Federal Regulations, Vol. 40, 2006, revised March 12, 2007.

2. SAMPLE HOLDING TIME

- 2.1 6 hours at 4°C; best if analyzed within one hour of sample collection.

3. SAMPLE COLLECTION

- 3.1 Samples are to be collected in sterile 500mL bottles containing sodium thiosulfate dechlorinating agent (see Inorganic Laboratory Technician SOP). When sample is collected, leave ample air space in the bottle (at least 2.5cm) to facilitate mixing of sample upon examination. Keep sample bottle closed until time of collection. Fill container without rinsing, then cap immediately.

4. APPARATUS

- 4.1 Incubator - Maintain stable temperature of $35 \pm 0.5^{\circ}\text{C}$. Include precision thermometer, with bulb immersed in liquid (glycerine, water or mineral oil). Calibrate thermometer against NIST-certified reference thermometer **annually** and retain written documentation. Check and record temperature twice daily, with readings at least four hours apart.
- 4.2 Water Bath **with cover** - Maintain stable temperature of $44.5 \pm 0.2^{\circ}\text{C}$, with precision thermometer immersed in water. Calibrate thermometer against NIST-certified reference thermometer **annually** and retain written documentation. Check and record temperature twice daily, with readings at least four hours apart.

- 4.3 Autoclave - Provides uniform temperature of 121°C and pressure of 15 psi. Test sterilization performance **monthly** with a spore ampule included with the load. Check temperature **weekly** using a high temperature recording mercury thermometer. Check autoclave timer **quarterly** using a stopwatch to ensure that temperature and pressure are maintained for the set time. Keep records of spore check, temperature and timer checks in the autoclave logbook. Use heat-sensitive tape where appropriate to identify materials that have been sterilized. Record date, time in, time out, sterilization time, and load type for each run. All loads except media must be autoclaved for 30 minutes.
- 4.4 Refrigerator - Maintains temperature at $4 \pm 2^{\circ}\text{C}$. Calibrate thermometer against NIST-certified reference thermometer **annually** and retain written documentation. Check and record temperature in log daily.
- 4.5 pH Meter - Calibrated before use according to manufacturer's specifications.
- 4.6 Alcohol Burner.
- 4.7 Pipets - TD serological, sterile, 1mL and 10mL.
- 4.8 Milk dilution bottles - Marked at 99 mL volume, screw cap with rubber liner.
- 4.9 Culture tubes, 20 x 150 mm or similar.
- 4.10 Fermentation vials (also known as Durham tubes), 12 x 35 mm or similar.
- 4.11 Culture tube closures, #20.
- 4.12 Culture tube racks, plastic, autoclavable.
- 4.13 Inoculating Loop - At least 3mm diameter, sterile disposable bulk pack.
- 4.14 Petri dishes, 15 x 100mm, sterile plastic.
- 4.15 Sterile sample bottles, 500mL - Polypropylene, autoclavable, containing 0.4 mL of 10% $\text{Na}_2\text{S}_2\text{O}_3$ dechlorinating agent. Lab Technician is responsible for preparing bottles. See Lab Technician SOP for details.
- 4.16 Dispenser, 10 mL – For dispensing liquid media into culture tubes. Check accuracy with a graduated cylinder.

5. MEDIA AND REAGENTS

Commercially prepared media is **preferred** (HACH tubes; see below for cat #s). Holding time is **three months** from date of manufacture for media in screw-cap tubes and stored in refrigerator; take out overnight at room temperature before use. Make sure to check pH and positive/negative QC control organisms **for EACH NEW BATCH/LOT Number** and record in Media logbook. **For all media prepared from**

dry powder in laboratory: Powdered medium must not be past mfr's. expiration date *or* internal expiration date, which is 6 months past date of opening, whichever comes first.

- 5.1 **(10 mL samples only) Lauryl Tryptose Broth, Concentrated (Hach LTB Concentrated Tubes, #21014-15, 15/pk, \$21.50/pk).** Or, to make double strength LTB in laboratory, follow manufacturer's directions for preparation. Suspend 71.2g in 1L DI water and warm to dissolve completely. Dispense 10 mL portions to culture tubes containing fermentation vials. Place closures on tubes and sterilize in autoclave for **exactly 15 minutes** at 15psi and 121°C. **Total time in autoclave should not exceed 45 minutes.** Final pH should be 6.8 ± 0.2 . Record date, pH, lot #, amount of dry medium used and volume made in Media logbook. Holding time for loosely capped lab-made sterile media is **2 weeks when stored in refrigerator; take out overnight at room temperature before use.** Check each new batch prepared for QC. Use positive (*Enterobacter aerogenes*) and negative (*Pseudomonas aeruginosa*) control cultures, and check sterility. Document this QC in the Media logbook.
- 5.2 **Lauryl Tryptose Broth, Single Strength (Hach LTB Single Strength Tubes, #21623-15, 15/pk, \$20.90/pk).** To make in laboratory, follow manufacturer's directions for preparation. Suspend 35.6g in 1L DI water and warm to dissolve completely. Proceed as above in Section 5.1.
- 5.3 **Brilliant Green Bile 2% (BGB) (Hach BGB Tubes, #322-15, 15/pk, \$20.90/pk).** To make in laboratory, follow manufacturer's directions for preparation. Suspend 40g in 1L DI water and warm to dissolve completely. Proceed as above in Section 5.1, except final pH should be 7.2 ± 0.2 .
- 5.4 **EC Medium (Hach EC Medium Tubes, #14104-15, 15/pk \$19.90/pk).** To make in laboratory, follow manufacturer's directions for preparation. Suspend 37g in 1L DI water and warm to dissolve completely. Proceed as above in Section 5.1, except final pH should be 6.9 ± 0.2 . For QC, use positive (*Escherichia coli*) and negative (*Enterobacter aerogenes*) control cultures, and check sterility.

SAFETY NOTE: Hach BGB and EC medium tubes are known to frequently have their caps stuck on more tightly than LTB tubes. This can be hazardous if a tube breaks when additional force is applied to remove a stuck cap. Always wear surgical gloves or other hand protection when removing all Hach tube caps, and avoid using excessive force.

- 5.5 **m-Endo Agar LES** - Follow manufacturer's directions for preparation. Suspend 51g in 1L DI water containing 20mL non-denatured ethanol and heat in a beaker of boiling water to dissolve completely. **Do not autoclave.** Cool to 45-50°C and aseptically dispense 11mL aliquots into 15 x100 mm petri dishes and allow to solidify. Final pH should be 7.2 ± 0.2 . Record date, pH, lot#, amount of dry medium used and volume prepared in Media logbook. Check QC using positive / negative controls (*Enterobacter aerogenes* / *Pseudomonas aeruginosa*). Store poured agar plates in a sealed bag in the refrigerator at 4 C for up to 2 weeks.

