

METHOD 8325

SOLVENT EXTRACTABLE NONVOLATILE COMPOUNDS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY/PARTICLE BEAM/MASS SPECTROMETRY (HPLC/PB/MS)

1.0 SCOPE AND APPLICATION

1.1 This method describes the use of high performance liquid chromatography (HPLC), coupled with particle beam (PB) mass spectrometry (MS), for the determination of benzidines and nitrogen-containing pesticides in water and wastewater. The following compounds can be determined by this method:

Compound	CAS No. ^a
Benzidine	92-87-5
Benzoylprop ethyl	33878-50-1
Carbaryl	63-25-2
o-Chlorophenyl thiourea	5344-82-1
3,3'-Dichlorobenzidine	91-94-1
3,3'-Dimethoxybenzidine	119-90-4
3,3'-Dimethylbenzidine	612-82-8
Diuron	330-54-1
Linuron (Lorox)	330-55-2
Monuron	150-68-5
Rotenone	83-79-4
Siduron	1982-49-6

^a Chemical Abstract Service Registry Number

1.2 The method also may be appropriate for the analysis of benzidines and nitrogen-containing pesticides in non-aqueous matrices. The method may be applicable to other compounds that can be extracted from a sample with methylene chloride and are amenable to separation on a reverse phase liquid chromatography column and transferable to the mass spectrometer with a particle beam interface.

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

1.4 Tables 4 - 6 present method detection limits (MDLs) for the target compounds, ranging from 2 to 25 µg/L. The MDLs are compound- and matrix-dependent.

1.5 This method is restricted to use by, or under the supervision of, analysts experienced in the use of HPLC and skilled in the interpretation of particle beam mass spectrometry. Each analyst must demonstrate the ability to generate acceptable results with this method.

2.0 SUMMARY OF METHOD

2.1 The target compounds for this method must be extracted from the sample matrix prior to analysis.

2.1.1 Benzidines and nitrogen-containing pesticides are extracted from aqueous matrices at a neutral pH with methylene chloride, using a separatory funnel (Method 3510), a continuous liquid-liquid extractor (Method 3520), or other suitable technique.

2.1.2 Solid samples are extracted using Methods 3540 (Soxhlet), 3541 (Automated Soxhlet), 3550 (Ultrasonic extraction), or other suitable technique.

2.2 An aliquot of the sample extract is introduced into the HPLC instrument and a gradient elution program is used to chromatographically separate the target analytes, using reverse-phase liquid chromatography.

2.3 Once separated, the analytes are transferred to the mass spectrometer via a particle beam HPLC/MS interface. Quantitation is performed using an external standard approach.

2.4 An optional internal standard quantitation procedure is included for samples which contain coeluting compounds or where matrix interferences preclude the use of the external standard procedure.

2.5 The use of ultraviolet/visible (UV/VIS) detection is an appropriate option for the analysis of routine samples, whose general composition has been previously determined.

3.0 INTERFERENCES

3.1 Refer to Methods 3500 and 8000 for general discussions of interferences with the sample extraction and chromatographic separation procedures.

3.2 Although this method relies on mass spectrometric detection, which can distinguish between chromatographically co-eluting compounds on the basis of their masses, co-elution of two or more compounds will adversely affect method performance. When two compounds coelute, the transport efficiency of both compounds through the particle beam interface generally improves, and the ion abundances observed in the mass spectrometer increase. The degree of signal enhancement by coelution is compound-dependent.

3.2.1 This coelution effect invalidates the calibration curve and, if not recognized, will result in incorrect quantitative measurements. Procedures are given in this method to check for co-eluting compounds, and must be followed to preclude inaccurate measurements.

3.2.2 An optional internal standard calibration procedure has been included for use in instances of severe co-elution or matrix interferences.

3.3 A major source of potential contamination is HPLC columns which may contain silicon compounds and other contaminants that could prevent the determination of method analytes. Generally, contaminants will be leached from the columns into mobile phase and produce a variable background. Figure 1 shows unacceptable background contamination from a column with stationary phase bleed.

3.4 Contamination may occur when a sample containing low analyte concentrations is analyzed immediately after a sample containing relatively high analyte concentrations. After analysis of a sample containing high analyte concentrations, one or more method blanks should be analyzed. Normally, with HPLC, this is not a problem unless the sample concentrations are at the percent level.

4.0 APPARATUS AND MATERIALS

4.1 High performance liquid chromatograph (HPLC) - An analytical system with programmable solvent delivery system and all necessary accessories including 5 μ L injection loop, analytical columns, purging gases, etc. The solvent delivery system must be capable, at a minimum, of handling a binary solvent system, and must be able to accurately deliver flow rates between 0.20 - 0.40 mL/min. Pulse dampening is recommended, but not required. The chromatographic system must be able to be interfaced with a mass spectrometer (MS). An autoinjector is recommended and should be capable of accurately delivering 1 - 10 μ L injections without affecting the chromatography.

