METHOD 526. DETERMINATION OF SELECTED SEMIVOLATILE ORGANIC COMPOUNDS IN DRINKING WATER BY SOLID PHASE EXTRACTION AND CAPILLARY COLUMN GAS CHROMATOGRAPHY/ MASS SPECTROMETRY (GC/MS)

Revision 1.0

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METHOD 526

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1. SCOPE AND APPLICATION

1.1 This is a gas chromatography/mass spectrometry (GC/MS) method for the determination of selected semivolatile organic compounds in raw and finished drinking waters. This method is applicable to the organic compounds listed below, which are efficiently extracted from water using a polystyrene divinylbenzene solid phase sorbent, and are sufficiently volatile and thermally stable for gas chromatography. Accuracy, precision, and method detection limit (MDL) data have been generated in reagent water, finished ground and surface water for the following compounds:

Analyte	Chemical Abstract Services Registry Number
Acetochlor	34256-82-1
Cyanazine	21725-46-2
Diazinon	61790-53-2
2,4-Dichlorophenol	120-83-2
1,2-Diphenylhydrazine	122-66-7
Disulfoton	298-04-4
Fonofos	944-22-9
Nitrobenzene	98-95-3
Prometon	1610-18-0
Terbufos	13071-79-9
2,4,6-Trichlorophenol	88-06-2

1.2 MDLs are compound, instrument, and matrix dependent. The MDL is defined as the statistically calculated minimum concentration that can be measured with 99% confidence that the reported value is greater than zero.[1] Experimentally determined MDLs for the above listed analytes are provided in Section 17, Table 3. The MDL differs from, and is lower than, the minimum reporting limit (MRL) (Sect. 3.17). Precision and accuracy were evaluated at 0.5 and 20 ug/L. Precision and accuracy data and sample

holding time data are presented Section 17, Tables 4 through 8. Analyte retention times are in Section 17, Table 2.

1.3 This method is restricted to use by or under the supervision of analysts skilled in solid-phase extractions (SPE) and GC/MS analysis.

2. SUMMARY OF METHOD

2.1 A 1 liter water sample is passed through a SPE disk or cartridge containing polystyrenedivinylbenzene (SDVB) to extract the target analytes and surrogate compounds. The extract is dried by passing through a column of anhydrous sodium sulfate and is concentrated by blowdown with nitrogen to a volume of about 0.7 mL. Internal standards are added and the extract is diluted to a final volume of 1 mL. Components are separated chromatographically by injecting an aliquot of the extract onto a gas chromatograph equipped with a high resolution fused silica capillary column. The analytes pass from the capillary column into a mass spectrometer where the they are identified by comparing their measured mass spectra and retention times to reference spectra and retention times collected for the same compounds. Instrument specific reference spectra and retention times for analytes are obtained by the analyses of calibration standards under the same GC/MS conditions used for samples. The concentration of each identified component is measured by relating the MS response of the compound's quantitation ion to the internal standard's quantitation ion MS response.

3. DEFINITIONS

3.1 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, 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 Matrix Duplicate.

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

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

3.4 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.

3.5 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, sample preservatives, internal standards, and surrogates that are used in the extraction batch. The LRB is analyzed is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

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

3.7 LABORATORY FORTIFIED SAMPLE MATRIX (LFM) – An aliquot of an environmental sample to which known quantities of the method analytes and all the preservation compounds are added in the laboratory. The LFM 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 LFM corrected for background concentrations.

3.8 LABORATORY FORTIFIED SAMPLE MATRIX DUPLICATE (LFMD) – A second aliquot of the Field Sample used to prepare the LFM which is fortified, extracted and analyzed identically. The LFMD is used instead of the Field Duplicate to access method precision and accuracy when the occurrence of target analytes is low.

3.9 LABORATORY DUPLICATES (LD1 and LD2) – Two aliquots of the same sample taken in the laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 indicate precision associated with laboratory procedures, but not with sample collection, preservation, and storage procedures.

3.10 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.

3.11 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.

3.12 PRIMARY DILUTION STANDARD SOLUTION (PDS) – A solution containing method analytes prepared in the laboratory from stock standard solutions and diluted as needed to prepare calibration solutions and other needed analyte solutions.

3.13 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.

3.14 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.

3.15 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 standard integrity.

3.16 METHOD DETECTION LIMIT (MDL) – The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero (Section 9.2.4). This is a statistical determination of precision. Accurate quantitation is not expected at this level.[1]

3.17 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 continuing calibration check standard for that analyte, and can only be used if acceptable quality control criteria for this standard are met.

3.18 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.

4. INTERFERENCES

4.1 All glassware must be meticulously cleaned. Wash glassware with detergent and tap water, rinse with tap water, followed by reagent water. Non-volumetric glassware can be heated in a muffle furnace at 400 °C for 2 hours as a substitute for a solvent rinse. Volumetric glassware should not be heated in an oven above 120 °C.

4.2 Method interferences may be caused by contaminants in solvents, reagents (including reagent water), sample bottles and caps, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in the chromatograms. All items such as these must be routinely demonstrated to be free from interferences (less than $^1/_3$ the MRL for each target analyte) under the conditions of the analysis by analyzing laboratory reagent blanks as described in Section 9.4. **Subtracting blank values from sample results is not permitted.**

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

depending upon the nature of the water. Water samples high in total organic carbon may have elevated baseline or interfering peaks.

4.4 Relatively large quantities of the buffer and preservatives (Sect. 8.1) are added to sample bottles. The potential for trace-level organic contaminants in these reagents exist. Interferences from these sources should be monitored by analysis of laboratory reagent blanks, particularly when new lots of reagents are acquired.

4.5 Benzophenone was an interferent recovered at low level from one manufacturer's lot of tris(hydroxymethyl)aminomethane hydrochloride. The spectra of benzophenone and 1,2-diphenylhydrazine are very similar, sharing the major m/z ions of 51, 77, 105, 152, and 182. At first glance, the benzophenone may appear to be a contaminant in the LRB. Given that the two compounds share common ions, their chromatographic peaks must be resolved.

4.6 During method development, one lot of anhydrous sodium sulfate was found to add an agent to the extract that, when injected, caused complete loss of prometon recovery and caused severe chromatographic tailing of the phenols. It is very important to check a new sodium sulfate lot before general use. When only one or two samples with the interfering agent were injected, recovery of good chromatographic performance was possible by removing the first meter of the capillary column and replacing the deactivated glass inlet liner.

4.7 Solid phase cartridges and disks and their associated extraction devices have been observed to be a source of interferences. The analysis of field and laboratory reagent blanks can provide important information regarding the presence or absence of such interferences. Brands and lots of solid phase extraction devices should be tested to ensure that contamination will not preclude analyte identification and quantitation.

4.8 Analyte carryover may occur when a relatively "clean" sample is analyzed immediately after a sample containing relatively high concentrations of compounds. Syringes and splitless injection port liners must be cleaned carefully or replaced as needed. 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.

4.9 Silicone compounds may be leached from punctured autosampler vial septa, particularly when particles of the septa sit in the vial. These silicone compounds should have no effect on the analysis, but the analyst should be aware of this potential problem.

5. SAFETY

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

method. A reference file of MSDSs should be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available.[2-4]

5.2 Pure standard materials and stock standard solutions of these compounds should be handled with suitable protection to skin and eyes, and care should be taken not to breath the vapors or ingest the materials.

6. <u>EQUIPMENT AND SUPPLIES</u> (All specifications are suggested. Catalog numbers are included for illustration only.)

6.1 SAMPLE CONTAINERS – 1 liter glass bottles fitted with polytetrafluoroethylene (PTFE) lined screw caps.

6.2 VIALS – Various sizes of amber glass vials with PTFE lined screw caps for storing standard solutions and extracts. Crimp-top glass autosampler vials, 2 mL, with PTFE faced septa.

6.3 VOLUMETRIC FLASKS – Class A, suggested sizes include 1, 5, and 10 mL, for preparation of standards and dilution of extract to final volume.

6.4 GRADUATED CYLINDERS – Suggested sizes include 5, 10, and 250 mL.

6.5 MICRO SYRINGES – Suggested sizes include 25, 50, 100, 250, 500, and 1000 mL.

6.6 DRYING COLUMN – The drying column must be able to contain 5 to 7 g of anhydrous sodium sulfate. The drying column should not leach interfering compounds or irreversibly adsorb target analytes. Any small column may be used, such as a glass pipet with glass wool plug.

6.7 CONICAL CENTRIFUGE TUBES – 15 mL, or other glassware suitable for collection of the eluate from the cartridge or disk after extraction.

6.8 COLLECTION TUBES OR VIALS – 25 mL or larger, or other glassware suitable for collecting extract from drying tube. Conical centrifuge tubes, 50 mL, with graduations (VWR #: 21049-063) were used to develop this method.

6.9 ANALYTICAL BALANCE – Capable of weighing to the nearest 0.0001 g.

6.10 SOLID PHASE EXTRACTION (SPE) APPARATUS USING CARTRIDGES

6.10.1 SOLID PHASE EXTRACTION CARTRIDGES – 6 mL, packed with 500 mg (125 um d_p) of polystyrene divinylbenzene (SDVB) sorbent phase (Varian Bond Elut ENV phase; cat.#:1225-5011 or equivalent).

6.10.2 SAMPLE RESERVOIR AND TRANSFER TUBE– Sample reservoirs (VWR

cat.#: JT7120-3 or equivalent) with a volume of about 75 mL are attached to the cartridges to hold the water sample. An alternative method is using transfer tubes (Supelco "Visiprep"; cat.#: 57275 or equivalent) which transfer the sample directly from the sample container to the SPE cartridge.

6.10.3 VACUUM EXTRACTION MANIFOLD – With flow/vacuum control (Supelco cat.#: 57044 or equivalent). The use of replaceable needles or valve liners may be used to prevent cross contamination.

6.10.4 REMOTE VACUUM GAUGE/BLEED VALVE ASSEMBLY – To monitor and adjust vacuum pressure delivered to the vacuum manifold (Supelco cat.#: 57161-U or equivalent)

6.10.5 An automatic or robotic system, designed for use with SPE cartridges, may be used as long as 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. All extraction and elution steps must be the same as in the manual procedure. Extraction or elution steps may not be changed or omitted to accommodate the use of an automated system.

6.11 SOLID PHASE EXTRACTION (SPE) APPARATUS USING DISKS

6.11.1 SOLID PHASE EXTRACTION DISKS – 47 mm diameter and 0.5 mm thick, manufactured with a polystyrene divinylbenzene (SDVB) sorbent phase (Varian cat. #: 1214-5010 or equivalent). Larger disks may be used as long as the QC performance criteria outlined in Section 9 are met.

