Technical Report

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

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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.

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

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

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