METHOD 553

DETERMINATION OF BENZIDINES AND NITROGEN-CONTAINING PESTICIDES IN WATER BY LIQUID-LIQUID EXTRACTION OR LIQUID-SOLID EXTRACTION AND REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY/ PARTICLE BEAM/MASS SPECTROMETRY

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1.0 SCOPE AND APPLICATION

1.1 This is a general purpose method that provides procedures for determination of benzidines and nitrogen-containing pesticides in water and wastewater. The method is applicable to a wide range of compounds that are efficiently partitioned from a water sample into methylene chloride or onto a liquid-solid extraction device. The compounds must also be amenable to separation on a reverse phase liquid chromatography column and transferable to the mass spectrometer with a particle beam interface. Particulate bound organic matter will not be partitioned onto the liquid-solid extraction system, and more than trace levels of particulates in the water may disrupt the partitioning process. The compounds listed below are potential method analytes and single-laboratory accuracy and precision data have been determined for the compounds as described in Section 13.0. The specific analytical conditions given in the method are applicable to those compounds for which accuracy and precision data are given. Other analytes (Section 1.2) may require slight adjustments of analytical conditions. A laboratory may use this method to identify and measure additional analytes after the laboratory obtains acceptable (defined in Section 9.0) accuracy and precision data for each added analyte.

Compound	Abbreviation	MW[1]	Chemical Abstracts Service Registry Number (CASRN)
benzidine	BZ	184	92-87-5
benzoylprop ethyl	BP	365	33878-50-1
caffeine	CF	194	58-08-2
carbaryl	CL	201	63-25-2
o-chlorophenyl thiourea	PT	186	5344-82-1
3,3'-dichlorobenzidine	DB	252	91-94-1
3,3'-dimethoxybenzidine	MB	244	119-90-4
3,3'-dimethylbenzidine	LB	212	612-82-8
diuron	DI	232	330-54-1
ethylene thiourea	ET	102	96-45-7
linuron (Lorox)	LI	248	330-55-2
monuron	MO	198	150-68-5
rotenone	RO	394	83-79-4
siduron	SI	232	1982-49-6

[1]Monoisotopic molecular weight calculated from the atomic masses of the isotopes with the smallest masses.

1.2 Preliminary investigation indicates that the following compounds may be amenable to this method: Aldicarb sulfone, Carbofuran, Methiocarb, Methomyl (Lannate), Mexacarbate (Zectran), and N-(1-Naphthyl) thiourea. Caffeine, Ethylene thiourea and o-Chlorophenyl thiourea have been successfully analyzed by HPLC/PB/MS, but have not been successfully extracted from a water matrix.

1.3 Method detection limit (MDL) is defined as the statistically calculated minimum amount that can be measured with 99% confidence that the reported value is greater than zero[1]. The MDL is compound dependent and is particularly dependent on extraction efficiency and sample matrix. For the analytes listed in Tables 3-5, the estimated MDLs range from 2-30 µg/L.

2.0 SUMMARY OF METHOD

2.1 Organic compound analytes and surrogates are extracted from 1 L of water sample by liquid-liquid extraction (LLE) with methylene chloride or by passing 1 L of sample water through a cartridge or disk containing a solid inorganic matrix coated with a chemically bonded C_{18} organic phase or a neutral polystyrene/divinylbenzene polymer (liquid-solid extraction, LSE). If LLE is used, the analytes are concentrated in methanol by evaporation of the methylene chloride and addition of methanol (solvent exchange). If LSE is used, the analytes are eluted from the LSE cartridge or disk with a small quantity of methanol and concentrated further by evaporation of some of the solvent. The sample components are separated, identified, and measured by injecting an aliquot of the concentrated methanol solution into a high performance liquid chromatograph (HPLC) containing a reverse phase HPLC column and interfaced to a mass spectrometer (MS) with a particle beam (PB) interface. Compounds eluting from the HPLC column are identified by comparing their measured mass spectra and retention times to reference spectra and retention times in a data base. Reference spectra and retention times for analytes are obtained by measurement of calibration standards under the same conditions used for samples. The concentration of each identified component is measured by relating the MS response of the quantitation ion produced by that compound to the MS response of the quantitation ion produced by the same compound in a calibration standard (external standard). Surrogate analytes, whose concentrations are known in every sample, are measured with the same external standard calibration procedure. An optional isotope dilution procedure is included for samples which contain interfering matrix or coeluting compounds.

3.0 DEFINITIONS

3.1 External Standard (ES) -- A pure analyte(s) that is measured in an experiment separate from the experiment used to measure the analyte(s) in the sample. The signal observed for a known quantity of the pure external standard(s) is used to calibrate the instrument response for the corresponding analyte(s).

The instrument response is used to calculate the concentrations of the analyte(s) in the sample.

3.2 Surrogate Analyte (SA) -- A pure analyte(s), which is extremely unlikely to be found in any sample, and which is added to a sample aliquot in known amount(s) before extraction and is measured with the same procedures used to measure other sample components. The purpose of a surrogate analyte is to monitor method performance with each sample.

3.3 Laboratory Duplicates (LD1 and LD2) -- Two aliquots of the same sample taken in the laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 indicate the precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.4 Field Duplicates (FD1 and FD2) -- Two separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analyses of FD1 and FD2 give a measure of the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.

3.5 Laboratory Reagent Blank (LRB) -- An aliquot of reagent water or other blank matrix that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.6 Field Reagent Blank (FRB) -- An aliquot of reagent water or other blank matrix that is placed in a sample container in the laboratory and treated as a sample in all respects, including shipment to the sampling site, exposure to sampling site conditions, storage, preservation and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are present in the field environment.

3.7 Instrument Performance Check Solution (IPC) -- A solution of one or more method analytes, surrogates, internal standards, or other test substances used to evaluate the performance of the instrument system with respect to a defined set of method criteria.

3.8 Laboratory Fortified Blank (LFB) -- An aliquot of reagent water or other blank matrix to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

3.9 Laboratory Fortified Sample Matrix (LFM) -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.10 Stock Standard Solution (SSS) -- A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

3.11 Primary Dilution Standard Solution (PDS) -- A solution of several analytes prepared in the laboratory from stock standard solutions and diluted as needed to prepare calibration solutions and other needed analyte solutions.

3.12 Calibration Standard (CAL) -- A solution prepared from the primary dilution standard solution or stock standard solutions and the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.13 Quality Control Sample (QCS) -- A solution of method analytes of known concentrations which is used to fortify an aliquot of LRB or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.

3.14 Instrument Detection Limit (IDL) -- The minimum quantity of analyte or the concentration equivalent which gives an analyte signal equal to three times the standard deviation of the background signal at the selected wavelength, mass, retention time, absorbance line, etc.

4.0 INTERFERENCES

4.1 When two compounds coelute, the transport efficiency of both compounds through the particle beam interface generally improves and enhanced ion abundances are observed in the mass spectrometer[2]. The degree of signal enhancement by coelution is compound dependent. This coelution effect invalidates the external calibration curve and, if not recognized, will result in incorrect concentration measurements. Procedures given in this method to check for coeluting compounds must be followed to preclude inaccurate measurements (Sections 10.2.6.5 and 12.1). An optional isotope dilution calibration procedure has been included for use when interfering matrix or coeluting compounds are present.

4.2 During analysis, major contaminant sources are reagents, chromatography columns, and liquid-solid extraction columns or disks. Analyses of field and laboratory reagent blanks provide information about the presence of contaminants.

4.3 Interfering contamination may occur when a sample containing low concentrations of compounds is analyzed immediately after a sample containing relatively high concentrations of compounds. Syringes, injectors, and other equipment must be cleaned carefully or replaced as needed. After analysis of a sample containing high concentrations of compounds, a laboratory reagent blank should be analyzed to ensure that accurate values are obtained for the next sample.

5.0 SAFETY

5.1 The toxicity or carcinogenicity of chemicals used in this method has not been precisely defined; each chemical should be treated as a potential health hazard, and exposure to these chemicals should be minimized. Each laboratory is responsible for maintaining awareness of procedures and regulations for safe handling of chemicals used in this method[3-5].

5.2 Some method analytes have been tentatively classified as known or suspected human or mammalian carcinogens. Pure standard materials and stock standard solutions of all analytes should be handled with suitable protection to skin, eyes, etc.

6.0 EQUIPMENT AND SUPPLIES

6.1 All glassware must be meticulously cleaned. This may be accomplished by washing with detergent and water, rinsing with water, distilled water, or solvents, air-drying, and heating (where appropriate) in an oven. Volumetric glassware is never heated.

6.2 Sample Containers -- 1 L or 1 qt amber glass bottles fitted with a Teflon-lined screw cap. (Bottles in which high purity solvents were received can be used as sample containers without additional cleaning if they have been handled carefully to avoid contamination during use and after use of original contents.)

6.3 Separatory Funnels -- 2 L and 100 mL with a Teflon stopcock.

6.4 Liquid Chromatography Column Reservoirs -- Pear-shaped 100 mL or 125 mL vessels without a stopcock but with a ground glass outlet sized to fit the liquid-solid extraction column. (Lab Glass, Inc., Part No. ML-700-706S, with a 24/40 top outer joint and a 14/35 bottom inner joint, or equivalent.) A 14/35 outlet joint fits some commercial cartridges.

6.5 Syringe Needles -- No. 18 or 20 stainless steel.

6.6 Vacuum Flasks -- 1 L or 2 L with solid rubber stoppers.

6.7 Volumetric Flasks -- Various sizes.

6.8 Laboratory or Aspirator Vacuum System -- Sufficient capacity to maintain a slight vacuum of 13 cm (5 in) of mercury in the vacuum flask.

6.9 Micro Syringes -- Various sizes.

6.10 Vials -- Various sizes of amber vials with Teflon-lined screw caps.

6.11 Drying Column -- 0.6 cm x 40 cm with 10 mL graduated collection vial.

6.12 Concentrator Tube -- Kuderna-Danish (K-D) 10 mL graduated with ground glass stoppers.

6.13 Analytical Balance -- Capable of weighing 0.0001 g accurately.

6.14 Liquid Chromatography Column -- A 15-25 cm x 2 mm (i.d.) stainless steel tube (e.g., Waters C-18 Novapak or equivalent) packed with silica particles (4-10 µm) with octadecyldimethylsilyl (C-18) groups chemically bonded to the silica surface. Residual acidic sites should be blocked (endcapped) with methyl or other non-polar groups and the stationary phase must be bonded to the solid support to minimize column bleed. Column selection for minimum bleeding is strongly recommended. The column must be conditioned over night before each use by pumping a 75-100% v/v acetonitrile: water solution through it at a rate of about 0.05 mL/min. Other packings and column sizes may be used if equivalent or better performance can be achieved.

