METHOD 515.4 DETERMINATION OF CHLORINATED ACIDS IN DRINKING WATER BY LIQUID-LIQUID MICROEXTRACTION, DERIVATIZATION, AND FAST GAS CHROMATOGRAPHY WITH ELECTRON CAPTURE DETECTION

Revision 1.0

April 2000

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METHOD 515.4 DETERMINATION OF CHLORINATED ACIDS IN DRINKING WATER BY LIQUID-LIQUID MICROEXTRACTION, DERIVATIZATION, AND FAST GAS CHROMATOGRAPHY WITH ELECTRON CAPTURE DETECTION

1. SCOPE AND APPLICATION

1.1 This is a fast gas chromatography (GC) method for the determination of chlorinated acids in drinking waters. Accuracy, precision, and Detection Limit data have been generated for the following compounds in reagent water and finished ground and surface waters.

Analyte	Chemical Abstracts Service Registry Number
Acifluorfen[(a)]	50594-66-6
Bentazon	25057-89-0
Chloramben	133-90-4
2,4-D	94-75-7
Dalapon	75-99-0
2,4-DB	94-82-6
Dacthal acid metabolites[(b)]	
Dicamba	1918-00-9
3,5-Dichlorobenzoic acid	51-36-5
Dichlorprop	120-36-5
Dinoseb	88-85-7
Pentachlorophenol	87-86-5
Picloram	1918-02-1
2,4,5-T	93-76-5
2,4,5-TP (Silvex)	93-72-1
Quinclorac	84087-01-4
[(a)] The herbicide Lactofen will be quantitated as Acifluorfen as their structures represent different esters of the same acid herbicide.	
[(b)] Dacthal mono-acid and di-acid metabolites are included in method scope; Dacthal di-acid was used for validation studies.	

1.2 This method is also applicable to the determination of salts and esters of analyte acids. The form of each acid is not distinguished by this method. Results are calculated and reported for each listed analyte as the total free acid. This method is able to quantify the mono- and di-acid forms of Dacthal but not the parent compound.

1.3 Detection Limits are compound, instrument, and matrix dependent. The Detection Limit is defined as the statistically calculated minimum amount that can be measured with 99% confidence that the reported value is greater than zero.[1] Experimentally determined Detection Limits for the above listed analytes are provided in Section 17, Table 4. The Detection Limit differs from, and is usually lower than (but never above), the minimum reporting level (MRL) (Sect. 3.17). The concentration range for target analytes in this method was evaluated between 0.5 ug/L and 20 ug/L for a 40-mL sample. Precision and accuracy data are presented in Section 17, Tables 5 - 10.

1.4 This method is restricted to use by or under the supervision of analysts skilled in liquid-liquid extractions, derivatization procedures and the use of GC and interpretation of gas chromatograms. The method was developed using fast GC but "conventional" GC may be used as long as the laboratory meets the requirements of the IDC (Sect. 9.2).

2. SUMMARY OF METHOD

2.1 A 40-mL volume of sample is adjusted to pH ≥ 12 with 4 N sodium hydroxide and allowed to sit for one hour at room temperature to hydrolyze derivatives. Following hydrolysis, a wash step using a hexane:MtBE mixture is performed as a sample cleanup and to remove Dacthal. The aqueous sample is then acidified with sulfuric acid to a pH of less than 1 and extracted with 4-mL of methyl tert-butyl ether (MtBE). The chlorinated acids that have been partitioned into the MtBE are then converted to methyl esters by derivatization with diazomethane. The target esters are separated and identified by fast capillary column gas chromatography using an electron capture detector (GC/ECD). Analytes are quantified using a procedural standard calibration technique with an internal standard.

NOTE: since many of the analytes contained in this method are applied as a variety of esters and salts, it is imperative to hydrolyze them to the parent acid prior to extraction.

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 lots of solvents, surrogate solution, and fortifying solutions. Required QC samples include Laboratory Reagent 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 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 DETECTION LIMIT – 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 quantified 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 standard for that analyte, and can only be used if acceptable quality control criteria for this standard are met.

3.18 PROCEDURAL STANDARD CALIBRATION – A calibration method where aqueous calibration standards are prepared and processed (e.g., purged, extracted, and/or derivatized) in exactly the same manner as a sample. All steps in the process from addition of sampling preservatives through instrumental analyses are included in the calibration. Using procedural standard calibration compensates for any inefficiencies in the processing procedure.

3.19 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. A final rinse with solvents may be needed. In place of a solvent rinse, non-volumetric glassware can be muffled at 400 °C for 2 hours. Volumetric glassware should not be heated in an oven above 120 °C. Store inverted or capped with aluminum foil.

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) under the conditions of the analysis by analyzing laboratory reagent blanks as described in Section 9. **Subtracting blank values from sample results is not permitted.**

4.2.1 During method development it was found that sodium sulfate from several sources contained multiple interferences. After screening several brands, it was found that a grade suitable for pesticide residue analysis provided the best results. If the suitability of the available sodium sulfate is in question, extract and analyze a laboratory reagent blank (section 3.5) to test for interferences prior to sample processing.

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

4.3.1 During method development a contaminant was found in local ground water and surface water that interfered with the analysis of Dalapon on the primary column. Due to this interferent, it was necessary to carefully optimize the chromatographic conditions (see Table 1). A slow initial temperature program was needed to ensure separation of the interferent from Dalapon. Analyte identifications should be confirmed using the confirmation column specified in Table 2 or another column that is dissimilar to the primary column or by GC/MS if the concentrations are sufficient.

4.4 Interferences by phthalate esters can pose a major problem in pesticide analysis when using an electron capture detector (ECD). These compounds generally appear in the chromatogram as large peaks. Common flexible plastics contain varying amounts of phthalates that are easily extracted or leached during laboratory operations. Cross contamination of clean glassware routinely occurs when plastics are handled during extraction steps, especially when solvent-wetted surfaces are handled. Interferences from phthalates can best be minimized by avoiding the use of plastics in the laboratory. Exhaustive purification of reagents and glassware may be required to eliminate background phthalate contamination.[2,3]

5. SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined. Each chemical compound should be treated as a potential health hazard, and exposure to these chemicals should be minimized. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of MSDSs should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available.[4-6]

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

5.3 The toxicity of the extraction solvent, MTBE, has not been well defined. Susceptible individuals may experience adverse affects upon skin contact or inhalation of vapors. Therefore protective clothing and gloves should be used and MTBE should be used only in a chemical fume hood or glove box. The same precaution applies to pure standard materials.

5.4 Diazomethane is a toxic carcinogen which can explode under certain conditions. The following precautions must be followed.

5.4.1 Use the diazomethane generator behind a safety shield in a well ventilated

fume hood. Under no circumstances can the generator be heated above 90°C, and all grinding surfaces such as ground glass joints, sleeve bearings and glass stirrers must be avoided. To minimize safety hazards, the diazomethane generator apparatus used in the esterification procedure (Sect 11.2) produces micro molar amounts of diazomethane in solution. If the procedure is followed carefully, no possibility for explosion exists.

5.5 Although hydrogen can be safely used as a carrier gas, the potential for fire or explosion does exist if the gas system is mishandled. If you are unsure of the safety guidelines for using hydrogen as a carrier gas, seek advice from your instrument manufacturer regarding its use.

6. APPARATUS AND EQUIPMENT

6.1 SAMPLE CONTAINERS – Amber glass bottles, approximately 40 mL, fitted with PTFE (polytetrafluoroethylene) lined screw caps.

6.2 EXTRACTION VIALS – 60-mL clear glass vials with PTFE lined screw caps.

6.3 AUTOSAMPLER VIALS – 2.0-mL vials with screw or crimp cap and a PTFE faced seal.

6.4 STANDARD SOLUTION STORAGE CONTAINERS – 10 to 20-mL amber glass vials with PTFE lined screw caps.

6.5 CLEAR VIALS – 7-mL glass, disposable, with PTFE lined screw caps for extract drying and derivatization.

6.6 PASTEUR PIPETTES – Glass, disposable.

6.7 PIPETTES – Class A, 2.0-mL and 4.0-mL glass, or adjustable volume dispensers.

6.8 VOLUMETRIC FLASKS – Class A, suggested sizes include 5 mL, 10 mL, 100 mL.

6.9 MICRO SYRINGES – Various sizes.

6.10 BALANCE – Analytical, capable of weighing to the nearest 0.0001 g.

6.11 DIAZOMETHANE GENERATOR – See Figure 4 for a diagram of an all glass system custom made for these validation studies. Micro molar generators are also available from commercial sources (Aldrich Cat.#: Z10,889-8 or equivalent).

