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METHOD 521 DETERMINATION OF NITROSAMINES IN DRINKING WATER BY SOLID PHASE EXTRACTION AND CAPILLARY COLUMN GAS CHROMATOGRAPHY WITH LARGE VOLUME INJECTION AND CHEMICAL IONIZATION TANDEM MASS SPECTROMETRY (MS/MS)

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METHOD 521

DETERMINATION OF NITROSAMINES IN DRINKING WATER BY SOLID PHASE EXTRACTION AND CAPILLARY COLUMN GAS CHROMATOGRAPHY WITH LARGE VOLUME INJECTION AND CHEMICAL IONIZATION TANDEM MASS SPECTROMETRY (MS/MS)

1. SCOPE AND APPLICATION

1.1 This method provides procedures for the determination of nitrosamines in finished drinking water. The method may be applicable to untreated source waters and other types of water samples, but it has not been evaluated for these uses. The method is applicable to nitrosamines that are efficiently partitioned from the water sample onto a solid phase extraction (SPE) sorbent, and sufficiently volatile and thermally stable for gas chromatography. The method includes the following compounds:

Analyte	Chemical Abstract Services (CAS) Registry Number
N-Nitrosodimethylamine (NDMA)	62-75-9
N-Nitrosomethylethylamine (NMEA)	10595-95-6
N-Nitrosodiethylamine (NDEA)	55-18-5
N-Nitrosodi-n-propylamine (NDPA)	621-64-7
N-Nitrosodi-n-butylamine (NDBA)	924-16-3
N-Nitrosopyrollidine (NPYR)	930-55-2
N-Nitrosopiperidine (NPIP)	100-75-4

1.2 The Minimum Reporting Level (MRL) is the lowest analyte concentration that meets Data Quality Objectives (DQOs) that are developed based on the intended use of this method. The lowest concentration MRL (LCMRL) is a single laboratory determination of the lowest true concentration for which a future recovery is expected, with 99 percent confidence, to be between 50 and 150 percent recovery. Single laboratory LCMRLs for analytes in this method range from 1.2-2.1 ng/L, and are listed in Table 1. The procedure used to determine LCMRLs is described elsewhere.[1]

1.3 Laboratories using this method are not required to determine LCMRLs for the method analytes, but must demonstrate that their laboratory MRLs meet the requirements in Section 9.2.4.

1.4 Detection limit (DL) is defined as the statistically calculated minimum concentration that can be measured with 99% confidence that the reported value is greater than zero.[2] The DL is compound dependent and is dependent on extraction efficiency, sample matrix, fortification concentration, and instrument performance. Determining the DL for analytes in this method is optional (Sect. 9.2.5). DLs for method analytes range from 0.26-0.66 ng/L, and are listed in Table 1.

1.5 This method should be performed only by or under the supervision of analysts with experience in solid phase extractions and chemical ionization GC/MS/MS analyses.

2. SUMMARY OF METHOD

Analytes and a surrogate analyte are extracted by passing a 0.5-L water sample through a solid phase extraction (SPE) cartridge containing 2 g of 80-120 mesh coconut charcoal (Sect. 7.2.7). The organic compounds are eluted from the solid phase with a small quantity of methylene chloride.

The methylene chloride extract is dried and concentrated, and an internal standard is added. The sample components are separated, identified, and measured by injecting an aliquot of the concentrated extract onto the fused silica capillary column of a GC/MS/MS system equipped with a large volume injector injector (LVI), and operated in the chemical ionization (CI) mode. Because the CI reagent gas used (methanol or acetonitrile) produces a mass spectrum with only one significant ion, identification and quantitation are performed in the MS/MS mode.

Compounds eluting from the GC column are identified by comparing their product ion mass spectra and retention times to reference spectra and retention times in a user created database. Reference spectra and retention times for analytes are obtained by the measurement of calibration standards under the same conditions used for samples. The concentration of each identified component is measured by an internal standard procedure, i.e. relating the product ion response of the analyte to the product ion response of the compound that is used as an internal standard. A surrogate analyte, whose concentration is known in every sample, is measured with the same internal standard calibration procedure.

3. DEFINITIONS

3.1 ANALYSIS BATCH -- A set of samples analyzed on the same instrument during a 24 hour period that begins and ends with the analysis of the appropriate Continuing

Calibration Check (CCC) standards. Additional CCCs may be required depending on the length of the analysis batch and/or the number of Field Samples.

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

3.3 COLLISIONALLY ACTIVATED DISSOCIATION (CAD) -- The process of converting the precursor ion's translational energy into internal energy by collisions with neutral gas molecules to bring about dissociation into product ions.

3.4 CONTINUING CALIBRATION CHECK (CCC) -- A calibration standard containing one or more method analytes, which is analyzed periodically to verify the accuracy of the existing calibration for those analytes.

3.5 DETECTION LIMIT (DL) -- The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero. This is a statistical determination (Sect. 9.2.5), and accurate quantitation is not expected at this level.[2]

3.6 EXTRACTION BATCH -- A set of up to 20 field samples (not including QC samples) extracted together by the same person(s) during a work day using the same lot of solid phase extraction devices and solvents, surrogate solution, and fortifying solutions. Required QC samples for each extraction batch include: Laboratory Reagent Blank, Laboratory Fortified Blank, Laboratory Fortified Sample Matrix, and either a Field Duplicate or Laboratory Fortified Sample Matrix Duplicate.

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. Analyses of FD1 and FD2 give a measure of the precision associated with sample collection, preservation, and storage, as well as with laboratory procedures.

3.8 INTERNAL STANDARD (IS) -- A pure analyte(s) added to a sample, extract, or standard solution in known amount(s) and used to measure the relative responses of other method analytes and surrogates that are components of the same solution. The internal standard must be an analyte that is not a sample component.

3.9 LABORATORY FORTIFIED BLANK (LFB) -- An aliquot of reagent water or other blank matrix to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, including the use of sample

preservatives, 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 FORTIFIED SAMPLE MATRIX (LFSM) -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFSM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFSM corrected for background concentrations.

3.11 LABORATORY FORTIFIED SAMPLE MATRIX DUPLICATE (LFSMD) -- A second aliquot of the Field Sample, or duplicate Field Sample, that is used to prepare the LFSM. The LFSMD is fortified, extracted and analyzed identically to the LFSM. The LFSMD is used instead of the Laboratory Duplicate to assess method precision when the occurrence of target analytes is low.

3.12 LABORATORY REAGENT BLANK (LRB) -- An aliquot of reagent water or other blank matrix that is treated exactly as a sample, including exposure to all glassware, equipment, solvents, reagents, internal standards, surrogates, and sample preservatives that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.13 LARGE VOLUME INJECTION (LVI) – An injection into a gas chromatograph that is larger than the typical 1 or 2 µL injection used for hot vaporizing injectors. A specialized injector and autosampler are usually required. The upper limit of the injection size is dependent upon the instrumentation used, and the exact mechanism utilized to accommodate the larger solvent volume. The type of LVI described in this method uses a temperature programmed vaporizing (PTV) injector (Sect. 3.20).

3.14 LOWEST CONCENTRATION MINIMUM REPORTING LEVEL (LCMRL) – The single laboratory LCMRL is the lowest true concentration for which the future recovery is predicted to fall, with high confidence (99 percent), between 50 and 150 percent recovery.[1]

3.15 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.

3.16 MINIMUM REPORTING LEVEL (MRL) -- The minimum concentration that can be reported as a quantitated value for a target analyte in a sample following analysis. This defined concentration can be no lower than the concentration of the lowest

calibration standard for that analyte, and can only be used if acceptable quality control criteria for the analyte at this concentration are met. A procedure for verifying a laboratory's MRL is provided in Section 9.2.4.

3.17 PRECURSOR ION -- For the purpose of this method, the precursor ion is the protonated molecule ($[M + H]^+$) of the target analyte. In MS/MS, the precursor ion is mass selected and fragmented to produce distinctive product ions of smaller mass.

3.18 PRIMARY DILUTION STANDARD SOLUTION (PDS) -- A solution of several analytes prepared in the laboratory from stock standard solutions and diluted as needed to prepare calibration solutions and other needed analyte solutions.

3.19 PRODUCT ION -- For the purpose of this method, a product ion is one of the fragment ions produced in MS/MS by collisionally activated dissociation of the precursor ion.

3.20 PROGRAMMED TEMPERATURE VAPORIZING INJECTOR (PTV) -- A GC injector capable of rapid heating. Typical use of a PTV injector involves introducing the sample with the injector cool, then rapidly heating it at 100-200 °C per minute to volatilize the analytes onto the GC column. One advantage of this type of injection is that thermally labile analytes in a mixture can be transferred to the GC column at a lower temperature than in conventional hot injections. It is also useful for large volume injections.

3.21 QUALITY CONTROL SAMPLE (QCS) -- A solution of method analytes of known concentrations that is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.

3.22 STOCK STANDARD SOLUTION (SSS) -- A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

3.23 SURROGATE ANALYTE (SUR) -- A pure analyte, which is extremely unlikely to be found in any sample, and which is added to a sample aliquot in a known amount before extraction or other processing, and is measured with the same procedures used to measure other sample components. The purpose of the SUR is to monitor method performance with each sample.

4. INTERFERENCES

4.1 During analysis, major contaminant sources are reagents and SPE devices. Analyses of laboratory reagent blanks provide information about the presence of

contaminants. Solid phase extraction devices described in this method have two potential sources of contamination, both the solid phase sorbent and the polypropylene cartridge that it is packed in. Manufacturers' brands and lot numbers of SPE materials should be monitored and tracked to ensure that contamination will not preclude analyte identification and quantitation.

4.2 Nitrosamines may be present in trace amounts in rubber products. The analyst should be aware that repeated injections from autosampler vials with PTFE (polytetrafluoroethylene, otherwise known as Teflon®) coated rubber septa may introduce method analytes into the sample extracts.

4.3 Interfering contamination may occur when a sample containing low concentrations of compounds is analyzed immediately after a sample containing relatively high concentrations of compounds. Injection port liners must be replaced as needed (cleaning and deactivation by the analyst is not recommended). After analysis of a sample containing high concentrations of compounds, a laboratory reagent blank should be analyzed to ensure that accurate values are obtained for the next sample. In the case of automated analysis, the analyst may not be aware of high concentration samples until after an entire batch is analyzed. In this situation, the analyst should carefully review data from samples analyzed immediately after high concentration samples, and reanalyze them if necessary.

4.4 Reagent water must be free of method analytes. Because NDMA and other nitrosamines can leach from rubber products, rubber components must be avoided in the reagent water system. Reagent water contamination with NDMA has been reported in the literature.[3] It is strongly recommended that the reagent water system include an ultraviolet module. Ultraviolet light is capable of destroying trace amounts of nitrosamines that may be in the water. During the development of this method, a Millipore Milli-Q A10 system was used. Reagent water may be successfully stored in glass bottles with PTFE lined screw caps.