6.15 Guard column of similar packing used in the analytical column is recommended.

6.16 Liquid Chromatograph/Mass Spectrometer/Data System (LC/MS/DS)

6.16.1 The LC must accurately maintain flow rates between 0.20-0.40 mL/min while performing a gradient elution from 100% Solvent A to 100% Solvent B. Pulse dampening is recommended but not required. An autoinjector is highly desirable and should be capable of accurately delivering 1-10 µL injections without affecting the chromatography.

6.16.2 The system should include a post-column mixing tee and an additional LC pump for post-column addition of acetonitrile at a constant rate of 0.1-0.7 mL/min.

6.16.3 The particle beam LC/MS interface must reduce the system pressure to a level fully compatible with the generation of classical electron ionization (EI) mass spectra, i.e., about 1×10^{-6} to 1×10^{-4} Torr, while delivering sufficient quantities of analytes to the conventional EI source

to meet sensitivity, accuracy, and precision requirements. All significant background components with mass greater than 62 Daltons should be removed to a level that does not produce ions greater than a relative abundance of 10% in the mass spectra of the analytes.

6.16.4 The mass spectrometer must be capable of electron ionization at a nominal electron energy of 70 eV. The spectrometer must be capable of scanning from 45-500 amu with a complete scan cycle time (including scan overhead) of 1.5 seconds or less. (Scan cycle time = Total MS data acquisition time in seconds divided by number of scans in the chromatogram). The spectrometer must produce a mass spectrum that meets all criteria in Table 1 when 500 ng or less of DFTPPO (Section 7.11) is introduced into the LC. An average spectrum across the DFTPPO LC peak may be used to test instrument performance.

6.16.5 An interfaced data system is required to acquire, store, reduce, and output mass spectral data. The computer software should have the capability of processing stored LC/MS data by integration of the ion abundance of any specific ion between specified time or scan number limits, construction of a first or second order regression calibration curves, calculation of response factors as defined in Section 10.2.9, calculation of response factor statistics (mean and standard deviation), and calculation of concentrations of analytes from the calibration curve or the equation in Section 12.0.

6.17 Millipore Standard Filter Apparatus, All Glass -- This will be used if the disks are to be used to carry out the extraction instead of cartridges.

7.0 REAGENTS AND STANDARDS

7.1 Helium Nebulizer/Carrier Gas -- As contaminant free as possible.

7.2 Liquid-Solid Extraction (LSE) Materials

7.2.1 Cartridges are inert non-leaching plastic, for example polypropylene, or glass and must not contain contaminants that leach into methanol. The cartridges are packed with various amounts of sorbents such as C_{18} or a neutral polystyrene/divinylbenzene polymer. The packing must have a narrow size distribution and must not leach organic compounds into methanol. One liter of water should pass through the cartridge in about two hours with the assistance of a slight vacuum of about 13 cm (5 in) of mercury. Faster flow rates are acceptable if equivalent accuracy and precision are obtained. Robotic systems typically pump the sample through a cartridge in less than two hours. These systems are also acceptable if equivalent accuracy and precision are obtained. Section 9.0 and Tables 4 and 5 provide criteria for acceptable LSE cartridges which are available from several commercial suppliers.

7.2.2 Extraction disks (Empore) -- Are thin filter-shaped materials with C_{18} modified silica, or neutral polystyrene/divinylbenzene polymer, impregnated in a Teflon or other inert matrix. As with cartridges, the disks should not contain any organic compounds, either from the Teflon or the bonded silica, which will leach into the methanol eluant. One liter of reagent water should pass through the disks in 5-20 minutes using a vacuum of about 66 cm (26 in) of mercury. Section 9.0 provides criteria for acceptable LSE disks which are available commercially.

7.3 Solvents

7.3.1 Acetonitrile, methylene chloride, and methanol -- HPLC grade and pesticide quality or equivalent.

7.3.2 Reagent water -- Water in which an interferant is not observed at the MDL of the compound of interest. Prepare reagent water by passing tap water through a filter bed containing about 0.5 kg of activated carbon or by using a water purification system. Store in clean, narrow-mouth bottles with Teflon-lined septa and screw caps.

7.4 Hydrochloric Acid -- Concentrated.

7.5 Sodium sulfate -- Anhydrous.

7.6 Reducing Agents for Chlorinated Water -- Sodium sulfite, sodium thiosulfate or sodium arsenite.

7.7 Ammonium Acetate, Sodium Chloride, and Sodium Hydroxide (1N) -- ACS reagent grade.

7.8 Stock Standard Solutions (SSS) -- Individual solutions of analytes, surrogates, and isotopically labelled analogues of the analytes may be purchased as certified solutions or prepared from pure materials. To prepare, add 10 mg (weighed on an analytical balance to 0.1 mg) of the pure material to 1.9 mL of methanol or acetonitrile in a 2 mL volumetric flask, dilute to the mark, and transfer the solution to an amber glass vial. Certain analytes, such as 3,3'-dimethoxybenzidine, may require dilution in 50% v/v acetonitrile or methanol: water solution. If the analytical standard is available only in quantities smaller than 10 mg, reduce the volume of solvent accordingly. If compound purity is certified by the supplier at >96%, the weighed amount can be used without correction to calculate the concentration of the solution (5 µg/µL). Store the amber vials in a freezer at <0°C.

7.8.1 Benzidines as the free base or as acid chlorides may be used for calibration purposes. However, the concentration of the standard must be calculated as the free base.

7.9 Primary Dilution Standard Solution (PDS) -- The stock standard solutions are used to prepare a primary dilution standard solution that contains multiple analytes. The recommended solvent for this dilution is a 50% v/v acetonitrile:water mixture. Aliquots of each of the stock standard solutions are combined to produce the primary dilution in which the concentration of the analytes is at least equal to the concentration of the most concentrated calibration solution. Store the primary dilution standard solution in an amber vial in a freezer at <0°C, and check frequently for signs of deterioration or evaporation, especially just before preparing calibration solutions.

7.10 Fortification Solution of Surrogates -- The analyst should monitor the performance of the extraction, cleanup (when used), and analytical system and the effectiveness of the method in dealing with each sample matrix by spiking each sample, standard, and blank with one or two surrogates recommended to encompass the range of the gradient elution program used in this method. The compounds recommended as surrogates for the analysis of benzidines and nitrogen-containing pesticides are benzidine-D_8 (DBZ), caffeine-$^{15}N_2$ (NCF), 3,3'-dichlorobenzidine-D_6 (DCB), and bis-(perfluorophenyl)-phenylphosphine oxide (OD) unless their unlabelled counterpart is being analyzed or they will be used for isotope dilution calibration (Abbreviations in parentheses are used in Figure 4). Prepare a solution of the surrogates in methanol or acetonitrile at a concentration of 5 mg/mL of each. Other surrogates may be included in this solution as needed. (A 10 µL aliquot of this solution added to 1 L of water gives a concentration of 50 µg/L of each surrogate). Store the surrogate fortifying solution in an amber vial in a freezer at <0ºC.

7.11 MS Performance Check Solution -- Prepare a 100 ng/µL solution of bis-(perfluorophenyl)-phenylphosphine oxide (DFTPPO) in acetonitrile. Store this solution in an amber vial in a freezer at <0°C. DFTPPO is not currently commercially available. For this method development work, DFTPPO was synthesized from bis-(perfluorophenyl) phenyl phosphine (DFTPP) in solution by adding a slight excess of hydrogen peroxide (DFTPP + $H_2O_2 \rightarrow$ DFTPPO + H_2O). The solvent was removed and the resulting crystals were thoroughly washed with water to remove any residual hydrogen peroxide. It is critical to remove all residual hydrogen peroxide before adding the DFTPPO to the CAL solution. Any residual hydrogen peroxide will degrade some analytes.

7.12 Calibration Solutions (CAL1-CAL6) -- Prepare a series of six concentration calibration solutions in acetonitrile which contain all analytes at concentrations of 2, 5, 10, 25, 50, and 100 times the instrument detection limit of each compound with a constant concentration of each surrogate in each CAL solution. This calibration range may be optimized by the operator, but each analyte must be bracketed by at least two calibration points. CAL1 through CAL6 are prepared by combining appropriate aliquots of the primary dilution standard solution (Section 7.9) and the fortification solution of surrogates (Section 7.10). DFTPPO may be added to one or more CAL solutions to verify MS tune (See Section 10.3.1.). Store these solutions in amber vials in a freezer at <0°C. Check these solutions quarterly for signs of deterioration.

7.12.1 For isotope dilution calibration, prepare the calibration solutions as described above with the addition of one coeluting isotopically labelled analog for each analyte of interest. The concentration for each coeluting labelled standard should be approximately 25-50 times the instrument detection limit of the analyte of interest and must be constant in all calibration solutions (CAL1 through CAL6). These solutions permit the relative response (unlabelled to labelled) to be measured as a function of the amount of analyte injected. If more than one labelled compound is used, one spiking solution containing all labelled compounds should be prepared.

7.13 Mobile Phase -- Solvent A is a 75:25 v/v water:acetonitrile solution containing ammonium acetate at a concentration of 0.01 M. This composition is used to eliminate biological activity in the A Phase. Solvent B is acetonitrile. Both solvents are degassed in an ultrasonic bath under reduced pressure and maintained by purging with a low flow of helium.

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 Sample Collection -- When sampling from a water tap, open the tap and allow the system to flush until the water temperature has stabilized (usually about two to five minutes). Adjust the flow to about 500 mL/min and collect samples from the flowing stream. Keep samples sealed from collection time until analysis. When sampling from an open body of water, fill the sample container with water from a representative area. Sampling equipment, including automatic samplers, must be free of plastic tubing, gaskets, and other parts that may leach analytes into water. Automatic samplers that composite samples over time must use refrigerated glass sample containers.

8.2 Sample Dechlorination and Preservation -- All samples should be iced or refrigerated at 4°C from the time of collection until extraction. Residual chlorine should be reduced at the sampling site by addition of a reducing agent. Add 40-50 mg of sodium sulfite or sodium thiosulfate (these may be added as solids with stirring until dissolved) to each liter of water.

