METHOD 8321A

SOLVENT EXTRACTABLE NONVOLATILE COMPOUNDS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY/THERMOSPRAY/MASS SPECTROMETRY (HPLC/TS/MS) OR ULTRAVIOLET (UV) DETECTION

1.0 SCOPE AND APPLICATION

1.1 This method covers the use of high performance liquid chromatography (HPLC), coupled with either thermospray-mass spectrometry (TS-MS), and/or ultraviolet (UV), for the determination of disperse azo dyes, organophosphorus compounds, and tris(2,3-dibromopropyl)phosphate, chlorinated phenoxyacid compounds and their esters, and carbamates in wastewater, ground water, and soil/sediment matrices. Data are also provided for chlorophenoxy acid herbicides in fly ash (Table 15), however, recoveries for most compounds are very poor indicating poor extraction efficiency for these analytes using the extraction procedure included in this method. Additionally, it may apply to other non-volatile compounds that are solvent extractable, are amenable to HPLC, and are ionizable under thermospray introduction for mass spectrometric detection or may be determined by a UV detector. The following compounds can be determined by this method:

Compound Name	CAS No.[a]
Azo Dyes	
Disperse Red 1	2872-52-8
Disperse Red 5	3769-57-1
Disperse Red 13	126038-78-6
Disperse Yellow 5	6439-53-8
Disperse Orange 3	730-40-5
Disperse Orange 30	5261-31-4
Disperse Brown 1	17464-91-4
Solvent Red 3	6535-42-8
Solvent Red 23	85-86-9
Anthraquinone Dyes	
Disperse Blue 3	2475-46-9
Disperse Blue 14	2475-44-7
Disperse Red 60	17418-58-5
Coumarin Dyes	
Fluorescent Brighteners	
Fluorescent Brightener 61	8066-05-5
Fluorescent Brightener 236	3333-62-8
Alkaloids	
Caffeine	58-08-2
Strychnine	57-24-9

Compound Name	CAS No.[a]
Organophosphorus Compounds	
Methomyl	16752-77-5
Thiofanox	39196-18-4
Famphur	52-85-7
Asulam	3337-71-1
Dichlorvos	62-73-7
Dimethoate	60-51-5
Disulfoton	298-04-4
Fensulfothion	115-90-2
Merphos	150-50-5
Methyl parathion	298-00-0
Monocrotophos	919-44-8
Naled	300-76-5
Phorate	298-02-2
Trichlorfon	52-68-6
Tris(2,3-dibromopropyl) phosphate (Tris-BP)	126-72-7
Chlorinated Phenoxyacid Compounds	
Dalapon	75-99-0
Dicamba	1918-00-9
2,4-D	94-75-7
MCPA	94-74-6
MCPP	7085-19-0
Dichlorprop	120-36-5
2,4,5-T	93-76-5
Silvex (2,4,5-TP)	93-72-1
Dinoseb	88-85-7
2,4-DB	94-82-6
2,4-D, butoxyethanol ester	1929-73-3
2,4-D, ethylhexyl ester	1928-43-4
2,4,5-T, butyl ester	93-79-8
2,4,5-T, butoxyethanol ester	2545-59-7
Carbamates	
Aldicarb*	116-06-3
Adicarb sulfone	1646-88-4
Aldicarb sulfoxide	1646-87-3
Aminocarb	2032-59-9
Barban	101-27-9
Benomyl	17804-35-2
Bromacil	314-40-9
Bendiocarb*	22781-23-3
Carbaryl*	63-25-2
Carbendazim*	10605-21-7
3-Hydroxycarbofuran	16655-82-6
Carbofuran*	1563-66-2

Compound Name	CAS No.[a]
Carbamates (continued)	
Chloroxuron	1982-47-4
Chloropropham	101-21-3
Diuron*	330-54-1
Fenuron	101-42-8
Fluometuron	2164-17-2
Linuron*	330-55-2
Methiocarb	2032-65-7
Methomyl*	16752-77-5
Mexacarbate	315-18-4
Monuron	150-68-5
Neburon	555-37-3
Oxamyl*	23135-22-0
Propachlor	1918-16-7
Propham	122-42-9
Propoxur	114-26-1
Siduron	1982-49-6
Tebuthiuron	34014-18-1

[a] Chemical Abstract Service Registry Number.

* These carbamates were tested in a multi-laboratory evaluation; all others were tested in a single-laboratory evaluation.

1.2 This method may be applicable to the analysis of other non-volatile or semivolatile compounds.

1.3 Tris-BP has been classified as a carcinogen. Purified standard material and stock standard solutions should be handled in a hood.

1.4 Method 8321 is designed to detect the chlorinated phenoxyacid compounds (free acid form) and their esters without the use of hydrolysis and esterification in the extraction procedure, although hydrolysis to the acid form will simplify quantitation.

1.5 The compounds were chosen for analysis by HPLC/MS because they have been designated as problem compounds that are hard to analyze by traditional chromatographic methods (e.g., gas chromatography). The sensitivity of this method is dependent upon the level of interferants within a given matrix, and varies with compound class and even with compounds within that class. Additionally, the limit of detection (LOD) is dependent upon the mode of operation of the mass spectrometer. For example, the LOD for caffeine in the selected reaction monitoring (SRM) mode is 45 pg of standard injected (10 µL injection), while for Disperse Red 1 the LOD is 180 pg. The LOD for caffeine under single quadrupole scanning is 84 pg and is 600 pg for Disperse Red 1 under similar scanning conditions.

1.6 The experimentally determined limits of detection (LOD) for the target analytes are presented in Tables 3, 10, 13, and 14. For further compound identification, MS/MS (CAD - Collision Activated Dissociation) can be used as an optional extension of this method.

1.7 This method is restricted to use by, or under the supervision of, analysts experienced in the use of high performance liquid chromatographs using mass spectrometers or ultraviolet detectors. Analysts should also be skilled in the interpretation of liquid chromatograms and mass spectra. Each analyst must demonstrate the ability to generate acceptable results with this method.

2.0 SUMMARY OF METHOD

2.1 This method provides reverse phase high performance liquid chromatographic (RP/HPLC) and thermospray (TS) mass spectrometric (MS) conditions and/or ultraviolet (UV) conditions for the detection of the target analytes. Quantitative analysis is performed by TS/MS, using either an external or internal standard approach. Sample extracts can be analyzed by direct injection into the thermospray or onto a liquid chromatographic- thermospray interface. A gradient elution program is used on the chromatograph to separate the compounds. Detection is achieved both by negative ionization (discharge electrode) and positive ionization, with a single quadrupole mass spectrometer. Since this method is based on an HPLC technique, the use of ultraviolet (UV) detection is optional on routine samples.

2.2 Prior to the use of this method, appropriate sample preparation techniques must be used.

2.2.1 Samples for analysis of chlorinated phenoxyacid compounds are prepared by a modification of Method 8151 (see Sec. 7.1.2). In general, one liter of aqueous sample or fifty grams of solid sample are pH adjusted, extracted with diethyl ether, concentrated and solvent exchanged to acetonitrile.

2.2.2 Samples for analysis of the other target analytes are prepared by established extraction techniques. In general, water samples are extracted at a neutral pH with methylene chloride, using an appropriate 3500 series method. An appropriate 3500 series method using methylene chloride/acetone (1:1) is used for solid samples. A micro-extraction technique is included for the extraction of Tris-BP from aqueous and non-aqueous matrices.

2.2.3 For carbamates one liter aqueous samples or forty grams of solid sample are methylene chloride extracted (refer to appropriate 3500 series method), concentrated (preferably using a rotary evaporator with adapter) and solvent exchanged with methanol.

2.3 An optional thermospray-mass spectrometry/mass spectrometry (TS-MS/MS) confirmatory method is provided. Confirmation is obtained by using MS/MS Collision Activated Dissociation (CAD) or wire-repeller CAD.

3.0 INTERFERENCES

3.1 Refer to Methods 3500, 3600, 8000 and 8151.

3.2 The use of Florisil Column Cleanup (Method 3620) has been demonstrated to yield recoveries less than 85% for some of the compounds in this method, and is therefore not recommended for all compounds. Refer to Table 2 of Method 3620 for recoveries of organophosphorus compounds as a function of Florisil fractions.

3.3 Compounds with high proton affinity may mask some of the target analytes. Therefore, an HPLC must be used as a chromatographic separator, for quantitative analysis.

3.4 Analytical difficulties encountered with specific organophosphorus compounds, as applied in this method, may include (but are not limited to) the following:

3.4.1 Methyl parathion shows some minor degradation upon analysis.

3.4.2 Naled can undergo debromination to form dichlorvos.

3.4.3 Merphos often contains contamination from merphos oxide. Oxidation of merphos can occur during storage, and possibly upon introduction into the mass spectrometer.

Refer to Method 8141 for other compound problems as related to the various extraction methods.

3.5 The chlorinated phenoxy acid compounds, being strong organic acids, react readily with alkaline substances and may be lost during analysis. Therefore, glassware and glass wool must be acid-rinsed, and sodium sulfate must be acidified with sulfuric acid prior to use to avoid this possibility.

3.6 Due to the reactivity of the chlorinated herbicides, the standards must be prepared in acetonitrile. Methylation will occur slowly, if prepared in methanol.

3.7 Benomyl is known to quickly degrade to carbendazim in the environment (Reference 21).

3.8 Solvents, reagents, glassware, and other sample processing hardware may yield discrete artifacts or elevated baselines, or both, causing misinterpretation of chromatograms or spectra. All of these materials must be demonstrated to be free from interferences under the conditions of the analysis by running reagent blanks. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required.

3.9 Interferants co-extracted from the sample will vary considerably from source to source. Retention times of target analytes must be verified by using reference standards.

3.10 The optional use of HPLC/MS/MS methods aids in the confirmation of specific analytes. These methods are less subject to chemical noise than other mass spectrometric methods.

4.0 APPARATUS AND MATERIALS

4.1 HPLC/MS

4.1.1 High Performance Liquid Chromatograph (HPLC) - An analytical system with programmable solvent delivery system and all required accessories including injection loop (with a minimum 10-µL loop volume), analytical columns, purging gases, etc. The solvent delivery system must be capable, at a minimum, of a binary solvent system. The chromatographic system must be capable of interfacing with a Mass Spectrometer (MS).

4.1.1.1 HPLC Post-Column Addition Pump - A pump for post column addition should be used. Ideally, this pump should be a syringe pump, and does not have to be capable of solvent programming.

4.1.1.2 Recommended HPLC Columns - A guard column and an analytical column are required.

4.1.1.2.1 Guard Column - C_{18} reverse phase guard column, 10 mm x 2.6 mm ID, 0.5 µm frit, or equivalent.

4.1.1.2.2 Analytical Column - C_{18} reverse phase column, 100 mm x 2 mm ID, 5 µm particle size of ODS-Hypersil; or C_8 reversed phase column, 100 mm x 2 mm ID, 3 µm particle size of MOS2-Hypersil, or equivalent.

4.1.2 HPLC/MS interface(s)

4.1.2.1 Micromixer - 10-µL, interfaces HPLC column system with HPLC post-column addition solvent system.

4.1.2.2 Interface - Thermospray ionization interface and source that will give acceptable calibration response for each analyte of interest at the concentration required. The source must be capable of generating both positive and negative ions, and have a discharge electrode or filament.

4.1.3 Mass spectrometer system - A single quadrupole mass spectrometer capable of scanning from 1 to 1000 amu. The spectrometer must also be capable of scanning from 150 to 450 amu in 1.5 sec. or less, using 70 volts (nominal) electron energy in the positive or negative electron impact modes. In addition, the mass spectrometer must be capable of producing a calibrated mass spectrum for PEG 400, 600, or 800 (see Sec. 5.14) or other compounds used as calibrants.

4.1.3.1 Optional triple quadrupole mass spectrometer - capable of generating daughter ion spectra with a collision gas in the second quadrupole and operation in the single quadrupole mode.