5. SAFETY

5.1 The toxicity or carcinogenicity of many of the chemicals used in this method have 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 awareness of OSHA regulations regarding safe handling of chemicals used in this method. Each laboratory should maintain a file of applicable MSDSs.

5.2 The analytes listed in this method have been classified as known or suspected human or mammalian carcinogens.[4,5] Pure standard materials and stock standard solutions of these compounds should be handled with suitable protection to skin, eyes, etc.[6-8]

6. EQUIPMENT AND SUPPLIES (References to specific brands or catalog numbers are included for illustration only, and do not imply endorsement of the product.)

6.1 GLASSWARE -- All glassware must be meticulously cleaned. This may be accomplished by washing with detergent and water, rinsing with water, distilled water, and solvent rinsing or heating (where appropriate) at 400 °C for 2 h in a muffle furnace. Volumetric glassware should never be heated to the temperatures obtained in a muffle furnace.

6.2 SAMPLE CONTAINERS -- 1-L, 1-qt, 1-pt or 0.5-L amber glass bottles fitted with PTFE lined polypropylene screw caps. Amber bottles are highly recommended since some of the method analytes are very sensitive to light and will degrade upon exposure. Clear glass bottles may be used only if they are wrapped in foil, or samples are stored in boxes that prevent exposure to light.

6.3 VIALS -- Screw cap or crimp top amber glass autosampler vials with PTFE faced septa for storing standards and extracts.

6.4 VOLUMETRIC FLASKS -- Class A, various sizes used for preparation of standards.

6.5 MICRO SYRINGES -- Various sizes.

6.6 BALANCE -- Analytical, capable of accurately weighing to 0.0001 g.

6.7 DRYING COLUMN -- The drying tube should contain about 5 to 7 grams of anhydrous sodium sulfate to remove residual water from the extract. During method development, 6-mL glass reaction tubes with PTFE frits (Supelco cat. # 504394 or equivalent) were used. Any small tube may be used, such as a syringe barrel, a glass dropper, etc. as long as no particulate sodium sulfate passes through the column into the extract.

6.8 CONICAL CENTRIFUGE TUBES -- 60 mL, used to collect extract after it is passed through anhydrous sodium sulfate and prior to concentration to final volume. Other glassware suitable for this purpose may be used.

6.9 SOLID PHASE EXTRACTION (SPE) APPARATUS

6.9.1 EXTRACTION CARTRIDGES -- 6-mL polypropylene tubes, to be packed with 1.8 to 2.2 g of coconut charcoal (Sect. 7.2.7.1). Glass tubes are also available, and may be used. These tubes are not needed if prepacked SPE cartridges are used (Sect. 7.2.7.2).

6.9.2 VACUUM EXTRACTION MANIFOLD -- Equipped with flow/vacuum control (Supelco cat. #57250-U or equivalent). The use of disposable PTFE liners to prevent cross contamination may be used.

6.9.3 SAMPLE DELIVERY SYSTEM -- Use of a transfer tube system (Supelco "Visiprep", cat. #57275 or equivalent), which transfers the sample directly from the sample container to the SPE cartridge, is recommended. Sample reservoirs, which attach to the cartridge, may be used, although they hold only a limited volume of sample.

6.9.4 CONICAL CENTRIFUGE TUBES -- 15 mL, or other glassware suitable for elution of the sample from the cartridge after extraction.

6.9.5 LABORATORY OR ASPIRATOR VACUUM SYSTEM -- Sufficient capacity to maintain a vacuum of approximately 25 cm (10 in) of mercury.

6.9.6 An automatic or robotic system designed for use with SPE cartridges may be used if all quality control requirements discussed in Section 9 are met. Automated systems may use either vacuum or positive pressure to process samples and solvents through the cartridge. <u>All extraction and elution steps must be the same as in the manual procedure. Extraction and/or elution steps may not be changed or omitted to accommodate the use of an automated system.</u>

6.10 FUSED SILICA CAPILLARY GAS CHROMATOGRAPHY COLUMN -- Any capillary column that provides adequate resolution, capacity, accuracy, and precision can be used. Medium polarity, low bleed columns are recommended for use with this method to provide adequate chromatography and minimize column bleed. Deactivated injection port liners are recommended.

6.10.1. Column - 30 m × 0.25 mm i.d. fused silica capillary column coated with a 1.0 micron bonded film of polyphenylmethylsilicone, (Restek Rtx 5SIL MS or equivalent).
NOTE: Although other columns may be used, shorter or thinner film columns are not recommended for use with ion trap GC/MS/MS systems because the chromatographic peak widths will be insufficient to obtain a sufficient number of MS/MS scans during peak elution.

6.11 GAS CHROMATOGRAPH/MASS SPECTROMETER/DATA SYSTEM (GC/MS/MS/DS)

6.11.1 The GC must be capable of temperature programming and should be equipped for large volume injection if low detection limits and MRLs are

required. The data presented in this method in Section 17 were obtained using 20-µL injections.

6.11.2 Deactivated injection port liners should be used, and it is highly recommended that they be replaced when necessary with a new deactivated liner. Cleaning and deactivation of injection port liners by the analyst is not recommended. The frequency of replacement will vary by the type of instrument used and the type and number of samples analyzed.

In general, packed injection port liners may be useful in performing large volume injections. The data demonstrated in this method were obtained with an injection port liner containing a Carbofrit ® (Restek). The positioning of the Carbofrit ® is specific to the instrument and liner used. On the Varian 1078 and 1079 injectors, 2.5 cm from the top of the liner is appropriate.

6.11.3 The tandem mass spectrometer may be either a triple quadrupole or an ion trap. However, during the method development, only ion trap mass spectrometers were used. The MS/MS must be capable of chemical ionization using either methanol or acetonitrile as the reagent gas. The mass spectrometer must be capable of scanning at a minimum from 40 to 160 daltons with a complete scan cycle time (including scan overhead) of 0.5 sec or less. The scan time must be set so that all analytes have a minimum of 5 scans across the chromatographic peak, when operated in MS/MS mode. Seven to ten scans across chromatographic peaks are strongly recommended.

6.11.4 An interfaced data system is required to acquire, store, reduce, and output mass spectral data. The computer software should have the capability of processing stored GC/MS/MS data by recognizing a GC peak within any given retention time window. The software must also allow integration of the ion abundance of any specific ion between specified time or scan number limits, calculation of response factors as defined in Section 10.2.3 or construction of a linear regression calibration curve, and calculation of analyte concentrations.

7. **REAGENTS AND STANDARDS** (References to specific brands or catalog numbers are included for illustration only, and do not imply endorsement of the product.)

7.1 GASES

7.1.1 CARBON DIOXIDE OR LIQUID NITROGEN -- Used to cool the injector for large volume injections.

7.1.2 HELIUM -- Carrier gas, purity as recommended by the GC/MS/MS manufacturer.

7.1.3 NITROGEN -- Ultra High Purity or equivalent, used to concentrate sample extracts.

7.2 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. Solvents should be HPLC grade or better. 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.

7.2.1 METHANOL (CH_3OH, CAS# 67-56-1) -- High purity, demonstrated to be free of analytes and interferences.

7.2.2 ACETONITRILE (CH_3CN, CAS#75-05-8) -- High purity, demonstrated to be free of analytes and interferences.

7.2.3 METHYLENE CHLORIDE (Dichloromethane, CH_2Cl_2, CAS# 75-09-2) -- High purity, demonstrated to be free of analytes and interferences.

7.2.4 REAGENT WATER -- Purified water which does not contain any measurable quantities of any target analytes or interfering compounds greater than 1/3 the MRL for each compound of interest. Refer to Section 4.4 for a description of how reagent water was obtained during method development.

7.2.5 SODIUM SULFATE, ANHYDROUS (Na_2SO_4, CAS# 7757-82-6) -- Should be Soxhlet extracted with methylene chloride for a minimum of 4 h or heated to 400 °C for 2 h in a muffle furnace. Store in a tightly sealed glass container. For the development of this method, a 10 to 60 mesh size was used. Finer mesh sized particles may slow the filtering process.

7.2.6 SODIUM THIOSULFATE ($Na_2S_2O_3$, CAS# 7772-98-7) -- Dechlorinating agent, used to eliminate residual chlorine in samples.

7.2.7 SOLID PHASE EXTRACTION MATERIAL -- The packing needed for solid phase extraction in this method consists of activated coconut charcoal, 80 to120 mesh size.

7.2.7.1 The product used for most of the method development was a 50:50 mix of 80/100 and 100/120 mesh obtained from Anspec of Ohio (Anspec, part # OV5487S).[9] It is recommended that the charcoal be Soxhlet extracted with methylene chloride for a minimum of 8 h, then air dried to evaporate the methylene chloride (keeping the air drying time minimal) and stored in a tightly sealed container. Pack 1.8 to 2.2 grams of the dry coconut charcoal in 6-mL polypropylene cartridges, using 20-micron polypropylene frits at the top and bottom of the tubes. As the tubes are being filled, periodically tap them on a hard surface to ensure even packing, without voids. Pack the cartridges just prior to use or store the packed tubes in a tightly sealed glass container. Glass SPE tubes may also be used as described in Section 6.9.1.

7.2.7.2 Pre-packed coconut charcoal cartridges ($\leq$100 mesh) were also used, and are commercially available (Restek cat. # 26032).

7.2.7.3 NOTE: SPE carbon disks (47 mm) were evaluated during method development, and determined to have insufficient capacity to retain all of the nitrosamine analytes using a 0.5-L water sample. Commercial brands of prepacked carbon SPE cartridges that contained carbon other than coconut charcoal, such as graphitized carbon, were also found to be unsatisfactory for this method.

7.3 STANDARD SOLUTIONS -- Standard solutions may be prepared from certified, commercially available solutions or from neat compounds. Compounds used to prepare solutions must be 96% pure or greater. The weight may be used without correction for purity 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. The majority of the method development work was done with commercially obtained stock standard solutions, which are readily available from most suppliers of environmental standards (Accustandard, Ultra, Supelco).

7.3.1 ANALYTE STOCK STANDARD SOLUTION -- If preparing from neat material, accurately weigh approximately 25 to 35 mg of pure material to the nearest 0.1 mg into a tared, 5-mL volumetric flask. Dilute to the mark with methylene chloride. Repeat for each target analyte. A serial dilution of these stock standards, made in methylene chloride, may be needed prior to making the final methylene chloride or water primary dilution standards. Alternatively, a stock standard mix of target analytes may be purchased commercially. For the development of this method, stock standards of 1000 or 2000 µg/mL were used to make primary dilution standards.

