CAC-Benzotriazole-1.1
Benzotriazole in Drinking Water by Liquid Chromatography/ Tandem Mass Spectrometry (LC/MS/MS)

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Determination of Benzotriazole in Drinking Water by Liquid Chromatography / Tandem Mass Spectrometry (LC/MS/MS)

1. Scope:

This method describes the determination of 1H-Benzotriazole in drinking water samples using direct aqueous injection liquid chromatography / tandem mass spectrometry (LC/MS/MS). It is validated for use on untreated and treated drinking water samples collected from water treatment plants, with a single laboratory detection limit of 0.17ppb.

2. Principle:

A 1 mL aliquot of the sample is spiked with an isotopically labelled internal standard prior to analysis by LC/MS/MS. The internal standard serves to correct for variations in instrument response, and matrix effects, ensuring accurate quantification. A 50 µL injection of the prepared sample is introduced into an LC system, equipped with a Phenyl-Hexyl column. Identification of Benzotriazole is achieved by comparing the mass spectra and retention times to the reference data for the calibration standards. The concentration of Benzotriazole is calculated with the internal standard technique.

3. Definitions:

3.1. ANALYSIS BATCH – A set of samples that are analyzed on the same instrument during a 24-hour period that begins and ends with the analysis of the appropriate Continuing Calibration Check (CCC) standards. Additional CCCs may be required depending on the length of the Analysis Batch and the number of field samples.

3.2. CALIBRATION STANDARDS – Solutions of Benzotriazole that are prepared from the Primary Dilution Standards. The calibration standards are used to calibrate the instrument response with respect to analyte concentration.

3.3. CONTINUING CALIBRATION CHECK (CCC) – A calibration standard which is analyzed periodically to verify the accuracy of the existing calibration curve.

3.4. INTERNAL STANDARD (IS or ISTD) – A pure compound that is added to all standard solutions and samples in a known amount and used to measure the relative response of other method analytes that are components of the same solution. The internal standard must respond similarly to the method analyte, has no potential to be present in water samples, and not be a method analyte. For this method, d_4-1H-Benzotriazole (Benzotriazole-d_4) was used.

3.5. LABORATORY FORTIFIED BLANK (LFB) - An aliquot of reagent water fortified with a known quantity of Benzotriazole. The LFB is prepared to match the analytical procedure for field samples. The LFB is used during the Initial Demonstration of Capability (IDC) to verify method performance for precision and accuracy. The LFB is also a required QC element with each Analysis Batch. The results of the LFB verify method performance in the absence of sample matrix.

3.6. LABORATORY FORTIFIED SAMPLE MATRIX (LFSM) - An aliquot of a field sample to which known quantity of Benzotriazole is added. The purpose of the LFSM is to determine the bias contribution of the sample matrix to the analytical results.

3.7. LABORATORY FORTIFIED SAMPLE MATRIX DUPLICATE (LFSMD) – A second aliquot of the same field sample used to prepare the LFSM, fortified and analyzed in the same Analysis Batch as the LFSM. The LFSMD is used to verify method precision in sample matrices.

3.8. LABORATORY REAGENT BLANK (LRB) - An aliquot of reagent water prepared to match the sample processing procedures. The LRB is used to check if Benzotriazole or other interferents are introduced from sample containers, processing equipment, or the reagents of the assay.

3.9. LOWEST CONCENTRATION MINIMUM REPORTING LEVEL (LCMRL) - The single-laboratory LCMRL is the lowest spiking concentration such that the probability of spike recovery in the 50 to 150% range is at least 99%.

3.10. MINIMUM REPORTING LEVEL (MRL) - The minimum concentration that can be reported by a laboratory as a quantified value for Benzotriazole in a sample following analysis. This concentration must meet the criteria defined in Section 9.1.3 and must be no lower than the concentration of the lowest calibration standard.

3.11. PRIMARY DILUTION STANDARD (PDS) - A solution of Benzotriazole in 90:10 reagent water: acetonitrile. The PDS solutions are used to prepare calibration standards and to fortify the QC samples.

3.12. QUALITY CONTROL SAMPLE (QCS) - A solution containing Benzotriazole at a known concentration that is obtained from a source different from the source of calibration standards. The purpose of the QCS is to verify the accuracy of the primary calibration standards.

3.13. REAGENT WATER - Purified water that does not contain any measurable quantity of Benzotriazole or interfering compounds at or above 1/3 the MRL.

3.14. STOCK STANDARD SOLUTION - a concentrated standard that is purchased from a commercial source with a certificate of analysis. The concentrated standard may also be prepared from neat reference material.

4. Interferences:

None observed during validation

5. Safety:

5.1. Read the Safety Data Sheet for all materials before use.
5.2. All general laboratory safety rules for sample preparation and analysis shall be followed.
5.3. All solvents should be handled with care in a ventilated area.