4.1.4 Data System - A computer system that allows the continuous acquisition and storage on machine-readable media of all mass spectra obtained throughout the duration of the chromatographic program must be interfaced to the mass spectrometer. The computer must have software that allows any MS data file to be searched for ions of a specified mass, and such ion abundances to be plotted versus time or scan number. This type of plot is defined as an Extracted Ion Current Profile (EICP). Software must also be available that allows integration of the abundances in any EICP between specified time or scan-number limits. There must be computer software available to operate the specific modes of the mass spectrometer.

4.2 HPLC with UV detector - An analytical system with solvent programmable pumping system for at least a binary solvent system, and all required accessories including syringes, 10-µL injection loop, analytical columns, purging gases, etc. An automatic injector is optional, but is useful for multiple samples. The columns specified in Sec. 4.1.1.2 are also used with this system.

4.2.1 If the UV detector is to be used in tandem with the thermospray interface, then the detector cell must be capable of withstanding high pressures (up to 6000 psi). However, the UV detector may be attached to an HPLC independent of the HPLC/TS/MS and in that case standard HPLC pressures are acceptable.

4.3 Purification Equipment for Azo Dye Standards

4.3.1 Soxhlet extraction apparatus.

4.3.2 Extraction thimbles, single thickness, 43 x 123 mm.

4.3.3 Filter paper, 9.0 cm (Whatman qualitative No. 1 or equivalent).

4.3.4 Silica-gel column - 3 in. x 8 in., packed with Silica gel (Type 60, EM reagent 70/230 mesh).

4.4 Extraction equipment for Chlorinated Phenoxyacid Compounds

4.4.1 Erlenmeyer flasks - 500-mL wide-mouth Pyrex®, 500-mL Pyrex®, with 24/40 ground glass joint, 1000-mL Pyrex®.

4.4.2 Separatory funnel - 2000-mL.

4.4.3 Graduated cylinder - 1000-mL.

4.4.4 Funnel - 75 mm diameter.

4.4.5 Wrist shaker - Burrell Model 75 or equivalent.

4.4.6 pH meter.

4.5 Kuderna-Danish (K-D) apparatus (optional).

4.5.1 Concentrator tube - 10-mL graduated (Kontes K-570050-1025 or equivalent). A ground glass stopper is used to prevent evaporation of extracts.

4.5.2 Evaporation flask - 500-mL (Kontes K-570001-500 or equivalent). Attach to concentrator tube with springs, clamps, or equivalent.

4.5.3 Snyder column - Two-ball micro (Kontes K-569001-0219 or equivalent).

4.5.4 Springs - 1/2 in. (Kontes K-662750 or equivalent).

NOTE: The following glassware is recommended for the purpose of solvent recovery during the concentration procedures requiring the use of Kuderna-Danish evaporative concentrators. Incorporation of this apparatus may be required by State or local municipality regulations that govern air emissions of volatile organics. EPA recommends the incorporation of this type of reclamation system as a method to implement an emissions reduction program. Solvent recovery is a means to conform with waste minimization and pollution prevention initiatives.

4.5.5 Solvent vapor recovery system (Kontes K-545000-1006 or K-547300-0000, Ace Glass 6614-30, or equivalent).

4.6 Disposable serological pipets - 5 mL x 1/10, 5.5 mm ID.

4.7 Collection tube - 15-mL conical, graduated (Kimble No. 45165 or equivalent).

4.8 Vials - 5-mL conical, glass, with polytetrafluoroethylene (PTFE)-lined screw-caps or crimp tops.

4.9 Glass wool - Supelco No. 2-0411 or equivalent.

4.10 Microsyringes - 100-µL, 50-µL, 10-µL (Hamilton 701 N or equivalent), and 50 µL (Blunted, Hamilton 705SNR or equivalent).

4.11 Rotary evaporator - Equipped with 1000-mL receiving flask.

4.12 Balances - Analytical, 0.0001 g, Top-loading, 0.01 g.

4.13 Volumetric flasks, Class A - 10-mL to 1000-mL.

4.14 Graduated cylinder - 100-mL.

4.15 Separatory funnel - 250-mL.

4.16 Separatory funnel - 2-liter, with PTFE stopcock.

4.17 Concentrator adaptor (optional- for carbamate extraction).

5.0 REAGENTS

5.1 Reagent grade chemicals shall be used in all tests. 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 ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

5.2 Organic free reagent water. All references to water in this method refer to organic-free reagent water, as defined in Chapter One.

5.3 Sodium sulfate (granular, anhydrous), Na_2SO_4. Purify by heating at 400°C for 4 hours in a shallow tray, or by precleaning the sodium sulfate with methylene chloride.

5.4 Ammonium acetate, NH_4OOCCH_3, solution (0.1 M). Filter through a 0.45 micron membrane filter (Millipore HA or equivalent).

5.5 Acetic acid, CH_3CO_2H

5.6 Sulfuric acid solution

5.6.1 (1:1, v/v) - Slowly add 50 mL H_2SO_4 (sp. gr. 1.84) to 50 mL of water.

5.6.2 (1:3, v/v) - slowly add 25 mL H_2SO_4 (sp. gr. 1.84) to 75 mL of water.

5.7 Argon gas, 99+% pure.

5.8 Solvents

5.8.1 Methylene chloride, CH_2Cl_2 - Pesticide quality or equivalent.

5.8.2 Toluene, $C_6H_5CH_3$ - Pesticide quality or equivalent.

5.8.3 Acetone, CH_3COCH_3 - Pesticide quality or equivalent.

5.8.4 Diethyl Ether, $C_2H_5OC_2H_5$ - Pesticide quality or equivalent. Must be free of peroxides as indicated by test strips (EM Quant, or equivalent). Procedures for removal of peroxides are provided with the test strips. After cleanup, 20 mL of ethyl alcohol preservative must be added to each liter of ether.

5.8.5 Methanol, CH_3OH - HPLC quality or equivalent.

5.8.6 Acetonitrile, CH_3CN - HPLC quality or equivalent.

5.8.7 Ethyl acetate $CH_3CO_2C_2H_5$ - Pesticide quality or equivalent.

5.9 Standard Materials - pure standard materials or certified solutions of each analyte targeted for analysis. Disperse azo dyes must be purified before use according to Sec. 5.10.

5.10 Disperse Azo Dye Purification

5.10.1 Two procedures are involved. The first step is the Soxhlet extraction of the dye for 24 hours with toluene and evaporation of the liquid extract to dryness, using a rotary evaporator. The solid is then recrystallized from toluene, and dried in an oven at approximately 100°C. If this step does not give the required purity, column chromatography should be employed. Load the solid onto a 3 x 8 inch silica gel column (Sec. 4.3.4), and elute with diethyl ether. Separate impurities chromatographically, and collect the major dye fraction.

5.11 Stock standard solutions - Can be prepared from pure standard materials or can be purchased as certified solutions. Commercially-prepared stock standards can be used if they are verified against EPA standards. If EPA standards are not available for verification, then standards certified by the manufacturer and verified against a standard made from pure material is acceptable.

5.11.1 Prepare stock standard solutions by accurately weighing 0.0100 g of pure material. Dissolve the material in methanol or other suitable solvent (e.g., prepare Tris-BP in ethyl acetate), and dilute to known volume in a volumetric flask.

NOTE: Due to the reactivity of the chlorinated herbicides, the standards must be prepared in acetonitrile. Methylation will occur if prepared in methanol.

If compound purity is certified at 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards can be used at any concentration if they are certified by the manufacturer or by an independent source.

5.11.2 Transfer the stock standard solutions into glass vials with PTFE-lined screw-caps or crimp-tops. Store at 4°C and protect from light. Stock standard solutions should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards.

5.12 Calibration standards - A minimum of five different concentrations for each parameter of interest should be prepared through dilution of the stock standards with methanol (or other suitable solvent). One of these concentrations should be near, but above, the MDL. The remaining concentrations should correspond to the expected range of concentrations found in real samples, or should define the working range of the HPLC-UV/VIS or HPLC-TS/MS. Calibration standards

must be replaced after one or two months, or sooner if comparison with check standards indicates a problem.

5.13 Surrogate standards - The analyst should monitor the performance of the extraction, cleanup (when used), and analytical system, along with the effectiveness of the method in dealing with each sample matrix, by spiking each sample, standard, and blank with one or two surrogates (e.g., organophosphorus or chlorinated phenoxyacid compounds not expected to be present in the sample).

5.14 HPLC/MS tuning standard - Polyethylene glycol 400 (PEG-400), PEG-600, or PEG-800 are recommended as tuning standards. However, analysts may use other tuning standards as recommended by the instrument manufacturer or other documented source. If one of the PEG solutions is used, dilute to 10 percent (v/v) in methanol. Which PEG is used will depend upon analyte molecular weight range: m.w. <500, use PEG-400; m.w. >500, use PEG-600 or PEG-800.

5.15 Internal standards - When the internal standard calibration option is used, it is recommended that analysts use stable-isotope labeled compounds of the same chemical class when they are available (e.g., 13C6-carbofuran may be used as an internal standard in the analysis of carbamates).

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 See the introductory material to this chapter, Organic Analytes, Sec. 4.1.

7.0 PROCEDURE

7.1 Sample preparation - Samples for analysis of disperse azo dyes and organophosphorus compounds must be prepared by an appropriate 3500 series method prior to HPLC/MS analysis.:

Samples for the analysis of Tris(2,3-dibromopropyl)phosphate wastewater must be prepared according to Sec. 7.1.1 prior to HPLC/MS analysis. Samples for the analysis of chlorinated phenoxyacid compounds and their esters should be prepared according to Sec. 7.1.2 prior to HPLC/MS analysis.

7.1.1 Microextraction for Tris-BP:

7.1.1.1 Solid Samples

7.1.1.1.1 Weigh a 1-gram portion of the sample into a tared beaker. If the sample appears moist, add an equivalent amount of anhydrous sodium sulfate and mix well. Add 100 µL of Tris-BP (approximate concentration 1000 mg/L) to the sample selected for spiking; the amount added should result in a final concentration of 100 ng/µL in the 1-mL extract.

7.1.1.1.2 Remove the glass wool plug from a disposable serological pipet. Insert a 1 cm plug of clean silane treated glass wool to the bottom (narrow end) of the pipet. Pack 2 cm of anhydrous sodium sulfate onto the top of the glass wool. Wash pipet and contents with 3 - 5 mL of methanol.

7.1.1.1.3 Pack the sample into the pipet prepared according to Sec. 7.1.1.1.2. If packing material has dried, wet with a few mL of methanol first, then pack sample into the pipet.

7.1.1.1.4 Extract the sample with 3 mL of methanol followed by 4 mL of 50% (v/v) methanol/methylene chloride (rinse the sample beaker with each volume of extraction solvent prior to adding it to the pipet containing the sample). Collect the extract in a 15-mL graduated glass tube.

7.1.1.1.5 Evaporate the extract to 1 mL using the nitrogen blowdown technique (Sec. 7.1.1.1.6). Record the volume. It may not be possible to evaporate some sludge samples to a reasonable concentration.

7.1.1.1.6 Nitrogen Blowdown Technique

7.1.1.1.6.1 Place the concentrator tube in a warm water bath (approximately 35°C) and evaporate the solvent volume to the required level using a gentle stream of clean, dry nitrogen (filtered through a column of activated carbon).

CAUTION: Do not use plasticized tubing between the carbon trap and the sample.

7.1.1.1.6.2 The internal wall of the tube must be rinsed down several times with methylene chloride during the operation. During evaporation, the solvent level in the tube must be positioned to prevent water from condensing into the sample (i.e., the solvent level should be below the level of the water bath).Under normal operating conditions, the extract should not be allowed to become dry. Proceed to Sec. 7.1.1.1.7.

7.1.1.1.7 Transfer the extract to a glass vial with a PTFE-lined screw-cap or crimp-top and store refrigerated at 4°C. Proceed with HPLC analysis.

7.1.1.1.8 Determination of percent dry weight - In certain cases, sample results are desired based on a dry weight basis. When such data are desired, or required, a portion of sample for this determination should be weighed out at the same time as the portion used for analytical determination.

WARNING: The drying oven should be contained in a hood or vented. Significant laboratory contamination may result from drying a heavily contaminated hazardous waste sample.