8.3 Holding Time -- Samples must be extracted within seven days and the extracts analyzed within 30 days of sample collection. Extracts should be stored in an amber vial in a freezer at <0°C.

8.4 Field Blanks

8.4.1 Processing of a field reagent blank (FRB) is recommended along with each sample set, which is composed of the samples collected from the same general sample site at approximately the same time. At the laboratory, fill a sample container with reagent water, seal, and ship to the sampling site along with the empty sample containers. Return the FRB to the laboratory with filled sample bottles.

9.0 QUALITY CONTROL

9.1 Quality control (QC) requirements are the initial demonstration of laboratory capability followed by regular analyses of LRBs, LFBs, and laboratory fortified matrix samples. The laboratory must maintain records to document the quality of the data generated. Additional QC practices are recommended.

9.2 Initial demonstration of low system background and acceptable particle size and packing. Before any samples are analyzed, or any time a new supply of LSE cartridges or disks is received from a supplier, or a new column is installed, it must be demonstrated that a LRB is reasonably free of contamination that would prevent the determination of any analyte of concern. In this same experiment it must be demonstrated that the particle size and packing of the LSE cartridge are acceptable. Consistent flow rate is an indication of acceptable particle size distribution and packing.

9.2.1 A source of potential contamination may be the liquid-solid extraction (LSE) cartridges and disks and columns which may contain silicon compounds and other contaminants that could prevent the determination of method analytes. Generally, contaminants will be leached from the cartridges, disks, or columns into the solvent and produce a variable background. If the background contamination is sufficient to prevent accurate and precise analyses, this condition must be corrected before proceeding with the initial demonstration. Figure 1 shows unacceptable background contamination from a column with stationary phase bleed.

9.2.2 Other sources of background contamination are solvents, reagents, and glassware. Background contamination must be reduced to an acceptable level before proceeding with the next section. In general, background for method analytes should be below the MDL.

9.2.3 One liter of water should pass through the cartridge in about two hours (faster flow rates are acceptable if precision and accuracy are acceptable) with a partial vacuum of about 13 cm (5 in) of mercury. The extraction time should not vary unreasonably among LSE cartridges. Robotic systems typically pump the sample through a cartridge in less than two hours. These systems are also acceptable if equivalent accuracy and precision are obtained. Extraction disks may be used at a faster flow rate (See Section 7.2.2).

9.3 Initial Demonstration of Laboratory Accuracy and Precision -- Analyze five to seven replicates of a LFB containing each analyte of concern at a concentration in the range of 10-50 times the instrument detection limits (see regulations and maximum contaminant levels for guidance on appropriate concentrations).

9.3.1 Prepare each replicate by adding an appropriate aliquot of the PDS, or another certified quality control sample, to reagent water. Analyze each replicate according to the procedures described in Section 11.0 and on a schedule that results in the analyses of all replicates with 48 hours.

9.3.2 Calculate the measured concentration of each analyte in each replicate, the mean concentration of each analyte in all replicates, and mean accuracy (as mean percentage of true value) for each analyte, and the precision (as relative standard deviation, RSD) of the measurements for each analyte. Calculate the MDL of each analyte using the referenced procedures[1].

9.3.3 For each analyte and surrogate, the mean accuracy, expressed as a percentage of the true value, should be 70-130% and the RSD should be <30%. The MDLs should be sufficient to detect analytes at the regulatory levels. If these criteria are not met for an analyte, take remedial action and repeat the measurements for that analyte to demonstrate acceptable performance before samples are analyzed.

9.3.4 Develop and maintain a system of control charts to plot the precision and accuracy of analyte and surrogate measurements as a function of time. Charting of surrogate recoveries is an especially valuable activity since these are present in every sample and the analytical results will form a significant record of data quality.

9.4 Laboratory Reagent Blanks (LRBs) -- With each batch of samples processed as a group within a work shift, analyze a laboratory reagent blank to determine the background system contamination. Any time a new batch of LSE cartridges or disks is used, or new supplies of other reagents are used, repeat the demonstration of low background described in Section 9.2.

9.5 With each batch of samples processed as a group within a work shift, analyze a single LFB containing each analyte of concern at a concentration as determined in Sect. 9.3. Evaluate the accuracy of the measurements (Section 9.3.3), and estimate whether acceptable MDLs can be obtained. If acceptable accuracy and MDLs cannot be achieved, the problem must be located and corrected before further samples are analyzed. Add these results to the ongoing control charts to document data quality.

9.6 Determine that the sample matrix does not contain materials that adversely affect method performance. This is accomplished by analyzing replicates of laboratory fortified matrix samples and ascertaining that the precision, accuracy, and MDLs of analytes are in the same range as obtained with LFBs. If a variety of different sample matrices are analyzed regularly, for example, drinking water from groundwater and surface water sources, matrix independence should be established for each.

9.7 With each set of field samples a FRB should be analyzed. The results of these analyses will help define contamination resulting from field sampling and transportation activities.

9.8 At least quarterly, replicates of LFBs should be analyzed to determine the precision of the laboratory measurements. Add these results to the ongoing control charts to document data quality.

9.9 At least quarterly, analyze a QCS from an external source. If measured analyte concentrations are not of acceptable accuracy (Section 9.3.3), check the entire analytical procedure to locate and correct the problem source.

9.10 Numerous other specific QC measures are incorporated into other parts of this procedure, and serve to alert the analyst to potential problems.

10.0 CALIBRATION AND STANDARDIZATION

10.1 Demonstration and documentation of acceptable initial calibration and system optimization are required before any samples are analyzed and is required intermittently during sample analysis as indicated by results of continuing calibration checks. After initial calibration is successful, a continuing calibration check is required at the beginning of each eight-hour period during which analyses are performed. Additional periodic calibration checks are good laboratory practice.

10.2 Initial Calibration

10.2.1 Optimize the interface according to the manufacturer's instructions. This usually is accomplished on initial installation by flow injection with caffeine or benzidine and should utilize a mobile phase of 50% v/v acetonitrile: water. Major maintenance may require reoptimization.

10.2.2 Calibrate the MS mass and abundance scales using the calibration compound and manual (not automated) ion source tuning procedures specified by the manufacturer. Calibration must be accomplished while a 50% v/v acetonitrile: water mixture is pumped through the LC column and the optimized particle beam interface. For optimum long-term stability and precision, interface and ion source parameters should be set near the center of a broad signal plateau rather than at the peak of a sharp maximum (sharp maxima vary short term with particle beam interfaces and gradient elution conditions).

10.2.3 Fine tune the interface by making a series of injections into the LC column of a medium level CAL standard and adjusting the operating parameters until optimum sensitivity and precision are obtained for the maximum number of target compounds[6]. Suggested additional operating conditions are:

mobile phase purge: helium at 30 mL/min continuous,

mobile phase flow rate: 0.3 mL/min through the column,

gradient elution: Hold for one minute at 25% acetonitrile, then linearly program to ≈ 70% acetonitrile in 29 minutes, start data acquisition immediately,

post-column addition: acetonitrile at 0.1-0.7 mL/min, depending on the interface requirements. Maintain a minimum of 30% acetonitrile in the interface to improve system precision and possibly sensitivity,

desolvation chamber temperature: 45-80°C,

ion source temperature: 250-290°C,

electron energy: 70 eV, and

scan range: 62-465 amu at 1-2 sec/scan.

10.2.4 The medium level standard (CAL) used in Section 10.2.3 should contain DFTPPO, or separately inject into the LC a 5-µL aliquot of the 100 ng/µL DFTPPO solution and acquire a mass spectrum that includes data form/z 62-465. Use LC conditions that produce a narrow (at least 10 scans per peak) symmetrical peak. If the spectrum does not meet all criteria (Table 1), the MS ion source must be retuned and adjusted to meet all criteria before proceeding with calibration. An average spectrum across the LC peak may be used to evaluate the performance of the system. Figure 2 represents the average composite spectrum obtained for DFTPPO from a multilaboratory study involving five different particle beam interfaces from 13 laboratories.

10.2.5 Inject a 5 µL aliquot of a medium concentration calibration solution, for example 50 ng/µL, and acquire and store data from m/z 62-465 with a total cycle time (including scan overhead time) of 1.5 seconds or less. Cycle time should be adjusted to measure at least 10 spectra during the elution of each LC peak.

10.2.6 Performance criteria for the medium calibration -- Examine the stored LC/MS data with the data system software. Figure 3 shows an acceptable total ion chromatogram.

10.2.6.1 LC performance. 3,3'-dimethyl- and 3,3'-dimethoxybenzidine should be separated by a valley whose height is less than 25% of the average peak height of these two compounds. If the valley between them exceeds 25%, modify the gradient. If this fails, the LC column requires maintenance. (See Section 10.3.6)

10.2.6.2 Peak tailing -- Examine a total ion chromatogram and determine the degree of peak tailing. Severe tailing indicates a major problem and system maintenance is required to correct the problem. (See Section 10.3.6)

10.2.6.3 MS sensitivity -- Signal/noise in any analyte mass spectrum should be at least 3:1.

10.2.6.4 Column bleed -- Figure 1 shows an unacceptable chromatogram with column bleed. Figure 3 is the mass spectrum of dimethyloctadecylsilanol, a common stationary phase bleed product. If unacceptable column bleed is present, the column must be changed or conditioned to produce an acceptable background (Figure 4).

10.2.6.5 Coeluting compounds -- Compounds which coelute cannot be measured accurately because of carrier effects in the particle beam interface. Peaks must be examined carefully for coeluting substances and if coeluting compounds are present at greater than 10% the concentration of the target compound, conditions must be adjusted to resolve the components, the target compound must be flagged as positively biased, or isotope dilution calibration should be used.

10.2.7 If all performance criteria are met, inject a 5 µL aliquot of each of the other CAL solutions using the same LC/MS conditions.

10.2.8 The general method of calibration (external) is a second order regression of integrated ion abundances of the quantitation ions (Table 2) as a function of amount injected. For second order regression, a sufficient number of calibration points must be obtained to accurately determine the equation of the curve. For some individual analytes over a short concentration range, reasonable linearity may be observed and response factors may be used. Calculate response factors using the equation below. Second order regressions and response factor calculations are supported in acceptable LC/MS data system software (Section 6.16.5), and many other software programs.

$$RF = \frac{(A_x)}{(Q_x)}$$

where: A_x = integrated abundance of the quantitation ion of the analyte.
Q_x = quantity of analyte injected in ng or concentration units.