6. Equipment and Supplies:

6.1. Micropipettes with disposable tips (10 – 1000 µL)
6.2. Electronic repeating pipette with disposable tips
6.3. Vortex vibrating mixer.
6.4. Polypropylene (PP) centrifuge tubes (15mL) with caps.
6.5. Autosampler vials with caps
6.6. Miscellaneous glassware as needed
6.7. Analytical Balance, Mettler XP26 or equivalent
6.8. Weigh boats
6.9. Liquid Chromatography Tandem Mass Spectrometry System (LC/MS/MS)

6.9.1. LC System

6.9.1.1. Binary Pumps

This method was developed using a Sciex Exion LC pump. Any pump capable of supplying the correct mobile phase percentages and flow may be used.

6.9.1.2. Autosampler

This method was developed using a Sciex Exion LC autosampler. Any autosampler equipped with a cooling compartment capable of reaching 15°C and of drawing the appropriate sample volume may be used.

6.9.1.3. Analytical Column

This method was developed using a Phenomenex Kinetex 2.6 µm Phenyl-Hexyl, LC column 100 x 3 mm (00D-4495-Y0). Any column that provides adequate resolution, peak shape, capacity, accuracy and precision (Section 9), and does not exacerbate suppression or enhancement of analyte responses may be used. Also, any column compartment capable of reaching 40°C may be used.

6.9.2. Tandem Mass Spectrometer

6.9.2.1. This method was developed using an ABSciex 6500 +. Any mass spectrometer that provides adequate sensitivity may be used.

6. Reagents and Standards:

7.1. Method Analyte Standard
1H-Benzotriazole (Benzotriazole) CAS# 95-14-7
HPC Standards Inc. 676968 used during method development

7.1.1. Preparation of Primary Dilution Standards

7.1.1.1. Benzotriazole Stock Solution purchased from HPC Standards Inc. (or another certified source) at a concentration of 100µg/mL.

7.1.1.2. The 100µg/mL standard is diluted to 200ppb, 20ppb, and 2ppb using 90:10 reagent water:acetonitrile. These standards are used for the PDS.

7.1.1.3. Other dilutions may be required depending on the concentration of the stock solution.

7.1.1.4. PDS should be prepared every 6 months or sooner. Even though stability times for solutions are suggested in this method, laboratories should use standard QC practices to determine when their standards need to be replaced.

7.1.2. Preparing Stock Solutions from neat reference material

7.1.2.1. Taking purity into account, weigh enough neat material so that 10mg ±0.1 mg of Benzotriazole is weighed onto a tared weigh boat.

7.1.2.2. Transfer weighed standard to a volumetric flask, by placing the weigh boat onto a pre-solvent rinsed 10mL volumetric flask fitted with a funnel

7.1.2.3. Rinse down the solid with acetonitrile up to the flask shoulder. Ensure all solids have been transferred into the volumetric flask.

7.1.2.4. Sonicate for about 5 minutes to ensure solid is dissolved.

7.1.2.5. Fill to the mark with acetonitrile.

7.1.2.6. Cap and invert several times to mix.

7.1.2.7. This prepares a 1mg/mL solution of Benzotriazole

7.1.2.7. Proceed with preparation of Primary Dilution Standard as described above in Section 7.1.1.

7.2. Internal Standard

d_4-1H-Benzotriazole (Benzotriazole-d_4) CAS# 1185072-03-0
HPC Standards Inc. 681255 used during method development

7.2.1. Preparation of Internal Standard Primary Dilution Solution

7.2.1.1. d_4-1H-Benzotriazole Stock Standard is purchased from HPC Standards Inc. (or another certified source) at a concentration of 100µg/mL.

7.2.1.2. The 100µg/mL ISTD Stock Standard is diluted to 200ppb and then 20ppb using 90:10 reagent water:acetonitrile. The 20ppb standard is used in calibration standards, spikes, and sample preparation (other concentrations of ISTD may be used so long as the amount of ISTD in the samples is the same as the amount of ISTD in the calibration standards).

7.2.1.3. Other dilutions may be required depending on the concentration of the stock solutions.

7.2.1.3. Internal standard PDS should be prepared every 6 months or sooner. Even though stability times for solutions are suggested in this method, laboratories should use standard QC practices to determine when their standards need to be replaced.

7.3. Calibration Standards

7.3.1. Prepare a series of calibration standards of at least six levels by diluting the analyte PDS into 90:10 reagent water:acetonitrile. The lowest calibration standards must be at or below the MRL. The calibration standards may also be used as Continuing Calibration Checks (CCCs). Using the PDS solutions, add a constant amount of internal standard to each calibration standard. The concentration of the internal standard should match the concentration of the internal standard in the samples.

