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

SW-846 is not intended to be an analytical training manual. Therefore, method procedures are written based on the assumption that they will be performed by analysts who are formally trained in at least the basic principles of chemical analysis and in the use of the subject technology.

In addition, SW-846 methods, with the exception of required method use for the analysis of method-defined parameters, are intended to be guidance methods which contain general information on how to perform an analytical procedure or technique which a laboratory can use as a basic starting point for generating its own detailed Standard Operating Procedure (SOP), either for its own general use or for a specific project application. The performance data included in this method are for guidance purposes only, and are not intended to be and must not be used as absolute QC acceptance criteria for purposes of laboratory accreditation.

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 have been determined by this method, although not all of the compounds are amenable to UV detection:

Analyte	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

Analyte	CAS No.[a]
Organophosphorus Compounds	
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
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
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
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

Analyte	CAS No.[a]
Bromacil	314-40-9
Butylate	2008-41-5
Carbaryl*	63-25-2
Carbendazim*	10605-21-7
Carbofuran*	1563-66-2
Carbosulfan	55285-14-8
Chloropropham	101-21-3
Chloroxuron	1982-47-4
m-Cumenyl methyl carbamate	64-00-6
Diuron*	330-54-1
EPTC	759-94-4
Fenuron	101-42-8
Fluometuron	2164-17-2
Formetanate hydrochloride	23422-53-9
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
o-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

[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 This method 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 chlorophenoxyacid 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, should 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
o-Phenylenediamine	Y	Y
m-Cumenyl methyl carbamate	Y	
Oxamyl	Y	
Physostigmine	Y	
Promecarb	Y	
Prosulfocarb	Y	
Thiodicarb	Y	
Triallate	Y	

Analyte	UV/Vis at 254 nm	UV/Vis at 280 nm
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 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 personnel appropriately experienced and trained 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 on the liquid chromatograph 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 may cause problems with 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 prepared by a modification of Method 8151 (see Sec. 11.3 of this method) or other appropriate extraction technique. In general, the pH of an aqueous or a solid sample is adjusted to less than 2, and the sample is extracted with diethyl ether and 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 equipped with an 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 need concentration and solvent exchange prior to analysis.

2.2.4 A micro-extraction technique for the extraction of tris (2,3-dibromopropyl) phosphate (abbreviated in this method as tris-BP) from aqueous and non-aqueous matrices is included in this method (see Sec. 11.2). See the safety warning in Sec. 5.2 regarding this compound.

2.3 An optional mass spectrometry/mass spectrometry (MS/MS) confirmation procedure is provided in this method (see Sec. 11.11). That procedure employs MS/MS collision activated dissociation (CAD) or wire-repeller CAD.

3.0 DEFINITIONS

Refer to Chapter One and the manufacturer's instructions for definitions that may be relevant to this procedure.

4.0 INTERFERENCES

4.1 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 analyzing method blanks. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be necessary. Refer to each method to be used for specific guidance on quality control procedures and to Chapter Four for general guidance on the cleaning of glassware.

4.2 Also refer to Methods 3500, 3600, 8000 and 8151 for discussions of interferences.

4.3 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 Method 3620 for recoveries of organophosphorus compounds as a function of Florisil® fractions.

4.4 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. 11.11.1), an HPLC must be used to perform the chromatographic separations necessary for quantitative analyses.

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

4.5.1 Methyl parathion shows some minor degradation during analysis.

4.5.2 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.

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

4.5.4 The water solubility of DDVP is 10 g/L at 20 EC, and as a result, recovery of this compound by solvent extraction from aqueous solutions is poor.

4.5.5 Trichloron can rearrange and undergo dehydrochlorination (loss of HCl) in all types of media, forming 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.

4.6 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.

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

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

4.9 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.

4.10 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.

4.11 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 Reference 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.

5.0 SAFETY

5.1 This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of the chemicals listed in this method. A reference file of material safety data sheets (MSDSs) should be available to all personnel involved in these analyses.

5.2 Tris-BP has been classified as a carcinogen. Purified standard material and stock standard solutions should be handled in a hood. Also, see Sec 11.2 for a description of micro-extraction procedures that may be applied to samples containing tris-BP. These procedures have been provided because 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.

6.0 EQUIPMENT AND SUPPLIES

The mention of trade names or commercial products in this manual is for illustrative purposes only, and does not constitute an EPA endorsement or exclusive recommendation for use. The products and instrument settings cited in SW-846 methods represent those products and settings used during method development or subsequently evaluated by the Agency. Glassware, reagents, supplies, equipment, and settings other than those listed in this manual may be employed provided that method performance appropriate for the intended application has been demonstrated and documented.

This section does not list common laboratory glassware (e.g., beakers and flasks).

6.1 HPLC/MS

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

6.1.1 High-performance liquid chromatograph (HPLC) -- An analytical system equipped with programmable solvent delivery system and all necessary accessories, including injection loop (with a minimum 10-µL 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).