- 5.6 Sodium Thiosulfate Solution, 10% - Lab Technician is responsible for preparing. Refer to Lab Technician SOP for directions.
- 5.7 Phosphate Buffer - Lab Technician is responsible for preparing buffered dilution water. Refer to Lab Technician SOP for directions.
- 5.8 Pure cultures of *Pseudomonas aeruginosa*, *Escherichia coli* and *Enterobacter aerogenes* - Transfer cultures to nutrient agar slants at least monthly. Preferably prepare new fresh cultures monthly.
- 5.9 Nutrient Agar - Follow manufacturers directions for preparation. Suspend 23g in 1L DI water and heat in a boiling water bath while stirring to dissolve completely. Sterilize in autoclave for 15 minutes at 15psi and 121°C. **Total time in autoclave should not exceed 45 minutes.** Sterilize required number of 16 x 125 mm screw-cap tubes. Aseptically dispense about 8 mL tempered agar into each sterile tube and allow to cool in a slant position. The length of the agar surface should be about 6.5 cm. After agar has solidified, store tubes in refrigerator until use. Final pH should be 6.8±0.2. Record date, pH, lot#, amount of dry medium used and volume prepared in Media logbook. Holding time for sterile agar slants is 3 months when stored at 4 C.

6. PROCEDURES

6.1 Presumptive Test

- 6.1.1 Arrange LTB tubes as follows for each sample of non-potable water to be tested:

First Row - Five tubes of **concentrated or double-strength** LTB.

Second Row - Five tubes of single-strength LTB.

Third Row - Five tubes of single-strength LTB.

Be sure to retain the adhesive label showing the lot # and manufacture date (preferably attached to the tube rack) for documentation, and log lot # and lab calculated expiration date (see sec. 5 above) with every sample in the data book.

Note: If media has been refrigerated, allow to warm to room temperature overnight before use.

NOTE: For **DRINKING WATER** sample, use 10 tubes of **concentrated or double strength LTB. (100 mL sample to be tested).**

- 6.1.2 Shake sample container and dilutions vigorously 30 times in an up-down motion. Inoculate first row of tubes with 10mL each of sample. Inoculate second row with 1mL each of sample and inoculate third row of tubes with 0.1mL each of sample. **In order to keep track of which row of tubes contains which sample volume, label the rows "10 mL," "1 mL," and**

“.1 mL.” Serially dilute original sample if needed. If using the commercially prepared HACH tubes, invert the tubes **once** after inoculation to remove any trapped air bubbles. Check tubes again after several hours for trapped air bubbles.

- 6.1.3 Incubate inoculated tubes at $35 \pm 0.5^\circ\text{C}$. **Be sure to allow adequate space for air circulation between racks of tubes in the incubator.** After 24 ± 2 hours, swirl each tube gently and examine it for growth and gas production. If no gas production is observed, reincubate and reexamine at the end of 48 ± 3 hours. Record presence or absence of growth, gas and acid production.
- 6.1.4 Production of gas within 48 ± 3 hours constitutes a positive presumptive reaction. Submit all positive presumptive tubes to the confirmation phase. Absence of gas at the end of 48 ± 3 hours constitutes a negative test and may be discarded.
- 6.1.5 **For DRINKING WATER samples ONLY. Negative Invalidation using the Total Coliform Rule. All samples showing a turbid culture (i.e. heavy growth) in the absence of gas/acid production are invalidated and another sample must be collected from the same location within 24 hours. (If the lab performs confirmed test on turbid culture and confirmed test is total coliform positive, sample is reported as such, but if total coliform-negative, sample is invalidated).**
- 6.1.6 **When MTF test is used on Drinking Water samples that have a history of confluent growth or TNTC by the MF procedure, all presumptive tubes with heavy growth without gas production must be submitted to the confirmed test and fecal coliform test to check for coliform suppression.**

6.2 Confirmed Test

- 6.2.1 Submit all presumptive tubes showing heavy growth and gas production after 24 to 48 hours to the confirmed phase.
- 6.2.2 Gently shake positive presumptive tubes to resuspend the organisms. With a sterile inoculating loop, transfer one loopful of culture to each of parallel tubes containing BGB broth and EC medium. Repeat for all other positive presumptive tubes.
- 6.2.3 Incubate the inoculated BGB broth tubes for 48 ± 3 hours at $35 \pm 0.5^\circ\text{C}$. Formation of gas at any time within 48 ± 3 hours constitutes a positive confirmed test. Calculate the MPN value from the number of positive BGB broth tubes. See Table 9221.IV. MPN Index on page 9-52 of the 20th edition of *Standard Methods*.

- 6.2.4 Incubate inoculated EC medium tubes in a **covered** water bath at $44.5 \pm 0.2^\circ\text{C}$ for 24 ± 2 hours. **Bath must remain covered at all times except when inserting or removing racks of fermentation tubes.** Maintain a sufficient water depth in the water bath incubator to immerse tubes to upper level of the medium (**Hach screw-cap tubes may be completely submerged in a rack with a lead donut on top**). Gas production with growth within 24 hours is considered a positive fecal coliform reaction. Failure to produce gas (with little or no growth) constitutes a negative reaction. Use Table 9221.IV to calculate MPN.
- 6.2.5 **Negative and positive results for each presumptive and confirmatory (including EC medium) tube are to be logged into the data book every 24 hours. For every sample, be sure to log sample ID, date sampled, dilution (if any), date and time presumptive incubation began, and date and time of reading/transfer and beginning of incubation of confirmatory media. Note that all entries in data book should have the above documentation for start ("In at") time, 24 hours, and 48 hours at minimum. Samples that require confirmation will potentially need 72- and 96-hour readings as well.**

6.3 Completed Test

To establish the presence of coliform bacteria and to provide quality control data, use the completed test on at least 10% of positive confirmed tubes or a **minimum of once per quarter**.

Completed Test is not required for Source / Surface water samples.

Source / Surface water samples that produce turbid growth in the absence of gas / acid production in LTB are invalidated and another sample obtained, which may be tested with another method, i.e. MF or Colilert.