6.11.2 SPE DISK EXTRACTION GLASSWARE – funnel, PTFE coated support screen, PTFE gasket, base, and clamp used to support SPE disks and contain samples during extraction. May be purchased as a set (Fisher cat. #:K971100-0047 or equivalent) or separately.

6.11.3 VACUUM EXTRACTION MANIFOLD – Designed to accommodate extraction glassware and disks (Varian cat.#: 1214-6001 or equivalent).

6.11.4 An automatic robotic system for disks as described in Section 6.10.5.

6.12 EXTRACT CONCENTRATION SYSTEM – Extracts are concentrated by blowdown with nitrogen using water bath set to 40°C (Meyer N-Evap, Model 111, Organomation Associates, Inc. or equivalent).

6.13 LABORATORY OR ASPIRATOR VACUUM SYSTEM – Sufficient capacity to maintain a vacuum of about 10 inches of mercury for cartridges. A greater vacuum of 15 to 25 inches of mercury may be used with disks.

6.14 GAS CHROMATOGRAPH/MASS SPECTROMETER (GC/MS) INSTRUMENTATION

6.14.1 FUSED SILICA CAPILLARY GC COLUMN – 30 m x 0.25 mm i.d. fused silica capillary column coated with a 0.25 um bonded film of poly(dimethylsiloxy)poly(1,4-bis(dimethylsiloxy)phenylene)siloxane (J&W DB-5MS or equivalent). Any capillary column that provides adequate resolution, capacity, accuracy, and precision as summarized in Section 17 may be used. A nonpolar, low-bleed column is recommended for use with this method to provide adequate chromatography and minimize column bleed.

6.14.2 GC INJECTOR AND OVEN – Capable of temperature programming and equipped for split/splitless injection. Target compounds included in this method are subject to thermal breakdown in the injector port, which increases when the injector is not properly deactivated or at excessive temperatures. The injection system must not allow analytes to contact hot stainless steel or other metal surfaces that promote decomposition. The performance data in Section 17 was obtained by hot, splitless injection using a 4 mm i.d. glass, deactivated liner (Restek cat.#: 20772). Other injection techniques such as temperature programmed injections, cold on-column injections and large volume injections may be used if the QC criteria in Sections 9 and 10 are met. Equipment designed appropriately for these alternate types of injections must be used if these options are selected.

6.14.3 GC/MS INTERFACE – Interface should allow the capillary column or transfer line exit to be placed within a few mm of the ion source. Other interfaces, like jet separators, are acceptable as long as the system has adequate sensitivity and QC performance criteria are met.

6.14.4 MASS SPECTROMETER – The MS must be capable of electron ionization at a nominal electron energy of 70 eV to produce positive ions. The spectrometer must be capable of scanning at a minimum from 45 to 450 amu with a complete scan cycle time (including scan overhead) of 1.0 second or less. (Scan cycle time = total MS data acquisition time in seconds divided by number of scans in the chromatogram). The spectrometer must produce a mass spectrum that meets all criteria in Table 1 when a solution containing approximately 5 ng of DFTPP is injected into the GC/MS. Use a single spectrum at the apex 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. The scan time should be set so that all analytes have a minimum of five scans across the chromatographic peak. Seven to ten scans across chromatographic peaks are preferred.

6.14.5 DATA SYSTEM – An interfaced data system is required to acquire, store, and output mass spectral data. The computer software should have the capability of

processing stored GC/MS data by recognizing a GC 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 be able to calculate relative response factors, construct a linear regression or quadratic calibration curve, and calculate analyte concentrations.

7. REAGENTS AND STANDARDS

7.1 REAGENTS AND SOLVENTS – Reagent grade or better chemicals should be used in all tests. 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, provided it is first determined that the reagent is of sufficiently high purity to permit its use without lessening the quality of the determination.

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

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

7.1.3 METHANOL (CAS# 67-56-1) – High purity, demonstrated to be free of analytes and interferences (B&J Brand GC$^{2®}$, Capillary GC/GC-MS Grade or equivalent).

7.1.4 ETHYL ACETATE (CAS# 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 METHYLENE CHLORIDE (CAS# 75-09-02) – 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 (CAS# 7757-82-6) – Soxhlet extracted with methylene chloride for a minimum of four hours or heated to 400^{o}C for two hours in a muffle furnace. One lot of "ACS grade" anhydrous sodium sulfate had a contaminant that degraded the capillary column so that analyte recoveries were unacceptable. An "ACS grade, suitable for pesticide residue analysis," or equivalent, of anhydrous sodium sulfate is recommended.

7.1.7 SAMPLE PRESERVATION REAGENTS – These preservatives are solids at room temperature and may be added to the sample bottle before shipment to the field.

7.1.7.1 BUFFER SALT MIX, pH 7 – The sample must be buffered to pH 7 with two components: 1) tris(hydroxymethyl)aminomethane, also called Tris,

0.47 g (CAS# 77-86-1, ACS Reagent Grade or equivalent); and 2) tris(hydroxymethyl)aminomethane hydrochloride, also called Tris HCl, 7.28 g (CAS# 1185-53-1, ACS Reagent Grade or equivalent). Alternately, 7.75 g of a commercial buffer crystal mixture, that is blended in proportion to the amounts given above, can be used.

7.1.7.2 L-ASCORBIC ACID (CAS# 50-81-7) – Ascorbic acid reduces "free chlorine" at the time of sample collection (ACS Reagent Grade or equivalent).

7.1.7.3 ETHYLENEDIAMINETETRAACETIC ACID, TRISODIUM SALT (Trisodium EDTA, CAS# 10378-22-0) – Trisodium EDTA is added to inhibit metal-catalyzed hydrolysis of analytes. The trisodium salt is used instead of the disodium salt because the trisodium salt solution pH is closer to the desired pH of 7 (Sigma cat.#: ED3SS or equivalent).

7.1.7.4 DIAZOLIDINYL UREA (DZU) (CAS# 78491-02-8) – DZU is added to inhibit microbial growth. DZU (Sigma cat.#: D-5146) is commonly used as a preservative in cosmetics such as skin lotion.

7.2 STANDARD SOLUTIONS – When a compound purity is assayed to be 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Solution concentrations listed in this section were used to develop this method and are included as an example. Standards for sample fortification generally should be prepared in the smallest volume that can be accurately measured to minimize the addition of excess organic solvent to aqueous samples. **Even though stability times for standard solutions are suggested in the following sections, laboratories should used standard QC practices to determine when their standards need to be replaced.**

7.2.1 INTERNAL STANDARD SOLUTIONS – This method uses three internal standard compounds listed in the table below.

Internal Standards	CAS#	FW
acenaphthene-d_{10}	15067-26-2	164.3
phenanthrene-d_{10}	1517-22-2	188.3
chrysene-d_{12}	1719-03-5	240.4

7.2.1.1 INTERNAL STANDARD PRIMARY DILUTION STANDARD (500 ug/mL) – Prepare or purchase commercially the Internal Standard Primary Dilution Standard (PDS) at a concentration of 500 ug/mL. If prepared from neat or solid standards, this solution requires the preparation of a more concentrated stock standard similar to the procedure followed for the

analyte stock (Sect. 7.2.3.1). The solvent for the Internal Standard PDS may be acetone or ethyl acetate. The Internal Standard PDS has been shown to be stable for 1 year in amber glass screw cap vials when stored at !10°C or less.

7.2.1.2 INTERNAL STANDARD EXTRACT FORTIFICATION SOLUTION (50 ug/mL) – Dilute a portion of the Internal Standard PDS (500 ug/mL) (Sect. 7.2.1.1) to a concentration of 50 ug/mL in ethyl acetate and use this solution to fortify the final 1 mL extracts (Sect. 11.6). The Internal Extract Fortification Solution has been shown to be stable in amber glass screw cap vials for 6 months when stored at !10°C or less.

7.2.2 SURROGATE (SUR) ANALYTE STANDARD SOLUTIONS – The two surrogates for this method are listed in the table below.

Surrogates	CAS #	FW
1,3-dimethyl-2-nitrobenzene	81-20-9	151.2
triphenylphosphate	115-86-6	326.3

7.2.2.1 SUR STOCK SOLUTION (~ 4 to 10 mg/mL) – Surrogate Stock Solutions may be purchased commercially or prepared from neat materials. The solvent for the SUR Stock Solution may be acetone or ethyl acetate. These solutions have been shown to be stable for one year when stored in amber glass containers at -10°C or less.

7.2.2.2 SUR PRIMARY DILUTION STANDARD (SUR PDS) (500 ug/mL) – The 500 ug/mL SUR PDS may be purchased commercially or prepared by volumetric dilution of the SUR Stock Solutions (Sect. 7.2.2.1) in acetone or ethyl acetate The PDS has been shown to be stable for one year when stored in amber glass screw cap vials at !10°C or less. This solution is used to make the 50 ug/mL solution for sample fortification and also to prepare calibration solutions.

7.2.2.3 SUR SAMPLE FORTIFICATION SOLUTION (50 ug/mL) – Dilute the 500 ug/mL SUR PDS in methanol to make a 50 ug/mL sample fortification solution. Add 100 uL of this 50 ug/mL solution to each 1 liter water sample before extraction to give a concentration of 5 ug/L of each surrogate. This solution has been shown to be stable for six months when stored in amber glass screw cap vials at !10°C or less.

7.2.3 ANALYTE STANDARD SOLUTIONS – Obtain the analytes listed in the table in Section 1.1 as neat or solid standards or as commercially prepared ampulized solutions from a reputable standard manufacturer. Prepare the Analyte Stock and

Primary Dilution Standards as described below.

7.2.3.1 ANALYTE STOCK STANDARD SOLUTIONS (1 to 10 mg/mL) – Analyte standards may be purchased commercially as ampulized solutions or prepared from neat materials. Stock standards have been shown to be stable for one year when stored in amber glass screw cap vials at !10°C or less.

7.2.3.2 ANALYTE PRIMARY DILUTION STANDARDS (200 ug/mL and 20 ug/mL) – Prepare the 200 ug/mL Analyte PDS by volumetric dilution of the Analyte Stock Standard Solution (Sect. 7.2.3.1) in ethyl acetate to make a 200 ug/mL solution. The 20 ug/mL Analyte PDS can be made by a volumetric dilution of the 200 ug/mL Analyte PDS in ethyl acetate. The Analyte PDSs are used to prepare calibration and fortification standards. They have been shown to be stable for six months when stored in an amber glass screw cap vial at !10°C or less. Check frequently for signs of evaporation, especially before preparing calibration solutions.

