

METHOD 8321B

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 both thermospray-mass spectrometry (TS-MS) and an ultraviolet (UV) detector, for the determination of a variety of solvent-extractable nonvolatile compounds, including dyes, organophosphorus compounds, phenoxyacid herbicides, and carbamates. The following compounds may be determined by this method, although not all of the compounds are amenable to UV detection:

Analyte	CAS No. ^a
<u>Azo Dyes</u>	
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
<u>Anthraquinone Dyes</u>	
Disperse Blue 3	2475-46-9
Disperse Blue 14	2475-44-7
Disperse Red 60	17418-58-5
<u>Organophosphorus Compounds</u>	
Methomyl	16752-77-5
Thiofanox	39196-18-4
Famphur	52-85-7
Asulam	3337-71-1
Dichlorvos (DDVP)	62-73-7
Dimethoate	60-51-5
Disulfoton	298-04-4
Fensulfothion	115-90-2
Merphos	150-50-5
Parathion methyl	298-00-0

Analyte	CAS No. ^a
Monocrotophos	6923-22-4
Naled	300-76-5
Phorate	298-02-2
Trichlorfon	52-68-6
Tris (2,3-dibromopropyl) phosphate (tris-BP)	126-72-7
<u>Chlorinated Phenoxyacid Compounds</u>	
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
<u>Carbamates and related compounds</u>	
Aldicarb*	116-06-3
Aldicarb sulfone	1646-88-4
Aldicarb sulfoxide	1646-87-3
Aminocarb	2032-59-9
Barban	101-27-9
Benomyl	17804-35-2
Bendiocarb*	22781-23-3
Bromacil	314-40-9
Butylate	2008-41-5
Carbaryl*	63-25-2
Carbendazim*	10605-21-7
Carbofuran*	1563-66-2
Carbosulfan	55285-14-8
Chloroxuron	1982-47-4
Chloroprotham	101-21-3

Analyte	CAS No. ^a
<i>m</i> -Cumenyl methyl carbamate	64-00-6
Diuron*	330-54-1
EPTC	759-94-4
Fenuron	101-42-8
Formetanate hydrochloride	23422-53-9
Fluometuron	2164-17-2
3-Hydroxycarbofuran	16655-82-6
Linuron*	330-55-2
Methiocarb	2032-65-7
Methomyl*	16752-77-5
Metolcarb	1129-41-5
Mexacarbate	315-18-4
Molinate	2212-67-1
Monuron	150-68-5
Neburon	555-37-3
Oxamyl*	23135-22-0
Pebulate	1114-71-2
<i>o</i> -Phenylenediamine	95-54-5
Physostigmine	57-47-6
Physostigmine salicylate	57-64-7
Promecarb	2631-37-0
Propham	122-42-9
Propoxur	114-26-1
Prosulfocarb	52888-80-9
Siduron	1982-49-6
Tebuthiuron	34014-18-1
Thiodicarb	59669-26-0
Thiophanate-methyl	23564-05-8
Triallate	2303-17-5
Vernolate	1929-77-7

^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 The compounds listed in this method were chosen for analysis by HPLC/MS because they have been designated as problem compounds that are hard to analyze by gas chromatographic methods. The sensitivity of this method is dependent upon the level of interferants within a given matrix, and varies with compound class and even by compound within a class. Additionally, the sensitivity is dependent upon the mode of operation of the mass spectrometer, with the selected reaction monitoring (SRM) mode providing greater sensitivity than single quadrupole scanning.

1.3 This method may be applicable to the analysis of other nonvolatile or semivolatile compounds that are solvent-extractable, are amenable to HPLC, and can be ionized under thermospray introduction for mass spectrometric detection or can be determined by a UV detector. Table 3 lists 10 other classes of compounds that may be amenable to analysis by HPLC/TS/MS.

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 is recommended because it will simplify quantitation.

1.5 Data are also provided for the determination of chlorophenoxy acid herbicides in fly ash (Table 12), however, recoveries for most compounds are very low, indicating poor extraction efficiency for these analytes using the extraction procedure included in this method. Therefore, other extraction procedures, such as those described in the various 3500 Series methods for solid samples, may be more effective.

1.6 All of the carbamates and related compounds listed in Sec. 1.1 may be analyzed by HPLC with MS detection. The following carbamates and related compounds also have been evaluated using HPLC with UV detection at two wavelengths. Some compounds can be determined at both UV wavelengths, and some at only one of the two wavelengths.

Analyte	UV/Vis at 254 nm	UV/Vis at 280 nm
Barban	Y	Y
Benomyl	Y	Y
Carbaryl	Y	Y
Carbofuran phenol	Y	Y
Carbosulfan	Y	Y
Formetanate hydrochloride	Y	Y
Methiocarb	Y	Y
Mexacarbate	Y	Y
Propham	Y	Y
Thiophanate-methyl	Y	Y
Carbendazim	Y	Y
<i>o</i> -Phenylenediamine	Y	Y
<i>m</i> -Cumenyl methyl carbamate	Y	
Oxamyl	Y	
Physostigmine	Y	
Promecarb	Y	

Analyte	UV/Vis at 254 nm	UV/Vis at 280 nm
Prosulfocarb	Y	
Thiodicarb	Y	
Triallate	Y	
Bendiocarb		Y
Carbofuran		Y
Physostigmine salicylate		Y
Propoxur		Y

1.7 For further compound identification, MS/MS with collision-activated dissociation (CAD) can be used as an optional extension of this method.

1.8 Prior to employing this method, analysts are advised to consult the base method for each type of procedure that may be employed in the overall analysis (e.g., Methods 3500, 3600, 5000, and 8000) for additional information on quality control procedures, development of QC acceptance criteria, calculations, and general guidance. Analysts also should consult the disclaimer statement at the front of the manual and the information in Chapter Two, Sec. 2.1, for guidance on the intended flexibility in the choice of methods, apparatus, materials, reagents, and supplies, and on the responsibilities of the analyst for demonstrating that the techniques employed are appropriate for the analytes of interest, in the matrix of interest, and at the levels of concern.

In addition, analysts and data users are advised that, except where explicitly specified in a regulation, the use of SW-846 methods is *not* mandatory in response to Federal testing requirements. The information contained in this method is provided by EPA as guidance to be used by the analyst and the regulated community in making judgments necessary to generate results that meet the data quality objectives for the intended application.

1.9 This method is restricted to use by, or under the supervision of, analysts experienced in the use of high performance liquid chromatography 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 reversed-phase high performance liquid chromatographic (RP/HPLC) and thermospray (TS) mass spectrometric (MS) conditions and ultraviolet (UV) conditions for the detection of the target analytes.

2.1.1 Sample extracts can be analyzed by direct injection into the thermospray or onto a liquid chromatographic-thermospray interface.

2.1.2 A gradient elution program is used to separate the compounds.

2.1.3 Quantitative analysis may be performed by either TS/MS or UV detection, using either an external or internal standard approach. TS/MS detection may be performed in either a negative ionization (discharge electrode) mode or a positive ionization mode, with a single quadrupole mass spectrometer.

2.1.4 In some cases, the thermospray interface may introduce variability that leads to less precise quantitation. In such instances, the MS response may be used to identify the analytes of interest while the quantitative results are derived from the response of the UV detector.

2.2 Prior to analysis, appropriate sample preparation techniques must be used.

2.2.1 Samples for analysis of chlorinated phenoxyacid compounds may be extracted by a modification of Method 8151 (see Sec. 7.3) or other appropriate extraction technique. In general, the pH of an aqueous sample or a solid sample is adjusted to less than 2, and the sample is extracted with diethyl ether, concentrated, and the solvent exchanged to acetonitrile. Aqueous samples may also be extracted using solid-phase extraction after a pH adjustment, as described in Method 3535.

2.2.2 For carbamates, both aqueous and solid samples may be extracted with methylene chloride using a 3500 series method or other appropriate extraction technique, concentrated (preferably using a rotary evaporator with adapter) and the solvent is exchanged to methanol.

2.2.3 Samples for analysis of the other target analytes may be prepared by appropriate extraction techniques. In general, water samples may be extracted at a neutral pH with methylene chloride, using a 3500 series method or other appropriate extraction technique. Solid samples may be extracted with a mixture of methylene chloride/acetone (1:1), using a 3500 series method or other appropriate extraction technique. Extracts may require concentration and solvent exchange prior to analysis.