6.12 GAS CHROMATOGRAPH – Capillary GC (Hewlett Packard Model 6890 or

equivalent), if the fast GC option is used the modifications should include a high pressure (≥ 50 psi) split/splitless injector, fast temperature ramp oven (50 °C/minute) and a low volume (150 µL) micro ECD detector. Additionally, a data system capable of fast sampling (20 points/peak) is required.

6.13 PRIMARY GC COLUMN – RTX-1701, 180 um i.d., fused silica capillary with chemically bonded (14% cyanopropylphenyl-methylpolysiloxane), or equivalent bonded, fused silica column.

6.14 CONFIRMATION GC COLUMN – DB-5, 180 um i.d., fused silica capillary with chemically bonded (5% phenyl-methylpolysiloxane), or equivalent bonded, fused silica column.

7. REAGENTS AND STANDARDS

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

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

7.1.2 METHYL tert-BUTYL ETHER (MtBE) – High purity, demonstrated to be free from analytes and interferences (HPLC grade or better).

7.1.3 ACETONE – High purity, demonstrated to be free from analytes and interferences (HPLC grade or better).

7.1.4 CARBITOL (DIETHYLENE GLYCOL MONOETHYL ETHER) – High purity, demonstrated to be free from analytes and interferences (HPLC grade or better).

7.1.5 HEXANE:MtBE (90:10, v/v) WASH SOLVENT – High purity, unpreserved, demonstrated to be free from analytes and interferences (HPLC grade or better).

7.1.6 HEXANE – High purity, demonstrated to be free from analytes and interferences (HPLC grade or better).

7.1.7 SODIUM SULFATE, Na2SO4 – Pesticide grade, granular, anhydrous. Interferences have been observed when lower quality grades have been used. If interferences are observed, it may be necessary to heat the sodium sulfate in a shallow tray at 400 °C for up to 4 hr to remove phthalates and other interfering organic substances. Alternatively, it can be extracted with methylene chloride in a Soxhlet apparatus for 48 hr. Store in a capped glass bottle rather than a plastic container.

7.1.8 ACIDIFIED SODIUM SULFATE – Acidify by slurrying 100 g of muffled sodium sulfate with enough ethyl ether to just cover the solid. Add 0.5-mL concentrated sulfuric acid dropwise while mixing thoroughly. Remove the ether under vacuum. Mix 1 g of the resulting solid with 5-mL of reagent water and measure the pH of the mixture. The pH must be below pH 4. Store in a desiccator or at 100°C to keep the reagent dry.

7.1.9 COPPER II SULFATE PENTAHYDRATE, $CUS0_4$-$5H_20$ – ACS reagent grade or better.

7.1.10 4 N SODIUM HYDROXIDE SOLUTION – Dissolve 16 g sodium hydroxide (NaOH) pellets (ACS grade or equivalent) in reagent water and dilute to 100 mL.

7.1.11 POTASSIUM HYDROXIDE SOLUTION (37%, w/v) – Dissolve 37 g of potassium hydroxide (KOH) pellets (ACS grade or equivalent) in reagent water and dilute to 100 mL.

7.1.12 SODIUM SULFITE, Na_2S0_3 – ACS reagent grade, used as a dechlorinating agent in this method.

7.1.13 DIAZALD SOLUTION – Prepare a solution containing 5 g diazald (ACS reagent grade) in 50 mL of a 50:50 (v/v) mixture of MtBE and carbitol. This solution is stable for one month or longer when stored at 4 °C in an amber bottle with a PTFE lined screw cap.

7.1.14 SULFURIC ACID – Concentrated, ACS reagent grade.

7.1.15 SILICA GEL – ACS reagent grade, 35-60 mesh.

7.1.16 HYDROGEN – 99.999% pure or better, GC carrier gas.

7.1.17 NITROGEN – 99.999% pure or better, ECD make-up gas.

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 organic solvent to aqueous samples. **Laboratories should use standard QC procedures to determine when Standard Solutions described in this section need to be replaced.**

7.2.1 INTERNAL STANDARD SOLUTIONS – 4,4'-Dibromooctafluorobiphenyl, 99+% is used as an internal standard for the method. This compound has been shown to be an effective internal standard for the method analytes, but other compounds may be used if the QC requirements in Section 9 are met.

7.2.1.1 INTERNAL STANDARD STOCK SOLUTION (2.0 mg/mL) – Prepare an internal standard a stock solution by accurately weighing approximately 0.0200 g of neat 4,4'- dibromooctafluorobiphenyl material. Dissolve the neat material in MtBE and dilute to volume in a 10-mL volumetric flask. Transfer the solution to an amber glass vial with a PTFE-lined screw cap and store at ≤ 0 °C. The resulting concentration of the stock internal standard solution will be approximately 2.0 mg/mL.

7.2.1.2 INTERNAL STANDARD PRIMARY DILUTION STANDARD (2.5 ug/mL) – Prepare a internal standard fortification solution at approximately 2.5 ug/mL by the addition of 12.5 uL of the stock standard to 10 mL of MtBE. Transfer the primary dilution to an amber glass vial with a PTFE lined screw cap and store at ≤ 0 °C. The solution should be replaced when ongoing QC indicates a problem.

7.2.1.3 MtBE EXTRACTION SOLVENT WITH INTERNAL STANDARD (2.5 ug/mL) – The internal standard 4,4'-dibromooctafluorobiphenyl is added to the extraction solvent prior to analyte extraction to compensate for any volumetric differences encountered during sample processing. This solution should be made fresh prior to extraction. The addition of 1 mL of the primary dilution standard (2.5 ug/mL) to 99-mL MtBE results in a final internal standard concentration of 25 ng/mL. This solution is used to extract the samples (Sect. 11.1).

7.2.2 SURROGATE (SUR) ANALYTE STANDARD SOLUTION – 2,4-Dichlorophenylacetic acid (99+%) is used as a surrogate compound in this method to evaluate the extraction and derivatization procedures. This compound has been shown to be an effective surrogate for the method analytes, but other compounds may be used if the QC requirements in Section

9 are met.

7.2.2.1 SURROGATE STOCK SOLUTION (1.0 mg/mL) – Prepare a surrogate stock standard solution of 2,4-dichlorophenylacetic acid by weighing 0.0100 g of neat material. Dissolve the neat material in acetone and dilute to volume in a 10-mL volumetric flask. Transfer the solution to an amber glass vial with a PTFE lined screw cap and store at ≤ 0 °C. The resulting concentration of the stock surrogate solution will be 1.0 mg/mL.

7.2.2.2 SURROGATE PRIMARY DILUTION STANDARD/SUR SAMPLE FORTIFICATION SOLUTION (100 ug/mL) – Prepare a primary dilution standard at approximately 100 ug/mL by the addition of 1 mL of the stock standard to 10 mL of acetone. Transfer the primary dilution to an amber glass vial with a PTFE lined screw cap and store at ≤ 0 °C. Addition of 10 ul, of the primary dilution standard to the 40-mL aqueous sample results in a surrogate concentration of 25 ng/mL. The solution should be replaced when ongoing QC indicates a problem.

7.2.3 ANALYTE STANDARD SOLUTIONS – Obtain the analytes listed in the table in Section 1.1 as neat or solid free acid standards or as commercially prepared ampulized solutions from a reputable standard manufacturer. The use of pre-methylated standards is not allowed for the preparation of analyte standards. Prepare the Analyte Stock and Primary Dilutions Standards as described below.

7.2.3.1 ANALYTE STOCK STANDARD SOLUTION – Prepare separate stock standard solutions for each analyte of interest at a concentration of 1-5 mg/mL in acetone. Method analytes may be obtained as neat materials or ampulized solutions (> 99% purity) from a number of commercial suppliers. These stock standard solutions should be stored at ≤ 0 °C.

