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High Throughput Determination of Tetramine in Drinking Water by Solid Phase Extraction and Isotope Dilution Gas Chromatography/Mass Spectrometry (GC/MS)

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HIGH THROUGHPUT DETERMINATION OF TETRAMINE IN DRINKING WATER BY SOLID PHASE EXTRACTION AND ISOTOPE DILUTION GAS CHROMATOGRAPHY/MASS SPECTROMETRY (GC/MS)

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Disclaimer

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Foreword

The National Homeland Security Research Center (NHSRC), part of the U.S. Environmental Protection Agency's (EPA's) Office of Research and Development, is focused on developing and delivering scientifically sound, reliable, and responsive products. These products are designed to address homeland security information gaps and research needs that support the Agency's mission of protecting public health and the environment. A portion of NHSRC's research is directed at decontamination of indoor surfaces, outdoor areas, and water infrastructure. This research is conducted as part of EPA's response to chemical, biological, and radiological (CBR) contamination incidents. NHSRC has been charged with delivering tools and methodologies (e.g. sampling and analytical methods, sample collection protocols) that enable the rapid characterization of indoor and outdoor areas, and water systems following terrorist attacks, and more broadly, natural and manmade disasters.

The *Selected Analytical Methods for Environmental Remediation and Recovery (SAM)*, formerly referred to as the *Standardized Analytical Methods for Environmental Restoration Following Homeland Security Events,* is a compendium of methods that informs sample collection and analysis during the response to an incident. SAM can be used by public and private laboratories which are analyzing a large number of samples associated with chemical, biological, or radiological contamination. Even though some of the analytes in SAM already have existing analytical methods, others are in need of improvements that enhance analytical capability and meet more rigorous performance criteria. Furthermore, not all of the analytical methods listed in the SAM document address all possible matrices (e.g., water, soil, air, glass) encountered in sample collection following an incident. Some of the analytical methods in SAM have been verified in a single laboratory, but most still need to undergo verification with respect to a specific contaminant in association with a specific matrix.

The sampling and analytical procedure (SAP) presented herein, describes a single laboratory developed method for the high throughput determination of tetramethylene disulfotetramine in drinking water by solid phase extraction and isotope dilution gas chromatography/mass spectrometry. Performance data for this method have been generated in a single lab but the method has not been studied jointly or independently by multiple labs. This method, which will be included in the SAM, is expected to provide the Water Laboratory Alliance, as part of EPA's Environmental Response Laboratory Network, with a more reliable and faster means of analyte collection and measurement.

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Acronyms

CAS ..... chemical abstract service
CCC ..... continuing calibration check
CDC ..... Centers for Disease Control and Prevention
CR ..... confirmation ratio
CTMDL ..... Chemical Terrorism Methods Development Laboratory
DL ..... detection limit
EI ..... electron ionization
FD ..... field duplicate
GC ..... gas chromatography
HR_{pir} ..... half range for the predicted interval of results
HPLC ..... high performance liquid chromatography
i.d. ..... inside diameter
IDC ..... initial demonstration of capability
IS ..... standard
ISTD ..... internal standard primary dilution standard
LD50 ..... median lethal dose
LFB ..... laboratory fortified blank
LFSM ..... laboratory fortified sample matrix
LRB ..... laboratory reagent blank
MRL ..... minimum reporting level
MS ..... mass spectrometer
MSDS ..... Material Safety Data Sheet
m/z ..... mass to charge ratio
NHSRC ..... National Homeland Security Research Center
OSHA ..... Occupational Safety and Health Administration
PIR ..... mean prediction interval of result ± half range for the predicted interval of results
QC ..... quality control
RPD ..... relative percent difference
SAM ..... *Selected Analytical Methods for Environmental Remediation and Recovery Restoration Following Homeland Security Events*
SAP ..... Sampling and analytical procedure
Sect. ..... section
SIM ..... selective ion monitoring
SPE ..... solid phase extraction
SS ..... standard solution
TETS ..... tetramine
TOC ..... Total Organic Carbon

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HIGH THROUGHPUT DETERMINATION OF TETRAMINE IN DRINKING WATER

1. SCOPE AND APPLICATION

1.1. This is a single laboratory developed isotope dilution gas chromatography/mass spectrometer (GC/MS) method for the determination of tetramethylene disulfotetramine (tetramine, TETS, Chemical Abstract Services Registry Number 80-12-6). This method, including QC requirements, is designed to support site specific clean-up goals of environmental restoration activities following a homeland security incident involving this analyte.

1.2. Significance: Although banned in the United States, an accidental tetramine poisoning has been reported in New York City and several intentional poisonings have been reported in other countries, primarily in China [1, 2]. Low levels of exposure can be deadly and the human oral LD_{50} (median lethal dose) has been reported to be as low as 0.1 mg/kg [2, 3]. Because tetramine is an odorless, tasteless white powder that easily dissolves in water but not absorbed through skin, the most common route of tetramine exposure is by ingestion [2]. Symptoms of mild tetramine poisoning may include headache, dizziness, fatigue, weakness, lethargy, nausea, vomiting, perioral paresthesias (numbness around the mouth), and anorexia while high levels of exposures are characterized by seizures, coma and death [2]. Symptoms may begin 0.5-13 hours post exposure [2].

1.3. The use of 96-well plates for the solid phase extraction (SPE) produces two key benefits. The 96-well plates allow for extensive automation of the method, thereby enabling high throughput of samples, as might be required during environmental restoration. Additionally, the use of this format results in the ability to perform isotope dilution by enabling the economical addition of isotopically labeled tetramine as an internal standard to the sample prior to extraction.

1.4. Isotopically labeled tetramine is added equally to all unknowns, quality controls, and calibration standards. In addition to enabling accurate quantitation, isotopically labeled tetramine also accounts for and resolves some of the QC issues surrounding analysis, including analysis efficiency and sample loss, in the intended use of this analyte. The overall QC approach utilizing quantitation and confirmation ions as well as an isotopically labeled analyte greatly increases confidence that tetramine, and not another molecule with similar fragmentation patterns, is being quantitated during analysis.

1.5. This method was adapted from one that was initially developed by the Centers for Disease Control and Prevention (CDC), in the National Center for Environmental Health (NCEH), Division of Laboratory Sciences (DLS), Emergency Response and Air Toxicants Branch, in the Chemical Terrorism Methods Development Laboratory (CTMDL) for the determination and quantitation of tetramine in

human urine. For the adapted method, accuracy and precision data have been generated in reagent water, and in finished ground and surface waters that use chlorine and/or chloramine as residual disinfectants.

1.6. The QC approach in this method conforms to CTMDL standards for clinical samples, and is presented here in terms more familiar to drinking water laboratories. Methods developed by CTMDL are distributed to the CDC's laboratory network, and the QC approach included in these methods, while single lab verified by the CTMDL lab, is designed to be sufficiently rigorous that network labs can successfully perform the method.

1.7. The minimum reporting level (MRL) is the lowest analyte concentration that meets data quality objectives for the intended use of the method, e.g., to meet site-specific remediation goals. Laboratories will need to demonstrate that their laboratory MRL meets the requirements described in Section 9.2.4.

1.8. Determining the detection limit (DL) is optional (Sect. 9.2.6). Detection limit is defined as the statistically calculated minimum concentration that can be measured with 99% confidence that the reported value is greater than zero.

1.9. This method is intended for use by analysts skilled in the performance of solid phase extractions, the operation of GC/MS instruments, and the interpretation of the associated data.

1.10. This method has been verified using only the conditions and equipment specified in the method. Alteration of this method is not recommended.

2. SUMMARY OF METHOD

2.1. A 50-mL water sample is collected, and a preservative and/or dechlorinating agent are optionally added as required by site-specific conditions. (The data in Table 8.1 suggest that the presence or choice of the additive does not affect the results.) An aliquot is pipetted into a well of a preconditioned 96-well solid phase extraction plate, and the isotopically labeled tetramine is added. Following a wash step, tetramine is then eluted in acetonitrile. The extract is concentrated to dryness under nitrogen and heat, and then adjusted to a 100 µL volume in acetonitrile. Tetramine is separated from the sample matrix and identified by GC/MS analysis, operated in SIM mode or equivalent. Analyte identification is accomplished by comparing the acquired mass spectra, including ion ratios, and retention times to reference spectra and retention times for calibration standards acquired under identical GC/MS conditions. Quantitation is performed using the internal standard technique. Utilization of an isotopically labeled internal standard provides a high degree of accuracy and precision for sample quantitation by accounting for analyte recovery and analytical efficiency.