- 6.3.1 Using aseptic technique, streak one LES Endo agar plate from each tube of BGB broth showing gas. Streak plates in a manner to insure presence of some discrete colonies separated by at least 0.5cm. Incubate plates inverted at $35 \pm 0.5^\circ\text{C}$ for 24 ± 2 hours.
- 6.3.2 The colonies developing on LES Endo agar are defined as *typical* (pink to dark red with a green metallic sheen); *atypical* (pink, red, white, or colorless colonies without sheen) after 24 hours incubation; or *negative* (all others).
- 6.3.3 From each LES Endo agar plate, pick one or more well-isolated coliform colonies, and transfer to single strength LTB. Incubate at $35 \pm 0.5^\circ\text{C}$ for 24 ± 2 hours. If gas is not produced within 24 hours, reincubate and examine again at 48 ± 3 hours.
- 6.3.4 Perform a gram-stain test on positive colonies from LES Endo agar plates. Consult *Standard Methods* for the procedure. The gram stain may be omitted from the Completed Test for **Drinking Water** samples only

because the chances of gram-positive bacteria and spore-forming organisms surviving this selective screening procedure are infrequent in drinking water.

6.4 Interpretation:

- 6.4.1 Formation of gas in the secondary tube of LTB within 48 hours and demonstration of gram-negative, nonspore-forming, rod-shaped bacteria constitutes a positive result for the completed test, demonstrating the presence of a member of the coliform group.

7. COMPUTING AND RECORDING OF MPN

- 7.1 To calculate coliform density, compute in terms of the most probable number (MPN).
- 7.2 For combinations of positive and negative results when five 10mL, five 1.0mL, and five 0.1mL volumes of sample are tested, use Table 9221.IV on page 9-52 of the 20th edition of *Standard Methods*. **For Drinking Water samples, with 10 tubes of 10 mL each, use Table 9221.III.**
- 7.3 When the series of decimal dilutions is different from the table, select the MPN value from Table 9222.IV for the combination of positive tubes and calculate according to the following formula:

$$\frac{\text{MPN value (from table)} \times 10}{\begin{array}{l} \text{Largest volume tested in dilution} \\ \text{Series used for MPN determination} \end{array}} = \text{MPN} / 100 \text{ mL}$$

- 7.4 The MPN for combinations not appearing in the table, or for other combinations of tubes or dilutions, MPN may be estimated by the Thomas Formula.

$$\text{MPN}/100 \text{ mL} = \frac{\text{no. of positive tubes} \times 100}{\begin{array}{l} \sqrt{\text{mL sample in}} \times \sqrt{\text{mL sample in}} \\ \text{negative tubes} \quad \quad \text{all tubes} \end{array}}$$

SOP PROCEDURE CHANGE
FOR COLIFORMS BY MULTIPLE TUBE FERMENTATION TECHNIQUE

CHANGE	REASON	DATE	INITIALS
Revision #2	Corrective action for audit of 12/02	2/1/03	JAB

Added medium need to be taken out from refrigerator overnight before use.		7/28/05	FC
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Included language preferring, and inserted safety-related language regarding uncapping Hach pre-made vials		9/19/06	JMD
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Added notes under 6.1.1 to retain label showing lot # and manufacture date attached to tube rack; under 6.1.2 to label tube rows with sample volume; under 6.1.3 to allow air circulation space between tube racks in incubator; and added 6.2.5 to clarify intermediate date/time data logging requirements in data book.		11/10/08	JMD
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Revised 4.2 and 6.2.4 to reflect a covered water bath for fecal coliforms.		12/3/08	JMD
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ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

FECAL COLIFORM ANALYSIS (Membrane Filter Method)

File Name: M:\SOP\INORGNIC\MF_FECAL.doc
Revision: 2

Effective Date: 02/01/2003
Supersedes: 1 (11/04/1996)

NOTE: This SOP is for WASTEWATER and other NON-POTABLE water samples ONLY! Amendments have been added when applicable for DRINKING WATER samples that must conform to the TOTAL COLIFORM RULE.

1. REFERENCES

- 1.1 *Standard Methods for the Analysis of Water and Wastewater*, 18th, 19th, and 20th ed., APHA, AWWA, WEF., Section 9222D.
- 1.2 Manual for the Certification of Laboratories Analyzing Drinking Water, EPA 815-B-97-001, March 1997.
- 1.3 Code of Federal Regulations, Vol. 40, 2001.

2. HOLDING TIME

- 2.1 6 Hours @ 4°C; best if analyzed within one hour of sample collection.

3. INTERFERENCES

- 3.1 Samples high in turbidity; samples low in coliform bacteria and high in non-coliform bacteria.

4. APPARATUS

- 4.1 Water Bath - Maintain stable temperature of $44.5 \pm 0.2^{\circ}\text{C}$, with precision thermometer immersed in water. Calibrate thermometer against NIST-certified reference thermometer **annually** and retain written documentation. Check and record temperature twice daily, with readings at least four hours apart..
- 4.2 Waterproof plastic bags, *Whirl-Pak*™ or equivalent.
- 4.3 Refer to Total Coliform MF SOP, Sections 4.2 to 4.15 and 4.17 to 4.18 for additional required apparatus.
- 4.4 Membrane Filters - Sterile, white, grid-marked, 47 mm diameter, 0.7 ± 0.02 um pore size, certified by manufacturer for water analysis.

5. MEDIA AND REAGENTS

- 5.1 m-FC Broth with rosolic acid – We normally use HACH *PourRite*™ broth ampules, catalog number 24285-20. When ordering, state “All same lot number” and “Manufacture date must be no more than 7 days before ship date.” ELAP requires expiration date to be manufacture date plus three months. Store continuously at 4 C before use. Check each new lot with positive (*Escherichia coli*) and negative (*Enterobacter arogenes*) control cultures, and for sterility.
- Also verify acceptable pH for each lot. Document this QC in the Media logbook.

Procedure for making up broth from dry medium: (Note: Powdered medium must not be past mfr's. expiration date *or* internal expiration date, which is 6 months past date of opening, whichever comes first.) Add 100mL DI water to 3.7g dehydrate m-FC medium in a 250-mL screw-cap Erlenmeyer flask. In a separate flask, add 10mL 0.02N NaOH to 100mg Rosolic Acid dehydrate to produce a 1% Rosolic Acid solution. Pipet 1mL of 1% Rosolic Acid solution into rehydrated m-FC broth. Loosely cap and place in boiling water bath. Heat medium 3 - 5 minutes. Bring to point of boiling, but do not allow to boil. Then promptly remove and cool. Final pH must be between 7.2 and 7.6. Medium may be refrigerated at 4°C for 96 hours maximum. Record date, pH, lot #, amount of dry medium used and volume made in Media log book. Also check performance with positive and negative controls as mentioned above.

- 5.2 EC Medium - Refer to manufacturers directions and OCWD SOP for Fecal Coliforms by Multiple Tube Fermentation Technique for preparation details.
- 5.3 Pure Cultures of *E. coli* and *Enterobacter aerogenes* - Transfer cultures to nutrient agar slants at least monthly. Preferably prepare fresh pure cultures monthly.
- 5.4 Refer to Total Coliform MF SOP, Sections 5.5, 5.6 and 5.8 for additional required media and reagents.