6.1.2 HPLC post-column addition pump -- If post-column addition of reagents is employed, a pump is needed. 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. 11.6.1).

6.1.3 HPLC/MS interface

6.1.3.1 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. (See Figure 1.)

6.1.3.2 Micromixer -- 10-µL, connects HPLC column system with HPLC post-column addition solvent system, if post-column addition is used.

6.1.4 Mass spectrometer system

6.1.4.1 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. 7.15) or other compounds used for mass calibration.

6.1.4.2 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.

6.1.5 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.

6.2 HPLC equipped with UV detector

An analytical system equipped with a solvent-programmable pumping system for at least a binary solvent system, and all necessary 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 listed in Sec. 6.3 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.

6.3 HPLC columns

A guard column and an analytical column are necessary.

The columns listed in this section were those used in developing the method. The listing of these columns in this method is not intended to exclude the use of other columns that are available or that may be developed. Laboratories may use these columns or other columns, of other dimensions and/or packed with different stationary phases, provided that the laboratories document method performance data (e.g., chromatographic resolution, analyte breakdown, and sensitivity) that provide analytical performance that is appropriate for the intended application.

6.3.1 Guard column -- C_{18} reversed-phase guard column, 10 mm x 2.6 mm ID, 0.5-µm frit, or equivalent. The guard column should utilize the same or a similar stationary phase as the analytical column.

6.3.2 Analytical column -- C_{18} reversed-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

6.4 Purification equipment for azo dye standards

6.4.1 Soxhlet extraction apparatus

6.4.2 Extraction thimbles -- single thickness, 43 x 123 mm

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

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

6.5 Extraction equipment for chlorinated phenoxyacid compounds

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

6.5.2 Separatory funnel -- 2000-mL

6.5.3 Graduated cylinder -- 1000-mL

6.5.4 Funnel -- 75-mm diameter

6.5.5 Wrist shaker -- Burrell Model 75 or equivalent

6.5.6 pH meter

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

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

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

6.6.3 Two-ball micro-Snyder column

6.6.4 Springs -- ½-in.

6.6.5 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 Federal, 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.

6.7 Disposable serological pipets

6.8 Collection tube -- 15-mL conical, graduated

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

6.10 Silanized glass wool

6.11 Microsyringes -- 100-µL, 50-µL, 10-µL (Hamilton 701 N or equivalent), and 50-µL blunted (Hamilton 705SNR or equivalent)

6.12 Rotary evaporator -- Equipped with 1000-mL receiving flask

6.13 Balances -- Analytical capable of weighing to 0.0001 g, and top-loading capable of weighing to 0.01 g

6.14 Volumetric flasks, Class A -- 10-mL to 1000-mL

6.15 Graduated cylinder -- 100-mL

6.16 Separatory funnel -- 250-mL

6.17 Separatory funnel -- 2-L, fitted with PTFE stopcock

6.18 Concentrator adaptor (optional) -- for carbamate extraction

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

7.0 REAGENTS AND STANDARDS

7.1 Reagent-grade chemicals must be used in all tests. Unless otherwise indicated, it is intended that all reagents 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. Reagents should be stored in glass to prevent the leaching of contaminants from plastic containers.

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

7.3 Sodium sulfate (granular, anhydrous), Na_2SO_4. Purify by heating at 400 EC for 4 hr in a shallow tray, or by precleaning with methylene chloride.

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

7.5 Acetic acid, CH_3CO_2H (1% v/v and 0.1N)

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

7.7 Argon gas, 99+% pure

7.8 Extraction solvents

Samples should be extracted using a solvent system that gives optimum, reproducible recovery of the analytes of interest from the sample matrix, at the concentrations of interest. The choice of extraction solvent will depend on the analytes of interest and no single solvent is universally applicable to all analyte groups. Whatever solvent system 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 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.

Unless otherwise noted, all solvents must be HPLC-grade or equivalent. Solvents may be degassed prior to use.

7.8.1 Methylene chloride, CH_2Cl_2

7.8.2 Toluene, $C_6H_5CH_3$

7.8.3 Acetone, CH_3COCH_3

7.8.4 Diethyl ether, $C_2H_5OC_2H_5$ -- 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.

7.8.5 Methanol, CH_3OH - HPLC-grade or equivalent

7.8.6 Acetonitrile, CH_3CN - HPLC-grade or equivalent

7.8.7 Ethyl acetate, $CH_3CO_2C_2H_5$

7.9 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. 7.10.

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

7.10 Disperse azo dye purification

Two procedures are involved. The first step is the Soxhlet extraction of the dye for 24 hr 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 EC. If this step does not give the necessary purity, column chromatography should be employed. Load the solid onto a silica gel column (Sec. 6.4.4), and elute with diethyl ether. Separate the impurities using chromatography, and collect the major dye fraction.

7.11 Standard solutions

The following sections describe the preparation of stock, intermediate, and working standards for the compounds of interest. This discussion is provided as an example, and other approaches and concentrations of the target compounds may be used, as appropriate for the intended application. See Method 8000 for additional information on the preparation of calibration standards.