4.1.1 HPLC Columns - An analytical column is needed, and a guard column is highly recommended.

4.1.1.1 Analytical Column - Reverse phase column, C₁₈ chemically bonded to 4-10 μ m silica particles, 150 - 200 mm x 2 mm, (Waters C-18 Novapak or equivalent). 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. Select a column that exhibits minimal bleeding. New columns must be conditioned overnight before use by pumping a 75 - 100% v/v acetonitrile:water solution through the column at a rate of about 0.05 mL/min. Other packings and column sizes may be used if appropriate performance can be achieved.

4.1.1.2 Guard Column - Packing similar to that used in analytical column.

4.1.2 HPLC/MS interface - The particle beam HPLC/MS interface must reduce the ion source pressure to a level compatible with the generation of classical electron ionization (EI) mass spectra, i.e., about 1×10^{-4} - 1×10^{-6} Torr, while delivering sufficient quantities of analytes to the conventional EI source to meet sensitivity, accuracy, and precision requirements. The concentrations of background components with masses greater than 62 Daltons should be reduced to levels that do not produce ions greater than a relative abundance of 10% in the mass spectra of the analytes.

4.2 Mass spectrometer system - The mass spectrometer must be capable of electron ionization at a nominal electron energy of 70 eV. The spectrometer should be capable of scanning from 45 to 500 amu in 1.5 seconds or less (including scan overhead). The spectrometer should produce a mass spectrum that meets the criteria in Table 1 when 500 ng or less of DFTPPO are introduced into the HPLC.

4.3 Data system - A computer system must be interfaced to the mass spectrometer, and must be capable of the continuous acquisition and storage on machine-readable media of all mass spectra obtained throughout the duration of the chromatographic program. The computer software must be capable of searching any HPLC/MS data file for ions of a specified mass and plotting such abundance data versus time or scan number.

4.4 Volumetric flasks - Class A, in various sizes, for preparation of standards.

4.5 Vials - 10-mL amber glass vials with polytetrafluoroethylene (PTFE)-lined screw caps or crimp tops.

4.6 Analytical balance - capable of weighing 0.0001 g.

4.7 Extract filtration apparatus

4.7.1 Syringe - 10-mL, with Luer-Lok fitting.

4.7.2 Syringe filter assembly, disposable - 0.45 μm pore size PTFE filter in filter assembly with Luer-Lok fitting (Gelman Acrodisc, or equivalent).

5.0 REAGENTS

5.1 Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

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

5.3 Solvents - All solvents must be HPLC-grade or equivalent.

5.3.1 Acetonitrile, CH_3CN

5.3.2 Methanol, CH_3OH

5.3.3 Ammonium acetate, $\text{NH}_4\text{OOCCH}_3$, (0.01M in water).

5.4 Mobile phase - Two mobile phase solutions are needed, and are designated Solvent A and Solvent B. Degas both solvents in an ultrasonic bath under reduced pressure and maintain by purging with a low flow of helium.

5.4.1 Solvent A is a water:acetonitrile solution (75/25, v/v) containing ammonium acetate at a concentration of 0.01M.

5.4.2 Solvent B is 100 % acetonitrile.

5.5 Stock standard solutions - Stock solutions may be prepared from pure standard materials or purchased as certified solutions. Commercially-prepared stock standards may be used at any concentration if they are certified by the manufacturer.

5.5.1 Prepare stock standard solutions by accurately weighing 0.0100 g of pure material in a volumetric flask. Dilute to known volume in a volumetric flask. If compound purity is certified at 96% or greater, the weight may be used without correction to calculate the concentration of the stock standard. Commercially-prepared stock standards may be used at any concentration if they are certified by the manufacturer or by an independent source.

5.5.1.1 Dissolve benzidines and nitrogen-containing pesticides in methanol, acetonitrile, or organic-free reagent water.

5.5.1.2 Certain analytes, such as 3,3'-dimethoxybenzidine, may require dilution in 50% (v/v) acetonitrile:water or methanol:water solution.

5.5.1.3 Benzidines may be used for calibration purposes in the free base or acid chlorides forms. However, the concentration of the standard should be calculated as the free base.

5.5.2 Transfer the stock standard solutions into amber bottles with PTFE-lined screw-caps or crimp tops. Store at -10°C or less and protect from light. Stock standard solutions should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.

5.6 Surrogate spiking solution - The recommended surrogates are benzidine- D_8 , caffeine- $^{15}\text{N}_2$, 3,3'-dichlorobenzidine- D_6 , and bis(perfluorophenyl)-phenylphosphine oxide. 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 $\mu\text{g}/\text{L}$ of each surrogate). Store the surrogate spiking solution in an amber vial in a freezer at -10°C or less.

5.7 MS performance check solution - Prepare a 100 ng/ μL solution of DFTPPO in acetonitrile. Store this solution in an amber vial in a freezer at -10°C or less.