2.2.4 A micro-extraction technique for the extraction of tris-BP from aqueous and non-aqueous matrices is included in this method (see Sec. 7.2). See the warning in Sec. 5.9 regarding this compound.

2.3 An optional mass spectrometry/mass spectrometry (MS/MS) confirmation procedure is provided in this method (see Sec. 7.11). That procedure employs 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 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.

3.3 The use of Florisil column cleanup in 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 6 of Method 3620 for recoveries of organophosphorus compounds as a function of Florisil fractions.

3.4 Compounds with high proton affinity may mask the MS response of some of the target analytes. Therefore, except when the thermospray MS/MS system is used for rapid screening of samples (see Sec. 7.11.1), an HPLC must be used to perform the chromatographic separations necessary for quantitative analyses.

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

3.5.1 Methyl parathion shows some minor degradation during analysis.

3.5.2 Naled can undergo debromination to form dichlorvos (DDVP). This reaction may occur during sample preparation and extraction, and the extent may depend on the nature of the sample matrix. The analyst should consider the potential for debromination of Naled when this compound is to be determined.

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

3.5.4 The water solubility of dichlorvos (DDVP) is 10 g/L at 20°C, and as a result, recovery of the this compound by solvent extraction from aqueous solutions is poor.

3.5.5 Trichloron rearranges and undergoes dehydrochlorination (loss of HCl) in acidic, neutral, or basic media, forming dichlorvos (DDVP). When either of these compounds are to be determined, the analyst should be aware of the possibility of this rearrangement in order to prevent misidentifications.

3.6 The chlorinated phenoxyacid 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.

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

3.8 Benomyl quickly degrades to carbendazim in the environment (Reference 7).

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 aids in the confirmation of specific analytes. These methods are less subject to chemical noise than other mass spectrometric methods.

3.11 It is unlikely that all 45 of the carbamates and related compounds that are listed in Sec. 1.1 can be determined in a single analysis. Based on a single-laboratory evaluation of 31 of the analytes (see Ref. 11), the following analytes are known to co-elute under the HPLC conditions described in this method.

Detector	Coeluting analytes	
MS	benomyl	carbendazim
	pebulate	vernolate
	physostigmine	physostigmine salicylate
UV/Vis at 254 nm	benomyl	carbendazim
	carbaryl	carbofuran phenol
	oxamyl	formetanate hydrochloride
	methiocarb/mexacarbate/promecarb/barban	
UV/Vis at 280 nm	benomyl	carbendazim
	barban	mexacarbate
	formetanate hydrochloride	physostigmine salicylate
	carbaryl	carbofuran phenol
	bendiocarb/propoxur/carbofuran	

The differences between the groups of coeluting compounds for the two UV wavelengths are due to the fact that some of the coeluting compounds respond at one wavelength but do not respond at the other. The differences between the MS detector and the UV detectors is the result of the ability to resolve the mass spectra of some of the co-eluting compound, but not their UV responses.

4.0 APPARATUS AND MATERIALS

4.1 HPLC/MS

The following apparatus and materials are necessary for the use of the HPLC/MS portions of this method.

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- μ L loop volume), analytical columns, purging gases, etc. At a minimum, the solvent delivery system must be capable of delivering a binary solvent system. The chromatographic system must be capable of being interfaced with a mass spectrometer (MS).

4.1.2 HPLC post-column addition pump - If post-column addition of reagents is employed, a pump is required. Ideally, this pump should be a syringe pump, and does not have to be capable of solvent programming. It is also possible to add the ionization reagents to the solvents and not perform post-column addition (see Sec. 7.6.1).

4.1.3 HPLC/MS interface

4.1.3.1 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.

4.1.3.2 Micromixer - 10- μ L, connects HPLC column system with HPLC post-column addition solvent system, if post-column addition is used.

4.1.4 Mass spectrometer system

4.1.4.1 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 810 (see Sec. 5.14) or other compounds used for mass calibration.

4.1.4.2 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.

4.1.5 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 connected 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- μ L injection loop, analytical columns, purging gases, etc. An automatic injector is optional, but is useful for multiple samples. The columns specified in Sec. 4.3 are also used with this system.

At a minimum, the UV detector must be capable of operating at the two wavelengths described in this procedure, e.g., 254 and 280 nm. Either conventional (photomultiplier tube) detectors or photodiode array detectors may be employed. 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/MS and, in that case, standard HPLC pressures are acceptable.

4.3 HPLC columns

A guard column and an analytical column are necessary. The columns listed in this section were those used to develop the method. The mention of these columns is not intended to exclude

the use of other columns that are available or that may be developed. Laboratories may use columns of other dimensions and/or packed with different stationary phases, provided that they document method performance data (e.g., chromatographic resolution, analyte breakdown, and quantitation limits) that provide analytical performance that is appropriate for the intended application.

4.3.1 Guard column - C₁₈ reversed-phase guard column, 10 mm x 2.6 mm ID, 0.5- μ m frit, or equivalent. The guard column should utilize the same or a similar stationary phase as the analytical column.

4.3.2 Analytical column - C₁₈ reversed-phase column, 100 mm x 2 mm ID, 5- μ m particle size of ODS-Hypersil, C₈ reversed-phase column, 100 mm x 2 mm ID, 3- μ m particle size of MOS2-Hypersil, or equivalent.

4.4 Purification equipment for azo dye standards

4.4.1 Soxhlet extraction apparatus

4.4.2 Extraction thimbles - single thickness, 43 x 123 mm

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

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

4.5 Extraction equipment for chlorinated phenoxyacid compounds

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

4.5.2 Separatory funnel - 2000-mL.

4.5.3 Graduated cylinder - 1000-mL.

4.5.4 Funnel - 75-mm diameter.

4.5.5 Wrist shaker - Burrell Model 75 or equivalent.

4.5.6 pH meter.

4.6 Kuderna-Danish (K-D) apparatus (optional)

4.6.1 Concentrator tube - 10-mL graduated. A ground-glass stopper is used to prevent evaporation of extracts.

4.6.2 Evaporation flask - 500-mL. Attach to concentrator tube with springs, clamps, or equivalent.

4.6.3 Two-ball micro-Snyder column

4.6.4 Springs - 1/2-in.

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

NOTE: This 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.

4.7 Disposable serological pipets

4.8 Collection tube - 15-mL conical, graduated.

4.9 Vials - 5-mL conical, glass, with PTFE-lined screw-caps or crimp tops.

4.10 Silanized glass wool

4.11 Microsyringes - 100- μ L, 50- μ L, 10- μ L (Hamilton 701 N or equivalent), and 50- μ L (blunted, Hamilton 705SNR or equivalent).

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

4.13 Balances - Analytical, 0.0001 g, top-loading, 0.01 g.

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

4.15 Graduated cylinder - 100-mL.

4.16 Separatory funnel - 250-mL.

4.17 Separatory funnel - 2-L, with PTFE stopcock.

4.18 Concentrator adaptor (optional) - for carbamate extraction.

4.19 Nitrogen evaporation apparatus - N-Evap Analytical Evaporator Model 111, Organomation Association Inc., Northborough, MA, or equivalent.

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, $\text{NH}_4\text{OOCCH}_3$, solution (0.1 M). Filter through a 0.45- μm membrane filter (Millipore HA or equivalent).

5.5 Acetic acid, $\text{CH}_3\text{CO}_2\text{H}$

5.6 Sulfuric acid solution - 1:1, v/v. Prepare by slowly adding 50 mL H_2SO_4 (sp. gr. 1.84) to 50 mL of water.

5.7 Argon gas, 99+ % pure.

5.8 Solvents - Unless otherwise noted, all solvents must be pesticide quality or equivalent.

5.8.1 Methylene chloride, CH_2Cl_2

5.8.2 Toluene, $\text{C}_6\text{H}_5\text{CH}_3$

5.8.3 Acetone, CH_3COCH_3

5.8.4 Diethyl Ether, $\text{C}_2\text{H}_5\text{OC}_2\text{H}_5$ - 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, $\text{CH}_3\text{CO}_2\text{C}_2\text{H}_5$

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.