7.4. Water, MS grade, Fisher Optima LC/MS or equivalent

7.5. Acetonitrile, Fisher Optima or equivalent

7.6. Aqueous Mobile Phase: water with 10mM ammonium formate and 0.05% formic acid
ThermoFisher Scientific MB1231 used during method development

7.7. Organic Mobile Phase: methanol with 10mM ammonium formate and 0.05% formic acid
ThermoFisher Scientific MB1221 used during method development

8. Sample Collection and Storage:

8.1. Samples should be collected in amber glass bottles

8.1.1. Sampler should wear nitrile gloves while filling and sealing the sample bottles, using a new pair of nitrile gloves at each sample site.

8.1.2. To collect the sample, open the tap and allow the system to flush until the water temperature has stabilized or allowed to flow for a minimum of 5 minutes before sampling to ensure that the sample reflects the water quality of the source. Collect the samples from the flowing system. After collecting the sample, cap the bottle and place the sample bottles into an ice chest with wet ice and keep them cool from time of collection until extraction.

8.2. SAMPLE SHIPMENT AND STORAGE – Samples must be chilled during shipment. Samples must be confirmed to be at or below 10 °C when they are received at the laboratory. In the laboratory, samples must be stored at or below 4 °C and protected from light. Do not freeze.

8.3. SAMPLE HOLDING TIMES – Analyze samples as soon as possible. Samples that are collected and stored as described in Section 8 must be analyzed within 28 days of collection. A storage stability study confirmed Benzotriazole is stable for 28 days (see Table 9).

9. Quality Control:

9.1. INITIAL DEMONSTRATION OF CAPABILITY (IDC)

9.1.1. DEMONSTRATION OF PRECISION AND ACCURACY - Prepare seven replicate LFBs, fortified with Benzotriazole near the midpoint of the linear range of the calibration curve. LFBs must be processed in a single Analysis Batch. The Analysis Batch must also include the LRBs from section 9.1.2. The percentage relative standard deviation (%RSD) for the LFBs must be ≤20. The mean recovery for the LFBs must be ≥70% and ≤130%.

9.1.2. DEMONSTRATION OF LOW SYSTEM BACKGROUND - Included in the Analysis Batch in section 9.1.1, prepare five LRBs. The results for each LRB must be less than one third the MRL.

9.1.3. MINIMUM REPORTING LIMIT (MRL) CONFIRMATION - The Minimum Reporting Limit (MRL) for Benzotriazole by this method was confirmed during single laboratory validation to be 0.5ppb.

Laboratories must confirm the MRL concentration proposed for their laboratory following the procedure outlined below.

Analyze seven samples spiked at the proposed MRL. The results of these spikes must meet the following requirements for the Prediction Interval of Results (PIR). If the criteria are not met, the MRL is set too low and must be determined again at a higher concentration

Half Range=3.963*S, where S is the standard deviation and 3.963 is a constant for seven replicates.

Upper PIR Limit=$\frac{Mean+HR}{Spiked\ Conc.} x100\% \leq 150\%$

Lower PIR Limit=$\frac{Mean-HR}{Spiked\ Conc.} x100\% \geq 50\%$

9.1.4. QUALITY CONTROL SAMPLE (QCS) - Analyze a mid-level QCS prepared as in section 9.2.4, to confirm the accuracy of the calibration standards. The QCS must be ±30% of the true value.

9.2. ANALYSIS BATCH QC REQUIREMENTS

9.2.1. LABORATORY REAGENT BLANK (LRB) - For each Analysis Batch, include one LRB. The Benzotriazole concentration must be less than one third the MRL. If the concentration is greater than or equal to that level, any positive results from that Analysis Batch are invalid.

9.2.2. LABORATORY FORTIFIED BLANK (LFB) - A LFB must be included in each Analysis Batch. The concentration of the LFB must rotate between low, medium, and high concentrations from batch to batch. The percent recovery for each LFB must be ±50% if at or below two times the MRL and ±30% if greater than or equal to two times above the MRL, else the entire Analysis Batch is invalid.

9.2.3. LABORATORY FORTIFIED SAMPLE MATRIX / LABORATORY FORTIFIED SAMPLE MATRIX DUPLICATE – A LFSM and LFSMD is required with each Analysis Batch. The native background concentration must be determined from a separate field sample. The source of the sets should be distributed among the various water sources for the laboratory over time.

9.2.3.1. Three separate aliquots of a field sample are required, one to determine the native background concentration and one-each for the LFSM and LFSMD. Homogenize the sample before separating into three vials. Fortify the LFSM and LFSMD near the center of the calibration curve.

9.2.3.2. Calculate the mean percent recovery for each LFSM and LFSMD set:

$$\%R = \frac{(A-B)}{C} \times 100\%$$

A= mean measured concentration of set
B= measured native background
C= fortification concentration

9.2.3.3. The mean percent recovery for each set must be ≥50% or ≤150% if spiked at or less than two times the MRL or ≥70% or ≤130% if spiked at greater than two times the MRL. If the percent recovery is outside this range, and the performance of the LFBs is in control for the same batch, the recovery may be matrix biased. Mark the result for the sample from which the LFSM was prepared as “suspect-matrix”.