5.8 Calibration solutions

This method describes two types of calibration procedures that may be applied to the target compounds: external standard calibration, and internal standard calibration. Each procedure requires separate calibration standards. In addition, the performance characteristics of the HPLC/PB/MS system indicate that it may be necessary to employ a second order regression for calibration purposes, unless a very narrow calibration range is chosen. See Method 8000 for additional information on non-linear calibration techniques.

5.8.1 For external standard calibration, prepare calibration standards for all target compounds and surrogates in acetonitrile. DFTPPO may be added to one or more calibration solutions to verify MS tune (see Sec. 7.3). Store these solutions in amber vials at -10°C or less. Check these solutions at least quarterly for signs of deterioration.

5.8.2 Internal standard calibration requires the use of suitable internal standards (see Method 8000). Ideally, stable, isotopically-labeled, analogs of the target compounds should be used. These labeled compounds are included in the calibration standards and must also be added to each sample extract immediately prior to analysis. Prepare the calibration standards in a fashion similar to that for external standard calibration, but include each internal standard in each of the calibration standards.

The concentration of the internal standards should be 50 - 100 times the lowest concentration of the unlabeled target compounds. In addition, the concentration of the internal standards does not vary with the concentrations of the target compounds, but is held constant. Store these solutions in amber vials at -10°C or less. Check these solutions at least quarterly for signs of deterioration.

5.9 Internal standard spiking solution - This solution is required when internal standard quantitation is used. Prepare a solution containing each of the internal standards that will be used for quantitation of target compounds (see Sec. 5.8.2) in methanol. The concentration of this solution must be such that a 1- μ L volume of the spiking solution added to a 1-mL final extract will result in a concentration of each internal standard that is equal to the concentration of the internal standard in the calibration standards in Sec. 5.8.2. Store this solution in an amber vial at -10°C or less. Check this solution at least quarterly for signs of deterioration. This solution is not necessary if only external standard calibration will be used.

5.10 Sodium chloride, NaCl - granular, used during sample extraction.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

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

6.2 Samples should be extracted within 7 days and analyzed within 30 days of extraction. Extracts should be stored in amber vials at -10°C or less.

7.0 PROCEDURE

7.1 Samples may be extracted by Method 3510 (separatory funnel), Method 3520 (continuous extractor), Method 3535 (solid-phase extraction), or other appropriate technique. Prior to extraction, add a 10- μ L aliquot of the surrogate spiking solution and 100 g of sodium chloride to the sample, and adjust the pH of the sample to 7.0. Samples of other matrices should be extracted by an appropriate sample preparation technique. The concentration of surrogates in the sample should be 20-50 times the method detection limit. Concentrate the extract to 1 mL, and exchange the solvent to methanol, following the procedures in the extraction method.

7.2 Establish chromatographic, particle beam interface, and mass spectrometer conditions, using the following conditions as guidance.

Mobile phase purge:	Helium at 30 mL/min, continuous
Mobile phase flow rate:	0.25 - 0.3 mL/min through the column
Gradient elution:	Hold for 1 min at 25% acetonitrile (Solvent A), then program linearly to about 70% acetonitrile (60% Solvent B) in 29 min. Start data acquisition immediately.
Desolvation chamber temperature:	45 - 80°C
Ion source temperature:	250 - 290°C
Electron energy:	70 eV
Scan range:	62 to 465 amu, at \leq 1.5 sec/scan

NOTE: Post-column addition is an option that improves system precision and, thereby, may improve sensitivity. Post-column flow rates depend on the requirements of the interface and may range from 0.1 to 0.7 mL/min of acetonitrile. Maintain a minimum of 30% acetonitrile in the interface.

Analyte-specific chromatographic conditions are also shown in Table 2. (The particle beam interface conditions will depend on the type of nebulizer).

7.2.1 The analyst should follow the manufacturer's recommended conditions for their interface's optimum performance. The interface is usually optimized during initial installation by flow injection with caffeine or benzidine, and should utilize a mobile phase of acetonitrile/water (50/50, v/v). Major maintenance may require re-optimization.

7.2.2 Fine tune the interface by making a series of injections into the HPLC column of a medium concentration calibration standard and adjusting the operating conditions (Sec. 7.2) until optimum sensitivity and precision are obtained for the maximum number of target compounds.

7.3 Initial calibration

7.3.1 Once the operating conditions have been established, calibrate the MS mass and abundance scales using DFTPPO to meet the recommended criteria in Table 1.

7.3.2 Inject a medium concentration standard containing DFTPPO, or separately inject into the HPLC a 5- μ L aliquot of the 100 ng/ μ L DFTPPO solution and acquire a mass spectrum. Use HPLC conditions that produce a narrow (at least ten scans per peak) symmetrical peak. If the spectrum does not meet the 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 HPLC peak may be used to evaluate the performance of the system.

Manual (not automated) ion source tuning procedures specified by the manufacturer should be employed during tuning. Mass calibration should be accomplished while an acetonitrile/water (50/50, v/v) mixture is pumped through the HPLC 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 exhibit short-term variations with particle beam interfaces and gradient elution conditions).