7.2.3.1.1 For analytes which are solids in their pure form, prepare stock standard solutions by accurately weighing approximately 0.01 to 0.05 grams of pure material in a 10-mL volumetric flask. Dilute to volume with acetone.

7.2.3.1.2 For analytes which are liquid in their pure form at room temperature, place about 9.8 mL of acetone into a 10-mL volumetric flask. Allow the flask to stand, unstoppered, for about 10 minutes to allow solvent film to evaporate from

the inner walls of the volumetric, and weigh to the nearest 0.1 mg. Use a 10-uL syringe and immediately add 10.0 uL of standard material to the flask by keeping the syringe needle just above the surface of the acetone. Be sure that the standard material falls dropwise directly into the acetone without contacting the inner wall of the volumetric. Calculate the concentration in milligrams per milliliter from the net gain in weight. Dilute to volume, stopper, then mix by inverting the flask several times.

7.2.3.2 PRIMARY DILUTION STANDARD (PDS) – Prepare the primary dilution standard solution by combining and diluting stock standard solutions with acetone. This primary dilution standard solution should be stored at ≤ 0 °C. As a guideline to the analyst, the analyte concentrations used in the primary dilution standard solution during method development are given below.

Analyte	Concentration (ug/mL)
Acifluorfen	5.0
Bentazon	10
Chloramben	5.0
2,4-D	10
Dalapon	10
2,4-DB	10
Dacthal acid metabolites	5.0
Dicamba	5.0
3,5-Dichlorobenzoic acid	5.0
Dichlorprop	10
Dinoseb	10
Pentachlorophenol	1.0
Picloram	5.0
2,4,5-T	2.5
2,4,5-TP (Silvex)	2.5
Quinclorac	5.0

This primary dilution standard is used to fortify reagent water for calibration standards. The lowest calibration standard concentration must be at or below the MRL of each analyte. The concentrations of the other standards should define a range containing the expected sample concentrations or the working range of the detector.

7.2.4 CALIBRATION STANDARDS (CAL) – A five-point calibration curve is to be prepared by fortifying reagent water with the primary dilution standard. A designated amount of each calibration standard in acetone is spiked into separate 40-mL aliquots of reagent water to produce a calibration curve ranging from below or at the MRL to approximately 10-20 times the lowest calibration level. These aqueous calibration standards should be treated like samples and therefore require the addition of all preservation and other reagents. They are extracted by the procedure described in Section 11. The calibration standard solutions in acetone should be stored at ≤ 0 °C.

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[7] using amber glass containers with PTFE lined screw caps and capacities of at least 40 mL.

8.1.2 Add sodium sulfite crystals (approximately 2 mg/40 mL of sample) to the sample bottle prior to collecting the sample. This reagent eliminates residual chlorine in the sample.

8.2. SAMPLE COLLECTION

8.2.1 Fill sample bottles but take care not to flush out the sodium sulfite. Because the target analytes of this method are not volatile, it is not necessary to ensure that the sample bottles are completely headspace free.

8.2.2 When sampling from a 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.

8.2.3 When sampling from an open body of water, fill a 1 quart wide-mouth bottle or 1 liter beaker with sample from a representative area, and carefully fill sample vials from the container.

8.2.4 After collecting the sample, seal the bottle and agitate by hand for 15 seconds.

8.3 SAMPLE SHIPMENT AND STORAGE – All samples should be iced 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 and protected from light until extraction. Samples should not be frozen.

8.4 SAMPLE AND EXTRACT HOLDING TIMES – Because of the several pH adjustments made to the samples in the course of this method, the addition of organic or inorganic biocides, including hydrochloric acid, have been omitted. The analyst should be aware of the potential for the biological degradation of the analytes. Accordingly, samples should be extracted as soon as possible. Samples must be extracted within 14 days. Extracts must be stored at ≤ 0 °C or less and protected from light in glass vials with PTFE lined caps. Holding time studies indicate that the analytes are stable for up to 21 days in the extracts.

9. QUALITY CONTROL

9.1 Quality control (QC) requirements include the Initial Demonstration of Capability (Sect. 17, Table 13), the determination of the Detection Limit, and subsequent analysis in each analysis batch of a Laboratory Reagent Blank (LRB), Continuing Calibration Check Standards (CCC), 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 13 and 14. These criteria are considered the minimum acceptable QC criteria, and laboratories are encouraged to institute additional QC practices to meet their specific needs.

9.1.1 Process all quality control samples through all steps of Section 11, including hydrolysis and methylation. Sample preservatives as described in Section 8.1 must be added prior to extracting and analyzing the quality control samples.

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 13.

9.2.1 INITIAL DEMONSTRATION OF LOW SYSTEM BACKGROUND – Before any samples are analyzed, it must be demonstrated that a laboratory reagent blank (LRB) is reasonably free of contamination and that the criteria in Section 9.4 are met. Process all quality control samples through all all steps of the method (Sect. 9.1.1).

9.2.2 INITIAL DEMONSTRATION OF PRECISION – Prepare, extract, and analyze 4-7 replicate LFBs fortified at 5 ug/L, or near the mid-range of the initial calibration curve. Process all quality control samples through all all steps of the method (Sect. 9.1.1). 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 ± 20% of the true value.

9.2.4 DETECTION LIMIT – Prepare, extract and analyze at least seven replicate LFBs at a concentration estimated to be near the Detection Limit, 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. Process all quality control samples through all all steps of the method (Sect. 9.1.1). The fortification level may be estimated by selecting a concentration with a signal of 2 to 5 times the noise level but must be at or below the laboratory's MRL (Sect. 9.3). The appropriate concentration will be dependent upon the sensitivity of the GC/ECD system being used. Sample preservatives as described in Section 8.1 must be added to these samples. Calculate the Detection Limit using the equation

$$\text{Detection Limit} = St_{(n - 1, 1 - alpha = 0.99)}$$

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: Calculated Detection Limits need only be less than 1/3 of the laboratory's MRL to be considered acceptable. Do not subtract blank values when performing Detection Limit calculations. The Detection Limit is a statistical determination of precision only.[1] If the Detection Limit replicates are fortified at a low enough concentration, it is likely that they will not meet precision and accuracy criteria, and may result in a calculated Detection Limit that is higher than the fortified concentration

9.2.5 METHOD MODIFICATIONS – The analyst is permitted to modify GC columns, GC conditions, 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 the threshold concentration of an analyte that a laboratory can expect to accurately quantitate in an unknown sample. The MRL should not be established at an analyte concentration that is less than either three times the Detection Limit or a concentration which would yield a response less than a signal-to-noise (S/N) ratio of five. Depending upon the study's data quality objectives it may be set at a higher concentration. **Although the lowest calibration standard must be at or 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 standard prepared in the same extraction batch as the samples of interest that contains all compounds of interest and is extracted in the same manner as the procedural standards used to prepare the initial calibration curve. Calibration checks, prepared with the samples being analyzed, are required at the beginning of each day that samples are analyzed, after every ten samples, and at the end of any group of sample analyses. See Section 10.3 for concentration requirements, frequency requirements, and acceptance criteria.

9.6 LABORATORY FORTIFIED BLANK (LFB) – Since this method utilizes procedural calibration standards, which are fortified reagent waters, there is no difference between the LFB and the continuing calibration check standard. Consequently, the analysis of an LFB is not required ; however the acronym LFB is used for clarity in the IDC.

9.7 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.

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

9.7.2 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 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.

9.8 SURROGATE RECOVERY – The surrogate standard is fortified into the aqueous portion of all samples, LRBs, and LFMs and LFMDs prior to extraction. It is 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.

9.8.1 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.

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

9.8.3 If the extract reanalysis fails the 70-130% recovery criterion, the analyst should check the calibration by injecting the last calibration standard that passed. If the calibration standard fails the criteria of Section 9.8.1, recalibration is in order per Section 10.2. If 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 to inform the data user that the results are suspect due to surrogate recovery.

9.9 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. 9.10). 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.