7.12 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.

7.12.1 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.

7.12.2 Transfer the stock standard solutions into glass vials fitted with PTFE-lined screw-caps or crimp-tops. Store at #6 EC and protect from light. Stock standard

solutions should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards.

7.13 Calibration standards

A minimum of five different concentrations for each parameter of interest should be prepared through dilution of the stock standard solutions 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 performance 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 should be replaced after one or two months, or sooner if comparison with check standards indicates a problem. See Method 8000 for additional information regarding the preparation of calibration standards.

7.14 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).

7.15 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.

7.16 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., $^{13}C_6$-carbofuran may be used as an internal standard in the analysis of carbamates).

7.17 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, because 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.

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

See the introductory material to Chapter Four, "Organic Analytes."

9.0 QUALITY CONTROL

9.1 Refer to Chapter One for guidance on quality assurance (QA) and quality control (QC) protocols. When inconsistencies exist between QC guidelines, method-specific QC criteria take precedence over both technique-specific criteria and those criteria given in Chapter One, and technique-specific QC criteria take precedence over the criteria in Chapter One. Any effort involving the collection of analytical data should include development of a structured and systematic planning document, such as a Quality Assurance Project Plan (QAPP) or a Sampling and Analysis Plan (SAP), which translates project objectives and specifications into directions for those that will implement the project and assess the results. Each laboratory should maintain a formal quality assurance program. The laboratory should also maintain records to document the quality of the data generated. All data sheets and quality control data should be maintained for reference or inspection.

9.2 Refer to Method 8000 for specific determinative method QC procedures. Refer to Method 3500 for QC procedures to ensure the proper operation of the various sample preparation techniques. If an extract cleanup procedure is performed, refer to Method 3600 for the appropriate QC procedures. Any more specific QC procedures provided in this method will supersede those noted in Methods 8000, 3500, or 3600.

9.3 The 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. 11.8.3.

9.4 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. If an autosampler is used to perform sample dilutions, before using the autosampler to dilute samples, the laboratory should satisfy itself that those dilutions are of equivalent or better accuracy than is achieved by an experienced analyst performing manual dilutions. The laboratory must also repeat the demonstration of proficiency whenever new staff members are trained or significant changes in instrumentation are made. See Method 8000 for information on how to accomplish a demonstration of proficiency.

9.5 Initially, before processing any samples, the analyst should demonstrate that all parts of the equipment in contact with the sample and reagents are interference-free. This is accomplished through the analysis of a method blank. As a continuing check, each time samples are extracted, cleaned up, and analyzed, and when there is a change in reagents, a method blank should be prepared and analyzed for the compounds of interest as a safeguard against chronic laboratory contamination. The blanks should be carried through all stages of sample preparation and analysis. If a peak is observed within the retention time window of any analyte that would prevent the determination of that analyte, determine the source and eliminate it, if possible, before processing samples. The blanks should be carried through all stages of sample preparation and analysis. When new reagents or chemicals are received, the laboratory should monitor the preparation and/or analysis blanks associated with samples for any signs of contamination. It is not necessary to test every new batch of reagents or chemicals prior to sample preparation if the source shows no prior problems. However, if reagents are changed during a preparation batch, separate blanks need to be prepared for each set of reagents.

9.6 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, method sensitivity). At a minimum, this should include the analysis of QC samples including a method blank, a matrix spike, a duplicate, and a laboratory control sample (LCS) in each analytical batch, the addition of surrogates to each field sample and QC sample when surrogates are used. Any method blanks, matrix spike samples, and replicate samples should be subjected to the same analytical procedures (Sec. 11.0) as those used on actual samples.

9.6.1 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 a matrix spike and a duplicate analysis of an unspiked field sample. If samples are not expected to contain target analytes, the laboratories should use a matrix spike and matrix spike duplicate pair. Consult Method 8000 for information on developing acceptance criteria for the MS/MSD.

9.6.2 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 appropriate. 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. Consult Method 8000 for information on developing acceptance criteria for the LCS.

9.6.3 Also see Method 8000 for the details on carrying out sample quality control procedures for preparation and analysis. In-house method performance criteria for evaluating method performance should be developed using the guidance found in Method 8000.

9.7 Surrogate recoveries

If surrogates are used, the laboratory should evaluate surrogate recovery data from individual samples versus the surrogate control limits developed by the laboratory. See Method 8000 for information on evaluating surrogate data and developing and updating surrogate limits. Procedures for evaluating the recoveries of multiple surrogates and the associated corrective actions should be defined in an approved project plan.

9.8 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.

10.0 CALIBRATION AND STANDARDIZATION

See Sec 11.8 for information on calibration and standardization.

11.0 PROCEDURE

11.1 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 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.

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

11.1.2 Samples for the analysis of tris-BP must be prepared according to Sec. 11.2, prior to HPLC/MS analysis, unless the analyst can demonstrate appropriate performance for the intended application using other techniques.