7.3.3 System performance criteria for the medium concentration standard - Evaluate the stored HPLC/MS data with the data system software and verify that the HPLC/PB/MS system meets the following performance criteria.

7.3.3.1 HPLC performance - 3,3'-dimethylbenzidine 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 HPLC column requires maintenance. See Sec. 7.4.6.

7.3.3.2 Peak tailing - Examine a total ion chromatogram and examine the degree of peak tailing. Severe tailing indicates a major problem and system maintenance is required to correct the problem. See Sec. 7.4.6

7.3.3.3 MS sensitivity - The signal-to-noise ratio for any compound's spectrum should be at least 3:1.

7.3.3.4 Column bleed - Figure 1 shows an unacceptable chromatogram with column bleed. Figure 2 shows an acceptable ion chromatogram. Figure 3 is the mass spectrum of dimethyloctadecyl-silanol, a common stationary phase bleed product. If unacceptable column bleed is present, the column must be changed or conditioned to produce an acceptable background.

7.3.3.5 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% of the concentration of the target compound, either conditions must be adjusted to resolve the components, or internal standard calibration must be used.

7.3.4 Once optimized, the same instrument operating conditions must be used for the analysis of all calibration standards, samples, blanks, etc.

7.3.5 Once all the performance criteria are met, inject a 5- μ L aliquot of each of the other calibration standards using the same HPLC/MS conditions.

7.3.5.1 The general method of calibration is a second order regression of integrated ion abundances of the quantitation ions (Table 3) 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. (See Method 8000 for the appropriate number of standards to be employed for a non-linear calibration). Non-linear calibration models can be applied to either the external standard or the internal standard calibration approaches described here.

7.3.5.2 For some analytes the instrument response may be linear over a narrow concentration range. In these instances, an average calibration factor (external standard) or average response factor (internal standard) may be employed for sample quantitation (see Method 8000).

7.3.6 If a linear calibration model is used, calculate the mean calibration factor or response factor for each analyte, including the surrogates, as described in Method 8000. Calculate the standard deviation (SD) and the relative standard deviation (RSD) as well. The RSD of an analyte or surrogate must be less than or equal to 20%, if the linear model is to be applied. Otherwise, proceed as described in Method 8000.

7.4 Calibration verification

Prior to sample analysis, verify the MS tune and initial calibration at the beginning of each 8-hour analysis shift using the following procedure:

7.4.1 Inject a 5- μ L aliquot of the DFTPPO solution or a mid-level calibration standard containing 500 ng of DFTPPO, and acquire a mass spectrum that includes data for m/z 62-465. If the spectrum does not meet the criteria in Table 1, the MS must be retuned to meet the criteria before proceeding with the continuing calibration check.

7.4.2 Inject a 5- μ L aliquot of a medium concentration calibration solution and analyze with the same conditions used during the initial calibration.

7.4.3 Demonstrate acceptable performance for the criteria shown in Sec. 7.3.3.

7.4.4 Using the initial calibration (either linear or non-linear, external standard or internal standard), calculate the concentrations in the medium concentration calibration solution and compare the results to the known values in the calibration solution. If calculated concentrations deviate by more than 20% from known values, adjust the instrument and inject the standard again. If the calibration cannot be verified with the second injection, then a new

initial calibration must be performed after taking corrective actions such as those described in Sec. 7.9.

7.5 Sample Analysis

7.5.1 The column should be conditioned overnight before each use by pumping a acetonitrile:water (70% v/v) solution through it at a rate of about 0.05 mL/min.

7.5.2 Filter the extract through a 0.45 μm filter. If internal standard calibration is employed, add 10 μL of the internal standard spiking solution to the 1-mL final extract immediately before injection.

7.5.3 Analyze a 5- μL aliquot of the extract, using the operating conditions established in Secs. 7.2 and 7.3.

7.6 Qualitative identification

The qualitative identification of compounds determined by this method is based on retention time and on comparison of the sample mass spectrum, after background correction, with characteristic ions in a reference mass spectrum. The reference mass spectrum must be generated by the laboratory using the conditions of this method. The characteristic ions from the reference mass spectrum are defined as the three ions of greatest relative intensity, or any ions over 30% relative intensity, if less than three such ions occur in the reference spectrum. Compounds are identified when the following criteria are met.

7.6.1 The intensities of the characteristic ions of a compound must maximize in the same scan or within one scan of each other. Selection of a peak by a data system target compound search routine where the search is based on the presence of a target chromatographic peak containing ions specific for the target compound at a compound-specific retention time will be accepted as meeting this criterion.

7.6.2 The retention time of the sample component is within $\pm 10\%$ of the retention time of the standard.

7.6.3 The relative intensities of the characteristic ions agree within 20% of the relative intensities of these ions in the reference spectrum. (Example: For an ion with an abundance of 50% in the reference spectrum, the corresponding abundance in a sample spectrum can range between 30% and 70%.)

