Method 523: Determination of Triazine Pesticides and their Degradates in Drinking Water by Gas Chromatography/Mass Spectrometry (GC/MS)

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METHOD 523 DETERMINATION OF TRIAZINE PESTICIDES AND THEIR DEGRADATES IN DRINKING WATER BY GAS CHROMATOGRAPHY/MASS SPECTROMETRY (GC/MS)

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1. SCOPE AND APPLICATION

1.1 This is a gas chromatography/mass spectrometry (GC/MS) method for the determination of triazine pesticides and their degradation products in finished drinking waters. Precision and accuracy data have been generated for the method analytes in reagent water, drinking water from a groundwater source, and drinking water from a surface water source. The single laboratory Lowest Concentration Minimum Reporting Level (LCMRL) has also been determined in reagent water. The following compounds can be determined using this method:

Analyte	Chemical Abstract Services Registry Number (CASRN)
Atrazine	1912-24-9
Atrazine-desethyl	6190-65-4
Atrazine-desethyl-desisopropyl	3397-62-4
Atrazine-desisopropyl	1007-28-9
Cyanazine	21725-46-2
Propazine	139-40-2
Simazine	122-34-9
Terbuthylazine-desethyl	30125-63-4
Terbuthylazine	5915-41-3
Prometon	1610-18-0
Prometryn	7287-19-6
Ametryn	834-12-8
Simetryn	1014-70-6

1.2 The MS conditions described in this method were developed using a time-of-flight (TOF) GC/MS system. The method was validated at a second laboratory that used a quadrupole-based GC/MS system.

1.3 The single laboratory LCMRL is the lowest spiking concentration such that the probability of spike recovery in the 50% to 150% range is at least 99%. Single laboratory LCMRLs for the analytes in this method ranged from 0.40 to 2.1 micrograms per liter (µg/L), and are listed in Table 4 (all Tables are found in Section 17). The procedure used to determine the LCMRL is described elsewhere.[1]

1.4 Laboratories using this method are not required to determine an LCMRL, but they must demonstrate that the Minimum Reporting Level (MRL) for each analyte meets the requirements described in Section 9.2.4.

1.5 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 dependent on sample matrix, fortification concentration, and instrument performance. Determining the DL for analytes in this method is optional (Sect. 9.2.6). DLs for method analytes fortified into RW ranged from 0.10 to 0.69 µg/L. These values are presented in Table 4.

1.6 This method is intended for use by analysts skilled in solid-phase extractions (SPE), the operation of GC/MS instrumentation, and the interpretation of the associated data.

1.7 METHOD FLEXIBILITY – In recognition of technological advances in analytical instrumentation and techniques, the laboratory is permitted to modify the GC and MS conditions. **Changes may not be made to sample collection and preservation (Sect. 8), to the sample extraction procedure (Sect. 11.3), or to the quality control (QC) requirements (Sect. 9)**. Method modifications must be considered only to improve method performance. Modifications that are introduced in the interest of reducing cost or sample processing time, but result in poorer method performance, may not be used. In all cases where method modifications are proposed, the analyst must perform the procedures outlined in the Initial Demonstration of Capability [(IDC), Sect. 9.2], verify that all QC acceptance criteria in this method (Tables 10 and 11) are met, and that method performance can be verified in a real sample matrix (Sect. 9.4).

2. SUMMARY OF METHOD

2.1 Samples are pH adjusted and dechlorinated with ammonium acetate ($NH_4C_2H_3O_2$) and protected from microbial degradation using 2-chloroacetamide during sample collection. Samples are fortified with isotopically enriched surrogates [Atrazine-desethyl-desisopropyl($^{13}C_3$), Atrazine-desisopropyl-d_5(ethyl-d_5), Cyanazine-d_5(*N*-ethyl-d_5), and Simazine-d_{10}(diethyl-d_{10})] just prior to extraction. Analytes are extracted from a 250-milliliter (mL) sample aliquot using 250-milligram (mg) carbon cartridges. After extraction, the cartridges are dewatered with a small volume of methanol (MeOH) and then eluted with 2 mL of ethyl acetate followed by two, 6-mL aliquots of 9:1 (v:v) dichloromethane/methanol (DCM/MeOH). The extracts are dried using anhydrous sodium sulfate (Na_2SO_4) and concentrated using a stream of nitrogen gas. Isotopically labeled internal standards (IS) [Atrazine-d_5(ethyl-d_5) and Atrazine-desethyl-d_7(isopropyl-d_7)] are added, and the extracts brought to a final 1.0-mL volume. Extracts are analyzed using a GC/MS operated in full scan mode. Method analytes are identified by comparing retention times and the acquired mass spectra to retention times and reference spectra for calibration standards acquired under identical GC/MS conditions. The concentration of each method analyte is determined using the IS technique.

3. DEFINITIONS

3.1 ANALYSIS BATCH – A set of samples that is 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 of the method analytes prepared from the primary dilution standard(s) (PDS) and stock standard solution(s), which includes the ISs and SURs. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.3 CONTINUING CALIBRATION CHECK (CCC) – A CAL containing the method analytes, surrogates, and ISs, which is analyzed periodically to verify the accuracy of the existing calibration.

3.4 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. 9.2.6), and accurate quantitation is not expected at this level.

3.5 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 SPE devices, solvents, surrogate solution, and fortifying solutions. Required QC samples include Laboratory Reagent Blank, Laboratory Fortified Blank, Laboratory Fortified Matrix, and either a Field Duplicate or Laboratory Fortified Sample Matrix Duplicate.

3.6 FIELD DUPLICATES (FD1 and FD2) – Separate samples collected at the same time, and shipped and stored under identical conditions. Method precision, including the contribution from sample collection procedures, is estimated from the analysis of FDs. For the purposes of this method, Field Duplicates are necessary to conduct repeat analyses if the original field sample is lost, or to conduct repeat analyses in the case of QC failures associated with the analysis of the original field sample. Field Duplicates are used to prepare Laboratory Fortified Sample Matrix (Sect. 3.9) and Laboratory Fortified Sample Matrix Duplicate (Sect. 3.10) QC samples.

3.7 INTERNAL STANDARD (IS) – A pure compound added to all standard solutions, field samples and QC samples in a known amount. Each internal standard is assigned to a specific analyte or multiple analytes, and is used to measure relative response.

3.8 LABORATORY FORTIFIED BLANK (LFB) – An aliquot of reagent water to which known quantities of the method analytes are added. The LFB is extracted and analyzed as a sample including use of the preservation procedures in Section 8. The LFB is used during the IDC to verify method performance for precision and accuracy, and as an ongoing QC element.

3.9 LABORATORY FORTIFIED SAMPLE MATRIX (LFSM) – A Field Duplicate to which known quantities of the method analytes are added. The LFSM is extracted and analyzed as a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results.

3.10 LABORATORY FORTIFIED SAMPLE MATRIX DUPLICATE (LFSMD) – A second Field Duplicate of the same field sample used to prepare the LFSM which is fortified identically to the LFSM. The LFSMD is used instead of the FD to assess method precision and accuracy when the occurrence of the method analytes at a concentration greater than the MRL is infrequent.

3.11 LABORATORY REAGENT BLANK (LRB) – An aliquot of RW that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, sample preservatives, ISs, and SURs associated with the Extraction Batch. The LRB is used to determine if method analytes or other interferences are introduced via the laboratory environment, the reagents, or the apparatus.

3.12 LOWEST CONCENTRATION MINIMUM REPORTING LEVEL (LCMRL) – The single-laboratory LCMRL is the lowest spiking concentration such that the probability of spike recovery in the 50 and 150% range is at least 99% .[1]

3.13 MATERIAL SAFETY DATA SHEETS (MSDS) – Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire and reactivity data, storage instructions, spill response procedures, and handling precautions.

3.14 MINIMUM REPORTING LEVEL (MRL) – The minimum concentration that can be reported by a laboratory as a quantified value for the method analyte in a sample following analysis. This concentration must meet the criteria defined in Section 9.2.4 and must be no lower than the concentration of the lowest CAL for the method analyte. A laboratory may be required to demonstrate a specific MRL by a regulatory body if this method is being performed for compliance purposes.

3.15 PRIMARY DILUTION STANDARD (PDS) – A solution containing the method analytes (or internal standards or surrogate analytes) prepared in the laboratory from Stock Standard Solutions and diluted as needed to prepare calibration standards and sample fortification solutions.

3.16 QUALITY CONTROL SAMPLE (QCS) – A solution containing the method analytes at a known concentration, which is obtained from a source external to the laboratory and different from the source of calibration standards. The purpose of the QCS is to verify the accuracy of the primary calibration standards.

3.17 REAGENT WATER (RW) – Purified water that does not contain any measurable quantity of the method analytes or interfering compounds at or above 1/3 the MRL.

3.18 STOCK STANDARD SOLUTION – A concentrated solution containing one or more of the method analytes that is prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source, so that the concentration and purity of analytes are traceable to certificates of analysis.

3.19 SURROGATE ANALYTE (SUR) – A pure analyte, extremely unlikely to be found in any sample, that is added to a sample aliquot in a known amount before extraction, and which 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 All glassware must be meticulously cleaned. Wash glassware with detergent and tap water, rinse with tap water, followed by RW. Volumetric glassware must be solvent rinsed after washing and dried in a low temperature oven [<120 degrees Centigrade (°C)] or air-dried. Non-volumetric glassware may be heated in a muffle furnace at 400 °C for two hours as a substitute for a solvent rinse.

4.2 Method interferences may be caused by contaminants in solvents, reagents (including RW), sample bottles and caps, and other sample processing hardware. These interferences may lead to discrete artifacts and/or elevated baselines in the chromatograms. All laboratory reagents and equipment must be routinely demonstrated to be free from interferences (less than 1/3 the MRL for the method analytes) under the conditions of the analysis. This may be accomplished by analyzing LRBs as described in Section 9.3.1.

4.3 Preservatives (Sect. 8.1) are added to samples to ensure the stability of method analytes during shipping and storage prior to analysis. The potential exists for trace-level organic contaminants in these reagents. Interferences from these sources must be evaluated by analysis of LRBs, particularly when new lots of reagents are acquired.

4.4 SPE cartridges may be a source of interferences. The analysis of LRBs can provide important information regarding the presence or absence of such interferences. Brands and lots of SPE devices must be tested to ensure that contamination does not preclude analyte identification and quantitation.

4.5 Silicone compounds may be leached from punctured septa of autosampler vials. This can occur after repeated injections from the same autosampler vial. These silicone compounds, which appear as regularly spaced chromatographic peaks with similar fragmentation patterns, could unnecessarily complicate the total ion chromatograms and may interfere with the identification of the method analytes.

4.6 Matrix interferences are caused by contaminants that are present in the sample. The extent of matrix interferences will vary considerably from source to source depending upon the nature of the water. The analysis of Laboratory Fortified Sample Matrix (Sect. 9.3.7) provides evidence for the presence (or absence) of matrix effects.