11.1.3 Samples for the analysis of chlorinated phenoxyacid compounds and their esters should be prepared according to Sec. 11.3, 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.

11.2 Microextraction 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 reduce the exposure of the analyst to samples or standards containing this compound over the extraction of larger sample weights or volumes.

11.2.1 Solid samples

11.2.1.1 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 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.

11.2.1.2 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.

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

11.2.1.4 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.

11.2.1.5 Concentrate the extract to 1 mL using the nitrogen evaporation technique (see Sec. 11.5). Record the volume. (It may not be possible to evaporate some sludge samples to a 1-mL final volume.)

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

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

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

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

NOTE: This dry weight determination also applies to the use of other extraction techniques for solids (e.g., a 3500 series method), when results are to be calculated on a dry weight basis.

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

11.2.2 Aqueous samples

11.2.2.1 Using a 100-mL graduated cylinder, measure 100 mL of sample and transfer it to a 250-mL separatory funnel. Add 200 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.

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

WARNING: 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.

11.2.2.3 Allow the organic layer to separate from the water phase for a minimum of 10 min. 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 Method 3510.

11.2.2.4 Collect the extract in a 15-mL graduated glass tube. Concentrate the extract to 1 mL, using nitrogen evaporation (see Sec. 11.5).

11.3 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 necessary. However, it is recommended that to determine all of the phenoxyacid moieties as the acid, hydrolysis to the acid form should be performed in order to simplify quantitation. Sec. 11.3.1 presents an outline of the procedure with the appropriate changes necessary for determination by this method. Sec. 11.3.2 describes the extraction procedure for aqueous samples. TCLP leachates may be extracted using solid-phase extraction, as described in Method 3535.

11.3.1 Extraction of solid samples

11.3.1.1 Add 50 g of soil/sediment sample to a 500-mL, wide-mouth Erlenmeyer flask. Add spiking solutions, if needed. Mix well and allow to stand for 15 min. Add 50 mL of organic-free reagent water and stir for 30 min. 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 min, 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.

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

11.3.1.3 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 min and the acetone-ether extract decanted.

11.3.1.4 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.

11.3.1.5 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 min and allow the layers to separate. Collect the aqueous phase in a clean beaker, and collect the extract phase (top layer) in a 500-mL Erlenmeyer flask equipped 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.

11.3.1.6 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 hr.

CAUTION: 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-hr drying time is a minimum; and the extracts may be held overnight in contact with the sodium sulfate.

11.3.1.7 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. 11.5).

11.3.2 Extraction of aqueous samples

11.3.2.1 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.

11.3.2.2 Add 150 mL of diethyl ether to the sample bottle, seal, and shake for 30 sec to rinse the walls. Transfer the solvent wash to the separatory funnel and extract the sample by shaking the funnel for 2 min with periodic venting to release excess pressure. Allow the organic layer to separate from the water layer for a minimum of 10 min. 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.

11.3.2.3 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.)

11.3.2.4 Proceed to Sec. 11.5 for drying, K-D concentration, solvent exchange, and final volume adjustment.

11.4 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.

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

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

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

11.4.4 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.

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

11.5 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.

11.5.1 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. 6.6.5) 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 EC - 65 EC) 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 necessary, to complete the concentration in 15 - 20 min. 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 min.

11.5.2 Solvent exchange

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

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

11.5.2.2 Reduce the extract volume according to Sec. 11.5.3 and adjust the final volume to 1 mL (or other volume necessary to achieve the required sensitivity).

11.5.3 Micro-concentration by nitrogen evaporation

11.5.3.1 Place the concentrator tube in a warm water bath (approximately 35 EC) and evaporate the solvent volume to the designated 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.

11.5.3.2 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 should 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.

11.5.4 Transfer the extract to a glass vial fitted with a PTFE-lined screw-cap or crimp-top and store refrigerated at #6 EC. Proceed with HPLC analysis.

11.6 HPLC chromatographic conditions

11.6.1 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.

11.6.2 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.

11.6.3 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.

11.7 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 analyses. This evaluation may be based on the structures of the analytes or on analyses conducted. 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.

11.7.1 Positive ionization mode conditions

Discharge electrode	Off
Filament	On or off (optional, analyte-dependent)
Mass range	150 - 450 amu (analyte-dependent, expect 1 - 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 equipped with wire repeller.

11.7.2 Negative ionization mode conditions

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

11.7.3 Thermospray temperatures

Vaporizer control	110 - 130 EC
Vaporizer tip	200 - 215 EC
Jet	210 - 220 EC
Source block	230 - 265 EC. (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.)

11.7.4 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 the 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.

11.8 Calibration

11.8.1 Tuning the mass spectrometer system

When an MS detector is employed, the detector must be tuned at the beginning of each 12-hr analytical shift using the procedures outlined in Secs. 11.8.1.1 and 11.8.1.2, to ensure correct mass assignments, sensitivity, and resolution. Tuning may be accomplished using polyethylene glycol (PEG) 400, 600, or 810 (see Sec. 7.15) 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.