7.1.1.1.9 Immediately after weighing the sample for extraction, weigh 5-10 g of the sample into a tared crucible. Determine the % dry weight of the sample by drying overnight at 105°C. Allow to cool in a desiccator before weighing:

$$\% \text{ dry weight} = \frac{\text{g of dry sample}}{\text{g of sample}} \times 100$$

7.1.1.2 Aqueous Samples

7.1.1.2.1 Using a 100-mL graduated cylinder, measure 100 mL of sample and transfer it to a 250-mL separatory funnel. Add 200 µL of Tris-BP (approximate concentration 1000 mg/L) to the sample selected for spiking; the amount added should result in a final concentration of 200 ng/µL in the 1-mL extract.

7.1.1.2.2 Add 10 mL of methylene chloride to the separatory funnel. Seal and shake the separatory funnel three times, approximately 30 seconds each time, with periodic venting to release excess pressure.

NOTE: Methylene chloride creates excessive pressure rapidly; therefore, initial venting should be done immediately after the separatory funnel has been sealed and shaken once. Methylene chloride is a suspected carcinogen, use necessary safety precautions.

7.1.1.2.3 Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the size of the solvent layer, the analyst must employ mechanical techniques to complete phase separation. See Section 7.5, Method 3510.

7.1.1.2.4 Collect the extract in a 15-mL graduated glass tube. Proceed as in Sec. 7.1.1.1.5.

7.1.2 Extraction for chlorinated phenoxyacid compounds - Preparation of soil, sediment, and other solid samples must follow Method 8151, with the exception of no hydrolysis or esterification. (However, if the analyst desires to determine all of the phenoxyacid moieties as the acid, hydrolysis may be performed.) Sec. 7.1.2.1 presents an outline of the procedure with the appropriate changes necessary for determination by Method 8321. Sec. 7.1.2.2 describes the extraction procedure for aqueous samples.

7.1.2.1 Extraction of solid samples

7.1.2.1.1 Add 50 g of soil/sediment sample to a 500-mL, wide mouth Erlenmeyer. Add spiking solutions if required, mix well and allow to stand for 15 minutes. Add 50 mL of organic-free reagent water and stir for 30 minutes. Determine the pH of the sample with a glass electrode and pH meter, while stirring. Adjust the pH to 2 with cold H_2SO_4 (1:1) and monitor the pH for 15 minutes, with stirring. If necessary, add additional H_2SO_4 until the pH remains at 2.

7.1.2.1.2 Add 20 mL of acetone to the flask, and mix the contents with the wrist shaker for 20 minutes. Add 80 mL of diethyl ether to the same flask, and shake again for 20 minutes. Decant the extract and measure the volume of solvent recovered.

7.1.2.1.3 Extract the sample twice more using 20 mL of acetone followed by 80 mL of diethyl ether. After addition of each solvent, the mixture should be shaken with the wrist shaker for 10 minutes and the acetone-ether extract decanted.

7.1.2.1.4 After the third extraction, the volume of extract recovered should be at least 75% of the volume of added solvent. If this is not the case, additional extractions may be necessary. Combine the extracts in a 2000 mL separatory funnel containing 250 mL of 5% acidified sodium sulfate. If an emulsion forms, slowly add 5 g of acidified sodium sulfate (anhydrous) until the solvent-water mixture separates. A quantity of acidified sodium sulfate equal to the weight of the sample may be added, if necessary.

7.1.2.1.5 Check the pH of the extract. If it is not at or below pH 2, add more concentrated HCl until the extract is stabilized at the desired pH. Gently mix the contents of the separatory funnel for 1 minute and allow the layers to separate. Collect the aqueous phase in a clean beaker, and the extract phase (top layer) in a 500 mL ground-glass Erlenmeyer flask. Place the aqueous phase back into the separatory funnel and re-extract using 25 mL of diethyl ether. Allow the layers to separate and discard the aqueous layer. Combine the ether extracts in the 500 mL Erlenmeyer flask.

7.1.2.1.6 Add 45 - 50 g acidified anhydrous sodium sulfate to the combined ether extracts. Allow the extract to remain in contact with the sodium sulfate for approximately 2 hours.

NOTE: The drying step is very critical. Any moisture remaining in the ether will result in low recoveries. The amount of sodium sulfate used is adequate if some free flowing crystals are visible when swirling the flask. If all of the sodium sulfate solidifies in a cake, add a few additional grams of acidified sodium sulfate and again test by swirling. The 2 hour drying time is a minimum; however, the extracts may be held overnight in contact with the sodium sulfate.

7.1.2.1.7 Transfer the ether extract, through a funnel plugged with acid-washed glass wool, into a 500-mL K-D flask equipped with a 10 mL concentrator tube. Use a glass rod to crush caked sodium sulfate during the transfer. Rinse the Erlenmeyer flask and column with 20-30 mL of diethyl ether to complete the quantitative transfer. Reduce the volume of the extract using the macro K-D technique (Sec. 7.1.2.1.8).

7.1.2.1.8 Add one or two clean boiling chips to the flask and attach a three ball macro-Snyder column. Attach the solvent vapor recovery glassware (condenser and collection device) (Sec. 4.5.5) to the Snyder column of the K-D apparatus following manufacturer's instructions. Prewet the Snyder column by adding about 1 mL of diethyl ether to the top. Place the apparatus on a hot water bath (60°-65°C) so that the concentrator tube is partially immersed in the hot water and the entire lower rounded surface of the flask is bathed in vapor. Adjust the vertical position of the apparatus and the water temperature, as required, to complete the concentration in 15-20 minutes. At the proper rate of distillation the balls of the column will actively chatter, but the chambers will not flood. When the apparent volume of liquid reaches 5 mL, remove the K-D apparatus from the water bath and allow it to drain and cool for at least 10 minutes.

7.1.2.1.9 Exchange the solvent of the extract to acetonitrile by quantitatively transferring the extract with acetonitrile to a blow-down apparatus. Add a total of 5 mL acetonitrile. Reduce the extract volume according to Sec. 7.1.1.1.6, and adjust the final volume to 1 mL.

7.1.2.2 Preparation of aqueous samples

7.1.2.2.1 Using a 1000-mL graduated cylinder, measure 1 liter (nominal) of sample, record the sample volume to the nearest 5 mL, and transfer it to a separatory funnel. If high concentrations are anticipated, a smaller volume may be used and then diluted with organic-free reagent water to 1 liter. Adjust the pH to less than 2 with sulfuric acid (1:1).

7.1.2.2.2 Add 150 mL of diethyl ether to the sample bottle, seal, and shake for 30 seconds to rinse the walls. Transfer the solvent wash to the separatory funnel and extract the sample by shaking the funnel for 2 minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water layer for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the size of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, and may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Drain the aqueous phase into a 1000-mL Erlenmeyer flask.

7.1.2.2.3 Repeat the extraction two more times using 100 mL of diethyl ether each time. Combine the extracts in a 500 mL Erlenmeyer flask. (Rinse the 1000-mL flask with each additional aliquot of extracting solvent to make a quantitative transfer.)

7.1.2.2.4 Proceed to Sec. 7.1.2.1.6 (drying, K-D concentration, solvent exchange, and final volume adjustment).

7.1.3 Extraction for carbamates - Preparation of soil, sediment, and other solid samples must follow an appropriate 3500 series method.

7.1.3.1 Forty gram quantities are extracted with methylene chloride using an appropriate 3500 series method.

7.1.3.2 Concentration steps can be achieved using a rotary evaporator or K-D, to 5-10 mL volumes.

7.1.3.3 Final concentration and solvent exchange to 1-mL final volume of methanol, can be done preferably using an adaptor on the rotary evaporator. If an adaptor is unavailable, the final concentration can be achieved using a gentle stream of nitrogen, in a fume hood.

7.1.4 Extraction for carbamates - Preparation of aqueous samples must follow an appropriate 3500 series method.

7.1.4.1 One liter quantities are extracted with methylene chloride using an appropriate 3500 series method.

7.1.4.2 Final concentration and exchange to methanol is the same as applied in Secs. 7.1.3.2 and 7.1.3.3.

7.2 Prior to HPLC analysis, the extraction solvent must be exchanged to methanol or acetonitrile (Sec. 7.1.2.1.9). The exchange is performed using the K-D procedures listed in all of the extraction methods.

7.3 HPLC Chromatographic Conditions:

7.3.1 Analyte-specific chromatographic conditions are shown in Table 1. Chromatographic conditions which are not analyte-specific are as follows:

Flow rate:	0.4 mL/min
Post-column mobile phase:	0.1 M ammonium acetate (1% methanol) (0.1 M ammonium acetate for phenoxyacid compounds)
Post-column flow rate:	0.8 mL/min

7.3.2 If there is a chromatographic problem from compound retention when analyzing for disperse azo dyes, organophosphorus compounds, and tris(2,3-dibromopropyl)phosphate, a 2% constant flow of methylene chloride may be applied as needed. Methylene chloride/aqueous methanol solutions must be used with caution as HPLC eluants. Acetic acid (1%), another mobile phase modifier, can be used with compounds with acid functional groups.

7.3.3 A total flow rate of 1.0 to 1.5 mL/min is necessary to maintain thermospray ionization.

7.3.4 Retention times for organophosphorus compounds on the specified analytical column are presented in Table 9.

7.4 Recommended HPLC/Thermospray/MS operating conditions: Prior to analysis of samples, the analyst should evaluate the relative sensitivity of the target compounds to each ionization mode to determine which may provide better sensitivity during analyses. This evaluation may be based on the structures of the analytes or by conducting analyses in each of the two ionization modes. See Sec. 7.5.2.6 for a discussion of the issue.

7.4.1 Positive Ionization mode

Repeller (wire or plate, optional): 170 to 250 v (sensitivity optimized). See Figure 2 for schematic of source with wire repeller.

Discharge electrode:	off
Filament:	on or off (optional, analyte dependent)
Mass range:	150 to 450 amu (analyte dependent, expect 1 to 18 amu higher than molecular weight of the compound).
Scan time:	1.50 sec/scan.

7.4.2 Negative Ionization mode

Discharge electrode:	on
Filament:	off
Mass Range:	135 to 450 amu
Scan time:	1.50 sec/scan.

7.4.3 Thermospray temperatures:

Vaporizer control:	110°C to 130°C.
Vaporizer tip:	200°C to 215°C.
Jet:	210°C to 220°C.
Source block:	230°C to 265°C. (Some compounds may degrade in the source block at higher temperatures, operator should use knowledge of chemical properties to estimate proper source temperature).

7.4.4 Sample injection volume: 20 to 100 µL is normally used. The injection loop must be overfilled by, minimally, a factor of two (e.g., 20 µL sample used to overfill a 10 µL injection loop) when manual injections are performed. If solids are present in the extract, allow them to settle or centrifuge the extract and withdraw the injection volume from the clear layer.

7.5 Calibration:

7.5.1 Thermospray/MS system - Must be hardware-tuned on quadrupole 1 (and quadrupole 3 for triple quadrupoles) for accurate mass assignment, sensitivity, and resolution. It is recommended that this be accomplished using polyethylene glycol (PEG) 400, 600, or 800 (see Sec. 5.14) which has average molecular weights of 400, 600, and 800, respectively. Analysts may use other tuning standards as recommended by the instrument manufacturer or other documented source. If PEGs are used, a mixture of these PEGs can be made such that it will approximate the expected working mass range for the analyses. Use PEG 400 for analysis of chlorinated phenoxyacid compounds. The PEG is introduced via the thermospray interface, circumventing the HPLC.

7.5.1.1 The mass calibration parameters are as follows:

<u>for PEG 400 and 600</u>	<u>for PEG 800</u>
Mass range: 15 to 765 amu	Mass range: 15 to 900 amu
Scan time: 0.5 to 5.0 sec/scan	Scan time: 0.5 to 5.0 sec/scan

Approximately 100 scans should be acquired, with 2 to 3 injections made. The scan with the best fit to the accurate mass table (see Tables 7 and 8) should be used as the calibration table. If calibrants other than PEG are used, the mass range should be from 15 amu to approximately 20 amu higher than the highest mass used for calibration. A scan time should be chosen which will give a least 6 scans across the calibrant peak.