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

5.10 Disperse azo dye purification

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 silica gel column (Sec. 4.4.4), and elute with diethyl ether. Separate the impurities using chromatography, and collect the major dye fraction.

5.11 Stock standard solutions - Standards may be prepared from pure standard materials or may be purchased as certified solutions. Commercially-prepared stock standards may be used if they are certified by the manufacturer and verified against a standard made from pure material.

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 standards are 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). At least one of the calibration standards should correspond to a sample concentration at or below that necessary to meet the data quality objectives of the project. 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 or HPLC-TS/MS system (see Method 8000). 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 810 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 of the analytes of interest. For molecular weights less than 500, use PEG 400, and for molecular weights greater than 500, use PEG 600 or PEG 810.

5.15 Internal standards - When the internal standard calibration option is used for HPLC/MS analyses, it is recommended that analysts use stable isotopically-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).

5.16 Matrix spiking standards - Consult Method 3500 for information on matrix spiking solutions. Prepare a solution containing the analytes of interest in a suitable solvent.

NOTE: The form of the compounds used for spiking should be identical to the form of the target analytes. For the phenoxyacid herbicides in particular, use the acid form of the acid analytes, not the ester form or an ether, as use of these other forms will not represent the performance of the overall extraction, cleanup, and determinative methods relative to the

target analytes. Conversely, when the ester forms are of the analytes of interest, e.g., 2,4-D, butoxyethanol ester, use the ester form of the analyte for preparing matrix spiking solutions.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

See the introductory material to Chapter Four, Organic Analytes, Sec. 4.1.

7.0 PROCEDURE

7.1 Sample preparation

Prior to analysis, samples must be extracted using an appropriate 3500 series method, the specific procedures described in this method, or another extraction procedure appropriate for the sample matrix. The analyst should choose among the possible extraction procedures based on the nature of the samples and the sensitivity required for the intended application.

Whatever extraction procedure is employed, *including* those specifically listed in this method, the analyst *must* demonstrate adequate performance for the analytes of interest, at the levels of interest. At a minimum, such a demonstration will encompass the initial demonstration of proficiency described in Sec. 8.2 of Method 3500, using a clean reference matrix. Method 8000 describes procedures that may be used to develop performance criteria for such demonstrations as well as for matrix spike and laboratory control sample results.

7.1.1 Samples for the analysis of disperse azo dyes and organophosphorus compounds must be prepared by an appropriate 3500 series method, or another appropriate extraction procedure, prior to HPLC/MS analysis.

7.1.2 Samples for the analysis of tris (2,3-dibromopropyl)phosphate (tris-BP) must be prepared according to Sec. 7.2, prior to HPLC/MS analysis, unless the analyst can demonstrate appropriate performance for the intended application using other techniques.

7.1.3 Samples for the analysis of chlorinated phenoxyacid compounds and their esters should be prepared according to Sec. 7.3, or other appropriate technique, prior to HPLC/MS analysis. TCLP leachates to be analyzed for the phenoxyacid herbicides may also be prepared using solid-phase extraction (SPE), as described in Method 3535.

7.2 Micro-extraction of tris-BP

The following sections describe micro-extraction procedures that may be applied to samples containing tris-BP. These procedures have been provided because tris-BP is a known carcinogen, and the micro-extraction procedures should minimize the exposure of the analyst to samples or standards containing this compound, relative to the extraction of larger sample weights or volumes.

7.2.1 Solid samples

7.2.1.1 Weigh a 1-g 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 μ L 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/ μ L in the 1-mL extract.

7.2.1.2 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 on top of the glass wool. Wash the pipet and its contents with 3 - 5 mL of methanol.

7.2.1.3 Pack the sample into the pipet. If the packing material has dried, begin by wetting it with a few mL of methanol, then pack sample into the pipet.

7.2.1.4 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.

7.2.1.5 Concentrate the extract to 1 mL using the nitrogen evaporation technique (see Sec. 7.5). Record the volume. (It may not be possible to evaporate some sludge samples to a 1-mL final volume).

7.2.1.6 Determination of percent dry weight - When sample results are to be calculated on a dry weight basis, a second portion of sample should be weighed at the same time as the portion used for analytical determination.

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

Immediately after weighing the sample for extraction, weigh 5 - 10 g of the sample into a tared crucible. Dry this aliquot overnight at 105°C. Allow to cool in a desiccator before weighing. Calculate the % dry weight as follows:

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

This oven-dried aliquot is not used for the extraction and should be disposed of appropriately once the dry weight has been determined.

7.2.2 Aqueous samples

7.2.2.1 Using a 100-mL graduated cylinder, measure 100 mL of sample and transfer it to a 250-mL separatory funnel. Add 200 μ L 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/ μ L in the 1-mL extract.

7.2.2.2 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.

7.2.2.3 Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between the 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 of Method 3510.

7.2.2.4 Collect the extract in a 15-mL graduated glass tube. Concentrate the extract to 1 mL, using nitrogen evaporation (see Sec. 7.5).

7.3 Extraction for chlorinated phenoxyacid compounds

Preparation of soil, sediment, and other solid samples should follow the procedures outlined in Method 8151, or other appropriate technique, except that no hydrolysis or esterification generally is performed. However, if the analyst desires to determine all of the phenoxyacid moieties as the acid, hydrolysis should be performed, because it will simplify quantitation. Sec. 7.3.1 presents an outline of the procedure with the appropriate changes necessary for determination by this method. Sec. 7.3.2 describes the extraction procedure for aqueous samples. TCLP leachates may be extracted using solid-phase extraction, as described in Method 3535.

7.3.1 Extraction of solid samples

7.3.1.1 Add 50 g of soil/sediment sample to a 500-mL, wide-mouth Erlenmeyer flask. 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 dilute (1:1) H_2SO_4 and monitor the pH for 15 minutes, with stirring. If necessary, add additional H_2SO_4 until the pH remains at 2. Other sample weights and reagent volumes may be employed, provided that the laboratory can demonstrate acceptable sensitivity and performance for the intended application.

7.3.1.2 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.

7.3.1.3 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.

7.3.1.4 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.

7.3.1.5 Check the pH of the extract. If it is not at or below pH 2, add more dilute H_2SO_4 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 Erlenmeyer flask with a ground-glass stopper. 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.

7.3.1.6 Add 45 - 50 g of 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 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.

7.3.1.7 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 (see Sec. 7.5).

7.3.2 Extraction of aqueous samples

7.3.2.1 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 2 with dilute (1:1) H_2SO_4 . Other sample weights and reagent volumes may be employed, provided that the laboratory can demonstrate acceptable sensitivity and performance for the intended application.

7.3.2.2 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 the 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.

7.3.2.3 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 extraction solvent to make a quantitative transfer.)

7.3.2.4 Proceed to Sec. 7.5 for drying, K-D concentration, solvent exchange, and final volume adjustment.

7.4 Extraction of carbamates

Preparation of aqueous, soil, sediment, and other solid samples may be accomplished using an appropriate 3500 series method. The following sections provide general considerations.

7.4.1 One-liter aqueous samples may be extracted with methylene chloride using an appropriate 3500 series method.

7.4.2 Forty-gram quantities of solid samples may be extracted with methylene chloride using an appropriate 3500 series method.

7.4.3 Concentration steps may be performed using a rotary evaporator or K-D, reducing the volume of the final extract to 5 - 10 mL.

7.4.4 Final concentration of the extract and exchanging the solvent to a 1-mL final volume of methanol may be accomplished using an adaptor on the rotary evaporator. If an adaptor is unavailable, the final concentration may be performed using nitrogen evaporation, in a fume hood.

7.4.5 Other sample weights and reagent volumes may be employed, provided that the laboratory can demonstrate acceptable sensitivity and performance for the intended application.

7.5 Extract concentration techniques

Two procedures are provided for the concentration of extracts: macro-concentration by Kuderna-Danish (K-D) and micro-concentration by nitrogen evaporation.

7.5.1 Macro-concentration by K-D

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. 4.6.5) 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.

7.5.2 Solvent exchange

Prior to analysis, exchange the solvent to methanol or acetonitrile.