4.7 This method uses a number of isotopically labeled ISs and SUR analytes. These standards must be determined to be sufficiently free of the unlabeled parent molecule to permit accurate quantitation of field samples. This is verified during the IDC via analysis of LRBs.

5. SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined. Each chemical must 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.[3,4] The OSHA laboratory standards can be found on line at http://www.osha.gov/SLTC/laboratories/standards.html. A reference file of MSDSs should be made available to all personnel involved in the chemical analysis.

5.2 Pure standard materials and stock standard solutions of the method compounds should be handled with suitable protection for skin, eyes, etc.[5]

6. EQUIPMENT AND SUPPLIES

References to specific brands or catalog numbers are included as examples only and do not imply endorsement of the product. These references do not preclude the use of other vendors or supplies.

6.1 SAMPLE CONTAINERS – Clean, amber bottles (250 mL or larger) fitted with polytetrafluoroethylene (PTFE)-faced silicone septa and polypropylene screw caps (I-Chem Cat. No. S249-0250 or equivalent).

6.2 VIALS – Amber, 2-mL glass autosampler vials with PTFE-faced septa (Fisher Cat. No. 03-375-19B or equivalent).

6.3 MICRO SYRINGES – Suggested sizes include 10, 25, 50, 100, 250, and 500 µL.

6.4 VOLUMETRIC FLASKS – Class A, suggested sizes include 5, 10, 50, 100, 200, and 500 mL for preparation of reagents and standards.

6.5 VOLUMETRIC PIPETTES – Class A, suggested sizes include 2, 3, 4, 6, and 10 mL.

6.6 ANALYTICAL BALANCE – Capable of weighing to the nearest 0.0001 gram.

6.7 SPE VACUUM MANIFOLD AND SAMPLE TRANSFER LINES – Supelco Visi-Prep™ Part No. 57044 or equivalent, and Supelco Visi-Prep™ Part No. 57275 or equivalent.

6.8 SPE CARTRIDGES – Cartridges packed with 250 mg of graphitized, non-porous carbon (Supelco ENVI-Carb™, Cat. No. 57092 or equivalent).

6.9 AUTOMATED EXTRACTORS – An automated 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 cartridges. 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.

6.10 DISPOSABLE PASTEUR PIPETTES – Nine-inch borosilicate glass (Fisher Cat. No. 13-678-20C or equivalent).

6.11 DRYING COLUMN – The drying column must be able to contain 3 g of anhydrous Na_2SO_4, plus several milliliters of eluate. The drying column must not leach interfering compounds or irreversibly adsorb method analytes. Any small glass column may be used, such as a glass pipette with glass wool plug (Chase Scientific Glass, Inc., P1005, 4.5-mL Monstr-Pette, Fisher Part No. 22-378-893 or equivalent).

6.12 EXTRACT CONCENTRATION SYSTEM – Extracts are concentrated by blowdown with nitrogen gas using a water bath set at 35° to 40 °C (Meyer N-Evap, Model 111, Organomation Associates, Inc., or equivalent). Other automated concentration devices may be used.

6.13 LABORATORY OR ASPIRATOR VACUUM SYSTEM – Sufficient capacity to maintain a vacuum of approximately 15 to 25 inches of mercury.

6.14 GRADUATED CONICAL SCREW-CAP TEST TUBES – Fifteen-mL capacity (Corning Part No. 8082-15, Fisher Part No. 05-538-30A, or equivalent), used to collect the eluate from the carbon cartridges. Also, 40-mL capacity (Kimble Part No. 45200-40, Fisher Part No. 05-538-33B or equivalent), used to collect eluates from the drying columns.

6.15 GAS CHROMATOGRAPHY MASS SPECTROMETRY SYSTEM (GC/MS)

6.15.1 FUSED SILICA CAPILLARY GC COLUMN – Fused silica capillary column [20-meter x 0.18-millimeter (mm) inside diameter (i.d.)] coated with a 0.20-µm bonded film of poly methylphenyl siloxane (Restek Rtx®-50 or equivalent). Any column that provides adequate resolution, peak shape, capacity (Sect. 10.2.2.1 and 10.2.2.2), and accuracy and precision (Sect. 9) may be used. A mid-polarity, low-bleed column is recommended for use with this method to provide appropriate selectivity, and to minimize mass spectrometric background.

6.15.2 GC INJECTOR AND OVEN – Equipped for split/splitless injection (Agilent 6890 GC with Agilent 7683 autosampler or equivalent). Some of the analytes included in this method are very polar and/or subject to thermal breakdown in the injection port. This effect increases when the injector is not properly deactivated or operated at excessive temperatures. The injection system must not allow analytes to contact hot stainless steel or other active surfaces that promote decomposition. The performance data in Section 17 were obtained using hot, splitless injection with a 2-mm-i.d. quartz liner (Restek Cat. No. 20914). Other injection techniques such as temperature programmed injections, cold on-column injections and large volume injections may

be used if the QC criteria in Section 9 are met. Commercially available inlet systems, specifically designed for these alternate types of injections, must be used if these options are selected. The GC system must provide consistent sample injection volumes and be capable of performing temperature programming at a constant flow rate, constant linear velocity, or constant pressure.

6.15.2.1 GC SYRINGE – During method development, a 5.0-µL syringe was used to inject 0.5-µL aliquots of standards and extracts (Agilent Cat. No. 5181-1273 or equivalent).

6.15.3 GC/MS INTERFACE – Interface must allow the capillary column or transfer line exit to be placed within a few millimeters of the ion source.

6.15.4 MASS SPECTROMETER (MS) – The MS must be capable of electron ionization and collecting spectra in positive ion mode. The instrument must be capable of obtaining at least five scans during the chromatographic peaks. Ten to fifteen scans across chromatographic peaks are recommended. The spectrometer must produce a mass spectrum that meets all criteria in Table 1 when a solution containing approximately five nanograms (ng) of decafluorotriphenyl phosphine (DFTPP) is injected into the GC/MS.

6.15.5 DATA SYSTEM – An interfaced data system is required to acquire, store, and output MS data. The computer software must have the capability of processing stored GC/MS data by recognizing a chromatographic peak within a given retention time window. The software must allow integration of the ion abundance of any specific ion between specified time or scan number limits. The software must also allow construction of linear or second-order regression calibration curves, and calculation of concentrations using the internal standard technique.

7. REAGENTS AND STANDARDS

7.1 REAGENTS AND SOLVENTS – Reagent-grade or better chemicals must be used. Unless otherwise indicated, it is intended that all reagents will conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society (ACS), where such specifications are available. Other grades may be used, as long as the reagent is of sufficiently high purity to permit its use without negatively affecting data quality.

7.1.1 HELIUM – 99.999% or better, GC carrier gas.

7.1.2 REAGENT WATER – Purified water, which does not contain any measurable quantities of any method analytes or interfering compounds at or above 1/3 the MRL for each compound of interest.

7.1.3 METHANOL – (MeOH) (CASRN 67-56-1) – High purity, demonstrated to be free of analytes and interferences (Fisher GC Resolv®-grade or equivalent).

7.1.4 ETHYL ACETATE (EtOAc) (CASRN 141-78-6) – High purity, demonstrated to be free of analytes and interferences (B & J Brand GC$^{2®}$, Capillary GC/GC-MS-grade or equivalent).

7.1.5 DICHLOROMETHANE (DCM), also known as METHYLENE CHLORIDE ($MeCl_2$) (CASRN 75-09-2) – High purity, demonstrated to be free of analytes and interferences (B & J Brand GC$^{2®}$, Capillary GC/GC-MS-grade or equivalent).

7.1.6 SODIUM SULFATE, ANHYDROUS (CASRN 7757-82-6) – Soxhlet extracted with DCM for a minimum of four hours or heated to 400 °C for two hours in a muffle furnace. An "ACS grade, suitable for pesticide residue analysis," or equivalent, of anhydrous Na_2SO_4 is recommended (Fisher, Cat. No. S415-10S or equivalent).

7.1.7 DICHLOROMETHANE/METHANOL 9:1 – Elution solvent. To make 1 L, add 900 mL of DCM to 100 mL of MeOH and mix thoroughly. Store in an amber glass container.

7.1.8 DICHLOROMETHANE/ETHYL ACETATE 3:1 – Drying column rinse solvent. To make 1 L, add 750 mL of DCM to 250 mL of EtOAc and mix thoroughly. Store in an amber glass container.

7.1.9 AMMONIUM ACETATE (CASRN 631-61-8) – High purity, demonstrated to be free of analytes and interferences (Sigma Aldrich, Cat. No. A7262 or equivalent).

7.1.10 2.5 M AMMONIUM ACETATE CONCENTRATED STOCK SOLUTION (192 g/L) – Used to sequester free available chlorine and to buffer field samples. To prepare 200 mL of solution, add 38.5 g ammonium acetate to a 200-mL volumetric flask, then add RW to the mark and mix well.

7.1.11 2-CHLOROACETAMIDE ($ClCH_2CONH_2$) (CASRN 79-07-2) – Used as an anti-microbial agent. High purity, demonstrated to be free of analytes and interferences (Sigma Cat. No. C0267 or equivalent).

7.2 STANDARD SOLUTIONS – When a compound's 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 only as examples**. Stock standard solutions are estimated to be stable for at least six months if stored at -10 °C or colder. Any fortified or dilute solutions made from the stock standards are stable for at least 60 days provided they are stored at a temperature ≤-10 °C and the stock standard solutions have not exceeded their six month stability period. **Although estimated stability times for standard solutions are given, laboratories should use accepted QC practices to determine when their standards need to be replaced.**

7.2.1 INTERNAL STANDARD SOLUTIONS – This method uses isotopically enriched ISs, specifically Atrazine-d_5(ethyl-d_5) (CASRN 163165-75-1; CDN Isotopes Cat.

No. D4389 or equivalent) and Atrazine-desethyl-d_7(isopropyl-d_7) (CASRN 6190-65-4; CDN Isotopes Cat. No. D5639 or equivalent).

7.2.1.1 INTERNAL STANDARD STOCK SOLUTIONS (1000 µg/mL) – Prepare the stock standards individually by weighing 10 mg of the solid materials (Atrazine-d_5 and Atrazine-desethyl-d_7) into tared 10-mL volumetric flasks and dilute to volume with EtOAc. Sonication may be required to achieve full dissolution of the solid materials. Alternatively, commercially produced standard solutions may be used.