7.5.1.2 The low mass range from 15 to 100 amu is covered by the ions from the ammonium acetate buffer used in the thermospray process: NH_4^+ (18 amu), $NH_4^+ H_2O$ (36), $CH_3OH\ NH_4^+$ (50)(methanol), or $CH_3\ CN\ NH_4^+$ (59)(acetonitrile) and $CH_3\ OOH\ NH_4^+$ (78) (acetic acid). The appearance of the m/z 50 or 59 ion depends upon the use of methanol or acetonitrile as the organic modifier. The higher mass range is covered by the ammonium ion adducts of the various ethylene glycols (e.g., $H(OCH_2CH_2)_nOH$ where n=4, gives the $H(OCH_2CH_2)_4OH\ NH_4^+$ ion at m/z 212).

7.5.2 Liquid Chromatograph

7.5.2.1 Prepare calibration standards as outlined in Sec. 5.12.

7.5.2.2 Choose the proper ionization conditions, as outlined in Sec. 7.4. Inject each calibration standard onto the HPLC, using the chromatographic conditions outlined in Table 1. Refer to Sec. 7.0 of Method 8000 for guidance on external and internal calibration options and calibration acceptance criteria. A correlation coefficient (r^2) of at least 0.97 should be used for chlorinated phenoxyacid analytes. In most cases the $(M^+H)^+$ and $(M^+NH_4)^+$ adduct ions are the only ions of significant abundance. For example, Table 9 lists the retention times and the major ions (>5%) present in the positive ionization thermospray single quadrupole spectra of the organophosphorus compounds.

7.5.2.2.1 The use of selective ion monitoring (SIM) is acceptable in situations requiring detection limits below the normal range of full spectra analysis. However, SIM may provide a lesser degree of confidence in the compound identification unless multiple ions are monitored for each compound.

7.5.2.2.2 The use of selective reaction monitoring (SRM) is also acceptable when using triple-quad MS/MS and enhanced sensitivity is needed.

7.5.2.3 If HPLC-UV detection is also being used, calibrate the instrument by preparing calibration standards as outlined in Sec. 5.12, and injecting each calibration standard onto the HPLC using the chromatographic conditions outlined in Table 1. Integrate the area under the full chromatographic peak for each concentration. Quantitation by HPLC-UV may be preferred if it is known that sample interference and/or analyte coelution are not a problem.

7.5.2.4 For the methods specified in Secs. 7.5.2.2 and 7.5.2.3, the retention time of the chromatographic peak is an important variable in analyte identification. Therefore, the ratio of the retention time of the sample analyte to the standard analyte should be 1.0 - 0.1.

7.5.2.5 The concentration of the sample analyte will be determined by using the calibration curves determined in Secs. 7.5.2.2 and 7.5.2.3. These calibration curves must be generated on the same day as each sample is analyzed. Samples whose concentrations exceed the standard calibration range should be diluted to fall within the range.

7.5.2.6 When using MS or MS/MS, and when it is appropriate for the compounds of interest and the project objectives, determinations of both positive and negative ionization analyses may be done on each sample extract. However, some groups of target compounds will have much better sensitivity using either positive or negative ionization, making a single analysis practical (e.g., carbamates are generally more sensitive to the positive ionization mode and phenoxyacids are generally more sensitive to the negative ionization mode). Prior to analysis of samples, the analyst should evaluate the relative sensitivity of the target compounds to each ionization mode to determine which may provide better sensitivity during analyses. This evaluation may be based on the structures of the analytes or by conducting analyses in each of the two ionization modes.

7.5.2.7 Refer to Method 8000 for further information on calculating sample concentrations and QC parameters such as accuracy and precision.

7.5.2.8 Precision can also be calculated from the ratio of response (area) to the amount injected; this is defined as the calibration factor (CF) for each standard concentration. If the percent relative standard deviation (%RSD) of the CF is less than 20 percent over the working range, linearity through the origin can be assumed, and the average calibration factor can be used in place of a calibration curve. The CF and %RSD can be calculated as follows:

CF = Total Area of Peak/Mass injected (ng)

$\%RSD = SD/\overline{CF} \times 100$

where:

SD = Standard deviation between CFs

$\overline{CF}$ = Average CF

7.6 Sample Analysis

7.6.1 Once the LC/MS system has been calibrated as outlined in Sec. 7.5, then it is ready for sample analysis. It is recommended that the samples be initially analyzed in the negative ionization mode. If low levels of compounds are suspected then the samples should also be screened in the positive ionization mode.

7.6.1.1 A blank injection (methanol) must be analyzed after the standard(s) analyses, in order to determine any residual contamination of the Thermospray/HPLC/MS system.

7.6.1.2 If performing manual injections, take an appropriate aliquot of the sample as per Sec. 7.4.4. Start the HPLC gradient elution, load and inject the sample aliquot, and start the mass spectrometer data system analysis.

7.6.1.3 If using an autoinjector, ensure that it is set up properly according to the manufacturer's instructions and that all samples and standards are loaded in the proper order. Start the autoinjector, the HPLC gradient elution, and the mass spectrometer data system.

7.7 Calculations

7.7.1 Using the external or internal standard calibration procedure (Method 8000), determine the identity and quantity of each component peak in the sample reconstructed ion chromatogram which corresponds to the compounds used for calibration processes. See Method 8000 for calculation equations.

7.7.2 The retention time of the chromatographic peak is an important parameter for the identity of the analyte. However, because matrix interferences can change chromatographic column conditions, the retention times are not as significant, and the mass spectra confirmations are important criteria for analyte identification.

7.8 Optional Thermospray HPLC/MS/MS confirmation

7.8.1 With respect to this method, MS/MS shall be defined as the daughter ion collision activated dissociation acquisition with quadrupole one set on one mass (parent ion), quadrupole two pressurized with argon and with a higher offset voltage than normal, and quadrupole three set to scan desired mass range.

7.8.2 Since the thermospray process often generates only one or two ions per compound, the use of MS/MS is a more specific mode of operation yielding molecular structural information. In this mode, fast screening of samples can be accomplished through direct injection of the sample into the thermospray.

7.8.3 For MS/MS experiments, the first quadrupole should be set to the protonated molecule or ammoniated adduct of the analyte of interest. The third quadrupole should be set to scan from 30 amu to just above the mass region of the protonated molecule.

7.8.4 The collision gas pressure (Ar) should be set at about 1.0 mTorr and the collision energy at 20 eV. If these parameters fail to give considerable fragmentation, they may be raised above these settings to create more and stronger collisions.

7.8.5 For analytical determinations, the base peak of the collision spectrum shall be taken as the quantification ion. For extra specificity, a second ion should be chosen as a backup quantification ion.

7.8.6 Generate a calibration curve as outlined in Sec. 7.5.2.

7.8.7 MS/MS contamination and interferences

7.8.7.1 If the MS/MS mode is to be used without chromatographic separation (fast screening), method blank analysis must show that the sample preparation and analysis procedures are free of contamination by the analyte of interest or by interfering compounds. Refer to Sec. 8.0 of Method 8000 for guidance on acceptable method blank performance. If contamination is detected in the method blank above acceptable limits, repreparation and reanalysis of the affected samples is necessary.

7.8.7.2 The MS/MS spectra of a calibration standard and the sample can be compared and the ratios of the three major (most intense) ions examined. These ratios should be approximately the same unless there is an interference. If an interference appears, chromatography must be utilized.

7.8.7.3 The signal of the target analyte in a sample may be suppressed by co-extracted interferences which do not give a signal in the monitored ions. In order to monitor such signal suppression, an internal standard may be spiked into all standard, blank, and sample extracts at a consistent concentration prior to analysis. The internal standard may be any compound which responds well in the appropriate ionization mode and which is not likely to be found in nature. (Note: d5-Atrazine has been used successfully for positive ion analysis, while d3-2,6-dinitrotoluene has been used successfully for negative ion analysis.) The amount spike should be chosen such that the signal produced is at least 100 times the noise level for the appropriate ion. The signal of the internal standard should be monitored. Reanalysis is required for any sample in which the internal standard peak height varies by more than 30% from the average internal standard height obtained during the five-point calibration. If reanalysis

confirms this variance in signal, the sample should be reanalyzed using a chromatographic separation. Quantitation of analyte concentration may be performed using this internal standard. External standard quantitation is also allowed.

7.8.8 For unknown concentrations, the total area of the quantitation ion(s) is calculated and the calibration curves generated as in Sec. 7.5 are used to attain an injected weight number.

7.8.9 MS/MS techniques can also be used to perform structural analysis on ions represented by unassigned m/z ratios. The procedure for compounds of unknown structures is to set up a CAD experiment on the ion of interest. The spectrum generated from this experiment will reflect the structure of the compound by its fragmentation pattern. A trained mass spectroscopist and some history of the sample are usually needed to interpret the spectrum. (CAD experiments on actual standards of the expected compound are necessary for confirmation or denial of that substance.)

7.9 Optional wire-repeller CAD confirmation

7.9.1 See Figure 3 for the correct position of the wire-repeller in the thermospray source block.

7.9.2 Once the wire-repeller is inserted into the thermospray flow, the voltage can be increased to approximately 500 - 700 v. Enough voltage is necessary to create fragment ions, but not so much that shorting occurs.

7.9.3 Continue as outlined in Sec. 7.6.

8.0 QUALITY CONTROL

8.1 Refer to Chapter One and Method 8000 for specific quality control (QC) procedures. Each laboratory should maintain a formal quality assurance program. The laboratory should also maintain records to document the quality of the data generated.

8.2 Quality control procedures necessary to evaluate the HPLC system operation are found in Method 8000, Sec. 7.0 and includes evaluation of retention time windows, calibration verification and chromatographic analysis of samples. Check the performance of the entire analytical system daily using data gathered from analyses of blanks, standards, and replicate samples.

8.2.1 See Sec. 7.5.2.8 for HPLC/MS parameters for standard calibration curve %RSD limits.

8.2.2 See Sec. 7.5.2.4 regarding retention time window QC limits.

8.2.3 If any of the chromatographic QC limits are not met, the analyst should examine the LC system for:

- Leaks,
- Proper pressure delivery,
- A dirty guard column; may need replacing or repacking, and
- Possible partial thermospray plugging.

Any of the above items will necessitate shutting down the HPLC/TS system, making repairs and/or replacements, and then restarting the analyses. The calibration standard should be reanalyzed before any sample analyses, as described in Sec. 7.5.

8.2.4 The experience of the analyst performing liquid chromatography is invaluable to the success of the method. Each day that analysis is performed, the daily calibration standard should be evaluated to determine if the chromatographic system is operating properly. If any changes are made to the system (e.g., column change), the system must be recalibrated.

8.3 Initial Demonstration of Proficiency - Each laboratory must demonstrate initial proficiency with each sample preparation and determinative method combination it utilizes, by generating data of acceptable accuracy and precision for target analytes in a clean matrix. The laboratory must also repeat the following operations whenever new staff are trained or significant changes in instrumentation are made. See Method 8000, Sec. 8.0 for information on how to accomplish this demonstration.

8.4 Sample Quality Control for Preparation and Analysis - The laboratory must also have procedures for documenting the effect of the matrix on method performance (precision, accuracy, and detection limit). At a minimum, this includes the analysis of QC samples including a method blank, matrix spike, a duplicate, and a laboratory control sample (LCS) in each analytical batch and the addition of surrogates to each field sample and QC sample.

8.4.1 Documenting the effect of the matrix should include the analysis of at least one matrix spike and one duplicate unspiked sample or one matrix spike/matrix spike duplicate pair. The decision on whether to prepare and analyze duplicate samples or a matrix spike/matrix spike duplicate must be based on a knowledge of the samples in the sample batch. If samples are expected to contain target analytes, then laboratories may use one matrix spike and a duplicate analysis of an unspiked field sample. If samples are not expected to contain target analytes, laboratories should use a matrix spike and matrix spike duplicate pair.

8.4.2 A Laboratory Control Sample (LCS) should be included with each analytical batch. The LCS consists of an aliquot of a clean (control) matrix similar to the sample matrix and of the same weight or volume. The LCS is spiked with the same analytes at the same concentrations as the matrix spike. When the results of the matrix spike analysis indicate a potential problem due to the sample matrix itself, the LCS results are used to verify that the laboratory can perform the analysis in a clean matrix.