9.9.1 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. 7.2.3.2). Select a spiking concentration that is at least twice the matrix background concentration, if known. Use historical data and rotate through the designated concentrations when selecting a fortifying concentration.

9.9.2 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.

9.9.3 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 150% 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.

9.9.4 If an LFMD is analyzed instead of a Field Duplicate (Sect. 9.10), calculate the relative percent difference (RPD) for duplicate LFMs (LFM and LFMD) using the equation

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

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.

9.10 FIELD DUPLICATES (FD1 AND FD2) – Within each extraction batch, a minimum of one Field Duplicate (FD) or LFMD (Sect. 9.9) 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 will not require the analysis of a Field Duplicate.

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

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

9.10.2 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.

9.11 QUALITY CONTROL SAMPLES (QCS) – A QCS sample should be analyzed each time that new Primary Dilution Standards (Sect. 7.2.3.2) are prepared. The source of the QCS sample should ideally be a second vendor. If a second vendor is not available then a different lot of the standard should be used. Although the use of pre-methylated standards is prohibited for preparing analyte standard solutions, pre-methylated standards may be used to prepare the QCS. 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 After initial calibration is successful, a Continuing Calibration Check (CCC) is required at the beginning and end of each analysis batch, and after every tenth sample (Sect. 10.3). Because this is a procedural standard method, the analyst will need to make a decision to include an appropriate number of CCCs or an entire initial calibration curve with each extraction batch. Initial calibration should be repeated each time a major instrument modification or maintenance is performed.

10.2 INITIAL CALIBRATION

10.2.1 Establish GC operating parameters equivalent to the suggested specifications in Section 17, Table 1. The GC system must be calibrated using the internal standard (IS) technique. Other columns or conditions may be used if equivalent or better performance can be demonstrated.

10.2.2 Prepare a set of at least 5 calibration standards as described in Section 7.2.4. The lowest concentration of calibration standard must be at or below the MRL, which will depend on system sensitivity.

10.2.3 CALIBRATION – Use the GC data system software to generate a linear regression or quadratic calibration curve using the internal standard. The analyst may choose whether or not to force zero to obtain a curve that best fits the data. Examples of common GC system calibration curve options are: 1) A_x / A_{is} vs Q_x / Q_{is} and 2) RRF vs A_x / A_{is}.

where:

A_x = integrated peak area of the analyte,
A_{is} = integrated peak area of the 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.

10.2.4 As an alternative, concentrations may be calculated through the use of average relative response factor (RRF). Calculate the RRFs using the equation

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

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 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.

10.3 CONTINUING CALIBRATION CHECK (CCC) – An appropriate number of CCCs must be prepared with each extraction batch. The CCC verifies the initial calibration at the beginning and end of each group of analyses, and after every 10^{th} sample during analyses. In this context, a "sample" is considered to be a field sample. LRBs, 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 check standard solution prepared with the extraction batch and analyze with the same conditions used during the initial calibration.

10.3.2 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 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.

11. PROCEDURE

11.1 SAMPLE EXTRACTION AND HYDROLYSIS

11.1.1 Remove the samples from storage (Sect. 8.3) and allow them to equilibrate to room temperature.

11.1.2 Place 40 mL of the water sample into a precleaned 60-mL, glass vial with a PTFE lined screw cap using a graduated cylinder.

11.1.3 Add 10 uL of surrogate standard (100 ug/mL, 2,4-dichlorophenylacetic acid in acetone per Section 7.2.2) to the aqueous sample.

11.1.4 Add 1 mL, of the 4 N NaOH solution prepared in Section 7.1.10 to each glass vial. Check the pH of the sample with pH paper or a pH meter. If the sample does not have a pH greater than or equal to 12, adjust the pH by adding more 4 N NaOH solution. Let the sample sit at room temperature for 1 hour, shaking the contents periodically.

NOTE: Since many of the herbicides contained in this method are applied as a variety of esters and salts, it is vital to hydrolyze them to the parent acid prior to extraction. This step must be included in the analysis of all extracted field samples, LRBs, LFMs and calibration standards. Failure to perform this step may result in data that are biased low for some targets in field samples.[8]

11.1.5 Following hydrolysis, add 5 mL of (90:10, v:v) hexane:MtBE (Section 7.1.5) and shake vigorously for three minutes. Allow the phases to separate for approximately 5 minutes then remove and discard the top hexane/MtBE layer. This wash aids in sample cleanup and removes any Dacthal from the sample which would interfere with the quantitation of the Dacthal metabolites.

11.1.6 Adjust the pH to approximately 1 by adding concentrated sulfuric acid. Cap, shake and then check the pH with a pH meter or narrow range pH paper. Add additional sulfuric acid as needed to properly adjust the pH.

11.1.7 Quickly add approximately 2 g of copper II sulfate pentahydrate and shake until dissolved. This colors the aqueous phase blue and allows the analyst to better distinguish between the aqueous phase and the organic phase in this micro extraction.

11.1.8 Quickly add approximately 16 g of muffled sodium sulfate (Sect. 7.1.7) and shake until almost all is dissolved. Sodium sulfate is added to increase the ionic strength of the aqueous phase and thus further drive the chlorophenoxy

acids into the organic phase. The addition of salt also decreases the solubility of MtBE in the aqueous phase and allows greater volumetric recovery. The addition of this salt and the copper II sulfate pentahydrate should be done quickly so that the heat generated from the addition of the acid (Section 11.1.6) will help dissolve the salts.

11.1.9 Add exactly 4.0-mL MtBE and shake vigorously for three minutes.

11.1.10 Allow the phases to separate for approximately 5 minutes.

11.2 SAMPLE METHYLATION WITH DIAZOMETHANE

11.2.1 GENERATION OF DIAZOMETHANE

11.2.1.1 Assemble the diazomethane generator (Figure 4) in a hood. The collection vessel is a 10- or 15-mL, glass vial equipped with a PTFE lined screw cap that is maintained at 0 - 5 °C.

11.2.1.2 Add a sufficient amount of MtBE (approximately 7 mL) to tube 1 to cover the first impinger. Add 10 mL of MtBE to the collection vial. Set the nitrogen flow at 5-10 mL/min. Add 4-mL Diazald solution (Sect. 7.1.13) and 3 mL of 37% KOH solution (Sect. 7.1.11) to the second impinger. Connect the tubing as shown and allow the nitrogen flow to purge the diazomethane from the reaction vessel into the collection vial for 30 minutes. Cap the vial when collection is complete and maintain at 0-5 °C. When stored at 0-5 °C, this diazomethane solution may be used over a period of 72 hours.

11.2.1.3 Several commercial sources of glassware are available for diazomethane generation. These include a mini Diazald apparatus from Aldrich (Cat. #:Z10,889-8).

11.2.2 Using a Pasteur pipette, transfer the sample extract (upper MtBE layer) to a 7-mL, screw cap vial. Add 0.6 g acidified sodium sulfate (Sect. 7.1.8) and shake. This step is included to dry the MtBE extract.

11.2.3 Using a Pasteur pipette, transfer the extract to a second, 7-mL glass vial.

11.2.4 Add 250 uL of the diazomethane solution prepared in Section 11.2.1 to each vial. The contents of the vial should remain slightly yellow in color indicating an excess of diazomethane. Additional diazomethane may be added if necessary. Let the esterification reaction proceed for 30 minutes.

11.2.5 Remove any unreacted diazomethane by adding 0.1 g of silica gel. Effervescence (evolution of nitrogen) is an indication that excess diazomethane was present. Allow the extracts to sit for 0.5 hour.

11.2.6 Transfer the extract to an autosampler vial. A duplicate vial may be filled using the excess extract.

11.2.7 Analyze the sample extracts as soon as possible. The sample extract may be stored up to 21 days if kept at 0 °C or less. Keep the extracts away from light in amber glass vials with PTFE lined caps.

11.3 GAS CHROMATOGRAPHY

11.3.1 If the fast GC option is used, several important changes from "conventional GC" should be made to aid in the rapid analysis of the analytes. The instrument should have a fast temperature ramp (50 °C/minute) oven and a high pressure (≥ 50 psi) split/splitless injector. When using columns with diameters less than 180 um the instrument should be equipped with a low volume (150 µL) micro ECD. Additionally, the column diameter and film thickness should be decreased and the carrier gas should be changed to hydrogen.