6. PROCEDURE

- 6.1 Disinfect work area with 70% alcohol before and after performing each separate activity. Wipe neck of m-FC broth ampule with 70% alcohol and carefully break ampule. Pour contents of ampule onto absorbent pad of sterile petri dish, or, if broth was made up from dry medium, pipet 1.8 mL onto each sterile absorbent pad. Sterilize forceps. Aseptically open and transfer sterile membrane filter to filter base and attach funnel to base. Follow general membrane filtration procedures outlined in OCWD Standard Operating Procedure for Total Coliforms by Membrane Filtration. After filtration and placement of membrane filter on petri dish, place dish inside waterproof bag and seal. Submerge bags, with petri dishes inverted, in water bath within 30 minutes of filtration. Incubate 24 hours at $44.5 \pm 0.2^{\circ}\text{C}$. After the required incubation period, remove petri dish from water bath. Place bottom of dish on microscope field. Examine and count all blue colonies.

7. CALCULATION OF FECAL COLIFORM DENSITY

- 7.1 Fecal Coliform Colonies/100 mL = $\frac{\text{Coliform colonies counted} \times 100}{\text{mL of sample filtered}}$
- 7.2 The minimum/maximum range for colony forming units on the membrane filter is 20 - 60 coliform colonies. Fecal coliform colonies on m-FC medium are various shades of blue. Nonfecal coliform colonies are gray to cream-colored.
- 7.3 **Fecal Coliform Verification: For water samples OTHER than drinking water quality**
- 7.3.1 Verify a minimum of 10 typical and atypical colonies per month. Verify by transferring growth from each colony to Lauryl Tryptose Broth. Incubate 24 - 48 hours at 35°C. Confirm gas-positive LTB tubes at 24 and 48 hours by inoculating a loopful of growth into EC Medium tubes and incubate for 24 hours at 44.5°C. Cultures that produce gas in EC Medium are interpreted as verified fecal coliform colonies.
- 7.4 **Drinking Water samples**
- 7.4.1 Required sample volume to be tested is 100 mL.
- 7.4.2 Follow verification procedure from Section 7.3.1.
- 7.4.3 Promptly notify proper authority of positive fecal coliforms. Notification record kept.

8. QUALITY CONTROL

- 8.1 Check sterility, positive and negative controls, and pH on each new lot of media used, and record in Media Prep logbook.
- 8.2 See SOP for Total Coliform Analysis for additional information.

9. TROUBLESHOOTING

- 9.1 Possible sources of poor results include:
- 9.1.1 Bacterial contamination at any stage of the analysis procedure. Insure sterility at all stages by using proper aseptic technique.
- 9.1.2 Power outage or water bath failure, causing temperature to go outside of acceptable range. Review documentation.
- 9.1.3 Heat-damaged or incorrectly prepared media. Remake.

CHANGE	REASON	DATE	INITIALS
Revised.	Corrective action for audit of 12/02	2/1/03	JAB

CHANGE	REASON	DATE	INITIALS
Revised.	Corrective action for audit of 12/02	2/1/03	JAB

Page 4 of 4

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

HETEROTROPHIC PLATE COUNT POUR PLATE AND SPREAD PLATE METHODS

File Name: M:\SOP\INORGNIC\HETERO.PLC.doc
Revision: 5

Effective Date: 10/8/2007
Supersedes: 4 (10/23/2006)

1. REFERENCES

- 1.1 *Standard Methods for the Analysis of Water and Wastewater*, 18th, 19th and 20th ed., APHA, AWWA, WEF., Sections 9215A, B, and C.
- 1.2 Manual for the Certification of Laboratories Analyzing Drinking Water, EPA 815-B-97-001, March 1997.
- 1.3 Code of Federal Regulations, Vol. 40, 2006, revised March 12, 2007.

2. PRESERVATION

- 2.1 Refrigerate @ 4°C

3. HOLDING TIME

- 3.1 8 hrs MAXIMUM; Analyze as soon as possible.

4. APPARATUS

- 4.1 15 x 100 mm plastic disposable Petri dishes.
- 4.2 1 mL sterile plastic disposable pipets.
- 4.3 10 mL sterile plastic disposable pipets.
- 4.4 15 mL screw-capped culture tubes.
- 4.5 Incubator - Capable of maintaining a stable temperature of $35 \pm 0.5^{\circ}\text{C}$. Include precision thermometer, with bulb immersed in liquid (glycerine, water or mineral oil). Calibrate thermometer against NIST-certified reference thermometer **annually** and retain written documentation. Check and record temperature twice daily, with readings at least four hours apart.
Note: This incubator is for samples using Pour Plate Method, Section 9215B.

- 4.6 Incubator - Capable of maintaining a stable temperature of $28 \pm 0.5^{\circ}\text{C}$. Calibrate thermometer against NIST-certified reference thermometer **annually** and retain written documentation. Check and record temperature twice daily, with readings at least four hours apart.

Note: This incubator is for samples using Spread Plate Method, Section 9215C.

- 4.7 250-mL screw-capped Erlenmeyer flasks.
- 4.8 160-mL milk dilution bottles.
- 4.9 Quebec dark field colony counter.
- 4.10 Glass rod. Bend 4-mm diameter fire-polished glass rod, 200 mm in length, 45° about 40 mm from one end. Sterilize before using.

5. MEDIA PREPARATION

NEW—Use R2A Agar for both Pour and Spread Plate Methods, per *Standard Methods*: Follow manufacturer's directions for preparation: Suspend 18.2 g dry powder (do not use powdered medium past manufacturer's expiration date or internal expiration date, which is 6 months past date of opening, whichever comes first) in 1 L DI water and heat in a boiling water bath, allowing media to boil for one minute while stirring to dissolve completely. Sterilize in autoclave loosely capped for 15 minutes at 15 psi and 121°C . Total time in autoclave should not exceed 45 minutes. Final pH should be 7.2 ± 0.2 . Record date, pH, lot #, amount of dry powder used and volume made in Media logbook.