8.4.3 See Method 8000, Sec. 8.0 for the details on carrying out sample quality control procedures for preparation and analysis.

8.5 Surrogate recoveries - The laboratory must evaluate surrogate recovery data from individual samples versus the surrogate control limits developed by the laboratory. See Method 8000, Sec. 8.0 for information on evaluating surrogate data and developing and updating surrogate limits.

8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Whenever possible, the laboratory should analyze standard reference materials and participate in relevant performance evaluation studies.

9.0 METHOD PERFORMANCE

9.1 Single operator accuracy and precision studies have been conducted using spiked sediment, wastewater, sludge, and water samples. The results are presented in Tables 4, 5, 6, 11, 12, 15, 20 and 21. Tables 4, 5, and 6 provide single-laboratory data for Disperse Red 1, Table 11 with organophosphorus pesticides, Table 12 with Tris-BP, Table 15 with chlorophenoxyacid herbicides and Tables 20 and 21 with carbamates.

9.2 LODs should be calculated for the known analytes, on each instrument to be used. Tables 3, 10, and 13 list limits of detection (LOD) and/or estimated quantitation limits (EQL) that are typical with this method.

9.2.1 The LODs presented in this method were calculated by analyzing three replicates of four standard concentrations, with the lowest concentration being near the instrument detection limit. A linear regression was performed on the data set to calculate the slope and intercept. Three times the standard deviation (3σ) of the lowest standard amount, along with the calculated slope and intercept, was used to find the LOD. The LOD was not calculated using the specifications in Chapter One, but according to the ACS guidelines specified in Reference 4.

9.2.2 Table 17 presents a comparison of the LODs from Method 8151 and Method 8321 for the chlorinated phenoxyacid compounds.

9.3 Table 16 presents multi-laboratory accuracy and precision data for the chlorinated phenoxyacid herbicides. The data summary is based on data from three laboratories that analyzed duplicate solvent solutions at each concentration specified in the Table.

9.4 Tables 22 and 23 present the multi-laboratory accuracy and precision data for the carbamates. The data summary is based on data from nine laboratories that analyzed triplicate solvent solutions at each concentration level specified in the Tables.

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TABLE 1

RECOMMENDED HPLC CHROMATOGRAPHIC CONDITIONS

Initial Mobile Phase (%)	Initial Time (min)	Final Gradient (linear) (min)	Final Mobile Phase (%)	Time (min)
Analytes:				
Organophosphorus Compounds				
50/50 (water/methanol)	0	10	100 (methanol)	5
Azo Dyes (e.g., Disperse Red 1)				
50/50 (water/CH_3CN)	0	5	100 (CH_3CN)	5
Tris(2,3-dibromopropyl)phosphate				
50/50 (water/methanol)	0	10	100 (methanol)	5
Chlorinated phenoxyacid compounds				
75/25 (A/methanol)	2	15	40/60 (A/methanol)	
40/60 (A/methanol)	3	5	75/25 (A/methanol)	10

Where A = 0.1 M ammonium acetate (1% acetic acid)

Carbamates

Option A:

Time (min)	Mobile phase A (percent)	Mobile phase B (percent)
0	95	5
30	20	80
35	0	100
40	95	5
45	95	5

Where A = 5 mM ammonium acetate with 0.1 M acetic acid, and
B = methanol
With optional post-column addition of 0.5 M ammonium acetate.

Carbamates (continued)

Option B:

Time (min)	Mobile phase A (percent)	Mobile phase B (percent)
0	95	5
30	0	100
35	0	100
40	95	5
45	95	5

Where A = water with 0.1 M ammonium acetate with 1% acetic acid
B = methanol with 0.1 M ammonium acetate with 1% acetic acid
With optional post-column addition of 0.1 M ammonium acetate.

TABLE 2

COMPOUNDS AMENABLE TO THERMOSPRAY MASS SPECTROMETRY

Disperse Azo Dyes	Alkaloids
Methine Dyes	Aromatic ureas
Arylmethane Dyes	Amides
Coumarin Dyes	Amines
Anthraquinone Dyes	Amino acids
Xanthene Dyes	Organophosphorus Compounds
Flame retardants	Chlorinated Phenoxyacid Compounds
Carbamates	

TABLE 3

LIMITS OF DETECTION AND METHOD SENSITIVITIES
FOR DISPERSE RED 1 AND CAFFEINE

Compound	Mode	LOD pg	EQL(7s) pg	EQL(10s) pg
Disperse Red 1	SRM	180	420	600
	Single Quad	600	1400	2000
	CAD	2,000	4700	6700
Caffeine	SRM	45	115	150
	Single Quad	84	200	280
	CAD	240	560	800

EQL = Estimated Quantitation Limit

Data from Reference 16.

TABLE 4

PRECISION AND ACCURACY COMPARISONS OF MS AND MS/MS WITH HPLC/UV FOR ORGANIC-FREE REAGENT WATER SPIKED WITH DISPERSE RED 1

	Percent Recovery			
Sample	HPLC/UV	MS	CAD	SRM
Spike 1	82.2 ± 0.2	92.5 ± 3.7	87.6 ± 4.6	95.5 ± 17.1
Spike 2	87.4 ± 0.6	90.2 ± 4.7	90.4 ± 9.9	90.0 ± 5.9
RPD	6.1%	2.5%	3.2%	5.9%

Data from Reference 16.

TABLE 5

PRECISION AND ACCURACY COMPARISONS OF MS AND MS/MS WITH HPLC/UV FOR MUNICIPAL WASTEWATER SPIKED WITH DISPERSE RED 1

	Percent Recovery		
Sample	HPLC/UV	MS	CAD
Spike 1	93.4 ± 0.3	102.0 ± 31	82.7 ± 13
Spike 2	96.2 ± 0.1	79.7 ± 15	83.7 ± 5.2
RPD	3.0%	25%	1.2%

Data from Reference 16.

TABLE 6

RESULTS FROM ANALYSES OF ACTIVATED SLUDGE PROCESS WASTEWATER

	Recovery of Disperse Red 1 (mg/L)		
Sample	HPLC/UV	MS	CAD
5 mg/L Spiking Concentration			
1	0.721 ± 0.003	0.664 ± 0.030	0.796 ± 0.008
1-D	0.731 ± 0.021	0.600 ± 0.068	0.768 ± 0.093
2	0.279 ± 0.000	0.253 ± 0.052	0.301 ± 0.042
3	0.482 ± 0.001	0.449 ± 0.016	0.510 ± 0.091
RPD	1.3%	10.1%	3.6%
0 mg/L Spiking Concentration			
1	0.000	0.005 ± 0.0007	<0.001
1-D	0.000	0.006 ± 0.001	<0.001
2	0.000	0.002 ± 0.0003	<0.001
3	0.000	0.003 ± 0.0004	<0.001
RPD	--	18.2%	--

Data from Reference 16.

TABLE 7

CALIBRATION MASSES AND % RELATIVE ABUNDANCES
OF PEG 400

Mass	% Relative Abundances[a]
18.0	32.3
35.06	13.5
36.04	40.5
50.06	94.6
77.04	27.0
168.12	5.4
212.14	10.3
256.17	17.6
300.20	27.0
344.22	45.9
388.25	64.9
432.28	100
476.30	94.6
520.33	81.1
564.35	67.6
608.38	32.4
652.41	16.2
653.41	4.1
696.43	8.1
697.44	2.7

[a] Intensity is normalized to mass 432.

TABLE 8

CALIBRATION MASSES AND % RELATIVE ABUNDANCES
OF PEG 600

Mass Abundances[a]	% Relative
18.0	4.7
36.04	11.4
50.06	64.9
77.04	17.5
168.12	9.3
212.14	43.9
256.17	56.1
300.20	22.8
344.22	28.1
388.25	38.6
432.28	54.4
476.30	64.9
520.33	86.0
564.35	100
608.38	63.2
652.41	17.5
653.41	5.6
696.43	1.8

[a] Intensity is normalized to mass 564.

TABLE 9

RETENTION TIMES AND THERMOSPRAY MASS SPECTRA
OF ORGANOPHOSPHORUS COMPOUNDS

Compound	Retention Time (minutes)	Mass Spectra (% Relative Abundance)[a]
Monocrotophos	1:09	241 (100), 224 (14)
Trichlorfon	1:22	274 (100), 257 (19), 238 (19)
Dimethoate	1:28	230 (100), 247 (20)
Dichlorvos	4:40	238 (100), 221 (40)
Naled	9:16	398 (100), 381 (23), 238 (5), 221 (2)
Fensulfothion	9:52	326 (10), 309 (100)
Methyl parathion	10:52	281 (100), 264 (8), 251 (21), 234 (48)
Phorate	13:30	278 (4), 261 (100)
Disulfoton	13:55	292 (10), 275 (100)
Merphos	18:51	315 (100), 299 (15)

[a] For molecules containing Cl, Br and S, only the base peak of the isotopic cluster is listed.

Data from Reference 17.

TABLE 10

PRECISION AND LIMITS OF DETECTION FOR
ORGANOPHOSPHORUS COMPOUND STANDARDS

Compound	Ion	Standard Quantitation Concentration (ng/µL)	%RSD	MDL (ng)
Dichlorvos	238	2	16	
		12.5	13	
		25	5.7	
		50	4.2	4
Dimethoate	230	2	2.2	
		12.5	4.2	
		25	13	
		50	7.3	2
Phorate	261	2	0.84	
		12.5	14	
		25	7.1	
		50	4.0	2
Disulfoton	275	2	2.2	
		12.5	14	
		25	6.7	
		50	3.0	1
Fensulfothion	309	2	4.1	
		12.5	9.2	
		25	9.8	
		50	2.5	0.4
Naled	398	2	9.5	
		12.5	9.6	
		25	5.2	
		50	6.3	0.2
Merphos	299	2	5.5	
		12.5	17	
		25	3.9	
		50	5.3	1
Methyl parathion	281	2	--	
		12.5	7.1	
		25	4.8	
		50	1.5	30

Data from Reference 17.

TABLE 11

SINGLE OPERATOR ACCURACY AND PRECISION FOR LOW CONCENTRATION DRINKING WATER (A), LOW CONCENTRATION SOIL (B), MEDIUM CONCENTRATION DRINKING WATER (C), MEDIUM CONCENTRATION SEDIMENT (D)

Compound	Average Recovery (%)	Standard Deviation	Spike Amount	Range of Recovery (%)	Number of Analyses
A			µg/L		
Dimethoate	70	7.7	5	85 - 54	15
Dichlorvos	40	12	5	64 - 14	15
Naled	0.5	1.0	5	2 - 0	15
Fensulfothion	112	3.3	5	119 - 106	15
Methyl parathion	50	28	10	105 - 0	15
Phorate	16	35	5	86 - 0	15
Disulfoton	3.5	8	5	19 - 0	15
Merphos	237	25	5	287 - 187	15
B			µg/g		
Dimethoate	16	4	50	24 - 7	15
Dichlorvos	ND		50		15
Naled	ND		50		15
Fensulfothion	45	5	50	56 - 34	15
Methyl parathion	ND		100		15
Phorate	78	15	50	109 - 48	15
Disulfoton	36	7	50	49 - 22	15
Merphos	118	19	50	155 - 81	15
C			µg/L		
Dimethoate	52	4	50	61 - 43	12
Dichlorvos	146	29	50	204 - 89	12
Naled	4	3	50	9 - 0	12
Fensulfothion	65	7	50	79 - 51	12
Methyl parathion	85	24	100	133 - 37	12
Phorate	10	15	50	41 - 0	12
Disulfoton	2	1	50	4 - 0	12
Merphos	101	13	50	126 - 75	12
D			mg/kg		
Dimethoate	74	8.5	2	91 - 57	15
Dichlorvos	166	25	2	216 - 115	15
Naled	ND		2		15
Fensulfothion	72	8.6	2	90 - 55	15
Methyl parathion	84	9	3	102 - 66	15
Phorate	58	6	2	70 - 46	15
Disulfoton	56	5	2	66 - 47	15
Merphos	78	4	2	86 - 70	12

Data from Reference 17.