7.2.1.2 INTERNAL STANDARD PRIMARY DILUTION STANDARD (IS PDS) (200 µg/mL) – Prepare the IS PDS by adding enough of each internal standard stock solution to a volumetric flask partially filled with EtOAc to make the final concentrations 200 µg/mL when filled to the mark with EtOAc. During method development, addition of 10 microliters (µL) of the IS PDS to each 1.0-mL extract produced a final concentration of 2.0 µg/mL. Analysts are permitted to use other PDS concentrations and volumes provided all field samples and calibration standards contain the same amount of IS, the concentration of the IS added provides adequate signal to maintain precision, and the volume added has a negligible effect on the final concentration. Analysts are NOT permitted to use alternate internal standards.

7.2.2 SURROGATE STOCK STANDARD SOLUTION (1500, 500, or 100 µg/mL) – This method uses isotopically enriched compounds for surrogate standards, specifically Atrazine-desethyl-desisopropyl($^{13}C_3$) (Cambridge Isotope Labs, Cat. No. CLM-7528-0), Atrazine-desisopropyl-d_5(ethyl-d_5) (CDN Isotopes Cat. No. D6456), Cyanazine-d_5(*N*-ethyl-d_5) (CASRN 3397-62-4; CDN Isotopes Cat. No. D6136), and Simazine-d_{10}(diethyl-d_{10}) (CASRN 220621-39-6; CDN Isotopes Cat. No. D5654). Concentrations were chosen based on the solubility of these analytes in EtOAc, on their relative instrument response, and in the case of Atrazine-desethyl-desisopropyl ($^{13}C_3$), on other important chromatographic considerations (Sect. 10.2.2.2). Prepare single component SUR stock standards by weighing out approximately 15 mg Cyanazine-d_5, and 5.0 mg of Atrazine-desisopropyl-d_5 and Simazine-d_{10} solid materials using an analytical balance into separate, tared 10-mL volumetric flasks. Dilute to volume with EtOAc. Atrazine-desethyl-desisopropyl ($^{13}C_3$) surrogate is much less soluble in EtOAc and is prepared by weighing out approximately 5.0 mg of the solid using an analytical balance into a tared 50-mL volumetric flask and diluting to volume with EtOAc; the Atrazine-desethyl-desisopropyl ($^{13}C_3$) used during method development was custom synthesized by Cambridge Laboratories. Analysts are NOT permitted to use alternate surrogates.

7.2.2.1 SURROGATE PRIMARY DILUTION STANDARDS (SUR PDS) – Prepare a single SUR PDS that contains Simazine-d_{10} and Atrazine-desisopropyl-d_5 at 200 µg/mL, and Cyanazine-d_5 at 500 µg/mL by making appropriate dilutions of the SUR stock standard solutions into ethyl acetate.

During method development, the Atrazine-desethyl-desisopropyl ($^{13}C_3$) SUR stock was used without further dilution to fortify samples prior to extraction (Sect. 11.2.2) and to prepare calibration standards (Sect. 7.2.4).

7.2.3 ANALYTE STANDARD SOLUTIONS – Obtain the analytes listed in the table in Section 1.1 as neat standards. Prepare the analyte stock and Primary Dilution Standards as described below. Alternatively, commercially produced standard solutions may be used.

7.2.3.1 ANALYTE STOCK STANDARD – Prepare the stock standards individually by weighing 10-20 mg of the solid materials using an analytical balance into tared 5-mL, 10-mL, or larger volumetric flasks and diluting to volume with EtOAc. Atrazine-desethyl-desisopropyl exhibits limited solubility in EtOAc and must be prepared at a concentration of ≤100 µg/mL. The table below summarizes the concentration of the analyte stock standards used during method development. In some cases, sonication is required to achieve full dissolution of the neat materials.

Analyte	Analyte Stock Standard* (µg/mL)	Manufacturer, Part No.**
Atrazine	2000	Chem Service, PS-380
Atrazine-desethyl	1000	Chem Service, MET-380B
Atrazine-desisopropyl	500	Chem Service, MET-58A
Atrazine-desethyl-desisopropyl	100	Chem Service, MET-58C
Cyanazine	2000	Chem Service, PS-387
Propazine	2000	Chem Service, PS-385
Simazine	500	Chem Service, PS-58
Terbuthylazine	2000	Chem Service, PS-413
Terbuthylazine-desethyl	850	Chem Service, MET-413A
Prometon	1200	Chem Service, PS-386
Prometryn	900	Chem Service, PS-384
Ametryn	1200	Chem Service, PS-383
Simetryn	840	Chem Service, PS-381

* Analyte stock standard concentrations used during method development. Some analytes are near their solubility limits at the storage temperature and required sonication to dissolve.

** Other manufacturer's standards are allowed as long as they have acceptable purity.

7.2.3.2 ANALYTE PRIMARY DILUTION STANDARD (50 µg/mL) – The analyte PDS is used to prepare the CALs and to fortify the LFBs, LFSMs and LFSMDs with the method analytes. The analyte PDS is prepared by adding appropriate volumes of the of the analyte stock solutions (except Atrazine-desethyl-desisopropyl) into a single volumetric flask and diluting to volume such that the final concentration is 50 µg/mL. Because the concentration of Atrazine-desethyl-desisopropyl in the analyte stock is low due to its limited

solubility in EtOAc, use the analyte stock directly as an analyte PDS solution in the steps that follow.

7.2.4 CALIBRATION STANDARDS – Prepare a calibration curve of at least five levels from dilutions of the analyte PDS in EtOAc. The preparation scheme, with concentrations of CALs that were used to collect data in Section 17, is presented in the table below. A constant concentration of each IS and SUR analyte (in the range of 1 to 5 µg/mL) is added to each calibration solution. For example, add 20 µL of the IS PDS (Sect. 7.2.1.2) and 20 µL of the multi-component SUR PDS (Sect. 7.2.2.1) to each CAL. For the surrogate Atrazine-desethyl-desisopropyl ($^{13}C_3$), add 20 µL of the SUR stock standard (Sect. 7.2.2). The lowest concentration CAL must be at or below the MRL. The CAL standards may also be used as CCCs. The standards must be stored at -10 ºC or lower.

CAL Level	Analyte PDS* (µg/mL)	Analyte PDS Volume** (µL)	Final CAL Volume (mL)	CAL (µg/mL)
1	50	4	2.00	0.10
2	50	8	2.00	0.20
3	50	12	2.00	0.30
4	50	20	2.00	0.50
5	50	30	2.00	0.75
6	50	40	2.00	1.00
7	50	80	2.00	2.00
8	50	200	2.00	5.00

* For atrazine-desethyl-desisopropyl, the analyte stock standard was used at a concentration of 100 µg/mL.

** For atrazine-desethyl-desisopropyl, ½ of the volume reported in this table was used.

7.2.5 GC/MS TUNE CHECK SOLUTION (5 µg/mL) – Prepare a DFTPP (CASRN 5074-71-5) solution in DCM. DFTPP is more stable in DCM than in acetone or EtOAc. Store this solution in an amber glass screw cap vial at -10 °C or lower.

8. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 SAMPLE COLLECTION

8.1.1 Prior to shipment to the field, the ammonium acetate solution and 2-chloroacetamide must be added to each sample bottle. 250-mL sample bottles are recommended. For this sample volume, add 2.0 mL of the ammonium acetate concentrated stock (Sect. 7.1.10) and 500 mg of 2-chloroacetamide. These reagents may be added in the field. However, the preservatives must be added to the container prior to sample collection. If other collection volumes are used, adjust the amount of the preservatives so that the final concentrations of ammonium acetate and 2-chloroacetamide in the sample containers are 1.5 g/L (20 millimolar, mM) and 2.0 g/L, respectively. Cap the bottles to avoid loss of the preservation reagents.

NOTE: The extraction procedure was developed using a 250-mL sample volume. Although the method performed well at this volume in matrices containing high levels of total organic carbon (TOC), larger extraction volumes could conceivably cause breakthrough for field samples with high TOC levels. For this reason, sample size must not exceed the recommended volume by more than 10%. Smaller sample volumes, although not subject to breakthrough, may decrease method sensitivity making it more difficult to confirm the MRL (Sect. 9.2.4).

8.1.2 When sampling from a cold water tap, open the tap and allow the system to flush until the water temperature has stabilized (usually 3 to 5 minutes). Collect a representative sample from the flowing system using a beaker of appropriate size. Use this bulk sample to generate individual samples as needed. Transfer a volume of at least 245 mL into each collection container, cap the container, and invert it several times to mix the sample with the preservatives. Care must be taken not to overfill the bottle and flush out the preservation reagents. Samples do not need to be headspace free.

8.1.3 When sampling from an open body of water, fill a beaker with water sampled from a representative area. Use this bulk sample to generate individual samples as needed.

8.2 FIELD DUPLICATES – Collect enough Field Duplicates to fulfill QC requirements for LFSMs and LFSMDs (at least three identical samples).

8.3 SAMPLE SHIPMENT AND STORAGE – Samples must be chilled during shipment and must not exceed 10 °C during the first 48 hours after collection. Samples must be confirmed to be at or below 10 °C when they are received at the laboratory. In the laboratory, samples must be stored at or below 6 °C until extraction. Samples must not be frozen.

8.4 SAMPLE HOLDING TIMES – Samples should be analyzed as soon as possible. Samples that are collected and stored as described in Sections 8.1 and 8.3 may be stored prior to analysis for a maximum of 28 days. Extracts may be held for a maximum of 28 days prior to analysis, if they are stored at -10 °C or lower.

9. QUALITY CONTROL

9.1 QC requirements include the IDC and ongoing QC requirements. 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 in Section 17, Tables 10 and 11. These QC requirements are considered the minimum acceptable QC criteria. Laboratories are encouraged to institute additional QC practices to meet their specific needs.

9.2 INITIAL DEMONSTRATION OF CAPABILITY – The IDC must be successfully performed prior to analyzing any field samples. Prior to conducting the IDC, the analyst must meet the calibration requirements outlined in Section 10. Requirements for the IDC are described in the following sections and are summarized in Table 10.

9.2.1 DEMONSTRATION OF LOW SYSTEM BACKGROUND – Analyze an LRB. Confirm that the blank is free of contamination as defined in Section 9.3.1.

9.2.2 DEMONSTRATION OF PRECISION – Prepare, extract and analyze four to seven replicate LFBs. Fortify these samples near the midrange of the initial calibration curve. Ammonium acetate and 2-chloroacetamide must be added to the samples as described in Section 8.1. The percent relative standard deviation (RSD) of the concentrations of the replicate analyses must be ≤20% for all analytes.

$$\%\ \text{RSD} = \frac{\text{Standard Deviation of Measured Concentrations}}{\text{Average Concentration}} \times 100$$

9.2.3 DEMONSTRATION OF ACCURACY – Calculate the average percent recovery using the same set of replicate data generated for Section 9.2.2. The average recovery of the replicate analyses must be within ±30% of the true value.

$$\%\ \text{Recovery} = \frac{\text{Average Measured Concentration}}{\text{Fortified Concentration}} \times 100$$

9.2.4 MINIMUM REPORTING LEVEL (MRL) CONFIRMATION – Establish a target concentration for the MRL based on the intended use of the method. Analyze an initial calibration following the procedures in Section 10.2. The lowest CAL used to establish the initial calibration (as well as the low-level CCC) 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.