7.2.4 CALIBRATION SOLUTIONS – Prepare a calibration curve of at least 5 CAL levels over the concentration range of interest from dilutions of the Analyte PDSs in ethyl acetate. All calibration solutions should contain at least 80% ethyl acetate so that gas chromatographic performance is not compromised. The lowest concentration of calibration standard must be at or below the MRL. The level of the MRL will depend on system sensitivity. A constant concentration of each internal standard and surrogate (in the range of 2 to 5 ng/uL) is added to each CAL. For instance, for method development work, 50 uL of the 500 ug/mL Internal Standard PDS and 50 uL of the 500 ug/mL SUR PDS were added to each CAL level standard for final concentrations of 5 ug/mL. The calibration solutions have been shown to be stable for six months when stored in an amber glass screw cap vial at !10°C or less.

7.2.5 ANALYTE FORTIFICATION SOLUTIONS (0.05 to 5.0 ug/mL) – The Analyte Fortification Solutions contain all method analytes of interest in methanol. They are prepared by dilution of the Analyte PDSs (200 ug/mL or 20 ug/mL). These solutions are used to fortify the LFBs, the LFMs and LFMDs with method analytes. It is recommended that three concentrations be prepared so that the fortification levels can be rotated. The Analyte Fortification Solutions have been shown to be stable for six months when stored in an amber glass screw cap vial at !10°C or less.

7.2.6 GC/MS TUNE CHECK SOLUTION (5 ug/mL) – Prepare a Decafluorotriphenylphosphine (DFTPP) solution in methylene chloride. DFTPP is more stable in methylene chloride than in acetone or ethyl acetate. Store this solution in an amber glass screw cap vial at !10°C or less.

8. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 SAMPLE BOTTLE PREPARATION

8.1.1 Grab samples must be collected in accordance with conventional sampling practices [5] using a 1 liter or 1 quart amber or clear glass bottle fitted with PTFE-lined screw-caps.

8.1.2 Preservation reagents, listed in the table below, are added to each sample bottle prior to shipment to the field.

Compound	Amount	Purpose
L-Ascorbic Acid	0.10 g/L	Dechlorination
Ethylenediaminetetraacetic acid trisodium salt	0.35 g/L	Inhibit metal-catalyzed hydrolysis of targets
Diazolidinyl Urea	1.0 g/L	Microbial inhibitor
***Tris(hydroxymethyl)aminomethane**	0.47 g/L	First component of pH 7 buffer mixture
***Tris(hydroxymethyl)aminomethane hydrochloride**	7.28 g/L	Second component of pH 7 buffer mixture

*Alternately, 7.75 g of a commercial buffer crystal mixture, that is blended in the proportions given in the table, can be used (Sect. 7.1.7.1).

8.1.2.1 Residual chlorine must be reduced at the time of sample collection with 100 mg of ascorbic acid per liter. Sodium thiosulfate and sodium sulfite cannot be used because they were found to degrade target analytes. In addition, while ammonium chloride is effective in converting free chlorine to chloramines, the chloramines also caused target compound loss.

8.1.2.2 Ethylenediaminetetraacetic acid, trisodium salt (trisodium EDTA) (0.35 g) must be added to inhibit metal-catalyzed hydrolysis of the target analytes, principally terbufos, disulfoton, diazinon, fonofos, and cyanazine.

8.1.2.3 Diazolidinyl urea (1.0 g) is added to inhibit microbial degradation of analytes. Diazolidinyl urea is used in cosmetics such as skin lotion. The antimicrobial activity of diazolidinyl urea has been proposed as due to protein alkylation of sulfhydryl groups and the ability to release formaldehyde.[6] Plate count studies conducted during method development indicated that it was effective in inhibiting microbial degradation for extended periods.

8.1.2.4 The sample must be buffered to pH 7 to reduce the acid and base catalyzed

hydrolysis of target analytes. The pH buffer has two components: tris(hydroxymethyl)aminomethane (0.47 g) and tris(hydroxymethyl)aminomethane hydrochloride (7.28 g). A commercially prepared combination of these two compounds can be purchased as pre-mixed crystals. When using the pH 7 pre-mixed crystals, add 7.75 g per liter of water sample.

8.2 SAMPLE COLLECTION

8.2.1 When sampling from a cold water tap, remove the aerator so that no air bubbles will be trapped in the sample. Open the tap and allow the system to flush until the water temperature has stabilized (usually about 3-5 minutes). Collect samples from the flowing system.

8.2.2 When sampling from an open body of water, fill a 1 quart wide-mouth bottle or 1 L beaker with water sampled from a representative area, and carefully fill sample bottles from the container. Sampling equipment, including automatic samplers, must be free of plastic tubing, gaskets, and other parts that may leach interfering analytes into the water sample.

8.2.3 Fill sample bottles, taking care not to flush out the sample preservation reagents. Samples do not need to be collected headspace free.

8.2.4 After collecting the sample, cap the bottle and agitate by hand until preservatives are dissolved. Keep the sample sealed from time of collection until extraction.

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 should 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.

8.4 SAMPLE AND EXTRACT HOLDING TIMES – Results of the sample storage stability study (Sect. 17, Table 7) indicated that most compounds listed in this method have adequate stability for 14 days when collected, dechlorinated, preserved, shipped and stored as described in Sections 8.1, 8.2, and 8.3. At 14 days, even the most unstable compound, terbufos, was recovered above 60 %. Water samples should be extracted as soon as possible, but must be extracted within 14 days. The extract storage stability study must be stored at 0°C or less and analyzed within 28 days after extraction. The extract storage stability study data are presented in Section 17, Table 8.

9. QUALITY CONTROL

9.1 Quality control (QC) requirements include the Initial Demonstration of Capability (Sect. 17, Table 9), the determination of the MDL, and subsequent analysis in each analysis batch of a Laboratory Reagent Blank (LRB), Continuing Calibration Check Standards

(CCC), a Laboratory Fortified Blank (LFB), a Laboratory Fortified Sample Matrix (LFM), and either a Laboratory Fortified Sample Matrix Duplicate (LFMD) or a Field Duplicate Sample. This section details the specific requirements for each QC parameter. The QC criteria discussed in the following sections are summarized in Section 17, Tables 9 and 10. These criteria are considered the minimum acceptable QC criteria, and laboratories are encouraged to institute additional QC practices to meet their specific needs.

9.2 INITIAL DEMONSTRATION OF CAPABILITY (IDC) – Requirements for the Initial Demonstration of Capability are described in the following sections and summarized in Section 17, Table 9.

9.2.1 INITIAL DEMONSTRATION OF LOW SYSTEM BACKGROUND – Any time a new lot of solid phase extraction (SPE) cartridges or disks is used, it must be demonstrated that a laboratory reagent blank (Sect. 9.4) is reasonably free of contamination and that the criteria in Section 9.4 are met.

9.2.2 INITIAL DEMONSTRATION OF PRECISION – Prepare, extract, and analyze 4-7 replicate LFBs fortified near the midrange of the initial calibration curve according to the procedure described in Section 11. Sample preservatives as described in Section 8.1 must be added to these samples. The relative standard deviation (RSD) of the results of the replicate analyses must be less than 20%.

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

9.2.4 MDL DETERMINATION – Prepare, extract and analyze at least seven replicate LFBs at a concentration estimated to be near the MDL, over a period of at least three days (both extraction and analysis should be conducted over at least three days) using the procedure described in Section 11. The fortification level may be estimated by selecting a concentration with a signal of 2 to 5 times the noise level. The appropriate concentration will be dependent upon the sensitivity of the GC/MS system being used. Sample preservatives as described in Section 8.1 must be added to these samples. Calculate the MDL using the equation

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

where
$t_{(n - 1, 1 - alpha = 0.99)}$ = Students t value for the 99% confidence level with n-1 degrees of freedom,
n = number of replicates, and
S = standard deviation of replicate analyses.

NOTE: Do not subtract blank values when performing MDL calculations. The

MDL is a statistical determination of precision only.[1] If the MDL replicates are fortified at a low enough concentration, it is likely that they will not meet precision and accuracy criteria.

9.2.5 METHOD MODIFICATIONS – The analyst is permitted to modify GC columns, GC conditions, evaporation techniques, internal standards or surrogate standards, but each time such method modifications are made, the analyst must repeat the procedures of the IDC (Sect. 9.2).

9.3 MINIMUM REPORTING LEVEL (MRL) – The MRL is a threshold concentration of an analyte that a laboratory can expect to accurately quantitate in an unknown sample. The MRL should be established at an analyte concentration that is either greater than three times the MDL or at an analyte concentration which would yield a response greater than a signal-to-noise ratio of five. **Although the lowest calibration standard for an analyte may be below the MRL, the MRL must never be established at a concentration lower than the lowest calibration standard.**

9.4 LABORATORY REAGENT BLANK (LRB) – An LRB is required with each extraction batch (Sect. 3.1) of samples to determine the background system contamination. If the LRB produces a peak within the retention time window of any analyte 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 analytes must be below 1/3 of the MRL. If the target 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 in the extraction batch.

9.5 CONTINUING CALIBRATION CHECK (CCC) – A CCC is a standard prepared with all compounds of interest which is analyzed during the analysis batch to ensure the stability of the instrument initial calibration. This calibration check is required at the beginning of each day that samples are analyzed, after every ten injections, 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) – An LFB is required with each extraction batch (Sect. 3.6). The fortified concentration of the LFB should be rotated between low, medium, and high concentrations from day to day. The low concentration LFB must be as near as practical to, but no more than two times the MRL. Similarly, the high concentration LFB should 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 50-160% of the true value. Results of the medium and high-level LFB analyses must be 70-130% of the true value. If the LFB results do not meet these criteria for target analytes, then all data for the problem analyte(s) must be considered invalid for all samples in the extraction batch.

9.7 MS TUNE CHECK – A complete description of the MS tune check is in Section 10.2.1. This 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). In this method daily DFTPP analysis is not required.

9.8 INTERNAL STANDARDS (IS) – The analyst must monitor the peak area of each internal standard in all injections during each analysis day. The IS response (as indicated by peak area) for any chromatographic run must not deviate by more than ±50% from the average area measured during the initial calibration for that IS. A poor injection could cause the IS area to exceed these criteria. Inject a second aliquot of the suspect extract to determine whether the failure is due to poor injection or instrument response drift.