9.2.3.4. Calculate the relative percent difference (RPD):

$$RPD = \frac{|LFSMD - LFSM|}{(LFSMD + LFSM)/2} \times 100\%$$

9.2.3.5. The RPD for each set must be ≤50% when at or less than two times the MRL or ≤30% when greater than two times the MRL. If the RPD is outside this range, and the performance of the LFBs is in control for the same batch, the precision may be matrix biased. Mark the result for the sample from which the LFSMD was prepared as “suspect-matrix”.

9.2.4. QUALITY CONTROL SAMPLE (QCS) - A QCS must be analyzed during the IDC, and again with each new set of calibration standards. The Benzotriazole used for the QCS must be procured from a source that is independent of the source of the Stock Standard. The concentration of the QCS should be near the center of the calibration curve. The percent recovery of the QCS must be ±30% of the true value.

9.2.5. CONTINUING CALIBRATION CHECK (CCC) – Analyze CCC standards at the beginning of each Analysis Batch, after every ten samples, and at the end of the Analysis Batch. See Section 10.5 for concentration requirements and acceptance criteria.

9.2.6. INTERNAL STANDARD (IS or ISTD) – The analyst must monitor the peak areas of the internal standard in all injections of the Analysis Batch. The internal standard responses (as indicated by peak areas) for any chromatographic run must not deviate by more than ±50% from the average areas measured during the initial calibration for the internal standards. If the IS areas in a chromatographic run do not meet these criteria, check the corresponding IS of the most recent CCC and proceed as follows:

9.2.6.1. IS Failure in Sample but not CCC: if the IS criterion is met in the CCC but not in the sample, reanalyze the sample in the same or subsequent Analysis Batch. If reanalysis produces an acceptable IS response, report results for that injection. If the IS area count fails to meet acceptance criterion in the repeat analysis but still passes the most recent CCC, report the sample results as “suspect/matrix.”

9.2.6.2. IS Failure in Sample and CCC: If both the original sample and the CCC fail the IS criterion, take corrective action (see section 10.6). It might be helpful to check the integrity of the IS solution and the fortification technique before reanalyzing the sample in a subsequent Analysis Batch. After corrective action, re-inject the sample in a subsequent Analysis Batch. If the IS area fails to meet the acceptance criterion in the repeat sample analysis, but passes in the most recent CCC, report the sample results as “suspect/matrix.”

9.2.7. RETENTION TIME (RT) – The retention time for each field sample in an Analysis Batch must be ± 0.1 minutes from the RT of the calibration standards of that batch.

9.2.8. ION RATIO – The ion ratio between the quantitation and confirming ions of samples in an Analysis Batch shall be ≤ 30% when compared to the ratios of the standards of that batch

10. Calibration and Standardization:

10.1. Mass Calibration

10.1.1. Calibrate the mass spectrometer as specified by the manufacturer.

10.2. MS/MS Optimization

10.2.1. Each LC/MS/MS system will have different optimal conditions, depending on source geometry and system design. Follow manufacturer recommendations for tuning the instrument.

10.2.2. During the development of this method, instrumental parameters were optimized for the precursor and product ions listed in Table 3. Alternate transitions are permitted so long as the product ions chosen are the most abundant, free from interference ions available. All required QC requirements as well as desired sensitivity, selectivity and calibration curve linearity must pass.

10.2.3. Optimize the response of the precursor and product ions for Benzotriazole and Benzotriazole-d_4 according to manufacturer recommendations. The MS parameters used during method development are listed in Table 2. The MS/MS parameters determined during method development are listed in Table 3.

10.3. Chromatographic Conditions

10.3.1. Establish LC method parameters to optimize peak shape. The LC parameters used during method development can be found in Table 1. Modifying conditions (i.e. mobile phase composition, and LC column) are allowed only if the QC criteria in section 9.0 are still satisfied.

10.3.2. No carry over of Benzotriazole was observed during validation but steps should be taken to minimize Benzotriazole background from the LC system components and mobile phases. Verify that no carryover occurs after the highest calibration point.

10.4. Initial Calibration

10.4.1. The calibration standard curve must contain at least 6 non-zero standards, the lowest calibration standard must be at or below the MRL. The method development calibration curve consisted of 8 levels: 0.2, 0.5, 1, 2, 5, 10, 20, 50ppb and were prepared in 90:10 reagent water: acetonitrile.

10.4.2. Fit the calibration points with either a linear or quadratic regression, calibration must be done using peak areas and the internal standard technique. During method development, a quadratic calibration curve weighted (1/X) was used.

10.4.3. Validate the initial calibration by calculating the concentration of Benzotriazole as an unknown at each calibration level. For calibration levels ≤MRL, results must be within ±50% of the true value. All other calibration levels must be within ±30% of their true values. Relative Standard Error must be 15% or less.

$$\%RSE = 100 \times \sqrt{\sum_{i=1}^{n} \left[\frac{x_i' - x_i}{x_i}\right]^2 / (n-p)}$$

10.4.4. If validation of the initial calibration is not successful, reanalyze the calibration standards or restrict the calibration range. If the cause of failure is due to contamination or degradation of the calibration standards, prepare fresh calibration standards and repeat the initial calibration steps.