9.2.4.1 Fortify, extract and analyze seven replicate LFBs at or below the target MRL concentration. Ammonium acetate and 2-chloroacetamide must be added to the samples as described in Section 8.1. Calculate the mean (*Mean*) and standard deviation (*S*) for these replicates. Determine the half range for the prediction interval of results (HR_{PIR}) using the equation

$$HR_{PIR} = 3.963S$$

where S is the standard deviation, and 3.963 is a constant value for seven replicates.[1]

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

The Upper PIR Limit must be ≤150% recovery.

$$\frac{Mean + HR_{PIR}}{Fortified\,Concentration} \times 100 \leq 150\%$$

The Lower PIR Limit must be ≥50% recovery.

$$\frac{Mean - HR_{PIR}}{Fortified\,Concentration} \times 100 \geq 50\%$$

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

NOTE: These equations are only valid for seven replicate samples.

9.2.5 QUALITY CONTROL SAMPLE – Analyze a mid-level QCS (Sect. 9.3.9) to confirm the accuracy of the primary CALs.

9.2.6 DETECTION LIMIT 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 ascertain whether DL determination is required based upon the intended use of the data.*

Analyses for this procedure must be done over at least three days (both the sample extraction and the GC analyses must be done over a period of at least three days). Prepare at least seven replicate LFBs. Use the solutions described in Section 7.2 to fortify at a concentration estimated to be near the DL. This concentration may be estimated by selecting a concentration at two to five times the noise level. Ammonium acetate and 2-chloroacetamide must be added to the samples as described in Section 8.1. Process the seven replicates through all steps in Section 11.

NOTE: If an MRL confirmation data set meets these requirements, DLs 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 ONGOING QC REQUIREMENTS – This section describes the ongoing QC procedures that must be followed when processing and analyzing field samples. Table 11 summarizes these requirements.

9.3.1 LABORATORY REAGENT BLANK – An LRB is required with each Extraction Batch. 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 from analytes or contaminants that interfere with the measurement of method analytes must be less than 1/3 the MRL. If the method analytes are detected in the LRB at concentrations equal to or greater than this level, then all data for the problem analyte(s) must be considered invalid for all samples that yielded a positive result.

NOTE: Subtracting blank values from sample results is not permitted.

NOTE: Although quantitative data below the MRL may not be accurate enough for data reporting, such data are useful in determining the magnitude of any background interference. Therefore, blank contamination levels may be estimated by extrapolation, when the concentration is below the MRL.

9.3.2 CONTINUING CALIBRATION CHECK – Analyze CCC standards at the beginning of each Analysis Batch, after every ten field samples, and at the end of the Analysis Batch. See Section 10.3 for concentration requirements and acceptance criteria.

9.3.3 LABORATORY FORTIFIED BLANK – An LFB is required with each Extraction Batch. The fortified concentration of the LFB must be rotated between low, medium, and high concentrations from batch to batch. The low concentration LFB must be at or below the MRL. Similarly, the high concentration LFB must be near the high end of the calibration range established during the initial calibration (Sect. 10.2). Results of the low-level LFB analyses must be within $\pm$50 of the true value. Results of the medium and high-level LFB analyses must be within $\pm$30% of the true value. If the LFB results do not meet these criteria, then all data for the problem analyte(s) must be considered invalid for all samples in the Extraction Batch.

9.3.4 MS TUNE CHECK – The procedure for conducting the MS Tune Check is found in Section 10.2.1. Acceptance criteria for the MS Tune Check are summarized in Section 17, Table 1. The MS Tune Check must be performed each time a major change is made to the mass spectrometer, and prior to establishing and/or re-establishing an initial calibration (Sect. 10.2). Daily DFTPP analysis is not required.

9.3.5 INTERNAL STANDARDS – The analyst must monitor the peak areas of the ISs in all injections of the Analysis Batch. The IS response (peak area) in any chromato-

graphic 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 an IS area in a chromatographic run does not meet these criteria, examine the areas of the ISs in the CCCs, and take corrective action such as recalibration, verifying the integrity of the IS solution, and servicing the GC/MS system. Reanalyze the extract in a subsequent Analysis Batch.

9.3.5.1 If the reinjected aliquot produces an acceptable IS response, report results from that analysis.

9.3.5.2 If the reinjected extract fails again, extraction of the sample may need to be repeated provided a sample is available and still within the holding time. Otherwise, report results obtained from the reinjected extract, but annotate as "suspect/IS recovery." Alternatively, collect a new sample and reanalyze.

9.3.6 SURROGATE RECOVERY – The surrogate analytes are fortified into all field samples and QC samples prior to extraction. Calculate the percent recovery (%R) for each surrogate using the equation

$$\%R = \left(\frac{A}{B}\right) \times 100$$

where A = calculated surrogate concentration for the QC or field sample, and B = fortified concentration of the surrogate analyte.

9.3.6.1 Surrogate recovery must be in the range of 70 to 130%. When a surrogate fails to meet this criterion, evaluate the recovery of the surrogates in the CCCs, the integrity of the CAL solutions, and take corrective action such as recalibration and servicing the GC/MS system. Reanalyze the extract in a subsequent Analysis Batch.

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

9.3.6.3 If the extract reanalysis fails the 70 to 130% recovery criterion after corrective action, extraction of the sample must be repeated provided a sample is available and still within the holding time. If the re-extracted sample also fails the recovery criterion, report all data for that sample as "suspect/surrogate recovery." Alternatively, collect a new sample and reanalyze.

9.3.7 LABORATORY FORTIFIED SAMPLE MATRIX – A minimum of one LFSM is required in each Extraction Batch. The native concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured value in the LFSM corrected for the native concentrations. If a variety of different sample

matrices are analyzed regularly, for example drinking water from groundwater and surface water sources, performance data must be collected for each source.

9.3.7.1 Prepare the LFSM by fortifying a Field Duplicate with appropriate amounts of the analyte PDS (Sect. 7.2.3.2) and DACT PDS (Sect, 7.2.3.1). Select a spiking concentration that is greater than or equal to the native background concentration, if known. Selecting a duplicate aliquot of a sample that has already been analyzed aids in the selection of an appropriate spiking level. If this is not possible, use historical data and rotate through low, medium and high calibration concentrations when selecting a fortifying concentration.

9.3.7.2 Calculate the %R using the equation

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

A = measured concentration in the fortified sample
B = measured concentration in the unfortified sample
C = fortification concentration.

9.3.7.3 Recoveries for samples fortified at concentrations near or at the MRL (within a factor of two times the MRL concentration) must be within ±50% of the true value. Recoveries for samples fortified at all other concentrations must be within ±30% of the true value. If the accuracy for any analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control in the CCCs and the LFB, the recovery is judged matrix biased. The result for that analyte in the unfortified sample is labeled "suspect/matrix."

NOTE: In order to obtain meaningful recovery results, correct the measured values in the LFSM and LFSMD for the native levels in the unfortified samples, even if the native values are less than the MRL. This situation and the LRB are the only permitted uses of analyte results below the MRL.

9.3.8 FIELD DUPLICATE OR LABORATORY FORTIFIED SAMPLE MATRIX DUPLICATE – A minimum of one FD or LFSMD is required in each Extraction Batch. If method analytes are not routinely observed in field samples, analyze an LFSMD rather than an FD.

9.3.8.1 Calculate the relative percent difference (RPD) for duplicate measurements (FD_1 and FD_2) using the equation

$$RPD = \frac{|FD_1 - FD_2|}{(FD_1 + FD_2)/2} \times 100$$

9.3.8.2 RPDs for Field Duplicates must be ≤30%. Greater variability may be observed when Field Duplicates have analyte concentrations that are near or at the MRL (within a factor of two times the MRL concentration). At these concentrations, Field Duplicates must have RPDs that are ≤50%. If the RPD for any analyte falls outside the designated range, and the laboratory performance for the analyte is shown to be in control in the CCC and the LFB, the precision is judged matrix influenced. The result from the unfortified sample is labeled "suspect/matrix."

9.3.8.3 If an LFSMD is analyzed instead of an FD, calculate the RPD for duplicate LFSMs (LFSM and LFSMD) using the equation

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

9.3.8.4 RPDs for duplicate LFSMs must be ≤30%. Greater variability may be observed when fortified LFSMs have analyte concentrations that are near or at the MRL (within a factor of two times the MRL concentration). LFSMs at these concentrations must have RPDs that are ≤50%. If the RPD for any analyte falls outside the designated range, and the laboratory performance for the analyte is shown to be in control in the CCC and the LFB, the precision is judged matrix influenced. The result from the unfortified sample is labeled "suspect/matrix."

9.3.9 QUALITY CONTROL SAMPLE – A QCS is required if an alternate commercial source is available for the method analytes. A QCS must be evaluated as part of the IDC (Sect. 9.2.5) and each time new stock standard solutions are prepared. If standards are prepared infrequently, analyze a QCS at least quarterly. The QCS must be fortified near the midpoint of the calibration range and analyzed as a CCC. The acceptance criteria for the QCS are the same as for the mid- and high-level CCCs (Sect. 10.3.2). If the accuracy for any analyte fails the recovery criterion, check the standard preparation process, stock standard sources, and the purity of neat materials used to prepare the stock standards to locate and correct the problem.

9.4 METHOD MODIFICATION QC REQUIREMENTS - The analyst is permitted to modify the GC column, GC conditions and MS conditions. The analyst is not permitted to modify sample collection and preservation, sample extraction conditions, or QC requirements of the method.

9.4.1 Each time method modifications are made, the analyst must repeat the procedures of the IDC (Sect. 9.2) and verify that all QC criteria can be met in ongoing QC samples (Sect. 9.3).

9.4.2 The analyst is also required to evaluate and document method performance for the proposed method modifications in real matrices that span the range of waters that the laboratory analyzes. This additional step is required because modifications that perform acceptably in the IDC, which is conducted in RW, could fail ongoing method QC requirements in real matrices due to common method interferences. If, for example, the laboratory analyzes finished waters from both surface and groundwater municipalities, this requirement can be accomplished by assessing precision and accuracy (Sects. 9.2.2 and 9.2.3) in a surface water with moderate to high TOC (e.g., 2 mg/L or greater) and a hard groundwater (e.g., hardness greater than 250 mg/L).

9.4.3 The results of Sections 9.4.1 and 9.4.2 must be appropriately documented by the analyst and must be independently verified by the laboratory's quality assurance officer prior to analyzing field samples. When implementing method modifications, it is the responsibility of the laboratory to closely review the results of ongoing QC, and in particular, results associated with the CCCs (Sect. 10.3) and the IS area counts (Sect. 9.3.5). If repeated failures are noted, the modification must be abandoned.