11.8.1.1 The mass calibration parameters are as follows:

PEG 400 and 600		PEG 810	
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.

11.8.1.2 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
$NH_4^+ \cdot H_2O$	36 amu
$CH_3OH \cdot NH_4^+$	50 amu (methanol)
$CH_3CN \cdot NH_4^+$	59 amu (acetonitrile)
$CH_3OOH \cdot NH_4^+$	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., $H(OCH_2CH_2)_nOH$ where n=4, gives the $H(OCH_2CH_2)_4OH \cdot NH_4^+$ ion at m/z 212).

11.8.2 Liquid chromatographic system

11.8.2.1 Choose the proper ionization conditions for the MS detector, as outlined in Sec. 11.7. When UV detection is employed in conjunction with the MS detector, establish appropriate operating conditions for the UV detector.

11.8.2.2 Prepare at least five calibration standards (see Sec. 7.11 and Method 8000). Inject each calibration standard onto the HPLC, using the chromatographic conditions outlined in Table 1. Refer to Method 8000 for guidance on external and internal calibration options and calibration acceptance criteria. In most cases the $(M^+H)^+$ and $(M^+NH_4)^+$ adduct ions are the only ions of significant abundance. For example, Table 9 lists example retention times and the major ions (>5%) present in the positive ionization thermospray single quadrupole spectra of the organophosphorus compounds. (The retention times listed in Table 9 are provided for illustrative purposes only. Each laboratory must determine retention times and retention time widows for their specific application of the method.)

11.8.2.3 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.

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

11.8.2.5 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.

11. 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.

11.8.3 Calibration verification

At the beginning of each 12-hr analytical shift, the response of the instrument system must be verified by the analysis of the MS tuning (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. 11.8.1 and Method 8000 for information on performing these demonstrations and the acceptance criteria that should be employed.

11.9 Sample analysis

Once the LC system has been calibrated as outlined in Sec. 11.8, 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.

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

11.9.2 If performing manual injections, take an appropriate aliquot of the sample as per Sec. 11.7.4. Start the HPLC gradient elution, load and inject the sample aliquot, and start the mass spectrometer data system.

11.9.3 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.

11.9.4 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.

11.9.5 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.

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

11.10 Calculations

11.10.1 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.

11.10.2 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.

11.10.3 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.

11.11 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.

11.11.1 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).

11.11.2 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.

11.11.3 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.

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

11.11.5 Perform an initial calibration, as outlined in Sec. 11.8.

11.11.6 MS/MS contamination and interferences

11.11.6.1 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 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.

11.11.6.2 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.

11.11.6.3 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-d_5 has been used successfully for positive ion analysis, while 2,6-dinitrotoluene-d_3 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 necessary 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.

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

11.11.8 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.

11.12 Optional wire-repeller CAD confirmation

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

11.12.2 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.

11.12.3 Continue as outlined in Sec. 11.9.

12.0 DATA ANALYSIS AND CALCULATIONS

See Sec. 11.10 and Method 8000 for information on data analysis and calculations.

13.0 METHOD PERFORMANCE

13.1 Performance data and related information are provided in SW-846 methods only as examples and guidance. The data do not represent required performance criteria for users of the methods. Instead, performance criteria should be developed on a project-specific basis, and the laboratory should establish in-house QC performance criteria for the application of this method. <u>These performance data are not intended to be and must not be used as absolute QC acceptance criteria for purposes of laboratory accreditation.</u>

13.2 Single-operator accuracy and precision studies have been conducted using spiked sediment, wastewater, sludge, and water samples. Tables 4, 5, and 6 provide example single-laboratory data for Disperse Red 1. Table 10 provides the data for organophosphorus pesticides, Table 11 provides the data for tris-BP, Table 12 provides the data for chlorophenoxyacid herbicides, and Tables 14 and 15 provide the data for carbamates. These data are provided for guidance purposes only.

13.3 Table 13 presents example 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 listed in the table. These data are provided for guidance purposes only.

13.4 Tables 16 and 17 present example 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 listed in the tables. These data are provided for guidance purposes only.

13.5 Table 18 provides example data for the solid-phase extraction of 2,4-D and 2,4,5-TP spiked into TCLP buffers at two different levels. These data are provided for guidance purposes only.

13.6 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. These data are provided for guidance purposes only. All data are taken from Reference 11.

14.0 POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity and/or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operations. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

14.2 For information about pollution prevention that may be applicable to laboratories and research institutions consult *Less is Better: Laboratory Chemical Management for Waste Reduction* available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th St., N.W. Washington, D.C. 20036, http://www.acs.org.

15.0 WASTE MANAGEMENT

The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management, consult *The Waste Management Manual for Laboratory Personnel* available from the American Chemical Society at the address listed in Sec. 14.2.