10.2.9 If response factors are used (i.e., linear calibration with the line going through the origin), calculate the mean RF from the analyses of the six CAL solutions for each analyte and surrogate. Calculate the standard deviation (SD) and the relative standard deviation (RSD) from each mean (M): RSD = 100 (SD / M). If the RSD of any analyte or surrogate mean RF exceeds 20%, either analyze additional aliquots of appropriate CAL solutions to obtain an acceptable RSD of RFs over the entire concentration range, take action to improve LC / MS performance, or use the second order regression calibration. (See Section 10.2.8)

10.3 Continuing Calibration Check -- Verify the MS tune and initial calibration at the beginning of each eight-hour work shift during which analyses are performed using the following procedure:

10.3.1 Inject a 5 µL aliquot of the 100 ng / µL DFTPPO solution (this may be contained in the medium level CAL solution used in Section 10.3.2) and acquire a mass spectrum that includes data for m / z 62-465. If the spectrum does not meet all criteria (Table 1), the MS must be retuned and adjusted to meet all criteria before proceeding with the continuing calibration check.

10.3.2 Inject a 5 µL aliquot of a medium level CAL solution and analyze with the same conditions used during the initial calibration. One or more additional CAL solutions should be analyzed.

10.3.3 Demonstrate acceptable performance for the criteria shown in Section 10.2.6.

10.3.4 Determine that the absolute areas of the quantitation ions of the external standards and surrogate(s) have not changed by more than 20% from the areas measured during initial calibration. If these areas have changed by more than 20%, recalibration and other adjustments are necessary. These adjustments may require cleaning of the MS ion source, or other maintenance as indicated in Section 10.3.6, and recalibration. Control charts are useful aids in documenting system sensitivity changes.

10.3.5 Using the previously generated second order regression curve, calculate the concentrations in the medium level CAL solution and compare the results to the known values in the CAL solution. If calculated concentrations deviate by more than 20% from known values, recalibration of the system with the six CAL solutions is required. If response factors were used, calculate the RF for each analyte and surrogate from the data measured in the continuing calibration check. The RF for each analyte and surrogate must be within 20% of the mean value measured in the initial calibration.

10.3.6 Some possible remedial actions -- Major maintenance such as cleaning an ion source, cleaning quadrupole rods, etc. require returning to the initial calibration step.

10.3.6.1 Check and adjust LC and/or MS operating conditions; check the MS resolution, and calibrate the mass scale.

10.3.6.2 Replace the mobile phases with fresh solvents. Verify that the combined flow rate from the LC and post-column addition pumps is constant.

10.3.6.3 Flush the LC column with acetonitrile.

10.3.6.4 Replace LC column; this action will cause a change in retention times.

10.3.6.5 Prepare fresh CAL solutions and repeat the initial calibration step.

10.3.6.6 Clean the MS ion source, entrance lens, and rods (if a quadrupole).

10.3.6.7 Replace any components that leak.

10.3.6.8 Replace the MS electron multiplier or any other faulty components.

10.3.6.9 Clean the interface to eliminate plugged components and/or replace skimmers according to the manufacturer's instructions.

10.3.6.10 If automated peak areas are being used, verify values by manual integration.

10.3.6.11 Increasing ion source temperature can reduce peak tailing, but excessive ion source temperature can affect the quality of the spectra for some compounds.

10.3.6.12 Air leaks into the interface may affect the quality of the spectra (e.g., DFTPPO), especially when ion source temperatures are operated in excess of 280°.

10.4 Calibration with Isotope Dilution (Optional) -- For samples with interfering matrix or coeluting peaks, the most reliable method for quantitation is the use of coeluting isotope labelled internal standards[7]. Isotope dilution calibration will be limited by the availability and cost of the labelled species and the requirement that each analyte must coelute with the labelled internal standard. Because the labelled internal standard must coelute with the analyte, the

quantitation ion for the internal standard must be larger than that of the analyte and not present in the analyte's mass spectrum. In addition, it must be verified that the labelled internal standard is not contaminated by its unlabelled counterpart.

10.4.1 A calibration curve encompassing the concentration range is prepared for each compound to be determined. The relative response (analyte integrated ion abundances to labelled integrated ion abundance) vs. amount of analyte injected is plotted using linear regression. A minimum of five data points are employed for this type of calibration.

10.4.2 To calibrate, inject a 5.0 µL aliquot of each of the calibration standards (Section 7.12.1) and compute the relative response (analyte integrated ion abundances to labelled compound integrated ion abundance). Plot this versus the amount of analyte injected by linear regression. This plotted line or the equation of this line should be used for quantitative calculations. Unless this line goes through the origin, the response factors at each point will not be constant and therefore, average response factors cannot be used. These calculations are supported in acceptable LC/MS data system software (Section 6.15.5), and in many other software programs.

10.4.3 Follow Section 10.3 to verify calibration at the beginning of each eight-hour work shift by injecting a 5.0 µL aliquot of a medium CAL solution and analyze it with the same conditions used during the initial calibration. Using the previously generated first order regression line (relative response versus amount of analyte injected), calculate the concentrations in the medium level CAL solution and compare the results to the known values in the CAL solution. If calculated concentrations deviate by more than 20% from known values, recalibration of the system with the CAL solutions, containing the isotopically labelled analogues, is re-quired.

11.0 PROCEDURE

11.1 The extraction procedure depends on the analytes selected and the nature of the sample. LSE (cartridge or disk) is limited to particulate-free water, e.g., drinking water. Consult Tables 3-5 to determine which analytes are amenable to liquid-solid and liquid-liquid extractions. Section 11.2 provides the LSE procedure using cartridges and Section 11.3 provides the LSE procedure using disks. Section 11.4 provides the procedure for LLE. After the extraction is complete, proceed to Section 11.5 to continue with the method.

11.2 Liquid-Solid Extraction (LSE) Procedure Using Cartridges -- This procedure may be manual or automated.

11.2.1 Set up the extraction apparatus shown in Figure 5. The reservoir is not required but recommended for convenient operation. Water drains from the reservoir through the LSE cartridge and into a syringe needle which is inserted through a rubber stopper into the suction flask. A slight vacuum of 13 cm (5 in) of mercury is used during all operations with the apparatus. The pressure used is critical as a vacuum greater than 13 cm may result in poor precision. About two hours is required to draw a liter of water through the cartridge, but faster flow rates are acceptable if precision and accuracy are acceptable. The use of robotic extraction systems is acceptable if equivalent MDLs, precision and accuracy are obtained.

11.2.2 Mark the water meniscus on the side of the sample bottle for later determination of the sample volume. A 1 L sample is recommended. Pour the water sample into the 2 L separatory funnel with the stopcock closed. Adjust the pH to 7.0 by the dropwise addition of hydrochloric acid or 1 N sodium hydroxide. Residual chlorine must not be present, as a reducing agent should have been added at the time of sampling. For extractions using C_{18} cartridges, add 0.01 M ammonium acetate (0.77 g in 1 L) to the water sample and mix until homogeneous. Add a 10 µL aliquot of the fortification solution for surrogates, and mix until homogeneous. The concentration of surrogates in the water should be 10-50 times the instrument detection limit.

11.2.3 Flush each cartridge with two 10 mL aliquots of methanol, letting the cartridge drain dry after each flush. This solvent flush may be accomplished by adding methanol directly to the solvent reservoir in Figure 5. Add 10 mL of reagent water to the solvent reservoir, but before the reagent water level drops below the top edge of the packing in the LSE cartridge, open the stopcock of the separatory funnel and begin adding sample water to the solvent reservoir. Close the stopcock when an adequate amount of sample is in the reservoir.

11.2.4 Periodically open the stopcock and drain a portion of the sample water into the solvent reservoir. The water sample will drain into the cartridge, and from the exit into the suction flask. Maintain the packing material in the cartridge immersed in water at all times. Wash the separatory funnel and cartridge with 10 mL of reagent water, and draw air through the cartridge for 10 minutes.

11.2.5 Transfer the LSE cartridge to the elution apparatus shown in Figure 5B. Wash the 2 L separatory funnel with 15 mL of methanol, close the stopcock of the 100 mL separatory funnel of the elution apparatus, and elute the cartridge with two 7.5 mL aliquots of the methanol washings. Concentrate the extract to the desired volume under a gentle stream of nitrogen. Record the exact volume of the extract.

11.2.5.1 If isotope dilution calibration is used, spike the extract with the isotopically labelled standards prior to solvent evaporation. The concentration of these isotopically labelled compounds after the desired extract volume is reached should be the same as the concentration in each CAL solution.

11.2.6 Determine the original sample volume by refilling the sample bottle to the mark and transferring the liquid to a 1000 mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11.3 Liquid-Solid Extraction (LSE) Procedure Using Disks -- This procedure may be manual or automated.

11.3.1 Mark the water meniscus on the side of the sample bottle for later determination of the sample volume. A 1 L sample is recommended. Pour the water sample into the 2 L separatory funnel with the stopcock closed. Adjust the pH to 7.0 by the dropwise addition of hydrochloric acid or 1 N sodium hydroxide. Residual chlorine must not be present because a reducing agent should have been added at the time of sampling. For extractions using C_{18} disks, add 0.01 M ammonium acetate (0.77 g in 1 L) to the water sample and mix until homogeneous. Add a 10 µL aliquot of the fortification solution for surrogates, and mix until homogeneous. The concentration of surrogates in the water should be 10-50 times the instrument detection limit.

11.3.2 Preparation of disks

11.3.2.1 Insert the disk into the 47 mm filter apparatus (See Figure 6). Wash and pre-wet the disk with 10 mL methanol (MeOH) by adding the MeOH to the disk and allowing it to soak for about a minute, then pulling most of the remaining MeOH through. A layer of MeOH must be left on the surface of the disk, which should not be allowed to go dry from this point until the end of the sample extraction. THIS IS A CRITICAL STEP FOR A UNIFORM FLOW AND GOOD RECOVERY.

11.3.2.2 Rinse the disk with 10 mL reagent water by adding the water to the disk and pulling most through, again leaving a layer of water on the surface of the disk.

11.3.3 Add the water sample to the reservoir and turn on the vacuum to begin the extraction. Full aspirator vacuum may be used. Particulate-free water may pass through the disk in as little as 10 minutes or less. Extract the entire sample, draining as much water from the sample container as possible.

11.3.4 Remove the filtration top from the flask, but do not disassemble the reservoir and fritted base. Empty the water from the flask, and insert a suitable sample tube to contain the eluant. The only constraint on the sample tube is that it must fit around the drip tip of the fritted base. Reassemble the apparatus.