10. CALIBRATION AND STANDARDIZATION

10.1 Demonstration and documentation of acceptable analyte calibration is required before performing the IDC (Sect. 9.2) and prior to analyzing field samples. The MS Tune Check and the initial GC/MS calibration should be repeated each time a major instrument modification or maintenance is performed.

10.2 GC/MS INITIAL CALIBRATION – An initial calibration requires establishing proper GC/MS conditions, confirming the instrument meets the DFTPP tune check criteria, and the preparation and analysis of at least five CALs to determine the calibration curve. Calibration must be performed using peak areas and the IS technique. Calibration using peak heights and external standard calibration are not permitted.

10.2.1 MS TUNE/MS TUNE CHECK– 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. Inject five ng or less of DFTPP (Sect. 7.2.5) into the GC/MS system. Acquire a mass spectrum that includes data for mass/charge ratio (*m/z*) 45 to 450. Use a single spectrum of the DFTPP peak, an average spectrum of the three highest points of the peak, or an average spectrum across the entire peak to evaluate the performance of the system. Appropriate background subtraction is allowed; however, the background scan(s) must be chosen from the baseline prior to or after elution of the DFTPP peak. If the DFTPP mass spectrum does not meet all criteria in Table 1, the MS must be retuned and adjusted to meet all DFTPP criteria before proceeding with the initial calibration.

10.2.2 GC/MS INSTRUMENT CONDITIONS – Operating conditions used during method development are described below. GC/MS operating conditions are summarized in Table 2 (Sect. 17). Conditions different from those described may be used if the method modification QC criteria in Section 9.4 are met. Alternate conditions include

appropriate GC columns, temperature programs, MS conditions, and injection techniques and volume, such as cold on-column and direct injection port liners and/or large volume injection techniques. Commercially available GC inlets must be used for these alternate injection techniques.

10.2.2.1 A narrow bore (0.18-mm i.d.) GC column and a relatively fast temperature program can be used in order to minimize run time (total run time during method development was 12.2 minutes) without jeopardizing resolution. This chromatographic technique requires an MS capable of scanning and storing sufficient numbers of spectra per second in order to record the recommended number of scans per peak (e.g., at least 5, but 10 to 15 scans are recommended). Instruments that are not capable of achieving these fast scan rates must employ a larger-diameter column (e.g., 0.25-mm i.d.) In addition, instruments that are being used with relatively fast temperature programs must have GC oven heaters that are sufficiently powerful enough to perform fast temperature ramping.

10.2.2.2 The method analyte, Atrazine-desethyl-desisopropyl, is the fully dealkylated form of the parent and as a consequence is very polar. It is often referred to as diaminochlorotriazine (DACT) in the literature. Its polar nature and its low solubility in the EtOAc solvent and many GC stationary phases make this analyte very difficult to chromatograph. Several GC columns of varying polarity were investigated, as were various injection volumes. The recommended column is a mid-polarity column that has low bleed and performed well for this challenging analyte. Solvent overloading and/or high concentrations of the ^{13}C-labeled DACT surrogate can lead to overloading of the column phase. When this happens, the DACT peak is broad and asymmetric.

10.2.2.3 Many of the triazine degradates are polar and subject to breakdown and/or adsorption onto active sites in the inlet. A straight, 2-mm quartz liner was determined to be optimal for this method. Even with this liner, a number of analytes exhibited a loss in sensitivity at low concentrations.

10.2.3 CALIBRATION STANDARDS – Prepare a set of at least five CAL standards as described in Section 7.2.3. The lowest concentration of the CALs must be at or below the MRL. Additionally, field samples must be quantified using a calibration curve that spans the same concentration range used to collect the IDC data (Sect. 9.2), e.g., analysts are not permitted to use a restricted calibration range to meet the IDC criteria and then use a larger dynamic range during analysis of field samples.

10.2.4 CALIBRATION – Calibrate the GC/MS system using peak areas and the IS technique. Fit the calibration points with either a linear regression or quadratic regression (response vs. concentration). Weighting may be used. Forcing the calibration curve through the origin is not recommended. Suggested quantitation

ions and retention times for analytes obtained during method development are listed in Table 3.

10.2.5 CALIBRATION ACCEPTANCE CRITERIA – The calibration is validated by calculating the concentration of the analytes from each of the analyses used to generate the calibration curve using the regression equations. Calibration points that are ≤MRL should calculate to be ±50% of their true value. All other calibration points should calculate to be within ±30% of their true value. If these criteria cannot be met, the analyst will have difficulty meeting ongoing QC criteria. In this case, corrective action is recommended, such as reanalyzing the calibration standards, restricting the range of calibration, or performing instrument maintenance.

10.3 CONTINUING CALIBRATION CHECKS – Analyze a CCC to verify the initial calibration at the beginning of each Analysis Batch, after every tenth field sample, and at the end of each Analysis Batch. In this context, a "sample" is considered to be a field sample. The LRBs, LFBs, LFSMs, LFSMDs, FDs and CCCs are not counted as samples. The beginning CCC for each Analysis Batch must be at or below the MRL. This CCC verifies instrument sensitivity prior to the analysis of samples. Alternate subsequent CCCs between the remaining calibration standards.

10.3.1 Determine that the absolute areas of the quantitation ions of each of the ISs have not changed by more than ±50% from the average areas measured during the initial calibration, or more than ±30% from the most recent CCC. If IS areas have changed by more than this amount, remedial action is necessary (Sect. 10.3.3).

10.3.2 Calculate the concentration of each analyte in the CCC. Each analyte in the CCC fortified at ≤MRL must calculate to be within ±50% of its true value. At all other levels, each analyte, including the surrogate analytes, must calculate to be within ±30%. If these limits are exceeded, then all data for the failed analytes must be considered invalid. Any field samples analyzed since the last acceptable CCC that are still within holding time must be reanalyzed after an acceptable calibration has been restored, with one exception. If the CCC fails because the concentration of a surrogate or analyte is greater than 130% (150% for the low-level CCC) and no method analytes are detected in the field sample(s), non-detects may be reported without reanalysis.

10.3.3 REMEDIAL ACTION – Failure to meet CCC QC performance criteria requires remedial action. Maintenance such as clipping or replacing a GC column, replacing the inlet liner or the inlet seal, and cleaning the MS source may be required. Following major maintenance, the analyst should return to the initial calibration step (Sect. 10.2).

11. PROCEDURE

11.1 Important aspects of this analytical procedure include proper sample collection and storage (Sect. 8), ensuring that the instrument is properly calibrated (Sect. 10) and that all required

QC elements are included (Sect. 9). This section describes the procedure used to extract and analyze field and QC samples.

11.2 SAMPLE PREPARATION

11.2.1 Allow field samples to reach room temperature prior to extraction. Before extraction, mark the level of the sample on the outside of the sample bottle for later sample volume determination. If using weight to determine volume (Sect. 11.3.2.7), weigh the full sample bottle before extraction.

11.2.2 Add an aliquot of the SUR PDS to each sample to be extracted. For method development work, a 10-µL aliquot of the three-component SUR PDS (Sect. 7.2.2.1) and 10 µL of the atrazine-desethyl-desisopropyl($^{13}C_3$) SUR stock (Sect. 7.2.2) were added to each 250-mL field and QC sample.

11.2.3 Fortify LFBs, LFSMs, or LFSMDs, with an appropriate volume of analyte PDS (Sect. 7.2.3.2) and the atrazine-desethyl-desisopropyl stock standard (Sect. 7.2.3.1). Cap and invert each sample several times to mix.

11.2.4 Proceed with sample extraction using SPE carbon cartridges.

11.3 CARTRIDGE SPE PROCEDURE

11.3.1 CARTRIDGE CLEANING AND CONDITIONING – Proper cleaning and conditioning of the solid-phase sorbent can have a marked effect on method precision and accuracy.

11.3.1.1 Set up extraction columns on the SPE vacuum manifold. Using low vacuum (approximately 1 to 2 inches Hg), rinse each cartridge with two 6-mL aliquots of DCM, aspirating completely.

11.3.1.2 Rinse each cartridge with a 6-mL aliquot of MeOH, being careful not to let the bed to go dry. Follow this with a 6-mL aliquot of RW, again being careful not to let the bed go dry.

11.3.2 SAMPLE EXTRACTION

11.3.2.1 Add an additional 4 mL of RW to each cartridge and attach the sample transfer lines. This additional volume prevents the SPE cartridge bed from going dry while the dead volume in the transfer lines is being filled. Extract samples at a cartridge flow rate of approximately 10 mL/minute.

NOTE: Faster flow rates have not been tested and could cause breakthrough.

11.3.2.2 Dry the cartridges under high vacuum for 10 seconds. Release the vacuum, then add a 0.25-mL aliquot of MeOH to each cartridge. Draw the MeOH to

waste with brief vacuum, then dry the cartridges under high vacuum for 10 minutes.

11.3.2.3 Elute the analytes from the cartridges into 15-mL conical tubes with 2 mL of EtOAc followed by two, 6-mL aliquots of 9:1 DCM/MeOH. Allow the cartridge beds to briefly soak in solvent before drawing the solvent through the cartridges.

11.3.2.4 Dry the eluate by passing it through approximately 3 grams of anhydrous Na_2SO_4 collecting it in a 40-mL centrifuge tube. (A Pasteur pipette may be used for a drying column.) Pre-rinse the Na_2SO_4 with a 1-mL aliquot of 3:1 DCM/EtOAc. Rinse the 15-mL conical tube with another 1-mL aliquot of 3:1 DCM/EtOAc and pass this through the anhydrous Na_2SO_4 drying column, collecting it in the same 40-mL centrifuge tube. At this point in the procedure, the dried extracts may be stored overnight in the 40-mL tubes at -10 ºC, if desired.

11.3.2.5 Thermostat the 40-mL tubes at approximately 35 °C in a water bath, and blow down the eluates under a stream of nitrogen gas to less than 1 mL (but no less than ½ mL).

11.3.2.6 Transfer the concentrated eluates to 1-mL volumetric tubes. Rinse the conical tube with a small volume of EtOAc, and transfer the rinseate to the volumetric. Add IS solution, and adjust to volume. During method development, a 10-µL aliquot of the IS PDS (Sect. 7.2.1.2) was added to each extract. Transfer the extracts to autosampler vials and store in a freezer (≤-10 ºC).

11.3.2.7 SAMPLE VOLUME OR WEIGHT DETERMINATION – Use a graduated cylinder to measure the volume of water required to fill the original sample bottle to the mark made prior to extraction (Sect. 11.2.1). Determine the volume of each sample to the nearest 2 mL for use in the final calculations of analyte concentration (Sect. 12.3). If using weight to determine volume, reweigh the empty sample bottle. From the weight of the original sample bottle measured in Section 11.2.1, subtract the empty bottle weight.