7.5.2.1 Transfer the concentrator tube to a nitrogen evaporation device. Add a total of 5 mL of the final solvent of choice (methanol or acetonitrile).

7.5.2.2 Reduce the extract volume according to Sec. 7.5.3 and adjust the final volume to 1 mL (or other volume necessary to achieve the required sensitivity).

7.5.3 Micro-concentration by nitrogen evaporation

7.5.3.1 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.

7.5.3.2 The internal wall of the tube must be rinsed down several times with the final solvent 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.

7.5.4 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.

7.6 HPLC chromatographic conditions

7.6.1 Suggested mobile phases and elution gradients for some groups of analytes are shown in Tables 1 and 2. Analysts should also consult the instrument manufacturer's instructions. In the absence of specific suggestions, the following conditions may be a useful starting point.

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

Optimize the instrumental conditions for resolution of the target analytes and sensitivity. Post-column addition of the MS ionization reagents may not be necessary in all instances, and these reagents may be added to the elution solvents, provided that adequate performance can be demonstrated.

NOTE: Once established, the same operating conditions must be used for both calibrations and sample analyses.

7.6.2 If there is a chromatographic problem when analyzing disperse azo dyes, organophosphorus compounds, or tris-BP, apply a 2% constant flow of methylene chloride, 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.

7.6.3 A total flow rate of 1.0 - 1.5 mL/min may be necessary to maintain thermospray ionization, however, consult the instrument manufacturer's instructions and adjust the flow rate as needed.

7.7 Suggested 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 analysis. This evaluation may be based on the structures of the analytes or on analyses conducted in both ionization modes. Some groups of target compounds will have much better sensitivity using either positive or negative ionization (e.g., carbamates are generally more sensitive to the positive ionization mode and phenoxyacids are generally more sensitive to the negative ionization mode). When all the analytes of interest for a given application respond adequately in a given ionization mode, a single analysis using that mode may be employed.

7.7.1 Positive ionization mode conditions

Discharge electrode	Off
Filament	On or off (optional, analyte-dependent)
Mass range	150 - 450 amu (analyte-dependent, expect 1 to 18 amu higher than the molecular weight of the compound).
Scan time	1.50 sec/scan
Optional repeller wire or plate	170 - 250 v (sensitivity optimized). See Figure 2 for schematic of source with wire repeller.

7.7.2 Negative ionization mode conditions

Discharge electrode	On
Filament	Off
Mass range	135 - 450 amu
Scan time	1.50 sec/scan

7.7.3 Thermospray temperatures

Vaporizer control	110 - 130°C
Vaporizer tip	200 - 215°C
Jet	210 - 220°C
Source block	230 - 265°C

Some compounds may degrade in the source block at higher temperatures. The operator should use knowledge of the chemical properties of the analytes of interest to estimate proper source temperature.

7.7.4 Sample injection volume

An injection volume of 20 - 100 μL is normally used. The injection loop must be overfilled by at least a factor of two (e.g., 20 μL of sample is 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.

7.8 Calibration

7.8.1 Tuning the mass spectrometer system

When an MS detector is employed, the detector must be tuned at the beginning of each 12-hour analytical shift using the procedures outlined in Secs. 7.8.1.1 and 7.8.1.2, to ensure correct mass assignments, sensitivity, and resolution. Tuning may be accomplished using polyethylene glycol (PEG) 400, 600, or 810 (see Sec. 5.14), which have average molecular weights of 400, 600, and 810, 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. For triple quadrupole systems, both quadrupole 1 and quadrupole 3 are tuned.

7.8.1.1 The mass calibration parameters are as follows:

<u>PEG 400 and 600</u>		<u>PEG 810</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 or 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 a mass calibrant other than PEG is used, then the mass range should extend approximately 15 - 20 amu higher than the highest mass used for calibration. A scan time should be chosen which will give at least 6 scans across the calibrant peak.

7.8.1.2 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
$\text{NH}_4^+\cdot\text{H}_2\text{O}$	36 amu
$\text{CH}_3\text{OH}\cdot\text{NH}_4^+$	50 amu (methanol)
$\text{CH}_3\text{CN}\cdot\text{NH}_4^+$	59 amu (acetonitrile)
$\text{CH}_3\text{OOH}\cdot\text{NH}_4^+$	78 amu (acetic acid)

The appearance of m/z 50 or 59 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., $\text{H}(\text{OCH}_2\text{CH}_2)_n\text{OH}$ where $n=4$, gives the $\text{H}(\text{OCH}_2\text{CH}_2)_4\text{OH}\cdot\text{NH}_4^+$ ion at m/z 212).

7.8.2 Liquid chromatographic system

7.8.2.1 Choose the proper ionization conditions for the MS detector, as outlined in Sec. 7.7. When UV detection is employed in conjunction with the MS detector, establish appropriate operating conditions for the UV detector.

7.8.2.2 Prepare five calibration standards (see Sec. 5.12 and Method 8000). 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. In most cases the $(\text{M}+\text{H})^+$ and $(\text{M}+\text{NH}_4)^+$ 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.

7.8.2.3 Selective ion monitoring (SIM) may be used 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.

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

7.8.2.5 If UV detection is being used, 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, or when response of the MS detector is not sufficiently stable for quantitative analyses. In these instances, the MS response may be used for positive qualitative identification of the analytes while the UV response is used for quantitation.

7.8.2.6 The retention time of the chromatographic peak is an important parameter in analyte identification. Therefore, the relative retention time of the analyte (versus the internal standard) should be in the range of 0.9 to 1.1.

7.8.3 Calibration verification

At the beginning of each 12-hour analytical shift, the response of the instrument system must be verified by the analysis of the MS tuning standard (if the MS detector is used) and by the analysis of a calibration verification standard at the approximate mid-point of the initial calibration range. Consult Sec. 7.8.1 and Method 8000 for information on performing these demonstrations and the acceptance criteria that should be employed.

7.9 Sample analysis

Once the LC system has been calibrated as outlined in Sec. 7.8, sample analysis may begin, employing either the MS or UV detector, or both. Depending on the sensitivity necessary for a

given project, analyses may be conducted using the MS detector in either the positive or negative ionization modes. The positive ionization mode generally provides greater sensitivity, and may be more appropriate for samples containing very low concentrations of the analytes of interest. However, analysts are advised that some compounds may be detectable in only the negative ionization mode.

7.9.1 An instrument blank (methanol) should be analyzed after the standards, in order to demonstrate that the system is free from contamination.

7.9.2 If performing manual injections, take an appropriate aliquot of the sample as per Sec. 7.7.4. Start the HPLC gradient elution, load and inject the sample aliquot, and start the mass spectrometer data system analysis.

7.9.3 When using an autoinjector, ensure that it is set up 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 MS data system.

7.9.4 The concentration of the analyte is determined by using the initial calibration data (see Method 8000) from either the MS or UV detector response. Samples whose concentrations exceed the calibration range must be diluted to fall within the range.

7.9.5 When using MS or MS/MS, and when it is appropriate for the compounds of interest and the project objectives, determinations in both positive and negative ionization analyses may be done on each sample extract.

7.9.6 If only the UV detector is used, then consult Method 8000 for information on the confirmation of analyte identifications.

7.10 Calculations

7.10.1 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 calculations.

7.10.2 The retention time of the chromatographic peak is an important parameter in analyte identification. However, because matrix interferences can change chromatographic column conditions and thereby alter retention times, the absolute retention times are not as significant in analyte identification as relative retention times (when using internal standards). In addition, the mass spectral patterns are important criteria for analyte identification.

7.10.3 In instances when the TS/MS response exhibits higher variability, the MS response may be used to identify the analytes of interest while the quantitative results are derived from the response of the UV detector.

7.11 Optional MS/MS confirmation

With respect to this method, MS/MS is defined as daughter ion collision-activated dissociation acquisition, with quadrupole 1 set on one mass (parent ion), quadrupole 2 pressurized with argon and with a higher offset voltage than normal, and quadrupole 3 set to scan the desired mass range.

7.11.1 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, samples can be rapidly screened through direct injection of the sample into the thermospray (e.g., without using the HPLC to separate the sample components).