2.2. Compared to some drinking water methods (e.g. certain EPA 500 series methods), the initial laboratory demonstration of capability (IDC) is lengthier than some drinking water methods, the frequency of the on-going calibration is shorter, and the number of continuing calibration checks (CCC) is higher. Based on experience in the developer's lab, this QC approach ensures successful long-term implementation of the method in other labs, particularly when these methods are used infrequently (e.g. in emergency situations). Due to site-specific circumstances during an environmental remediation activity, e.g. in which sample throughput requirements exceed available lab capacity, a shorter initial demonstration of capability (IDC), changes to the on-going calibration frequency, and number of CCCs may be necessary and appropriate.. However, initial and ongoing QC requirements and acceptance criteria (see Section 9) should not be changed. Adopting steps, such as a replacing on-going recalibration with a calibration check only, to save time may result in higher QC failure rates and perhaps less accurate quantitation. Labs should discuss these increased risks with sample submitters before taking such steps.

3. DEFINITIONS

3.1. ANALYSIS BATCH – a sequence of samples, analyzed within a 24-hour period, including no more than 20 field samples in addition to all of the required QC samples (Sect. 9.3)

3.2. CALIBRATION STANDARD (CAL) – A solution prepared from the primary dilution standard solution and/or stock standard solution and the internal standard. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.3. CONFIRMATION ION – for this method, the second most abundant tetramine ion (See Confirmation Ratio, Sect. 3.4, below). The confirmation ion is used to calculate the confirmation ratio (Sect. 3.4)

3.4. CONFIRMATION RATIO (CR) - peak area produced by the confirmation ion divided by the peak area produced by the quantitation ion which serves as an additional QC measure of analyte selectivity

3.5. CONTINUING CALIBRATION CHECK (CCC) SOLUTION – a calibration solution containing the method analyte(s), which is extracted in the same manner as the samples and analyzed periodically to verify the accuracy of the existing calibration for those analyte(s)

3.6. DETECTION LIMIT (DL) – the minimum concentration of an analyte that can be identified, measured, and reported to be greater than zero with 99% confidence

3.7. FIELD DUPLICATES (FD1 and FD2) – two separate samples collected at the same time and place under identical circumstances, and treated exactly the same

throughout field and laboratory procedures to provide check the precision associated with sample collection, preservation, storage, and laboratory procedures

3.8. ISOTOPICALLY-LABELED INTERNAL STANDARD – a pure chemical added to an extract or to a standard solution in a known amount(s) and used to measure the relative response of other method analytes and surrogates that are components of the same solution.

3.9. LABORATORY FORTIFIED BLANK (LFB) – a volume of reagent water or other blank matrix to which known quantities of the method analytes and all the preservation reagents are added in the laboratory (Sect. 7.3.5.2) The LFB is analyzed exactly like a sample and its purpose is to determine whether the methodology is in control and whether the laboratory is capable of making accurate and precise measurements.

3.10. LABORATORY REAGENT BLANK (LRB) – an aliquot of reagent water that is treated exactly as a sample and used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus

3.11. MATERIAL SAFETY DATA SHEET (MSDS) – written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions

3.12. MINIMUM REPORTING LEVEL (MRL) – the minimum concentration qualified to be reported as a quantitated value for a method analyte in a sample following analysis (Sect. 9.2.4. for MRL verification procedure)

3.13. PRIMARY DILUTION STANDARD SOLUTION – a solution containing the analytes prepared in the laboratory from stock standard solutions and diluted as needed to prepare calibration solutions and other needed analyte solutions

3.14. QUANTITATION ION –for this method, the quantitation ion is the parent tetramine ion with a mass to charge ratio (m/z) of 240 (See Confirmation Ratio, Sect. 3.4, above)

3.15. SECOND SOURCE QUALITY CONTROL SAMPLES – materials obtained from a source different than the original and used to verify the accuracy of the existing calibration for those analytes

4. INTERFERENCES

4.1 Method interferences that can lead to discrete artifacts and/or elevated baselines in the chromatograms may be caused by contaminants in solvents, reagents

(including reagent water), sample bottles and caps, and other sample processing hardware. All such items must be routinely demonstrated to be free from interferences under the conditions of the analysis by analyzing laboratory reagent blanks. Subtracting blank values from sample results is not permitted.

4.2 Matrix interferences may be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature of the water.

4.3 Relatively high concentrations, in the mg/L range, of preservatives, antimicrobial agents, or dechlorinating agents might be added to sample collection vessels (Section 8.1.2). The potential exists for trace-level organic contaminants in these reagents. Interferences from these sources should be monitored by analysis of laboratory reagent blanks particularly when new lots of reagents are acquired.

4.4 Due to the nature of the matrix analyzed in this procedure, occasional interferences from unknown substances might be encountered. Interfering compounds can be recognized by deviations in the sample quantitation/conformation ratios from the calibration standard ratios and can also be monitored using appropriate LRBs. Any interference that results in QC failure (Sect. 9) results in rejection of the entire analysis batch. If repeating the analysis does not remove the interference with the reference standard, the results for that analyte are not reportable.

4.5 All glassware should be chemically cleaned before running this method. Wash glassware thoroughly with reagent-grade water followed by acetonitrile. Allow glass to dry completely before use. If the laboratory wishes to use a muffle oven for decontamination then the appropriate measures should be taken to assure that the muffle oven conditions are suitable to remove all traces of tetramine and other interferences.

4.6 Care should be taken at all times to prevent contamination of QC materials, standards, and samples.

4.7 Chromatographic separation of the analyte should be carefully monitored for unknown interferences. See Section 11.2.5 for analyte confirmation.

5. SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined. Each chemical should be treated as a potential health hazard, and exposure to these chemicals should be minimized. Each laboratory is responsible for maintaining an awareness of OSHA regulations regarding safe handling of chemicals used in this method. A reference file of MSDSs should be

made available to all personnel involved in the chemical analyses. Additional references to laboratory safety are available [4-6].

5.2 Tetramine is highly toxic, and the human oral LD_{50} has been reported to be as low as 0.1 mg/kg [2, 3]. Ingestion is the primary reported route of exposure, but all other routes of exposure (e.g. inhalation, dermal contact, and eye exposure) should be avoided. Follow universal safety precautions when performing this procedure, including the use of a lab coat, safety glasses, appropriate gloves, and a high quality-ventilated chemical fume hood and/or biological safety cabinet.

5.3 Avoid inhalation or dermal exposure to acetonitrile, which is used in the sample preparation steps.

5.4 Mechanical hazards when performing this procedure using standard safety practices are minimal. Read and follow the manufacturer's information regarding safe operation of the equipment. Avoid direct contact with the mechanical and electronic components of the gas chromatograph and mass spectrometer, unless all power to the instrument is off. Generally, maintenance and repair of mechanical and electronic components should be performed only by qualified technicians.

6. EQUIPMENT AND SUPPLIES

(It is important to note that specific brands or catalog numbers included in this section are examples only and do not imply endorsement of these particular products. These specific products were used during the validation of this method.)

6.1 MICRODISPENSERS – with adjustable volume (5-100 µL, 100-1000 µL) (Eppendorf Co., Westbury, NY or equivalent)

6.2 REPEATER PIPETTE – 4780 (Eppendorf Co., Westbury, NY or equivalent)

6.3 CONICAL AUTOSAMPLER VIALS – 300-µL vials (must be compatible with GC autosampler) especially for use as an alternative to 96 well plates

6.4 ANALYTICAL BALANCE – Capable of weighing to the nearest 0.0001 g

6.5 SOLID PHASE EXTRACTION (SPE) APPARATUS WITH 96 WELL PLATES

6.5.1 96-WELL SPE PLATE – Strata X 60-mg / 6-mL (PN# 8E-S100-UGB), available from Phenomenex (Torrence, CA) or equivalent

6.5.2 PLATE SHAKER (ThermoFisher Scientific, Waltham, MA or equivalent)

6.5.3 96-WELL LIQUID HANDLER – Use a 96-well liquid handler equipped with a solid-phase extraction manifold and vacuum system. These systems must be calibrated prior to use, according to vendor or laboratory specifications. In addition, these liquid handlers must be used during laboratory-method validation. The liquid handlers that have been used with this method in different laboratories have included the Tomtec® Quadra 3 SPE (Tomtec, Inc. Hamden, CT), the Caliper Zephyr, and the Caliper i1000 (Caliper Life Sciences, Hopkinton, MA). The selection of these liquid handlers has typically been based on cost and required sample throughput.