- 5.1 **Pour Plate Method (9215B):** Pour 100 mL dissolved agar into each of 10 milk dilution bottles. After sterilization (or after re-melting previously refrigerated agar in boiling water bath), temper melted agar at $44 - 46^{\circ}\text{C}$ in water bath before pouring. **Do not hold melted agar longer than 3 hours, and melt only once. Discard any unused agar in biohazard bag in trash so as not to clog sink drain.** Holding time for agar held in screw-cap bottles is 3 months in refrigerator at 4°C .
- 5.2 **Spread Plate Method (9215C):** heat in a screw-capped flask or bottle of sufficient size in boiling water bath while stirring to dissolve completely. After sterilization, temper melted agar at $44 - 46^{\circ}\text{C}$ in water bath before pouring. Aseptically pour 15 mL tempered agar into **each of a quantity of** sterile petri dishes and let agar solidify. Predry plates inverted so there is a 2 to 3 g water loss overnight with lids on. Use predried plates immediately after drying or store for up to 2 weeks in sealed plastic bags at 4°C .
- 5.3 Buffered dilution water – Lab aide is responsible for preparing buffered dilution water. Refer to Lab Aide SOP for directions.

6. PROCEDURES

6.1 **Method 9215B – Pour Plate Method.** This method is used for HPC testing of waters for compliance with Surface Water Treatment Rule. Select the dilution(s) so that the total number of colonies on a plate will be between 30 and 300. For most potable water samples, plates suitable for counting will be obtained by plating 1 mL and 0.1 mL undiluted sample and 1 mL of the 10^{-2} dilution.

- 6.1.1 Use a sterile pipet for initial and subsequent transfers from each container. When removing sample, do not insert pipets more than 2.5 cm below the surface of sample or dilution.
- 6.1.2 When discharging sample portions, hold pipet at a 45 degree angle with the tip touching the bottom of the petri dish. Use gentle blow out of remaining volume. Pipet 1 mL, 0.1 mL, or other suitable volume into petri dish before adding melted agar. Prepare at least one duplicate plate per each dilution used. After dispensing sample into plate, “pour” tempered agar (**pipet approx. 11 mL tempered agar into dish using a sterile disposable pipet**). **Mix carefully by sliding Petri dish in a figure-8 pattern on benchtop.**
- 6.1.3 Limit the number of samples to be plated in any one series so that no more than 20 minutes elapse between pipetting first sample and pouring of the last plate in the series. Pour at least 10 to 12 mL tempered agar into each dish. As each plate is poured, mix sample and agar thoroughly by rotating in a gentle figure eight motion. Let agar solidify, then invert plates and place in incubator.
- 6.1.4 Incubate pour plates at $35 \pm 0.5^{\circ}\text{C}$ for 48 hours. During incubation, maintain humidity within the incubator so moisture loss is no greater than 15 %. Stack plates no more than 4 high.
- 6.1.5 For QC, include an agar sterility control, a sterility control for dilution water (if used), and a room air control (< 15 cfu / plate for 15 minute exposure).

6.2 **Method 9215C – Spread Plate Method.**

- 6.2.1 Select the dilution(s) so that the total number of colonies on a plate will be between 30 and 300.
- 6.2.2 Pipet 0.1 or 0.5 mL sample onto the surface of predried agar plate. Using a sterile bent glass rod, distribute the inoculum evenly over the surface of the medium by rotating dish by hand or on a turntable. Be careful not to gouge the surface. Allow the inoculum to be completely absorbed into the medium before incubating.

- 6.2.3 Incubate spread plates at $28 \pm 0.5^{\circ}\text{C}$ for 7 days. Seal plates in plastic bags. During incubation maintain humidity within the incubator so that plates will have no moisture weight loss greater than 15 %. Place a container of water in the incubator to maintain humidity.

7. COUNTING AND RECORDING

- 7.1 Colonies are counted manually using a dark field colony counter. Consider only plates having 30 to 300 colonies in determining the plate count, except for plates inoculated with 1 mL of undiluted sample (drinking water and source/surface water). Compute bacterial count per milliliter of sample by multiplying the average number of colonies per plate by the reciprocal of the dilution used. Report as "CFU / mL". If there is no plate with 30 to 300 colonies, and one or more plates have more than 300 colonies, use the plate(s) having a count nearest 300 colonies. Compute the count by multiplying the average count per plate by the reciprocal of the dilution used and report as "Estimated CFU / mL". If plates from all dilutions of any sample have no colonies, report the count as less than one times the reciprocal of the corresponding lowest dilution. For example, if no colonies develop on the 1:100 dilution, report the count as <100 estimated CFU/mL.
- 7.2 If the number of colonies per plate far exceeds 300, do not report result as "too numerous to count" (TNTC). If there are fewer than 10 colonies/cm², count colonies in 13 squares of colony counter. If possible, select seven consecutive squares horizontally across the plate and six vertically, taking care not to count the same square twice. Multiply sum of the number of colonies in 13 square centimeters by 5 to compute the estimated colonies per plate. When there are more than 10 colonies/cm², count four squares, take an average count per square centimeter, then multiply by a factor of 57 for disposable plastic plates and 65 for glass plates. When bacterial counts are greater than 100 colonies/cm², report result as greater than (>) 5700 times reciprocal of highest dilution plated for plastic plates. Report as estimated CFU/mL.
- 7.3 If spreading colonies are encountered, count colonies on representative portions only when colonies are well distributed in spreader-free areas and the area covered by spreaders does not exceed one-half the plate area. If plates have excessive spreader growth, report as "spreaders". When plates are uncountable because of missed dilution, accidental dropping, contamination, or the control plates indicate that the medium or other material or labware was contaminated, report as "laboratory accident" (LA).

8. COMPUTING AND REPORTING COUNTS

- 8.1 Report all counts as "colony-forming units" (CFU). Include in the report the method used, incubation temperature and time, and the medium used
- 8.2 To compute the heterotrophic plate count, multiply the total number of colonies or

average number for duplicate plates by the reciprocal of the dilution used.

9. QUALITY CONTROL

- 9.1 All normal microbiological quality control procedures should be followed. In addition, agar and dilution blank controls and duplicates of all sample dilutions must be done for each set of samples analyzed. There should be no contamination indicated in the controls, and duplicates should agree within 10%.

10. TROUBLESHOOTING

- 10.1 Colonies found in the controls will point to improper sterilization or subsequent contamination of media and labware. Remake and request resampling in order to repeat analysis. Overheating of agar during initial preparation will potentially render it unusable

SOP PROCEDURE CHANGE
FOR HETEROTROPHIC PLATE COUNT - SPREAD PLATE METHOD

CHANGE	REASON	DATE	INITIALS
Revision #3.	Corrective action for audit of 12/02.	2/1/03	JAB

Revised section 5.1 to recommend autoclaving agar in smaller containers for later reuse.	10/23/06	JMD
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Revised section 5.1 to use R2A agar for both methods; 6.1.3 to clarify pouring directions, other minor changes.	10/8/07	JMD
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ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

IDEXX QUANTI-TRAY/2000 (Chromogenic/Fluorogenic MPN Method)

File Name: M:\SOP\INORGNIC\Quanti-Tray.doc
Revision: 2

Effective Date: 02/01/2003
Supersedes: Rev. 1 (05/25/2000)

1. REFERENCES

1.1 *Standard Methods*, 18th ed., # 9223 B.