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

9.8.2 If the internal standard area for the reinjected extract deviates greater than 50% from the initial calibration average, the analyst should check the continuing calibration check standards that ran before and after the sample. If the continuing calibration check fails the criteria of Section 9.5 and 10.3, recalibration is in order per Section 10. If the calibration standard is acceptable, extraction of the sample should be repeated provided the sample is still within holding time. Otherwise, report results obtained from the reinjected extract, but annotate as suspect.

9.9 SURROGATE RECOVERY – The surrogate standards are fortified into the aqueous portion of all samples, duplicates, LRBs, LFMs and LFMDs prior to extraction. Surrogates are also added to the calibration curve and calibration check standards. The surrogate is a means of assessing method performance from extraction to final chromatographic measurement.

9.9.1 When surrogate recovery from a sample, blank, or CCC is less than 70% or greater than 130%, check (1) calculations to locate possible errors, (2) standard solutions for degradation, (3) contamination, and (4) instrument performance. If those steps do not reveal the cause of the problem, reanalyze the extract.

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

9.9.3 If the extract reanalysis fails the 70-130% surrogate recovery criterion, the analyst should check the surrogate calibration by analyzing the most recently acceptable calibration standard. If the calibration standard fails the 70-130% surrogate recovery criteria of Section 9.9.1, recalibration is in order. If the surrogate recovery of the calibration standard is acceptable, extraction of the sample should be repeated, provided the sample is still within the holding time. If the sample re-extract also fails the recovery criterion, report all data for that sample as suspect/surrogate recovery.

9.9.4 The surrogate, 1,3-dimethyl-2-nitrobenzene, is used to track the recovery of nitrobenzene. The other surrogate, triphenylphosphate, monitors the recovery of all the other target analytes. If nitrobenzene is not included on the target analyte list, then the first surrogate, 1,3-dimethyl-2-nitrobenzene, does not need to be analyzed.

9.10 LABORATORY FORTIFIED SAMPLE MATRIX AND DUPLICATE (LFM AND LFMD) – Analyses of LFMs (Sect. 3.7) are required in each extraction batch and are used to determine that the sample matrix does not adversely affect method accuracy. If the occurrence of target analytes in the samples is infrequent, or if historical trends are unavailable, a second LFM, or LMFD, must be prepared, extracted, and analyzed from a duplicate field sample used to prepare the LFM to assess method precision. Extraction batches that contain LFMDs will not require the analysis of a Field Duplicate (Sect. 9.11). If a variety of different sample matrices are analyzed regularly, for example, drinking water from groundwater and surface water sources, method performance should be established for each. Over time, LFM data should be documented for all routine sample sources for the laboratory.

9.10.1 Within each extraction batch, a minimum of one field sample is fortified as an LFM for every 20 samples extracted. The LFM is prepared by spiking a sample with an appropriate amount of Analyte PDS (Sect. 7.2.5). Select a spiking concentration that is at least twice the matrix background concentration, if known. Use historical data or rotate through the designated concentrations to select a fortifying concentration. Selecting a duplicate bottle of a sample that has already been analyzed aids in the selection of appropriate spiking levels.

9.10.2 Calculate the percent recovery (R) for each analyte using the equation

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

where: A = measured concentration in the fortified sample,
B = measured concentration in the unfortified sample, and
C = fortification concentration.

9.10.3 Analyte recoveries may exhibit matrix bias. For samples fortified at or above their native concentration, recoveries should range between 70 and 130%, except for low-level fortification near or at the MRL where 50 to 160% recoveries are acceptable. If the accuracy of any analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control in the LFB, the recovery is judged to be matrix biased. The result for that analyte in the unfortified sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

9.10.4 If an LFMD is analyzed instead of a Field Duplicate (Sect. 9.11), calculate the

$$RPD = \frac{LFM - LFMD}{(LFM + LFMD)/2} * 100 \qquad \textbf{(1)}$$

relative percent difference (RPD) for duplicate LFMs (LFM and LFMD) using the equation RPDs for duplicate LFMs should fall in the range of ±30% for samples fortified at or above their native concentration. Greater variability may be observed when LFMs are spiked near the MRL. At the MRL, RPDs should fall in the range of ±50% for samples fortified at or above their native concentration. If the accuracy of any analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control in the LFB, the recovery is judged to be matrix biased. The result for that analyte in the unfortified sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

9.11 FIELD DUPLICATES (FD1 AND FD2) – Within each extraction batch, a minimum of one Field Duplicate (FD) or LFMD (Sect. 9.10) must be analyzed. FDs check the precision associated with sample collection, preservation, storage, and laboratory procedures. If target analytes are not routinely observed in field samples, a LFMD (Sect. 9.10) should be analyzed to substitute for this requirement. Extraction batches that contain LFMDs (Section 9.10) will not require the analysis of a Field Duplicate.

9.11.1 Calculate the relative percent difference (RPD) for duplicate measurements (FD1 and FD2) using the equation

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

9.11.2 RPDs for duplicates should be in the range of ±30%. Greater variability may be observed when analyte concentrations are near the MRL. At the MRL, RPDs should fall in the range of ±50%. If the accuracy of any analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control in the LFB, the recovery is judged to be matrix biased. The result for that analyte in the unfortified sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

9.12 QUALITY CONTROL SAMPLES (QCS) – Each time that new standards are prepared or a new calibration curve is run, analyze a QCS from a source different than the source of the calibration standards. The QCS may be injected as a calibration standard or fortified into reagent water and analyzed as a LFB. If the QCS is analyzed as a continuing

calibration, then the acceptance criteria are the same as for the CCC. If the QCS is analyzed as a LFB, then the acceptance criteria are the same as for an LFB. If measured analyte concentrations are not of acceptable accuracy, check the entire analytical procedure to locate and correct the problem source.

10. CALIBRATION AND STANDARDIZATION

10.1 Demonstration and documentation of acceptable mass spectrometer tune and initial calibration is required before any samples are analyzed. After the 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. Verification of mass spectrometer tune must be repeated each time a major instrument modification is made or maintenance is performed, and prior to analyte calibration.

10.2 INITIAL CALIBRATION

10.2.1 MS TUNE/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 5 ng or less of the DFTPP solution (Sect. 7.2.6) into the GC/MS system. Acquire a mass spectrum that includes data for m/z 45-450. Use a single spectrum at the apex 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. If the DFTPP mass spectrum does not meet all criteria in Table 1, the MS must be retuned and adjusted to meet all criteria before proceeding with the initial calibration.

10.2.2 INSTRUMENT CONDITIONS – Operating conditions are described below. Conditions different from those described may be used if QC criteria in Section 9 are met. Different conditions include alternate GC columns, temperature programs, and injection techniques, such as cold on-column and large volume injections. Equipment designed for alternate types of injections must be used if these options are selected.

10.2.2.1 Inject 1 uL into a hot, splitless injection port held at 210°C with a split delay of 1 min. The temperature program is as follows: initially hold at 55°C for one minute, then ramp at 8°C/ min to 320°C. Total run time is approximately 33 min. Begin data acquisition at 3.5 minutes.

Note: The GC was operated in a constant flow rate mode at a rate of 1.4 mL per minute and an initial head-pressure of 12.2 psi.

10.2.2.2 Many of the target compounds exhibit decreased sensitivity for low-level injections due to degradation or irreversible adsorption in the injector port. Deactivated glass or quartz inlet liners are recommended.

10.2.2.3 MS Detection and Sensitivity – Acquire and store data from m/z 45 to 450 with a total cycle time (including scan overhead time) of 1.0 second or less. Adjust the cycle time to measure at least five or more spectra during the elution of each GC peak. Seven to ten scans across each GC peak are recommended. The GC/MS/DS peak identification software must be able to recognize a GC peak in the appropriate retention time window for each of the compounds in the calibration solution, and make correct qualitative identifications.

10.2.3 CALIBRATION SOLUTIONS – Prepare a set of at least 5 calibration standards as described in Section 7.2.4. The lowest concentration of the calibration standard must be at or below the MRL, which will depend on system sensitivity. Acceptable calibration over a large dynamic range, greater than about 50 fold range, may require multiple calibration curves.

10.2.4 CALIBRATION – The system is calibrated using the internal standard technique. Concentrations may be calculated through the use of average relative response factor (RRF) or through the use of a calibration curve. Calculate the RRFs using the equation

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

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, and

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

Average RRF calibrations may only be used if the RRF values over the calibration range are relatively constant (<30% RSD). Average RRF is determined by calculating the mean RRF of a minimum of five calibration concentrations.

10.2.5 As an alternative to calculating average RRFs and applying the RSD test, 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 to obtain a curve that best fits the data. Examples of common GC/MS system calibration curve options are: 1) A_x / A_{is} vs Q_x / Q_{is} and 2) RRF vs A_x / A_{is}.

10.2.6 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 RRF. Each calibration point, except the

lowest point, for each analyte must calculate to be 70-130 % of its true value. The lowest point must calculate to be 50-150% of its true value. If this criteria cannot be met, reanalyze the calibration standards, restrict the range of calibration, or select an alternate method of calibration. The data presented in this method were obtained using quadratic fit (RRF vs. amount). 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 CCC verifies the initial calibration at the beginning and end of each group of analyses, and after every 10th sample during analyses. In this context, a "sample" is considered to be a field sample. LRBs, LFBs, LFMs, LFMDs and CCCs are not counted as samples. The beginning CCC for each analysis batch must be at or below 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. Subsequent CCCs should alternate between a medium and high concentration.

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 ± 50% from the areas measured during initial calibration. If any IS area has changed by more this amount, remedial action must be taken (Sect. 10.3.4). 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 ±30% of the true value. The calculated amount for the lowest calibration level for each analyte must be within ±50% of the true value. If these conditions do not exist, then all data for the problem analyte must be considered invalid, and remedial action (Sect. 10.3.4) 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 fails because the calculated concentration is greater than 130% (150% for the low-level CCC) for a particular target compound, and field sample extracts show no detection for that target compound, non-detects may be reported without re-analysis.

10.3.4 REMEDIAL ACTION – Failure to meet CCC QC performance criteria may require remedial action. Major maintenance such as cleaning an ion source, cleaning quadrapole rods, replacing filament assemblies, etc., require returning to the initial calibration step (Sect. 10.2).

11. PROCEDURE

11.1 Important aspects of this analytical procedure include proper preparation of laboratory glassware and sample containers (Sect. 4.1), and sample collection and storage (Sect. 8). This section describes the procedures for sample preparation, solid phase extraction (SPE) using cartridges or disks, and extract analysis.

