Technical Report

Multi-Laboratory Validation Study for Quantitation of Benzotriazole by CAC-Benzotriazole-1.0

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Table of Contents

List of Tables

1. Introduction:

1.1. Background

Benzotriazoles contain a five-member ring with three nitrogen atoms directly bonded to one another as substituents on a benzene ring. There are three primary uses for benzotriazoles: corrosion inhibitor, ultraviolet light stabilizer for plastics, and antifogging in photography. It is also used in electronic manufacturing, construction and coating. Because benzotriazoles are used in large quantities as corrosion inhibitors, it is mainly through this type of use that benzotriazoles become an environmental contaminant.

Benzotriazoles are increasingly recognized as an emerging contaminant, are endocrinal disruptors, and genotoxins. They are of interest in drinking water monitoring due to their persistence and mobility; they are highly water soluble and could migrate easily through soil into groundwater or surface waters used for drinking water supplies.

Specifically, 1-H Benzotriazole (Benzotriazole), the focus of this study, is a heterocyclic compound with the chemical formula C6H5N3, CAS# 95-14-7, and a molar mass of 119.1 g/mol. It is in the benzotriazole class of compounds used as a corrosion inhibitor for metals like copper, aluminum, and zinc. Because many drinking water pipes are made of copper, Benzotriazole is an emerging health hazard concern regarding drinking water.

The State Water Resources Control Board, Division of Drinking Water (DDW) selected this compound to conduct method development and validation which includes accuracy, precision, limit of detection, method reporting limits, linearity, selectivity/specificity, robustness, stability, quality control procedure, and this Multi-Laboratory Validation Study.

1.2. Method Summary

The analytical method development for this study was validated by the California Department of Food and Agriculture, Center for Analytical Chemistry (CAC) and the single laboratory validation results are summarized in the following section. Refinements were made to that method based on the comments and results of the participating four laboratories in the Multi-Laboratory Validation Study (MLVS). Those updates are released as CAC-Benzotriazole-1.1. This complete method is attached to this report as Appendix A.

The analytical method includes sample preparation and sample analysis procedure for source and treated surface and groundwater. The limited sample preparation includes adding a deuterated internal standard (1-H Benzotriazole d4 or Benzotriazole-d4) to samples prior to a direct injection. The method utilized liquid chromatography tandem mass spectrometry (LCMS/MS) for the quantitative analysis of Benzotriazole.

1.3. Summary of the Single-Laboratory Study

The single-laboratory validation was performed by CAC. This laboratory was originally contracted by the State Water Resources Control Board, Division of Drinking Water (DDW) to develop a laboratory Standard Operation Procedure (SOP) for this study with a couple of goals:

- Identify and quantify Benzotriazole in drinking water
    - The study generated method performance data for aqueous matrix samples (surface water, source and treated) and of those spiked and analyzed in the single-laboratory study, most had recoveries between 70-130% with three data points landing above 130% recovery at 131%,135%, and 137% with a Relative Standard Deviation (RSD) percent of 11.9%. The single-laboratory validation results demonstrated that this method could identify and quantify Benzotriazole.
    - Using simply a direct injection method, the single-laboratory validation proposed a minimum reporting limit (MRL) of 0.5ppb Benzotriazole after calculating an estimated lowest concentration minimum reporting limit (LCMRL) of 0.44ppb Benzotriazole. The MRL was confirmed with an Upper PIR Limit of 124% and a Lower PIR Limit of 81%.
- Implementation of the method at a typical mid-sized full-service testing laboratory
    - Because the required instrumentation for this method has become commonplace in most of the full-service laboratories, the results of the single-laboratory study demonstrate that this goal is achievable. Also, the limited sample preparation involved in this method makes for an easy to implement method in any laboratory. The multi-laboratory validation study will determine how well a typical full-service laboratory can perform the method.

2. Study Management, Objectives, Design, and Implementation:

2.1. Study Management

DDW communicated with laboratories to recruit participants for the study and the ones listed in Table 2-1 were selected by DDW to participate in this MLVS. Four laboratories (three commercial contract laboratories and one municipal water district laboratory) are amongst the participating laboratories. All laboratories contributed to the analysis of aqueous matrices in this report (surface drinking water, treated and untreated sources). For the purposes of this study, the laboratories were randomly assigned numbers, which were used to maintain the anonymity of the results.