2. HOLDING TIME

2.1 6 Hours @ 4°C; best if analyzed within one hour of sample collection.

3. APPARATUS

- 3.1 Incubator - Maintain stable temperature of $35 \pm 0.5^\circ\text{C}$. Include precision thermometer, with bulb immersed in liquid (glycerine, water or mineral oil). Calibrate thermometer against NIST-certified reference thermometer annually and retain written documentation. Check and record temperature twice daily.
- 3.2 Autoclave - Provides uniform temperature of 121°C and pressure of 15 psi. Test sterilization performance **monthly** with a spore ampule included with a load. Check temperature **weekly** using a high temperature recording mercury thermometer. Check autoclave timer **quarterly** against a stopwatch to ensure that temperature and pressure are maintained for the set time. Use heat-sensitive tape where appropriate to identify materials that have been sterilized. Record date, time in, time out, sterilization time, and load type for each run in autoclave logbook. Also record autoclave performance checks in logbook. All loads except media must be autoclaved for 30 minutes.
- 3.3 Refrigerator - Maintains temperature at $4 \pm 2^\circ\text{C}$. Calibrate thermometer against NIST-certified reference thermometer annually and retain written documentation. Check and record temperature in log daily.
- 3.4 IDEXX UV lamp for reading tray fluorescence. 365-366 nm. 6 W bulb.

- 3.5 IDEXX Quanti-Tray sealing unit. Check performance **monthly** by adding dye (bromocresol purple) to water and pouring into tray. Seal tray and check for leakage of dye outside of wells.
- 3.6 Sterile Sample Bottles – either IDEXX pre-sterilized or polypropylene, 500 mL, containing 0.4 mL of 10% Na₂S₂O₃ dechlorinating agent. (See LabAide SOP for procedures on preparing bacti sample bottles).
- 3.7 IDEXX Color Comparator.

4. MEDIA AND REAGENTS

- 4.1 IDEXX Colilert Reagent Ampules. Check each new lot for auto-fluorescence by dispensing into a IDEXX sample bottle containing sterile DI water. Observe under UV light, checking for fluorescence. Discard any lots that auto-fluoresce.
- 4.2 Sterile DI water dispensed into 99 mL Dilution Bottles. Do not use buffered water when making sample dilutions.

5. PROCEDURE

Disinfect work area with 70% alcohol before and after performing each separate activity.

Drinking Water – 100 mL sample into 1 Quanti-Tray 2000.

Drinking Water – If no enumeration is required, 100 mL sample into 1 bottle.

Source/Surface Water – Enumeration Only – Quanti-Tray 2000.

- 5.1 Shake sample bottle vigorously 25 times in up-down motion. Aseptically transfer 100 mL to IDEXX sample bottle. Add one IDEXX Colilert powder ampule to bottle and close. Shake bottle 25 times.
- 5.2 Gently pull foil tab of IDEXX Quanti-Tray to separate the foil from the tray. Avoid touching the inside of the foil or tray. Pour the reagent/sample mixture directly into the Quanti-Tray, avoiding contact with the foil tab. Tap the small wells 2-3 times to release any air bubbles. Allow foam to settle. Place the sample-filled Quanti-Tray onto the Quanti-Tray/2000 rubber insert of the Quanti-Tray Sealer with the well side (plastic) of the Quanti-Tray facing down. Seal according to Sealer instructions. Place sealed Quanti-Trays into incubator. Allow the temperature of the samples to reach 35C (about 30 minutes) before starting the incubation time. Incubate at 35 ± 0.5°C for 24 hours.

6. INTERPRETATION AND REPORTING

- 6.1 Total Coliform Bacteria: After the minimum proper incubation period, examine the wells for the appropriate color change. ONPG is hydrolyzed by the bacterial enzyme to produce a yellow color. Samples are negative for total coliforms if no color is observed. If the chromogenic response is questionable after 24 hours, use the IDEXX color comparator. If the yellow color is less intense than the comparator, incubate those samples for an additional 4 hours (for a total of 28 hours). If the color intensifies beyond the comparator, the sample is total-coliform positive, if it does not, the sample is negative. Count the number of positive large/small wells and then refer to the IDEXX Quanti-Tray 2000 MPN table to find the Most Probable Number.
- 6.2 *Escherichia coli*: Examine positive total coliform wells for fluorescence using a long-wavelength (365-366 nm) ultraviolet lamp. Compare each tray against reference comparator. The presence of fluorescence is a positive indication for *E. coli*. If fluorescence is questionable, incubate for an additional 4 hours (for a total of 28 hours). Intensified fluorescence is a positive result. Count the number of positive large/small wells, the refer to table to find the Most Probable Number.

7. QUALITY CONTROL

- 7.1 Test each new lot of media for performance by inoculation with three control bacteria. Record the results in the Colilert Quanti-Tray logbook.
- 7.1.1 *Enterbacter aerogenes* – Used for Total Coliform.
- 7.1.2 *Escherichia coli*
- 7.1.3 *Pseudomonas aeruginosa* – Used for non-coliform.
- 7.2 As mentioned in 4.1, check each new lot of media for auto-fluorescence in sterile DI water. Record results in logbook.
- 7.3 Check expiration date of color comparator and reorder when necessary.
- 7.4 As mentioned in 3.5, on a **monthly** basis check Quanti-Tray for leakage by performing dye test. Record results in logbook.
- 7.5 QC-3 Bacteriological Proficiency Samples are to be analyzed quarterly.

SOP PROCEDURE CHANGE
IDEXX QUANTI-TRAY/2000

CHANGE	DATE	INITIALS
Revised	3/6/00	CAN
Revised. Corrective Action for 12/02 audit.	2/1/03	JAB

Total Coli.

ORANGE COUNTY WATER DISTRICT STANDARD OPERATING PROCEDURE

TOTAL COLIFORM ANALYSIS (Membrane Filter Method)

File Name: M:\SOP\INORGNIC\MF_TOTAL.doc
Revision: 3

Effective Date: 02/01/2003
Supersedes: 2 (03/06/2000)

NOTE: This SOP is for WASTEWATER and other NON-POTABLE water samples ONLY! Amendments have been added when applicable for DRINKING WATER samples that must conform to the TOTAL COLIFORM RULE.

1. REFERENCES

- 1.1 *Standard Methods for the Analysis of Water and Wastewater*, 18th, 19th, and 20th ed., APHA, AWWA, WEF., Section 9222B.
- 1.2 Manual for the Certification of Laboratories Analyzing Drinking Water, EPA 815-B-97-001, March 1997.
- 1.3 Code of Federal Regulations, Vol. 40, 2001.