7.6.4 Structural isomers that produce very similar mass spectra should be identified as individual isomers if they have sufficiently different HPLC retention times. Sufficient GC resolution is achieved if the height of the valley between two isomer peaks is less than 25% of the sum of the two peak heights. Otherwise, structural isomers are identified as isomeric pairs.

7.6.5 Identification is hampered when sample components are not resolved chromatographically and produce mass spectra containing ions contributed by more than one analyte. When HPLC peaks obviously represent more than one sample component (i.e., a broadened peak with shoulder(s) or a valley between two or more maxima), appropriate selection of analyte spectra and background spectra is important.

7.6.6 Examination of extracted ion current profiles of appropriate ions can aid in the selection of spectra, and in qualitative identification of compounds. When analytes coelute (i.e., only one chromatographic peak is apparent), the identification criteria may be met, but each analyte spectrum will contain extraneous ions contributed by the coeluting compound.

7.7 Quantitative Analysis

7.7.1 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. Peaks must be examined carefully for coeluting substances and if coeluting compounds are present at greater than 10% of the concentration of the target compound, either conditions must be adjusted to resolve the components, or the results for the target compound must be flagged as potentially positively biased.

7.7.2 Calculate the concentration of each analyte, using either the external standard or internal standard calibration. See Method 8000 for the specific equations to be employed for either the non-linear or linear calibration models.

7.7.3 If the response for any quantitation ion exceeds the initial calibration range of the HPLC/PB/MS system, the sample extract must be diluted and reanalyzed. When internal standard calibration is employed, additional internal standard must be added to the diluted extract to maintain the same concentration as in the calibration standards.

7.8 HPLC-UV/VIS Detection (optional)

7.8.1 Prepare calibration solutions as outlined in Sec. 5.8.

7.8.2 Inject 5 μ L of each calibration solution onto the HPLC, using the chromatographic conditions outlined in Secs. 7.2.1 and 7.2.2. Integrate the area under the full chromatographic peak at the optimum wavelength (or at 230 nm if that option is not available) for each target compound at each concentration.

7.8.3 The retention time of the chromatographic peak is an important criterion for analyte identification. Therefore, the ratio of the retention time of the sample analyte to the standard analyte should be 1.0 ± 0.1 .

7.8.4 Calculate calibration factors or response factors as described in Method 8000, for either external standard or internal standard calibration, and evaluate the calibration linearity as described in Method 8000.

7.8.5 Verify the calibration at the beginning of each 8-hour analytical shift, as described above.

7.8.6 Once the calibration has been verified, inject a 5- μ L aliquot of the sample extract, start the HPLC gradient elution, load and inject the sample aliquot, and begin data acquisition. Refer to Method 8000 for guidance on calculation of concentration.

7.9 Corrective Actions

When the initial calibration cannot be verified, one or more of the following corrective actions may be necessary.

7.9.1 Major maintenance such as cleaning an ion source, cleaning the entrance lens, quadrupole rods, etc., will require a new initial calibration.

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

7.9.3 Replace the mobile phases with fresh solvents. Verify that the flow rate from the HPLC pump is constant.

7.9.4 Flush the HPLC column with acetonitrile.

7.9.5 Replace the HPLC column. This action will cause a change in retention times.

7.9.6 Prepare fresh calibration solutions, and repeat the initial calibration step.

7.9.7 Replace any components that leak.

7.9.8 Replace the MS electron multiplier, or any other faulty components.

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

7.9.10 If peak areas are determined by the instrument software, verify values by manual integration.

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

7.9.12 Air leaks into the interface may effect the quality of the spectra (e.g., DFTPPO), especially when the ion source is operated at temperatures in excess of 280°C.

8.0 QUALITY CONTROL

8.1 Refer to Chapter One and Method 8000 for specific quality control (QC) procedures. Quality control procedures to ensure the proper operation of the various sample preparation techniques can be found in Method 3500. Each laboratory should maintain a formal quality assurance program. The laboratory should also maintain records to document the quality of the data generated.

8.2 Quality control procedures necessary to evaluate the HPLC system operation are found in Method 8000, Sec. 7.0 and includes evaluation of retention time windows, calibration verification and chromatographic analysis of samples. Necessary instrument QC is found in the following sections.

8.2.1 The HPLC/PB/MS system should be tuned to meet the DFTPPO criteria in Secs. 7.3.1 and 7.4.1.

8.2.2 There should be an initial calibration of the HPLC/PB/MS system as described in Sec. 7.3.

8.2.3 The HPLC/PB/MS system should meet the system performance criteria in Sec. 7.3.3, each 8 hours.

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

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

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

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

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

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

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

9.0 METHOD PERFORMANCE

9.1 Single laboratory accuracy and precision data for the benzidines and nitrogen-containing pesticides are presented in Tables 4 - 6. Five to seven 1-L aliquots of organic-free reagent water, containing approximately five times the MDL of each analyte, were analyzed with this procedure (Reference 1). The final extract volume was 0.5 mL for these determinations.