The CAC Quality Assurance unit (CAC-QA) managed the Proficiency Testing (PT) spiking and received all data packages from the laboratories. Analytical standards from two different sources were provided to the participating laboratories by CAC. A neat Benzotriazole standard was purchased for second source use, and CAC prepared the stock solution that was provided to the participating laboratories. CAC-QA, with an established operational set-up and expertise to conduct Proficiency Testing schemes, prepared the PT samples, and shipped as part of this study, while CAC prepared the aqueous sample matrices.

CAC served as the method consultant to the Multi-Laboratory Validation Study (MLVS).

Table 2-1: Participating Laboratories

Laboratory	Location
Eurofins	West Sacramento, CA
Irvine Ranch Water District	Irvine, CA
McCampbell Analytical, Inc.	Pittsburg, CA
Weck Laboratories, Inc.	Industry, CA

Laboratory numbers listed throughout this report are randomized and will not follow the order provided in Table 2-1.

2.2. Study Objective and Design

The focus of the MLVS is to generate the necessary data to document the precision and accuracy and overall performance of the analytical method for quantitation of Benzotriazole in aqueous matrices. The primary objectives of this MLVS are to:

- Obtain data from aqueous matrices that are representative of the method's intended use.
- Obtain data from laboratories that are representative of those likely to use the method, but that were not directly involved in the method development.
- Obtain feedback from laboratory users on the specifics of the method SOP.

- Use study data to evaluate the performance of the method.

A brief description of the key points of this study design include:

- At least four laboratories, one of which is a municipal surface water testing site
- Two aqueous matrix samples from source and treated ground water.
- Initial calibration of Benzotriazole by each laboratory.
- Initial Demonstration of Capability (IDOC) by each laboratory.
- Analyses of PT sample and matrix spike samples from each aqueous matrix.
- Data analysis, statistical validation, and compliance with acceptance criteria for participating laboratories results

This MLVS was conducted in two phases. The IDOC, which includes the initial calibration and verification of MRL, and the method evaluation in the chosen aqueous matrices.

2.3. Matrix and Sample Selection

During the single laboratory validation, surface and ground water, treated and untreated sources were tested without a noticeable matrix effect. Therefore, two ground water samples (one source and one treated) were used for this phase of the MLVS. These were chosen to be representative of the expected real-world matrices analyzed by this method. These samples were collected from the Citrus Heights Water District by DDW personnel and brought to CAC for analysis, spiking, and distribution to the participating laboratories.

The MLVS were designed so that for each sample the following would be analyzed: an unspiked sample, two replicates spiked at low concentration, and two replicates spiked at a mid-level concentration.

2.4. Selection of Spiking Levels and Aqueous Media

All the drinking water samples collected were screened for baseline Benzotriazole levels. No detectable amounts of Benzotriazole were observed above one-third of the single laboratory MRL. The low concentration spikes were chosen at the proposed MRL (0.5ppb) to ensure accurate detection and quantitation at the MRL. The mid-level concentration spike was chosen at 5ppb so as to fall well within the calibration curve. The calibration curve spanned from 0.2 ppb to 50ppb.

2.5. Preparation of Study Samples

Aliquots of ground drinking water for both treated and untreated sources were prepared as follows:
The amber glass sample bottles delivered by DDW were allowed to come to room temperature and contents were mixed by inverting several times to ensure homogeneity as described in the SOP by CAC staff. A 10mL aliquot was taken and transferred to a

12mL amber bottle. The water samples prepared and shipped by CAC were each prepared as one unspiked (blank) sample, duplicates at the low spike level, and duplicates at the mid-spike level. After spiking, they were mixed well, sealed and stored in a refrigerator until they were packaged and shipped to the participating laboratories. The samples were shipped in insulated boxes with blue-ice to keep them cold.

2.6. Proficiency Testing Samples

As part of the MLVS, PT Samples were sent to the participating volunteer laboratories. An unknown spiked sample, the level of which was chosen and prepared by CAC-QA was prepared in reagent water. The standard used for spiking was the second source standard. The PT sample was shipped along with the study sample spikes. PT samples were prepared by QA staff. These were analyzed by CAC's Research and Development staff. Data was submitted to CAC-QA and the results are included in Table 6-1.

2.7. Storage Stability Study

As part of the method development for Benzotriazole, a storage stability study was conducted. Both treated and raw drinking water samples were spiked in triplicate at a mid-level concentration. The samples were placed in the refrigerator to be brought out on days 0, 3, 7, 14, 21, and 28 and analyzed to determine percent recovery. The percent recovery for all samples on all days was greater than or equal to 95% but less than 105% resulting in the conclusion that Benzotriazole is stable for 28 days and thus samples may remain refrigerated and unanalyzed for that length of time without loss or degradation of analyte.