11.3.2 Use of Hydrogen Safely – Although hydrogen can be used safely as a carrier gas, the potential for fire or explosion does exist if the gas system is mishandled. If you are unsure of the safety guidelines for using hydrogen as a carrier gas, seek advice from your instrument manufacturer regarding its use.

11.3.3 Column Selection and Installation – During method development the RTX-1701 columns provided baseline resolution for the 2,4-DB /Chloramben pair (peaks 11 and 12 in Figure 1). Columns from other manufacturers were not as effective in separating these analytes. Strict attention must be paid to established column installation guidelines with regard to the proper cutting and placement of the capillary columns within the instrument. Additionally, a loss of response over time was noted for Acifluorifen and Dinoseb. Trimming approximately 1 meter from the head of the column restored the response for these analytes. If conditions in the laboratory necessitate frequent column trimming, a guard column is recommended.

11.4 ANALYSIS OF EXTRACTS

11.4.1 Establish operating conditions as described in Section 17, Table 1 (Table 2 if performing confirmation). Confirm that retention times, compound separation and resolution are similar to those summarized in Table 1 and Figure1.

11.4.2 Establish an appropriate retention time window for each target and surrogate to identify them in the QC and field samples. This should be based on measurements of actual retention time variation for each compound in standard solutions analyzed on the GC over the course of time. Plus or minus three times the standard deviation of the retention time for each compound while establishing the initial calibration and completing the IDC can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily on the determination of the appropriate retention window size.

11.4.3 Check system calibration by analyzing a CCC (Sect. 10.3) and calibrate the system by either the analysis of a calibration curve (Sect. 10. 2) or by confirming the initial calibration is still valid by analyzing a continuing calibration check as described in Section 10.3. Begin analyzing field and QC samples at their appropriate frequency by injecting the same size aliquots under the same conditions used to analyze the initial calibration.

11.4.4 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 MtBE containing the internal standard (Sect. 7.2.1.3), and the diluted extract injected. Acceptable surrogate performance (Sect. 9.8) 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 CALCULATION

12.1 Identify the method analytes in the sample chromatogram by comparing the retention time of the suspect peak to retention time of an analyte peak in a calibration standard. Surrogate retention times should be confirmed to be within acceptance limits (Sect. 11.4.2) even if no target compounds are detected.

12.2 Calculate the analyte concentrations using the initial calibration curve generated as described in Section 10.2. Quantitate only those values that fall between the MRL and the highest calibration standard. Samples with target analyte responses that exceed the highest standard require dilution and reanalysis (Sect. 11.4.4).

12.3 Analyte identifications should be confirmed using the confirmation column specified in Table 2 or another column that is dissimilar to the primary column or by GC/MS if the concentrations are sufficient.

12.4 Adjust the calculated concentrations of the detected analytes to reflect the initial

sample volume and any dilutions performed.

12.5 Analyte concentrations are reported in ug/L as the total free acid. Calculations should use all available digits of precision.

13. METHOD PERFORMANCE

13.1 PRECISION, ACCURACY, AND Detection Limits – Tables for these data are presented in Section 17. Single laboratory precision and accuracy data are presented in Tables 5 -10. Detection Limits are presented in Table 4 and were calculated using the formula presented in Section 9.2.4.

14. POLLUTION PREVENTION

14.1 This method utilizes liquid:liquid microextraction to extract 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 as compared to the use of large volumes of organic solvents in conventional liquid-liquid extractions.

14.2 For information about pollution prevention that may be applicable to laboratory operations, consult "Less is Better: Laboratory Chemical Management for Waste Reduction" available from the American Chemical Society's Department of Government Relations and Science Policy, 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: CHROMATOGRAPHIC CONDITIONS AND AVERAGE RETENTION TIME DATA FOR THE PRIMARY COLUMN (RTX-1701, 40m x 180 um i.d.)

Peak Number (Figure 1)	Compound	Average T_r (min)[a]	% RSD
1	Dalapon	7.06	0.017
2	3,5 Dichlorobenzoic acid	11.32	0.002
3	2,4-Dichlorophenylacetic acid (SUR)	11.80	0.002
4	Dicamba	11.91	0.002
5	Dichlorprop	12.21	0.002
6	4,4'-Dibromooctafluorobiphenyl (IS)	12.25	0.002
7	2,4-D	12.37	0.002
8	Pentachlorophenol	12.49	0.002
9	Silvex	12.70	0.002
10	2,4,5-T	12.90	0.002
11	2,4-DB	13.10	0.003
12	Chloramben	13.15	0.003
13	Dinoseb	13.44	0.003
14	Bentazon	13.60	0.003
15	Dacthal	13.68	0.002
16	Quinclorac	13.85	0.004
17	Picloram	14.02	0.003
18	Acifluorfen	15.20	0.004

[a] The average retention time represents the average of 7 runs.

Primary Column:

DB-1701, 40 m x 0.180 mm i.d., 0.20 µm film thickness, injector temp. 200 °C, liner 2 mm straight siltek® deactivated, injection volume 1 µL of 200 ng/mL (highest level component) standard, splitless injection hold to 1 min then purge @ 50 mL/min, detector temp 360 °C, detector make up gas nitrogen at 20 mL/minute. Temperature program: 45 °C initial, program at 5 °C/minute to 80 °C, Then 50 °C/minute to 220 °C, Then 20 °C/minute to 280 °C and Hold for 3 min, Data collection via HP GC Chemstation at a rate of 20Hz.

Carrier gas: Hydrogen (UHP)

Detector makeup gas: Nitrogen (UHP)

TABLE 2: CHROMATOGRAPHIC CONDITIONS AND AVERAGE RETENTION TIME DATA FOR THE CONFIRMATION COLUMN (DB-5, 40 m x 180 um i.d.)

Peak Number (Figure 1)	Compound	Average T_r (min)[a]	% RSD
1	Dalapon	5.04	0.057
2	3,5-Dichlorobenzoic acid	10.33	0.007
3	2,4-Dichlorophenylacetic acid (SUR)	11.31	0.004
4	Dicamba	11.46	0.006
5	Dichlorprop	12.19	0.006
6	4,4'-Dibromooctafluorobiphenyl (IS)	12.71	0.004
7	2,4-D	12.34	0.005
8	Pentachlorophenol	13.16	0.006
9	Silvex	13.29	0.004
10	2,4,5-T	13.48	0.005
11	2,4-DB	13.94	0.005
12	Chloramben	13.34	0.004
13	Dinoseb	14.01	0.004
14	Bentazon	14.28	0.006
15	Dacthal	15.00	0.004
16	Quinclorac	14.88	0.004
17	Picloram	14.59	0.004
18	Acifluorfen	16.38	0.003

[a] The average retention time represents the average of 7 runs.

Confirmation Column:

DB-5, 40 m x 0.180 mm i.d., 0.20 µm film thickness, injector temp. 200 °C, liner 2 mm straight siltek® deactivated, injection volume 1 µL of 100 ng/mL (highest level component) standard, splitless injection hold to 1 min then purge @ 50 mL/min, detector temp 360 °C, detector make up gas nitrogen at 20 mL/minute. Temperature program: 45 °C initial, program at 4 °C/minute to 80 °C then program at 30 °C/minute to 280 and Hold for 2 min, Data collection via HP GC Chemstation at a rate of 20Hz.

Carrier gas: Hydrogen (UHP)

Detector makeup gas: Nitrogen (UHP)

TABLE 3: CHROMATOGRAPHIC CONDITIONS AND AVERAGE RETENTION TIME DATA FOR A CONVENTIONAL PRIMARY COLUMN (DB-5, 30 m x 250 um i.d.)