10.5. Continuing Calibration Checks (CCCs)

10.5.1. Analyze a CCC at the beginning of each Analysis Batch to verify the calibration, after every 10th sample, and at the end of the Batch. The first CCC must be at the MRL to verify initial instrument sensitivity.

Subsequent CCCs should alternate between mid- and high-level calibration standards.

10.5.2. Verify that the peak areas of the quantitation ion of the internal standard have not changed by more than ±50% of the average areas measured in the initial calibration. If the internal standard peaks areas are not within the limits, see Section 10.6 for corrective actions.

10.5.3. Calculate the concentration for Benzotriazole in each CCC. Those fortified at the MRL must be ±50% of the true value. The mid- and high-calibration levels must be within ±30% of their true values. If the limits are exceeded, then any samples run since the last passing CCC must be reanalyzed after an acceptable calibration has been reestablished.

10.6. Corrective Action

10.6.1. Failure to meet CCC QC criteria necessitates corrective action. Performance may be restored by flushing the column with 100% ACN. After this or other minor corrective steps, check the calibration with both an MRL- and mid-level CCC. If failures continue, more major changes may be necessary, such as replacing the LC column or MS/MS system service. If major maintenance is performed, return to initial calibration (Section 10.4) before proceeding.

11. Procedure:

11.1. Preparation of blank and fortified samples

11.1.1. Allow standards to come to room temperature.

11.1.2. LRB: Add 1mL of reagent water to an autosampler vial followed by 25µL of 20ppb IS (Benzotriazole-d_4). Cap, then vortex to mix well.

11.1.3. LFB / LFSM / LFSMD: Add required reagent water or field sample water to achieve the correct spike level to a 15mL polypropylene centrifuge tube with cap. Spike appropriate amount of Benzotriazole. For example, for a 5ppb LFB, 9.5 mL of reagent water is added to a centrifuge tube. 0.25mL of 200ppb Benzotriazole solution is then spiked into the water. Cap centrifuge tube and vortex to mix well. A 1mL aliquot is then taken and added to an autosampler vial followed by 25µL of 20ppb ISTD (other concentrations of ISTD may be used so long as the concentration of ISTD in the sample is the same as the ISTD concentration in the standards). Cap, then vortex to mix well.

11.2. Test sample preparation.

11.2.1. Allow sample to come to room temperature

11.2.2. Aliquot 1mL of sample into an autosampler vial

11.2.3. Add 25µL of 20ppb ISTD (other concentrations of ISTD may be used so long as the ISTD concentration in the sample is the same as the ISTD concentration in the standards).

11.2.4. Cap, then vortex to mix well

11.3. Sample Analysis

11.3.1. Set instrument parameter to MS/MS operating conditions per the procedures in Section 10.2 and chromatographic conditions per Section 10.3. Establish a valid initial calibration following the procedures in Section 10.4 or confirm that the existing calibration is still valid by analyzing a low-level CCC. Analyze field and QC samples in a properly sequenced Analysis Batch as described in Section 11.4.

11.3.2. The analyst must ensure that the method analyte elutes entirely within the assigned window during each Analysis Batch, see Section 12.1. Make this observation by viewing the quantitation ion for each analyte in the CCCs analyzed during an Analysis Batch. If an analyte peak drifts out of the assigned window, then data for the analyte is invalid in all injections acquired since the last valid CCC.

11.4. Analysis Batch Sequence

11.4.1. An Analysis Batch is a sequence of samples, analyzed within a 24-hour period, of no more than 20 field samples and includes all required QC samples (LRB, CCCs, LFSM, and LFSMD). The required QC samples are not included in the maximum total of 20 field samples. LC-MS/MS conditions for the Analysis Batch must be the same as those used during calibration.

11.4.2. ANALYZE INITIAL CCC – After a valid calibration is established, begin every Analysis Batch by analyzing an initial CCC at or below the MRL. This initial CCC must be within ±50% of the true value and must pass the IS area criteria (see 10.5.2.). The initial CCC confirms that the calibration is still valid. Failure to meet the QC criteria may indicate that recalibration is required prior to analyzing samples. After the initial CCC, continue the Analysis Batch by analyzing an LRB, followed by field and QC samples at appropriate frequencies (see 9.2).

11.3.3. ANALYZE FINAL CCC – A final CCC completes the Analysis Batch. The acquisition start time of the final CCC must be within 24 hours of the acquisition start time of the initial low-level CCC at the beginning of the Analysis Batch. More than one Analysis Batch within a 24-hour period is permitted

12. Data Analysis and Calculations:

12.1. ESTABLISH RETENTION TIME WINDOW – Establish an appropriate retention time window for the analyte to identify them in the resulting chromatograms. Base this assignment on measurements of actual retention time variation for each compound in standard solutions over the course of time. The suggested variation is plus or minus three times the standard deviation of the retention time for each compound for a series of injections. The injections from the initial calibration and from the ICD (Section 9.1) may be used to calculate the retention time window.