A tandem storage stability study was conducted with samples containing a chlorine quencher (ascorbic acid) to evaluate the need for such an additive. It was determined that a chlorine quencher was not needed as the percentage recoveries of samples with and without the chlorine quencher were comparable throughout the 28 days.

3. Data Management, Data Validation, and Data Rules for Statistical Analysis:

3.1. Programmatic Overview

The data management process involved documented, thoroughly reviewed and approved instructions, meetings, consultation and communication when required, and review of laboratory packages. A “kickoff” meeting over Teams was scheduled to explain the expectations for the MLVS to the participating laboratories. Laboratories were given the method prior to the meeting in preparation and given the opportunity to ask clarifying questions or concerns. They were also encouraged to ask questions via email throughout the MLVS. A written instructional of the MLVS guidelines and passing criteria was also provided as well as a data reporting excel sheet template to ensure uniform reporting. See Appendix B for a copy of the excel sheet template.

3.2. Data Management

All raw data and reporting forms were submitted electronically by the laboratories to CAC-QA. The laboratories were given an Excel reporting template where they would enter their data and calculate results such as percent recovery and %RSD. The Excel template had locked password protected cells to prevent accidental corruption or unintended changes. The laboratories also submitted any pertinent instrument reports such as chromatograms.

3.3. Data Validation

All data packages were reviewed for completeness and compliance with the requirement of the MLVS Method. CAC-QA performed the review process.

The data validation process included examining the submitted data for meeting passing MLVS Method criteria. Passing criteria for this MLVS are summarized in the Guidelines for Interlaboratory Validation of Method CAC-Benzotriazole-1.0 and are included as Appendix C.

Laboratories were instructed by the SOP to analyze seven Lab Fortified Blanks (LFB) for Precision and Accuracy (P&A) in one analysis batch which was to include the five lab reagent blanks for demonstration of low system background. Laboratories 1 and 2 analyzed these samples in three or more batches.

4. Calibration and Quantification:

Water sample extracts were analyzed by LC-MS/MS. The mass spectrometer underwent mass calibration to ensure the accuracy of the mass to charge ration (m/z) values assigned to the instrument per the manufacturer's instructions. After the mass calibration had been verified, an eight-point calibration was performed using quantitative standards.

4.1. Mass Calibration and Mass Calibration Verification

Each laboratory performed mass calibration and mass calibration verification in accordance with their respective instrument manufacturer's instructions. The laboratories were instructed to determine precursor and product ion masses to one decimal place. All laboratories used the following transitions: 120->65 (for quantitation), 120->92 (for confirmation).

4.2. Initial Calibration

To provide each laboratory with the target analyte, CAC procured standards from HPC Standards Inc and LGC Standards, two commercial standards vendors. By providing the standards to all laboratories, the variability in the study results which may have resulted in the variability in standard preparation from each laboratory was significantly reduced. This also increased the effectiveness in terms of direct costs or the time factor to each laboratory for their participation. The standards provided by CAC were used by the laboratories to create all calibration, calibration verification, and spiking solutions used in the MLV.

Each laboratory calibrated their LC-MS/MS instrument using a series of calibration standards like the calibration standards listed in the MLVS method; 0.2, 0.5, 1, 2, 5, 10, 20, and 50ppb. Laboratory 4 used calibration curves with their lowest standard at 0.25ppb. A minimum of six calibration standards was required for a valid analysis with the lowest calibration standard being at or below the MRL. The laboratories were allowed to use a linear or quadratic regression using peak areas and the internal standard technique. The MLVS method outlines calibration and quantification using an internal standard where Benzotriazole's response is compared to the isotopically labeled Benzotriazole-d4 (HPC Standards Inc., item# 681254).

Using the internal standard technique, participating laboratories needed to generate a linear or quadratic calibration curve. 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. Regression coefficients, r or r^2, were required to pass the following criteria: $r>0.995$, $r^2>0.990$. The relative standard error of the calibration curve needed to be less than or equal to 15%.

An initial calibration was required to be submitted by each laboratory as part of the IDOC prior to receiving spikes/PT samples. Laboratory number 1 excluded points from one of

their calibration curves used for their IDOC. Laboratory number 2 reported results from a failing curve with an r = 0.98865 in their IDOC and excluded points from three calibration curves. The remaining laboratories included all points. The results from the failing curve were not excluded but will be flagged as estimates. The data that is affected by this failing curve are three LFB used for precision and accuracy, one MB used to verify low system background, and one MRL spike used to verify their MRL. Since one MRL spike is amongst these estimated values, the upper and lower PIR for Laboratory number 2 also be considered an estimate

4.3. Calibration Verification

Continuing Calibration Checks (CCCs) were analyzed at the beginning of each analysis batch to verify the calibration and at the end to verify continued calibration. The first CCC must be at the MRL to verify initial instrument sensitivity. Subsequent CCCs should alternate between mid- and high-level calibration standards. CCCs fortified at the MRL must be within 50% of the true value. Mid- and high-calibration levels must be within 30% of the true value. All laboratories had passing CCCs even at the MRL. Data submitted from all laboratories met this criterion indicating the MLVS calibration verification percent recovery criteria is routinely achievable.