9.1.1 Method detection limits (MDLs) are presented in Tables 4 - 6.

9.1.2 A multi-laboratory (12 laboratories) validation of the determinative step was done for four of the analytes (benzidine, 3,3'-dimethoxybenzidine, 3,3'-dimethylbenzidine, 3,3'-dichlorobenzidine). Table 7 provides 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.

10.0 REFERENCES

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TABLE 1

ION ABUNDANCE CRITERIA FOR BIS(PERFLUOROPHENYL)PHENYLPHOSPHINE
(DECAFLUOROTRIPHENYLPHOSPHINE OXIDE, DFTPPO)

m/z	Relative Abundance	Purpose of Specification 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 The primary use of all the ions is to check the mass calibration of the mass spectrometer. The second use of these ions are the mass resolution checks, including the natural isotope abundance ratios. The correct setting of the baseline threshold is indicated by the presence of low intensity ions, and is the third use of this test. Finally, the ion abundance ranges may provide some standardization to fragmentation patterns of the target compounds.

TABLE 2

RECOMMENDED HPLC CHROMATOGRAPHIC CONDITIONS
FOR BENZIDINES AND NITROGEN-CONTAINING PESTICIDES

Initial Mobile Phase (v/v %)	Initial Time (min)	Gradient Time	Final Mobile Phase (v/v %)
75/25 (water ¹ /CH ₃ CN)	1	29	30/70 (water ¹ /CH ₃ CN)

¹ Water contains 0.01M ammonium acetate.

TABLE 3

RETENTION TIME DATA AND QUANTITATION IONS FOR TARGET COMPOUNDS

Compound	Retention Time System 1 ^a	Retention Time System 2 ^b	Quantitation Ion
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 Table 1.

^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 Sec. 7.2.

^c These compounds cannot be used as surrogates if their unlabeled analogs are present (see Sec. 3.2).

TABLE 4

ACCURACY AND PRECISION DATA FROM SIX DETERMINATIONS OF THE TARGET COMPOUNDS IN ORGANIC-FREE REAGENT WATER USING LIQUID-LIQUID EXTRACTION

Compound	True Conc. (µg/L)	Mean Observed Conc. (µg/L)	Std. Dev. (µg/L)	RSD (%)	Mean Accuracy (% of True)	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

* Not recovered

TABLE 5

ACCURACY AND PRECISION DATA FROM SEVEN DETERMINATIONS OF THE TARGET COMPOUNDS IN ORGANIC-FREE REAGENT WATER USING SOLID-PHASE EXTRACTION (C₁₈ CARTRIDGE)^a

Compound	True Conc. (µg/L)	Mean Observed Conc. (µg/L)	Std. Dev. (µg/L)	RSD (%)	Mean Accuracy (% of True)	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

^a Reagent water contained 0.01 M ammonium acetate.

* Not recovered.

TABLE 6

ACCURACY AND PRECISION DATA FROM SIX DETERMINATIONS OF THE TARGET COMPOUNDS IN ORGANIC-FREE REAGENT WATER USING SOLID-PHASE EXTRACTION (NEUTRAL POLYSTYRENE/DIVINYLBENZENE POLYMER DISK)

Compound	True Conc. (µg/L)	Mean Observed Conc. (µg/L)	Std. Dev. (µg/L)	RSD (%)	Mean Accuracy (% of True)	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

* Not recovered.

TABLE 7

MEAN RECOVERIES, MULTI-LABORATORY PRECISION AND ESTIMATES OF SINGLE ANALYST PRECISION FOR THE MEASUREMENTS OF FOUR BENZIDINES BY LC/PB/MS

Compound	<u>10 µg/L Test Conc.</u>			<u>100 µg/L Test Conc.</u>		
	Recovery (%)	RSD Multi-lab	RSD Single Analyst	Recovery (%)	RSD Multi-lab	RSD Single Analyst
Benzidine	96	10	5.6	97	10	9.1
3,3'-Dimethoxybenzidine	104	20	18	95	10	7.0
3,3'-Dimethylbenzidine	98	14	10	97	8.6	4.9
3,3'-Dichlorobenzidine	96	18	9.4	97	9.1	4.6