16.0 REFERENCES

1. L. D. Betowski, T. L. Jones, "The Analysis of Organophosphorus Pesticide Samples by HPLC/MS and HPLC/MS/MS," *Environmental Science and Technology*, 1988.

2. "Development and Evaluations of an LC/MS/MS Protocol," EPA/600/X-86/328, Dec. 1986.

3. "An LC/MS Performance Evaluation Study of Organophosphorus Pesticides," EPA/600/X-89/006, Jan. 1989.

4. "A Performance Evaluation Study of a Liquid Chromatography/Mass Spectrometry Method for Tris-(2,3-Dibromopropyl) Phosphate," EPA/600/X-89/135, June 1989.

5. "Liquid Chromatography/Mass Spectrometry Performance Evaluation of Chlorinated Phenoxyacid Herbicides and Their Esters," EPA/600/X-89/176, July 1989.

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. L. Somasundaram, and J.R. Coates, Ed., "Pesticide Transformation Products Fate and Significance in the Environment," ACS Symposium Series 459, Ch. 13, 1991.

8. APPL, Inc., Fresno, CA, "Single-Laboratory Evaluation of Carbamates."

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. C. Markell, "3M Data Submission to EPA," letter to B. Lesnik, June 27, 1995.

11. Science Applications International Corporation, "Carbamates Method Evaluation Report," report for EPA Contract 68-W6-0068, August 25, 1998.

17.0 TABLES, DIAGRAMS, FLOW CHARTS, AND VALIDATION DATA

The following pages contain the tables and figures referenced by this method.

TABLE 1

SUGGESTED HPLC CHROMATOGRAPHIC CONDITIONS

Analyte Class	Initial Mobile Phase (%)	Initial Time (min)	Linear 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_3CN)	0	5	100 (% CH_3CN)	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
or	40/60 (0.1 M NH4 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

EXAMPLE 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.

These data are provided for guidance purposes only.

TABLE 5

EXAMPLE 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.
These data are provided for guidance purposes only.

TABLE 6

EXAMPLE 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.
These data are provided for guidance purposes only.

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.

The retention times listed in this table are provided for illustrative purposes only. Each laboratory must determine retention times and retention time widows for their specific application of the method.

TABLE 10

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

Matrix and Compound	Spike Conc.	Mean Rec. (%)	Std. Dev.	Recovery Range (%)	# Analyses
Low concentration drinking water	(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
Low concentration soil	(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 and Compound	Spike Conc.	Mean Rec. (%)	Std. Dev.	Recovery Range (%)	# Analyses
Medium concentration drinking water	(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
Medium concentration sediment	(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.
These data are provided for guidance purposes only.

TABLE 11

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

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

Data from Reference 4.
These data are provided for guidance purposes only.

TABLE 12

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

Matrix and Compound	Spike Conc.	Mean Recovery (%)	Std. Dev.	Recovery Range (%)	# Analyses
Low concentration drinking water	(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
High concentration drinking water	(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)

Matrix and Compound	Spike Conc.	Mean Recovery (%)	Std. Dev.	Recovery Range (%)	# Analyses
Low concentration sand	(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
High concentration sand	(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)

Matrix and Compound	Spike Conc.	Mean Recovery (%)	Std. Dev.	Recovery Range (%)	# Analyses
Low concentration municipal ash	(mg/kg)				
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
High concentration municipal ash	(mg/kg)				
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. These data are provided for guidance purposes only.
All recoveries are in negative ionization mode, except for 2,4-D, ester.
ND = Not detected.

TABLE 13

EXAMPLE 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. These data are provided for guidance purposes only.

TABLE 14

EXAMPLE SINGLE-LABORATORY RECOVERY
AND PRECISION DATA FOR CARBAMATES SPIKED IN 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
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 standard 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.

Data from Reference 8. These data are provided for guidance purposes only.

TABLE 15

EXAMPLE 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
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.

Data from Reference 8. These data are provided for guidance purposes only.

TABLE 16

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

	Percent Recovery		
Analyte	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. These data are provided for guidance purposes only.

TABLE 17

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

	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.
Data from Reference 9. These data are provided for guidance purposes only.

TABLE 18

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

		Buffer 1		Buffer 2	
Compound	Spike Conc. (µg/L)	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. These data are provided for guidance purposes only.

TABLE 19

EXAMPLE 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
m-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	--	--	--	--
o-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	--	--
Promecarb	45	68.1	11.4	--	--	****91.1	7.3
Propham	45	65.6	14.2	90.1	13.3	79.9	12.0

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
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. These data are provided for guidance purposes only.

TABLE 20

EXAMPLE 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
m-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	--	--	--	--
o-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
Propoxur	1100	98.6	16.2	**89.4	5.5	--	--

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
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. These data are provided for guidance purposes only.