4.4. Quality Control Samples (QCS)

Benzotriazole standards were provided from two different sources. One source was to be used for the Quality Control Sample. The QCS is a mid-level standard prepared from a source separate from the calibration curve and is used to confirm the accuracy of the calibration standards. The QCS must be within 30% of the true value. All laboratories had passing QCS values indicating accurate calibration curves for this MLVS.

Table 4-1: QCS Results

MLVS Laboratory number	QCS Results	
	Amount spiked (ppb)	% Recovery
1	10	118.0
2	5	98.9
3	5	100.5
3*	5*	98.8*
4	1	101.1

*Laboratory number three re-ran IDOC

5. Initial Demonstration of Capabilities:

In addition to performing an initial calibration, laboratories submitted an IDOC. The IDOC included MRL confirmation, precision and accuracy results, and a system background check.

5.1. Method Reporting Limit Confirmation

The Minimum Reporting Limit for Benzotriazole by this method was confirmed during the single laboratory validation to be 0.5ppb. Participating laboratories were required to determine and verify an MRL for their laboratory by spiking seven reagent water samples at the proposed MRL. The results of these spikes must pass the upper and lower Prediction Interval of Results (PIR) criteria.

All laboratories used 0.5ppb as their proposed MRL and all laboratories had passing PIR values. Laboratory 2 reported the widest PIR value range with the upper PIR 141.3% and the lower PIR 51.9%.

5.2. Precision and Accuracy (P&A) Results

Participating laboratories were required to spike seven replicate Laboratory Fortified Blanks (LFB) at 5ppb Benzotriazole using reagent water samples. These spikes were prepared and analyzed in the same manner as study samples. A relative standard deviation percent of less than 20% and a mean percent recovery of 70-130% is the passing criteria. The lowest reported percent recovery is 83.3% reported by Laboratory 2 which also had the highest relative standard deviation percent at 7.72%. Still, the results for all laboratories are well within the passing criteria.

5.3. Acceptability of System Background

Five laboratory reagent blanks were to be run in the same batch as the P&A samples. A native Benzotriazole of no more than one-third the MRL was required. No laboratory reported values above this amount.

Table 5-1: IDOC Results

MLVS Laboratory number	Initial Calibration	IDOC					
		P&A		System background	MRL confirmation		QCS
		Mean %	%RSD	Mean ppb	Upper PIR	Lower PIR	% Recovery
1	Pass	102.7	0.92	0.043**	121.6	78.9	118.0
2	Pass	83.3[a]	7.72[a]	0[a]	141.3[a]	51.9[a]	98.9
3	Pass	102.2	1.18	0.014	108.1	98.3	98.8
3*	Pass*	103.8*	5.07*	0.016*	107.2*	99.9*	100.5*
4	Pass	93.0	3.81	0	110.7	85.5	101.1

*Data from the rerun
** not true peaks, ion ratio fails
a = estimate – results reported from a failing curve, $r^2 < 0.990$

6. Water Matrix Results:

A total of ten samples were created and shipped to each participating laboratory as described in Section 2 of this report. The water matrix used was drinking ground water, treated and untreated, from Citrus Heights Water District. A PT reagent water sample was also created and shipped.

6.1. Benzotriazole Concentrations in Unspiked Matrices

Each laboratory received one bottle each containing about 10mL of unspiked treated water and untreated water. Table 6-1 summarizes the amounts reported. All but one laboratory reported none detected. Laboratory 3 reported results for Benzotriazole that were far below their lowest calibration standard but had confirming ion ratios for the samples.

Given that only one participating laboratory was able to determine a Benzotriazole amount indicates that real-world laboratories would have difficulty identifying Benzotriazole at lower concentration levels than those determined by this method. An objective of this method was to obtain data from laboratories that are representative of those likely to use the method. Since most laboratories reported none detect for unspiked samples shows that this method's reporting limit is in alignment with what most laboratories would be able to detect.