Peak Number (Figure 3)	Compound	T_r (min)
1	Dalapon	14.28
2	3,5-Dichlorobenzoic acid	20.35
3	2,4-Dichlorophenylacetic acid (SUR)	20.98
4	Dicamba	21.10
5	Dichlorprop	21.46
6	4,4'-Dibromooctafluorobiphenyl (IS)	21.53
7	2,4-D	21.64
8	Pentachlorophenol	21.81
9	Silvex	22.03
10	2,4,5-T	22.27
11	2,4-DB	22.51
12	Chloramben	22.55
13	Dinoseb	22.94
14	Bentazon	23.17
15	Dacthal	23.30
16	Quinclorac	23.55
17	Picloram	23.82
18	Acifluorfen	25.90

Conventional Primary Column:

DB-1701, 30 m x 0.250 mm i.d., 0.25 µm film thickness, injector temp. 200 °C, liner 2 mm straight deactivated, injection volume 1 µL of 100 ng/mL (highest level component) standard, splitless injection hold to .5 min then purge @ 30 mL/min, detector temp 290 °C, detector make up gas P-5 at 60 mL/minute. Temperature program: 45 °C initial, hold 10 min then program at 5 °C/minute to 70 °C, Then 30 °C/minute to 260 °C, and Hold for 5 min, Data collection via Turbochrom 4.1

Carrier gas: Helium (UHP)

Detector makeup gas: 5% methane/argon (UHP)

TABLE 4: DETECTION LIMITS IN REAGENT WATER

Compound	Fortification Level (ug/L)	Primary Column Detection Limit[a] (ug/L)	Secondary Column Detection Limit[a] (ug/L)
Dalapon	0.100	0.054	0.074
3,5-Dichlorobenzoic acid	0.050	0.212	0.049
Dicamba	0.050	0.032	0.042
Dichlorprop	0.100	0.433	0.12
2,4-D	0.100	0.055	0.066
Pentachlorophenol	0.010	0.014	0.084
Silvex	0.025	0.033	0.018
2,4,5-T	0.025	0.024	0.033
2,4-DB	0.100	0.246	0.182
Chloramben	0.050	0.057	0.083
Dinoseb	0.100	0.166	0.081
Bentazon	0.100	0.064	0.185
Dacthal	0.050	0.113	0.105
Quinclorac	0.050	0.080	0.113
Picloram	0.050	0.076	0.055
Acifluorfen	0.250	0.307	0.092

[a]Detection Limits were extracted and analyzed over 3 days for 9 replicates following the procedure outlined in Section 9.

TABLE 5: LOW LEVEL PRECISION AND ACCURACY IN REAGENT WATER

Compound	Fortification Level (ug/L)	Mean % Recovery	% RSD (n=7)	S/N Ratio[a]
Dalapon	1.0	108	2.2	578
3,5-Dichlorobenzoic acid	0.50	117	16	9
Dicamba	0.50	97	3.7	35
Dichlorprop	1.0	95	5.7	19
2,4-D	1.0	98	5.2	23
Pentachlorophenol	0.10	76	7.4	21
Silvex	0.25	84	20	9
2,4,5-T	0.25	96	7.3	16
2,4-DB	1.0	97	8.4	6
Chloramben	0.50	90	9.0	113
Dinoseb	1.0	103	6.7	8
Bentazon	1.0	100	2.1	41
Dacthal	0.50	92	6.5	100
Quinclorac	0.10	106	9.3	20
Picloram	1.0	92	2.6	23
Acifluorfen	0.50	77	5.9	80
2,4-Dichlorophenylacetic acid (SUR)	25	104	8.0	-

[a]Signal-to-noise ratios were calculated for each target compound 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.

TABLE 6: MID LEVEL PRECISION AND ACCURACY IN REAGENT WATER

Compound	Fortification Level (ug/L)	Mean % Recovery	% RSD (n=7)
Dalapon	10	107	2.3
3,5-Dichlorobenzoic acid	5.0	96	2.3
Dicamba	5.0	102	1.5
Dichlorprop	10	107	0.6
2,4-D	10	106	1.4
Pentachlorophenol	1.0	103	1.4
Silvex	2.5	107	4.4
2,4,5-T	2.5	105	1.3
2,4-DB	10	93	1.6
Chloramben	5.0	105	2.9
Dinoseb	10	119	4.6
Bentazon	10	98	0.7
Dacthal	5.0	100	1.9
Quinclorac	1.0	101	2.9
Picloram	10	99	3.1
Acifluorfen	5.0	107	4.5
2,4-Dichlorophenylacetic acid (SUR)	25	112	1.3

TABLE 7: LOW LEVEL PRECISION AND ACCURACY IN FORTIFIED CHLORINATED SURFACE WATER

Compound	Fortification Level (ug/L)	Mean % Recovery	% RSD (n=7)
Dalapon	1.0	104	2.1
3,5-Dichlorobenzoic acid	0.50	110	3.0
Dicamba	0.50	93	4.4
Dichlorprop	1.0	93	11
2,4-D	1.0	97	5.2
Pentachlorophenol	0.10	86	3.2
Silvex	0.25	103	4.3
2,4,5-T	0.25	100	3.5
2,4-DB	1.0	104	5.9
Chloramben	0.50	93	2.0
Dinoseb	1.0	99	1.2
Bentazon	1.0	91	2.2
Dacthal	0.50	92	4.6
Quinclorac	0.10	96	15
Picloram	1.0	102	2.4
Acifluorfen	0.50	98	4.1
2,4-Dichlorophenylacetic acid (SUR)	25	107	1.5

TABLE 8: MID LEVEL PRECISION AND ACCURACY IN FORTIFIED CHLORINATED SURFACE WATER

Compound	Fortification Level (ug/L)	Mean % Recovery	% RSD (n=7)
Dalapon	10	100	2.5
3,5-Dichlorobenzoic acid	5.0	104	2.6
Dicamba	5.0	102	2.9
Dichlorprop	10	101	1.2
2,4-D	10	101	1.2
Pentachlorophenol	1.0	100	1.0
Silvex	2.5	101	1.0
2,4,5-T	2.5	100	1.6
2,4-DB	10	101	2.3
Chloramben	5.0	102	2.6
Dinoseb	10	101	1.6
Bentazon	10	98	1.8
Dacthal	5.0	99	2.0
Quinclorac	1.0	105	2.5
Picloram	10	107	3.2
Acifluorfen	5.0	92	2.4
2,4-Dichlorophenylacetic acid (SUR)	25	106	1.4

TABLE 9: LOW LEVEL PRECISION AND ACCURACY IN FORTIFIED CHLORINATED GROUND WATER

Compound	Fortification Level (ug/L)	Mean % Recovery	% RSD (n=7)
Dalapon	1.0	105	1.5
3,5-Dichlorobenzoic acid	0.50	104	6.0
Dicamba	0.50	100	2.2
Dichlorprop	1.0	109	3.5
2,4-D	1.0	108	10
Pentachlorophenol	0.10	103	17
Silvex	0.25	96	3.0
2,4,5-T	0.25	91	3.4
2,4-DB	1.0	94	3.1
Chloramben	0.50	88	2.7
Dinoseb	1.0	100	4.0
Bentazon	1.0	101	2.8
Dacthal	0.50	62[a]	9.9
Quinclorac	0.10	107	5.7
Picloram	1.0	97	2.8
Acifluorfen	0.50	92	4.2
2,4-Dichlorophenylacetic acid (SUR)	25	95	3.1

[a]Dacthal was present in the chlorinated surface water at about 1/5th the fortification level. This was taken into account in the determination of the Mean % Recovery.