11.3.5 Add 5 mL MeOH to the sample bottle, and rinse the inside walls thoroughly. Allow the MeOH to settle to the bottom of the bottle, and transfer to the disk with a disposable pipet, rinsing the sides of the glass filtration reservoir in the process. Pull about half of the MeOH through the disk, release the vacuum, and allow the disk to soak for a minute. Pull the remaining MeOH through the disk.

11.3.6 Repeat the above step twice. Concentrate the combined extracts to the desired volume under a gentle stream of nitrogen. Record the exact volume of the extract. (Preliminary investigation indicates that acetonitrile is a better extraction solvent for rotenone when extracting water, containing high levels of particulate matter, with LSE disks.)

11.3.6.1 If isotope dilution calibration is used, spike the extract with the isotopically labelled standards prior to solvent evaporation. The concentration of these isotopically labelled compounds after the desired extract volume is reached should be the same as the concentration in each CAL solution.

11.3.7 Determine the original sample volume by refilling the sample bottle to the mark and transferring the liquid to a 1000 mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11.4 Liquid-Liquid Extraction (LLE) Procedure

11.4.1 Mark the water meniscus on the side of the sample bottle for later determination of the sample volume. A 1 L sample is recommended. Pour the water sample into a 2 L separatory funnel with the stopcock closed. Residual chlorine should not be present as a reducing agent should have been added at the time of sampling. Add a 10 µL aliquot of the fortification solution for surrogates, and mix until homogeneous. The concentration of surrogates in the water should be 10-50 times the instrument detection limit.

11.4.2 Adjust the pH of the sample to 7.0 by dropwise addition of hydrochloric acid or 1 N sodium hydroxide. Add 100 g of sodium chloride to the sample and shake to dissolve the salt.

11.4.3 Add 60 mL of methylene chloride to the sample bottle, shake, and transfer the solvent to the separatory funnel and extract the sample by vigorously shaking the funnel for 2 min with periodic venting to release

excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, mechanical techniques must be employed to complete the phase separation. The optimum technique depends on the sample, but may include stirring, filtration of the emulsion through glass wool, centrifuging, etc. Collect the methylene chloride extract in a 500 mL Erlenmeyer flask.

11.4.4 Add a second 60 mL volume of methylene chloride and repeat the extraction a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner.

11.4.5 Assemble a K-D concentrator by attaching a 10 mL concentrator tube to a 500 mL evaporative flask. Dry the extract by pouring it through a solvent-rinsed drying column containing about 10 cm of anhydrous sodium sulfate. Collect the extract in the K-D concentrator, and rinse the column with 20-30 mL of methylene chloride.

11.4.6 Add one or two clean boiling stones to the evaporative flask and attach a macro Snyder column. Pre-wet the Snyder column by adding about 1 mL of methylene chloride to the top. Place the K-D apparatus on a hot water bath, 65-70°C, so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15-20 minutes. At the proper rate of distillation, the balls of the column will actively chatter, but the chambers will not flood. When the apparent volume of the liquid reaches 2 mL, add 20 mL of methanol through the Snyder column using a syringe and needle. Raise the temperature of the hot water bath to 90°C, and concentrate the sample to about 2 mL. Concentrate the extract to the desired volume under a gentle stream of nitrogen. Record the exact volume of the concentrated extract.

11.4.6.1 If isotope dilution calibration is used, spike the extract with the isotopically labelled standards prior to solvent evaporation. The concentration of these isotopically labelled compounds after the desired extract volume is reached should be the same as the concentration in each CAL solution.

11.4.7 Determine the original sample volume by refilling the sample bottle to the mark and transferring the liquid to a 1000 mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11.5 Liquid Chromatography/Mass Spectrometry (LC/MS)

11.5.1 Analyze a 5 µL aliquot with the LC/MS system under the same conditions used for the initial and continuing calibrations (Section 10.2).

11.6 Identification of Analytes

11.6.1 At the conclusion of data acquisition, use the system software to display the chromatogram, mass spectra and retention times of the peaks in the chromatogram.

11.6.2 Identify a sample component by comparison of its mass spectrum (after background subtraction) to a reference spectrum in the user-created data base. The LC retention time of the sample component should be within 10 seconds of the time observed for that same compound when a calibration solution was analyzed. In general, all ions that are present above 10% relative abundance in the mass spectrum of the standard should be present in the mass spectrum of the sample component and should agree within absolute 20%. For example, if an ion has a relative abundance of 30% in the standard spectrum, its abundance in the sample spectrum should be in the range of 10-50%. Some ions, particularly the molecular ion, are of special importance, and should be evaluated even if they are below 10% relative abundance.

11.6.3 Use the data system software to examine the ion abundances of components of the chromatogram. If any ion abundance exceeds the system working range, dilute the sample aliquot and analyze the diluted aliquot.

11.6.4 Identification is hampered when sample components are not resolved chromatographically and produce mass spectra containing ions contributed by more than one analyte. When LC peaks obviously represent more than one sample component (i.e., broadened peak with shoulder(s) or vallies between two or more maxima), appropriate analyte spectra and background spectra can be selected by examining plots of characteristic ions for tentatively identified components. When analytes coelute (i.e., only one LC peak is apparent), the identification criteria can be met but each analyte spectrum will contain extraneous ions contributed by the coeluting compound.

11.6.5 Structural isomers that produce very similar mass spectra can be explicitly identified only if they have sufficiently different LC retention times. (See Section 10.2.6.1) Acceptable resolution is achieved if the height of the valley between two isomer peaks is less than 25% of the average height of the two peak heights. Otherwise, structural isomers are identified as isomeric pairs.

11.6.6 Background components appear in variable quantities in laboratory and field reagent blanks, and generally subtraction of the concentration in the blank from the concentration in the sample is not recommended because the concentration of the background in the blank is highly variable. If method analytes appear in the blank, then resample.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 Complete chromatographic resolution is necessary for accurate and precise measurements of analyte concentrations. Compounds which coelute cannot be measured accurately because of carrier effects in the particle beam interface[2]. Peaks must be examined carefully for coeluting substances and if coeluting compounds are present at greater than 10% the concentration of the target compound, either conditions must be adjusted to resolve the components, or the target compound must be removed from the list of quantitative analytes.

12.2 Use the LC/MS system software or other available proven software to compute the concentrations of the analytes and surrogates from the second order regression curves. Manual verification of automated integration is recommended.

12.2.1 For isotope dilution calculations, use the first order plot of relative response (analyte integrated ion abundances to labelled integrated ion abundance) vs. amount of analyte injected or the equation of the line to compute concentrations. If the plotted line does not go through the origin, response factors will not be constant at each calibration point; therefore, average response factors cannot be used.

12.3 If appropriate, calculate analyte and surrogate concentrations from response factors and the following equation.

$$C_x = \frac{(A_x)\ (V_e)}{(RF)\ (V)\ (V_i)}$$

where: C_x = Concentration of analyte or surrogate in µg/L in the water sample.
A_x = integrated abundance of the quantitation ion of the analyte in the sample.
V = original water sample volume in liters.
RF = mean response factor of analyte from the initial calibration.
V_e = volume of final extract in µL.
V_i = injection volume in µL.

13.0 METHOD PERFORMANCE

13.1 Single laboratory accuracy and precision data (Tables 3-5) for each listed analyte were obtained. Five to seven 1 L aliquots of reagent water containing approximately five times the MDL of each analyte were analyzed with this procedure. (For these experiments, the final extract volume was 0.5 mL.)

13.1.2 With these data, MDLs were calculated using the formula:

$$MDL = S\ t_{(n-1,1-alpha\ =\ 0.99)}$$

where: $t_{(n-1,1-alpha\ =\ 0.99)}$ = Student's t value for the 99% confidence level with n-1 degrees of freedom.
n = number of replicates.
S = standard deviation of replicate analyses.

13.2 A multilaboratory (12 laboratories) validation of the determinative step was done for four of the analytes (benzidine - BZ, 3,3'-dimethoxybenzidine - MB, 3,3'-dimethylbenzidine - LB, 3,3'-dichloro-benzidine - DB). Table 6 gives the results from this study for single laboratory precision, overall laboratory precision, and overall laboratory accuracy. The two concentration levels shown represent the two extremes of the concentration range studied.

14.0 POLLUTION PREVENTION

14.1 Although this method allows the use of either LLE or LSE, LSE is highly recommended whenever possible. Only small amounts of methanol are used with this procedure as compared to much larger amounts of methylene chloride used for LLE. All other compounds used are neat materials used to prepare standards and sample preservatives. All compounds are used in small amounts and pose minimal threat to the environment if properly disposed.

14.2 For information about pollution prevention that may be applicable to laboratory operations, consult "Less Is Better: Laboratory Chemical Management for Waste Reduction" available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036.

15.0 WASTE MANAGEMENT

15.1 There are generally no waste management problems involved with discarding spent or left over samples in this method since most often the sample matrix is drinking water. If a sample is analyzed which appears to be highly contaminated with chemicals, analyses should be carried out to assess the type and degree of contamination so that the samples may be discarded properly. All other expired standards should be discarded properly. It is the laboratory's responsibility to comply with all applicable regulations for waste disposal. The Agency requires that laboratory waste management practices be conducted

consistent with all applicable rules and regulations, and that laboratories protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Also, compliance is required with any sewage discharge permits and regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management, consult "The Waste Management Manual for Laboratory Personnel" also available from the American Chemical Society at the address in Section 14.2.

16.0 REFERENCES

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17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

TABLE 1. ION ABUNDANCE CRITERIA FOR BIS(PERFLUOROPHENYL)PHENYL-PHOSPHINE OXIDE (DECAFLUOROTRIPHENYLPHOSPHINE OXIDE, DFTPPO)

Mass (M/z)	Relative Abundance Criteria	Purpose of Checkpoint[1]
77	present, major ion	low mass sensitivity
168	present, major ion	mid-mass sensitivity
169	4-10% of 168	mid-mass resolution and isotope ratio
271	present, major ion	base peak
365	5-10% of base peak	baseline threshold check
438	present	important high mass fragment
458	present	molecular ion
459	15-24% of mass 458	high mass resolution and isotope ratio

[1]All ions are used primarily to check the mass measuring accuracy of the mass spectrometer and data system, and this is the most important part of the performance test. The resolution checks, which include natural abundance isotope ratios, constitute the next most important part of the performance test. The correct setting of the baseline threshold, as indicated by the presence of low intensity ions, is the next most important part of the performance test. Finally, the ion abundance ranges are designed to encourage some standardization of fragmentation patterns.