6.2. Matrix Spike Results

Spiked drinking water samples were analyzed in duplicate to demonstrate precision and accuracy on real-world samples. An objective of this study was to demonstrate performance of the method in real-world samples that contain target analytes.

As detailed in Section 2, the matrix spike samples were prepared and analyzed by each laboratory. The percentage recovery results are in Table 6-1. All results were within 70-130% of the amount spiked. The MRL level spike was at 0.5ppb and the mid-level spike was at 5ppb. The highest reported spike recovery is 122% by Laboratory 4 for a MRL spiked sample and the lowest spike recovery is 86.8% reported by Laboratory 2 for a mid-level spiked sample.

6.3. PT Results

Each laboratory received one 10mL PT sample. PT sample ensemble with matrices were packaged in a ThermoSafe box with ice packs ensuring standard integrity. All PT samples were shipped together, to ensure process integrity and uniformity. Participating laboratories were given a two-week period to submit results to the CAC-QAO. The results are presented in Table 6-1 and 6-2.
(Note: laboratory numbers listed below are randomized and do not follow the order provided in Table 2-1)

Table 6-1: Unspiked and Spiked Results

MLVS Laboratory number	Untreated %Recovery					Treated %Recovery				
	Unspiked	MRL		Mid-level		Unspiked	MRL		Mid-level	
		1	2	1	2		1	2	1	2
1	ND	119.9	110.3	115.5	116.8	ND	111.9	112.6	112.8	108.5
2	ND	103.2	100.6	97.9	104.5	ND	101.2	105.8	86.8	96.4
3	0.0153	113.3	111.7	111.0	111.1	0.0149	107.1	110.3	109.1	105.1
4	ND	122.0	121.8	107.3	107.6	ND	111.8	108.6	99.5	104.8

Table 6-2: PT Results

Laboratory number	Target Value in ppb	Recover Conc. In ppb	Percent Recovery
1	14.787	17.221	116%
2	14.787	14.634	99%
3	14.787	17.145	116%
4	14.787	15.723	106%

CAC PT results = 16.442 ppb

Target amount was used to evaluate the performance of the PT samples, and all four labs performed the tests satisfactorily. The overall recovery was 110% with an RSD of 7.5%. CDFA/CAC laboratory also conducted the PT analysis and reported a recovery of 111%. The data is not included in the calculation of the overall recovery.

7. Summary:

7.1. Preparatory Batch QC

7.1.1. Method Blank

Method blanks, also known as Laboratory Reagent Blanks (LRB) in this method, are included in the method to evaluate the potential for background contamination to be introduced during sample preparation in the laboratory. A Benzotriazole concentration in the LFB must be less than one third the MRL. The three laboratories that analyzed a LFB met this requirement. Laboratory number 4 did not analyze a LRB.

7.1.2. Laboratory Fortified Blanks Recovery (LFB)

Ongoing precision and recovery analyses (OPR), also known as Laboratory Fortified Blanks (LFB) in this method, were included in the method to evaluate the efficiency of the sample preparation process. A LFB was to be included in each preparation batch, which consisted of an aliquot of reagent water spiked with a known amount of Benzotriazole such that the final concentration of Benzotriazole in the LFB was greater than or equal to the MRL and less than or equal to the midpoint of the laboratory's calibration. This spike was prepared and analyzed in the exact same manner as study samples.

Laboratory number 2, in addition to an LFB, also prepared a Laboratory Fortified Blank Duplicate (LFBD). Although there are no method criteria for Relative Percent Difference (RPD) of LFB and LFBD, the RPD result for Laboratory 2 is 2.66.

Laboratory numbers 1 and 3 prepared Laboratory Fortified Sample Matrix (LFSM) and Laboratory Fortified Sample Matrix Duplicate (LFSMD). A LFSM and LFSMD are field samples, spiked with a known amount of Benzotriazole. This was not a requirement of this MLVS. However, the method criteria for LFSM and LFSMD is a RPD of ±50% when less than two times or equal to the MRL or ±30% when greater than two times the MRL. The RPD for Laboratory number 1 is 0.29 and RPD for Laboratory number 3 is 0.63. Laboratory 1 prepared their LFSM and LFSMD by spiking 5 ppb Benzotriazole on the PT sample. The result of their PT sample was 17.221ppb. 17.221ppb plus 5ppb gives a theoretical value of 22.22ppb. Laboratory 1 reported 22.275 and 22.339ppb for their LFSM and LFSMD respectively. Calculating percent recovery based on a 5 ppb spikes yields ~400% recovery. However, subtracting the incurred Benzotriazole amount of 17.221ppb in the PT sample results in 101 and 102% recovery for LFSM/LFSMD for Laboratory number 1. Laboratory number 3 used an unspiked water matrix sample. See Table 7.2. Laboratory number 4 did not analyze any LFB, LFBD, LFSM, or LFSMD. A LFB was a QC requirement for this MLVS.