TABLE 12

SINGLE OPERATOR ACCURACY AND PRECISION FOR MUNICIPAL WASTE WATER (A), DRINKING WATER (B), CHEMICAL SLUDGE WASTE (C)

Compound		Average Recovery (%)	Standard Deviation	Spike Amount (ng/µL)	Range of % Recovery	Number of Analyses
Tris-BP	(A)	25	8.0	2	41 - 9.0	15
	(B)	40	5.0	2	50 - 30	12
	(C)	63	11	100	84 - 42	8

Data from Reference 18.

TABLE 13

SINGLE OPERATOR EQL TABLE FOR TRIS-BP

Concentration (ng/µL)	Average Area	Standard Deviation	3*Std Dev.	7*Std Dev.	10*Std Dev.
50	2675	782	2347	5476	7823
100	5091	558			
150	7674	2090			
200	8379	2030			

LOD (ng/µL)	Lower EQL (ng/µL)	Upper EQL (ng/µL)
33	113	172

EQL = Estimated Quantitation Limit
Data from Reference 18.

TABLE 14

LIMITS OF DETECTION IN THE POSITIVE AND NEGATIVE ION MODES
FOR THE CHLORINATED PHENOXYACID HERBICIDES AND FOUR ESTERS

Compound	Positive Mode Quantitation LOD		Negative Mode Quantitation LOD	
	Ion	(ng)	Ion	(ng)
Dalapon	Not detected		141 $(M^{-}H)^{-}$	11
Dicamba	238 $(M^{+}NH_4)^{+}$	13	184 $(M^{-}HCl)^{-}$	3.0
2,4-D	238 $(M^{+}NH_4)^{+}$	2.9	184 $(M^{-}HCl)^{-}$	50
MCPA	218 $(M^{+}NH_4)^{+}$	120	199 $(M^{-}1)^{-}$	28
Dichlorprop	252 $(M^{+}NH_4)^{+}$	2.7	235 $(M^{-}1)^{-}$	25
MCPP	232 $(M^{+}NH_4)^{+}$	5.0	213 $(M^{-}1)^{-}$	12
2,4,5-T	272 $(M^{+}NH_4)^{+}$	170	218 $(M^{-}HCl)^{-}$	6.5
2,4,5-TP (Silvex)	286 $(M^{+}NH_4)^{+}$	160	269 $(M^{-}1)^{-}$	43
Dinoseb	228 $(M^{+}NH_4\text{-}NO)^{+}$	24	240 $(M)^{-}$	19
2,4-DB	266 $(M^{+}NH_4)^{+}$	3.4	247 $(M^{-}1)^{-}$	110
2,4-D,Butoxy ethanol ester	321 $(M^{+}H)^{+}$	1.4	185 $(M^{-}C_6H_{13}O_1)^{-}$	
2,4,5-T,Butoxy ethanol ester	372 $(M^{+}NH_4)^{+}$	0.6	195 $(M^{-}C_8H_{15}O_3)^{-}$	
2,4,5-T,Butyl ester	328 $(M^{+}NH_4)^{+}$	8.6	195 $(M^{-}C_6H_{11}O_2)^{-}$	
2,4-D,ethyl-hexyl ester	350 $(M^{+}NH_4)^{+}$	1.2	161 $(M^{-}C_{10}H_{19}O_3)^{-}$	

Data from Reference 19.

TABLE 15

SINGLE LABORATORY OPERATOR ACCURACY AND PRECISION
FOR THE CHLORINATED PHENOXYACID HERBICIDES

Compounds	Average[(a)] Recovery %	Standard Deviation	Spike Amount	Range of Recovery %	Number of Analyses
	LOW LEVEL DRINKING WATER		µg/L		
Dicamba	63	22	5	86 - 33	9
2,4-D	26	13	5	37 - 0	9
MCPA	60	23	5	92 - 37	9
MCPP	78	21	5	116 - 54	9
Dichlorprop	43	18	5	61 - 0	9
2,4,5-T	72	31	5	138 - 43	9
Silvex	62	14	5	88 - 46	9
2,4-DB	29	24	5	62 - 0	9
Dinoseb	73	11	5	85 - 49	9
Dalapon	ND	ND	5	ND	9
2,4-D,ester	73	17	5	104 - 48	9
	HIGH LEVEL DRINKING WATER		µg/L		
Dicamba	54	30	50	103 - 26	9
2,4-D	60	35	50	119 - 35	9
MCPA	67	41	50	128 - 32	9
MCPP	66	33	50	122 - 35	9
Dichlorprop	66	33	50	116 - 27	9
2,4,5-T	61	23	50	99 - 44	9
Silvex	74	35	50	132 - 45	9
2,4-DB	83	25	50	120 - 52	9
Dinoseb	91	10	50	102 - 76	9
Dalapon	43	9.6	50	56 - 31	6
2,4-D,ester	97	19	50	130 - 76	9
	LOW LEVEL SAND		µg/g		
Dicamba	117	26	0.1	147 - 82	10
2,4-D	147	23	0.1	180 -118	10
MCPA	167	79	0.1	280 - 78	10
MCPP	142	39	0.1	192 - 81	10
Dichlorprop	ND	ND	0.1	ND	10
2,4,5-T	134	27	0.1	171 - 99	10
Silvex	121	23	0.1	154 - 85	10
2,4-DB	199	86	0.1	245 - 0	10
Dinoseb	76	74	0.1	210 - 6	10
Dalapon	ND	ND	0.1	ND	10
2,4-D,ester	180	58	0.1	239 - 59	7

[(a)]All recoveries are in negative ionization mode, except for 2,4-D,ester.
ND = Not Detected.

TABLE 15 (continued)

SINGLE LABORATORY OPERATOR ACCURACY AND PRECISION
FOR THE CHLORINATED PHENOXYACID HERBICIDES

Compounds	Average[(a)] Recovery %	Standard Deviation	Spike Amount	Range of Recovery %	Number of Analyses
	HIGH LEVEL SAND		µg/g		
Dicamba	153	33	1	209 - 119	9
2,4-D	218	27	1	276 - 187	9
MCPA	143	30	1	205 - 111	9
MCPP	158	34	1	226 - 115	9
Dichlorprop	92	37	1	161 - 51	9
2,4,5-T	160	29	1	204 - 131	9
Silvex	176	34	1	225 - 141	9
2,4-DB	145	22	1	192 - 110	9
Dinoseb	114	28	1	140 - 65	9
Dalapon	287	86	1	418 - 166	9
2,4-D,ester	20	3.6	1	25 - 17	7
	LOW LEVEL MUNICIPAL ASH		µg/g		
Dicamba	83	22	0.1	104 - 48	9
2,4-D	ND	ND	0.1	ND	9
MCPA	ND	ND	0.1	ND	9
MCPP	ND	ND	0.1	ND	9
Dichlorprop	ND	ND	0.1	ND	9
2,4,5-T	27	25	0.1	60 - 0	9
Silvex	68	38	0.1	128 - 22	9
2,4-DB	ND	ND	0.1	ND	9
Dinoseb	44	13	0.1	65 - 26	9
Dalapon	ND	ND	0.1	ND	9
2,4-D,ester	29	23	0.1	53 - 0	6
	HIGH LEVEL MUNICIPAL ASH		µg/g		
Dicamba	66	21	1	96 - 41	9
2,4-D	8.7	4.8	1	21 - 5	9
MCPA	3.2	4.8	1	10 - 0	9
MCPP	10	4.3	1	16 - 4.7	9
Dichlorprop	ND	ND	1	ND	9
2,4,5-T	2.9	1.2	1	3.6 - 0	9
Silvex	6.0	3.1	1	12 - 2.8	9
2,4-DB	ND	ND	1	ND	9
Dinoseb	16	6.8	1	23 - 0	9
Dalapon	ND	ND	1	ND	9
2,4-D,ester	1.9	1.7	1	6.7 - 0	6

[(a)]All recoveries are in negative ionization mode, except for 2,4-D,ester.
ND = Not Detected.

TABLE 16

MULTI-LABORATORY ACCURACY AND PRECISION DATA
FOR THE CHLORINATED PHENOXYACID HERBICIDES

Compounds	Spiking Concentration	Mean (% Recovery)[a]	% Relative Standard Deviation[b]
		500 mg/L	
2,4,5-T		90	23
2,4,5-T,butoxy		90	29
2,4-D		86	17
2,4-DB		95	22
Dalapon		83	13
Dicamba		77	25
Dichlorprop		84	20
Dinoseb		78	15
MCPA		89	11
MCPP		86	12
Silvex		96	27
		50 mg/L	
2,4,5-T		62	68
2,4,5-T,butoxy		85	9
2,4-D		64	80
2,4-DB		104	28
Dalapon		121	99
Dicamba		90	23
Dichlorprop		96	15
Dinoseb		86	57
MCPA		96	20
MCPP		76	74
Silvex		65	71
		5 mg/L	
2,4,5-T		90	28
2,4,5-T,butoxy		99	17
2,4-D		103	31
2,4-DB		96	21
Dalapon		150	4
Dicamba		105	12
Dichlorprop		102	22
Dinoseb		108	30
MCPA		94	18
MCPP		98	15
Silvex		87	15

[a] Mean of duplicate data from 3 laboratories.
[b] % RSD of duplicate data from 3 laboratories.
Data from Reference 20.

TABLE 17

COMPARISON OF LODs: METHOD 8151 vs. METHOD 8321

Ionization Compound	Method 8151 Aqueous Samples GC/ECD EDL[a] (µg/L)	Method 8321 Aqueous Samples HPLC/MS/TS LOD (µg/L)	Mode
Dalapon	1.3	1.1	(-)
Dicamba	0.081	0.3	(-)
2,4-D	0.2	0.29	(+)
MCPA	0.056[b]	2.8	(-)
Dichlorprop	0.26	0.27	(+)
MCPP	0.09	0.50	(+)
2,4,5-T	0.08	0.65	(-)
2,4,5-TP (Silvex)	0.075	4.3	(-)
2,4-DB	0.8	0.34	(+)
Dinoseb	0.19	1.9	(-)

[a] EDL = estimated detection limit; defined as either the MDL, or a concentration of analyte in a sample yielding a peak in the final extract with signal-to-noise ratio of approximately 5, whichever value is higher.

[b] 40 CFR Part 136, Appendix B (49 FR 43234). Chromatography using wide-bore capillary column.

TABLE 18

SINGLE-LABORATORY METHOD DETECTION LIMIT DETERMINATION
AND PRECISION RESULTS - WATER[c]

Analyte	Average % Recovery	Standard Deviation	%RSD	MDL[b] µg/L
Aldicarb sulfoxide[a]	7.5	0.27	72.4	0.8
Aldicarb sulfone	88.4	0.44	50.3	1.3
Oxamyl	60.7	0.10	16.6	0.3
Methomyl	117	0.49	41.5	1.5
3-Hydroxycarbofuran[a]	37.4	0.25	65.4	0.8
Fenuron	104	0.20	19.3	0.6
Benomyl/Carbendazim	67.3	0.13	19.7	0.4
Aldicarb	93.7	0.46	49.6	1.4
Aminocarb	117	0.53	44.9	1.6
Carbofuran	94.2	0.17	17.7	0.5
Propoxur	106	0.32	30.4	1.0
Monuron	95.6	0.24	25.6	0.7
Bromacil	86.4	0.12	14.1	0.4
Tebuthiuron	106	0.17	16.1	0.5
Carbaryl	85.1	0.29	34.1	0.9
Fluometuron	89.1	0.19	21.7	0.6
Propham	84.2	0.15	17.3	0.4
Propachlor	98.5	0.16	16.0	0.5
Diuron	95.6	0.14	14.7	0.4
Siduron	105	0.27	25.9	0.8
Methiocarb	92.4	0.16	17.5	0.5
Barban	90.5	0.79	17.4	2.4
Linuron	97.7	0.19	19.5	0.6
Chloropropham	89.1	0.68	15.2	2.0
Mexacarbate	80.0	1.41	35.1	4.2
Chloroxuron	109	0.32	29.2	1.0
Neburon	92.5	0.14	14.9	0.4

[a] Values generated from internal response factor calculations.