7.11.2 When using MS/MS, quadrupole 1 should be set to the protonated molecule or ammoniated adduct of the analyte of interest. Quadrupole 3 should be set to scan from 30 amu to just above the mass region of the protonated molecule.

7.11.3 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, the settings may be increased to create more and stronger collisions.

7.11.4 For analytical determinations, the base peak of the collision spectrum shall be used as the quantitation ion. For extra specificity, a second ion should be chosen as a backup quantitation ion.

7.11.5 Perform an initial calibration, as outlined in Sec. 7.8.

7.11.6 MS/MS contamination and interferences

7.11.6.1 If the MS/MS mode is to be used without chromatographic separation (e.g., for rapid screening), then the 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, re-extraction and reanalysis of the affected samples are necessary.

7.11.6.2 The MS/MS spectra of a calibration standard and the sample should 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, chromatographic separation must be utilized.

7.11.6.3 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 standards, blanks, 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. (Atrazine-d₅ has been used successfully for positive ion analysis, while 2,6-dinitrotoluene-d₃ has been used successfully for negative ion analysis.) The amount spiked 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.

7.11.7 The total area of the quantitation ion(s) is calculated and the initial calibration is used to calculate sample results.

7.11.8 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 to confirm or deny that a substance is present.

7.12 Optional wire-repeller CAD confirmation

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

7.12.2 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.

7.12.3 Continue as outlined in Sec. 7.9.

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 and include evaluation of retention time windows, calibration verification and chromatographic analysis of samples. When the mass spectrometer is used as the detector, it must be tuned at the beginning of each 12-hour analytical shift. Check the performance of the entire analytical system daily using data gathered from analyses of blanks, standards, and replicate samples. 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.

Checking any of the above items will necessitate shutting down the HPLC/TS system, making repairs and/or replacements, and then restarting the analyses. A tuning standard and a calibration verification standard should be reanalyzed before any sample analyses, as described in Sec. 7.8.3.

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 and a laboratory control sample (LCS) in each analytical batch, the addition of surrogates to each field sample and QC sample, and routine analyses of matrix spike and matrix spike duplicate aliquots.

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. Tables 4, 5, and 6 provide single-laboratory data for Disperse Red 1. Table 10 provides the data for organophosphorus pesticides, Table 11 for tris-BP, Table 12 for chlorophenoxyacid herbicides, and Tables 14 and 15 for carbamates.

9.2 Table 13 presents multi-laboratory accuracy and precision data for the chlorinated phenoxyacid herbicides. Table 13 is based on data from three laboratories that performed replicate analyses of standard solutions at each concentration specified in the table.

9.3 Tables 16 and 17 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 specified in the tables.

9.4 Table 18 provides data for solid-phase extraction of 2,4-D and 2,4,5-TP spiked into TCLP buffers at two different levels.

9.5 A single-laboratory study of 31 carbamates and related compounds was conducted in 1998. Bulk quantities of a clay soil and the effluent from a publicly-owned treatment works (POTW) were collected, homogenized, and spiked with the carbamates. The spiking levels were chosen to be approximately 80% of the Universal Treatment Standard (UTS) values for wastewater and non-wastewater, rounded to two significant figures. The spiking levels for the effluents samples are shown in Table 19, and ranged from 22 to 45 µg/L. The spiking levels for the soil samples are shown in Table 20, and ranged from 110 to 4500 µg/kg.

The spiked material was divided into aliquots of suitable size for extraction and analysis. Effluent samples were extracted using continuous liquid-liquid extraction, as described in Method 3520. Soil samples were extracted using Soxhlet extraction, as described in Method 3540. Four aliquots of each sample matrix were prepared and analyzed using all three detector systems in the method. The mean recovery and the relative standard deviation (RSD) of each analyte for the effluent and soil samples are shown in Tables 19 and 20, respectively. All data are taken from Reference 11.

10.0 REFERENCES

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6. "An Interlaboratory Comparison of an SW-846 Method for the Analysis of the Chlorinated Phenoxyacid Herbicides by LC/MS", EPA/600/X-90/133, June 1990.
7. Somasundaram, L., and J.R. Coates, Ed., "Pesticide Transformation Products Fate and Significance in the Environment", ACS Symposium Series 459, Chapter 13, 1991.
8. Single-Laboratory Evaluation of Carbamates, APPL, Inc., Fresno, CA.
9. "Interlaboratory Calibration Study of a Thermospray-Liquid Chromatography/Mass Spectrometry (TS-LC/MS) Method for Selected Carbamate Pesticides," EPA/600/X-92/102, August 1992.

10. Markell, C., "3M Data Submission to EPA," letter to B. Lesnik, June 27, 1995.
11. "Carbamates Method Evaluation Report," report for EPA Contract 68-W6-0068, Science Applications International Corporation, Dunn Loring, VA 22027, August 25, 1998.

TABLE 1
SUGGESTED HPLC CHROMATOGRAPHIC CONDITIONS

Analyte Class	Initial Mobile Phase (%)	Initial Time (min)	Linear Gradient Time (min)	Final Mobile Phase (%)	Final Hold Time (min)
Organophosphorus compounds	50/50 (water/methanol)	0	10	100% methanol	5
Azo dyes	50/50 (water/CH ₃ CN)	0	5	100% CH ₃ CN	5
tris-BP	50/50 (water/methanol)	0	10	100% methanol	5
Chlorinated phenoxyacid compounds	75/25 (0.1 M NH ₄ acetate in 1% acetic acid/ methanol)	2	15	40/60 0.1 M NH ₄ acetate in 1% acetic acid/ methanol	0
<i>or</i>	40/60 (0.1 M NH ₄ acetate in 1% acetic acid/ methanol)	3	5	75/25 0.1 M NH ₄ acetate in 1% acetic acid/ methanol	10

The conditions listed above are simply suggested starting points and may be modified by the laboratory to improve separation, increase sensitivity, or accommodate other analytes or analyte classes, provided that the laboratory demonstrates performance appropriate for the intended application .

TABLE 2

SUGGESTED HPLC CHROMATOGRAPHIC CONDITIONS FOR CARBAMATES

	Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
Option 1	0	95	5
	30	20	80
	35	0	100
	40	95	5
	45	95	5

Mobile phase A = 5 mM ammonium acetate with 0.1 M acetic acid

Mobile phase B = methanol, with optional post-column addition of 0.5 M ammonium acetate

Option 2	0	95	5
	30	0	100
	35	0	100
	40	95	5
	45	95	5

Mobile phase A = water with 0.1 M ammonium acetate with 1% acetic acid

Mobile phase B = methanol with 0.1 M ammonium acetate and 1% acetic acid, with optional post-column addition of 0.1 M ammonium acetate

TABLE 3

OTHER COMPOUNDS AMENABLE TO HPLC/MS

Methine Dyes	Alkaloids
Arylmethane Dyes	Aromatic ureas
Coumarin Dyes	Amides
Xanthene Dyes	Amines
Flame retardants	Amino acids

TABLE 4

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

	Percent Recovery of Disperse Red 1			
	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 2.

TABLE 5

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

	Percent Recovery of Disperse Red 1		
	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 2.

TABLE 6

RESULTS FROM ANALYSES OF ACTIVATED SLUDGE PROCESS WASTEWATER SPIKED WITH DISPERSE RED 1

	Recovery of Disperse Red 1 (mg/L)		
	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%
Unspiked Sample			
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 2.

TABLE 7

CALIBRATION MASSES AND % RELATIVE ABUNDANCES OF PEG 400

Mass	% Relative Abundance ^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	100.0
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 Intensities are normalized to mass 432.

TABLE 8

CALIBRATION MASSES AND % RELATIVE ABUNDANCES OF PEG 600

Mass	% Relative Abundance ^a
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	100.0
608.38	63.2
652.41	17.5
653.41	5.6
696.43	1.8

^a Intensities are normalized to mass 564.

TABLE 9

EXAMPLE RETENTION TIMES AND MASS SPECTRA
OF ORGANOPHOSPHORUS COMPOUNDS

Compound	Retention Time (min)	Mass (% 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)
Parathion methyl	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 3.