7.3.2 ANALYTE PRIMARY DILUTION STANDARD SOLUTION IN METHYLENE CHLORIDE ($MeCl_2$ PDS) -- The $MeCl_2$ PDS is a mix of all of the analytes of interest prepared in methylene chloride and used for calibration standard preparation. Prepare the analyte $MeCl_2$ PDS by dilution of the target analyte stock standard(s). Add enough of each target stock standard (or commercial stock standard mix) to a volumetric flask partially filled with methylene chloride to make the final desired concentration when filled to the mark with methylene chloride. During method development, a 2.0-µg/mL solution was made by diluting a 2000-µg/mL stock standard solution. This PDS has been shown to be stable for at least 6 months when tightly capped and stored at -15 °C or less and protected from light.

7.3.3 ANALYTE PRIMARY DILUTION STANDARD SOLUTION IN WATER (Water PDS) -- A PDS of the method analytes prepared in water is used to fortify LFBs or LFSMs prior to extraction. When an $MeCl_2$ PDS (as prepared in Sect. 7.3.2) was used to fortify the samples, decreased recoveries for NDMA were observed. This problem was corrected with the use of a water PDS. To make this PDS, a 5-µL volume of a 2000-µg/mL methylene chloride stock standard (Sect. 7.3.1) was added to a 5-mL volume of reagent water for a 2.0-µg/mL water PDS. The water PDS has been shown to be stable for at least 45 days when tightly capped and stored at 4 °C, and protected from light. A lower concentration water PDS may be useful for fortifying water samples at low concentrations. For this method, a 100-ng/mL water PDS was also made by adding 5 µL of a methylene chloride stock standard into 100 mL of reagent water. All of the data demonstrated in this method was obtained using the solution prepared as described above. However, the analyst is permitted to modify the solvent that this solution is prepared in, as long as it is a water miscible solvent, and the QC criteria in Section 9 are met.

7.3.4 SURROGATE (SUR) STOCK STANDARD SOLUTION (N-Nitrosodimethylamine- d_6) -- It is recommended that this compound be obtained as a certified stock standard solution rather than neat material. During the development of this method, a commercially obtained 1000 µg/mL stock standard solution was used (Cambridge Isotopes).

7.3.5 SURROGATE PRIMARY DILUTION STANDARD SOLUTION IN METHYLENE CHLORIDE (SUR $MeCl_2$ PDS)-- The SUR $MeCl_2$ PDS is a dilution of the SUR stock standard (Sect. 7.3.4) prepared in methylene chloride and used for calibration standard preparation. Add enough of the SUR stock standard to a volumetric flask partially filled with methylene chloride to make a final concentration of 2.0 µg/mL when filled to the mark with methylene chloride.

7.3.6 SURROGATE PRIMARY DILUTION STANDARD SOLUTION IN WATER (SUR Water PDS) -- A PDS of the surrogate analyte prepared in water is used to fortify all water samples prior to extraction. See Section 7.3.3 for the reason a water PDS is used. During the development of this method, a SUR water PDS was made by adding 10 µL of a 1000 µg/mL methylene chloride stock standard (Sect. 7.3.4) to a 5-mL volume of reagent water for a 2.0 µg/mL SUR water PDS. This PDS has been shown to be stable for at least 45 days when tightly capped and stored at 4 °C, and protected from light. All of the data demonstrated in this method was obtained using the solution prepared as described above. However, the analyst is permitted to modify the solvent that this solution is prepared in, as long as it is a water miscible solvent, and the QC criteria in Section 9 are met.

7.3.7 INTERNAL STANDARD (IS) STOCK STANDARD SOLUTIONS

7.3.7.1 N-nitrosodi-n-propylamine-d_{14} (NDPA-d14) -- It is recommended that this compound be obtained as a certified stock standard solution rather than neat material. During the development of this method, a commercially obtained 1000 µg/mL stock standard solution was used (Cambridge Isotopes).

7.3.7.2 N-nitrosodiethylamine-d_{10} (NDEA-d10) (optional) -- This is an optional second internal standard that may be used to quantify early eluting peaks, since it elutes early in the chromatogram. At the time of method development this chemical was available only in neat form from CDN Isotopes. Prepare in methylene chloride at a suggested concentration of 1000 µg/mL. For long term storage of stock solutions, prepare in deuterated methylene chloride.

7.3.8 INTERNAL STANDARD PRIMARY DILUTION STANDARD SOLUTION -- The IS PDS is a dilution of the internal standard stock (Sect. 7.3.7) prepared in methylene chloride and used to fortify all sample extracts and calibration standards. Add enough of the internal standard stock solutions to a volumetric flask partially filled with methylene chloride to make a final concentration of 1.0 µg/mL when filled to the mark with methylene chloride.

7.3.9 CALIBRATION STANDARDS (CAL) -- For an initial calibration curve over a concentration range of 2 orders of magnitude, a minimum of at least 5 calibration standards are required. Prepare the calibration standards over the concentration range of interest from dilutions of the methylene chloride

PDSs (Sect. 7.3.2, 7.3.5, and 7.3.8). The lowest concentration of calibration standard must be at or below the MRL (Sect. 9.2.4), which will depend on system sensitivity. The surrogate standard may be added to all the CAL standards at the same concentration, or at the analyst's discretion, it may be varied to create a calibration curve. The calibration standards used for the development of this method were prepared as described in the following table. This table serves only as an example, and adjustments may be made to meet the needs of the laboratory. In this example, the surrogate standard is calibrated at a single concentration.

PREPARATION OF CALIBRATION (CAL) CURVE STANDARDS					
CAL Level	Vol. of 2.0 µg/mL Analyte PDS $MeCl_2$ (µL)	Vol. of 2.0 µg/mL SUR PDS $MeCl_2$ (µL)	Vol. of 1.0 µg/mL IS PDS (µL)	Final Vol. of CAL Std. (mL)	Final Conc. of Analytes (ng/mL)**
1	2.5	50.0	200.0	10.0	0.50
2	5.0	50.0	200.0	10.0	1.0
3	10.0	50.0	200.0	10.0	2.0
4	25.0	50.0	200.0	10.0	5.0
5	25.0	25.0	100.0	5.0	10.0
6	50.0	25.0	100.0	5.0	20.0
7	125.0	25.0	100.0	5.0	50.0

** Concentration of SUR is 10 ng/mL and concentration of IS is 20 ng/mL. Concentration is calculated in final volume.

8. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 SAMPLE COLLECTION -- When sampling from a water tap, open the tap and allow the system to flush until the water temperature has stabilized (usually about 2 min). Adjust the flow to about 500 mL/min and collect samples from the flowing stream. The sample should nearly fill the bottle, but does not need to be headspace free. If the dechlorination agent has been added to the bottle prior to sampling (Sect. 8.2), be careful not to rinse it out during sample collection. 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, must be free of plastic or rubber tubing, gaskets, and other parts that may leach interfering analytes into the water sample.

8.2 SAMPLE DECHLORINATION -- All samples must be dechlorinated at the time of collection. Add 80-100 mg sodium thiosulfate for each liter of water. The sodium thiosulfate may be added to the empty sampling bottles before they are transported to the sampling location. The material must be added as a solid, because aqueous solutions of sodium thiosulfate added to the bottles in advance of sampling are not stable and can be ineffective at dechlorinating the sample.

8.3 SAMPLE TRANSPORT AND STORAGE -- All samples should be iced during shipment and must not exceed 10 °C during the first 48 hours. Samples must be confirmed to be at or below 10 °C when they are received at the laboratory. Samples stored in the lab must be held at or below 6 °C until extraction, but should not be frozen. Chemical freeze packs may be used as a substitute for ice, provided that the required temperatures can be met.
NOTE: Samples that are significantly above 10 °C at the time of collection, may need to be iced or refrigerated for a period of time, in order to chill them prior to shipping. This will allow them to be shipped with sufficient ice to meet the above requirements.

8.4 AQUEOUS SAMPLE AND EXTRACT HOLDING TIMES -- Samples must be extracted within 14 days of collection. Sample extracts may be stored for up to 28 days after sample extraction, when stored in amber vials at -15 °C or less, and protected from light.

9. QUALITY CONTROL

9.1 QC requirements include the Initial Demonstration of Capability and ongoing QC requirements that must be met when preparing and analyzing Field Samples. This section describes each QC parameter, its required frequency, and the performance criteria that must be met in order to meet EPA quality objectives. The QC criteria discussed in the following sections are summarized at the conclusion of the method, in Section 17, Tables 8 and 9. The QC requirements described here are considered the minimum acceptable QC criteria. Laboratories are encouraged to institute additional QC practices to meet their specific needs.

9.1.1 METHOD MODIFICATIONS -- The analyst is permitted to modify GC columns, GC conditions, LVI techniques and CI and MS/MS parameters. Each time such method modifications are made, the analyst must repeat the procedures in Section 9.2.

9.2 INITIAL DEMONSTRATION OF CAPABILITY (IDC) -- Requirements for the initial demonstration of laboratory capability are described in the following sections and summarized in Table 8.

9.2.1 INITIAL DEMONSTRATION OF LOW SPE EXTRACTION BACKGROUND AND SYSTEM BACKGROUND -- Before any samples are analyzed, or any time a new lot or brand of solid phase extraction materials is received from a supplier, it must be demonstrated that a laboratory reagent blank (LRB) is reasonably free of any contamination that would prevent the determination of any analyte of concern (Sects. 9.2.1.2 and 9.4).

9.2.1.1 A source of potential contamination is the solid phase extraction materials which may contain phthalate esters, silicon compounds, and other contaminants that could interfere with the determination of method analytes. Although extraction media are generally made of inert materials, they may still contain extractable organic material. If the background contamination is sufficient to prevent accurate and precise measurements, the condition must be corrected before proceeding with the initial demonstration.

9.2.1.2 Other sources of background contamination are solvents, reagents (including reagent water), and glassware. Reagent water has been shown to be a potential cause of contamination in the analysis of nitrosamines.[3] Background contamination must be reduced to an acceptable level before proceeding with the IDP (Sect. 9.2.2). Background from method analytes and interferences must be $\leq$ 1/3 the MRL. If background contamination is an on-going or intermittent issue for any method analyte, the analyst must not attempt to verify an MRL less than the mean LRB concentration + 3σ, or three times the mean LRB concentration, whichever is greater.
NOTE: Although quantitative data below the MRL may not be reliably accurate enough for data reporting, such data are useful in determining an MRL cut off for analytes that are typically detected in LRBs. Therefore, blank contamination levels may be estimated by extrapolation, when the concentration is below the lowest calibration standard.

9.2.2 INITIAL DEMONSTRATION OF PRECISION (IDP) -- Establish an Initial Calibration following the procedure outlined in Section 10.2. Prepare 4-7 replicate LFBs fortified at 10-20 ng/L, or other mid-range concentration, using solutions described in Sections 7.3.3 and 7.3.6. Sodium thiosulfate must be added to these samples as described in Section 8.2. Extract and analyze these replicates according to the procedure described in Section 11.