[b] Method detection limit determinations are based on twenty water extractions. Aldicarb sulfoxide, Barban, Chloropropham, and Mexacarbate spike levels were at 5 µg/L. All other analytes were spiked at 1 µg/L. The method detection limit was determined by multiplying the standard deviation by 3. Quantitation was done using average linear regression values, unless otherwise indicated.

[c] Data from Reference 22.

TABLE 19

SINGLE-LABORATORY METHOD QUANTITATION LIMIT DETERMINATION
AND PRECISION RESULTS - SOIL[b]

Analyte	Average % Recovery	Standard Deviation	%RSD	MDL[a] µg/g
Aldicarb sulfoxide	66.9	0.0492	58.9	0.15
Aldicarb sulfone	118	0.0076	25.7	0.023
Oxamyl	89.6	0.0049	21.9	0.015
Methomyl	86.8	0.0051	23.6	0.015
3-Hydroxycarbofuran	103	0.0116	45.0	0.035
Fenuron	91.2	0.0049	21.6	0.015
Benomyl/Carbendazim	68.0	0.0082	47.0	0.025
Aldicarb	72.0	0.0056	30.1	0.017
Aminocarb	84.4	0.0082	38.7	0.025
Carbofuran	102	0.0083	32.7	0.025
Propoxur	95.2	0.0091	38.2	0.027
Monuron	107	0.0077	28.8	0.023
Bromacil	99.6	0.0069	27.5	0.021
Tebuthiuron	96.8	0.0071	29.5	0.021
Carbaryl	99.6	0.0054	21.7	0.016
Fluometuron	92.8	0.0035	15.1	0.011
Propham	100	0.0039	15.7	0.012
Propachlor	114	0.0037	13.0	0.011
Diuron	101	0.0060	23.8	0.018
Siduron	107	0.0063	23.7	0.019
Methiocarb	124	0.0054	17.5	0.016
Barban	108	0.0333	24.8	0.10
Linuron	113	0.0037	13.0	0.011
Chloropropham	104	0.0217	16.6	0.065
Mexacarbate	62.2	0.0119	15.3	0.036
Chloroxuron	97.6	0.0031	12.6	0.009
Neburon	110	0.0044	16.0	0.011

[a] Method detection limit determinations are based on twenty soil extractions. Aldicarb sulfoxide, Barban, Chloropropham, and Mexacarbate spike levels were at 0.125 µg/g. All other analytes were spiked at 0.025 µg/g. The method detection limit was determined by multiplying the standard deviation by 3. Quantitation was done using average linear regression values, unless otherwise indicated.

[b] Data from Reference 22.

TABLE 20

SINGLE-LABORATORY EVALUATION OF AVERAGE RECOVERY AND PRECISION DATA - WATER[c]

Analyte	Average % Recovery[b]	Standard Deviation	%RSD
Aldicarb sulfoxide	7.6	2.8	37.0
Aldicarb sulfone	56.0	27.1	48.5
Oxamyl[a]	38.9	17.9	45.9
Methomyl	52.0	19.6	37.7
3-Hydroxycarbofuran[a]	22.2	9.3	41.7
Fenuron	72.5	22.0	30.3
Benomyl/Carbendazim	47.3	14.7	31.0
Aldicarb	81.0	13.7	16.9
Aminocarb	109	38.3	35.1
Carbofuran	85.5	10.0	11.7
Propoxur	79.1	13.7	17.3
Monuron	91.8	11.3	12.3
Bromacil	87.6	12.1	13.8
Tebuthiuron	87.1	9.0	10.3
Carbaryl	82.1	13.5	16.5
Fluometuron	84.4	8.3	9.8
Propham	80.7	13.8	17.1
Propachlor	84.3	10.0	11.9
Diuron	90.8	14.1	15.6
Siduron	88.0	9.5	10.8
Methiocarb	93.3	12.8	13.8
Barban	88.1	11.2	12.7
Linuron	87.1	16.8	19.3
Chloropropham	94.9	15.3	16.1
Mexacarbate	79.8	12.9	16.2
Chloroxuron	106	24.9	23.5
Neburon	85.3	12.6	14.8

[a] Values generated from internal response factor calculations.

[b] Nine spikes were performed at three concentrations. The concentrations for Aldicarb sulfoxide, Barban, Chloropropham, and Mexacarbate spike levels were at 25 µg/L, 50 µg/L, and 100 µg/L. All other analyte concentrations were 5 µg/L, 10 µg/L, and 20 µg/L. One injection was disregarded as an outlier. The total number of spikes analyzed was 26. Quantitation was done using average linear regression values, unless otherwise indicated.

[c] Data from Reference 22.

TABLE 21

SINGLE-LABORATORY EVALUATION OF AVERAGE RECOVERY AND PRECISION DATA - SOIL[b]

Analyte	Average % Recovery[a]	Standard Deviation	%RSD
Aldicarb sulfoxide	66.9	31.3	46.7
Aldicarb sulfone	162	51.4	31.7
Oxamyl	78.9	46.1	58.5
Methomyl	84.9	25.8	30.4
3-Hydroxycarbofuran	105	36.3	34.5
Fenuron	91.9	16.7	18.1
Benomyl/Carbendazim	95.6	18.2	19.0
Aldicarb	97.9	17.0	17.4
Aminocarb	133	44.7	33.6
Carbofuran	109	14.4	13.2
Propoxur	104	16.5	15.9
Monuron	101	12.4	12.3
Bromacil	100	9.0	9.0
Tebuthiuron	104	11.9	11.5
Carbaryl	102	15.5	15.2
Fluometuron	94.5	15.7	16.7
Propham	92.8	12.0	12.9
Propachlor	94.6	10.3	10.9
Diuron	107	17.4	16.2
Siduron	100	12.0	12.0
Methiocarb	107	14.2	13.2
Barban	92.3	15.6	16.9
Linuron	104	13.6	13.1
Chloropropham	105	9.3	8.9
Mexacarbate	77.2	9.8	12.7
Chloroxuron	121	27.3	22.5
Neburon	92.1	16.5	17.9

[a] Nine spikes were performed at three concentrations. The concentrations for Aldicarb sulfoxide, Barban, Chloropropham, and Mexacarbate spike levels were at 0.625 µg/g, 1.25 µg/g, and 2.5 µg/g. All other analyte concentrations were 0.125 µg/g, 0.25 µg/g, and 0.50 µg/g. One injection was disregarded as an outlier. The total number of spikes analyzed was 26. Quantitation was done using average linear regression values.

[b] Data from Reference 22.

TABLE 22

MULTI-LABORATORY EVALUATION OF METHOD ACCURACY
(AFTER OUTLIER REMOVAL)[d]

Analyte	Percent Recovery		
	High-Concentration Samples[a]	Medium-Concentration Samples[b]	Low-Concentration Samples[c]
Aldicarb	98.7	110	52.0
Bendiocarb	81.4	95.0	52.0
Carbaryl	92.0	108	62.0
Carbendazim	125	138	128
Carbofuran	87.8	92.3	72.0
Diuron	79.9	98.8	66.0
Linuron	84.8	93.0	82.0
Methomyl	93.3	90.8	90.0
Oxamyl	83.8	88.0	98.0

[a] Three replicates per laboratory; eight to nine laboratories (per Table 26 of Reference 23). The true concentration is 90 mg/L per compound, except Carbendazim at 22.5 mg/L.

[b] Two replicates per laboratory; eight to nine laboratories (per Table 26 of Reference 23). The true concentration is 40 mg/L per compound except Carbendazim at 10 mg/L.

[c] Three replicates per laboratory; eight to nine laboratories (per Table 26 of Reference 23). The true concentration is 5 mg/L per compound, except Carbendazim at 1.25 mg/L.

[d] Data from Reference 23.

TABLE 23

MULTI-LABORATORY EVALUATION OF METHOD PRECISION
(AFTER OUTLIER REMOVAL)[a]

	High Concentration					Medium Concentration					Low Concentration				
Analyte	Avg.	s_r	s_R	$\%RSD_R$	$\%RSD_R$	Avg.	s_r	s_R	$\%RSD_r$	$\%RSD_R$	Avg.	s_r	s_R	$\%RSD_r$	$\%RSD_R$
Aldicarb	88.8	11.4	34.4	12.9	38.8	44.1	7.7	17.0	17.5	38.5	2.6	0.9	2.6	33.1	98.2
Bendiocarb	73.3	16.1	39.3	21.9	53.6	38.0	6.6	16.6	17.3	43.7	2.6	0.6	1.6	21.3	61.9
Carbaryl	82.8	11.7	34.0	14.2	41.1	43.1	3.0	15.7	7.0	36.4	3.1	0.7	2.3	23.3	75.8
Carbendazim	28.1	5.6	15.3	19.9	54.4	13.8	1.4	8.9	10.4	64.2	1.6	0.4	1.1	26.1	68.2
Carbofuran	79.0	16.7	35.2	21.2	44.5	36.9	5.0	16.3	13.6	44.3	3.6	0.9	3.3	25.2	91.6
Diuron	71.9	13.1	26.1	18.2	36.3	39.5	2.6	11.8	6.5	29.8	3.3	0.5	2.6	16.2	77.9
Linuron	76.3	8.3	32.5	10.9	42.6	37.2	3.9	13.4	10.5	35.9	4.1	0.6	2.1	15.7	51.4
Methomyl	84.0	10.8	29.4	12.9	35.0	36.3	2.8	15.0	7.8	41.2	4.5	0.7	4.1	15.3	92.9
Oxamyl	75.5	12.4	37.0	16.4	49.1	35.2	3.7	20.8	10.4	59.1	4.9	0.5	4.6	9.7	93.6
Average				16.5	43.9				11.2	43.7				20.7	79.1
Std. Dev.				4.0	7.1				4.1	11.2				7.1	16.3

s_r and s_R are the standard deviations for repeatability and reproducibility, respectively. RSD_r and RSD_R are the corresponding relative standard deviations for repeatability and reproducibility, respectively. The units for average, s_r and s_R are mg/L.

[a] Data from Reference 23.

FIGURE 1
SCHEMATIC OF THE THERMOSPRAY PROBE AND ION SOURCE

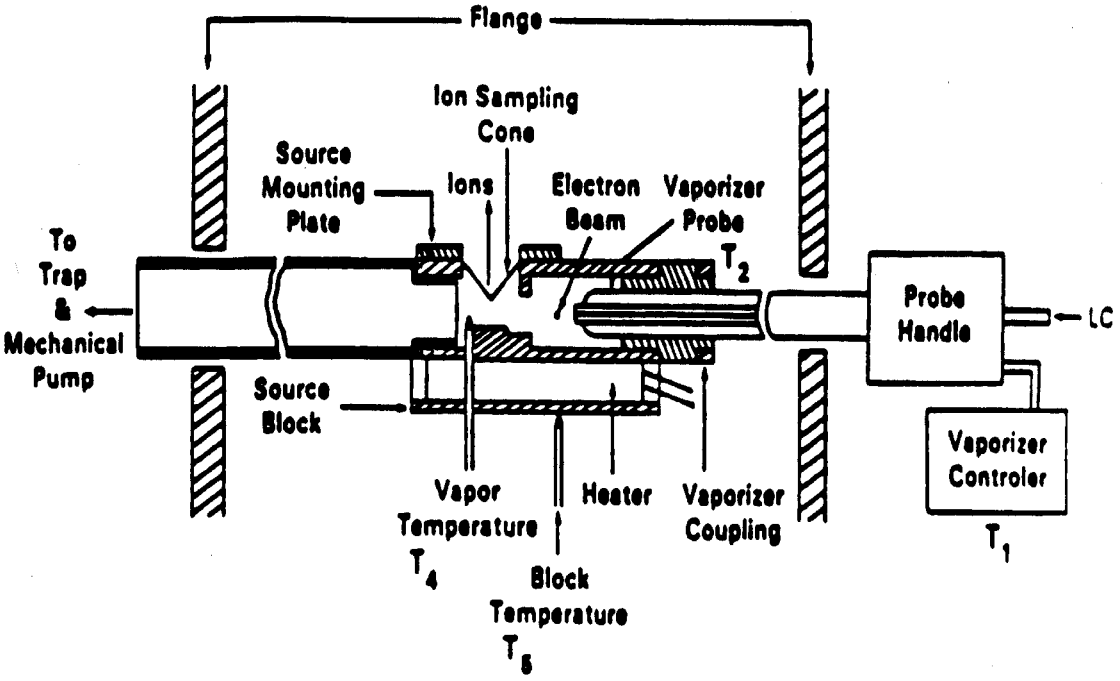


FIGURE 2
THERMOSPRAY SOURCE WITH WIRE-REPELLER
(High sensitivity configuration)