FIGURE 1

AN UNACCEPTABLE CHROMATOGRAM WITH COLUMN BLEED

C18 COLUMN FOLLOWING EXPOSURE TO AMMONIUM ACETATE

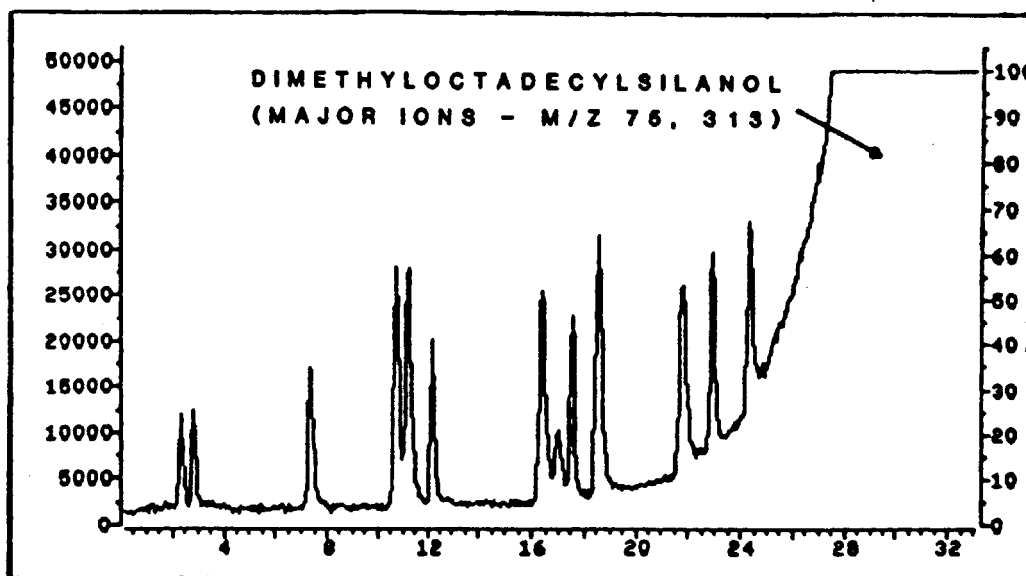


FIGURE 2

AN ACCEPTABLE CHROMATOGRAM FOLLOWING COLUMN FLUSHING

C18 COLUMN MAINTAINED WITH ACETONITRILE FLUSHING

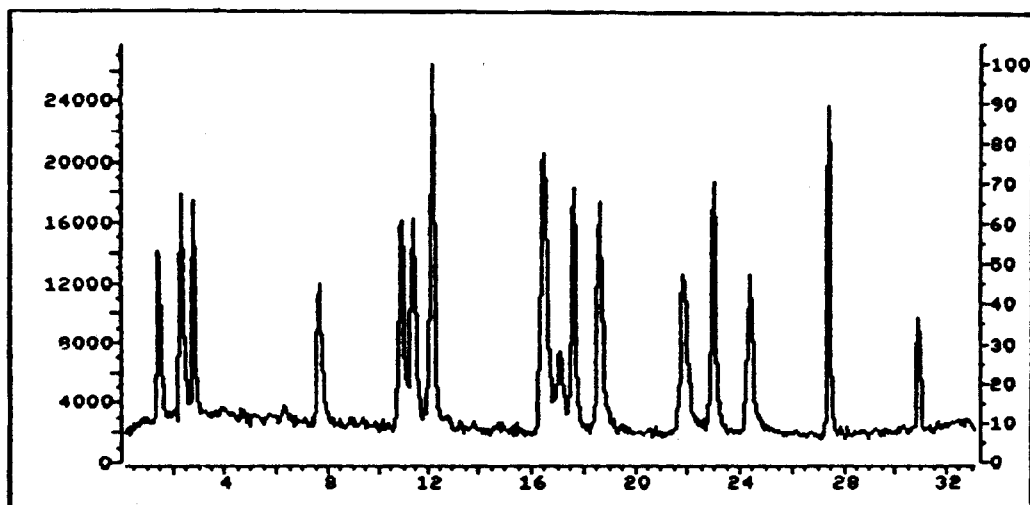


FIGURE 3

MASS SPECTRUM OF DIMETHYLOCTADECYL-SILANOL,