The relative standard deviation (RSD) of the results of the replicate analyses must be less than or equal to 20% for all method analytes and surrogates.

9.2.3 INITIAL DEMONSTRATION OF ACCURACY (IDA) -- Using the same set of replicate data generated for Section 9.2.2, calculate average recovery. The average recovery of the replicate values for all analytes and surrogates must be within 70-130% of the true value.

9.2.4 MINIMUM REPORTING LEVEL (MRL) CONFIRMATION -- Select a target concentration for the MRL based on the intended use of the method. The lowest calibration standard used to establish the Initial Calibration (as well as the low-level Continuing Calibration Check standard) must be at or below the concentration of the MRL. Establishing the MRL concentration too low may cause repeated failure of ongoing QC requirements. Confirm the MRL following the procedure outlined below. [1]

9.2.4.1 Fortify, extract, and analyze 7 replicate Laboratory Fortified Blanks at the proposed MRL concentration. These LFBs must contain sodium thiosulfate as described in Section 8.2. Calculate the mean (*Mean*) and standard deviation for these replicates. Determine the Half Range for the prediction interval of results (HR_{PIR}) 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 $\leq$ 150 percent recovery.

$$\frac{Mean + HR_{PIR}}{Fortified\ Concentration} x100 \leq 150\%$$

The Lower PIR Limit must be $\geq$ 50 percent recovery.

$$\frac{Mean - HR_{PIR}}{Fortified\ Concentration} x100 \geq 50\%$$

9.2.4.3 The MRL is validated if both the Upper and Lower PIR Limits meet the criteria described above. If these criteria are not met, the MRL has been set too low and must be determined again at a higher concentration.

9.2.5 DL DETERMINATION *(optional)* -- *While DL determination is not a specific requirement of this method, it may be required by various regulatory bodies associated with compliance monitoring. It is the responsibility of the laboratory to determine if DL determination is required based upon the intended use of the data.*

Replicate analyses for this procedure should be done over at least 3 days (both the sample extraction and the GC/MS/MS analyses should be done over at least 3 days). Prepare at least 7 replicate LFBs using solutions described in Section 7.3.3 and 7.3.6, at a concentration estimated to be near the DL. This concentration may be estimated by selecting a concentration at 2-5 times the noise level. The DLs in Table 1 were calculated from LFBs fortified at 1.0 ng/L; however, the appropriate concentration will be dependent upon the injection technique and the sensitivity of the GC/MS/MS system used. Sodium thiosulfate must be added to these samples as described in Section 8.2. Analyze the seven replicates through all steps of Section 11.
NOTE: If an MRL confirmation data set meets these requirements, a DL may be calculated from the MRL confirmation data, and no additional analyses are necessary.

Calculate the DL using the following equation:

$$DL = St_{(n - 1,\ 1 - alpha\ =\ 0.99)}$$

where:

$t_{(n - 1,1 - alpha = 0.99)}$ = Student's t value for the 99% confidence level with n-1 degrees of freedom
n = number of replicates
S = standard deviation of replicate analyses.

NOTE**:** Do not subtract blank values when performing DL calculations.

9.3 INITIAL QCS ANALYSIS – As part of, or immediately following the IDC, analyze a QCS as described in Section 9.11.

9.4 LABORATORY REAGENT BLANKS (LRB) -- With each extraction batch, analyze a laboratory reagent blank to determine the background system contamination. If, within the retention time window of any analyte, the LRB produces a peak that would prevent the determination of that 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 analytes or contaminants that interfere with the measurement of method analyses must be $\leq$ 1/3 the MRL. Any time a new batch of SPE materials is received, or new supplies of other reagents are used, repeat the demonstration of low background described in Section 9.2.1.

9.5 CONTINUING CALIBRATION CHECK (CCC) -- This calibration check is required at the beginning of each day that samples are analyzed, after every ten field samples, and at the end of any group of sample analyses. See Section 10.3 for concentration requirements and acceptance criteria.

9.6 LABORATORY FORTIFIED BLANK (LFB) -- With each extraction batch, extract and analyze an LFB containing each analyte of concern, using solutions described in Sections 7.3.3 and 7.3.6, and the dechlorination agent described in Section 8.2. If more than 20 field samples are included in a batch, analyze an LFB for every 20 samples. The fortified concentration of the LFB must be rotated between low, medium, and high concentrations from day to day. The low concentration LFB must be as near as practical to the MRL. Results of LFB analyses corresponding to the low fortification concentration for an analyte must be within 50-150% of the true value. Results of LFB analysis from medium and high level concentrations must be 70-130% of the true value for all analytes.

9.7 INTERNAL STANDARDS (IS) --The analyst must monitor the peak area of the IS in all injections during each analysis day. 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 average 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.

9.7.1 If the reinjected aliquot produces an acceptable internal standard response, report results for that aliquot.

9.7.2 If the reinjected extract fails again, the analyst should check the calibration by reanalyzing the most recently acceptable calibration standard. If the calibration standard fails the criteria of Section 10.3.3, recalibration is in order per Section 10.2.3. and 10.2.4. If the calibration standard is acceptable, extraction of the sample may need to be repeated provided the sample is still within the holding time. Otherwise, report results obtained

from the reinjected extract, but annotate as suspect. Alternatively, collect a new sample and re-analyze.

9.8 SURROGATE RECOVERY -- The surrogate standard is fortified into all calibration standards, samples, LFBs, LFSMs, LFSMDs, FDs and LRBs. The surrogate is a means of assessing method performance from extraction to final chromatographic measurement.

9.8.1 Surrogate recovery criteria are 70-130% of the fortified amount for the method surrogate. When surrogate recovery from a sample, blank, or CCC does not meet these criteria, check: (1) calculations to locate possible errors, (2) standard solutions for degradation, (3) contamination, and (4) instrument performance. Correct any problems that are identified. If these steps do not reveal the cause of the problem, reanalyze the extract.

9.8.2 If the extract reanalysis meets the surrogate recovery criterion, report only data for the reanalyzed extract.

9.8.3 If the extract reanalysis fails the recovery criterion, the analyst should check the calibration by reanalyzing the most recently acceptable calibration standard. If the calibration standard fails the criteria of Section 10.3.3, recalibration is in order per Section 10.2. If the calibration standard is acceptable, it may be necessary to extract another aliquot of sample if sample holding time has not been exceeded. If the sample re-extract also fails the recovery criterion, report all data for that sample as suspect, or obtain and analyze another sample.

9.9 LABORATORY FORTIFIED SAMPLE MATRIX (LFSM) -- Determine that the sample matrix does not contain materials that adversely affect method performance. This is accomplished by analyzing replicates of laboratory fortified matrix samples and ascertaining that the precision, accuracy, and detection limits of analytes are in the same range as obtained with laboratory fortified blanks. If a variety of different sample matrices are analyzed regularly, for example, drinking water from groundwater and surface water sources, LFSM data should be collected for each matrix. Over time, LFSM data should be documented for all routine sample sources for the laboratory.

9.9.1 Within each extraction batch, a minimum of one field sample is fortified as an LFSM for every 20 samples analyzed. The LFSM is prepared by spiking a sample with an appropriate amount of the fortification solutions described in Section 7.3.3 and 7.3.6. Select the spiking concentration that is greater than or equal to the matrix background concentration. Selecting a duplicate bottle of a sample that has already been analyzed, aids in the selection of

appropriate spiking levels. If this is not possible, use historical data or rotate through low, medium and high calibration concentrations to select a fortifying concentration.

9.9.2 Calculate the percent recovery (R) for each analyte, after correcting the measured fortified sample concentration, A, for the background concentration, B, measured in the unfortified sample, i.e.,

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

where C is the fortified concentration. Compare these values to control limits for LFBs (Sect. 9.6).

9.9.3 Recoveries may exhibit a matrix dependence. For samples fortified at or above their native concentration, recoveries should range between 70 and 130% for all method analytes. If the accuracy (percent recovery) of any analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control, the accuracy problem encountered with the fortified sample is judged to be matrix related, not system related. 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.

9.10 FIELD DUPLICATES (FD) -- Within each extraction batch, a minimum of one field sample must be analyzed in duplicate. If more than 20 samples are extracted in a batch, analyze a FD for each 20 samples. Duplicate sample analyses serve as a check on sampling and laboratory precision. If analytes are not routinely observed in field samples, LFSMDs must be analyzed to substitute for this requirement. Refer to Section 9.9.1 for guidance on spiking concentrations.

9.10.1 Calculate the relative percent difference (RPD) for duplicate measurements (FD1 and FD2) as shown below.

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

9.10.2 RPDs for FDs and LFSMDs should be ≤ 30%. Greater variability may be observed for target analytes whose concentrations are within a factor of 2 of the MRL. In this case, the RPDs should be ≤ 50%.

9.11 QUALITY CONTROL SAMPLE (QCS) -- Each time that new PDSs are prepared, analyze a QCS from an external source. If standards are prepared infrequently, analyze a QCS at least quarterly. The QCS may be injected as a calibration standard, or fortified into reagent water and analyzed as an LFB. If the QCS is analyzed as a calibration check standard, then the acceptance criteria are the same as for the CCC (Sect. 10.3.3). If the QCS is analyzed as an LFB, then the acceptance criteria are the same as for an LFB (Sect. 9.6). If measured analyte concentrations are not of acceptable accuracy, check the entire analytical procedure to locate and correct the problem.

10. CALIBRATION AND STANDARDIZATION

10.1 Demonstration and documentation of acceptable initial calibration is required before any samples are analyzed. After initial calibration is successful, a continuing calibration check is required at the beginning and end of each period in which analyses are performed, and after every tenth sample.

10.2 INITIAL CALIBRATION

10.2.1 INSTRUMENT SET-UP FOR LIQUID CI GC/MS/MS – The following description is a suggested sequence of steps for instrument set-up. Because of differences in instrumentation, it may be desirable or necessary to alter these steps. However, it is highly recommended that each part of the instrumental analysis be added in a stepwise fashion, i.e., first verify that the instrument works in the CI mode, then optimize the LVI, and then proceed to MS/MS analysis. It is likely that the method will be slightly less sensitive in the MS/MS mode than in standard MS. However, the use of MS/MS is required to provide sufficient specificity and minimize false positive results.

10.2.1.1 Mass calibrate the MS in EI mode using FC-43 or the tuning compound recommended by the MS manufacturer. A manifold temperature of 150 °C (Varian Saturn 4) is recommended. On the Varian Saturn 2000, the recommended manifold temperature is 50 °C, and the recommended trap temperature is 150 °C. On other instruments, the manufacturer's recommendation for instrument temperatures should be followed.

10.2.1.2 Introduce CI reagent (methanol or acetonitrile) into the MS, and adjust the level of reagent as directed by the MS manufacturer.

