APPENDIX K

QUALITY ASSURANCE PROCEDURES SUMMARY

UNIVERSITY OF GEORGIA INSTITUTE OF ECOLOGY

STABLE ISOTOPE/SOIL BIOLOGY LABORATORY
Analytical Chemistry Lab, Rm154
Institute of Ecology, Green Street
University of Georgia
Athens, Georgia 30602

February 28, 2007

This page intentionally left blank

Quality Assurance Measures

With respect to precision, accuracy, recovery, and blanks, UGA Laboratory adheres to the following procedures:

1) Precision:
To confirm precision UGA Laboratory runs multiple (20 +) injections of the exactly the same analyte. The variation in peak height is assessed, and cannot exceed the detection limit for a given manifold/concentration range setup. This roughly translates into 1/100th of the concentration of the measured standard (*e.g.*, 1mg/L (or 1000ug/L) standard should not vary more than +/- 10ug/L for a set of 20 measured standards.

2) Accuracy:
UGA Laboratory uses two different standards to determine accuracy. One standard is a QC standard available from Spex Certiprep that comes in a sealed glass ampoule, and comes with certified values for nitrate, phosphate and ammonium. The other standard is prepared from dry chemicals in the laboratory. The known concentrations of these two standards are compared the two measured values to determine acceptability of the run. For sample runs, one standard-containing samples is run for every 20 unknowns.

3) Recovery:
To determine % recovery, a known concentration of standard is analyzed and compared to the output concentration value. If, for example a 1mg/L concentration of analyte is used for the spike, and the output reads 0.9mg/L, it is interpreted as 90% recovery. Runs will not be accepted unless they match known concentration spikes within +/-2% or better. % Recovery is used when digesting nonlabile QC standards as a reference check for running with total persulfate N or P.

4) Blanks:
UGA incorporates blanks into the run setup of samples. Every 20 samples, a series of 6 blanks are run. The format is as follows: samples (n=20) followed by blank, the working standard, another blank, working standard again, two more consecutive blanks, then back to samples (n=20), and so on. The second working standard is tagged by the software to serve as a drift correction. The last blank is tagged by the software to serve as a baseline correction/reading.

General Quality Assurance measures carried out by UGA Laboratories are as follows:

- 1000 ppm standard stocks are made from appropriate dry reagents (KNO3, NaNO2, (NH4)2SO4, and KH2PO4) and are checked by analyzing Environmental Protection Agency certified Nutrient-1 quality control (QC) solutions of known analyte values. If the determined value of the QC solution differs from the known value by > 1%, the stock is discarded.
- Working standards are made daily from standard stocks
- Calibration of each run is accomplished by linear regression on a descending sequence of three working standards and a blank at the start of the run. Within-run drift correction is achieved by one recalibrant standard and baseline blank every twenty samples. Recovery check standards are included once for every twenty samples.

- Digests for total nitrogen and total phosphorus are also checked by digesting and analyzing E.P.A. Nutrient-2 QC solutions formulated to challenge digestion techniques alongside every batch of unknowns, one EPA-QC2 standard per twenty unknowns.
- Clients are referred to the colorimetric analysis instructions for methods of sample treatment that will ensure the best possible precision and accuracy.
    - Liquid samples must be free of turbidity and particulate matter. Any such substances must be removed before analysis by filtering or centrifugation.
    - Strongly colored samples may contribute confounding absorbance at the analytical wavelength.
    - Water samples which cannot be analyzed immediately after collection must be preserved for shipment. The E.P.A. publication Methods for Chemical Analysis of Water and Wastes lists acceptable preservation methods and holding times for many analytes

Standard Operating Procedures for Simultaneous Determination of Total Nitrogen and Total Phosphorus (USGS Methods I-4650-03 & I-2650-03)

Equipment

1) Chemical autoclave
2) 13x100 mm glass screw-cap culture tubes with teflon-lined caps. (Tubes are acid-washed in 20% HCl and muffled at 500 degrees C for two hours. Caps are acid-washed in 50% HCl)
3) autoclave-safe test tube racks
4) 100 ml, 200 ml or 500 ml acid-washed volumetric flask for oxidizing reagent (depending on how much reagent is needed)
5) 500 ml acid-washed volumetric flask for 3.75M NaOH stock
6) 1000 ul and 5000 ul automatic pipetters
7) weigh boats and clean chemical spatula

Reagents

1) fresh deionized H2O
2) low-N potassium peroxydisulfate (e.g. Fisher P282-100)
3) boric acid (e.g. Baker 0084-01)
4) low-N NaOH if stock is needed
5) EPA-certified Nutrient 2 quality control digest standard

NaOH stock:

Place ~350 ml diH2O in 500 ml volumetric on stir plate. Add 75.0 g NaOH. Stir to dissolve; remove stir bar and bring to volume. Cap with parafilm and invert to mix. Allow to stand ~30 minutes and recheck volume.

Oxidizing reagent:

Place clean volumetric of appropriate size on stir plate; into it rinse in the appropriate amounts of reagent from the table below with diH2O. Bring to about 80% of flask volume with diH2O and stir to dissolve; takes ~15 minutes on stir plate (gentle warming may help.) When dissolved, remove stir bar and bring to final volume with diH2O. Cap with parafilm and invert to mix. Allow to stand ~30 minutes and recheck volume.