TABLE 10

SINGLE-OPERATOR ACCURACY AND PRECISION FOR ORGANOPHOSPHORUS
COMPOUNDS SPIKED INTO DRINKING WATER, SOIL, AND SEDIMENT

Matrix	Compound	Spike Conc.	Mean Rec. (%)	Std. Dev.	Recovery Range (%)	# Analyses
<i>Low concentration drinking water</i>		(µg/L)				
	Dimethoate	5	70	7.7	85 - 54	15
	Dichlorvos	5	40	12	64 - 14	15
	Naled	5	0.5	1.0	2 - 0	15
	Fensulfothion	5	112	3.3	119 - 106	15
	Parathion methyl	10	50	28	105 - 0	15
	Phorate	5	16	35	86 - 0	15
	Disulfoton	5	3.5	8	19 - 0	15
	Merphos	5	237	25	287 - 187	15
<i>Low concentration soil</i>		(µg/kg)				
	Dimethoate	50	16	4	24 - 7	15
	Dichlorvos	50	ND			15
	Naled	50	ND			15
	Fensulfothion	50	45	5	56 - 34	15
	Parathion methyl	100	ND			15
	Phorate	50	78	15	109 - 48	15
	Disulfoton	50	36	7	49 - 22	15
	Merphos	50	118	19	155 - 81	15

TABLE 10
(continued)

Matrix	Compound	Spike Conc.	Mean Rec. (%)	Std. Dev.	Recovery Range (%)	# Analyses
<i>Medium concentration drinking water</i>		(µg/L)				
	Dimethoate	50	52	4	61 - 43	12
	Dichlorvos	50	146	29	204 - 89	12
	Naled	50	4	3	9 - 0	12
	Fensulfothion	50	65	7	79 - 51	12
	Parathion methyl	100	85	24	133 - 37	12
	Phorate	50	10	15	41 - 0	12
	Disulfoton	50	2	1	4 - 0	12
	Merphos	50	101	13	126 - 75	12
<i>Medium concentration sediment</i>		(mg/kg)				
	Dimethoate	2	74	8.5	91 - 57	15
	Dichlorvos	2	166	25	216 - 115	15
	Naled	2	ND			15
	Fensulfothion	2	72	8.6	90 - 55	15
	Parathion methyl	3	84	9	102 - 66	15
	Phorate	2	58	6	70 - 46	15
	Disulfoton	2	56	5	66 - 47	15
	Merphos	2	78	4	86 - 70	12

ND = Not detected

Data from Reference 3.

TABLE 11

SINGLE-OPERATOR ACCURACY AND PRECISION FOR TRIS-BP SPIKED INTO
MUNICIPAL WASTE WATER, DRINKING WATER, CHEMICAL SLUDGE

Matrix	Spike Conc.	Mean Rec. (%)	Std. Dev.	Recovery Range (%)	# Analyses
Municipal wastewater	2	25	8.0	41 - 9.0	15
Drinking water	2	40	5.0	50 - 30	12
Chemical sludge	100	63	11	84 - 42	8

Data from Reference 4.

TABLE 12

SINGLE-OPERATOR ACCURACY AND PRECISION FOR CHLORINATED PHENOXYACID
HERBICIDES SPIKED INTO DRINKING WATER, SAND, AND INCINERATOR ASH

Compound	Spike Conc.	Mean Recovery (%)	Std. Dev.	Recovery Range (%)	# Analyses
<i>Low concentration drinking water</i> (µg/L)					
Dicamba	5	63	22	86 - 33	9
2,4-D	5	26	13	37 - 0	9
MCPA	5	60	23	92 - 37	9
MCPP	5	78	21	116 - 54	9
Dichlorprop	5	43	18	61 - 0	9
2,4,5-T	5	72	31	138 - 43	9
Silvex	5	62	14	88 - 46	9
2,4-DB	5	29	24	62 - 0	9
Dinoseb	5	73	11	85 - 49	9
Dalapon	5	ND	ND	ND	9
2,4-D, ester	5	73	17	104 - 48	9
<i>High concentration drinking water</i> (µg/L)					
Dicamba	50	54	30	103 - 26	9
2,4-D	50	60	35	119 - 35	9
MCPA	50	67	41	128 - 32	9
MCPP	50	66	33	122 - 35	9
Dichlorprop	50	66	33	116 - 27	9
2,4,5-T	50	61	23	99 - 44	9
Silvex	50	74	35	132 - 45	9
2,4-DB	50	83	25	120 - 52	9
Dinoseb	50	91	10	102 - 76	9
Dalapon	50	43	9.6	56 - 31	9
2,4-D, ester	50	97	19	130 - 76	9

TABLE 12
(continued)

Compound	Spike Conc.	Mean Recovery (%)	Std. Dev.	Recovery Range (%)	# Analyses
<i>Low concentration sand</i>					
	(mg/kg)				
Dicamba	0.1	117	26	147 - 82	10
2,4-D	0.1	147	23	180 - 118	10
MCPA	0.1	167	79	280 - 78	10
MCPP	0.1	142	39	192 - 81	10
Dichlorprop	0.1	ND	ND	ND	10
2,4,5-T	0.1	134	27	171 - 99	10
Silvex	0.1	121	23	154 - 85	10
2,4-DB	0.1	199	86	245 - 0	10
Dinoseb	0.1	76	74	210 - 6	10
Dalapon	0.1	ND	ND	ND	10
2,4-D, ester	0.1	180	58	239 - 59	7
<i>High concentration sand</i>					
	(mg/kg)				
Dicamba	1	153	33	209 - 119	9
2,4-D	1	218	27	276 - 187	9
MCPA	1	143	30	205 - 111	9
MCPP	1	158	34	226 - 115	9
Dichlorprop	1	92	37	161 - 51	9
2,4,5-T	1	160	29	204 - 131	9
Silvex	1	176	34	225 - 141	9
2,4-DB	1	145	22	192 - 110	9
Dinoseb	1	114	28	140 - 65	9
Dalapon	1	287	86	418 - 166	9
2,4-D, ester	1	20	3.6	25 - 17	7

TABLE 12
(continued)

Compound	Spike Conc.	Mean Recovery (%)	Std. Dev.	Recovery Range (%)	# Analyses
<i>Low concentration municipal ash (mg/kg)</i>					
Dicamba	0.1	83	22	104 - 48	9
2,4-D	0.1	ND	ND	ND	9
MCPA	0.1	ND	ND	ND	9
MCPP	0.1	ND	ND	ND	9
Dichlorprop	0.1	ND	ND	ND	9
2,4,5-T	0.1	27	25	60 - 0	9
Silvex	0.1	68	38	128 - 22	9
2,4-DB	0.1	ND	ND	ND	9
Dinoseb	0.1	44	13	65 - 26	9
Dalapon	0.1	ND	ND	ND	9
2,4-D, ester	0.1	29	23	53 - 0	6
<i>High concentration municipal ash (mg/kg)</i>					
Dicamba	1	66	21	96 - 41	9
2,4-D	1	8.7	4.8	21 - 5	9
MCPA	1	3.2	4.8	10 - 0	9
MCPP	1	10	4.3	16 - 4.7	9
Dichlorprop	1	ND	ND	ND	9
2,4,5-T	1	2.9	1.2	3.6 - 0	9
Silvex	1	6.0	3.1	12 - 2.8	9
2,4-DB	1	ND	ND	ND	9
Dinoseb	1	16	6.8	23 - 0	9
Dalapon	1	ND	ND	ND	9
2,4-D, ester	1	1.9	1.7	6.7 - 0	6

Data are from Reference 5.

All recoveries are in negative ionization mode, except for 2,4-D, ester.

ND = Not detected.

TABLE 13

MULTI-LABORATORY ACCURACY AND PRECISION DATA FOR
CHLORINATED PHENOXYACID HERBICIDES SPIKED INTO WATER

Compound	Spike Concentration	Mean Recovery (%) ^a	RSD ^b	
2,4,5-T	500 mg/L	90	23	
2,4,5-T, butoxy ester		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	
2,4,5-T		50 mg/L	62	68
2,4,5-T, butoxy ester			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	

TABLE 13
(continued)

Compound	Spike Concentration	Mean Recovery (%) ^a	RSD ^b
2,4,5-T	5 mg/L	90	28
2,4,5-T, butoxy ester		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 the data from three laboratories. Each laboratory performed six analyses at each spike concentration.