10.2.1.3 Set the instrument acquisition parameters as necessary to collect data in the CI mode. Example parameters are included in Tables 2a and 2b. It is recommended that during initial instrument set-up, full scan CI data be acquired. The acquisition mass range should be 40-160 daltons with a total cycle time (including scan overhead time) of 0.5 sec or less. Cycle time must be adjusted to measure at least five or more scans during the elution of each GC peak. Seven to ten scans across each GC peak are recommended.
NOTE: On ion trap mass spectrometers, the scan time and the chromatographic peak width may need to be carefully evaluated to make sure that this requirement is met, because of the relatively slow scan speed in MS/MS mode.

10.2.1.4 Analyze a high concentration CAL standard (50-100 pg/µL) by injecting 1-2 µL into the GC in the splitless mode. The injection volume selected will depend upon the injector design. Suggested GC parameters are: injector temperature 250 °C, split activation time 1 min. A suggested GC oven temperature program is presented in Table 3.

10.2.1.5 Identify each analyte by its potonated molecular ion (precursor ion). Example retention times and precursor ions are presented in Table 4.

10.2.1.6 Optimize the LVI parameters according to the manufacturer's instructions. Because of the variety of instrumentation available for LVI, specific instructions are not discussed here. Some general literature references on LVI using a programmed-temperature vaporizing injector (PTV) have been cited.[10-12] Example parameters for LVI using a 20-µL injection and a PTV injector are given in Table 3. After LVI optimization, compare the peak areas from the conventional injection in Sect 10.2.1.4 to the LVI peak areas. For example, the response from a 1-µL injection of a 50-pg/µL standard should not vary dramatically from a 10-µL injection of a 5-pg/µL standard.

10.2.1.7 Create an MS/MS method by selecting a time window around each analyte, or in the case of closely eluting analytes, around each pair of analytes. For analyses using ion trap mass

spectrometers, it is recommended that no more than two compounds of interest be contained in an MS/MS time window. Due to multiple scan events in each time window, it may not be possible to achieve a sufficient number of scans during the elution of each chromatographic peak. A minimum of 5 scans must be obtained during the elution of each chromatographic peak; however, 7-10 are recommended.

10.2.1.8 Set parameters for the formation of product ions from the precursor ion. An example of these parameters and the values used to obtain the demonstration data are found in Tables 5a and 5b. The parameters listed in the tables were selected to produce product ion spectra with approximately 5% of the precursor ion remaining. Optimal parameters may be expected to vary from instrument to instrument.

10.2.1.9 Analyze a standard at a concentration estimated to be in the middle of the final calibration range. Create a database that includes the retention times, product ion spectra and quantitation ion response for each analyte, surrogate and internal standard. Suggested quantitation ions are listed in Table 4. Do not select the precursor ion (molecular ion) as the quantitation ion.

CAUTION: When acquiring MS/MS data, GC operating conditions must be carefully reproduced for each analysis to provide reproducible retention times. If this is not done, the correct ions will not be monitored at the appropriate times. As a precautionary measure, the chromatographic peaks in each window must not elute too close to the edge of the time window. As a minimum, there should be at least 5 sec between the edge of the time window and the beginning of an analyte peak.

10.2.2 CALIBRATION SOLUTIONS -- Prepare a set of calibration solutions as described in Section 7.3.9. When selecting calibration standard concentrations, the analyst should be aware that the MRL cannot be less than the lowest CAL. Use a minimum of 3 standards for a calibration range of 1 order of magnitude, and at least 5 standards for 2 orders of magnitude. During method development, typical calibration curves were generated from CAL standards of either 0.5 to 20 ng/mL (6 points) or 0.5 to 50 ng/mL (7 points). Data outside of the established calibration range should never be reported (Sect. 12.1).

10.2.3 CALIBRATION -- Concentrations may be calculated through the use of average response factor (RF) or through the use of a calibration curve. Average RF calibrations may only be used if the RF values over the calibration range are relatively constant. The RFs must be constant enough to meet the calibration acceptance criteria in Section 10.2.4.

Average RF is determined by calculating the mean RF of each calibration point.

$$RF = \frac{(A_x)(Q_{is})}{(A_{is})(Q_x)}$$

where:

A_x = integrated abundance (peak area) of the quantitation ion of the analyte.

A_{is} = integrated abundance (peak area) of the quantitation ion internal standard.

Q_x = quantity of analyte injected in ng or concentration units.

Q_{is} = quantity of internal standard injected in ng or concentration units.

As an alternative to calculating average RFs, use the GC/MS data system software to generate a linear regression or quadratic calibration curve. The analyst may choose whether or not to force zero, or whether weighting should be employed to obtain a curve that best fits the data. In general, forcing zero is not recommended. However, in situations where an analyte is typically detected in LRBs, forcing zero is recommended. Examples of common GC/MS system calibration curve options are: 1) A_x /A_{is} vs Q_x /Q_{is} and 2) RF vs A_x /A_{is}.

10.2.4 CALIBRATION ACCEPTANCE CRITERIA -- Acceptance criteria for the calibration of each analyte is determined by calculating the concentration of each analyte and surrogate in each of the analyses used to generate the calibration curve or average RF. Each calibration standard, except the lowest point, for each analyte should calculate to be 70-130% of its true value. The lowest point should calculate to be 50-150% of its true value. If these criteria cannot be met, reanalyze the calibration standards, or select an alternate method of calibration. The data in this method were generated using both quadratic fits and average RF, depending upon the analyte. Quadratic fit calibrations should be used with caution, because the non-linear area of the curve may not be reproducible.

10.3 CONTINUING CALIBRATION CHECK (CCC) -- The minimum daily calibration verification is as follows. Verify the initial calibration at the beginning and end of

each group of analyses, and after every tenth sample during analyses. (In this context, a "sample" is considered to be a field sample. LRBs, LFSMs, LFSMDs, LFBs and CCCs are not counted as samples.) The beginning CCC each day must be at or near the MRL in order to verify instrument sensitivity prior to any analyses. If standards have been prepared such that all low CAL points are not in the same CAL solution, it may be necessary to analyze two CAL solutions to meet this requirement. Alternatively, it may be cost effective to prepare or obtain a customized standard to meet this criteria. Subsequent CCCs must alternate between a medium and high concentration standard.

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 Determine that the absolute areas of the quantitation ions of the internal standards have not changed by more than 30% from the areas measured in the most recent continuing calibration check, and by more than 50% from the average areas measured during initial calibration. If either of the IS areas has changed by more than these amounts, adjustments must be made to restore system sensitivity. These adjustments may include cleaning of the MS ion source, or other maintenance as indicated in Section 10.3.4. Major instrument maintenance requires recalibration. Control charts are useful aids in documenting system sensitivity changes.

10.3.3 Calculate the concentration of each analyte and surrogate in the check standard. The calculated amount for each analyte for medium and high level CCCs must be within 70-130% of the true value. The calculated amount for the lowest calibration point for each analyte must be within 50-150% of the true value. If these conditions do not exist, remedial action should be taken which may require recalibration. Any field sample extracts that have been analyzed since the last acceptable calibration verification should be reanalyzed after adequate calibration has been restored, with the following exception. **If the continuing calibration check in the middle or at the end of an analysis batch fails because the calculated concentration is >130% of the true value, and field sample extracts showed no detection of method analytes, non-detects may be reported without re-analysis.**

10.3.4 Some possible remedial actions are: maintenance and/or recalibration of the MS, GC column and injection port maintenance, and preparation of new CAL standards. This list is not meant to be all inclusive. Major maintenance such as cleaning the MS, replacing filament assemblies, replacing the electron multiplier, changing the GC column, etc. require returning to the initial calibration step (Sect. 10.2). Changes in the GC injection or column may affect retention times. After maintenance, verify all retention times before recalibrating.

11. PROCEDURE

11.1 This procedure may be performed manually or in an automated mode (Sect. 6.9.6) using a robotic or automatic sample preparation device. If an automatic system is used to prepare samples, follow the manufacturer's operating instructions, but all extraction and elution steps must be the same as in the manual procedure. Extraction and/or elution steps may not be changed or omitted to accommodate the use of an automated system.

11.2 Important aspects of this analytical procedure include proper preparation of laboratory glassware and sample containers (Sect. 6.1 and 6.2), as well as sample collection and storage (Sect. 8). This section details the procedures for sample preparation, solid phase extraction (SPE) using cartridges, and extract analysis.

11.3 SAMPLE PREPARATION --

11.3.1 Mark the level of the sample on the bottle prior to extraction for later sample volume determination. The LRB and LFB may be prepared by filling a 0.5-L sample bottle with reagent water.

11.3.2 All field and QC samples must contain the dechlorinating agent, sodium thiosulfate, including the LRB and LFB. Verify that field samples were dechlorinated at the time of collection. Add 40-50 mg of sodium thiosulfate to the LRB and LFB at this time and swirl until dissolved.

11.3.3 If 1-qt or 1-L sample bottles were used, transfer 500 mL of the sample to a clean container.

11.3.4 ADDITION OF SURROGATE ANALYTE -- Add an aliquot of the water SUR Water PDS (Sect. 7.3.6) to all samples and mix by swirling the sample. Addition of 5.0 µL of a 2.0-µg/mL water SUR Water PDS to a 500-mL sample will result in a concentration of 20 ng/L.

11.3.5 FORTIFICATION WITH METHOD ANALYES -- If the sample is an LFB, LFSM, or LFSMD, add the necessary amount of analyte Water PDS (Sect.7.3.3). Swirl each sample to ensure all components are properly mixed.

11.3.6 Proceed with sample extraction.

11.4 SPE PROCEDURE -- Proper conditioning of the solid phase sorbent can have a marked effect on method precision and accuracy. This section describes the SPE procedure using a transfer tube system and SPE manifold as described in Section 6.9.

11.4.1 CARTRIDGE CONDITIONING --

11.4.1.1 Fill the cartridge with approximately 3 mL methylene chloride, turn on the vacuum, and pull the solvent through, aspirating completely. Repeat once.

11.4.1.2 Fill the cartridge with approximately 3 mL methanol, turn on the vacuum, and pull the solvent through, aspirating completely. Repeat once.

11.4.1.3 Fill the cartridge with approximately 3 mL methanol and elute with vacuum to just above the top frit - not allowing the cartridge to go dry at the end. From this point forward, do not allow the cartridge to go dry. Repeat once.

11.4.1.4 Fill the cartridge with approximately 3 mL reagent water, turn on the vacuum, and pull the water through, repeat 5 times, without allowing the cartridge to go dry in between washes or at the end.

11.4.2 SAMPLE EXTRACTION -- Attach a transfer tube from each sample bottle to each cartridge and then turn on the vacuum. Adjust the vacuum so that the approximate flow rate is 10 mL/min. After all of the sample has passed through each SPE cartridge, detach the reservoir and draw air through the cartridge for 10 minutes at full vacuum. Turn off and release the vacuum. Proceed immediately with cartridge elution.