TABLE 2. RETENTION TIME DATA AND QUANTITATION IONS FOR METHOD ANALYTES

Compound	Retention Time (min:sec) A[a]	Retention Time (min:sec) B[b]	Quantitation Ion (m/z)
benzidine	4.3	4.9	184
benzoylprop ethyl	24.8	31.3	105
caffeine	1.4	1.6	194
carbaryl	10.1	14.7	144
o-chlorophenyl thiourea	2.7	3.0	151
3,3'-dichlorobenzidine	16.6	22.7	252
3,3'-dimethoxybenzidine	8.1	11.5	244
3,3'-dimethylbenzidine	8.5	12.4	212
diuron	11.0	16.1	72
ethylene thiourea	1.2	1.4	102
linuron	16.0	21.9	161
rotenone	21.1	27.4	192
siduron	14.8	20.6	93
Surrogates:[c]			
benzidine-D_8	4.2	4.8	192
caffeine-$^{15}N_2$	1.3	1.6	196
3,3'-dichlorobenzidine-D_6	16.5	22.6	258
bis(perfluorophenyl)-phenylphosphine oxide	22.0	28.9	271

[a]These retention times were obtained on a Hewlett-Packard 1090 liquid chromatograph with a Waters C18 Novapak 15 cm x 2 mm column using gradient conditions given in Section 10.2.3.

[b]These retention times were obtained on a Waters 600 MS liquid chromatograph with a Waters C18 Novapak 15 cm x 2 mm column using gradient conditions given in Section 10.2.3.

[c]These compounds cannot be used if unlabelled compounds are present (See Section 4.1).

TABLE 3. ACCURACY AND PRECISION DATA FROM SIX DETERMINATIONS OF THE METHOD ANALYTES IN REAGENT WATER USING LIQUID-LIQUID EXTRACTION

Compound	True Conc. (µg/L)	Mean Observed Conc. (µg/L)	Std. Dev. (µg/L)	Rel. Std. Dev. (%)	Mean Method Accuracy (% of True Conc.)	Method Detection Limit (MDL) (µg/L)
benzidine	22.9	20.5	0.8	3.3	89.6	2.5
benzoylprop ethyl	32.5	33.0	1.1	3.3	101.6	3.7
caffeine	14.4	10.5	0.9	6.3	72.6	3.1
carbaryl	56.6	52.2	2.9	5.1	92.3	9.8
o-chlorophenyl thiourea	32.6	15.3	2.2	6.8	47.0	7.4*
3,3'-dichlorobenzidine	24.8	21.7	0.7	2.9	89.6	2.4
3,3'-dimethoxybenzidine	31.6	29.2	2.3	7.3	92.3	7.7
3,3'-dimethylbenzidine	31.7	31.8	1.0	3.1	100.4	3.3
diuron	25.0	26.2	1.3	5.1	104.8	4.4
ethylene thiourea	32.0	0.0	0.0	0.0	0.0	*
linuron	95.0	89.5	3.9	4.1	94.2	13.1
monuron	31.2	31.8	1.2	3.8	101.9	4.0
rotenone	50.3	44.9	9.4	18.8	89.3	31.6
siduron	27.9	29.6	1.4	5.2	106.3	4.7

*Recovery was not in the 70-130% range (See Section 9.3.3).

TABLE 4. ACCURACY AND PRECISION DATA FROM SEVEN DETERMINATIONS OF THE METHOD ANALYTES IN REAGENT WATER USING LIQUID-SOLID EXTRACTION (C_{18} LSE CARTRIDGE)

Compound	True Conc. (µg/L)	Mean Observed Conc. (µg/L)	Std. Dev. (µg/L)	Rel. Std. Dev. (%)	Mean Method Accuracy (% of True Conc.)	Method Detection Limit (MDL) (µg/L)
benzidine	22.9	12.2	1.7	13.7	53.2	5.3*
benzoylprop ethyl	32.5	29.3	2.0	6.9	90.2	6.3
caffeine	14.4	6.4	1.4	21.4	44.2	4.4*
carbaryl	56.6	53.9	1.8	3.3	95.2	5.7
o-chlorophenyl thiourea	32.6	0.0	0.0	0.0	0.0	*
3,3'-dichlorobenzidine	5.0	4.4	0.4	10.0	89.6	1.4
3,3'-dimethoxybenzidine	31.6	25.5	1.8	7.1	80.8	5.7
3,3'-dimethylbenzidine	31.7	31.4	1.0	3.1	99.0	3.0
diuron	25.0	24.4	1.4	5.6	97.6	4.4
ethylene thiourea	32.0	0.0	0.0	0.0	0.0	*
linuron	95.0	88.9	4.8	5.4	93.6	15.1
monuron	31.2	30.5	2.9	9.6	97.8	9.1
rotenone	50.3	45.0	2.4	5.4	89.6	7.5
siduron	27.9	24.8	2.0	7.9	88.9	6.3

*Recovery was not in the 70-130% range (See Section 9.3.3)

TABLE 5. ACCURACY AND PRECISION DATA FROM SIX DETERMINATIONS OF THE METHOD ANALYTES IN REAGENT WATER USING LIQUID-SOLID EXTRACTION (NEUTRAL POLYSTYRENE/DIVINYLBENZENE POLYMER LSE DISK)

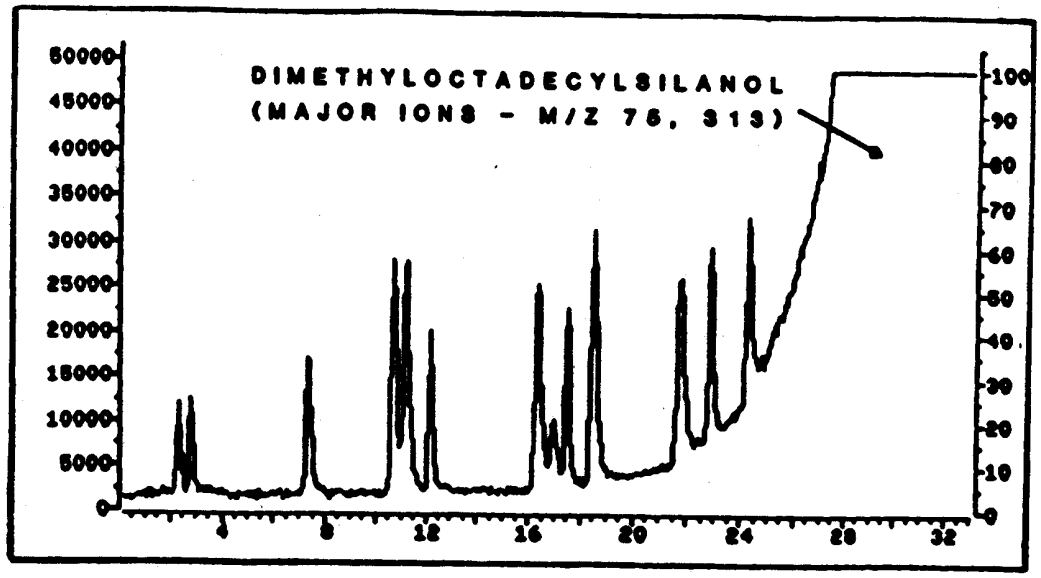
Compound	True Conc. (µg/L)	Mean Observed Conc. (µg/L)	Std. Dev. (µg/L)	Rel. Std. Dev. (%)	Mean Method Accuracy (% of True Conc.)	Method Detection Limit (MDL) (µg/L)
benzidine	22.9	24.7	2.4	9.8	108.0	8.1
benzoylprop ethyl	32.5	31.1	3.0	9.6	95.8	10.1
caffeine	14.4	0.7	0.5	72.5	5.2	1.8*
carbaryl	56.6	59.5	4.7	7.9	105.1	15.8
o-chlorophenyl thiourea	32.6	0.0	0.0	0.0	0.0	*
3,3'-dichlorobenzidine	5.0	5.0	0.5	9.4	101.7	1.6
3,3'-dimethoxybenzidine	31.6	32.8	2.2	6.7	103.8	7.4
3,3'-dimethylbenzidine	31.7	31.5	2.1	6.7	99.4	7.1
diuron	25.0	26.1	1.8	7.0	104.5	6.1
ethylene thiourea	32.0	0.0	0.0	0.0	0.0	*
linuron	95.0	97.9	8.7	9.0	103.0	29.3
monuron	31.2	34.4	2.5	7.3	110.4	8.4
rotenone	50.3	40.5	6.0	14.8	80.5	20.2
siduron	27.9	26.8	1.0	3.6	96.1	3.4

*Recovery was not in the 70-130% range (See Section 9.3.3).

TABLE 6. MEAN RECOVERIES, MULTILABORATORY PRECISION AND ESTIMATES OF SINGLE ANALYST PRECISION FOR THE MEASUREMENTS OF FOUR BENZIDINES BY LC/PB/MS

	10 µg/mL			100 µg/mL		
Compound	**Recovery (%)**	**RSD Multi-Lab**	**RSD Single Analyst**	**Recovery (%)**	**RSD Multi-Lab**	**RSD Single Analyst**
BZ	96	10	5.6	97	10	9.1
MB	104	20	18	95	10	7.0
LB	98	14	10	97	8.6	4.9
DB	96	18	9.4	97	9.1	4.6

C18 COLUMN FOLLOWING EXPOSURE TO AMMONIUM ACETATE



C18 COLUMN MAINTAINED WITH ACETONITRILE FLUSHING

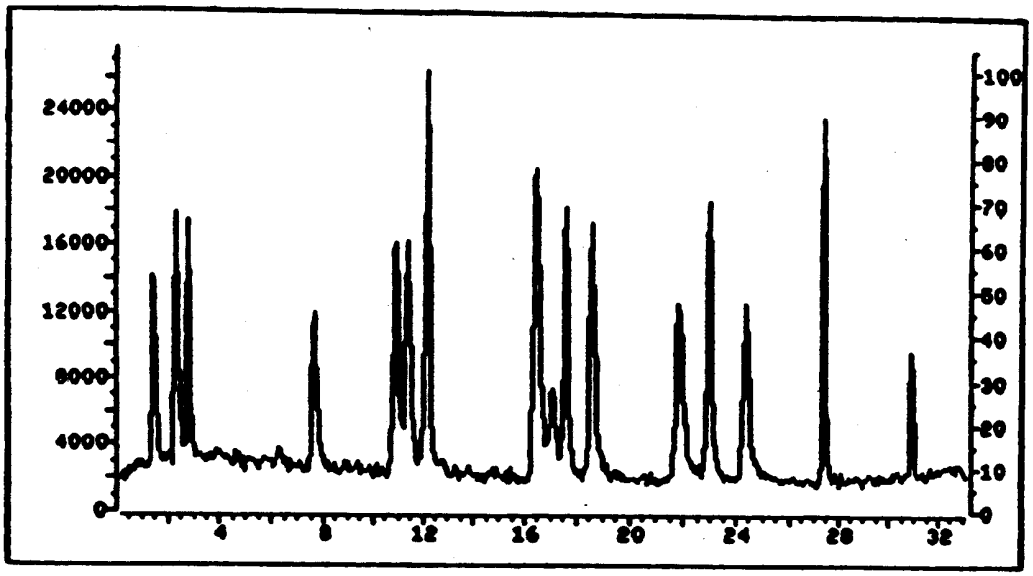


FIGURE 1. Unacceptable chromatogram with column bleed and acceptable chromatogram following column flushing.