11.4 ANALYSIS OF SAMPLE EXTRACTS

11.4.1 Establish GC/MS operating conditions equivalent to those summarized in Table 2 of Section 17. Confirm that compound separation and resolution are similar to those summarized in Table 3 and illustrated in Figure 1 (Sect. 17).

11.4.2 Establish a valid initial calibration following the procedures outlined in Section 10.2 or confirm that the calibration is still valid by analyzing a CCC as described in Section 10.3.

11.4.3 Analyze aliquots of field samples and QC samples. Analyze CCCs at the required frequency alternating concentrations as described in Section 10.3. The GC/MS conditions used to acquire the initial calibration must be used for all sample analyses. The Laboratory Reagent Blank should be the first sample analyzed after the opening CCC.

NOTE: Each Analysis Batch must begin with the analysis of a CCC at or below the MRL for each analyte that the laboratory intends to report. This is true whether or not an initial calibration is analyzed. After 24 hours or 20 field samples, the low-level CCC must be repeated to begin a new Analysis Batch. Do not count QC samples (LRBs, LFBs, FDs, LFSMs, LFSMDs) when calculating the frequency of CCCs that are required during an Analysis Batch.

12. DATA ANALYSIS AND CALCULATIONS

12.1 COMPOUND IDENTIFICATION – Establish an appropriate retention time window for each analyte to identify them in QC and field sample chromatograms. Base this assignment on measurements of actual retention time variation for each compound in standard solutions analyzed on the GC/MS over the course of time. The suggested variation is plus or minus three times the standard deviation of the retention time for each compound for a series of injections. The injections from the initial calibration and from the IDC (Sect. 9.2) may be used to calculate the retention time window. However, the experience of the analyst should weigh heavily on the determination of an appropriate range.

12.1.1 At the conclusion of data acquisition, use the same software settings established during the calibration procedure to identify peaks of interest in the predetermined retention time windows. Initially, identify an analyte by comparison of its retention time with that of the corresponding analyte peak in a recent initial calibration standard or CCC.

12.1.2 Some GC/MS programs use a spectrum matching criterion when collecting data in full scan mode based on the comparison of field sample spectra (after background subtraction if necessary) to reference spectra in the user-created database. This database must be created prior to conducting the IDC from spectra obtained for a mid-level to high-level calibration standard and updated as necessary. If available, this feature may be utilized as a secondary identification routine; however, the primary criterion must be based on the analyte retention time.

12.2 COMPOUND CONFIRMATION – In general, all ions that are present above 30% relative abundance in the mass spectrum of the user-generated database should be present in the mass spectrum of the sample component and should agree within an absolute 20% of the relative abundance in the reference spectrum. For example, if an ion has a relative abundance of 30% in the standard spectrum, its abundance in the sample spectrum should be in the range of 10 to 50%. Some ions, particularly the molecular ion, are of special importance, and should be evaluated even if they are below 30% relative abundance.

NOTE: Compound identification is more challenging when sample components are not resolved chromatographically and produce mass spectra containing ions contributed by more than one compound. When GC peaks obviously represent more than one sample component (i.e., broadened peak with shoulder(s) or valley between two or more maxima), appropriate analyte spectra and background spectra can be selected by examining individual spectra profiles during the peak to determine the characteristic ions. When analytes co-elute (i.e., only one GC peak is apparent), the identification criteria may be met but each analyte spectrum will contain extraneous ions contributed by the co-eluting compound.

12.3 COMPOUND QUANTITATION – Calculate analyte concentrations using the multipoint calibration established in Section 10.2. Report only those values that fall between the MRL and the highest CAL.

12.3.1 EXCEEDING CALIBRATION RANGE – The analyst must not extrapolate beyond the established calibration range. If an analyte result exceeds the range of the initial calibration curve, the extract may be diluted using EtOAc with the appropriate amount of IS added to match the original level, and the diluted extract reinjected. Acceptable surrogate performance must be determined from the undiluted sample extract. Incorporate the dilution factor into final concentration calculations. The resulting data must be documented as a dilution, and the reported MRLs must reflect the dilution factor.

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

12.3.3 Prior to reporting data, the chromatograms must be reviewed for any incorrect peak identifications or poor integrations.

12.3.4 Prior to reporting data, the laboratory is responsible for assuring that QC requirements have been met and that any appropriate qualifier is assigned.

13. METHOD PERFORMANCE

13.1 PRECISION, ACCURACY AND LOWEST CONCENTRATION MINIMUM REPORTING LIMITS – Table 4 presents the DL and LCMRL values obtained at EPA. LCMRLs were determined and calculated using a procedure described elsewhere.[1] Single laboratory precision and accuracy data are presented for three water matrices: RW (Table 5), chlorinated surface water (Table 6), and chlorinated groundwater (Table 7). Figure 1 displays a representative chromatogram fortified at a high concentration in chlorinated groundwater.

13.2 SECOND LABORATORY EVALUATION – 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 Ms. Diane Gregg and Ms. Meredith Clarage of the

U.S. EPA Region 6 Laboratory, Houston, TX, and Mr. Frederick Feyerherm of Agilent, Inc., for conducting the second laboratory evaluation.

13.3 SAMPLE STORAGE STABILITY STUDIES – Chlorinated surface water samples, fortified with method analytes at 5.0 µg/L, were preserved and stored over a 28-day period as specified in Section 8. The analyte recovery and the precision of three replicate analyses of the stored samples, conducted after 0, 7, 14, 21, and 28 days of storage are presented in Section 17, Table 8. These data were used to determine the 28-day holding time.

13.4 EXTRACT STORAGE STABILITY STUDIES – Extract storage stability studies were conducted for extracts obtained from a chlorinated surface water fortified at 5.0 µg/L. The precision and average analyte recovery of triplicate injections conducted after 0, 7, 14, 21, and 28 days of storage are presented in Table 9. These data were used to confirm the 28-day holding time.

13.5 PROBLEM COMPOUNDS

13.5.1 DACT: Atrazine-desethyl-desisopropyl or DACT is subject to hydrolysis at both high (>9) and low (<5) pH but has acceptable stability in neutral aqueous solutions. DACT is also degraded in the presence of free available chlorine, but stable in the presence of chloramines. Ammonium acetate mitigates both of these modes of loss during sample storage, and has sufficient buffer capacity at 20 mM to buffer finished groundwaters with relatively high pH that also have high buffer capacity. The low solubility of DACT in many common low-polarity GC phases (e.g., DB-1, DB-5) leads to phase overloading and distortion of the DACT peak shape at even modest column loadings. The recommendations for the Rtx®-50 GC column as well as a 0.5-µL injection volume are based on these factors. Even with this mid-polarity phase, coupled with a sub-sized injection volume, it is advisable to lower the fortification concentration of the $^{13}C_3$-DACT surrogate to 4 µg/L in field samples (or 1 µg/mL in the extract) to minimize peak distortion at higher calibration levels.

13.5.2 Cyanazine, which is the last eluting analyte, is prone to degradation in the injection port. It also has the highest LCMRL and more variable recovery than any other analyte in this method. For these reasons, a relatively high spike level of 20 µg/L for the surrogate, Cyanazine-d_5, is recommended.

13.6 MATRIX ENHANCED SENSITIVITY – Method analytes may exhibit "matrix-induced chromatographic response enhancement."[7-11] That is, compounds susceptible to GC inlet adsorption or thermal degradation suffer more breakdown when injected in a "cleaner" matrix. The injection of a "dirty" sample extract coats surfaces with matrix components and "protects" the problem compounds from decomposition or adsorption. As a result, a relatively greater response is observed for analytes in sample extracts than in calibration solutions. Compounds that exhibit this phenomenon often give analytical results that exceed 100% recovery in fortified extracts, especially at low concentrations. Analyte areas in CCCs may increase after the injection of several "real samples" compared to injections during a calibration sequence at the beginning of an Analysis Batch. If these symptoms are observed,

more frequent recalibration is recommended. The analyst may also choose to condition the injection port after maintenance or prior to starting an Analysis Batch by injecting a few aliquots of a field sample extract. Matrix effects are also mitigated by using a quartz inlet liner and by minimizing the liner volume as much as is practical (Sect. 6.15.2).

14. POLLUTION PREVENTION

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, 1155 16th Street N.W., Washington, D.C., 20036, or on-line at http://www.ups.edu/x7432.xml.

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 matrices of concern are finished drinking water or source 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. In addition, compliance is required with any sewage discharge permits and regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management, see the publications of the American Chemical Society's Laboratory Environment, Health & Safety Task Force on the Internet at http://membership.acs.org/c/ccs/publications.htm. Additional waste management information can be found in "Laboratory Waste Minimization and Pollution Prevention," Copyright © 1996 Battelle Seattle Research Center, which can be located at http://www.p2pays.org/ref/01/text/00779/ch05.htm.

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17. TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

TABLE 1. ION ABUNDANCE CRITERIA FOR BIS(PENTAFLUOROPHENYL)PHENYL PHOSPHINE, (DECAFLUOROTRIPHENYL PHOSPHINE, DFTPP)[a]

Mass (*m/z*)	Relative Abundance Criteria	Purpose of Checkpoint[b]
68	<2% of *m/z* 69	Low-mass resolution
69	Present	Low-mass resolution
70	<2% of *m/z* 69	Low-mass resolution
197	<2% of *m/z* 198	Mid-mass resolution
198	Present	Mid-mass resolution and sensitivity
199	5-9% of *m/z* 198	Mid-mass resolution and isotope ratio
365	>1% of base peak	Baseline threshold
441	<150% of *m/z* 443	High-mass resolution
442	Present	High-mass resolution and sensitivity
443	15-24% of *m/z* 442	High-mass resolution and isotope ratio

[a] These ion abundance criteria have been developed specifically for target compound analysis as described in this method. Adherence to these criteria may not produce spectra suitable for identifying unknowns by searching commercial mass spectral libraries. If the analyst intends to use data generated with this method to identify unknowns, adherence to stricter DFTPP criteria as published in previous methods[12] is suggested.

[b] All ions are used primarily to check the mass accuracy of the mass spectrometer and data system, and this is the most important part of the performance test. The three resolution checks, which include natural abundance isotope ratios, constitute the next most important part of the performance test, followed by the correct setting of the baseline threshold, as indicated by the presence of *m/z* 365.