FIGURE 1
SCHEMATIC OF THE THERMOSPRAY PROBE AND ION SOURCE

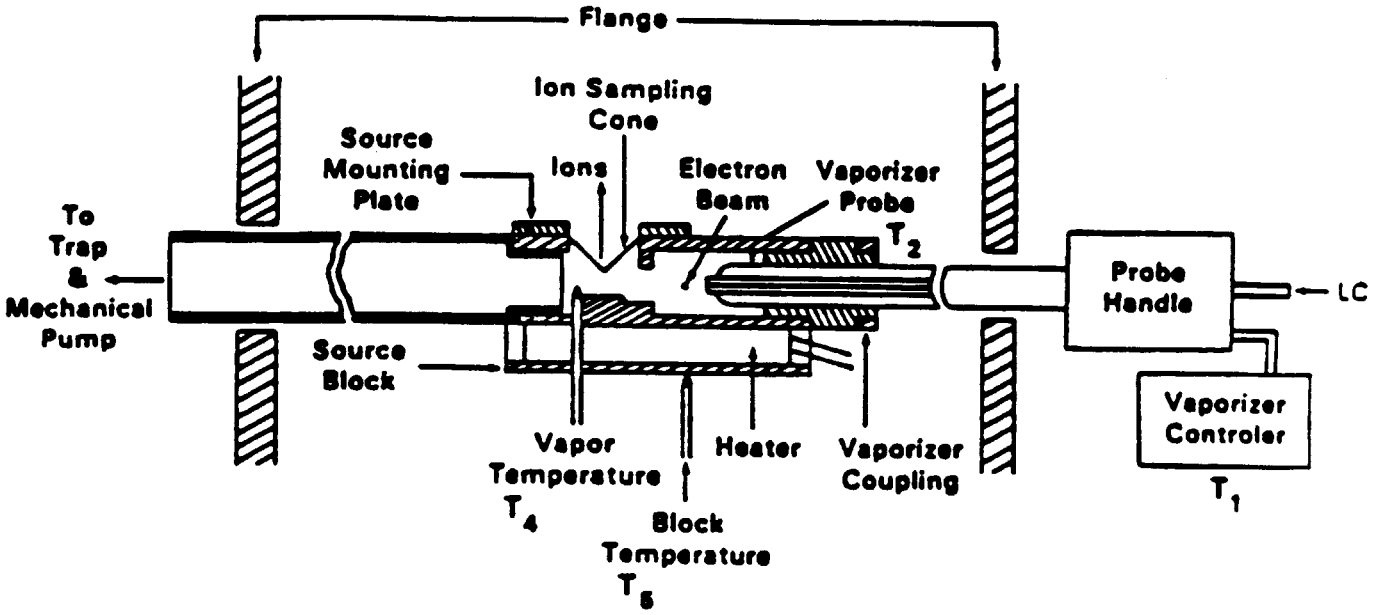


FIGURE 2
THERMOSPRAY SOURCE WITH WIRE-REPELLER
(High sensitivity configuration)