^b Relative standard deviation of the data from three laboratories.

Data from Reference 6.

TABLE 14

SINGLE-LABORATORY RECOVERY AND PRECISION DATA FOR
CARBAMATES SPIKED INTO WATER

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
Chloroprotham	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 standard response factor calculations.

^b Nine spikes were performed at three concentrations. The concentrations for Aldicarb sulfoxide, Barban, Chloroprotham, 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.
Data from Reference 8.

TABLE 15

SINGLE-LABORATORY RECOVERY AND PRECISION DATA FOR
CARBAMATES SPIKED INTO SOIL

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
Chloroprotham	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, Chloroprotham, 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.
Data from Reference 8.

TABLE 16

MULTI-LABORATORY EVALUATION OF METHOD ACCURACY FOR CARBAMATES
(AFTER OUTLIER REMOVAL)

Analyte	Percent Recovery		
	High-Concentration ^a	Medium-Concentration ^b	Low-Concentration ^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 9). The true concentration was 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 9). The true concentration was 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 9). The true concentration was 5 mg/L per compound, except Carbendazim at 1.25 mg/L.

Data from Reference 9.

TABLE 17

MULTI-LABORATORY EVALUATION OF METHOD PRECISION FOR CARBAMATES (AFTER OUTLIER REMOVAL)

Analyte	High Concentration					Medium Concentration					Low Concentration				
	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.
Data from Reference 9.

TABLE 18

SINGLE-LABORATORY RECOVERY DATA FOR SOLID-PHASE EXTRACTION
OF THE TOXICITY CHARACTERISTIC CHLORINATED HERBICIDES
FROM SPIKED TCLP BUFFERS

Compound	Spike Conc. (µg/L)	Buffer 1		Buffer 2	
		Recovery (%)	RSD	Recovery (%)	RSD
2,4-D	5,000	91	2	79	6
2,4,5-TP	500	93	9	92*	2*
2,4-D	20,000	100	3	99*	1*
2,4,5-TP	2000	103*	2*	78	7

Except where noted with an asterisk, all results are from seven replicates. Those marked with an asterisk are from three replicates.

Data are from Reference 10.

TABLE 19

SINGLE-LABORATORY ACCURACY AND PRECISION FOR CARBAMATES EXTRACTED
FROM AQUEOUS SAMPLES USING CONTINUOUS LIQUID-LIQUID EXTRACTION
(METHOD 3520)

Compound	Spike Conc. (µg/L)	Thermospray/MS		UV at 280 nm		UV at 254 nm	
		Mean Recovery	RSD	Mean Recovery	RSD	Mean Recovery	RSD
Aldicarb sulfone	45	38.5	17.6	--	--	--	--
Barban	45	78.7	10.7	*94.1	12.6	****91.1	7.3
Bendiocarb	45	70.5	7.4	**85.5	14.6	--	--
Benomyl	45	*44.9	9.6	***60.6	11.2	*61.5	5.8
Butylate	34	51.5	34.0	--	--	--	--
Carbaryl	5	78.5	6.0	****86.2	16.7	**85.5	14.5
Carbendazim	45	*44.9	9.6	***60.6	11.2	*61.5	5.8
Carbofuran	5	54.8	5.4	**85.5	14.6	--	--
Carbofuran phenol	45	--	--	86.2	16.7	85.5	14.5
Carbosulfan	22	65.7	11.1	75.8	14.9	190	4.2
<i>m</i> -Cumenyl-methyl	45	76.5	10.9	--	--	18.2	117
EPTC	34	59.3	41.3	--	--	--	--
Formetanate hydrochloride	45	22.2	17.9	9.3	13.0	49.1	9.2
Methiocarb	45	74.1	4.8	94.7	12.9	91.1	7.3
Methomyl	22	57.6	8.9	--	--	--	--
Metolcarb	45	64.9	10.1	--	--	--	--
Mexacarbate	45	70.4	8.5	94.1	12.6	91.1	7.3
Molinate	34	66.7	18.4	--	--	--	--
Oxamyl	45	48.3	20.2	--	--	49.1	9.2
Pebulate	34	**59.4	30.6	--	--	--	--
<i>o</i> -Phenylene diamine	45	--	--	10.6	59.9	24.5	21.6
Physostigimine	45	***47.4	11.6	--	--	71.0	9.8
Physostigimine Salicylate	45	***47.4	11.6	9.3	13.0	--	--

TABLE 19
(continued)

Compound	Spike Conc. (µg/L)	Thermospray/MS		UV at 280 nm		UV at 254 nm	
		Mean Recovery	RSD	Mean Recovery	RSD	Mean Recovery	RSD
Promecarb	45	68.1	11.4	--	--	****91.1	7.3
Propham	45	65.6	14.2	90.1	13.3	79.9	12.0
Propoxur	45	55.6	5.1	85.5	14.6	--	--
Prosulfocarb	34	63.6	10.2	--	--	132	11.3
Thiodicarb	15	72.2	30.3	--	--	61.9	27.6
Thiophanate-methyl	45	71.8	13.0	78.2	17.9	78.4	10.4
Triallate	34	71.0	8.5	--	--	86.1	13.3
Vernolate	34	59.4	30.6	--	--	--	--

The asterisks indicate the compounds that coelute from the HPLC column. Those marked with one asterisk (*) in the same column represent one group of compounds that elute together, those with two asterisks (**) are a second group, etc. For coeluting compounds, the same recovery and RSD values are reported for both compounds, since separate results cannot be determined.

Those compounds with a double hyphen (--) did not respond to the detector listed.

All data are taken from Reference 11.

TABLE 20

SINGLE-LABORATORY ACCURACY AND PRECISION FOR CARBAMATES EXTRACTED FROM SOIL SAMPLES USING SOXHLET EXTRACTION (METHOD 3540)

Compound	Spike Conc. (µg/L)	Thermospray/MS		UV at 280 nm		UV at 254 nm	
		Mean Recovery	RSD	Mean Recovery	RSD	Mean Recovery	RSD
Aldicarb sulfone	2200	126	10.0	--	--	--	--
Barban	1100	100	8.8	*98.3	12.9	****110	6.9
Bendiocarb	1100	99.1	11.0	**89.4	5.5	--	--
Benomyl	1100	*109	7.2	***115	4.7	*115	3.0
Butylate	1100	77.5	12.5				
Carbaryl	110	111	12.0	****92.7	10.8	**93.4	4.1
Carbendazim	1100	*109	7.2	***115	4.7	*115	3.0
Carbofuran	110	89.3	5.8	**89.4	5.5	--	--
Carbofuran phenol	1100	--	--	****92.7	10.8	**93.4	4.1
Carbosulfan	1100	104	15.0	115	10.8	132	118
<i>m</i> -Cumenyl-methyl	1100	102	9.0	--	--	92.1	44
EPTC	1100	84.7	10.6	--	--	--	--
Formetanate hydrochloride	1100	71.8	17.3	*****11.5	52.8	***45.9	8.7
Methiocarb	1100	101	6.4	107	28.7	****110	6.9
Methomyl	110	111	8.1	--	--	--	--
Metolcarb	1100	96.5	12.1	--	--	--	--
Mexacarbate	1100	102	8.0	*98.3	12.9	****110	6.9
Molinate	1100	85.5	2.3	--	--	--	--
Oxamyl	2200	140	7.0	--	--	***45.9	8.7
Pebulate	1100	**86.6	13.7	--	--	--	--
<i>o</i> -Phenylene diamine	4500	--	--	40.4	82.4	--	--
Physostigimine	1100	***4.5	13.1	--	--	5.3	122.9
Physostigimine Salicylate	1100	***4.5	13.1	*****11.5	52.8	--	--
Promecarb	1100	92.4	4.0	--	--	****110	6.9
Propham	1100	98.6	12.3	156	25.4	127	11.3

Table 20
(continued)

Compound	Spike Conc. (µg/L)	Thermospray/MS		UV at 280 nm		UV at 254 nm	
		Mean Recovery	RSD	Mean Recovery	RSD	Mean Recovery	RSD
Propoxur	1100	98.6	16.2	**89.4	5.5	--	--
Prosulfocarb	1100	112	17.0	--	--	130	26.2
Thiodicarb	1100	118	26.2	--	--	98.9	4.6
Thiophanate-methyl	1100	43.0	10.9	45.1	9.7	59.6	11.2
Triallate	1100	90.1	7.3	--	--	106	18.3
Vernolate	1100	**86.6	13.7	--	--	--	--

The asterisks indicate the compounds that coelute from the HPLC column. Those marked with one asterisk (*) in the same column represent one group of compounds that elute together, those with two asterisks (**) are a second group, etc. For coeluting compounds, the same recovery and RSD values are reported for both compounds, since separate results cannot be determined.