TABLE 10: MID LEVEL PRECISION AND ACCURACY IN FORTIFIED CHLORINATED GROUND WATER

Compound	Fortification Level (ug/L)	Mean % Recovery	% RSD (n=7)
Dalapon	10	97	5.5
3,5-Dichlorobenzoic acid	5.0	95	2.1
Dicamba	5.0	98	1.6
Dichlorprop	10	79	0.7
2,4-D	10	99	1.6
Pentachlorophenol	1.0	100	4.2
Silvex	2.5	97	1.6
2,4,5-T	2.5	97	1.5
2,4-DB	10	92	1.1
Chloramben	5.0	102	2.6
Dinoseb	10	105	2.6
Bentazon	10	101	1.8
Dacthal	5.0	99	1.2
Quinclorac	1.0	94	1.5
Picloram	10	103	2.5
Acifluorfen	5.0	84	2.5
2,4-Dichlorophenylacetic acid (SUR)	25	106	1.1

TABLE 11. SAMPLE HOLDING TIME DATA FOR SAMPLES FROM A CHLORINATED SURFACE WATER FORTIFIED WITH METHOD ANALYTES[a]

Compound	Day 0 % Rec	Day 2 % Rec	Day 7 % Rec	Day 14 % Rec
Dalapon	91	90	91	108
3,5-Dichlorobenzoic acid	98	97	85	91
Dicamba	92	92	96	99
Dichlorprop	89	88	91	95
2,4-D	88	85	87	92
Pentachlorophenol	92	85	86	93
Silvex	91	88	86	94
2,4,5-T	89	89	85	91
2,4-DB	87	84	86	93
Chloramben	86	86	88	100
Dinoseb	105	95	99	111
Bentazon	91	87	95	99
Dacthal	92	91	86	94
Quinclorac	90	80	84	91
Picloram	94	85	85	99
Acifluorfen	109	96	103	123

[a] All samples fortified at the same level used to collect the mid level precision and accuracy data (see Table 10 for example).

TABLE 12. EXTRACT HOLDING TIME DATA FOR SAMPLES FROM A CHLORINATED SURFACE WATER FORTIFIED WITH METHOD ANALYTES[a,b]

Compound	Initial [a, b] Injection %Rec	Day 21 Reinjection %Rec
Dalapon	100	89
3,5-Dichlorobenzoic acid	104	113
Dicamba	102	104
Dichlorprop	100	101
2,4-D	101	102
Pentachlorophenol	100	102
Silvex	101	103
2,4,5-T	99	102
2,4-DB	101	102
Chloramben	102	105
Dinoseb	99	81
Bentazon	98	100
Dacthal	99	103
Quinclorac	105	127
Picloram	107	98
Acifluorfen	92	104
2,4-Dichlorophenylacetic acid (SUR)	106	105

[a]Sample storage stability is expressed as a percent recovery (%Rec) value calculated as described in Section 9.9.2.

[b]All samples fortified at the same level used to collect the mid level precision and accuracy data (see Table 10 for example).

TABLE 13: 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 must be within ± 20% of true value.
Section 9.2.4	Detection Limit Determination	Over a period of three days, prepare a minimum of 7 replicate LFBs fortified at a concentration estimated to be near the Detection Limit. Analyze the replicates through all steps of the analysis. Calculate the Detection Limit using the equation in Section 9.2.4.	Must be ≤ 1/3 of the established MRL. Note: Data from Detection Limit replicates are not required to meet method precision and accuracy criteria. If the Detection Limit replicates are fortified at a low enough concentration, it is likely that they will not meet precision and accuracy criteria.

TABLE 14: QUALITY CONTROL REQUIREMENTS (SUMMARY)

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Section 10.2	Initial Calibration	Use internal standard calibration technique to generate a 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.
Sections 9.5 and 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.

TABLE 14: QUALITY CONTROL REQUIREMENTS (SUMMARY)

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Section 9.7	Internal Standard (IS)	4,4'-dibromooctafluoro-biphenyl is added to the extraction solvent.	Peak area counts for the IS in LFBs, LRBs, and sample extracts must be within 50-150% of the average peak area calculated during the initial calibration. If the IS does not meet criteria, corresponding target results are invalid.
Section 9.8	Surrogate Standard (SUR)	2,4-dichlorophenylacetic acid is added to all calibration standards and samples, including QC samples.	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 9.9	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 within ±30% at mid and high levels of fortification and within ±50% near MRL.
Section 9.10	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 within ±30% at mid and high levels of fortification and within ±50% near MRL.
Section 9.11	Quality Control Sample (QCS)	Analyzed when new Primary Dilution Standards (PDS) are prepared.	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	21 days with appropriate storage	Sample results are valid only if extracts are analyzed within extract hold time.

FIGURE 1
CHROMATOGRAM OF THE CHLORPHENOXY HERBICIDES ON A RTX-1701 COLUMN
CHROMATOGRAPHIC CONDITIONS ARE GIVEN IN TABLE 1.

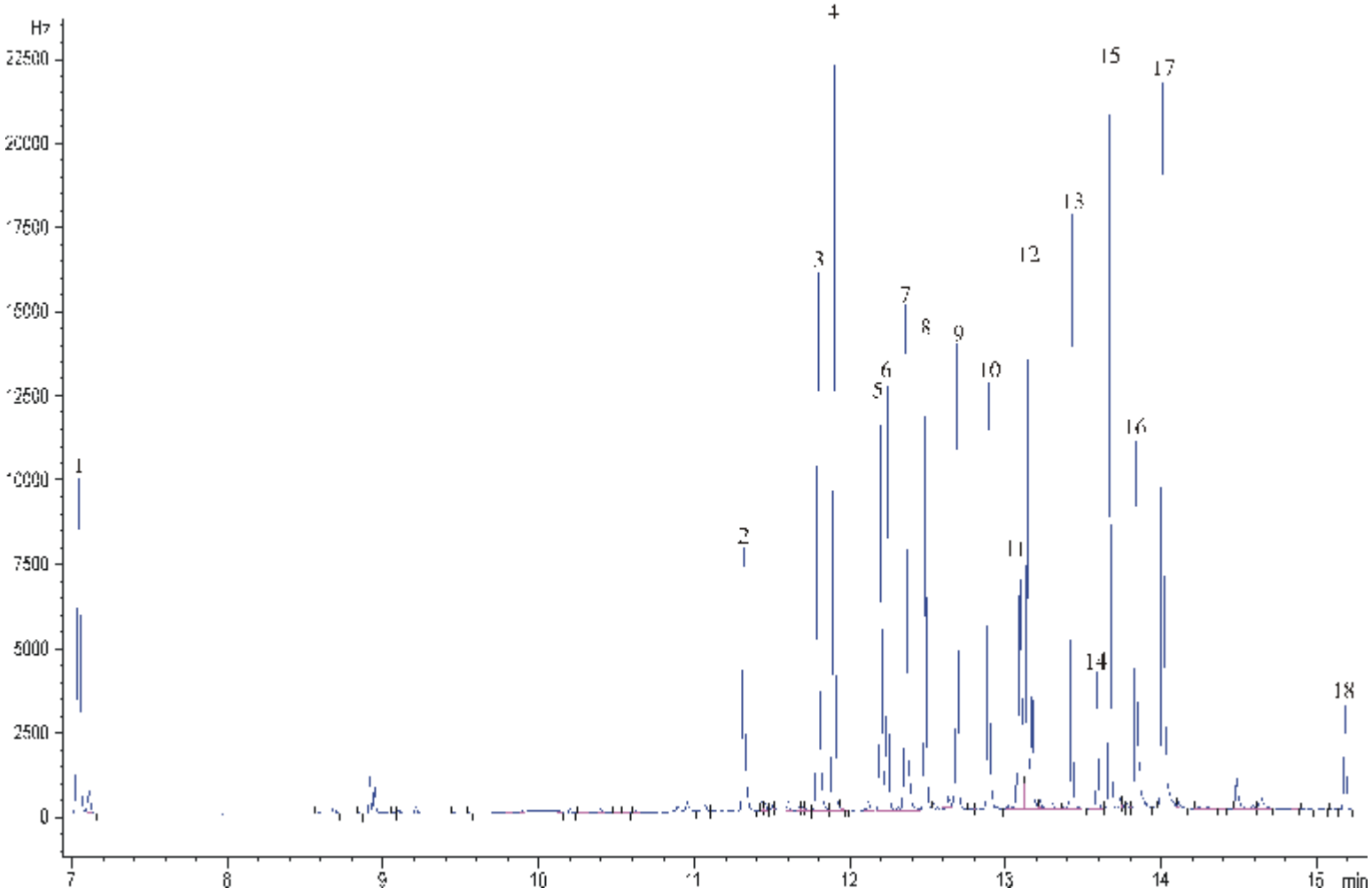

FIGURE 2
CHROMATOGRAM OF THE CHLORPHENOXY HERBICIDES ON A DB-5 COLUMN
CHROMATOGRAPHIC CONDITIONS ARE GIVEN IN TABLE 2.