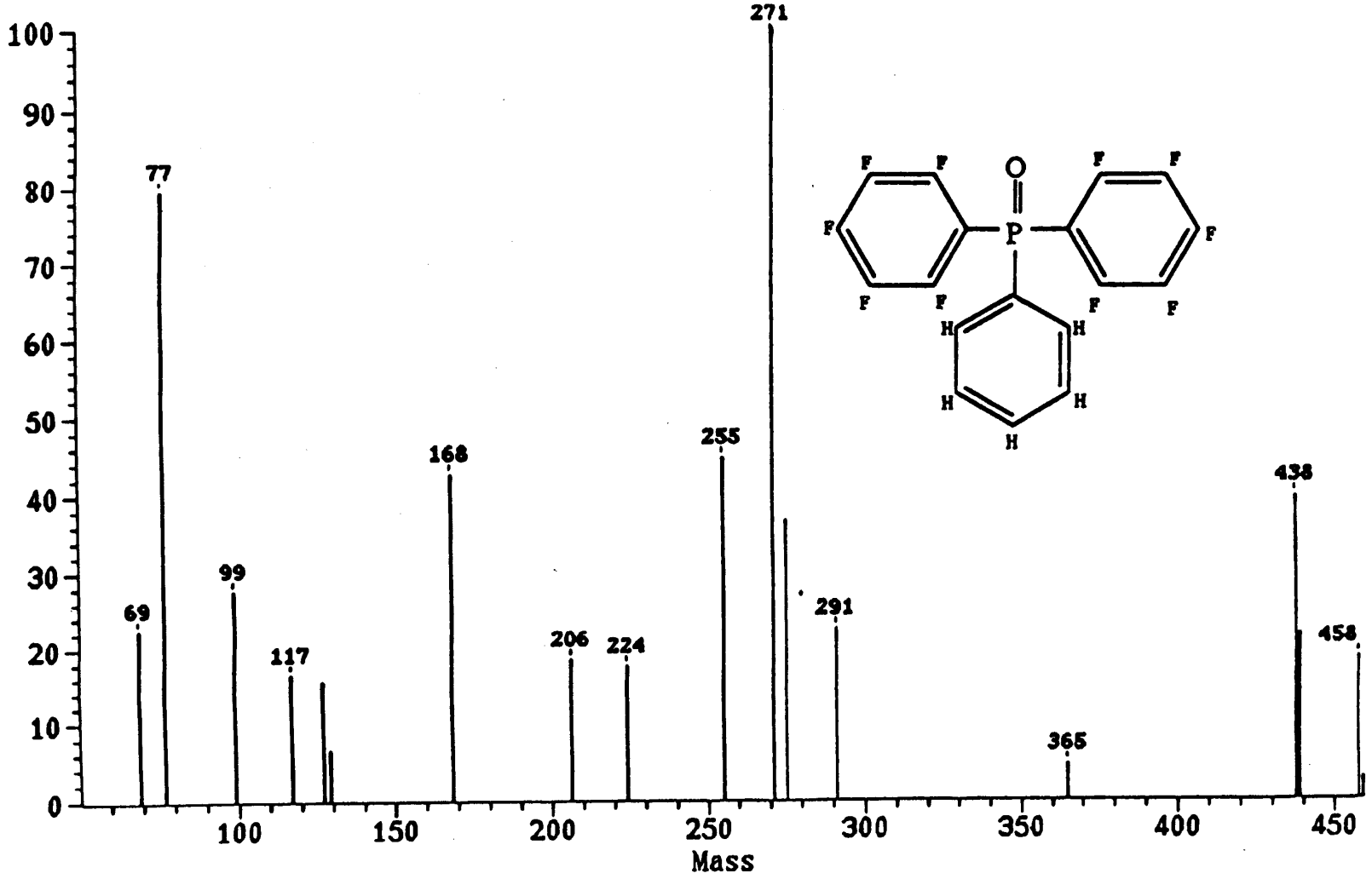


Figure 2. Average spectrum of DFTPPO from multilaboratory study.

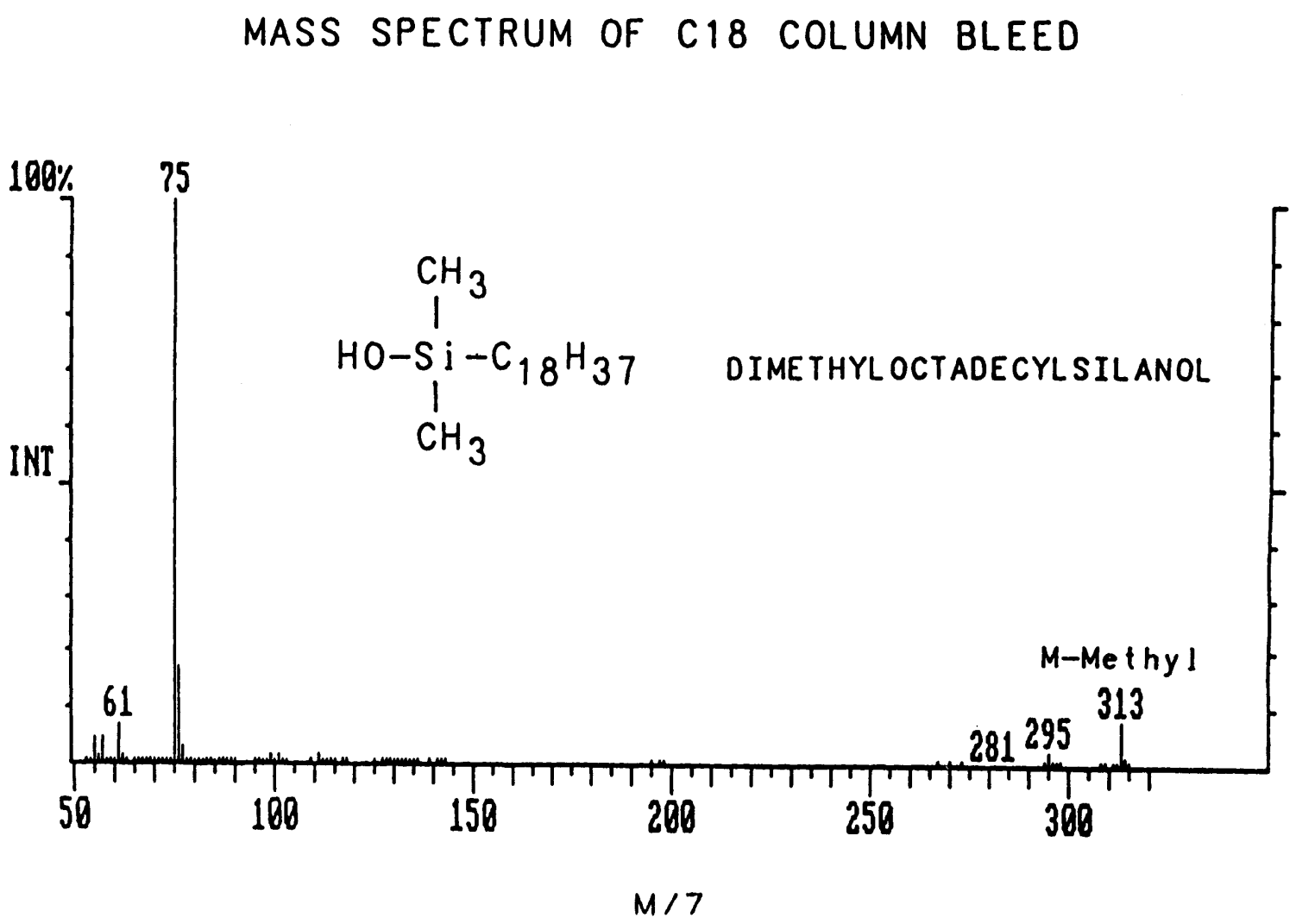


FIGURE 3. Mass spectrum of dimethyloctadecylsilanol, a common stationary phase bleed product.