Those compounds with a double hyphen (--) did not respond to the detector listed.

All data are taken from Reference 11.

FIGURE 1
SCHEMATIC OF THE THERMOSPRAY PROBE AND ION SOURCE

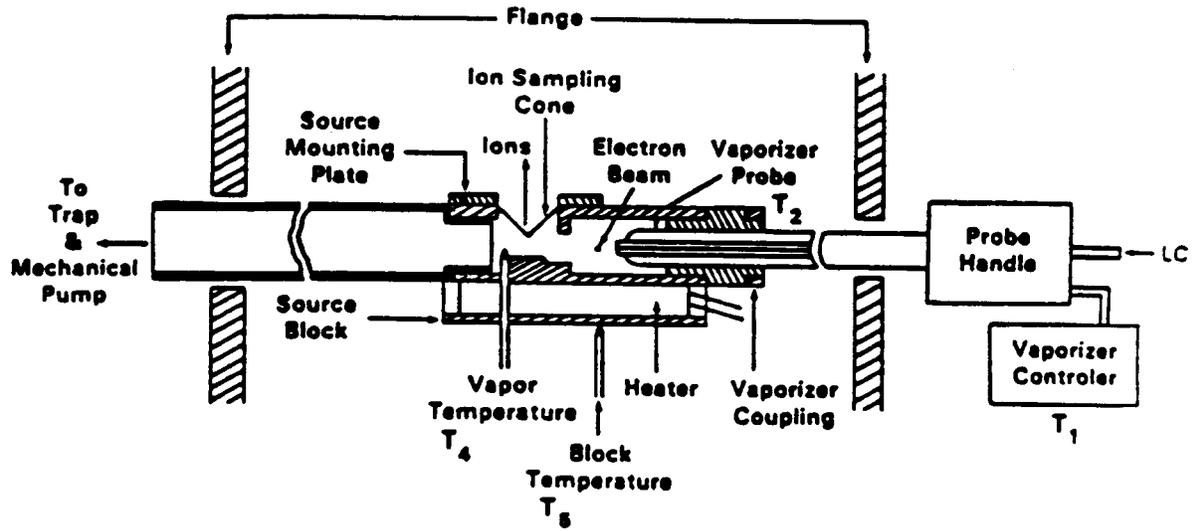


FIGURE 2
THERMOSPRAY SOURCE WITH WIRE-REPELLER
(High sensitivity configuration)

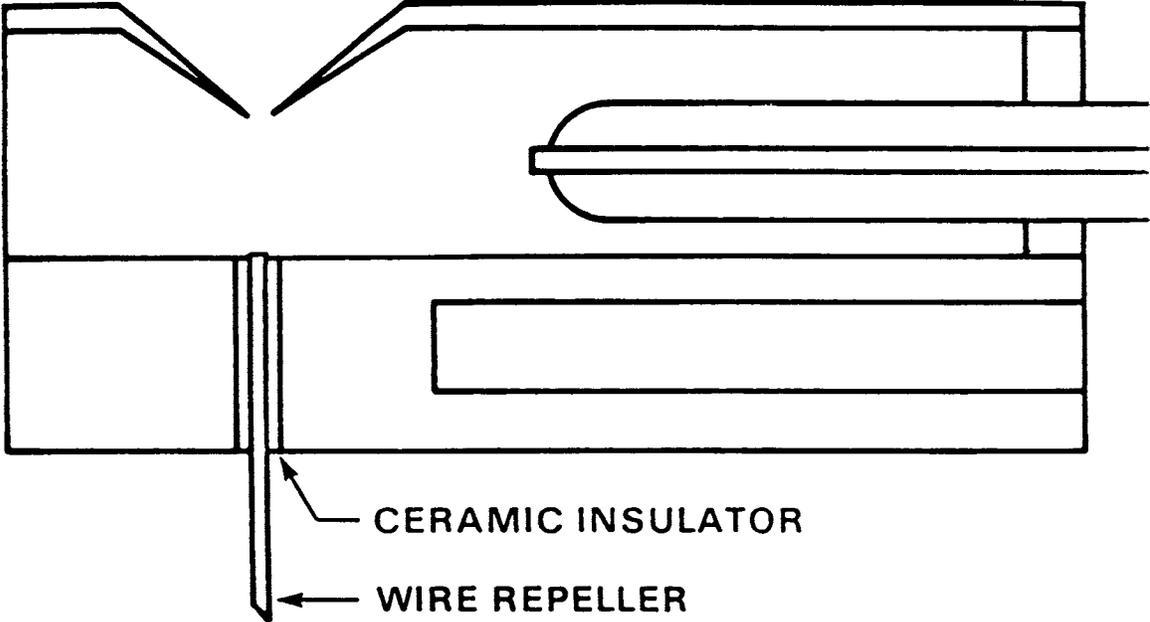
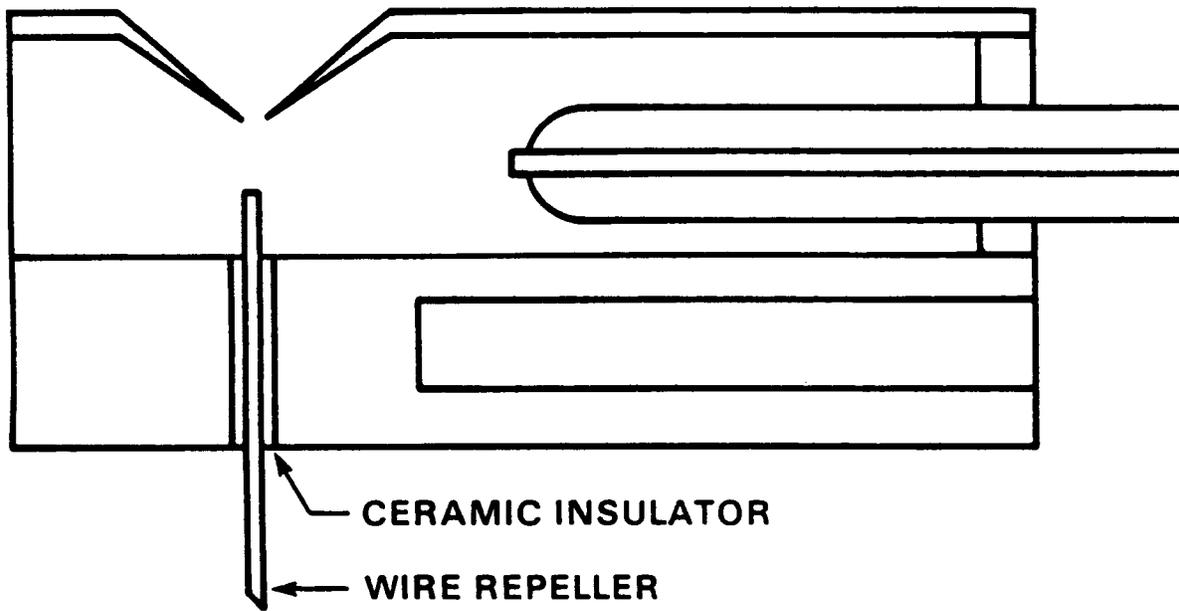


FIGURE 3
THERMOSPRAY SOURCE WITH WIRE-REPELLER
(CAD configuration)



METHOD 8321B
SOLVENT-EXTRACTABLE NONVOLATILE COMPOUNDS BY
HIGH PERFORMANCE LIQUID CHROMATOGRAPHY/THERMOSPRAY/MASS
SPECTROMETRY (HPLC/TS/MS) OR ULTRAVIOLET (UV) DETECTION