TABLE 2. GC/TOF-MS CONDITIONS (LECO Model Pegasus III, GC/TOF-MS)

Chromatographic Conditions

Column: Restek Rtx-50, 20 meters x 0.18 mm i.d., 0.20-µm film thickness
Injection Liner: 2-mm quartz
Injection Volume: 0.50 µL; Syringe: 5.0 µL
Injection Port Temperature: 260 °C
Split Delay: 0.33 minute
Carrier Gas: He, 1.0 mL/minute, constant flow, initial head-pressure 27 pounds per square inch
Temperature Program: initial oven temperature of 120 °C hold for 2 minutes, then ramp at 25 °C/minutes to 250 °C and hold for 5 minutes
Total Run Time: 12.2 minutes

TOF-MS Conditions

Scan Delay: 375 seconds
Scan End: 520 seconds
Mass Scan Range: *m/z* 45 to 260
Mass Defect: 0
Detector Voltage: 1600 volts
Acquisition Rate: 20 spectra/second
Source Temperature: 200 °C

TABLE 3. RETENTION TIMES, SUGGESTED QUANTITATION IONS, AND RECOMMENDED INTERNAL STANDARD REFERENCES

Analyte	Peak # Fig. 1	Ret. Time (minute)	Quan. Ion (*m/z*)	
Atrazine-desethyl-desisopropyl-$^{13}C_3$ (SUR)	1	6.44	148	1
Atrazine-desethyl-desisopropyl	2	6.44	145	1
Terbuthylazine-desethyl	3	6.61	186	1
Atrazine-desethyl-d_7 (IS#1)	4	6.62	176	(IS#1)
Atrazine-desethyl	5	6.65	172	1
Prometon	6	6.69	210	2
Atrazine-desisopropyl-d_5 (SUR)	7	6.71	178	1
Atrazine-desisopropyl	8	6.73	173	1
Propazine	9	6.76	214	2
Atrazine-d_5 (IS#2)	10	6.84	205	(IS#2)
Atrazine	11	6.86	200	2
Terbuthylazine	12	6.88	214	2
Simazine-d_{10} (SUR)	13	6.90	193	2
Simazine	14	6.95	201	2
Prometryn	15	7.53	241	2
Ametryn	16	7.64	227	2
Simetryn	17	7.74	213	2
Cyanazine-d_5 (SUR)	18	8.46	230	2
Cyanazine	19	8.49	225	2

TABLE 4. LOWEST CONCENTRATION MINIMUM REPORTING LEVELS (LCMRL) AND DETECTION LIMITS (DL)

Analyte	LCMRL Concentration Levels (µg/L)	LCMRL (µg/L)	DL (µg/L)
Atrazine-desethyl-desisopropyl	0.30, 0.40, 0.60, 0.80, 1.00, 1.20, 1.60	0.48	0.13
Terbuthylazine-desethyl	0.40, 0.60, 0.80, 1.00, 1.20, 1.60, 2.00	0.72	0.13
Atrazine-desethyl	0.30, 0.40, 0.60, 0.80, 1.00, 1.20, 1.60	0.60	0.10
Prometon	0.60, 0.80, 1.00, 1.20, 1.60, 2.00, 3.00	0.84	0.54
Atrazine-desisopropyl	0.40, 0.60, 0.80, 1.00, 1.20, 1.60, 2.00	0.65	0.19
Propazine	0.40, 0.60, 0.80, 1.00, 1.20, 1.60, 2.00	0.68	0.16
Atrazine	0.30, 0.40, 0.60, 0.80, 1.00, 1.20, 1.60	0.40	0.12
Terbuthylazine	0.40, 0.60, 0.80, 1.00, 1.20, 1.60, 2.00	0.45	0.14
Simazine	0.40, 0.60, 0.80, 1.00, 1.20, 1.60, 2.00	0.60	0.22
Prometryn	0.60, 0.80, 1.00, 1.20, 1.60, 2.00, 3.00	0.85	0.24
Ametryn	0.60, 0.80, 1.00, 1.20, 1.60, 2.00, 3.00	0.83	0.45
Simetryn	0.60, 0.80, 1.00, 1.20, 1.60, 2.00, 3.00	0.78	0.12
Cyanazine	0.80, 1.00, 1.20, 1.60, 2.00, 3.00, 4.00	2.1	0.69

TABLE 5. PRECISION AND ACCURACY OF METHOD ANALYTES FORTIFIED AT 3.0 µg/L AND 20 µg/L IN REAGENT WATER (N=7 SAMPLES)

Analyte	Fortified Conc. = 3.0 µg/L (n=7)		Fortified Conc. = 20 µg/L (n=7)	
	Mean % Recovery	Relative Standard Deviation	Mean % Recovery	Relative Standard Deviation
Atrazine-desethyl-desisopropyl	103	6.0	90.2	2.2
Terbuthylazine-desethyl	108	5.0	89.6	3.8
Atrazine-desethyl	105	2.7	93.9	2.0
Prometon	88.2	9.0	84.4	2.5
Atrazine-desisopropyl	103	3.5	93.1	2.2
Propazine	97.9	4.7	94.3	1.4
Atrazine	97.0	3.8	96.0	1.8
Terbuthylazine	101	5.0	96.4	1.4
Simazine	95.1	5.5	104	2.2
Prometryn	92.1	4.9	89.4	1.8
Ametryn	92.8	4.6	89.6	1.1
Simetryn	88.7	8.5	95.3	4.7
Cyanazine	96.8	10	94.9	2.5
Atrazine-desethyl-desisopropyl-$^{13}C_3$ (SUR) - 4 µg/L	98.3	5.5	103	3.1
Atrazine-desisopropyl-d_5 (SUR) - 8 µg/L	102	3.8	100	2.9
Simazine-d_{10} (SUR) - 8 µg/L	96.9	3.8	88.3	3.2
Cyanazine-d_5 (SUR) - 20 µg/L	92.5	2.8	81.9	2.4

TABLE 6. PRECISION AND ACCURACY OF METHOD ANALYTES FORTIFIED AT 3.0 µg/L AND 20 µg/L IN A CHLORINATED SURFACE WATER[a] (N=7 SAMPLES)

Analyte	Fortified Conc. = 3.0 µg/L (n=7)		Fortified Conc. = 20 µg/L (n=7)	
	Mean % Recovery	Relative Standard Deviation	Mean % Recovery	Relative Standard Deviation
Atrazine-desethyl-desisopropyl	97.8	1.4	102	2.2
Terbuthylazine-desethyl	97.8	2.5	98.9	6.7
Atrazine-desethyl	96.1	2.1	103	1.4
Prometon	97.7	5.9	97.7	1.8
Atrazine-desisopropyl	102	2.0	101	1.2
Propazine	101	3.8	98.2	1.1
Atrazine	104	4.4	101	1.4
Terbuthylazine	97.6	5.6	100	1.6
Simazine	102	4.7	100	4.3
Prometryn	97.7	5.6	95.5	0.75
Ametryn	102	11	95.5	1.3
Simetryn	95.4	4.3	98.9	4.1
Cyanazine	107	10	106	4.1
Atrazine-desethyl-desisopropyl-$^{13}C_3$ (SUR) - 4 µg/L	100	2.6	107	4.1
Atrazine-desisopropyl-d_5 (SUR) - 8 µg/L	106	3.6	101	2.0
Simazine-d_{10} (SUR) - 8 µg/L	103	4.8	102	3.4
Cyanazine-d_5 (SUR) - 20 µg/L	107	3.8	97.4	4.2

[a] Surface water physical parameters: pH = 7.3; hardness = 140 mg/L; free chlorine = 1.7 mg/L; total organic carbon = 4 mg/L.

TABLE 7. PRECISION AND ACCURACY OF METHOD ANALYTES FORTIFIED AT 1.2 µg/L AND 20 µg/L IN A CHLORINATED GROUNDWATER[a] (N=7 SAMPLES)

Analyte	Fortified Conc. = 1.2 µg/L (n=7)[b]		Fortified Conc. = 20 µg/L (n=7)	
	Mean % Recovery	Relative Standard Deviation	Mean % Recovery	Relative Standard Deviation
Atrazine-desethyl-desisopropyl	88.9	10	92.6	3.7
Terbuthylazine-desethyl	100	7.1	92.6	3.8
Atrazine-desethyl	97.6	2.6	93.8	4.2
Prometon	97.1	7.0	90.3	3.8
Atrazine-desisopropyl	100	3.8	93.8	3.9
Propazine	93.8	4.3	98.0	1.4
Atrazine	100	9.5	100	1.5
Terbuthylazine	91.4	4.1	98.9	1.7
Simazine	110	4.3	98.8	2.5
Prometryn	90.5	12	97.1	2.3
Ametryn	92.9	7.0	94.3	2.6
Simetryn	81.9	6.2	95.0	3.1
Cyanazine	102	8.2	97.9	5.3
Atrazine-desethyl-desisopropyl-$^{13}C_3$ (SUR) - 4 µg/L	84.6	9.2	101	3.5
Atrazine-desisopropyl-d_5 (SUR) - 8 µg/L	92.2	5.2	98.7	3.4
Simazine-d_{10} (SUR) - 8 µg/L	103	2.3	91.7	2.2
Cyanazine-d_5 (SUR) - 20 µg/L	108	5.9	85.8	4.2

[a] Ground water physical parameters: hardness = 325 mg/L; free chlorine = 0.5 mg/L.

[b] Atrazine-desethyl-desisopropyl was spiked at 2.0 µg/L for the low level.

TABLE 8. SAMPLE HOLDING TIME DATA FOR METHOD ANALYTES FORTIFIED AT 5.0 µg/L IN A CHLORINATED SURFACE WATER[a,b] (N=3 SAMPLES)

Analyte	Day 0		Day 7		Day 14		Day 21		Day 28	
	%R	%RSD	%R	%RSD	%R	%RSD	%R	%RSD	%R	%RSD
Atrazine-desethyl-desisopropyl	93.2	4.3	95.6	0.26	97.5	5.1	95.5	9.1	95.1	5.8
Terbuthylazine-desethyl	101	4.4	100	3.1	98.4	6.6	101	1.5	104	3.5
Atrazine-desethyl	98.0	1.4	99.3	3.2	100	1.6	98.3	1.8	95.9	4.8
Prometon	93.9	5.0	96.1	4.9	88.5	7.0	91.7	13	94.1	2.1
Atrazine-desisopropyl	98.7	0.4	98.5	1.2	96.3	1.7	101	2.2	92.4	2.5
Propazine	95.7	4.1	100	4.8	97.3	8.5	91.7	1.5	90.6	2.9
Atrazine	96.7	1.2	99.0	5.4	93.2	5.9	91.5	2.2	91.3	4.6
Terbuthylazine	97.1	1.7	97.1	5.9	87.8	5.2	93.6	8.0	98.0	2.1
Simazine	92.1	3.2	102	2.7	100	7.6	96.4	2.9	88.6	6.1
Prometryn	94.9	5.9	103	17	95.1	5.4	94.9	8.2	94.4	5.7
Ametryn	89.6	4.7	89.2	9.2	83.8	11	93.0	1.0	92.0	2.9
Simetryn	86.8	6.3	99.0	3.9	101	2.3	91.6	2.1	89.5	3.5
Cyanazine	98.1	4.4	109	4.4	96.4	0.80	100	10	99.0	8.3

[a] Surface water physical parameters: pH = 7.4; hardness = 140 mg/L; free chlorine = 2.2 mg/L.