7.2. Internal Standard (ISTD) Recovery Analyses

The labeled internal standard (Benzotriazole-d4) is added to the sample aliquot shortly before instrumental analysis. The response of the internal standard is used to calibrate Benzotriazole and to calculate recoveries. The response of the internal standard in the sample must be within 50-150% of its mean area in the calibration. All laboratories met this criterion.

Laboratory 3 opted to re-analyze their IDOC batch because the observed ISTD area count differed in their IDOC batch in comparison to their sample matrix/PT batch by a factor of about ten. An ISTD spiking error was suspected. However, the ISTD area, comparing standard curve and samples within the batch, had similar area counts. The data from the initial run and the re-run showed comparable results for Laboratory 3. Laboratory 1 had similar ISTD area count discrepancies between their IDOC batch and their sample matrix/PT batch. Laboratory 1 did not provide any re-run data.

7.3. Matrix Spike Analyses

The average minimum matrix spike recoveries for all participating laboratories was 86.8% and the average maximum matrix spike recoveries was 122%. The RSD for all matrix spike recoveries was 6.9%. This is consistent with matrix spike recoveries performed during the single laboratory validation which had a minimum spike recovery of 84% and a maximum percent recovery of 125%. The RSD for the single laboratory validation for matrix spikes was as low as 10% and as high as 23%. See Table 7-3 in this report and Table 8 in the method SOP.

7.4. Determination of Final QC Specifications for CAC-Benzotriazole-1.1

The initial QC acceptance criteria established in CAC-Benzotriazole-1.1 are validated by this MLVS. The final QC specifications remain unchanged as those established by this method

7.4.1. Final P&A

Precision and Accuracy criteria are 70-130% recovery and an RSD of less than or equal to 20%.

7.4.2. Final OPR (LFB)

The On-going precision and recovery, or LFB recoveries, criteria will remain the same at 50-150% for spikes at or below the MRL and 70-130% for all other spike levels. The relative percent difference between the Laboratory Fortified Blank and the Laboratory Fortified Blank Duplicate will be less than or equal to 30%.

7.4.3. Final ISTD

The ISTD percent recovery criteria is 50-150% of the true value.

Table 7-1: Method Blanks

Laboratory Number	Results
1	ND
2	ND
3	0.0116ppb
4	NA

Table 7-2: Laboratory Fortified Blank Results (also known as Ongoing Precision and Recovery) (LFB)

Laboratory number	LFB	% Recovery	LFBD	% Recovery	RPD	LFSM	% Recovery	LFSMD	% Recovery	RPD
1	5.056	101.1	--	--	--	22.275	445.5*** (spiked 5ppb)	22.339	446.8*** (spiked 5ppb)	0.29
2	5.189	103.8	5.053	101.1	2.66	--	--	--	--	--
3	5.0968	101.9	--	--	--	4.9855	99.7	5.0172	100.3	0.63
4	NA	NA	NA	NA	--	NA	NA	NA	NA	--

***Laboratory 1 used the PT sample for LFSM/LFSMD. Results for Laboratory 1 PT is 17.221ppb. Therefore, LFSM and LFSMD adjusted recoveries are 101 and 102% respectively.

Table 7-3: Matrix Spike Analyses (does not include PT results)

Number of Laboratories	Number of Results	Minimum % Recovery	Maximum % Recovery	Mean % Recovery	% RSD
4	32	86.8	122	108.3	6.9

8. Conclusions:

The objectives of this MLVS were achieved: validation of method CAC-Benzotriazole-1.1 and the development of a method that can be implemented at a typical mid-size environmental laboratory. Overall, the data generated during the MLVS demonstrated that CAC-Benzotriazole-1.1, as written, is unambiguous and robust enough to be performed by suitable laboratories using similar instruments of different manufacturers and models. The results generated by participating laboratories in this study routinely met the requirements stated in the method for:

- Mass calibration and mass calibration verification
- Initial calibration and calibration verification
- MRL verification
- Initial demonstration of capability (P&A, system blanks)
- Preparatory batch QC samples (LRB and LFB) and
- Quantitative analyte identification criteria (sample spikes and PT).