6.6 96-WELL NUNC DEEP WELL PLATE – 2000 mL plate (Nunc PN# 278752 or equivalent). Must be compatible with 96-well liquid handler described in Section 6.5.3.

6.7 EXTRACT CONCENTRATION SYSTEM. The 96-well plate requires a compatible dry-down step for sample pre-concentration following extraction. The TurboVap 96 concentrator evaporator workstation (Zymark® Corp., Hopkinton, MA) has proven to be well suited for this application, but other evaporator systems which result in equivalent method performance could be used instead.

6.8 GAS CHROMATOGRAPHY ELECTRON IONIZATION MASS SPECTROMETRY SYSTEM (GC/MS)

6.8.1 GC COLUMN – 30 m x 0.25-mm inside diameter (i.d.) fused silica capillary (5%-Phenyl)-methylpolysiloxane column coated with a 0.25um bonded film (Agilent HP-5ms [Agilent Technologies, Santa Clara, CA] or equivalent). A nonpolar, low-bleed column designed for GC/MS applications is recommended for use with this method to provide adequate chromatography and minimize column bleed.

6.8.2 GC SYSTEM – The GC system (e.g., Agilent 6890N GC or equivalent) must be equipped with an autosampler and injector and must provide consistent sample injection volumes. The system should also be capable of performing linear temperature gradients at a constant flow rate. The GC should be capable of being configured exactly as stated below:

Table 6-1. Gas Chromatograph (GC) Parameters

Parameter	Setting
GC Method	Constant flow at 1 mL/min Initial pressure: 10.5 psi Carrier Gas: Helium
Column type	HP5-ms (5% phenyl methyl siloxane), 30 m x 0.25 mm x 0.25 µm
Injection Volume	1 µL
Inlet liner	Splitless liner double taper, unpacked
Inlet Temperature	250°C
Injection mode	Splitless injection; purge flow to split vent 100 mL/min at 1 min; gas saver at 20 mL/min at 3 min
Autosampler Tray Temperature	Room temperature
Oven Program	Initial temperature 100°C Ramp 8°C/min to 200°C Ramp 50°C/min to 300°C Hold 300°C for 1.7 min
Typical retention time	Tetramine = 11.6 min
MS Scan Mode	Selected ion monitoring (SIM)
Ionization Type	Electron ionization (EI)
Dwell Time	100 msec per ion

6.8.3 MASS SPECTROMETER (MS) – The MS (Agilent 5973 Mass Selective Detector, Palo Alto, CA, or equivalent) must be capable of performing electron impact ionization with positive ion detection and must be configured for selected ion monitoring (SIM, or equivalent depending on MS type) with a dwell time of 100 msec per ion. The SIM ions monitored for this method should be set exactly as stated below:

Analyte	Ion (m/z)
Tetramine quantification ion	240
Tetramine confirmation ion	212
Tetramine internal standard	244

7. REAGENTS AND STANDARDS (These reagents were used during the validation of the method, and only these or their equivalent are acceptable for use.)

7.1 GASES, REAGENTS, AND SOLVENTS – Reagent grade or better chemicals should be used. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first determined that the reagent is of sufficiently high purity to permit its use without lessening the quality of the determination.

7.1.1 HELIUM - 99.9999% pure or better, GC carrier gas

7.1.2 REAGENT WATER – purified, deionized water which does not contain any measurable quantities of the method analyte or interfering compounds ([Tedia, Fairfield, OH] ® HPLC or equivalent grade water)

7.1.3 METHANOL - (CH_3OH, CAS#: 67-56-1) – high purity, demonstrated to be free of analytes and interferences (Tedia HPLC or equivalent)

7.1.4 ACETONITRILE - (CH_3CN, CAS#: 75-05-8) – high purity, demonstrated to be free of analytes and interferences (Tedia HPLC or equivalent)

7.1.5 SAMPLE PRESERVATION REAGENTS – One of the following sample preservation reagents may be required by site specific conditions:

7.1.5.1 AMMONIUM CHLORIDE (NH_4Cl, CAS#: 12125-02-9) – an additive used in sample collection (Sigma-Aldrich ACS grade or equivalent)

7.1.5.2 SODIUM THIOSULFATE ($Na_2S_2O_3$, CAS#: 7772-98-7) – an additive used in sample collection (Sigma-Aldrich ACS grade or equivalent)

7.1.5.3 SODIUM SULFITE (Na_2SO_3, CAS#: 7757-83-7) – an additive used in sample collection (Sigma-Aldrich ACS grade or equivalent)

7.1.5.4 ASCORBIC ACID ($C_6H_8O_6$, CAS#: 50-81-7) – an additive used in sample collection (Sigma-Aldrich ACS grade or equivalent)

7.1.5.5 AMMONIUM ACETATE ($CH_3CO_2NH_4$, CAS#: 631-61-8) – An additive used in sample collection (Sigma-Aldrich ACS grade or equivalent)

7.1.5.6 CITRIC ACID ($HOC(COOH)(CH_2COOH)_2$, CAS#: 77-92-9) – an additive used in sample collection (Sigma-Aldrich ACS grade or equivalent)

7.1.5.7 DIAZOLIDINYL UREA ($C_8H_{14}N_4O_7$, CAS#: 78491-02-8) – an additive used for sample collection (Sigma-Aldrich ACS grade or equivalent)

7.2 REAGENT PREPARATION

7.2.1 5% METHANOL IN WATER – A 5%/95% methanol/water solution is prepared through volumetric dilution with HPLC grade deionized water. Measure 10 mL of methanol using an appropriate pipette, volumetric flask, or graduated cylinder and pour into a clean, dry container with a capacity of 250 mL or more. Measure 190 mL of HPLC grade deionized water with a volumetric flask or graduated cylinder and pour into the same container with the methanol. Mix the solution well.

7.3 STANDARDS SOLUTIONS -- When a compound purity is assayed to be 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Solution concentrations listed in this section were used to develop this method and are included as an example. Standards for sample fortification generally should be prepared in the smallest volume that can be accurately measured to minimize the addition of excess organic solvent to aqueous samples. Store all calibration and control materials at either -20±5°C when not in use. **Even though stability times for standard solutions are suggested in the following sections, laboratories should use standard QC practices to determine when their standards need to be replaced.**

7.3.1 ISOTOPICALLY LABELLED INTERNAL STANDARD SOLUTIONS The internal standard used in this method is $^{13}C_4$-tetramine (Cambridge Isotopes, MA; catalog #CLM-8146-0). Note that in this method, the internal standard is a chemical that is structurally identical to the method analyte, but is substituted with ^{13}C. The isotopically-labeled internal standard has no potential to be present in water samples, and is not a method analyte. The internal standard is added to all samples, standards, and QC solutions as described in Section 11.1.3.

7.3.2 Prepare or purchase the internal standard at a concentration of 500 ng/mL. Steps for the preparation of this mixture are described below:

7.3.2.1 INTERNAL STANDARD STOCK SOLUTION - Accurately weigh approximately 20.1 mg of $^{13}C_4$-tetramine in a weigh boat and then transfer into a 200 mL volumetric flask. Add 100 mL of acetonitrile and mix well until dissolved. Dilute to the 200 mL mark with additional acetonitrile and mix well. The stock solution is stable for at least one year when stored at -20 ±5°C.

7.3.2.2 INTERNAL STANDARD PRIMARY DILUTION STANDARD (ISTD) (500 ng/mL) - Combine 50 µL of the internal standard stock solution with 9.95 mL of deionized water in a 15 mL polypropylene centrifuge tube (BD, Franklin Lakes, NJ) or equivalent. The stock solution is stable for at least one year when stored at -20±5°C.

7.3.3 ANALYTE STOCK STANDARD SOLUTIONS. Prepare or purchase three stock solutions using a reliable source of tetramine (Cambridge Isotopes, 50 Frontage Road, Andover, MA 01810. CAS #:80-12-6; unlabeled material product #ULM-8147 and labeled material product # CLM-8146 (13C4 label)). These stock solutions are stable for at least one year when stored at -20±5°C.