11.4.3 CARTRIDGE ELUTION -- Lift the extraction manifold top and insert a rack with collection tubes into the extraction tank to collect the extracts as they are eluted from the cartridges. Fill each cartridge with methylene chloride. Pull enough of the solvent into the cartridge at low vacuum to soak the sorbent. Turn off the vacuum and vent the system. Allow the sorbent to soak in methylene chloride for approximately 1 minute. Apply a low vacuum and pull the methylene chloride through the cartridge in a dropwise fashion into the collection tube. Continue to add methylene chloride to the cartridge as it is being drawn through until the volume of extract is about 12 or 13 mL, determined by the markings on the side of the collection tube.

11.4.4 Small amounts of residual water from the sample container and the SPE cartridge may form an immiscible layer with the extract. To eliminate residual water, pass the extract through the drying column (Sect. 6.7). The drying column is packed with approximately 5 to 7 grams of anhydrous sodium sulfate, and is pre-wetted with a small volume of methylene

chloride prior to passing the extract through it. Collect the dried extract in a clean centrifuge tube (Sect. 6.8). After passing the extract through the drying tube, wash the sodium sulfate with at least 3 mL methylene chloride and collect the solvent wash in the same collection tube. Concentrate the extract to approximately 0.9 mL in a water bath near room temperature (20 to 25 °C) under a gentle stream of nitrogen. It is strongly recommended that you do not concentrate the extract to less than 0.5 mL, as this may result in loss of analytes. Add the internal standard (Sect. 7.3.8). Adjust final volume to 1.0 mL with methylene chloride. This may be done by transferring the extract to a 1.0-mL volumetric and filling to the volume mark.
NOTE: Other types of evaporation/concentration equipment may be used, as long as all QC requirements in Section 9 are met.

11.5 Fill the sample bottle to the volume mark noted in Section11.3.1 with tap water. Transfer the tap water to a 1000 mL graduated cylinder, and measure the sample volume to the nearest 10 mL. Record this volume for later analyte concentration calculations. As an alternative to this process, the sample volume may be determined by the difference in weight between the full bottle (before extraction) and the empty bottle (after extraction). Assume a sample density of 1.0 g/mL.

11.6 Analyze an aliquot of the sample extract with the GC/MS/MS system under the same conditions used for the initial and continuing calibrations.

11.7 At the conclusion of data acquisition, use the same software that was used in the calibration procedure to identify peaks in predetermined retention time windows of interest. Use the data system software to examine the ion abundances of components of the chromatogram.

11.8 IDENTIFICATION OF ANALYTES IN TANDEM MASS SPECTROMETRY -- Identify a sample component by comparison of its product ion spectrum to a reference spectrum in the user-created data base. The GC retention time of a method analyte should be within 1-2 sec of the retention time observed for that same compound in the most recently analyzed CCC standard. Very early eluting compounds may exhibit more variability in retention time. Ideally, the width of the retention time window should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for an analyte. However, the experience of the analyst should weigh heavily in the interpretation of the chromatogram.

11.8.1 In general, all ions that are present above 30% relative abundance in the product ion mass spectrum of the standard should be present in the mass spectrum of the sample component and should agree within an

absolute 20%. For example, if an ion has a relative abundance of 30% in the product spectrum of the standard, its abundance in the sample product spectrum should be in the range of 10 to 50%. If the instrument parameters have been set as described in Section 10.2.1.8, the precursor ion will have a low relative abundance, and is not used as part of the analyte identification criteria.

12. DATA ANALYSIS AND CALCULATIONS

12.1 Complete chromatographic resolution is not necessary for accurate and precise measurements of analyte concentrations using MS/MS. In validating this method, concentrations were calculated by measuring the product ions listed in Table 4. Other ions may be selected at the discretion of the analyst. If the response of any analyte exceeds the calibration range established in Section 10, dilute the extract, add additional internal standard, and reanalyze. The resulting data should be documented as a dilution, with an increased MRL.

12.1.1 Calculate analyte and surrogate concentrations, using the multipoint calibration established in Section 10. Do not use daily calibration verification data to quantitate analytes in samples. Adjust final analyte concentrations to reflect the actual sample volume determined in Section 11.5.

12.1.2 Calculations must utilize all available digits of precision, but final reported concentrations should be rounded to an appropriate number of significant figures (one digit of uncertainty), typically two, and not more than three significant figures.
NOTE: Some data in Section 17 of this method are reported with more than 2 significant figures. This is done to better illustrate the method performance data.

13. METHOD PERFORMANCE

13.1 PRECISION, ACCURACY AND DLs -- Single laboratory DLs and LCMRLs are presented in Table 1. Single laboratory accuracy and precision data from both fortified reagent water and fortified matrices are presented in Tables 6 and 7. These data were obtained on a Varian Saturn 4 GC/MS/MS system, operated in the CI mode, using methanol as the CI reagent. Methanol was selected as the solvent used for the method demonstration data, since it can be more widely used in both new and vintage instrumentation. Acetonitrile can be used only in newer instruments. Consult the instrument manufacturer for liquid CI reagent choices.

13.2 EVALUATION OF ADDITIONAL NITROSAMINES -- N-Nitrosomorpholine was evaluated for inclusion into this method. It was not included in this method because of unresolved problems with background contamination.

13.3 ANALYTE STABILITY STUDIES

13.3.1 AQUEOUS SAMPLES -- Chlorinated drinking water samples, from a surface water source, were fortified with method analytes, and preserved and stored as required in Section 8. The average of replicate analyses (N=7) conducted on day 0, and at three additional time points up to and beyond 14 days are presented in Figure 1. These data document the 14 day holding time.

13.3.2 EXTRACTS -- Extracts from the first day of the holding time study described above were stored protected from light, at -15 °C, and analyzed in replicate (N=7) on day 0, and at six additional time points up to and beyond 28 days. The results of these analyses that document the 28 day holding time are presented in Figure 2.

13.4 SECOND LABORATORY DEMONSTRATION -- The performance of this method was demonstrated by a second laboratory, with results similar to those reported in Section 17. The authors wish to acknowledge the work of Mr. Ed Price and Mr. Mark Domino of Shaw Environmental Incorporated for their participation in the second laboratory demonstration.

14. POLLUTION PREVENTION

14.1 This method utilizes SPE technology to remove the analytes from water. It requires the use of very small volumes of organic solvent and very small quantities of pure analytes, thereby minimizing the potential hazards to both the analyst and the environment when compared with 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: Guide to Minimizing Waste in Laboratories" available from the American Chemical Society, on-line at http://membership.acs.org/c/ccs/pub_9.htm.

15. WASTE MANAGEMENT

15.1 The analytical procedures described in this method generate relatively small amounts of waste since only small amounts of reagents and solvents are used. The only matrix of concern is finished drinking water. However, the Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations, and that laboratories protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Also, compliance is required with any sewage discharge permits and regulations, particularly the hazardous waste identification rules and land disposal restrictions.

16. REFERENCES

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17. TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

Table 1. Detection Limits and Lowest Concentration Minimum Reporting Levels [a, b, c]

Analyte	DL (ng/L)	LCMRL (ng/L)
NDMA	0.28	1.6
NMEA	0.28	1.5
NDEA	0.26	2.1
NPYR	0.35	1.4
NDPA	0.32	1.2
NPIP	0.66	1.4
NDBA	0.36	1.4

a. DLs determination from LFBs fortified at 1.0 ng/L (N = 8).
b. LCMRLs determined from LFBs fortified at 1.0, 2.0, 3.0, and 4.0 ng/L (N = 5 or 6 at each concentration).
c. DL and LCMRL data were obtained on a Varian Saturn 4 GC/MS/MS.

Table 2a. Chemical Ionization Parameters for a Varian Saturn 4 GC/MS/MS Using Methanol as the CI Reagent

Automatic Reaction Control (ARC) ionization time (μsec)	100
CI maximum ionization time (μsec)	2000
CI maximum reaction time (μsec)	100
CI ion storage level (*m/z*)	20
Reagent ion eject amplitude (V)	10
CI reaction storage level (*m/z*)	15
CI background mass (*m/z*)	45
Emission current (μamps)	50
Electron Multiplier offset (V)	+100

Table 2b. Chemical Ionization Parameters for a Varian Saturn 2000 GC/MS/MS Using Methanol or Acetonitrile as the CI Reagent

CI storage level (*m/z*)	15-19
Ejection amplitude (*m/z*)	10-15
Background mass (*m/z*)	40
Maximum ionization time (μsec)	2000
Maximum reaction time (μsec)	100-120
Target total ion current (TIC) (counts)	5000
Prescan Ionization time (μsec)	100-200
Emission current (μamps)	50
Electron Multiplier offset (V)	+300

Table 3. Chromatographic Conditions including LVI Using a PTV Injector

Injector Temperature program

Varian Saturn 4			Varian Saturn 2000		
Temp (°C)	Rate (°C/min)	Time (min)	Temp (°C)	Rate (°C/min)	Time (min)
37	0	0.72	37	0	0.6
250	100	2.13	250	100	2.13
250	0	40	250	0	23.00

Injector Split Vent Program

Varian Saturn 4		Varian Saturn 2000	
Time (min)	Split status	Time (min)	Split status
0	open [a]	0	open [b]
0.70	closed	0.6	closed
2.00	open [a]	3.5	open [c]

a. split flow 100 mL/min
b. split ratio 25
c. split ratio 100

GC Oven Temperature Program

Varian Saturn 4			Varian Saturn 2000		
Temp (°C)	Rate (°C/min)	Hold Time (min)	Temp (°C)	Rate (°C/min)	Hold Time (min)
40	0	3.0	35	0	4.0
170	4.0	0	130	4.0	0
250	20.0	3.0	280	40	0.5

Table 4. Retention Times and Suggested Product Ions for Quantitation of Method Analytes in Tandem Mass Spectrometry (MS/MS) Mode [a, b]

Analyte	Retention Time (min)	Precursor Ion (*m/z*)	Product Ion/ Quantitation Ion (*m/z*)
NDMA	8.43	75	43 (56)
NMEA	11.76	89	61 (61)
NDEA	14.80	103	75 (75)
NPYR	22.34	101	55 (55)
NDPA	22.40	131	89 (89)
NPIP	24.25	115	69 (69)
NDBA	30.09	159	57 (103)
NDMA-d6 (surrogate)	8.34	81	46 (59)
NDEA-d10 (internal standard) [c]	14.63	113	81 (81)
NDPA-d14 (internal standard) [c]	22.07	145	97 (97)

a. Retention times obtained on Saturn 4 GC/MS/MS with the GC column and conditions described in Section 6.10, 6.11 and Tables 2 and 3. Absolute retention times may vary slightly with different instruments.
b. Product ions are those obtained on a Varian Saturn 4 or Saturn 2000 GC/MS/MS using methanol as the chemical ionization reagent gas. Ions in "( )" are product ions observed using acetonitrile as the CI reagent on the Saturn 2000. Other product ions may be used as appropriate when different instrumentation is used.
c. One or two internal standards may be used. If all analytes are determined, and only one IS is used, NDPA-d14 is recommended. If two ISs are used, use NDEA- d10 for the surrogate and the first three eluting analytes. Use NDPA-d14 for the rest of the analytes.