2. HOLDING TIME

- 2.1 6 Hours @ 4°C; best if analyzed within one hour of sample collection.

3. INTERFERENCES

- 3.1 Samples high in turbidity; samples low in coliform bacteria and high in non-coliform bacteria.

4. APPARATUS

- 4.1 Incubator - Maintain stable temperature of $35 \pm 0.5^{\circ}\text{C}$. Include precision thermometer, with bulb immersed in liquid (glycerine, water or mineral oil). Calibrate thermometer against NIST-certified reference thermometer **annually** and retain written documentation. Check and record temperature twice daily, with readings at least four hours apart.

- 4.2 Autoclave - Provides uniform temperature of 121°C and pressure of 15 psi. Test sterilization performance **monthly** with a spore ampule included with the load. Check temperature **weekly** using a high temperature recording mercury thermometer. Check autoclave timer **quarterly** using a stopwatch to ensure that temperature and pressure are maintained for the set time. Keep records of spore check, temperature and timer checks in the autoclave logbook. Use heat-sensitive tape where appropriate to identify materials that have been sterilized. Record date, time in, time out, sterilization time, and load type for each run. All loads except media must be autoclaved for 30 minutes.
- 4.3 Refrigerator - Maintains temperature at 4±2°C. Calibrate thermometer against NIST-certified reference thermometer annually and retain written documentation. Check and record temperature in log daily.
- 4.4 Microscope - Binocular, 10 to 15X magnification, illuminated by daylight type fluorescent ring lamp.
- 4.5 Hand tally counter.
- 4.6 Membrane Filtration Unit - Sterile, filter base and funnel, wrapped with aluminum foil until use.
- 4.7 Vacuum Source
- 4.8 Vacuum Flask and Manifold - With appropriate tubing, safety trap between flask and vacuum source.
- 4.9 pH Meter - Calibrated before use according to manufacturer's specifications.
- 4.10 Forceps - With smooth tips. 95-100% ethanol, in small beaker, for flaming forceps.
- 4.11 Alcohol Burner
- 4.12 Sterile, disposable TD Bacteriological or Mohr Pipets - Glass or plastic, in appropriate sizes.
- 4.13 Sterile Petri Dishes - 50 x 12 mm, tight-fitting lids, absorbent pads. Keep covered when storing opened packages in drawer.
- 4.14 Sterile Graduated Cylinder - 100 mL, covered with aluminum foil.
- 4.15 Milk Dilution Bottles - Marked at 99 mL volume, screw cap with rubber liner.

- 4.16 Membrane Filters - Sterile, white, grid-marked, 47 mm diameter, $0.45 \pm 0.02 \mu\text{m}$ pore size, certified by manufacturer for water analysis.
- 4.17 Inoculating Loop - At least 3 mm diameter, sterile disposable bulk pack.
- 4.18 Sterile Sample Bottles - Polypropylene, 500 mL, containing 0.4 mL of 10% $\text{Na}_2\text{S}_2\text{O}_3$ dechlorinating agent. Lab aide is responsible for preparing bottles. See Lab Aide SOP for details.

5. MEDIA AND REAGENTS

- 5.1 M-Endo Broth - We normally use HACH *PourRite*[™] m-Endo broth ampules, cat. # 23735-20. When ordering, state "All same lot number" and "Manufacture date must be no more than 7 days before ship date." ELAP requires expiration date to be manufacturing date + 3 months. Store continuously at 4°C before use. Check each new lot with positive (*Enterobacter aerogenes*) and negative (*Pseudomonas aeruginosa*) control cultures, and for sterility. Also verify acceptable pH for each lot. Document this QC in the Media logbook.

Procedure for making up broth from dry medium: (Note: Powdered medium must not be past mfrs expiration date *or* internal expiration date, which is 6 months past date of opening, whichever comes first). Weigh out 4.8 g dehydrate into weighing dish. first. Add 2 mL 95% ethanol (non-denatured) to 100 ml DI water in graduated cylinder. Decant approximately 20 mL to a 250 mL screw-cap Erlenmeyer flask. Empty contents of weighing dish into flask. Add remaining liquid. Swirl flask to disperse powder in solution. Place flask, loosely capped, in boiling water bath. Heat medium 3 to 5 minutes. Bring to point of boiling, but do not allow to boil. Remove and cool. Check pH and adjust to between 7.1 and 7.3 with sterile 1N NaOH. Medium may be stored at 4°C for 96 hours maximum. Record date, pH, lot #, amount of dry medium used and volume made in Media log book. Also check performance with the positive and negative controls as mentioned above.

- 5.2 Phosphate Buffer - Lab aide is responsible for preparing buffered dilution water and sterile rinse water. Refer to Lab Aide SOP for directions.
- 5.3 Lauryl Tryptose Broth - Refer to manufacturers directions and OCWD SOP for Total Coliforms by Multiple Tube Fermentation Technique for preparation details.
- 5.4 Brilliant Green Bile 2% - Refer to manufacturers directions and OCWD SOP for Total Coliforms by Multiple Tube Fermentation Technique for preparation details.
- 5.5 Sodium Thiosulfate Solution, 10% - Lab aide is responsible for preparing. Refer to Lab Aide SOP for directions.

- 5.6 Alcohol, 70% - Dilute 350 mL denatured ethyl or isopropyl alcohol to 500 ml with DI water. Use this for disinfection.
- 5.7 Pure cultures of *Pseudomonas aeruginosa* and *Enterobacter aerogenes* - Transfer cultures to nutrient agar slants at least monthly. Preferably prepare fresh pure cultures monthly.
- 5.8 Nutrient agar slants – Sterilize required number of 16 x 125 mm screw-cap tubes. Then prepare required amount of nutrient agar according to manufacturers directions. Sterilize in autoclave for 15 minutes. Aseptically dispense about 8 mL tempered agar into each sterile tube and allow to cool in a slant position. The length of the agar surface should be about 6.5 cm. After agar has solidified, store tubes in refrigerator until use.

6. PROCEDURE

- 6.1 Disinfect work area with 70% alcohol before and after performing each separate activity. Wipe neck of m-Endo broth ampule with 70% alcohol and carefully break ampule. Pour contents of ampule onto absorbent pad of sterile petri dish, or, if broth was made up from dry medium, pipet 1.8 mL onto each sterile absorbent pad. Sterilize forceps by dipping in alcohol and flaming. Aseptically open and transfer sterile membrane filter to filter base and attach funnel to base.

NOTE: A sterility check must be conducted on EACH funnel in use at the beginning and end of each filtration series. (Filtration series ends when 30 minutes or more elapse between sample filtrations.)