12.2. IDENTIFY PEAKS OF INTEREST – At the conclusion of data acquisition, use the same software settings established during the calibration procedure to identify peaks of interest in the predetermined retention time window. Confirm the identity of the analyte by comparison of its retention time with that of the corresponding analyte peak in an initial calibration standard.

12.3. CALCULATE ANALYTE CONCENTRATION – Calculate analyte concentrations using the multipoint calibration established in Section 10.4. Report only those values that fall between the MRL and the highest calibration standard.

12.4. ROUND CONCENTRATIONS - Calculations must use all available digits of precision, but final reported concentrations should be rounded to an appropriate number of significant figures (one digit of uncertainty), typically two, and not more than three significant figures.

12.5. EXCEEDING THE CALIBRATION RANGE – If a result exceeds the calibration curve, dilute the sample with 90:10 reagent water: acetonitrile and the appropriate amount of internal standard added to match the original level. Select a dilution factor to result in the diluted concentration being near midpoint of the calibration curve and reinject this diluted sample. Incorporate the dilution factor into final concentration calculations. The resulting data must be annotated as a dilution, and the reported MRL must reflect the dilution factor.

13. Method Performance:

13.1. EPA’s Alternate Testing Procedure (ATP) protocol was followed, where possible, for method development and validation.

13.2. Method Detection Limits (MDL) refers to the lowest concentration of the analyte that a method can report with 99% confidence that the measured concentration is distinguishable from method blank results. To determine the MDL, seven reagent water samples were spiked at 0.5ppb of Benzotriazole and processed through the entire method along with seven drinking water blanks. The standard deviation derived from the spiked sample recoveries was used to calculate the MDL using the following equation:

$MDL_S = (t)*(S)$
Where t is the Student single tailed t-test value for the 99% confidence level with n-1 degrees of freedom and S denotes the standard deviation obtained from n replicate analyses. For the n=7 replicates used to determine the MDL, t=3.143.

The MDL from the blanks (MDL_B) did not apply based on the EPA's procedure for having none of the method blanks give numerical results, none detected (ND). The results for the standard deviations and MDL are in Table 4.

13.3. The LCMRL fortification levels and calculated result are shown in Table 5. The single-laboratory LCMRL is 0.44ppb.

13.4. MRL passed the EPA confirmation criteria at 0.5ppb. The results for this MRL confirmation are in Table 6.

13.5. Method Validation consisted of the analysis of reagent water. These waters were spiked at five different levels (0.5, 1, 2, 5, and 10ppb) and analyzed in five separate data sets on separate days. Recoveries for these validation samples are shown in Table 7.

13.6. Precision and Accuracy spikes were performed on background water. Benzotriazole was spiked at a low level (0.6ppb) and a mid-level (5ppb) on one pre-treatment and one post-treatment sample from two different sites. The results are in Table 8.

13.7. STORAGE STABILITY STUDY - A storage stability study was completed. The storage stability study consisted of three replicates spiked at 5ppb tested over a 28-day period. Amber glass bottles containing background water; one pre-treatment water, one post-treatment water, and each with and without chlorine quencher (ascorbic acid) were spiked and stored in the refrigerator, and 1 mL aliquots of each were removed to be analyzed on days 0-28. A matrix blank and a matrix spike (5 ppb) were also analyzed on each analysis day and analyzed with the storage stability samples. This storage stability study shows Benzotriazole stability through day 28. No notable differences were observed with spike recoveries of samples with and without the chlorine quencher. A chlorine quencher is not required for this analysis. The results are shown in Table 9.

14. Pollution Prevention

14.1. For information about pollution prevention applicable to laboratory operations described in this method, consult: Less is Better, Guide to Minimizing Waste in Laboratories, a web-based resource available from the American Chemical Society at www.acs.org.

15. Waste Management

15.1. The Agency requires that laboratory waste management practices be consistent with all applicable rules and regulations, and that laboratories protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. In addition, compliance is required with any sewage discharge permits and regulations, particularly the hazardous waste identification rules and land disposal restrictions.