7.3.3.1 ANALYTE STOCK STANDARD SOLUTION 1 (SS1, 82.4 mg/L) – Accurately weigh approximately 20.6 mg of tetramine to a weigh boat and then into a 250 mL volumetric flask. Add 100 mL of acetonitrile and mix well until dissolved. Dilute to the 250 mL with additional acetonitrile and mix well.

7.3.3.2 ANALYTE STOCK STANDARD SOLUTION 2 (SS2, 206 ug/L) – Accurately transfer 25 µL of SS1 into a 10 mL volumetric flask. Dilute with acetonitrile to 10 mL mark and mix well.

7.3.3.3 ANALYTE STOCK STANDARD SOLUTION 3 (SS3, 8.24 mg/L) -- Accurately transfer 1 mL of SS1 into a 10 mL volumetric flask. Dilute with acetonitrile to the 10 mL mark and mix well.

7.3.4 CALIBRATION STANDARD STOCK SOLUTIONS – Prepare the calibration standard stock solutions from dilutions of the analyte stock solutions in reagent water containing any preservatives required by site-specific circumstances (See Sects 2.2 and 8.1.3). For this purpose, a Falcon polypropylene 50 mL centrifuge tube (BD, Franklin Lakes, NJ) may be used by quantitatively transferring the volumes of the respective solution listed in the table below to the tube, diluting to the 40 mL mark, and mixing well. (Note: Diluting to the 40 mL mark provided sufficient accuracy in the developer's lab. Other labs may wish to utilize alternate polypropylene vessels if they experience dilution related inaccuracies.) The calibration curve is composed of at least six concentrations. These calibration standard solutions are stable for at least one year when stored at -20±5°C.

7.3.4.1 PREPARATION OF CALIBRATION STANDARD STOCK SOLUTIONS – Calibrations standard stock solutions may be prepared using the volumes listed in Table 7-1 below. The concentrations, along with the numbers of solutions, are for illustration purposes only. Other concentrations may be required in practice to meet performance and QC goal. (See Sect. 10.3 for the number of calibration solutions required for calibration.)

Table 7-1. Calibration Standard Stock Solution Volumes

Concentration (ng/mL)	Total Volume (mL)	Stock Solution 3 (µL)	Stock Solution 2 (µL)	Stock Solution 1 (µL)
0				
0.5	40		97	
1	40		194	
2	40		388	
5	40	24		
10	40	49		
15	40	73		
25	40	121		
50	40			24
75	40			36
100	40			49
250	40			121

7.3.5 QUALITY CONTROL SOLUTIONS – There are several types of quality control solutions, some of which are identical in composition but serve different QC functions and hence may be referred to by different names in Section 9.

7.3.5.1 SECOND SOURCE QUALITY CONTROL SAMPLE – These samples are used to verify the accuracy of the calibration standard solutions (7.3.4) and are prepared the same way as the calibration standards. They are prepared from an analyte source different than the calibration standard solutions as described more completely in Section 9.3.7.

7.3.5.2 LABORATORY FORTIFIED BLANKS (LFBs) - LFBs are used throughout this method for various purposes. The LFB is analyzed exactly like a sample, and its purpose is to verify that the methodology is be competently replicated, and that the laboratory has the capability to make accurate and precise measurements. The two specific LFBs are required in this method are referred to as LFB-low and LFB-high, which relate to initial and ongoing QC. For the demonstration of the method in the developer's laboratory, the LFB-low and -high are 5 and 75 ng/mL, respectively, prepared as indicated in Table 7.1, in Section 7.3.4. In a particular lab, the LFBs should be selected from similar points in their calibration range (e.g., LFB-low should be around 10 times the MRL (Sect. 9.2.4) and LFB-high should be around 150 times the MRL.

The LFBs are inherently calibration standards and can be used to construct the calibration curve. However, the LFBs are specifically used

to develop QC criteria during the initial demonstration of capability (Sect. 9.2), and serve as an additional QC function during each analysis batch. The LFBs serve a similar, but generally more stringent, QC function as continuous calibration checks (Sect. 10.3).

7.3.5.3 LABORATORY REAGENT BLANK. This blank is prepared as a LFB with no analyte added (i.e., the 0 ng/mL in Table 7-1).

8. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 SAMPLE VESSEL PREPARATION COLLECTION

8.1.1 Samples can be collected in a 50-mL polypropylene vessel fitted with a flat-top polyethylene screw-cap (e.g., BD Falcon 50 mL centrifuge tube or equivalent).

8.1.2 The performance data for the method presented in Section 13 are presented without addition of preservatives. This is based on the stability of tetramine in the presence of preservatives suggested in Table 8-1, which suggests the analyte does not require sample preservation up to 28 days, particularly if tetramine is the sole analyte of interest in the sample.

8.1.3 However, vessels should be prepared before sample collection with appropriate preservative(s) (Table 8-2) required by site-specific circumstances, e.g., to fulfill the purpose(s) listed in the Table 8-2. Preservation through binding free chlorine or dechlorination may also be necessary if analytical artifacts are observed in the samples but not the LFBs. All initial and on-going QC requirements should be demonstrated for the preservatives added to the sample, particularly if added in combination. If tetramine is the only analyte determined, necessity of preservatives is expected to be a very rare event.

Table 8-1. Recoveries of Tetramine in Preservatives over Time (n=3)

Water Type	Preservative	Day 0		Day 7		Day 14		Day 28	
		4°C	25°C	4°C	25°C	4°C	25°C	4°C	25°C
Deionized	-	109 ± 3	102 ± 6	105 ± 14	112 ± 4	101 ± 2	103 ± 2	107 ± 4	108 ± 4
Chlorine	-	104 ± 7	105 ± 5	107 ± 9	111 ± 5	102 ± 1	105 ± 2	113 ± 4	118 ± 2
Monochloramine	-	108 ± 7	101 ± 9	100 ± 2	108 ± 4	101 ± 4	104 ± 1	110 ± 4	112 ± 10
Chlorine	Ammonium Chloride (0.1 g/L)	106 ± 7	106 ± 5	104 ± 5	113 ± 8	116 ± 4	103 ± 1	124 ± 6	112 ± 5
Chlorine	Sodium Thiosulfate (0.08 g/L)	107 ± 7	99 ± 3	96 ± 2	111 ± 5	100 ± 2	96 ± 2	111 ± 4	115 ± 9
Monochloramine	Sodium Thiosulfate (0.08 g/L)	109 ± 9	103 ± 4	99 ± 6	111 ± 5	113 ± 2	102 ± 2	115 ± 2	113 ± 4
Chlorine	Sodium Sulfite (0.05 g/L)	112 ± 17	105 ± 5	110 ± 6	105 ± 5	101 ± 2	105 ± 1	111 ± 4	109 ± 10
Chlorine	Ascorbic Acid (0.1 g/L)	111 ± 8	103 ± 6	101 ± 7	111 ± 10	104 ± 3	114 ± 3	114 ± 11	111 ± 4
Chlorine	Ammonium Acetate (1.5 g/L)	101 ± 8	99 ± 0	116 ± 6	111 ± 3	114 ± 2	106 ± 3	107 ± 2	110 ± 3
Deionized Water	Citric Acid (9.3 g/L)	109 ± 8	108 ± 6	104 ± 1	110 ± 5	95 ± 2	112 ± 4	114 ± 4	110 ± 2
Deionized Water	Diazolidinyl Urea (1 g/L)	100 ± 26	96 ± 5	102 ± 6	103 ± 2	99 ± 1	98 ± 10	109 ± 8	110 ± 6

Table 8-2. Preservative Concentrations and Purposes of Preservatives

Compound	Mass added to sample (mg)	Concentration in sample (g/L)	Purpose
Ammonium chloride	5	0.1	Binds free chlorine
Ammonium acetate	75	1.5	Binds free chlorine
Sodium thiosulfate	4	0.08	Dechlorinates free chlorine and chloramine
Sodium sulfite	2.5	0.05	Dechlorinates free chlorine and chloramine
Ascorbic acid	5	0.1	Dechlorinates free chlorine and chloramine
Citric acid	465	9.3	pH adjustment
Diazolidinyl urea	50	1	Microbial inhibitor

8.2 SAMPLE COLLECTION - When sampling from a water tap, samplers should request guidance about how long to flush the tap, if at all. Depending on site specific goals, incident managers may request that the tap not be flushed to minimize loss of contaminant. If incident managers do not specify a shorter time, flush until the water temperature has stabilized (approximately 3-5 minutes). Collect samples from the flowing stream. It may be convenient to collect a bulk sample in a polypropylene vessel from which to generate individual 50 mL samples. Keep samples sealed from collection time until analysis. When sampling from an open body of water, fill the sample container with water from a representative area. Sampling equipment, including automatic samplers, should be free of plastic tubing, gaskets, and other parts that may leach interfering analytes into the water sample.