- 6.1.1 Shake sample bottle vigorously 25 times in up-down motion. Measure desired volume of sample into funnel. Select sample volume that will produce between 20 and 80 coliform colonies and no greater than 200 colonies of all types. If sample volume is less than 20 ml, add 20 - 30 mL sterile buffer to funnel, then pipet sample onto buffer. Swirl funnel to mix. Sample volumes less than 1 mL should be diluted prior to filtration. (As example, for a sample volume of .01 mL, pipet 1 ml of full strength sample into a 99 ml dilution bottle. This is a 10^{-2} dilution. Then pipet 1 ml of 10^{-2} dilution to filter funnel containing 20 - 30 ml buffer and swirl to mix.) Have vacuum OFF when adding sample to funnel. Rinse funnel after filtration of each sample, using autoclaved wash bottle containing sterile buffer to rinse down funnel walls, followed by 2 to 3 rinses of 20 - 30 ml buffer from a sterile 50 mL graduate cylinder reserved for this use only. Turn off vacuum and remove funnel from base.
- 6.2 Aseptically remove membrane filter and place on filter pad. Use slight rolling motion, being careful not to trap air under the filter. Replace cover tightly.

- 6.3 Invert petri dish and incubate at $35 \pm 0.5^{\circ}\text{C}$ for 22 - 24 hours, in a humid (60% relative humidity) environment. After the required incubation period, turn dish right side up and remove lid. Place bottom of dish containing membrane filter in microscope field. Examine and count all colonies exhibiting a golden-green metallic sheen under fluorescent ring light. To facilitate counting, proceed from top to bottom and alternate left to right and right to left in a zigzag pattern.
- 6.4 Typical total coliform colonies are pink to dark-red in color with a distinctive metallic-green sheen surface. Count both typical and atypical coliform colonies. Atypical colonies can be dark red, mucoid or nucleated, but without sheen.

7. CALCULATION OF TOTAL COLIFORM DENSITY

- 7.1
$$\text{Total Coliform Colonies/100 mL} = \frac{\text{Coliform colonies counted} \times 100}{\text{mL of sample filtered}}$$
- 7.2 The minimum/maximum range for colony forming units on the membrane filter is 20 - 80 coliform colonies, and not more than 200 total colonies, coliform plus non-coliform. For counts greater than the upper limit of 80 colonies, use the count from the smallest filtration volume and report as ">(greater than) count/100 ml." If estimate of number of sheen colonies is not possible, report as ">(200 X dil. factor)" with "Colonies Too Numerous To Count" in the Remarks section, or, if confluent growth occurs, report as "Confluent Growth With (or Without) Coliforms." If no coliform colonies observed, report as "< 1 Coliform / 100 mL".
- 7.3 **Coliform Verification: For water samples OTHER than drinking water quality**

Verify 10 colonies from positive sample monthly, including both sheen and atypical colonies.

Transfer growth from each colony to a tube of Lauryl Tryptose broth and a tube of BGB broth simultaneously; incubate at $35 \pm 0.5^{\circ}\text{C}$ for 48 hours. Gas formed in Lauryl Tryptose broth and BGB broth within 48 hours verifies the colony as a coliform. Use this method also for confirming the presence of coliforms in all samples which produce confluent growth; either place the entire filter in a tube of LTB, or brush the entire filter with a sterile swab and inoculate into LTB. Gas production within 48 hours at $35 \pm 0.5^{\circ}\text{C}$, followed by gas production in BGB indicates coliforms are present.

7.4 VERIFICATION FOR DRINKING WATER SAMPLES AND THE TOTAL COLIFORM RULE

- 7.4.1 Required sample volume to be tested is 100 mL.
- 7.4.2 Positive results are based on the verification test. All Total Coliform positive samples must also be tested for the presence of either Fecal Coliform or *E. coli*. **Laboratory must promptly notify the proper authorities of any positive Total Coliform results. Notification record kept.**
- 7.4.3 For verification, the entire membrane surface is swabbed. The order of inoculation is EC broth, LTB broth, then BGB broth.
- 7.4.4 **Invalidation of POSITIVE Total Coliform Samples.**
 - 7.4.4.1 The laboratory establishes that improper sample analysis caused positive result.
 - 7.4.4.2 State, on basis of results of repeated sampling, determines that positive Total Coliform sample resulted from domestic or other non-distribution system plumbing problem.
 - 7.4.4.3 State has substantial grounds to believe that positive result is due to circumstances or conditions which does not reflect the water quality in the distribution system.
- 7.4.5 **Invalidation of NEGATIVE Total Coliform Samples.**
 - 7.4.5.1 Laboratory **MUST** invalidate negative total coliform results if the sample exhibits confluent growth or produces colonies too numerous to count with no coliforms detected. Ask for a resample.
 - 7.4.5.2 Sample is NOT invalid if the membrane filter contains at least one sheen colony.

8. QUALITY CONTROL

- 8.1 Each day, at beginning and end of each series of samples, a sterility check must be run with each filter funnel apparatus used (within a 30 minute use period). Filter 50 ml sterile buffer through membrane filter and incubate in MF-Endo broth. Note result and make appropriate entry in logbook.
- 8.2 Transfer 10% of positive colonies from MF to LTB and BGB for verification.

- 8.3 For **drinking water** samples, transfer 5 typical and 5 atypical positive samples from MF to LTB and BGB for verification. Also verify any positive samples for fecal coliforms. If no positive samples, test at least one known positive source water **quarterly**.
- 8.4 At the end of each week, have membrane filtration apparatus cleaned with detergent.
- 8.5 QC-3 Bacteriological Proficiency Samples are to be analyzed quarterly.
- 8.6 Check sterility of sample bottles from each autoclave run by putting 20 mL Tryptic Soy Broth (see *Standard Methods* for preparation instructions) in sterile sample bottle and incubating for 24 hours at $35 \pm 0.5^{\circ}\text{C}$. Turbidity indicates the bottle was not sterile. Record results in autoclave logbook. See also Lab Aide SOP.
- 8.7 Run water suitability test annually, or whenever a new lot of detergent is used.
- 8.8 Check sterility, positive and negative controls, and pH on each new lot of media used and record in Media Prep. logbook.

9. TROUBLESHOOTING

- 9.1 Possible sources of poor results include:
 - 9.1.1 Bacterial contamination at any stage of the analysis procedure. Insure sterility at all stages by using proper aseptic technique.
 - 9.1.2 Power outage or incubator failure, causing temperature to go outside of acceptable range. Review documentation.
 - 9.1.3 Heat-damaged or incorrectly prepared media. Remake.

CHANGE	REASON	DATE	INITIALS
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