16. References:

16.1. Definition and Procedure for the Determination of the Method Detection Limit, Revision 2. United States Environmental Protection Agency. December 2016.
16.2. Protocol for the Evaluation of Alternate Test Procedure for Organic and Inorganic Analytes in Drinking Water. United States Environmental Protection Agency. May 2023.
16.3. McGowin, Audrey PhD, Wiese, Jessica. Isolation of Benzotriazole and Analog Compounds in Wilmington Air Park Runoff Water Samples Via Solid-Phase Extraction, October 8, 2019.
16.4. Kloepfer, Achim, Jekel, Martin, Reemtsma, Thorsten. Determination of benzothiazoles from complex aqueous samples by liquid chromatography-mass spectrometry following solid-phase extraction. September 15, 2004.
16.5. Determination of polar 1H-benzotriazoles and benzothiazoles in water by solid-phase extraction and liquid chromatography LTQ FT Orbitrap mass spectrometry, KWR Watercycle Research Institute BTO 2009.020 February 2009.
16.6. Krasevec, Ida, Prosen, Helena. Solid-Phase Extraction of Polar Benzotriazoles as Environmental Pollutants: A Review. Published September 29, 2018.
16.7. Zhang, Zifeng, Ren, Nanqi, Li, Yi-Fan, Kunisue, Tatsuya, Gao, Dawen, Kannan, Kurunthachalam. Determination of Benzotriazole

and Benzophenone UV Filters in Sediment and Sewage Sludge. Environmental Science Technology. Published April 11, 2011.

16.8. Asimakopoulos, Alexandros G., Bletsou, anna A., Wu, Qian, Thomaidis, Nikolaos S., Kannan, Kurunthachalam. Determination of Benzotriazoles and Benzothiazoles in Human Urine by Liquid Chromatography-Tandem Mass Spectrometry, ACS Publications. Published December 4. 2012.

16.9. Carpinteiro, I., Abuin, B., Ramil, M., Rodriguez, I., Cela, R. Simultaneous determination of benzotriazole and benzothiazole derivatives in aqueous matrices by mixed-mode solid-phase extraction followed by liquid chromatography-tandem mass spectrometry, Anal Bioanal Chem (2012) 402:2471-2478

16.10. TZW: DVGW Water Technology Centre

16.11. Leedy, Clara. Detection of benzotriazole and related analogues in surface samples collected near an Ohia airpark. Wright State University, 2022.

16.12. EPA Document # 815-R-05-006 Statistical Protocol for the Determination of the Single-laboratory Lowest Concentration Minimum Reporting Level (LCMRL) and Validation of Laboratory Performance at or Below the Minimum Reporting Level (MRL). David Munch, Phillis Branson U.S. Environmental Protection Agency Office of Ground Water and Drinking Water Standards and Risk Management Division, Technical Support Center. November 2004.

17. Tables, Figures, and Method Performance Data

Table 1. HPLC Method Conditions*

Time (min)	%Organic Phase	Flow Rate (mL/min)
Initial	5	0.4
1	5	0.4
8	95	0.4
10	95	0.4
11	5	0.4
12	5	0.4

*Phenomenex Kinetex 2.6 µm Phenyl-Hexyl, LC column 100 x 3 mm. 50 µL injection into a 100 µL loop; run time 12 minutes.

Table 2. MS Method Conditions

MS Conditions for CAC (Sacramento, CA) AB Sciex 6500 +	
Polarity	Positive

Ion Spray Voltage	4500
Curtain Gas	30
Temperature	500°C
Ion Source Gas 1	60
Ion Source Gas 2	60

Table 3. Retention Times and MS/MS Method Conditions[a]

Analyte	RT (min)	Precursor Ion (m/z)[b]	Product Ion (m/z)[b]	Declustering Potential (V)	Collision Energy (V)[c]
Benzotriazole	5.22	**120**	**65**	**80**	**30**
		120	92	80	24
Benzotriazole-d_4	5.14	**124**	**69**	**80**	**30**
		124	96	80	24

[a] Quantitation Precursor and Product Ions are in bold

[b] Precursor and product ions listed in this table are nominal masses. During MS and MS/MS optimization, the analyst should determine precursor and product ion masses to one decimal place by locating the apex of the mass spectral peak (e.g., m/z 120.0→64.9 for Benzotriazole). These precursor and product ion masses (with at least one decimal place) should be used in the MS/MS method for all analyses.

[c] Nitrogen used as collision gas.

Table 4. The Determination of Method Detection Limit (MDL) in Reagent Water Spiked at 0.5 ppb.

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	SD	MDL
Spike	0.505	0.506	0.512	0.469	0.635	0.519	0.518	0.053	0.17
Blank	ND	ND	ND	ND	ND	ND	ND	-	-

EPA MDL definitions set the MDL to be the higher value of the MDL_S and MDL_B. Therefore, the Benzotriazole MDL= 0.17ppb.

Table 5. Lowest Concentration Minimum Reporting Limit for Benzotriazole

Analyte	Fortification levels, ng/mL	LCMRL
Benzotriazole	0, 0.17, 0.5, 0.68, 1, 2, 5, 10	0.44ppb

Table 6. The Confirmation of Method Reporting Limit (MRL) in Reagent Water Spiked at 0.5 ppb.

Sample	Conc. (ppb)
1	0.512237

2	0.522045
3	0.538971
4	0.551674
5	0.484872
6	0.491576
7	0.483537
Mean	0.51213
SD	0.026974
Half Range	0.106169
Upper Limit	124%
Lower Limit	81%

Lower Limit >50% and Upper Limit <150%
0.5 ppb PASSES as the MRL for Benzotriazole.