The suitability of CAC-Benzotriazole-1.1 to detect and quantify Benzotriazole in drinking water was successfully demonstrated through the analysis of real-world spiked samples. Method blank results demonstrated that there was negligible bias associated with background contamination. The P&A and OPR (LFB) recoveries (Tables 5-1, 7-1, and 7-2) associated with study samples were used to confirm QC acceptance criteria for the finalized method.

9. References:

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Appendix A: Standard Operating Procedure for CAC-Benzotriazole-1.1

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

Version 1.1
July 7, 2025

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This method was prepared under the contract with the State Water Resources Control Board, Division of Drinking Water 22-007-400.

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:

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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.

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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

Appendix B: Guidelines for MLVS of Method CAC-Benzotriazole-1.0

Guidelines for Interlaboratory Validation of Method CAC-Benzotriazole-1.0

This set of guidelines is written for the purpose of guiding the participating volunteering labs in the interlaboratory validation of method CAC-Benzotriazole-1.0: Benzotriazole, in Drinking Water by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS).

1. Initial Demonstration of Capability (IDOC)
    1.1. Labs will be provided with the SOP and stock solutions
        1.1.1. Upon receipt, verify the integrity of stock solutions:
            1.1.1.1. Box and ampules arrived undamaged
            1.1.1.2. Certificate of analysis included
            1.1.1.3. Expiration dates are valid
        1.1.2. Store according to certificate of analysis until ready for use.
    1.2. Prepare appropriate PDS levels as per the SOP in 90:10 reagent water:acetonitrile.
        1.2.1. 2ppb, 20ppb, and 200ppb levels for Benzotriazole
        1.2.2. 20ppb and 200ppb levels for ISTD
    1.3. Follow the SOP to perform the Initial Demonstration of Capability (IDOC)
    1.4. IDOC Data submission and competency verification: Submit IDOC data to ensure competency and address any procedural challenges before Water Matrix / PT sample distribution.

        1.4.1. Table 1: QC requirements summary.

Method Reference	**Requirement**	**Specification**	**Acceptance Criteria**
9.1.1	Demonstration of precision and accuracy	Analyze 7 replicate Laboratory Fortified Blanks (LFBs) at the midpoint of the calibration curve.	Percent relative standard deviation (RSD) ≤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.

Method Reference	**Requirement**	**Specification**	**Acceptance Criteria**
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

1.4.2. The initial proposed MRL should be 0.5 ppb based on the method development results.

1.4.3. MRL confirmation spikes can be made by diluting 250µL of a 20ppb standard with 9.75mL of reagent water.

2. Method Evaluation in Drinking Water
   2.1. Labs will be sent drinking water samples. Quantity and sources may vary but no more than 5 samples per source. Sources may be from a raw-surface source, raw-groundwater source, finished-surface source, or finished-ground water source. The samples will be spiked at a low level (MRL or near MRL level) and a mid-level spike. Additionally, a blank from each source will be analyzed.
   2.2. A Sample Handling Sheet will be provided, please fill it out for sample traceability.
   2.3. Analyze samples according to the SOP with all required QC.

3. Proficiency Testing
   3.1. After acceptance of IDOC data, each lab is sent one Proficiency Testing (PT) sample
      3.1.1. Analyze the sample as described in the SOP with all necessary QCs.

3.1.2. Table 3: Analysis Batch QC Summary

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
10	Initial Calibration	Use internal standard technique to generate linear	Analytes at or below the MRL must be within 50-150% of the true value. All other levels must be within

		or quadratic curve. Use at least 6 standard concentrations.	70-130% of the true value.RSE ≤ 15% preferred or $r^2 \geq 0.990$
9.2.1.	Laboratory Reagent Blank (LRB)	One per batch	Benzotriazole concentrations less than 1/3 MRL
9.2.2	Laboratory Fortified Blank (LFB)	One per batch. Run on a rotation of low, middle, and high concentrations.	Percent recovery ≥50% and ≤150% when spiked at or less than 2xMRL or ≥70% and ≤130% when spiked >2xMRL
9.2.3	Laboratory Fortified Sample Matrix (LFSM)/Laboratory Fortified Sample Matrix Duplicate (LFSMD)	Fortify near the center of calibration. One set in an Analysis Batch	Mean percent recovery of LFSM and LFSMD must be 70-130% of the true value
9.2.4	Quality Control Sample (QCS)	One per batch. Prepare near the center of curve and from a source independent of calibration standards.	Percent recovery ≥70% and ≤130%
9.2.5	Continuing Calibration Checks (CCC)	Initial CCC at MRL, CCC after every ten samples, and an ending CCC.	Percent recovery ±50% of true value for levels at MRL and ±30% of true value for all other levels
9.2.7	Retention Time (RT)	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 ratio between the quantitation and confirming fragments of field samples shall be ≤ 30% when compared to the ratio of standards	Ratio of field sample ≤ 30% ratio of standards