8.3 SAMPLE SHIPMENT AND STORAGE - Results of the sample storage stability study (Table 8-1) suggest that storage at 25°C produces results similar to reduced temperatures. As a matter of practice to ensure that the samples do not experience excessive temperature outside the stability range investigated, it is recommended that all samples be iced, frozen (-20°C), or refrigerated (4°C) from the time of collection until extraction. During method development, no significant differences were observed between standards that were frozen or refrigerated.

8.4 SAMPLE AND EXTRACT HOLDING TIMES – Results of the sample storage stability study (Table 8-1, n=3) suggest that tetramine has adequate stability for at least 28 days when collected, preserved, shipped, and stored as described in Sections 8.1, 8.2, and 8.3. As matter of practice, water samples should be

extracted as soon as possible but must be extracted within 28 days. Data generated during this study indicates that extracts are stable for at least 28 days when stored at 0 °C or lower. As matter of practice, analysis should occur as soon as possible.

9. QUALITY CONTROL

9.1 QC requirements include the initial demonstration of capability (IDC) and ongoing QC requirements that must be met when preparing and analyzing field samples. This section describes the QC parameters, their required frequencies, and the performance criteria that must be met in order to meet typical EPA quality objectives for drinking water analysis, although these objectives will be site specific during a remediation activity. These QC requirements are considered the minimum acceptable QC criteria in particular for this method which utilizes an isotopically labeled internal standard. Laboratories are encouraged to institute additional QC practices to meet specific needs [7].

9.2 INITIAL DEMONSTRATION OF CAPABILITY (IDC) – The IDC must be successfully performed prior to analyzing any field samples. Prior to conducting the IDC, the analyst must first generate an acceptable initial calibration following the procedure outlined in Section 10.2. It should be noted that the IDC is lengthier than some drinking water methods, but based on experience in the developer's lab, the IDC helps ensure successful long-term implementation of the method in a variety of other labs. Due to site-specific conditions during a environmental remediation activity, a shorter IDC may be necessary and appropriate. For example, a more minimal IDC could consist of: a) demonstration of low system background (Sect. 9.2.1); b) 4-7 same-day replicates fortified near the midrange of the initial calibration curve for precision and accuracy demonstration, combined with c) the MRL estimation described in Section 9.2.4. However, QC acceptance requirements, both initial (Sect. 9.2.1-9.2.4) and ongoing (Sect. 9.3) should not be changed, and a shorter IDC may result in higher QC failure rates and less accurate quantitation in some concentration ranges. Labs should consider these risks before choosing a shorter IDC.

9.2.1 INITIAL DEMONSTRATION OF LOW SYSTEM BACKGROUND – Any time a new lot of solvents, reagents, and autosampler vials/plates are used, it must be demonstrated that an LRB is reasonably free of contamination and that the criteria in Section 9.3.1 are met.

9.2.2 INITIAL DEMONSTRATION OF PRECISION – Prepare and analyze at least twenty replicates of both laboratory fortified blanks (LFB-high and LFB-low, see Sect. 7.3.5.2) over the course of at least 10 days. Any sample preservative, as described in Section 8.1.2, must be added to these samples. For the initial demonstration of precision, the coefficient of

variation for the concentrations of the replicate analyses must be less than 20%.

9.2.3 INITIAL DEMONSTRATION OF ACCURACY – Using the same set of replicate data generated for Section 9.2.2, calculate the mean recovery. For the initial demonstration of accuracy, the mean recovery of the replicate values must be within ± 30% of the true value.

9.2.4 MINIMUM REPORTING LEVEL (MRL) ESTIMATION – Because clean-up goals will be site specific, laboratories need to estimate a minimum reporting level so that incident managers can understand a specific laboratory's capabilities and can distribute samples to appropriate laboratories. Establishing the MRL concentration too low may cause repeated failure of ongoing QC requirements. If the IDC procedure (Sect. 9.2.1-9.2.3) is followed explicitly, establishing the MRL as the lowest standard is expected to ensure compliance with QC requirements, This is a result of the rigor of the QC requirements in the lengthy IDC (Sect. 9.2.1-9.2.3), especially those associated with the LFBs (see Sect. 10.3.3). If a shorter IDC is required by site specific conditions (see Sect. 2.2), the MRL should be confirmed with the procedure below.

9.2.4.1 Fortify and analyze seven replicate LFBs at the proposed MRL concentration. These LFBs must contain all method preservatives described in Section 8.1.2. Calculate the mean measured concentration (*Mean*) and standard deviation for the method analytes in these replicates. Determine the half range for the prediction interval of results (HR_{PIR}) for each analyte using the equation below:

$$HR_{PIR} = 3.963s$$

where s is the standard deviation and 3.963 is a constant value for seven replicates.

9.2.4.2 Confirm that the upper and lower limits for the prediction interval of result ($PIR = Mean \pm HR_{PIR}$) meet the upper and lower recovery limits as shown below:

The Upper PIR Limit must be ≤150% recovery.

$$\frac{Mean + HR_{PIR}}{FortifiedConcentration} \times 100\% \leq 150\%$$

The Lower PIR Limit must be ≥ 50% recovery.

$$\frac{Mean - HR_{PIR}}{FortifiedConcentration} \times 100\% \geq 50\%$$

9.2.4.3 The MRL is validated if both the upper and lower PIR limits meet the criteria described above (Sect. 9.2.4.2). If these criteria are not met, the MRL has been set too low and must be confirmed again at a higher concentration.

9.2.5 CALIBRATION CONFIRMATION – The calibration is confirmed by analysis of a second source quality control sample as described in Section 9.3.5

9.2.6 DETECTION LIMIT (DL). This is a statistical determination of precision and accurate quantitation is not expected at this level.Replicate analyses for this procedure should be done over at least three days (i.e., both the sample preparation and the LC/MS/MS analyses should be done over at least three days). At least seven replicate LFBs should be analyzed during this time period. The concentration may be estimated by selecting a concentration at two to five times the noise level.). The appropriate fortification concentrations will be dependent upon the sensitivity of the GC/MS system used. Any preservation reagents added in Section 8.1.2 must also be added to these samples. Note that the concentration for some IDC steps may be appropriate for DL determination, in which case the IDC data may be used to calculated the DL. (For example, for the results presented in Section 13, twenty replicate LFBs were analyzed over 10 days, e.g., three LFBs individually fortified on day one, two LFBs individually fortified on day two, and two LFBs individually fortified on day three, etc). Analyze the replicates through all steps of Section 11. Calculate the DL from the equation: DL = s x $t_{(n\text{-}1)}$

where:

s = standard deviation of replicate analysis, without subtraction of values of analyte free blanks

t = Student's t value for the 99% confidence level with n-1 degrees of freedom

n = number of replicates

9.3 ONGOING QC REQUIREMENTS -- This section summarizes the ongoing QC criteria that must be followed when processing and analyzing field samples. The required QC samples for an analysis batch include the laboratory reagent blank (LRB) and four continuing calibration check (CCC) solutions.

9.3.1 LABORATORY REAGENT BLANK (LRB) – An LRB is required with each analysis batch (Sect. 3.1) to confirm that potential background contaminants are not interfering with the identification or quantitation of method analytes. Running the LRB first may prevent unnecessary analysis if the LRB is invalid. Preparation of the LRB is described in Section 7.3.5. If the LRB produces a peak within the retention time window of the analyte that would prevent the determination of the analyte, determine the source of contamination and eliminate the interference before processing samples. Background contamination must be reduced to an acceptable level before proceeding. Background from method analyte or other contaminants that interfere with the measurement of method analyte must be below 1/3 of the MRL. Blank contamination is estimated by extrapolation, if the concentration is below the lowest calibration standard. This extrapolation procedure is not allowed for sample results as it may not meet data quality objectives. If the method analytes are detected in the LRB at concentrations equal to or greater than 1/3 the MRL, then all data for the problem analyte(s) must be considered invalid for all samples in the analysis batch.