11.2 SAMPLE BOTTLE PREPARATION

11.2.1 Samples are preserved, collected and stored as presented in Section 8. All field and QC samples must contain the preservatives listed in Section 8.1.2, including the LRB and LFB. 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. 11.3.7), weigh the bottle with collected sample before extraction.

11.2.2 Add an aliquot of the surrogate fortification solution to each sample to be extracted. For method development work, a 100 uL aliquot of the 50 ug/mL SUR Sample Fortification Solution (Sect. 7.2.2.3) was added to 1 L for a final concentration of 5.0 ug/L.

11.2.3 If the sample is an LFB, LFM, or LFMD, add the necessary amount of analyte fortification solution. Swirl each sample to ensure all components are mixed.

11.2.4 Proceed with sample extraction using either SPE cartridges (Sect. 11.3) or disks (Sect. 11.4).

11.3 CARTRIDGE SPE PROCEDURE – The cartridge extraction procedure is carried out in a manual mode or by using a robotic or automatic sample preparation device. This section describes a SPE manual procedure using the equipment outlined in Section 6.10. The manual mode of sample addition to cartridges is performed with a large reservoir attached to the cartridge or with a transfer tube from the sample bottle to the cartridge. Cartridge extraction data in Section 17 was collected using the transfer tube option described below.

11.3.1 CARTRIDGE CLEANUP – Attach the extraction cartridges to the vacuum manifold. Rinse each cartridge with a 5-mL aliquot of ethyl acetate, allowing the sorbent to soak in the ethyl acetate for about 1 minute by turning off the vacuum temporarily. Repeat with a 5-mL aliquot of methylene chloride ($MeCl_2$). Let the cartridge vacuum dry after each flush.

11.3.2 CARTRIDGE CONDITIONING – This conditioning step is critical for recovery of analytes and can have a marked effect on method precision and accuracy. If the cartridge goes dry during the conditioning phase, the conditioning must be started over. Once the conditioning has begun, the cartridge must not go dry until the last

portion of the sample passes because analyte and surrogate recoveries may be affected. The analyst should note premature drying of the solid phase, because the sample may require re-extraction due to low surrogate recoveries.

11.3.2.1 CONDITIONING WITH METHANOL – Rinse each cartridge with a 5-mL aliquot of methanol (MeOH), allowing the sorbent to soak for about 30 seconds by turning off the vacuum temporarily. From this point on, do not allow the cartridge to go dry until after extraction is complete. Drain most of the MeOH without going below the top of the cartridge packing and rinse again with a 5-mL aliquot of MeOH.

11.3.2.2 CONDITIONING WITH REAGENT WATER – Drain most of the MeOH and rinse the cartridge with two consecutive 5-mL aliquots of reagent water, being careful not to allow the water level to go below the cartridge packing. Turn off the vacuum. Fill the cartridge to the top with reagent water and attach a reservoir or a transfer tube (Sect. 6.10.2).

11.3.3 CARTRIDGE EXTRACTION – Prepare samples, including the QC samples, as specified in Section 11.2. The sample may be added to the cartridge using either a large reservoir attached to the cartridge or using a transfer tube from the sample bottle to the cartridge.

11.3.3.1 SAMPLE ADDITION USING RESERVOIRS – Attach a reservoir to the conditioned cartridge from Section 11.3.2. Fill the reservoir with sample and turn on the vacuum adding additional aliquots of sample until the entire 1 L sample is processed. Adjust the vacuum so that the flow rate is about 20 mL/min. Do not let the cartridge packing go dry before all the sample has been extracted. After all of the sample has passed through the SPE cartridge, draw air through the cartridge for 10 minutes at full vacuum (minus 10 to 15 inches Hg). If the cartridge is dried for period much longer than 10 minutes, there may be a loss of recovery for nitrobenzene and the surrogate 1,3-dimethyl-2-nitrobenzene. After drying, turn off and release vacuum.

11.3.3.2 SAMPLE ADDITION USING TRANSFER TUBES – Fit the PTFE transfer tube adapter securely to the conditioned cartridge. The screw on the adapter must be finger-tight, otherwise, air can leak and the cartridge may go dry. Place the weighted end of the transfer tube inside on the bottom of the sample bottle. Adjust the flow rate to about 20 mL/min. If the adapter is securely attached, the water level in the cartridge should drop only as much as the volume of the transfer tube and no more. Do not let the SPE sorbent go dry before all the sample has been extracted. After all the sample has passed through the SPE cartridge, draw air through the cartridge for 10 minutes at full vacuum (minus 10 to 15 inches Hg). If the cartridge is dried for a much longer period than 10

minutes, there may be a loss of recovery for nitrobenzene and the surrogate 1,3-dimethyl-2-nitrobenzene. After drying, turn off and release vacuum.

11.3.4 CARTRIDGE ELUTION – Keep reservoirs or transfer tubes attached. Lift the manifold top, place collection tubes into the extraction tank, and insert valve liners into the collection tubes for extract collection. Add 5 mL of ethyl acetate (EtAc) to the empty sample bottle and rotate the bottle on its side, rinsing the inside of the bottle. If using the cartridge reservoir method, pour the EtAc from the bottle into the cartridge reservoir and draw enough of the EtAc through the cartridge to soak the sorbent. If using the transfer tubes method, pull the EtAc through the PTFE transfer tubes and draw enough of the EtAc through the tubes into the cartridge to soak the sorbent. Turn off the vacuum and vent the system and allow the sorbent to soak in EtAc for approximately 30 seconds. Start a low vacuum (minus 2-4 in Hg) and pull the ethyl acetate through in a dropwise fashion into the collection tube. Repeat rinse with 5 mL $MeCl_2$. Take off reservoirs and transfer tubes and rinse the cartridge body with 2 to 3 mLs of 1:1 mixture of $MeCl_2$ and EtAc (1:1 $MeCl_2$/EtAc).

11.3.5 DRYING OF THE EXTRACT – Small amounts of residual water from the sample container and solid phase may form an immiscible layer with the solvent in the extract. Set up a drying column (Sect. 6.6) packed with about 5 grams of anhydrous sodium sulfate. Pre-rinse the sodium sulfate column with about 2 mL of 1:1 EtAc/$MeCl_2$. Place a clean collection tube that can hold at least 20 mL beneath the drying column. Add the extract to the column and follow with two, 3 mL aliquots of 1:1 EtAc/$MeCl_2$.

11.3.6 EXTRACT CONCENTRATION – Concentrate the extract to about 0.7 mL under a gentle stream of nitrogen in a warm water bath (at ~ 40°C). Do not blow down samples to less than 0.5 mL, because the most volatile compounds will show diminished recovery. Transfer the extract to a 1 mL volumetric flask and add the internal standard (method development used 100 uL of 50 ug/mL internal standard solution for an extract concentration of 5 ug/mL). Rinse the collection tube that held the dried extract with small amounts of EtAc and add to the volumetric flask to bring the volume up to the 1 mL mark. Transfer to autosampler vial.

11.3.7 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 volume to the nearest 10 mL for use in the final calculations of analyte concentration (Sect. 12.2). If using weight to determine volume, reweigh empty sample bottle. From the weight of the original sample bottle measured in Section 11.2.1, subtract the empty bottle weight. Use this value for analyte concentration calculations in Section 12.2.

11.4 DISK SPE PROCEDURE – The disk extraction procedure may be carried out in a manual mode or by using a robotic or automatic sample preparation device. This section describes the disk SPE procedure using the equipment outlined in Section 6.10 in its simplest, least expensive mode without the use of a robotics systems. The manual mode described below was used to collect data presented in Section 17.

11.4.1 SAMPLE PREPARATION – Prepare the sample as given in Section 11.2.

11.4.2 DISK CLEANUP – Assemble the extraction glassware onto the vacuum manifold, placing disks on a support screen between the funnel and base. Add a 5 mL aliquot of 1:1 mixture of ethyl acetate (EtAc) and methylene chloride ($MeCl_2$) (1:1 $MeCl_2$/EtAc), drawing about half through the disk, and allowing the solvent to soak the disk for about a minute. Draw the remaining solvent through the disk to waste until the disk is dry of solvent.

11.4.3 DISK CONDITIONING – The conditioning step is critical for recovery of analytes and can have a marked effect on method precision and accuracy. If the disk goes dry during the conditioning phase, the conditioning must be started over. Once the conditioning has begun, the disk must not go dry until the last portion of the sample passes, because analyte and surrogate recoveries may be affected. The analyst should note premature drying of the solid phase, because the sample may require re-extraction due to low surrogate recoveries. During conditioning, it is not unusual for the middle of the solid phase disk to form a wrinkle. This typically does not adversely affect extraction.

11.4.3.1 CONDITIONING WITH METHANOL – Add approximately 10 mL methanol to each disk. Pull about 1 mL of MeOH through the disk and turn off the vacuum temporarily to let the disk soak for about one minute. Draw most of the remaining MeOH through the disk, but leave a layer of MeOH on the surface of the disk. The disk must not be allowed to go dry from this point until the end of the sample extraction.

11.4.3.2 CONDITIONING WITH WATER – Follow the MeOH rinse with two, 10 mL aliquots of reagent water, being careful to keep the water level above the disk surface. Turn off the vacuum.

11.4.4 DISK EXTRACTION – Add sample to the extraction funnel containing the conditioned disk and turn on the vacuum. Do not let the disk go dry before all sample has been extracted. Drain as much water from the sample container as possible. After all of the sample has passed, draw air through the disk by maintaining full vacuum (minus 10-15 in Hg) for 10 minutes. If the disk is dried for a period much longer than 10 minutes, there will be a loss of recovery for nitrobenzene and the surrogate 1,3-dimethyl-2-nitrobenzene. After drying, turn off and release the vacuum.

11.4.5 DISK ELUTION – Detach the glassware base from the manifold without disassembling the funnel from the base. Dry the underside of the base. Insert collection tubes into the manifold to catch the extracts as they are eluted from the disk. The collection tube must fit around the drip tip of the base to ensure collection of all the eluent. Reattach the base to the manifold. Add 5 mL of ethyl acetate to the empty sample bottle and rinse the inside of the bottle. Transfer the ethyl acetate to the disk and, with vacuum, pull enough ethyl acetate into the disk to soak the sorbent, and allow the solvent to soak the disk for about one minute. Pull the remaining solvent slowly through the disk into the collection tube. Repeat the rinse with 5 mL $MeCl_2$. Rinse the SPE funnel surface once with a 2-3 mL aliquot of 1:1 EtAc/$MeCl_2$. Repeat this last rinse of the SPE funnel. Detach glassware from manifold and remove collection tube from the manifold.