Table 5a. MS/MS Parameters for the Varian Saturn 4 Using Methanol as the CI Reagent

Analyte	Segment #	Retention Time Window (min)	Scan range (*m/z*)	Scan rate (sec/scan)	CAD Amplitude (volts)	CAD rf (*m/z*)	CAD Time (msec)	Precursor Ion Isolation Time (msec)
NDMA	1	7.00-10.00	40-83	0.37	0.56	35.0	10	2
NDMA-d6 (surrogate)	1	7.00-10.00	40-83	0.37	0.50	35.0	10	2
NMEA	2	10.00-13.00	40-91	0.57	0.56	35.0	10	2
NDEA	3	13.00-15.50	39-114	0.39	0.46	35.0	10	2
NDEA-d10 (internal standard)	3	13.00-15.50	39-114	0.39	0.36	35.0	10	2
NDPA-d14 (internal standard)	4	15.50-22.17	48-147	0.62	0.46	35.0	10	2
NPYR	5	22.17-23.48	40-133	0.42	0.44	35.0	10	2
NDPA	5	22.17-23.48	40-133	0.42	0.44	35.0	10	2
NPIP	6	23.48-25.74	39-117	0.60	0.44	35.0	10	2
NDBA	7	25.74-32.74	55-161	0.62	0.44	35.0	10	2

Waveform type- Resonant**;** Default values for all other MS/MS parameters**:** Isolation window 3 amu, Low (DAC) offset 6 steps, High (DAC) offset 6 steps**,** Broadband amplitude 10 V, Modulation range 2 steps, Modulation rate 3000 µsec/step, Bandwidth 0 Hertz, Ejection Amplitude 20 V**;** Ionization RF 48 *m/z*

Table 5b. MS/MS Parameters for the Varian Saturn 2000 Using Methanol as the CI Reagent

Analyte	Segment #	Retention Time Window (min)	Scan range (*m/z*)	Scan rate (sec/scan)	Excitation Amplitude (volts)	Excitation Storage Level (*m/z*)	Excitation Time (msec)	Precursor Ion Isolation Time (msec)
NDMA	1	8.00-12.00	40-83	0.61	0.50	35.0	10	2
NDMA-d6 (surrogate)	1	8.00-12.00	40-83	0.61	0.50	35.0	10	2
NMEA	2	12.00-15.00	40-91	0.55	0.55	35.0	10	2
NDEA	3	15.00-18.00	39-114	0.62	0.46	35.0	10	2
NDEA-d10 (internal standard)	3	15.00-18.00	39-114	0.62	0.44	35.0	10	2
NDPA-d14 (internal standard)	4	18.00-23.21	48-147	0.56	0.48	48.0	10	2
NPYR	5	23.21-25.00	40-133	0.56	0.44	35.0	10	2
NDPA	5	23.21-25.00	40-133	0.56	0.44	35.0	10	2
NPIP	6	25.00-29.00	39-117	0.54	0.42	35.0	10	2
NDBA	7	29.00-32.00	55-161	0.57	0.40	35.0	10	2

Waveform type- Resonant**;** Default values for all other MS/MS parameters**:** Isolation window 3 amu, Low edge offset 6 steps, High edge offset 2 steps**,** High edge amplitude 30 V, Modulation range 2 steps, Modulation rate 3000 µsec/step, # of Frequencies 1, CAD frequency offset 0 Hertz**,** Ejection Amplitude 20 V**;** Ionization RF 48 *m/z*

Table 5c. MS/MS Parameters for the Varian Saturn 2000 Using Acetonitrile as the CI Reagent

Analyte	Segment #	Retention Time Window (min)	Scan range (*m/z*)	Scan rate (sec/scan)	Excitation Amplitude (volts)	Excitation Storage Level (*m/z*)	Excitation Time (msec)	Precursor Ion Isolation Time (msec)
NDMA	1	8.00-12.00	40-83	0.61	0.50	35.0	10	2
NDMA-d6 (surrogate)	1	8.00-12.00	40-83	0.61	0.50	35.0	10	2
NMEA	2	12.00-15.00	40-91	0.55	0.55	40.0	10	2
NDEA	3	15.00-18.00	39-114	0.62	0.46	40.0	10	2
NDEA-d10 (internal standard)	3	15.00-18.00	39-114	0.62	0.44	40.0	10	2
NDPA-d14 (internal standard)	4	18.00-23.21	48-147	0.56	0.48	40.0	10	2
NPYR	5	23.21-25.00	40-133	0.56	0.44	40.0	10	2
NDPA	5	23.21-25.00	40-133	0.56	0.44	40.0	10	2
NPIP	6	25.00-29.00	39-117	0.54	0.42	40.0	10	2
NDBA	7	29.00-32.00	55-161	0.57	0.40	40.0	10	2

Waveform type- Resonant**;** Default values for all other MS/MS parameters**:** Isolation window 3 amu, Low edge offset 6 steps, High edge offset 2 steps**,** High edge amplitude 30 V, Modulation range 2 steps, Modulation rate 3000 µsec/step, # of Frequencies 1, CAD frequency offset 0 Hertz**,** Ejection Amplitude 20 V**;** Ionization RF 48 *m/z*

Table 6. Precision and Accuracy Data Obtained from Fortified Reagent Water at Four Concentrations

Analyte	Fortified Concentration 2.0 ng/L (N=7)		Fortified Concentration 4.0 ng/L (N=6)		Fortified Concentration 10.0 ng/L (N=4)		Fortified Concentration 20.0 ng/L (N=6)	
	Accuracy (% rec)	Precision (RSD)	Accuracy (% rec)	Precision (RSD)	Accuracy (% rec)	Precision (RSD)	Accuracy (% rec)	Precision (RSD)
NDMA	94.7	12	92.7	11	88.7	3.8	89.5	7.8
NMEA	81.8	9.6	83.1	6.3	86.5	4.5	90.9	6.9
NDEA	84.6	9.0	92.0	14	87.5	9.1	95.6	11
NPYR	92.6	12	102	4.0	101	5.0	101	6.1
NDPA	81.7	8.0	87.6	7.3	97.0	10	87.1	7.7
NPIP	98.3	20	86.3	6.1	91.8	3.7	93.6	4.0
NDBA	85.2	16	82.2	7.7	86.4	9.4	104	8.7
NDMA-d6 (surrogate)	83.5	7.3	86.0	7.5	86.8	4.0	88.7	8.3

Fortified samples at 2.0 and 10.0 ng/L were analyzed using a single IS (NDPA-d14). Fortified samples at 4.0 and 20.0 ng/L were analyzed using two internal standards (NDEA-d10 and NDPA-14).

Table 7. Precision and Accuracy Data Obtained from Fortified Drinking Water Matrices Fortified at 20 ng/L N=6 for each matrix

Analyte	Surface Water [a]		Ground Water [b]		High TOC Water [c]	
	Accuracy (% recovery)	Precision (RSD)	Accuracy (% recovery)	Precision (RSD)	Accuracy (% recovery)	Precision (RSD)
NDMA	85.0	6.6	90.8	4.4	83.7	6.8
NMEA	81.9	6.2	91.0	7.2	81.4	4.5
NDEA	88.4	6.5	92.8	7.8	86.1	11.3
NPYR	85.2	6.5	92.7	4.4	88.4	6.6
NDPA	77.1	3.7	85.1	6.6	78.3	10.2
NPIP	81.6	6.7	87.3	2.0	80.6	6.0
NDBA	79.7	7.2	97.8	2.9	86.2	7.8
NDMA-d6 (surrogate)	82.9	8.4	91.5	8.2	77.2	5.5

a. Chlorinated drinking water from a river water source.
b. Chlorinated drinking water from a ground water source. Hardness = 400 mg/L as calcium carbonate.
c. Chlorinated drinking water from a high TOC surface water source. TOC = 14 mg/L.

Table 8. Initial Demonstration of Capability (IDC) Requirements (Summary)