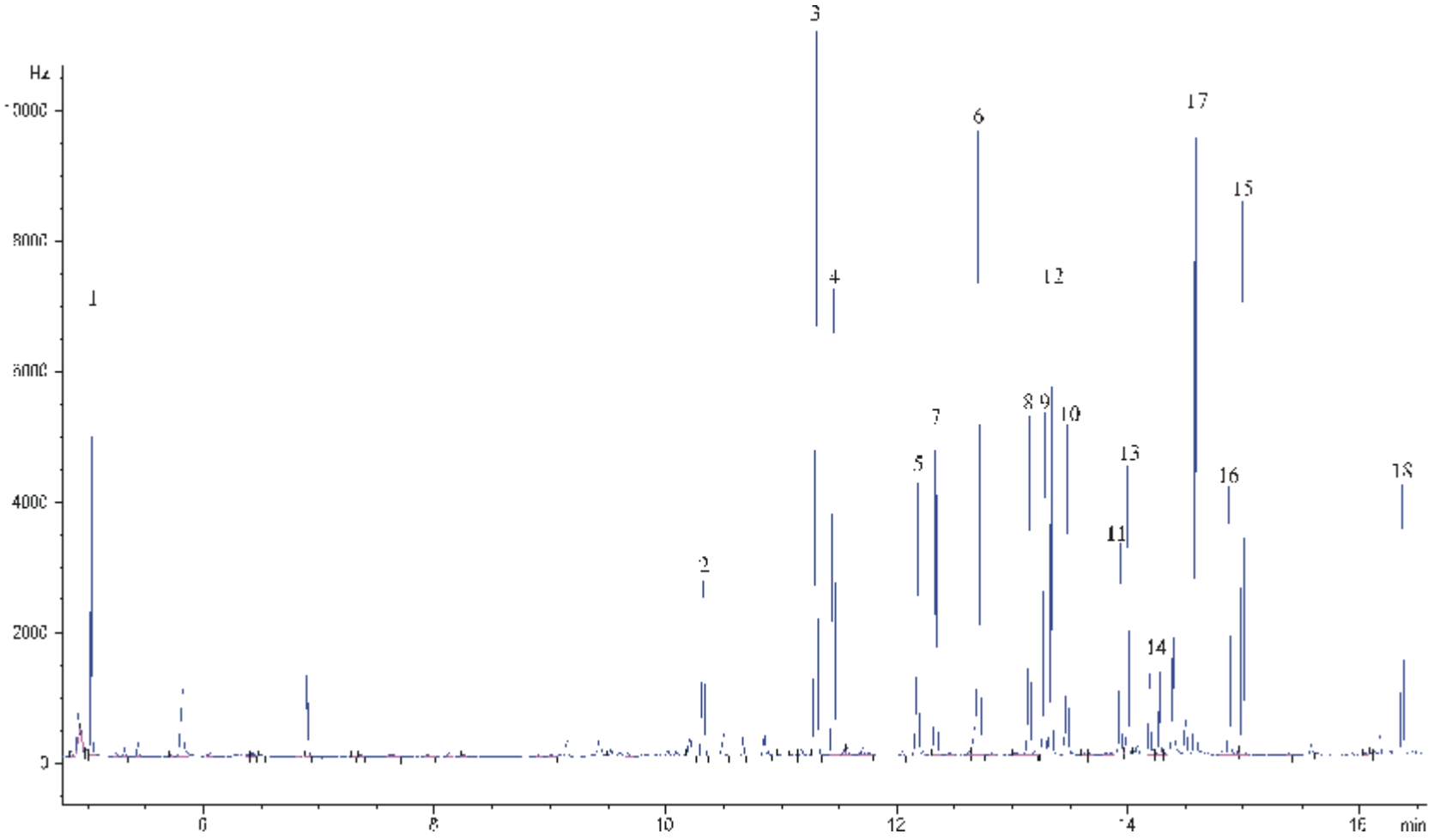

FIGURE 3

CHROMATOGRAM OF THE CHLORPHENOXY HERBICIDES ON A CONVENTIONAL PRIMARY COLUMN (30 m x 250 um) CHROMATOGRAPHIC CONDITIONS ARE GIVEN IN TABLE 3.

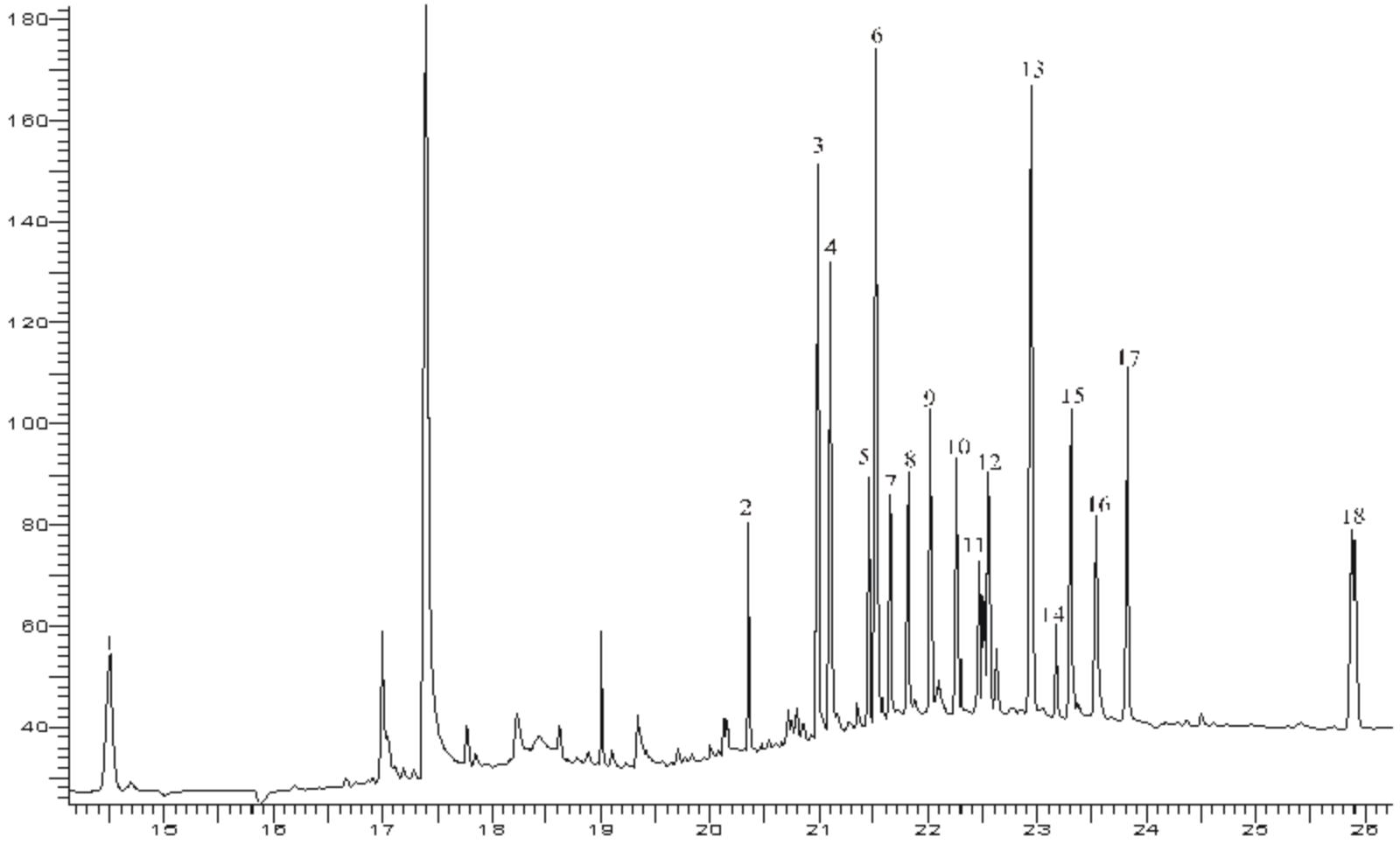

FIGURE 4

DIAZOMETHANE GENERATOR