11.4.6 DRYING OF THE EXTRACT – Proceed with drying the extract, Section 11.3.5.

11.4.7 EXTRACT CONCENTRATION – Proceed with extract concentration, Section 11.3.6.

11.4.8 SAMPLE VOLUME OR WEIGHT DETERMINATION – Proceed with sample volume or weight determination, Section 11.3.7.

11.5 ANALYSIS OF SAMPLE EXTRACTS

11.5.1 Establish operating conditions as described in Section 10.2.2. Confirm that retention times, compound separation and resolution are similar to those summarized in Table 2 and Figure 1.

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

11.5.3 Analyze aliquots of field and QC samples at appropriate frequencies (Sect. 9) with the GC/MS system using the conditions used for the initial and continuing calibrations. At the conclusion of data acquisition, use the same software that was used in the calibration procedure to tentatively identify peaks in predetermined retention time windows of interest. Use the data system software to examine the ion abundances of components of the chromatogram.

11.5.4 COMPOUND IDENTIFICATION – Identify a sample component by comparison of its mass spectrum (after background subtraction) to a reference spectrum in the user-created data base.

11.5.4.1 Establish an appropriate retention time window for each target analyte, internal standard and surrogate standard to identify them in QC and Field

Samples chromatograms. Ideally, the retention time window should be based on measurements of actual retention time variation for each compound in standard solutions collected on each 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 Initial Demonstration of Capability may be used to calculate a suggested window size. However, the experience of the analyst should weigh heavily on the determination of an appropriate retention window size.

11.5.4.2 In general, all ions that are present above 10% relative abundance in the 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 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 10% relative abundance.

11.5.5 EXCEEDING CALIBRATION RANGE – An 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 with ethyl acetate, with the appropriate amount of internal standard added to match the original level, and the diluted extract injected. Acceptable surrogate performance (Sect. 9.9) should be determined from the undiluted sample extract. Incorporate the dilution factor into final concentration calculations. The dilution will also affect analyte MRLs.

12. DATA ANALYSIS AND CALCULATIONS

12.1 Identify method analytes present in the field and QC samples as described in Section 11.8.4. Complete chromatographic resolution is not necessary for accurate and precise measurements of analyte concentrations if unique ions with adequate intensities are available for quantitation.

12.1.1 Identification is hampered when sample components are not resolved chromatographically and produce mass spectra containing ions contributed by more than one analyte. 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 plots of characteristic ions. When analytes coelute (i.e., only one GC peak is apparent), the identification criteria can be met but each analyte spectrum will contain extraneous ions contributed by the coeluting compound.

12.1.2 In validating this method, concentrations were calculated by measuring the

characteristic ions listed in Table 2. Other ions may be selected at the discretion of the analyst.

12.2 Calculate analyte and surrogate concentrations, using the multipoint calibration established in Section 10.2. Do not use daily continuing calibration check data to quantitate analytes in samples. Adjust the final analyte concentrations to reflect the actual sample volume or weight determined in Section 11.3.7 or 11.4.8. Field Sample extracts that require dilution should be treated as described in Section 11.5.5.

12.3 Calculations should utilize all available digits of precision, but final reported concentrations should be rounded to an appropriate number of significant figures.

13. METHOD PERFORMANCE

13.1 PRECISION, ACCURACY, AND MDLs – Method performance data are summarized in Section 17. Method detection limits (MDLs) are presented in Table 3 and were calculated using the formula in Section 9.2.4. Single laboratory precision and accuracy data are presented for reagent water (Sect. 17, Tables 4A and 4B), chlorinated "finished" ground water (Sect. 17, Tables 5A and 5B), and chlorinated "finished" surface water (Sect. 17, Tables 6A and 6B).

13.2 POTENTIAL PROBLEM COMPOUNDS

13.2.1 MATRIX ENHANCED SENSITIVITY – Cyanazine, and to a lesser extent 2,4,6-trichlorophenol and prometon, tend to exhibit "matrix-induced chromatographic response enhancement."[7-11] Compounds that exhibit this phenomenon often give analytical results that exceed 100 % recovery in fortified extracts at low concentration and in continuing calibration checks. More frequent recalibration is required. It has been proposed that these compounds are susceptible to GC inlet absorption or thermal degradation so that analytes degrade more 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. This effect is minimized by using deactivated injection liners (Sect. 10.2.2.2). The analyst may also choose to condition the injection port after maintenance by injecting a few aliquots of a field sample extract prior to establishing an initial calibration. Preparation of calibration standards in clean extracts is not allowed.

13.2.2 COMPOUND DEGRADATION – Method development work indicated that several of the target compounds were unstable when stored in water without preservation. There were various modes of loss. Hydrolysis of 1,2-diphenylhydrazine, terbufos, diazinon, disulfoton and cyanazine was accelerated at low and high pH. In addition, transition metal ions further catalyzed hydrolysis of terbufos, fonofos, and diazinon. Free chlorine and chloramines degraded 2,4-

dichlorophenol, terbufos, fonofos, diazinon, and disulfoton. When water samples were not properly preserved, after three days, there was more than 80% loss of some targets, initially fortified at 5 ppb. Sample preservation conditions (Sect. 8) have been carefully chosen to minimize analyte degradation to acceptable levels during the 14 day sample holding time.

13.2.3 Inlet liners and/or capillary GC columns that develop active sites can cause a complete loss of prometon and excessive tailing of 2,4-dichlorophenol and 2,4,6-trichlorophenol peaks in the chromatogram.

13.3 ANALYTE STABILITY STUDIES

13.3.1 FIELD SAMPLES – Chlorinated surface water samples, fortified with method analytes at 5.0 ug/L, were preserved and stored as required in Section 8. The average of triplicate analyses, conducted on days 0, 3, 7, and 14, are presented in Section 17, Table 7. These data document the 14-day sample holding time. It is advisable to extract as soon as possible because some compounds exhibit significant losses by 7 days.

13.3.2 EXTRACTS – Extracts from the day 0 extract holding time study described above were stored below 0 ^{o}C and analyzed on days 0, 6, 13, 20, and 32. The data presented in Section 17, Table 8, document the 28-day extract holding time.

14. POLLUTION PREVENTION

14.1 This method utilizes solid phase extraction 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 involved 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: Laboratory Chemical Management for Waste Reduction” available form the American Chemical Society’s Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036.

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. Also, 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, consult "The Waste Management Manual for Laboratory Personnel" also available from the American Chemical Society at the address in Section 14.2.

16 REFERENCES

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17. TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

TABLE 1. ION ABUNDANCE CRITERIA FOR BIS(PERFLUOROPHENYL)PHENYL PHOSPHINE, (DECAFLUOROTRIPHENYL PHOSPHINE, DFTPP)

Mass (M/z)	Relative Abundance Criteria	Purpose of Checkpoint[1]
51	10-80% of the base peak	Low-mass sensitivity
68	<2% of Mass 69	Low-mass resolution
70	<2% of Mass 69	Low-mass resolution
127	10-80% of the base peak	Low- to mid-mass resolution
197	<2% of Mass 98	Mid-mass resolution
198	Base peak or >50% of Mass 442	Mid-mass resolution and sensitivity
199	5-9% of Mass 198	Mid-mass resolution and isotope ratio
275	10-60% of the base peak	Mid- to high-mass sensitivity
365	>1% of the base peak	Baseline threshold
441	Present and <Mass 443	High-mass resolution
442	Base peak or >50% of Mass 198	High-mass resolution and sensitivity
443	15-24% of Mass 442	High-mass resolution and isotope ratio

[1]All ions are used primarily to check the mass measuring 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. The correct setting of the baseline threshold, as indicated by the presence of low intensity ions, is the next most important part of the performance test. Finally, the ion abundance ranges are designed to encourage some standardization to fragmentation patterns.

TABLE 2. RETENTION TIMES (RTs), SUGGESTED QUANTITATION IONS (QIs), AND INTERNAL STANDARD REFERENCE

Peak #[a]	Analyte	Peak Label in Figure #1	RT[b] (min)	Quanti-tation Ion	IS# Ref.
1	Nitrobenzene	1	6.33	77	1
2	2,4-Dichlorophenol	2	7.70	162	1
3	2,4,6-Trichlorophenol	4	10.81	196	1
4	1,2-Diphenylhydrazine	6	15.08	182	2
5	Prometon	7	16.64	225	2
6	Terbufos	8	17.08	231	2
7	Fonofos	9	17.14	246	2
8	Diazinon	11	17.29	179	2
9	Disulfoton	12	17.51	88	2
10	Acetochlor	13	18.39	146	2
11	Cyanazine	14	19.73	225	2
12	Acenaphthene-d_{10} (IS#1)	5	12.88	164	-
13	Phenanthrene-d_{10} (IS#2)	10	17.20	188	-
14	Chrysene-d_{12} (IS#3)	16	24.98	240	-
15	1,3-Dimethyl-2-Nitrobenzene (SURR)	3	7.98	151	1
16	Triphenylphosphate (SURR)	15	24.29	326	3

a- Number refers to peak number in Figure 1.
b- Column: 30 m X 0.25 mm i.d. DB5-MS (J&W), 0.25 um film thickness.

TABLE 3. METHOD DETECTION LIMITS IN REAGENT WATER FOR SDVB DISK AND CARTRIDGE EXTRACTION PROCEDURES

Compound	Disk Extraction		Cartridge Extraction	
	Spiking Conc. (ug/L)	MDL[a] (ug/L)	Spiking Conc. (ug/L)	MDL[a] (ug/L)
Nitrobenzene	0.05	0.015	0.20	0.09
2,4-Dichlorophenol	0.05	0.012	0.20	0.04
2,4,6-Trichlorophenol	0.05	0.012	0.20	0.14
1,2-Diphenylhydrazine	0.20	0.028	0.20	0.10
Prometon	0.10	0.035	0.20	0.14
Terbufos	0.05	0.017	0.20	0.05
Fonofos	0.05	0.022	0.20	0.06
Diazinon	0.10	0.015	0.20	0.03
Disulfoton	0.10	0.024	0.20	0.05
Acetochlor	0.05	0.015	0.20	0.10
Cyanazine	0.05	0.025	0.20	0.09

[a]Method detection limits samples were extracted and analyzed over 3 days for 7 replicates following the procedure outlined in Section 9.2.4.