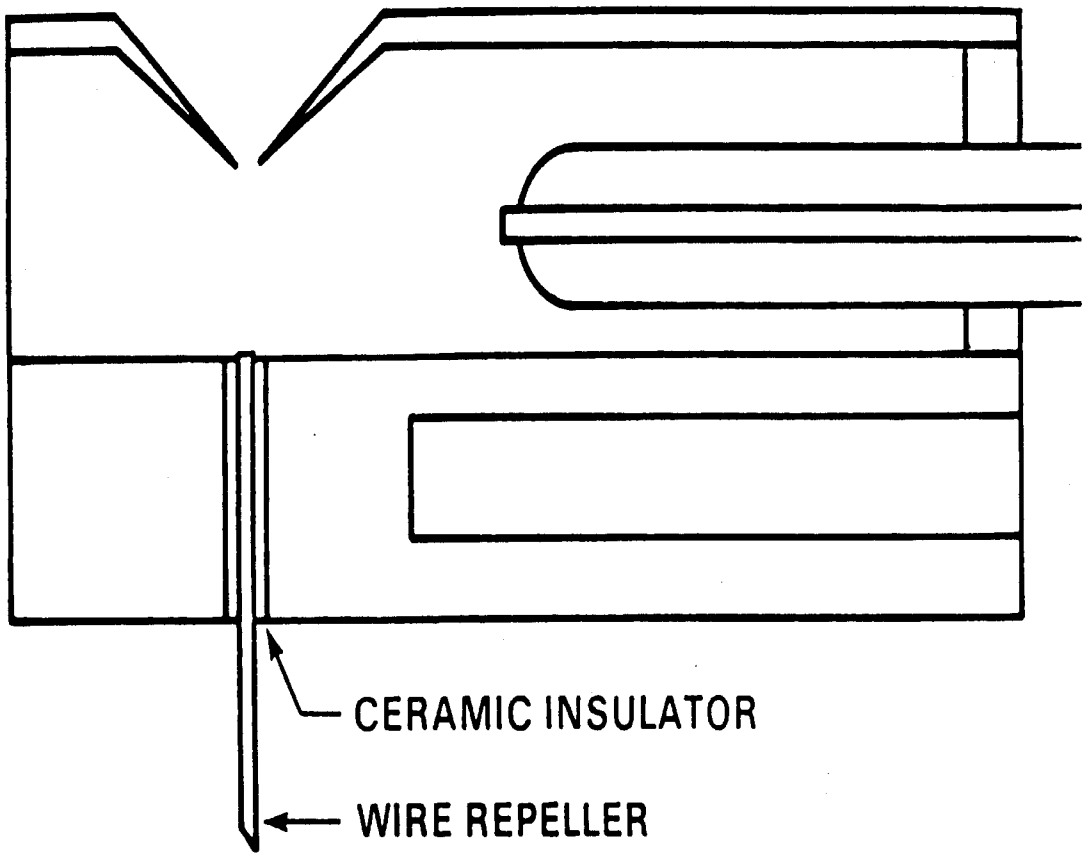
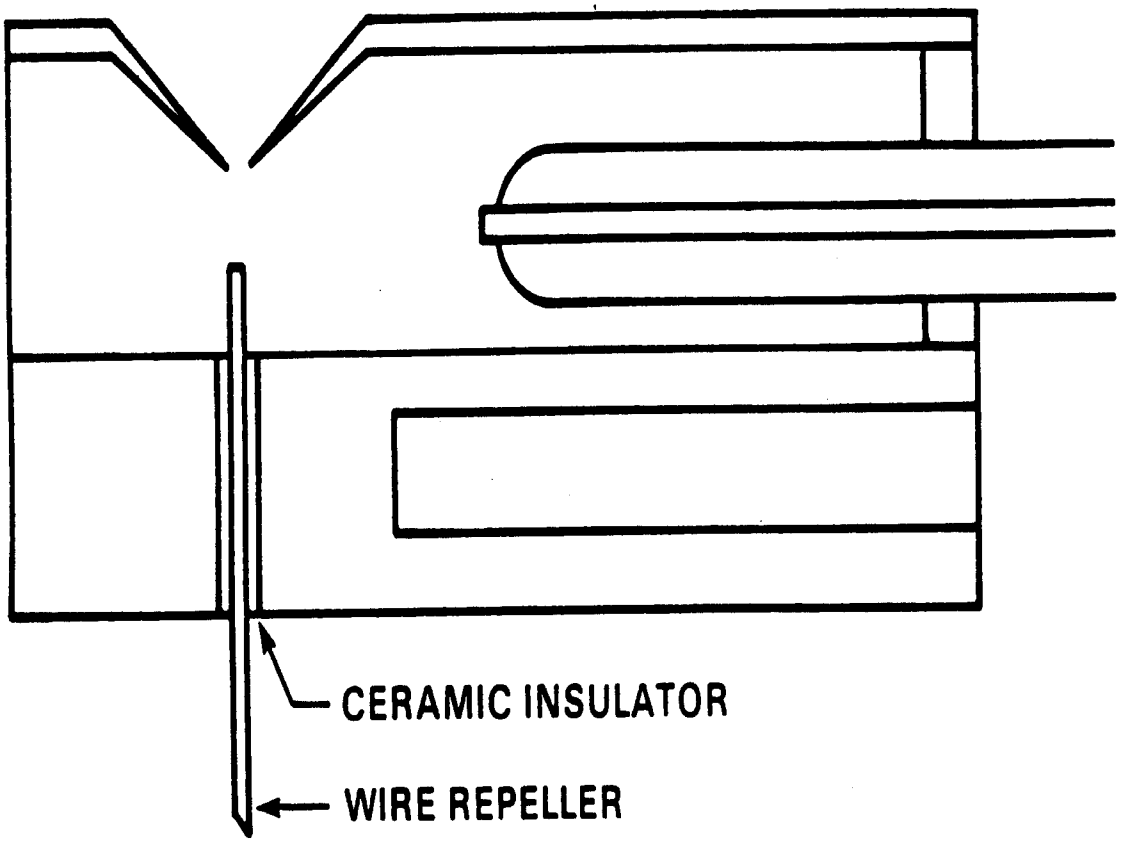
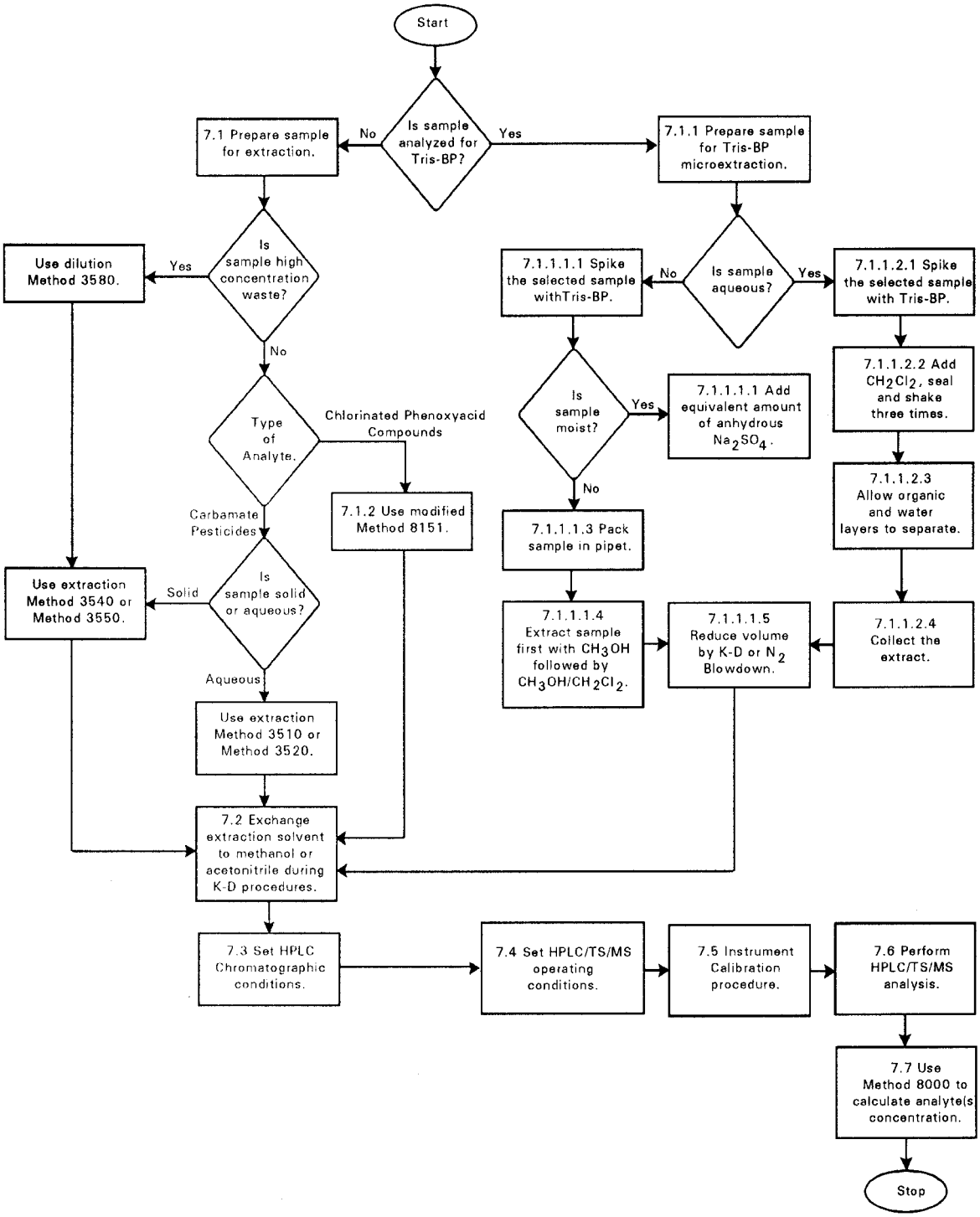


FIGURE 3
THERMOSPRAY SOURCE WITH WIRE-REPELLER
(CAD configuration)




Start
Is sample analyzed for Tris-BP?
No
Yes
7.1 Prepare sample for extraction.
7.1.1 Prepare sample for Tris-BP microextraction.
Is sample high concentration waste?
Yes
No
Use dilution Method 3580.
Type of Analyte.
Chlorinated Phenoxyacid Compounds
Carbamate Pesticides
7.1.2 Use modified Method 8151.
Is sample solid or aqueous?
Solid
Aqueous
Use extraction Method 3540 or Method 3550.
Use extraction Method 3510 or Method 3520.
7.2 Exchange extraction solvent to methanol or acetonitrile during K-D procedures.
7.3 Set HPLC Chromatographic conditions.
7.4 Set HPLC/TS/MS operating conditions.
7.5 Instrument Calibration procedure.
7.6 Perform HPLC/TS/MS analysis.
7.7 Use Method 8000 to calculate analyte(s) concentration.
Stop
Is sample aqueous?
No
Yes
7.1.1.1.1 Spike the selected sample withTris-BP.
7.1.1.2.1 Spike the selected sample with Tris-BP.
Is sample moist?
Yes
No
7.1.1.1.1 Add equivalent amount of anhydrous Na_2SO_4.
7.1.1.1.3 Pack sample in pipet.
7.1.1.2.2 Add CH_2Cl_2, seal and shake three times.
7.1.1.2.3 Allow organic and water layers to separate.
7.1.1.1.4 Extract sample first with CH_3OH followed by CH_3OH/CH_2Cl_2.
7.1.1.1.5 Reduce volume by K-D or N_2 Blowdown.
7.1.1.2.4 Collect the extract.