9.3.2 ONGOING CALIBRATION. The analytical system in recalibrated at the beginning of each analysis batch using the same analyte concentrations determined during the initial calibration. The acceptance criteria for the ongoing calibration is described in Section 10.2.5, except that removal of calibration points may result in too few calibration points and therefore an invalid calibration. The ongoing calibration is performed after the first two continuing calibration check (CCC) samples (Sec. 9.3.3) to allow for corrective action if the calibration fails. As mentioned in Sect. 2.2, in some well considered circumstances and in consultation with the sample submitter about increased QC and quantitation risk, it may be desirable to not perform the ongoing calibration (Sect. 9.3.2) and instead rely on CCC samples (as described in Sect. 9.3.3) to verify ongoing calibration. If so, the beginning CCC of each analysis batch must be at or below the MRL in order to verify instrument sensitivity prior to any analyses. Subsequent CCCs should alternate between a medium and high concentration calibration standard.

9.3.3 CONTINUING CALIBRATION CHECK (CCC) – CCC standards, containing the preservatives, if any, are analyzed at the beginning of each analysis batch, after every 20 field samples. Note that there are up to four CCCs depending on the IDC appropriate for the site specific circumstance. In the lengthier IDC described in Sect. 9.2, there are four CCCs: LFB-low and LFB-high, which are analyzed before the batch, and the lowest and highest calibration standards from the ongoing calibration (Sect 9.3.2), which are analyzed after the field samples. If this IDC approach is not appropriate, then there are at most two CCC standards, i.e. the calibration standards. Depending on site specific goals and tolerance of QC and

quantitation risk, it may acceptable to only run one of these calibration standards as the CCC before and after the batch. If so, the beginning CCC of each analysis batch must be at or below the MRL in order to verify instrument sensitivity prior to any analyses. Subsequent CCCs should alternate between a medium and high concentration calibration standard. See Section 10.3 for acceptance criteria for the various CCCs. Preparation of the CCCs is described in Section 7.3.5.

9.3.4 LABORATORY FORTIFIED BLANK (LFB) – Since this method utilizes procedural calibration standards, which are fortified reagent waters, there is no difference between the LFB and the CCC, except for the order in which they are run as part of an analysis batch and the corresponding QC acceptance criteria. The acronym LFB is used for clarity in the IDC.

9.3.5 SECOND SOURCE QUALITY CONTROL SAMPLES (QCS) – As part of the IDC (Sect. 9.2), each time a new analyte stock standard solution 1 (SS1, Sect. 7.3.3.1) is prepared, and at least quarterly, analyze a QCS sample from a source different from the source of the calibration standards. If a second vendor is not available, then a different lot of the standard should be used. The QCS should be prepared near the midpoint of the calibration range and analyzed as a CCC. Acceptance criteria for the QCS are identical to the CCCs; the calculated amount for each analyte must be ± 30% of the expected value. If measured analyte concentrations are not of acceptable accuracy, check the entire analytical procedure to locate and correct the problem.

9.3.6 INTERNAL STANDARD (IS) – The analyst must monitor the peak area of the IS in all injections during each analysis day. The IS peak area must meet the criteria in the both following two subsection

9.3.6.1 The internal standard should produce a peak area at least five times higher than the peak area of the quantitation ion of tetramine in the lowest concentration calibration solution. If it does not, the concentration of IS may not be as predicted. Prepare new calibrations solutions, QC samples, and field samples with an appropriately increased concentration of IS.

9.3.6.2 The IS response (peak area) in any chromatographic run must not deviate from the response in the most recent CCC by more than 30%, and must not deviate by more than 50% from the area measured during initial analyte calibration. If the IS area in a chromatographic run does not meet these criteria, inject a second aliquot of that extract.

9.3.6.2.1 If the reinjected aliquot produces an acceptable IS response, report results for that aliquot.

9.3.6.2.2 If the reinjected aliquot fails the IS criterion, the analyst should check the calibration by reanalyzing the most recently acceptable calibration standard. If the calibration standard fails the criteria of Section 10.3, recalibration is in order per Section 10.2. If the calibration standard is acceptable, report results obtained from the reinjected aliquot, but annotate as "suspect/IS recovery." Alternatively, prepare another aliquot of the sample as specified in Section 11.2 or collect a new sample and re-analyze.

9.3.7 LABORATORY FORTIFIED SAMPLE MATRIX (LFSM) and LFSM DUPLICATES – The isotopically labeled internal standard in this method also serves the role of the LFSM, which is used to determine that the sample matrix does not adversely affect method accuracy. In the context of application of this method for environmental restoration, it is not expected that there would be a native tetramine background concentration. Also, it is likely that the water samples will come from the same drinking water system, and hence the sample matrices from a single collection time will be very similar. Further, experience with the automated extraction equipment used suggests that if most failures in IS QC requirements result from failure of the automation equipment. This would correspond to LFSM failure, as well. Accordingly, neither LFSMs or duplicate LFSMs would be expected to yield additional information about influence of sample matrix on method accuracy, except for the unlikely case of a feature of the sampling/restoration plan that produces a co-eluting peak with identical chromatographic and mass spectral properties as tetramine. In this case, the lab should discuss with the submitter.

9.3.7.1 If an LFSM and LFSM is deemed necessary, calculate the relative percent difference (RPD) for duplicate LFSMs (LFSM and LFSMD) using the equation

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

9.3.7.2 Relative percent difference (RPD) for duplicate LFSMs should be ≤30% for samples fortified at or above their native concentration. Greater variability may be observed when LFSMs are fortified at analyte concentrations that are within a factor of two of the MRL. LFSMs fortified at these concentrations should have RPDs that are ≤50%. If the RPD of any analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control

in the CCC, the recovery is judged to be matrix biased. The result for that analyte in the unfortified sample is labeled "suspect/matrix" to inform the data user that the results are suspect due to matrix effects.

9.3.5 FIELD DUPLICATE (FD) – Field duplicates check the precision associated with sample collection, preservation, storage, and laboratory procedures. Some of these factors are out of control of the laboratory, and the rest are covered by other QC checks. Accordingly, results of any field duplicates requested should be discussed with the sample submitter if they do not meet the following criteria.

9.3.5.1 Calculate the relative percent difference (*RPD*) for duplicate samples (*FD1* and *FD2*) using the equation

$$RPD = \frac{|FD1 - FD2|}{(FD1 + FD2)/2} \times 100$$

9.3.5.2 RPDs for FDs should be ≤30%. Greater variability may be observed when FDs have analyte concentrations that are within a factor of two of the MRL. At these concentrations, FDs should have RPDs that are ≤50%. If the RPD of any analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control in the CCC, the recovery is judged to be biased. The result for that analyte in the unfortified sample is labeled "suspect/field duplicate bias" to inform the data user that the results are suspect due to field bias. (Note some other sources of lab bias may also be present.)

10. CALIBRATION AND STANDARDIZATION

10.1 All laboratory equipment should be calibrated according to manufacturer's protocols and equipment with expired calibrations should not be used. Demonstration and documentation of acceptable mass spectrometer tune and initial calibration is required before any samples are analyzed. After the initial calibration is successful, the instrument is recalibrated using the same conditions as the initial calibration before each analysis batch. After the batch, the lowest and highest calibration solutions are run as continuing calibration checks (CCC) Verification of mass spectrometer tune must be repeated each time a major instrument modification is made or maintenance is performed, and prior to analyte calibration.

10.2 INITIAL CALIBRATION

10.2.1 MS TUNE – Calibrate the mass and abundance scales of the MS with calibration compounds and procedures prescribed by the manufacturer with any modifications necessary to meet tuning requirements. For an Agilent MSD, some labs have experienced better results if following the automatic tune, they perform a manual tune to set the mass resolution to unit mass, the peak width to 0.50 ± 0.01 amu and the abundance for the ion at mass 69 to 500,000 ± 50,000 counts. For other instruments, follow manufacturer's protocols to tune the instrument.

10.2.2 INSTRUMENT CONDITIONS – Operational conditions are tabulated in Section 6.8.3. Alteration of the conditions is not recommended and would require redevelopment of QC criteria. Frequently reported problems can be avoided by: 1) checking that needle wash solutions are adequately filled and the injection syringe is functioning properly and 2) changing the septum and inlet liner as needed.