	100 ml	200 ml	250 ml	500 ml
persulfate	5.2 g	10.4 g	13 g	26 g
boric acid	3.12 g	6.24 g	7.8 g	15.6 g
NaOH stock	10 ml	20 ml	25 ml	50 ml

(This reagent may be stored 7 days at room temperature. Crystalizes when refrigerated.)

EPA Nutrient-2:

10 ml concentrate from ampoule in 1000 ml diH2O (or 5 ml concentrate in 500 ml.) yields 5.00 mg/liter total nitrogen and 1.50 mg/liter total phosphorus.

Procedure

1) bring samples to room temperature if chilled or frozen
2) make up fresh digest reagent, and NaOH stock if needed
3) obtain acid-washed, muffled digest tubes; label them.
4) for unknowns and EPA2 samples:

- on first pass through the sample set, pipette 5 ml sample into each labeled digest tube. Cap loosely to exclude dust.
- on second pass, pipette 1 ml oxidizing reagent into each digest tube. Cap tightly and mix well (invert several times.)

5) For reagent blanks, pipette only 1 ml oxidizing reagent into tube and cap tightly. (N.B., take care! Qualls (5) p.136: "For low level samples the variability in the reagent blanks determines the limit of detection, not the error associated with the NO3 and PO4 analyses themselves.")
6) Place capped tubes in autoclave, 30 minutes on liquid cycle. (= 30 minutes on "sterilize" in addition to all other cycle segments. If using pressure-cooker field method, time 30 minutes after coming to canning temperature in addition to warmup and cooldown times.)
7) After tubes are cool, add 5 ml diH2O to all reagent blank tubes so that the total volume of liquid in these tubes is the same as in the others. (N.B. Qualls (5) p.136: "Since distilled or deionized water contains significant N, the dilution water [for the blanks] is added after the digestion.")
8) Analyze digest-tube contents on Alpkem using the nitrate-nitrite and orthophosphate manifolds.

Post-analysis calculations

1) Take the mean of the reagent blank determined values. Throw out any that are >2 std. deviations above the mean (for nitrogen in particular this indicates that the tube cap has cracked during the autoclave step and admitted atmospheric N to the tube.)
2) To compensate for color absorption by the digest reagents, subtract the mean reagent blank N and P values from the Alpkem determined values for each unknown or EPA2 sample.
3) The effect of diluting the samples by the addition of digest reagents must be reversed:

$$df = \frac{\text{sample volume} + \text{reagent volume}}{\text{initial sample volume}}$$

In the case of the above procedure, where initial sample volume is 5 ml and reagent volume is 1 ml,

$$df = \frac{\text{5 ml sample} + \text{1 ml reagent}}{\text{5 ml sample}} = 1.2$$

Find the actual value of the undiluted sample by multiplying the determined value (after reagent blank subtraction) by the df.

True analyte concentration

=

(Alpkem raw determined value - rblank value)*(df)

Comments

1) Successful digests have pH in the range 5 to 8; incomplete digests are ~2. This can be checked with wide-range pH paper. We have observed no reliable correlation between final digest pH and the yellow color developed in some digests, so the color cannot be used to spot incomplete digests.
2) Instead of using reagent blanks, it is possible to digest the calibration standards (including water blanks, i.e. calibration standards of content zero), the D3 (recalibrant) and ref3 (reference check) and the W (baseline drift correction) cups. Thus with digest reagent in both samples and calibants, the reagent's contribution to total absorbance will be compensated for automatically. (This strategy is of course useable only if all samples in the run are using the same reagent/diluent ratio.)
It may be necessary to have different dilutions of the EPA2 QC standards for nitrate and phosphate to get both into the optimum manifold range (e.g. if the PO4 manifold range is 0.2-1 ppm while the expected TP content of EPA2 is 1.5 ppm PO4, the QC digests will be offscale for phosphate unless diluted.) Perform these dilutions before digestion and then use the same reagent/diluent ratio for everything.

References

1. D'Elia, C. F., P. A. Steudler, and N. Corwin. 1977.
   Determination of total nitrogen in aqueous samples using persulfate digestion. *Limnol. Oceanogr.* 22, 760-764.
2. Edwards, R. T. (no date).
   A semiautomated technique for the determination of total persulfate nitrogen and total persulfate phosphorus. (unpublished ms.)
3. Koroleff, F. 1983.
   Simultaneous oxidation of nitrogen and phosphorus compounds by persulfate. p.168-169. In K. Grasshoff, M. Eberhardt, and K. Kremling, eds., Methods of Seawater Analysis. 2nd ed., Verlag Chemie, Weinheimer, FRG.
4. Langner, C. L. and P. F. Hendrix. 1982.
   Evaluation of a persulfate digestion method for particulate nitrogen and phosphorus. *Water Res.* 16, 1451-1454.
5. Qualls, R. G. 1989.
   Determination of total nitrogen and phosphorus in water using persulfate oxidation: a modification for small sample volumes using the method of Koroleff (1983). Appendix A pp. 131-138. In The biogeochemical properties of dissolved organic matter in a hardwood forest ecosystem: their influence on the retention of nitrogen, phosphorus, and carbon. Ph.D. dissertation, University of Georgia Institute of Ecology, Athens, Georgia, USA. University Microfilms, Inc., no. DEX9003448