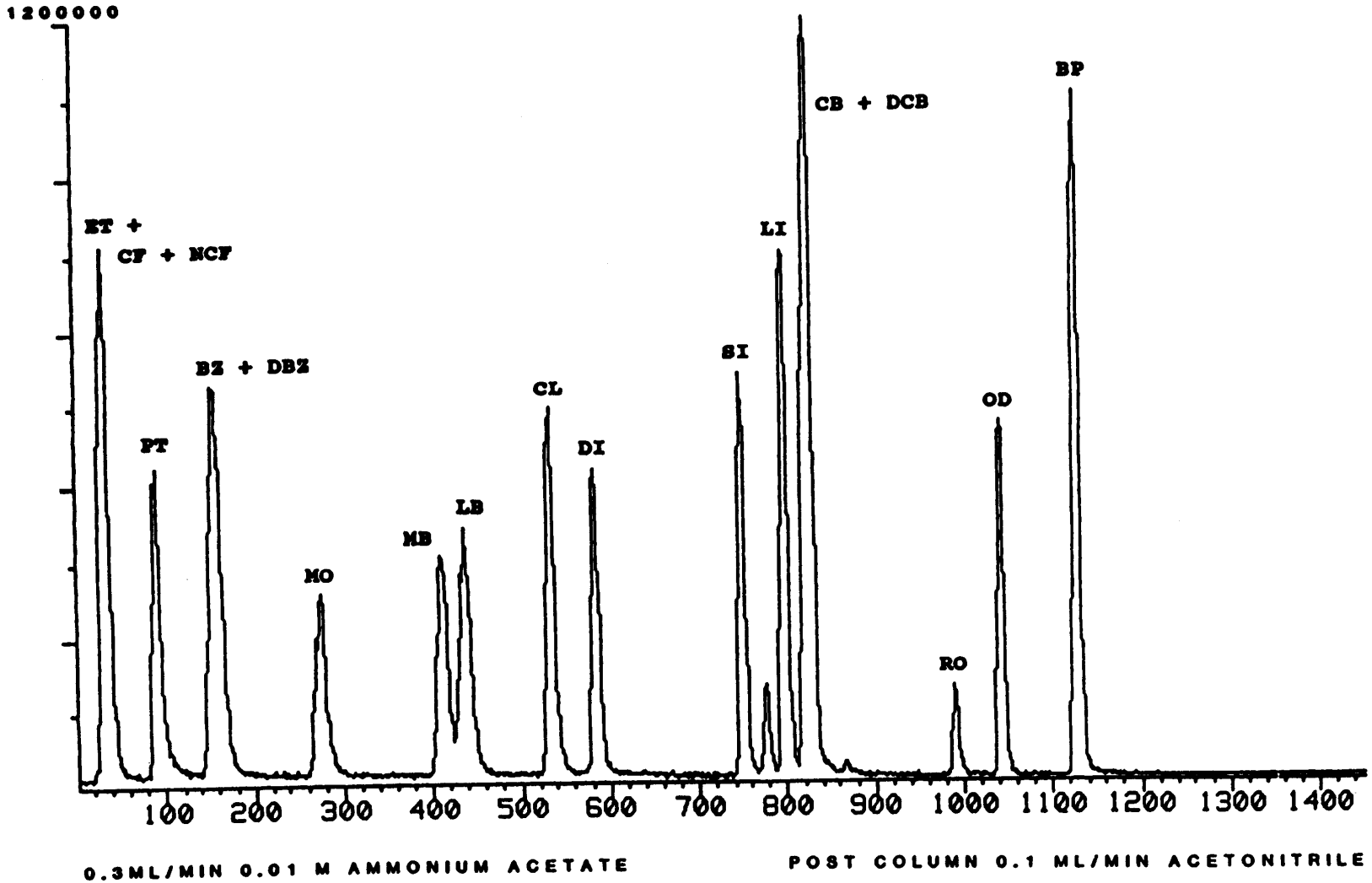


FIGURE 4. Total ion chromatogram of analytes and surrogates (140-950 ng injected).

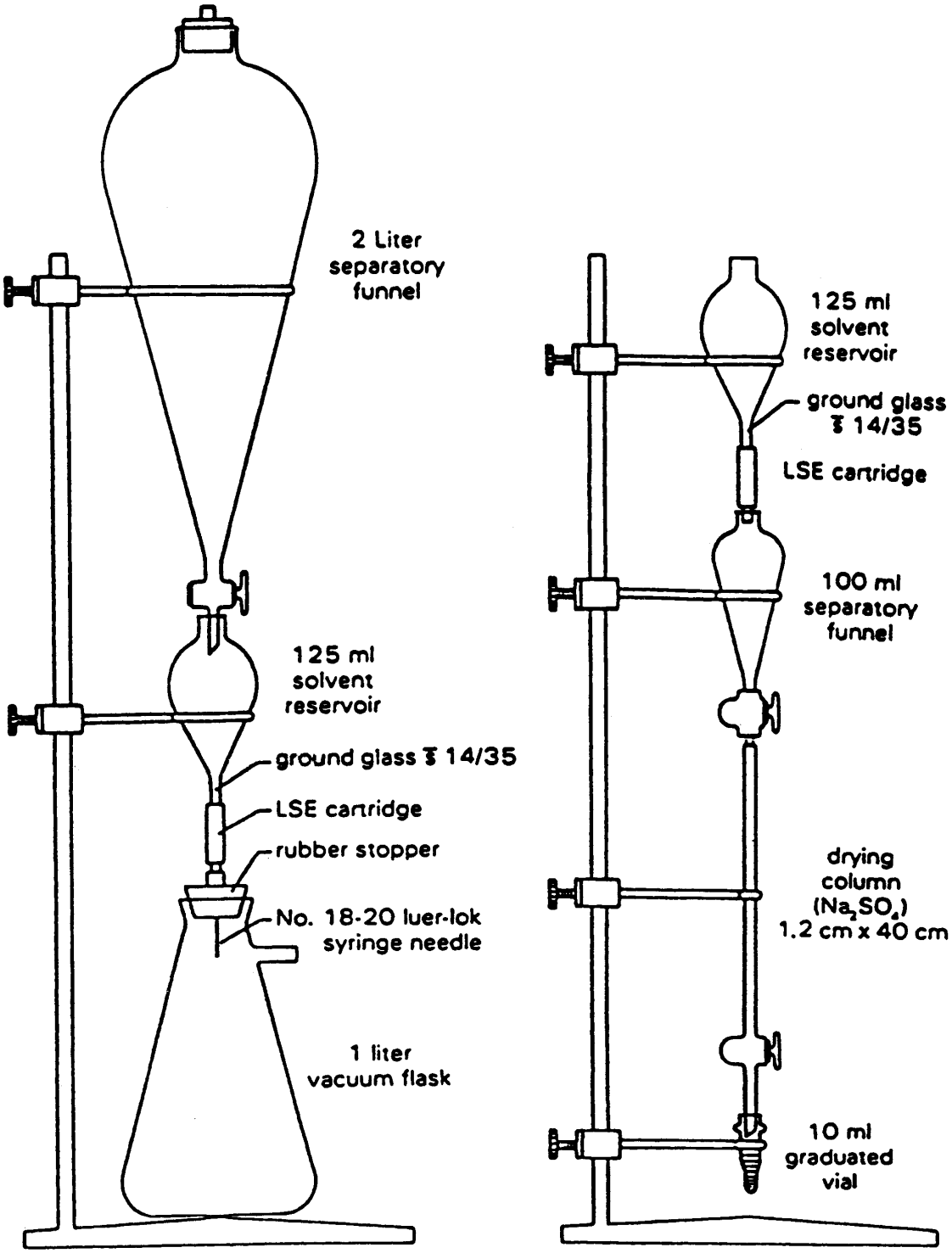


FIGURE 5. Schematic diagram of a liquid-solid extaction (LSE) apparatus.

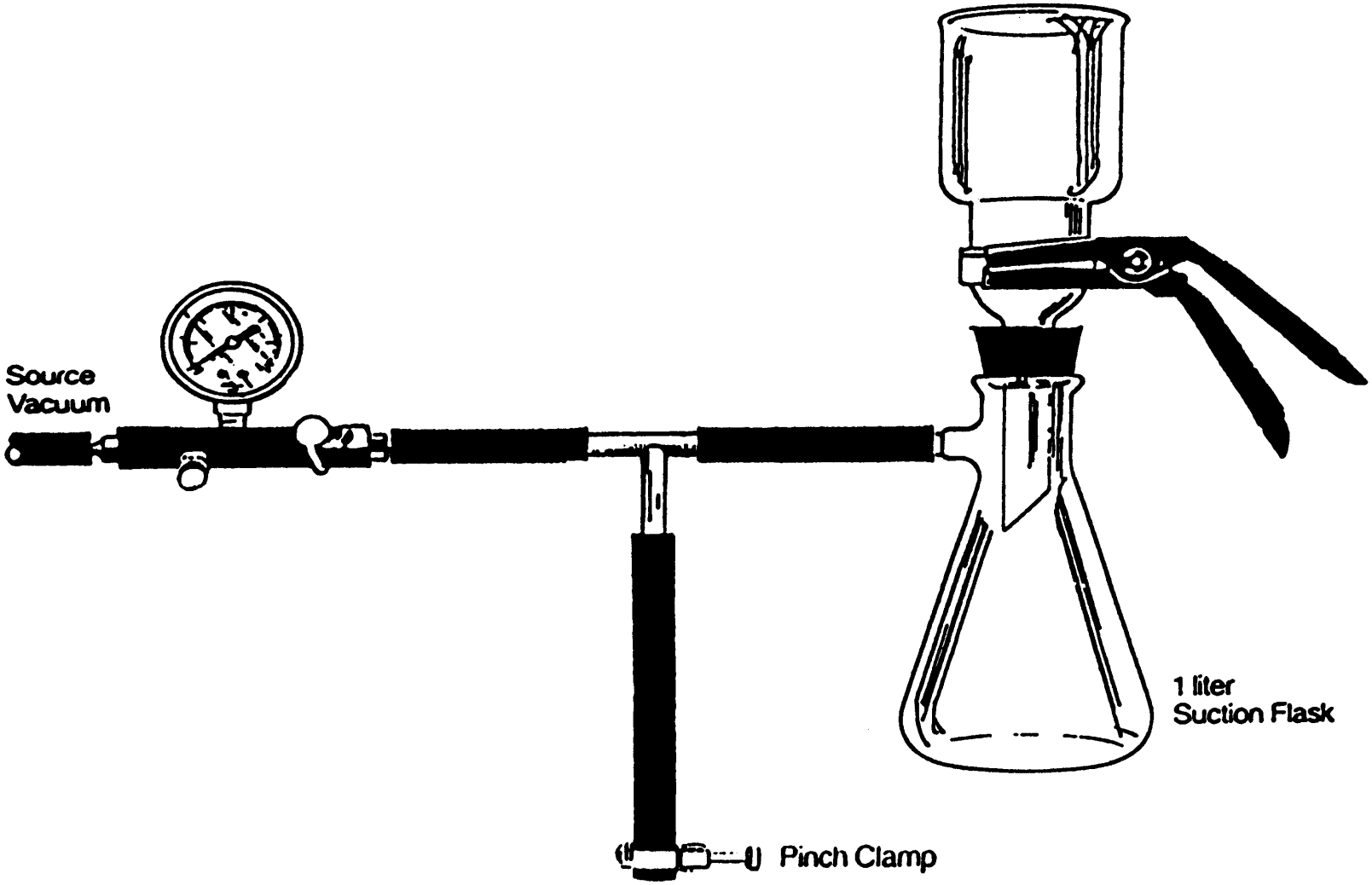


Figure 6. Schematic diagram of liquid-solid disk extraction apparatus.