TABLE 4A. PRECISION, ACCURACY AND SENSITIVITY DATA FOR METHOD ANALYTES FORTIFIED AT 0.5 AND 20 UG/L IN REAGENT WATER EXTRACTED WITH SDVB DISKS

Compound	Concentration= 0.5 ug/L, n=7			Concentration = 20 ug/L, n=7	
	Mean % Recovery	%RSD[a]	S/N Ratio [b]	Mean % Recovery	%RSD[a]
Nitrobenzene	106	3.8	159	81.5	5.6
2,4-Dichlorophenol	114	1.9	71	97.6	4.3
2,4,6-Trichlorophenol	136	2.3	134	104	3.4
1,2-Diphenylhydrazine	121	2.5	25	103	3.7
Prometon	138	2.3	42	101	3.8
Terbufos	111	2.7	71	91.4	4.0
Fonofos	104	2.0	138	106	4.4
Diazinon	101	2.5	14	98.3	4.7
Disulfoton	105	2.4	103	95.0	4.8
Acetochlor	124	2.9	67	98.9	4.0
Cyanazine	153	2.5	10	104	4.6
1,3-Dimethyl-2-Nitrobenzene (SUR)[c]	86.2	2.6	NC	84.2	4.6
Triphenylphosphate (SUR)[c]	106	2.6	NC	105	5.2

[a]Relative Standard Deviation = (Standard Deviation/Recovery)*100.
[b]Signal-to-noise ratios were calculated for each peak by dividing the peak height for each compound by the peak-to-peak noise, which was determined for each component from the method blank over a period of time equal to the full peak width in the target analyte's retention time window.
[c]Surrogate fortification concentration of all samples was 5 ug/L.

TABLE 4B. PRECISION AND ACCURACY DATA FOR METHOD ANALYTES FORTIFIED AT 0.5 AND 20 UG/L IN REAGENT WATER EXTRACTED WITH SDVB CARTRIDGES

Compound	Concentration = 0.5 ug/L, n=7		Concentration = 20 ug/L, n=7	
	Mean % Recovery	%RSD[a]	Mean % Recovery	%RSD[a]
Nitrobenzene	86.3	6.9	73.4	3.7
2,4-Dichlorophenol	104	7.3	83.8	3.3
2,4,6-Trichlorophenol	129	3.8	92.9	3.0
1,2-Diphenylhydrazine	96.9	16	123	3.2
Prometon	148	3.2	100	0.8
Terbufos	115	2.8	85.0	1.8
Fonofos	101	3.8	90.1	1.9
Diazinon	104	3.4	91.1	1.4
Disulfoton	106	3.0	85.5	1.7
Acetochlor	125	3.0	91.3	1.2
Cyanazine	147	5.6	101	1.2
1,3-Dimethyl-2-Nitrobenzene (SUR)[b]	81.7	4.1	80.2	3.0
Triphenylphosphate (SUR)[b]	107	7.5	108	3.0

[a]Relative Standard Deviation = (Standard Deviation/Recovery)*100.
[b]Surrogate fortification concentration of all samples was 5 ug/L.

TABLE 5A. PRECISION AND ACCURACY DATA FOR METHOD ANALYTES FORTIFIED AT 0.5, AND 20 UG/L IN GROUND WATER EXTRACTED WITH SDVB DISKS

Compound	Concentration = 0.5 ug/L, n = 7		Concentration = 20 ug/L, n = 7	
	Mean % Recovery	%RSD[a]	Mean % Recovery	%RSD[a]
Nitrobenzene	110	4.7	83.5	5.3
2,4-Dichlorophenol	113	3.0	96.5	5.0
2,4,6-Trichlorophenol	148	2.0	103	4.1
1,2-Diphenylhydrazine	134	3.8	102	4.0
Prometon	152	2.4	101	3.8
Terbufos	119	1.6	93.4	3.5
Fonofos	105	2.4	105	3.7
Diazinon	110	1.5	97.6	3.7
Disulfoton	113	2.5	95.1	4.2
Acetochlor	130	2.1	98.3	3.7
Cyanazine	163	2.3	104	3.8
1,3-Dimethyl-2-Nitrobenzene (SUR)[b]	86.3	4.8	83.8	5.5
Triphenylphosphate (SUR)[b]	105	2.9	106	4.0

[a]Relative Standard Deviation = (Standard Deviation/Recovery)*100.
[b]Surrogate fortification concentration of all samples was 5 ug/L.

TABLE 5B. PRECISION AND ACCURACY DATA FOR METHOD ANALYTES FORTIFIED AT 0.5, 5.0 AND 20 UG/L IN GROUND WATER EXTRACTED WITH SDVB CARTRIDGES

Compound	Concentration = 0.5 ug/L, n = 7		Concentration = 5.0 ug/L, n = 7		Concentration = 20 ug/L, n = 7	
	Mean % Recovery	%RSD[a]	Mean % Recovery	%RSD[a]	Mean % Recovery	%RSD[a]
Nitrobenzene	114	5.4	87.7	3.9	84.1	1.9
2,4-Dichlorophenol	110	3.4	89.8	3.9	89.1	2.2
2,4,6-Trichlorophenol	136	4.1	102	3.8	98.3	1.4
1,2-Diphenylhydrazine	122	4.3	84.8	3.2	90.4	3.7
Prometon	144	3.3	103	2.9	100	2.3
Terbufos	120	3.0	87.1	4.2	84.9	2.4
Fonofos	107	3.0	87.9	3.5	90.8	2.4
Diazinon	105	2.4	88.7	3.1	91.6	2.3
Disulfoton	108	3.8	93.3	3.7	88.6	3.1
Acetochlor	129	4.5	103	3.7	94.2	1.1
Cyanazine	160	3.1	106	3.6	98.6	3.6
1,3-Dimethyl-2-Nitrobenzene (SUR)[b]	86.7	6.3	86.3	3.3	84.1	4.6
Triphenylphosphate (SUR)[b]	119	4.3	123	3.4	111	14

[a]Relative Standard Deviation = (Standard Deviation/Recovery)*100.
[b]Surrogate fortification concentration of all samples was 5 ug/L.

TABLE 6A. PRECISION AND ACCURACY DATA FOR METHOD ANALYTES FORTIFIED AT 0.5, 5.0 AND 20 UG/L IN SURFACE WATER EXTRACTED WITH SDVB DISKS

Compound	Concentration = 0.5 ug/L, n = 7		Concentration = 5.0 ug/L, n = 7		Concentration = 20 ug/L, n = 7	
	Mean % Recovery	%RSD[a]	Mean % Recovery	%RSD[a]	Mean % Recovery	%RSD[a]
Nitrobenzene	105	3.8	72.4	4.4	76.7	4.1
2,4-Dichlorophenol	111	4.0	81.2	4.0	89.9	4.8
2,4,6-Trichlorophenol	143	2.8	90.8	4.2	92.4	4.0
1,2-Diphenylhydrazine	118	2.4	105	4.5	88.3	3.9
Prometon	136	3.6	102	3.1	89.5	3.9
Terbufos	109	3.0	85.7	4.6	80.2	3.4
Fonofos	98.9	3.8	83.0	4.2	89.1	4.0
Diazinon	102	3.6	83.7	3.5	86.1	4.1
Disulfoton	105	3.4	81.2	4.2	86.6	3.7
Acetochlor	124	3.4	89.2	3.6	89.4	3.4
Cyanazine	151	3.2	105	3.9	93.6	3.9
1,3-Dimethyl-2-Nitrobenzene (SUR)[b]	82.9	4.5	77.4	4.4	83.9	3.9
Triphenylphosphate (SUR)[b]	101	4.5	98.2	3.6	98.8	4.3

[a]Relative Standard Deviation = (Standard Deviation/Recovery)*100.
[b]Surrogate fortification concentration of all samples was 5 ug/L.

TABLE 6B. PRECISION AND ACCURACY DATA FOR METHOD ANALYTES FORTIFIED AT 0.5 AND 20 UG/L IN SURFACE WATER EXTRACTED WITH SDVB CARTRIDGES

Compound	Concentration = 0.5 ug/L, n = 7		Concentration = 20 ug/L, n = 7	
	Mean % Recovery	%RSD[a]	Mean % Recovery	%RSD[a]
Nitrobenzene	91.7	6.6	84.9	2.9
2,4-Dichlorophenol	113	4.9	89.7	2.2
2,4,6-Trichlorophenol	135	4.0	97.2	1.8
1,2-Diphenylhydrazine	107	9.9	93.7	2.3
Prometon	158	3.3	101	1.3
Terbufos	128	3.0	89.0	2.1
Fonofos	109	2.3	91.6	1.6
Diazinon	112	3.7	92.8	1.5
Disulfoton	109	3.0	90.0	1.6
Acetochlor	132	2.8	94.6	1.4
Cyanazine	158	1.7	99.9	1.0
1,3-Dimethyl-2-Nitrobenzene (SUR)[b]	83.0	5.3	85.1	3.8
Triphenylphosphate (SUR)[b]	104	1.9	99.1	1.60

[a]Relative Standard Deviation = (Standard Deviation/Recovery)*100.
[b]Surrogate fortification concentration of all samples was 5 ug/L.

TABLE 7. SAMPLE HOLDING TIME DATA[a] FOR SAMPLES FROM A CHLORINATED SURFACE WATER, FORTIFIED WITH METHOD ANALYTES AT 5 UG/L, AND PRESERVED ACCORDING TO SECTION 8.

Compound	Day 0 % Rec.	Day 3 % Rec.	Day 7 % Rec.	Day 14 % Rec.
Nitrobenzene	83.5	82.4	76.7	88.1
2,4-Dichlorophenol	97.2	92.6	86.9	98.1
2,4,6-Trichlorophenol	110	102	97.3	108
1,2-Diphenylhydrazine	98.8	93.9	87.5	97.2
Prometon	104	97.7	93.1	102
Terbufos	96.7	79.3	69.1	65.4
Fonofos	94.1	88.0	84.8	93.1
Diazinon	93.0	86.7	83.4	91.3
Disulfoton	90.8	84.7	81.5	89.1
Acetochlor	100	93.3	90.5	97.6
Cyanazine	126	119	112	125

[a]Storage stability is expressed as a percent recovery value. Each percent recovery value represents the mean of 3 replicate analyses. Relative Standard Deviations ([Standard Deviation/Recovery]*100) for replicate analyses were all less than 13.8 %.