4. Submit all data to CDFA/CAC QA unit.
    4.1. CDFA QA Supervisor, Sarva Balachandra, sarvamangala.gunjur@cdfa.ca.gov
    4.2. Use provided Report Template to report results for IDOC, water samples, and PT
        4.2.1. Additional sheets may be created for each batch
    4.3. In addition to the Report Template, please also submit:
        4.3.1. Instrument reports
        4.3.2. Chromatograms

****NOTE: The QC requirement tables in this document are the most up to date.

- LFB, LFMS/LFMSD, QCS are now correctly numbered 9.2.2, 9.2.3, and 9.2.4
- Table 11: 10 Initial Calibration has been updated to read ‘at least 6 standards’ and ‘RSE ≤ 15% preferred or r2 ≥ 0.990’.
- Section 9.2.2 changed from mid-level spike to spike needing to rotate between low, medium, and high levels.
- Added Sections 9.2.7 and 9.2.8 Retention Time and Ion Ratio in body of SOP and Table 11

Official revisions to the SOP will be made after the multi-laboratory study is completed.

Appendix C: Data Reporting Template for MLVS of Method CAC-Benzotriazole-1.0

Sample Handling Sheet

Method # : CAC-Benzotriazole-1.0
Analysis:
Matrix:

CDFA SampleID	LAB SampleID	prepared by/date:	Matrix ID	sample volume (mL)	spk soln. ID	Volume spiked (mL)	Final Volume (mL)		comments

QC Samples

QC SampleID	LAB SampleID	prepared by/date:	Matrix ID	sample volume (mL)	spk soln. ID	Volume spiked (mL)	Final Volume (mL)		comments
LRB			Reagent		n/a		1.00		
LFB			Reagent						
LFSM									
LFSMD									

Reagent water lot#: _____ Pipette ID: _____

Approved By: Mohammad Chehelamirani Rev. 7 01/08/2025

Lab: _____

Batch ID: _____

Primary Standard Vendor: _____

Primary Standard Lot#: _____

Primary Standard Exp: _____

Date Analyzed	SampleID	Sample Type	Spiked Conc. (ppb)	Calculated Conc. (ppb)	Recovery	ΔRetention Time (min)	ISTD Area Deviation (%)
					N/A		
					N/A		
					N/A		
					N/A		
					N/A		
					N/A		
					N/A		
					N/A		
					N/A		
					N/A		
					N/A		
					N/A		
					N/A		
					N/A		
					N/A		
					N/A		
					N/A		
					N/A		
					N/A		
					N/A		
					N/A		
					N/A		
					N/A		
					N/A		

QC requirements	Value
R or R^2	1.00
RSE	15.00
[a]RPD	15.00
[b]Upper PIR	150.00
[b]Lower PIR	50.00

[a]for batch LFSM/LFSMD pair

[b]for MRL determination only

Lab:
Batch ID:
Primary Standard Vendor:
Primary Standard Lot#:
Primary Standard Exp:

Method Reference 9.1.1 Demonstration of Accuracy and Precision

Date Analyzed	SampleID	Spiked Conc. (ppb)	Calculated Conc. (ppb)	Recovery
				#DIV/0!
				#DIV/0!
				#DIV/0!
				#DIV/0!
				#DIV/0!
				#DIV/0!
				#DIV/0!
		Mean Calc Conc (ppb) =	#DIV/0!	
		Mean Percent Recovery =		#DIV/0!
			STDev =	#DIV/0!
			% RSD =	#DIV/0!

Method Reference 9.1.2 Demonstration of Acceptable System Background

Date Analyzed	SampleID	Spiked Conc. (ppb)	Calculated Conc. (ppb)
		Mean Calc Conc. (ppb) =	#DIV/0!

Benzotriazole MRL = 0.5 ppb
1/3 MRL = 0.17 ppb

Method Reference 9.1.3 MRL Confirmation

Date Analyzed	Sample ID	Spiked Conc. (ppb)	Calculated Conc. (ppb)
		Mean Conc. (ppb)	#DIV/0!

Stdev = #DIV/0!
HR = #DIV/0!
UPIR Limit = #DIV/0! %
LPIR Limit = #DIV/0! %

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

Method Reference 9.1.4 Quality Control Sample (QCS)

Date Analyzed	SampleID	Spiked Conc. (ppb)	Calculated Conc. (ppb)	Recovery
				#DIV/0!