10.2.3 Prepare six calibration standards as described in Section 7.3.4. Note that as procedural calibration standards, they are processed through the procedure in Section 11, in which the isotopically labeled internal standard is added before extraction. In practice, the lowest concentration of the calibration standard must be at or below the MRL (Sect. 9.2.4), which will depend on system sensitivity. The lowest point on the calibration curve is close to the reported detection limit and the highest point is above the expected range of results. The remainder of the points are distributed between these two extremes, with the majority of points in the concentration range where most unknowns are expected to fall.

10.2.4 The GC/MS system is calibrated using the internal standard technique, as implemented by the data system software. Construct a calibration curve using at least a six-point curve of response ratios (i.e., ratio of calibration standard peak area to internal standard peak area). As the internal standard concentration is consistent among samples and calibrators, some labs have found it convenient to set it to a value of one instead of the actual concentration.

10.2.5 CALIBRATION ACCEPTANCE -- Calculate the slope and intercept of the calibration curve with 1/x weighting (or other appropriate weighting) by a linear least squares fit (or other appropriate calibration function). Evaluate the r^2 value for the curve, which must be greater than 0.990. Linearity of the standard curve should extend over the entire standard range. The intercept should not be significantly different from 0; if it is, the source of

the bias should be identified. Each calibration point, except the lowest point, for the analyte should calculate to be 70 to 130 percent of its true value. The lowest point should calculate to be 50 to 150 percent of its true value. If these criteria cannot be met, the analyst will have difficulty meeting ongoing QC criteria. If any standard is in error and does not fit the standard curve (i.e., the r^2 value for the curve is < 0.990), it can be removed from the calibration. No more than one standard may be discarded in any given calibration curve. If either the high or low standard is dropped, the reporting limits must be adjusted accordingly. The resulting r^2 value must be greater than 0.990.

10.3 CONTINUING CALIBRATION CHECKS (CCCs). As described in Sect 9.3.3, up to four CCCs are used in conjunction with each analysis batch. If applicable, LFB-low and LFB-high are run at the beginning of the batch, and the calibration solutions are run at the end. The LFBs serve to verify the initial IDC, and the calibration solutions verify the calibration generated at the start of the analysis. The LRBs, LFBs, and CCCs are not counted as the 20 samples that constitute an analysis batch.

10.3.1 Inject an aliquot of the appropriate concentration calibration solution and analyze with the same conditions used during the initial calibration.

10.3.2 Acceptance of the calibration solutions is based on the same criteria as described in Section 10.2.5. Failure to meet these criteria is a rare occurrence, and suggests maintenance of the GC/MS system is required.

10.3.3 Acceptance of the results of the LFB-Low and LFB-High is based on the Quality Control Limits (Sect. 10.3.3.1) established via the IDC. Acceptability of results for that entire analytical batch is dependent upon the agreement of the results from these control materials within established ranges. Quality Control Limits for the CCCs are based primarily on the standard deviation (σ_{n-1}, sigma) of the replicate analysis in the IDC (Sect. 9.2.2). Section 13.3 presents sample values for these parameters obtained in the developer's laboratory, in which 20 replicate analyses performed over no less than 10 days are used to establish the LFB-low and -high limits (Sect. 9.2.2). If the CCC results do not meet the following criteria, it is "out-of-control," and the cause of the failure must be determined and corrected. No results from the associated analytical batch may be reported. These criteria apply to non-zero analyte concentrations used to make the quality control solutions in section 7.3.5.1

10.3.3.1 If both of the LFB-Low and LFB-High results are within $2\sigma_{n-1}$ of the mean determined during the IDC, then accept the entire analytical batch. Otherwise, reject the entire analytical batch.

10.3.4 Common remedial actions if the CCCs fails to meet acceptable criteria

10.3.4.1 LOW ANALYTE RESPONSE – If the signal-to-noise of the low standard confirmation ion falls below 10, this indicates that the instrumental sensitivity, or SPE recovery, has fallen below acceptable limits. The following steps should be taken and the instrument sensitivity rechecked after each corrective action is performed. Once sensitivity has been reestablished, further steps are not necessary.

i. Re-extract the samples.
ii. If tailing is a significant issue, clip the GC column.
iii. Ensure the filament for the MS is still intact.
iv. Clean the mass spectrometer source
v. Clean the gas chromatograph inlet liner

10.3.4.2 Analyte in standards – If an inordinately large amount of analyte is measured in one of the calibration standards, but this is not seen in the remainder of the samples, this indicates a contamination of this particular sample. The source of this incident should be investigated to prevent repeat occurrences, but no further action is required. The contaminated calibration standard should be excluded when developing the calibration curve.

10.3.4.3 Analyte in all samples – If an inordinately large amount of analyte is present in all measurements for a particular day, it is likely that one or more of the spiking solutions are contaminated. If necessary, prepare new solutions.

11. PROCEDURE

11.1 SAMPLE PREPARATION

11.1.1 Samples are preserved, collected and stored as presented in Section 8. Allow samples to come to room temperature prior to analysis.

11.1.2 If using a TurboVap 96 evaporator system, set it to 65-75°C. Follow manufacturer's direction for other equipment.

Note: Steps 11.1.3 through 11.1.13 can be performed using an automated liquid handler or a manual pipettor with a manual 96-well manifold. However, data presented in this document was collected using an automated liquid handler.

11.1.3 Fill 96 plate wells.

- 11.1.3.1 Into each well of the 96-well Nunc deep well plate (Nalge Nunc International, Rochester, NY), add 50 µL of the isotopically-labeled internal standard (refer to section 7.3.2)
- 11.1.3.2 Into each sample well, add 1000 µL of sample.
- 11.1.3.3 Into each blank well, add 1000 µL of reagent water (for the LRB).
- 11.1.3.4 Into each calibration standard well, add 1000 µL of tetramine calibration standard stock solutions (refer to sections 7.3.4)
- 11.1.3.5 Into each quality control well, add 1000 µL of appropriate quality control material. (refer to section 7.3.5)

11.1.4 Mix on the plate shaker for 2 min or by other appropriate means.

11.1.5 Plate SPE procedure. For each well on the Nunc plate filled in Section 11.1.3, perform the following steps and do not let wells go dry for more than 1 minute:

- 11.1.5.1 Condition/preclean the selected well on the Phenomenex® Strata-X 60-mg SPE well plate (Phenomenex, Torrance, CA) with 1125 µL of 100% methanol.

- 11.1.5.2 Condition the SPE plate with 1125 µL of deionized water.

- 11.1.5.3 Load 1000 µL from the Nunc plate and draw through the SPE plate using positive or negative pressure.

- 11.1.5.4 Wash the SPE plate with 1125 µL of 5% methanol/95% water (Sect. 7.2.1).

- 11.1.5.5 Elute the sample with 800 µL of acetonitrile into a 96-well Nunc deep well plate.

11.1.6 Blow down the sample to dryness using nitrogen gas at 65-75°C. If using a TurboVap evaporator system, set the flow rate to 45 flow

units until approximately 50% has been evaporated. Then raise the flow rate to 75 flow units until dry. When using systems other than the TurboVap, set the flow rate for the blow down gas according to manufacturer's directions.

11.1.7 Add 100 µL of acetonitrile to reconstitute each sample and vortex.

11.1.8 Transfer the acetonitrile solution into appropriate autosampler vials or a 96-well autosampler plate.

11.2 ANALYSIS OF SAMPLE EXTRACTS

11.2.1 Establish operating conditions as described in Section 10.2.2.

11.2.2 Establish a valid initial calibration following the procedures outlined in Section 10.2 or confirm that the calibration is still valid by running both CCCs as described in Section 10.3. If establishing an initial calibration for the first time, complete the IDC as described in Section 9.2.

11.2.3 Set up the available automation equipment and software as specified by the manufacturer for batch analysis, paying particular attention to the following frequent stumbling blocks:

11.2.3.1 On the instrument computer, edit the automation software:
(a) Select the sample type.
(b) Identify the correct vial position.
(c) Name the sample. Due to large number of samples analyzable with the automation equipment, it is important that appropriate record keeping (e.g., database, notebooks, data files, etc.) should be used to track specimens.
(d) Enter information related to particular specimens into the software manually or by electronic transfer.
(e) Select the instrument control method.
(f) Identify the target path where the data will be stored.

11.2.3.2 Check to be sure that the number and positions of samples entered on the sequence set-up page correspond to the samples in the autosampler.