[b] %R = percent recovery; %RSD = percent relative standard deviation.

TABLE 9. EXTRACT HOLDING TIME DATA FOR METHOD ANALYTES FORTIFIED AT 5.0 µg/L IN A CHLORINATED SURFACE WATER[a,b] (N=3 SAMPLES)

Analyte	Day 0		Day 7		Day 14		Day 21		Day 28	
	%R	%RSD	%R	%RSD	%R	%RSD	%R	%RSD	%R	%RSD
Atrazine-desethyl-desisopropyl	93.2	4.3	91.9	3.7	97.6	2.6	95.5	2.5	94.8	1.9
Terbuthylazine-desethyl	101	4.4	95.9	2.3	94.0	2.1	95.9	6.4	102	2.7
Atrazine-desethyl	98.0	1.4	100	2.0	99.4	3.3	96.0	2.3	95.3	1.9
Prometon	93.9	5.0	98.5	0.46	93.1	4.5	94.3	6.5	93.7	2.5
Atrazine-desisopropyl	98.7	0.40	97.4	1.2	94.8	1.4	101	3.0	94.2	1.9
Propazine	95.7	4.1	102	7.5	96.9	2.0	94.5	1.7	90.3	4.2
Atrazine	96.7	1.2	100	2.6	97.1	1.8	95.7	1.7	93.4	1.6
Terbuthylazine	97.1	1.7	101	2.2	100	5.7	95.8	11	98.8	5.3
Simazine	92.1	3.2	100	3.4	97.6	3.1	99.2	1.6	97.0	3.1
Prometryn	94.9	5.9	102	0.88	93.1	9.5	104	5.0	100	3.4
Ametryn	89.6	4.7	97.7	4.6	97.2	4.8	98.6	6.2	93.9	10.
Simetryn	86.8	6.3	98.4	0.29	97.8	0.84	94.5	1.0	94.5	6.6
Cyanazine	98.1	4.4	97.7	0.31	102	8.4	108	8.4	102	7.2
Atrazine-desethyl-desisopropyl-$^{13}C_3$ (SUR)	100	4.1	99.3	7.5	104	3.2	96.2	5.2	100	6.5
Atrazine-desisopropyl-d_5 (SUR)	103	6.2	103	6.4	107	2.3	103	5.3	98.8	0.87
Simazine-d_{10} (SUR)	95.9	0.10	97.4	1.3	99.4	2.1	100	0.37	97.6	1.8
Cyanazine-d_5 (SUR)	101	3.6	97.9	1.7	103	6.2	103	4.0	104	4.0

[a] Surface water physical parameters: pH = 7.4; hardness = 140 mg/L; free chlorine = 2.2 mg/L.
[b] %R = percent recovery; %RSD = percent relative standard deviation.

TABLE 10. INITIAL DEMONSTRATION OF CAPABILITY (IDC) QUALITY CONTROL REQUIREMENTS

Method Reference	Requirement	Specification and Frequency	
Section 9.2.1	Demonstration of low system background	Analyze a Laboratory Reagent Blank (LRB) prior to any other IDC steps.	Demonstrate that all method analytes are <1/3 of the Minimum Reporting Level (MRL) and that possible interferences from reagents and glassware do not prevent the identification and quantitation of method analytes.
Section 9.2.2	Demonstration of precision	Extract and analyze four to seven replicate Laboratory Fortified Blanks (LFBs) fortified near the midrange concentration.	Percent relative standard deviation must be ≤20%
Section 9.2.3	Demonstration of accuracy	Calculate average recovery for replicates used in Section 9.2.2.	Mean recovery 70-130% of the true value
Section 9.2.4	MRL confirmation	Fortify, extract, and analyze seven replicate LFBs at the proposed MRL concentration. Calculate the mean and the half range (HR). Confirm that the upper prediction interval of results (PIR) and lower PIR (Sect. 9.2.4.2) meet the recovery criteria.	Upper PIR ≤ 150% Lower PIR ≥ 50%
Section 9.2.5	Quality Control Sample (QCS)	Analyze mid-level QCS sample.	Results must be 70-130% of the true value.

TABLE 11. ONGOING QUALITY CONTROL REQUIREMENTS

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Section 8.4	Sample holding time	28 days when processed and stored according to Sections 8.1 and 8.3.	Sample results are valid only if samples are analyzed within the sample holding time.
Section 9.3.1	Laboratory Reagent Blank (LRB)	One with each Extraction Batch	Demonstrate that all method analytes are below 1/3 the Minimum Reporting Level (MRL), and that possible interference from reagents and glassware do not prevent identification and quantitation of method analytes.
Section 9.3.3	Laboratory Fortified Blank (LFB)	One with each Extraction Batch Rotate the fortified concentrations between low, medium, and high levels.	Results of LFB analyses at medium and high fortifications must be 70-130% of the true value for each analyte and surrogate. Results of the low-level LFB must be 50-150% of the true value.
Section 9.3.5	Internal standard (IS)	Atrazine-d_5 and Atrazine-desethyl-d_7 are added to all standards and sample extracts.	Peak area counts for all ISs in field and QC sample extracts must be within ±50% of the average peak area calculated during the initial calibration and ±30% from the most recent continuing calibration check (CCC). If ISs do not meet these criteria, corresponding method results are invalid.
Section 9.3.6	Surrogates	Surrogates are added to all field and QC samples prior to extraction.	70-130% recovery
Section 9.3.7	Laboratory Fortified Sample Matrix (LFSM)	One LFSM per Extraction Batch. Fortify the LFSM with method analytes at a concentration close to but greater than the native concentrations (if known).	For LFSMs fortified at concentrations ≤2 x MRL, the result must be within 50-150% of the true value. All other LFSMs must be within 70-130% of the true value.
Section 9.3.8	Laboratory Fortified Sample Matrix Duplicate (LFSMD) or Field Duplicate (FD)	One LFSMD or FD with each Extraction Batch	For LFSMDs or FDs, RPDs must be ≤30% at middle and high levels of fortification and ≤50% at concentrations ≤2 x the MRL.
Section 9.3.9	Quality Control Sample (QCS)	Analyze mid-level QCS sample when new stock standard solutions are prepared and at least quarterly if stock standards are prepared less frequently.	Results must be 70-130% of the true value.

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Section 10.2	Initial calibration	Use the internal standard calibration technique to generate a linear or quadratic calibration curve. Use at least 5 standard concentrations. Validate the calibration curve as described in Section 10.2.5.	When each calibration standard is calculated as an unknown using the calibration curve, the lowest level standard must be within 50-150% of the true value. All other points must be within 70-130% of the true value.
Section 10.3	CCC	Verify initial calibration by analyzing a low-level CCC at the beginning of each Analysis Batch. Subsequent CCCs are required after every 10 field samples, and after the last field sample in a batch.	The lowest level CCC must be within 50-150% of the true value. All other points must be within 70-130% of the true value. Results for field samples that are not bracketed by acceptable CCCs are invalid.

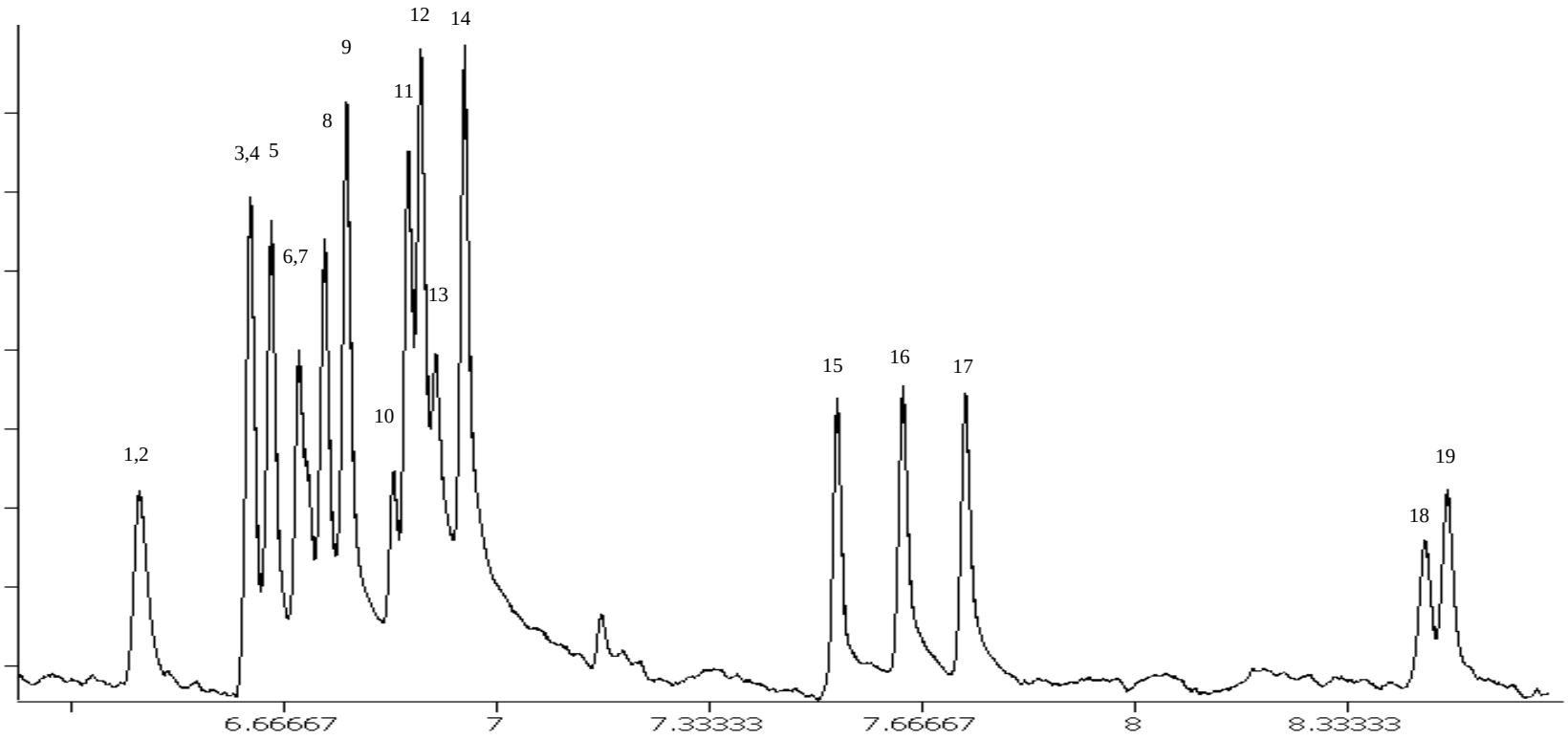

Figure 1. Example chromatogram recorded on the GC/TOF-MS for a chlorinated groundwater fortified with Method 523 analytes at 5 μg/L. Numbered peaks are identified in Table 3.