A COMMON STATIONARY PHASE BLEED PRODUCT

MASS SPECTRUM OF C18 COLUMN BLEED

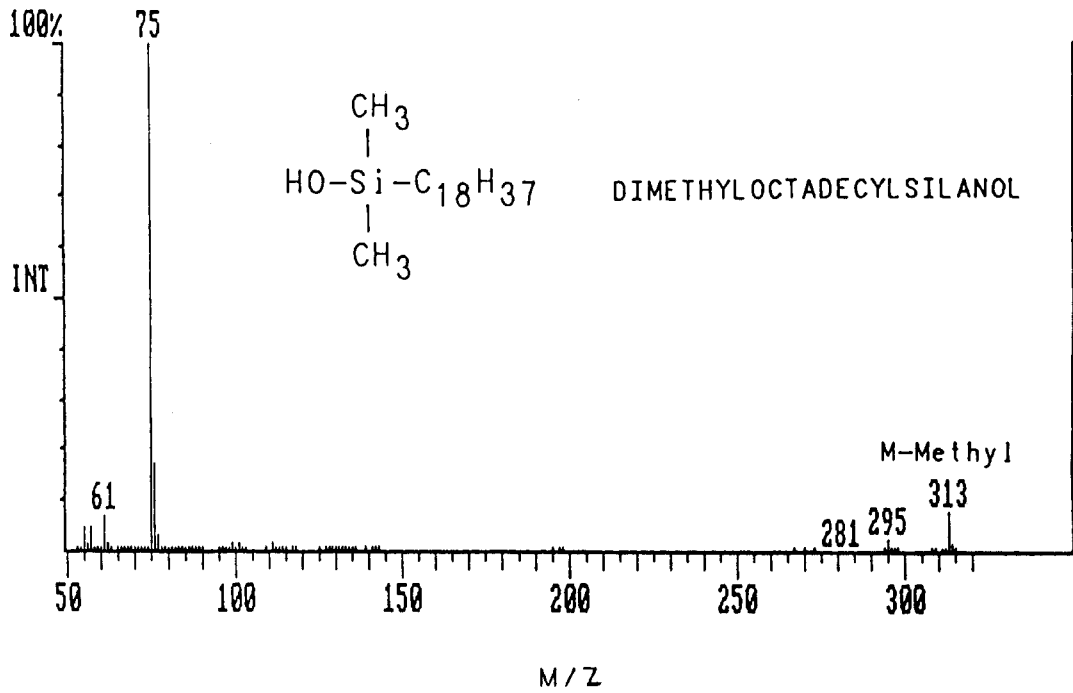
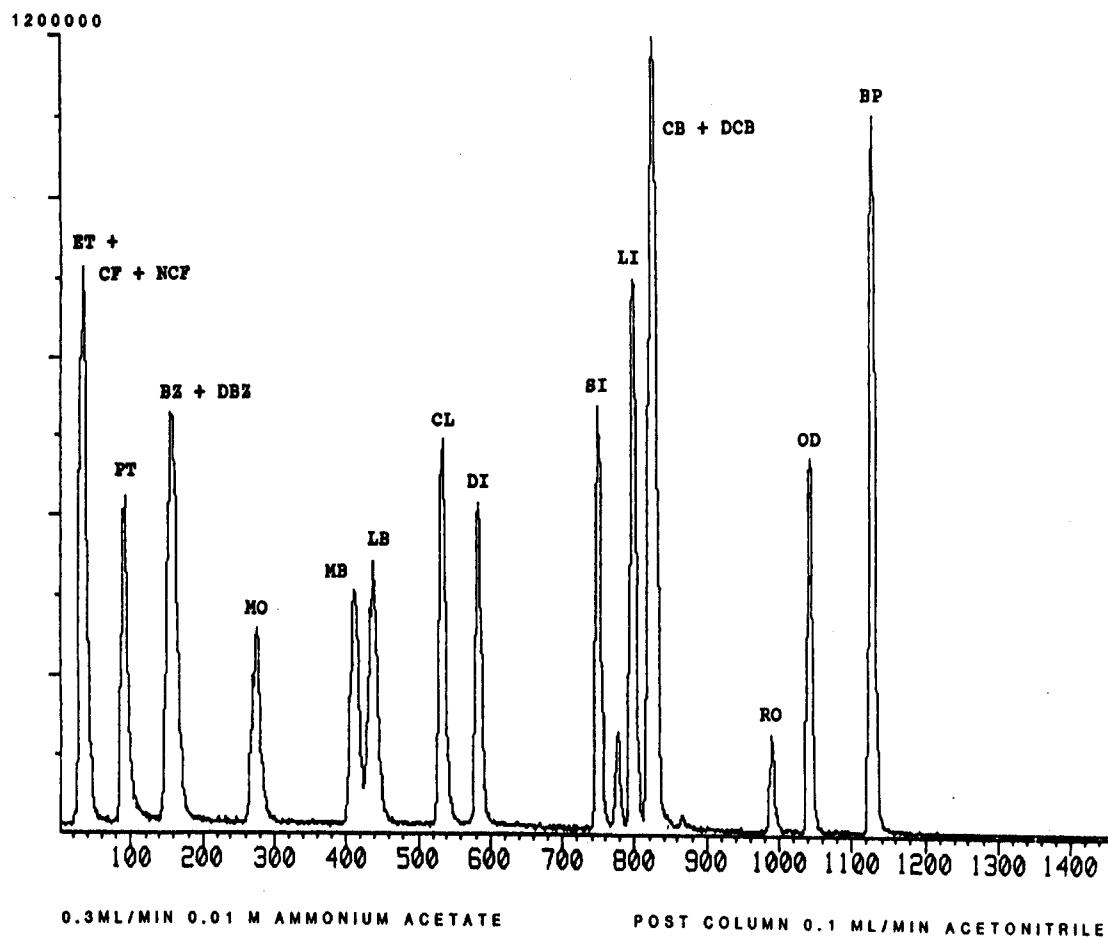


FIGURE 4

TOTAL ION CHROMATOGRAM OF ANALYTES AND SURROGATES
(140-950 ng Injected)



METHOD 8325

SOLVENT EXTRACTABLE NONVOLATILE COMPOUNDS BY
HIGH PERFORMANCE LIQUID CHROMATOGRAPHY/PARTICLE BEAM/MASS
SPECTROMETRY (HPLC/PB/MS)