11.2.4 Run the automation sequence to analyze the batch of aliquots of field and QC samples at appropriate frequencies (Sect. 9, 10.3). All field, QC, and calibration standards should be run using the same GC/MS conditions. At the conclusion of data acquisition, use the same software that was used in the calibration procedure to

identify the peaks in predetermined retention time windows of interest. Use the data system software to examine the ion abundances of components of the chromatogram.

11.2.5 COMPOUND IDENTIFICATION – The presumed tetramine peak in the sample must appear in the same retention time window as the isotopically-labeled internal standard (around 11.6 min in the developer's lab) and have similar chromatographic characteristics such as peak shape. This relies on expert judgment of the analyst since the retention times reported by the software are not always reliable. Identification of the peak as tetramine is then confirmed through calculating the confirmation ratio (CR), i.e., by dividing the response for m/z 240 by the response for m/z 212 of the presumed tetramine peak. Using the manufacturer's software or manually, compare the confirmation ratio of the peak from the sample with the mean of the CRs measured for the six calibration standards associated with that batch. The mean CR is the average CR from the calibration standards only and is batch dependent. The CR value for each sample should be within 30% of the mean. (CR value was 1.74 in the developer's lab for the IDC samples).

12. DATA ANALYSIS AND CALCULATIONS

12.1 Concentrations are calculated using the ions listed in Section 6.8.3. Use of other ions is not advised. If a particular instrument cannot produce the fragments listed in section 6.8.3, this instrument should not be used to run this method.

12.2 Calculate analyte concentrations using the ongoing multipoint calibration established in Section 9.3.2. Do not perform calibration using just the CCC or LFB-low and -high data to quantitate analytes in samples, although these samples might be part of the ongoing calibration curve.

12.3 All raw data files are quantified using the quantitation capabilities of the instrument software. The peaks are automatically integrated using the software-associated integration program, and the integration of each peak is reviewed and manually corrected as appropriate. This is particularly important for the calibration standards. The quality control samples (e.g., CCCs and LFBs) are quantified and evaluated against the calibration curve, and each field sample is then quantified against that calibration curve. The run data can be processed within instrument data analysis software and exported to external spreadsheets, per laboratory policy, generating

files containing the unknown and QC concentrations, retention times, standard curves, and other run information.

12.3.1 Results are generally reported to two significant digits. In addition to analytical measurements of unknowns, statistical results of measurement of blanks should accompany all results.

12.3.2 Check all sample and analytical data for transcription errors and overall validity after being entered into the instrument software database. Back up onto external media both the instrument and data storage databases according to laboratory guidelines.

13. METHOD PERFORMANCE

13.1 ANALYTICAL IDENTIFICATION–Analyte identification using the approach described in Section 11.2.5 resulted in no false positives or negatives for the samples reported below. There was very low background noise according to the signal-to-noise ratios for the *m/z* monitored.

13.2 SINGLE LABORATY MINIMUM REPORTING LEVELS and DETECTION LIMIT– The reportable range of results for tetramine is summarized below, along with the DL determined from the IDC procedure (n=20, >10 days) described previously. The lowest standard is used as the method reportable limit, and the DL calculated from the standard deviation of replicate measurements of that standard (in the case of Table 13-1, 0.059). The highest reportable limit is based on the highest linear standard.

Table 13-1. Method Performance

Compound	**Minimum reporting level (ng/mL)**	**Highest reportable limit (ng/mL)**	**Method DL (ng/mL)**
Tetramine (retention time = 11.6 min)	0.5	250	0.15

13.3 SINGLE LABORATORY ACCURACY AND PRECISION for LFBs – Single lab precision and accuracy data is represented in Table 13-2. Accuracy is defined as the mean of the measured concentration in the fortified samples divided by the fortification concentration, expressed as a percentage. Method accuracy was determined by analyzing LFBs at the two non-zero levels in Section 7.3 (i.e., LBF-low and –high) and twenty analyses for each of the two concentration levels were completed over a period of 28 days. The means, standard deviations, and relative standard deviations for the two LFBs are shown in Table 13-2. The means are less than one standard deviation from the known concentration.

Table 13-2. Single Lab Precision and Accuracy Data

Analyte	**Sample**	**Fortified Concentration** (µg/L)	**Mean of IDC Replicates** (µg/L)	**Standard Deviation** (µg/L)	**RSD (%)**	**Accuracy of Mean (%)**
Tetramine	LFB-low	5	5.03	0.28	5.6	100
	LFB-high	75	75.5	2.8	3.7	101

13.4 SINGLE LABORATORY RECOVERY AND PRECISION FOR TAP WATER MATRICES. Table 13-3 expresses percent mean recoveries for tetramine in several different chlorinated and chloraminated tap waters derived from the types of sources (i.e., ground or surface water) indicated. Water quality parameters describing these sources are indicated in the footnotes. Percent recoveries were determined by dividing the measured concentration by the spiked concentration (75 ug/L) (n=3 for each day). No tetramine or interferences were detected in the unspiked samples.

13.5 SAMPLE STORAGE STABILITY STUDIES – Table 13-3 also presents tetramine storage stability data. Samples were collected and stored as described in Section 8 and also at room temperature. No preservatives were added to the samples (See Sect. 8.1.2). The precision and average recovery of triplicate analyses was conducted on Days 0, 7, 14 and 28. These data support the maximum 28 day aqueous holding time specified in Section 8.4.

Table 13-3. Percent Recovery of Tetramine for Several Tap Water Matrices and Residual Disinfectants

Water Type	Day 0		Day 7		Day 14		Day 28	
	4°C	25°C	4°C	25°C	4°C	25°C	4°C	25°C
Ground Water 1[a] (chlorine)	103 ± 8	109 ± 3	105 ± 3	113 ± 4	113 ± 4	104 ± 5	122 ± 2	114 ± 7
Surface Water 2[b] (chloramine)	107 ± 8	100 ± 1	99 ± 3	114 ± 3	92 ± 3	105 ± 6	119 ± 6	111 ± 3
Surface Water 3[c] (chlorine)	110 ± 3	96 ± 0	102 ± 3	106 ± 6	108 ± 6	107 ± 5	111 ± 2	107 ± 2
Surface Water 4[d] (chloramine)	102 ± 2	94 ± 4	102 ± 1	107 ± 3	98 ± 7	98 ± 5	109 ± 2	114 ± 3
Surface Water 5[e] (chlorine)	102 ± 3	99 ± 7	117 ± 4	108 ± 3	108 ± 6	108 ± 6	112 ± 3	116 ± 8

[a]Total organic carbon (TOC) not detected in well-field; pH 7.6; hardness 350 mg/L; Chlorine 0.2-0.4 mg/L; (monthly averages)

[b]TOC 7.61 mg/L; pH 9.2; hardness 65 mg/L; Monochloramines 2.4 mg/L (monthly averages)

[c]TOC 2.0 mg/L; pH 7.3; hardness 135 mg/L; Chlorine 1 mg/L (monthly averages)

[d]TOC 2.3; pH 7.4; hardness 190 mg/L; Monochloramine 3.4 mg/L (monthly averages)

[e]TOC 1.0; pH 8.5; 130 mg/L; Chlorine 0.8 mg/L (monthly averages)

14. POLLUTION PREVENTION

14.1 This method utilizes solid phase extraction to extract analytes from water. It requires the use of reduced volumes of organic solvent and very small quantities of pure analytes, thereby minimizing the potential hazards to both the analyst and the environment as compared to the use of large volumes of organic solvents in conventional liquid-liquid extractions.

14.2 For information about pollution prevention that may be applicable to laboratory operations, consult "Less is Better: Laboratory Chemical Management for Waste Reduction" available from the American Chemical Society's Department of Government Relations and Science Policy on-line at http://portal.acs.org/portal/fileFetch/C/WPCP_012290/pdf/WPCP_012290.pdf (accessed May 2010).

15. WASTE MANAGEMENT

15.1 Dispose of waste materials in compliance with the laboratory chemical hygiene plan, as well as federal, state, and local regulations. Always dispose of solvents and reagents in an appropriate container clearly marked for waste products and temporarily store them in a chemical fume hood. Dispose of tetramine in an appropriate waste stream as well. Tetramine is not destroyed by autoclaving [8], so wash any other non-disposable glassware, empty ampoules, and/or apparatus before recycling or disposing of in an appropriate manner.

16. REFERENCES

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