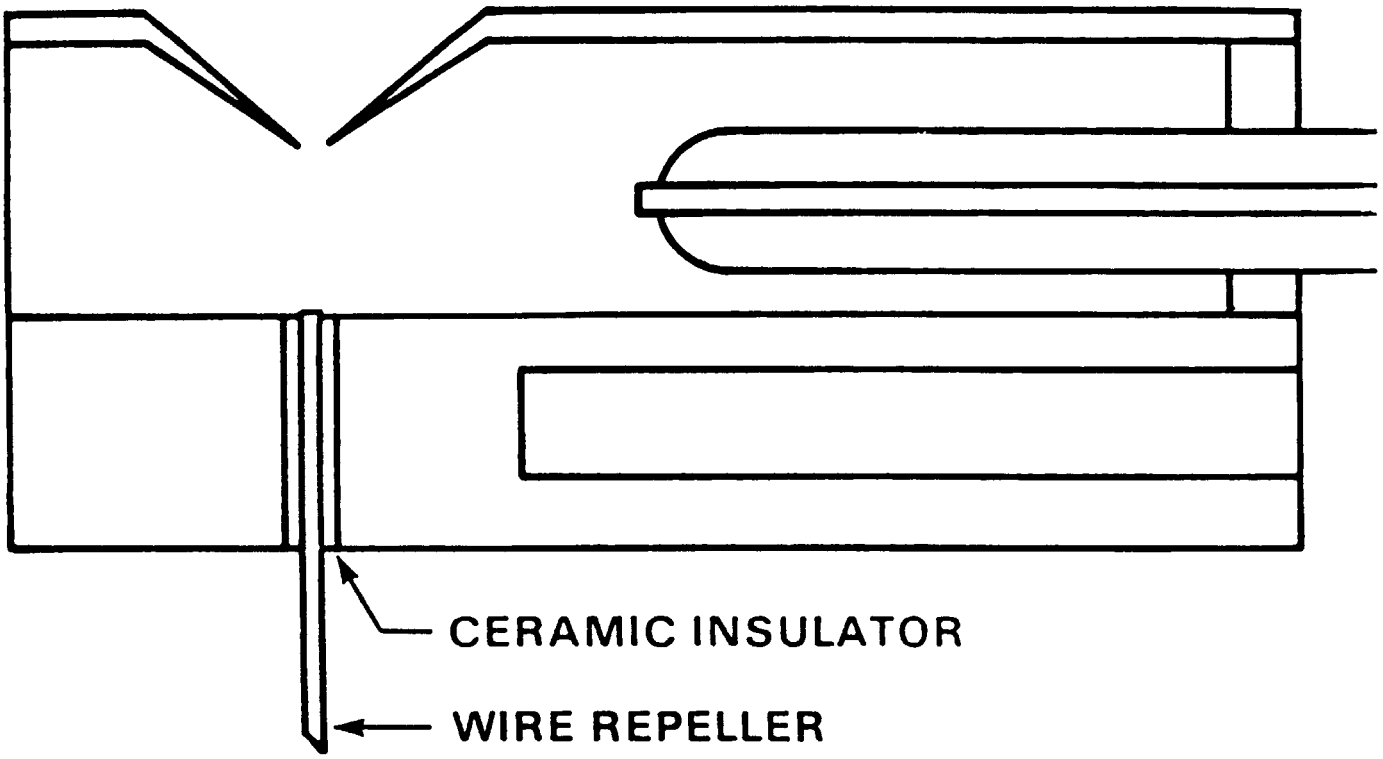
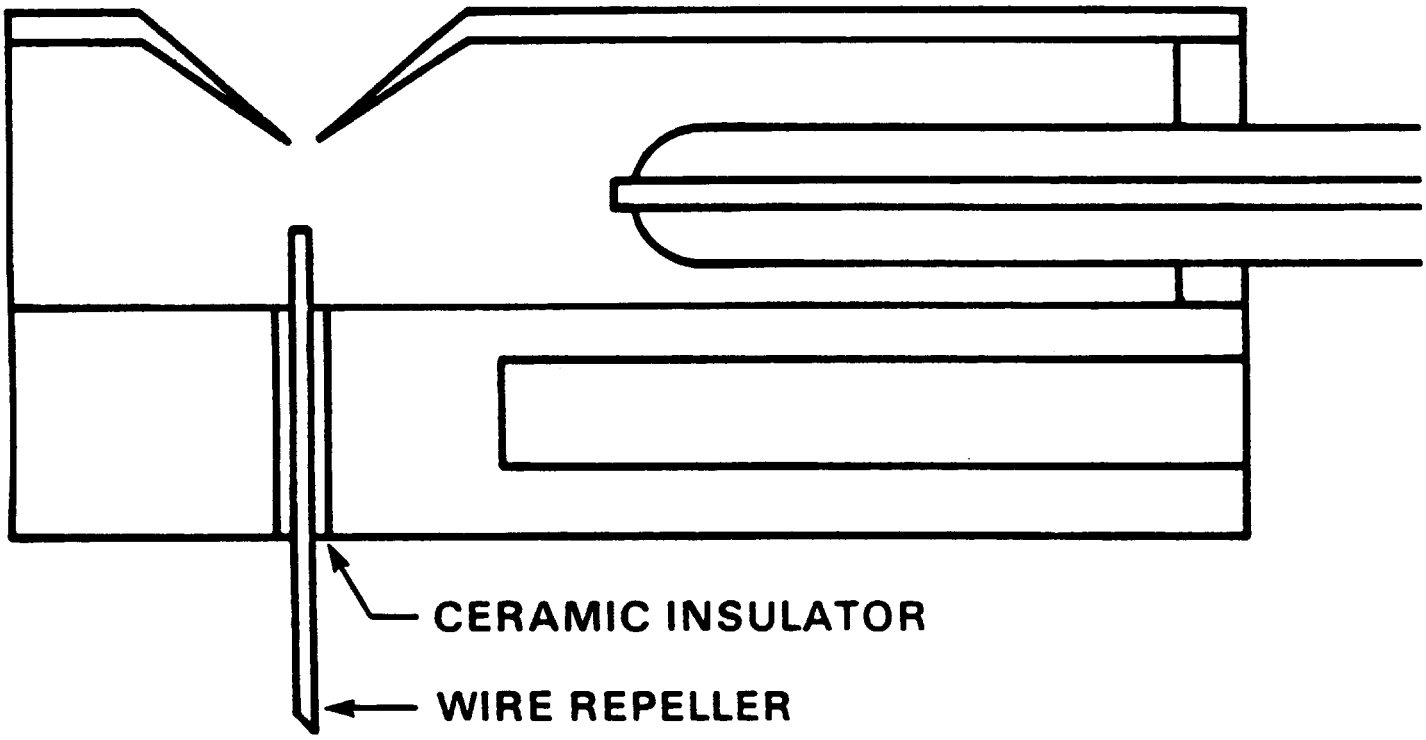


FIGURE 3 THERMOSPRAY SOURCE WITH WIRE-REPELLER
(CAD configuration)


CERAMIC INSULATOR
WIRE REPELLER