TABLE 8. EXTRACT HOLDING TIME DATA[a] FOR SAMPLES FROM A CHLORINATED SURFACE WATER, FORTIFIED WITH METHOD ANALYTES AT 5 UG/L, AND PRESERVED ACCORDING TO SECTION 8.

Compound	Day 0 % Rec.	Day 6 % Rec.	Day 13 % Rec.	Day 20 % Rec.	Day 32 % Rec.
Nitrobenzene	83.5	84.7	85.2	85.5	80.5
2,4-Dichlorophenol	97.2	97.5	96.2	96.7	95.9
2,4,6-Trichlorophenol	110	110	108	107	112
1,2-Diphenylhydrazine	98.8	97.9	97.9	97.0	98.3
Prometon	104	104	103	103	101
Terbufos	96.7	97.5	97.9	98.6	89.5
Fonofos	94.1	95.0	94.3	95.3	97.3
Diazinon	93.0	93.0	93.6	94.3	93.0
Disulfoton	90.8	91.9	92.9	94.6	93.5
Acetochlor	100	100	99.8	99.8	98.7
Cyanazine	126	125	124	122	123
1,3-Dimethyl-2-Nitrobenzene (SUR)[b]	79.3	78.5	78.7	78.5	77.1
Triphenylphosphate (SUR)[b]	97.9	96.6	96.7	97.8	93.4

[a]Extracts were stored at less than 0 °C and reinjected periodically. Each table value represents the mean of 3 replicate analyses. Relative Standard Deviations ([Standard Deviation/Recovery]*100) for replicate analyses were all less than 5.7 %.

TABLE 9. INITIAL DEMONSTRATION OF CAPABILITY (IDC) REQUIREMENTS

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Section 9.2.1	Initial Demonstration of Low Method Background	Analyze LRB prior to any other IDC steps	Demonstrate that all target analytes are below $^1/_3$ the reporting limit or lowest CAL standard, and that possible interference from extraction media do not prevent the identification and quantification of method analytes.
Section 9.2.2	Initial Demonstration of Precision (IDP)	Analyze 4-7 replicate LFBs fortified at midrange concentration.	%RSD must be #20%
Section 9.2.3	Initial Demonstration of Accuracy	Calculate average recovery for replicates used in IDP	Mean recovery 70-130% of true value.
Section 9.2.4	Method Detection Limit (MDL) Determination	Over a period of three days, prepare a minimum of 7 replicate LFBs fortified at a concentration estimated to be near the MDL. Analyze the replicates through all steps of the analysis. Calculate the MDL using the equation in Section 9.2.4.	Note: Data from MDL replicates are not required to meet method precision and accuracy criteria. If the MDL replicates are fortified at a low enough concentration, it is likely that they will not meet precision and accuracy criteria.

TABLE 10. QUALITY CONTROL REQUIREMENTS (SUMMARY)

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Section 10.2.1	MS Tune Check	Analyze DFTPP to verify MS tune each time the instrument is calibrated.	Criteria are given in Table 1.
Section 10.2	Initial Calibration	Use internal standard calibration technique to generate an average RRF or first or second order calibration curve. Use at least 5 standard concentrations.	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 except the lowest standard, which must be 50-150% of the true value.
Section 9.4	Laboratory Reagent Blank (LRB)	Daily, or with each extraction batch of up to 20 samples, whichever is more frequent.	Demonstrate that all target analytes are below $^1/_3$ the method reporting limit or lowest CAL standard, and that possible interferences do not prevent quantification of method analytes. If targets exceed $^1/_3$ the MRL, results for all subject analytes in extraction batch are invalid.
Section 10.3	Continuing Calibration Check (CCC)	Verify initial calibration by analyzing a calibration standard at the beginning of each analysis batch prior to analyzing samples, after every 10 samples, and after the last sample. Low CCC - near MRL Mid CCC - near midpoint in initial calibration curve High CCC - near highest calibration standard	1) The result for each analyte 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. 2) The peak area of internal standards must be 50-150% of the average peak area calculated during the initial calibration. Results for analytes that do not meet IS criteria or are not bracketed by acceptable CCCs are invalid.

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Section 9.6	Laboratory Fortified Blank (LFB)	One LFB is required daily or for each extraction batch of up to 20 field samples. Rotate the fortified concentration between low, medium, and high amounts.	Results of LFB analyses at medium and high fortification must be 70-130% of the true value for each analyte and surrogate. LFB Results of the low level LFB must be 50-160% of the true value.
Section 9.8	Internal Standard	Acenaphthene-d_{10} (IS#1) phenanthrene-d_{10} (IS#2), and chrysene-d_{12} (IS#3), are added to all standards and sample extracts.	Peak area counts for all ISs in LFBs, LRBs, and sample extracts must be within 50-150% of the average peak area calculated during the initial calibration. If ISs do not meet criteria, corresponding target results are invalid.
Section 9.9	Surrogate Standards	Surrogate standards, 1,3-dimethyl-2-nitrobenzene and triphenylphosphate, are added to all calibration standards and samples, including QC samples. If nitrobenzene is not to be included on the target list, then the surrogate 1,3-dimethyl-2-nitrobenzene is not required.	Surrogate recovery must be 70-130% of the true value. If surrogate fails this criterion, report all results for sample as suspect/surrogate recovery.
Section 9.10	Laboratory Fortified Sample Matrix (LFM) and Laboratory Fortified Matrix Duplicate (LFMD)	Analyze one LFM per analysis batch (20 samples or less) fortified with method analytes at a concentration close to but greater than the native concentration. LFMD should be used in place of Field Duplicate if frequency of detects for targets is low.	Recoveries at mid and high levels not within 70-130% or low-level recoveries not within 50-150% of the fortified amount may indicate a matrix effect. Target analyte RPDs for LFMD should be ±30% at mid and high levels of fortification and ±50% near MRL.
Section 9.11	Field Duplicates (FD)	Extract and analyze at least one FD with each extraction batch (20 samples or less). A LFMD may be substituted for a FD when the frequency of detects for target analytes is low.	Target analyte RPDs for FD should be ±30% at mid and high levels of fortification and ±50% near MRL.
Section 9.12	Quality Control Sample (QCS)	Analyzed QCS quarterly.	Results must be 70-130% of the expected value.

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Section 8.4	Sample Holding Time	14 days with appropriate preservation and storage	Sample results are valid only if samples are extracted within sample hold time.
Section 8.4	Extract Holding Time	28 days with appropriate storage	Sample results are valid only if extracts are analyzed within extract hold time.

FIGURE 1. EXAMPLE CHROMATOGRAM FOR METHOD 526. NUMBERED PEAKS ARE IDENTIFIED IN TABLE 2.

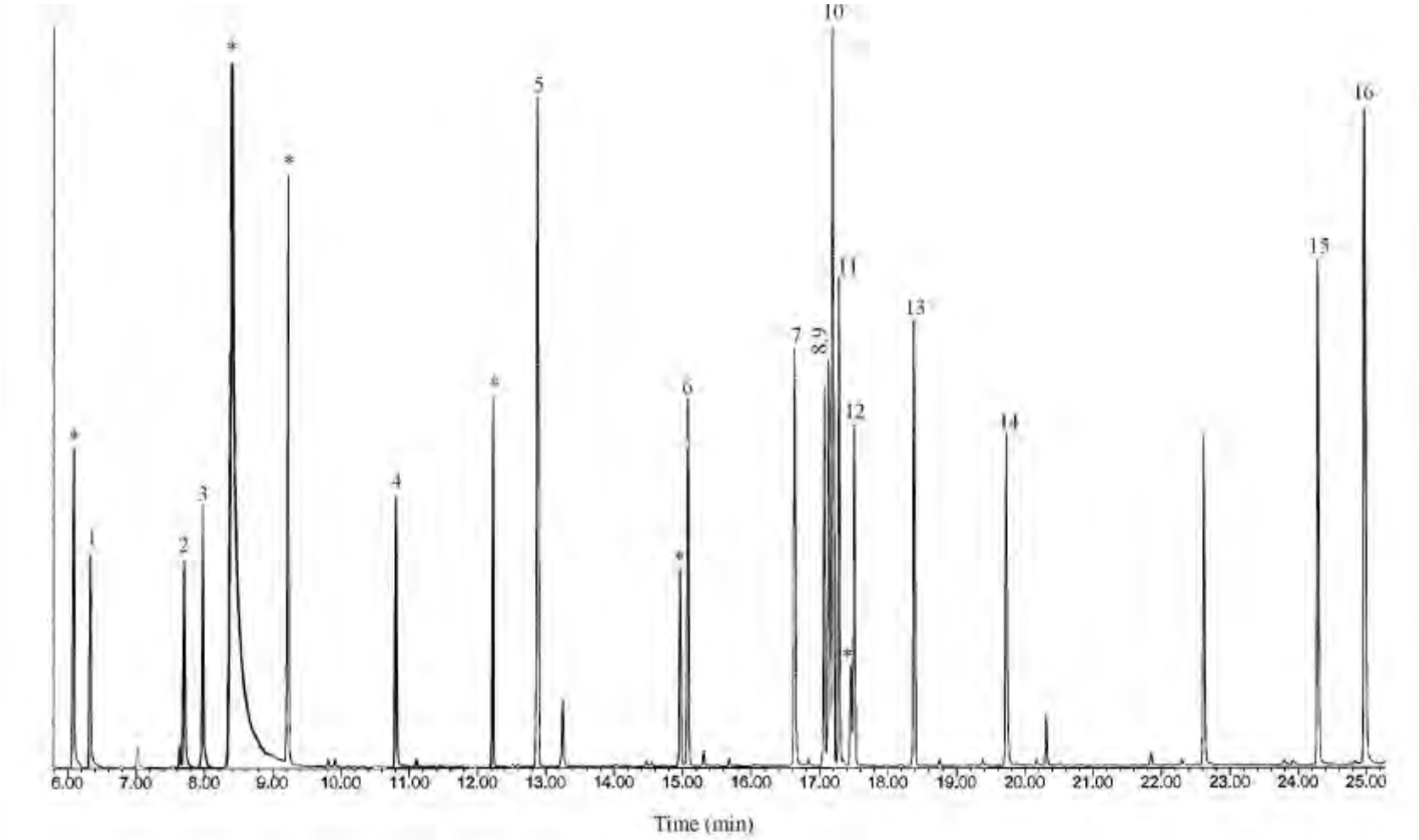

Figure 1: Total ion chromatogram of surface water sample extract with target compounds, internal standards, and surrogate standards fortified at 5 ppm level in extract. Peaks with asterisk (*) are interference associated with the use of diazolidinyl urea.