Table 7. Method Validation in Reagent Water

		Spike Level Recovery				
Water Source	Day	0.5 ppb	1 ppb	2 ppb	5 ppb	10 ppb
Reagent water	1	101%	103%	101%	103%	101%
	2	101%	95%	90%	101%	103%
	3	93%	107%	97%	107%	101%
	4	127%	106%	125%	112%	111%
	5	104%	131%	106%	135%	137%

Control Limits	
Mean	108%
SD	13%
RSD	11.9%
UCL	147%
LCL	69%

Table 8. Precision and Accuracy in Drinking Water before and after Treatment

Water type	Average %Recovery	%RSD

Sacramento finished	116	11
Sacramento raw	125	10
Fairbearn finished	123	20
Fairbearn raw	84	17

Spiked at 0.6ppb Benzotriazole

Water type	Average %Recovery	%RSD
Sacramento finished	123	23
Sacramento raw	119	13
Fairbearn finished	121	13
Fairbearn raw	92	17

Spiked at 5ppb Benzotriazole

Table 9. Storage Stability Study in Raw and Finished Drinking Water with and without chlorine quencher Spiked at 5 ppb.

	Spike Recovery					
	Day 0	Day 3	Day 7	Day 14	Day 21	Day 28
Raw	103%	97%	98%	95%	95%	99%
Finished	100%	95%	100%	96%	95%	99%
Raw w/quencher	100%	94%	101%	96%	94%	93%
Finished w/quencher	98%	98%	102%	95%	96%	99%

Table 10. Initial Demonstration of Capability (IDC) QC Requirements

Method Reference	**Requirement**	**Specification**	**Acceptance Criteria**
9.1.1	Demonstration of precision and accuracy	Analyze 7 replicate Laboratory Fortified Blanks (LFBs) at 5ppb.	Percent relative standard deviation ≤20%. Mean percent recovery ≥70% and ≤130%.
9.1.2	Demonstration of acceptable system background	Analyze 5 Laboratory Reagent Blanks (LRBs).	Benzotriazole concentration must be less than one third the Minimum Reporting

			Level (MRL) in each LRB.
9.1.3	MRL confirmation	Fortify and analyze 7 replicate LFBs at the proposed MRL concentration. Confirm that the Upper Prediction Interval of Results (PIR) and Lower PIR meet the recovery criteria.	Upper PIR ≤150% Lower PIR ≥50%
9.1.4	Quality Control Sample (QCS)	Prepare a QCS near the center of the calibration with Benzotriazole from a source independent from the calibration standards.	Percent recovery ≥70% and ≤130% of the true value

Table 11. Analysis Batch QC Requirements

Method Reference	**Requirement**	**Specification and Frequency**	**Acceptance Criteria**
10	Initial Calibration	Use internal standard technique to generate a linear or quadratic calibration curve. Use at least 6 standard concentrations. Evaluate the calibration curve as in (10.4)	Analytes at or below the MRL must be within 50-150% of the true value. All other levels must be within 70-130% of the true value. RSE ≤ 15% preferred or r^2 ≥ 0.990
9.2.1	Laboratory Reagent Blank (LRB)	Analyze one LRB per Analysis Batch.	Benzotriazole concentration must be less than one third the Minimum Reporting Level (MRL) in each LRB.
9.2.2	Laboratory Fortified Blank (LFB)	Reagent water fortified at low, middle, and high concentrations. Run on a rotational basis.	Percent recovery ≥50% and ≤150% of the true value when at or less than two times the MRL is used

			or ≥70% and ≤130% when greater than two times the MRL is used
9.2.3	Laboratory Fortified Sample Matrix (LFSM) and LFSM Duplicate	Fortify the LFSM and LFSMD near the center of the calibration curve. One set in an Analysis Batch	Mean percent recovery of LFSM and LFSMD pair ≥70% and ≤130%. Relative percent difference (RPD) ≤ 30%. Qualify results for samples failing these limits as “suspect–matrix”.
9.2.4	Quality Control Sample (QCS)	Assay 1 QCS for each new lot of calibration standards. Prepare the QCS near the center of calibration with Benzotriazole from a source independent of the calibration standards.	Percent recovery ≥70% and ≤130% of the true value.
9.2.5	Continuing Calibration Checks (CCC)	Calibration standards at MRL-. Mid-, and high-level concentrations.	Percent recovery for at MRL must be within 50% of the true value. All other levels must be within 30% of the true value
9.2.7	Retention Time (RT)	The retention time for each field sample in an Analysis Batch must be ± 0.1 min from the RT of the calibration standards of that batch.	Field Sample RT ± 0.1 min. of Calibration RT.
9.2.8.	Ion Ratio	The ion ratio between the quantitation and confirming ions shall of field samples shall be ≤ 30% when compared to the ion ratio of the standards	Ion ratio field sample ≤ 30% ion ratio of standards