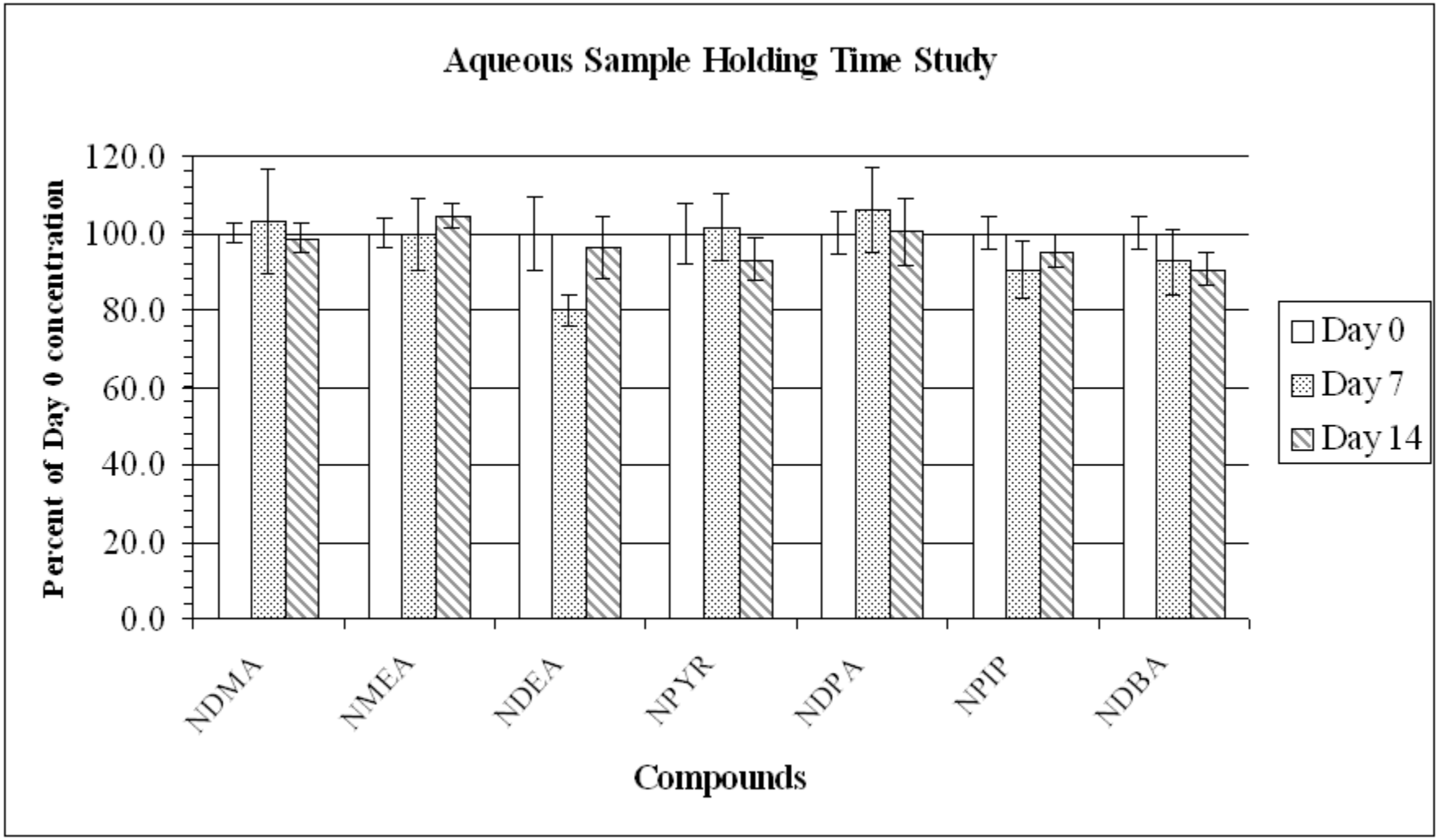
Method Ref.	Requirement	Specification and Frequency	Acceptance Criteria
Sect. 9.2.1	Initial Demonstration of Low Method Background	Analyze LRB prior to any other IDC steps, or anytime a new lot of SPE materials or reagents are used.	Demonstrate that all target analytes are ≤ 1/3 the MRL, and that possible interferences from extraction media do not prevent the identification and quantification of method analytes.
Sect. 9.2.2	Initial Demonstration of Precision (IDP)	Analyze 4-7 replicate LFBs fortified at 10-20 ng/L, or other mid-range concentration.	RSD must be ≤20% for all analytes.
Sect. 9.2.3	Initial Demonstration of Accuracy (IDA)	Calculate average recovery for replicates used in IDP	Mean recovery 70-130% of true value.
Sect. 9.2.4	Minimum Reporting Level (MRL) Confirmation	Fortify, extract and analyze 7 replicate LFBs at the proposed MRL. Use the equation provided to verify the MRL. Repeat after major instrument or operational changes.	See Section 9.2.4 for confirmation criteria.
Sect. 9.2.5	Detection Limit (DL) Determination (optional)	Over a period of three days, prepare a minimum of 7 replicate LFBs fortified at a concentration estimated to be near the DL. Analyze the replicates through all steps of the analysis. Calculate the DL using the equation in Section9.2.5.	**NOTE:** Data from DL replicates are <u>not required</u> to meet method precision and accuracy criteria. If the DL replicates are fortified at a low enough concentration, it is likely that they will not meet precision and accuracy criteria.
Sect. 9.3 and 9.11	Quality Control Sample (QCS)	Analyze a standard from a second source, as part of IDC, or immediately after completion of IDC.	If analyzed as a calibration sample, CCC criteria apply. If analyzed as an LFB, those criteria apply.

Table 9. Quality Control Requirements (Summary)

Method Ref.	Requirement	Specification and Frequency	Acceptance Criteria
Sect. 8.1 - Sect 8.4	Sample Collection, Preservation, and Holding Time	14 days, protected from light, with addition of sodium thiosulfate.	Iced or refrigerated at 10° C or less for up to 48 hours to allow time for shipping; refrigerated at 6° C or less, after arrival at the laboratory.
Sect. 8.4	Extract Holding Time	28 days	Stored at -15° C or less in amber vials. Protect from light.
Sect 9.4	Laboratory Reagent Blank (LRB)	One with each extraction batch of up to 20 field samples.	Demonstrate that all target analytes are ≤ 1/3 the MRL, and that possible interferences from extraction media or reagents do not prevent the identification and quantification of method analytes.
Sect. 9.6	Laboratory Fortified Blanks (LFB)	Analyze at least one LFB daily or one for each extraction batch of up to 20 field samples. Rotate the fortified concentration between low, medium and high amounts.	Results of LFB analyses must be 70-130% of the true value for each analyte and surrogate for all fortified concentrations greater than the lowest CAL point. Results of LFBs corresponding to the lowest CAL point must be 50-150% of the true value.
Sect. 9.7	Internal Standard	At least one internal standard is added to all calibration standards and extracts.	Peak area counts for the IS in LFBs, LRBs and sample extracts must be within 70-130% of the peak area in the most recent CCC, and 50-150% of average area in the initial calibration.
Sect. 9.8	Surrogate Standards	Surrogate standard is added to all calibration standards, samples, LFBs, LFSMs, LFSMDs, FDs, and LRBs.	Recovery for the surrogate in all calibration standards, LRB, LFB, LFSM, LFSMDs, FD and sample extracts must be 70-130% of the true value.

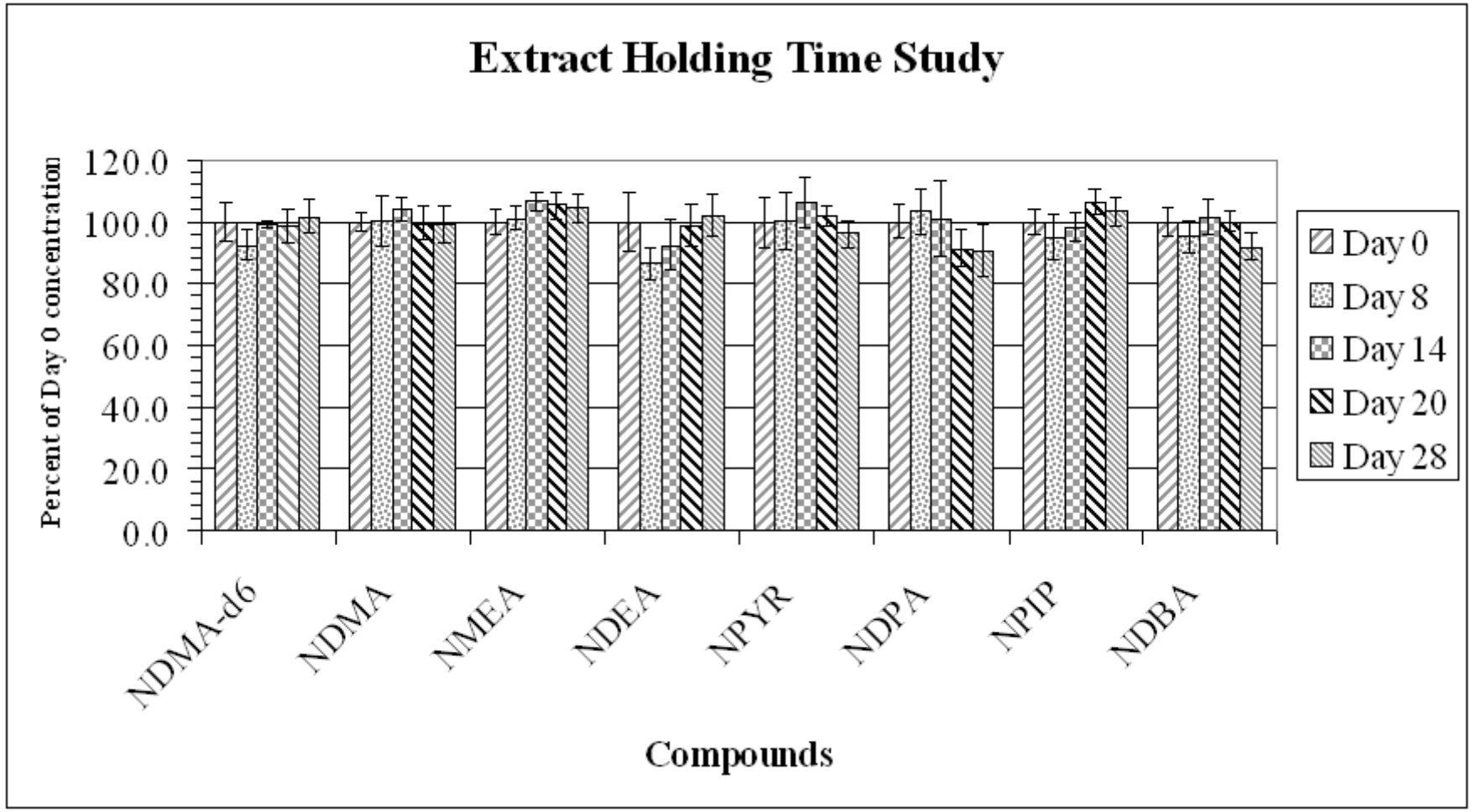
Method Ref.	Requirement	Specification and Frequency	Acceptance Criteria
Sect. 9.9	Laboratory Fortified Sample Matrix (LFSM)	Analyze one LFSM per extraction batch (20 field samples or less) fortified with method analytes at a concentration ≥ the native concentration.	Recoveries not within 70-130% of the fortified amount may indicate a matrix effect.
Sect. 9.10	Field Duplicates or Laboratory Fortified Sample Matrix Duplicates (LFSMD)	Analyze 1 FD for each 20 samples, or 1 per extraction batch, whichever is greater. See the referenced Section for a discussion of when LFSMDs should be analyzed.	Suggested RPD ≤30%.
Sect. 9.11	Quality Control Sample (QCS)	Analyze QCS whenever new standards are prepared, or at least quarterly.	If analyzed as a calibration sample, CCC criteria apply. If analyzed as an LFB, those criteria apply.
Sect. 10.2.	Initial Calibration	Use internal standard calibration technique to generate an average RF, or first or second order calibration curve for each analyte. Use a minimum of 3 standards for a calibration range of 1 order of magnitude, and at least 5 standards for 2 orders of magnitude.	When each calibration standard is calculated as an unknown using the calibration curve, the result must be 70-130% of the true value for all but the lowest standard. The lowest standard must be 50-150% of the true value.
Sect. 10.3	Continuing Calibration Check	Verify initial calibration by analyzing a calibration standard prior to analyzing samples, after every 10 samples, and after the last sample. Always analyze a low concentration (near the MRL) CCC at the beginning of the analysis period. Rotate through low, medium, and high concentration calibration standards to meet the CCC for every 10 samples requirement.	The result for each analyte and surrogate must be 70-130% of the true value for all concentrations except the lowest CAL point for each analyte. The lowest CAL point for each analyte must be 50-150% of the true value. The peak area of the IS must be within 70-130% of the peak area in the most recent CCC, and 50-150% of the average peak area calculated during initial calibration.

Figure 1.



Error bars represent 1 standard deviation. N=7 at each time point.

Figure 2.



Error bars represent 1 standard deviation. N=7 at each time point.