METHOD 8323

DETERMINATION OF ORGANOTINS BY MICRO-LIQUID CHROMATOGRAPHY- ELECTROSPRAY ION TRAP MASS SPECTROMETRY

1.0 SCOPE AND APPLICATION

1.1 This method covers the use of solid-phase extraction (SPE) discs, solvent extractions (for biological tissues), and micro-liquid chromatography (µLC) coupled with electrospray ion trap mass spectrometry (ES-ITMS) [this technique would also be applicable to ES-quadrupole mass spectrometry (ES-MS)] for the determination of organotins (as the cation) in waters and biological tissues. The following compounds can be determined by this method:

Compound Name	CAS No. [a]
Tributyltin chloride[b]	1461-22-9
Dibutyltin dichloride	683-18-1
Monobutyltin trichloride	1118-46-3
Triphenyltin chloride	668-34-8
Diphenyltin dichloride	1135-99-5
Monophenyltin trichloride	1124-19-2

[a] Chemical Abstract Service Registry Number.
[b] The organotins are listed as the chloride salt, however this method is designed to detect the free cation, whether from the chloride salt, oxide, etc., of the organotin.

1.2 Additionally, this method may be applicable to other non-volatile, water-soluble, polar, compounds that are amenable to extraction by SPE and are ionizable by µLC-ES-ITMS.

1.3 Method 8323 is designed to detect the organotin compounds (as the cation) without the use of hydrolysis and derivatization in the extraction procedure.

1.4 The compounds listed in this method were chosen for analysis by µLC-ES-ITMS because they have been designated as problem compounds that are hard to analyze by gas chromatographic methods. Currently (2002) no official Environmental Protection Agency (EPA) methodology exists for the determination of these compounds. The sensitivity of this method is dependent upon the level of interferants within a given matrix, and varies with compound class and even by compound within a class. Additionally, the sensitivity is dependent upon the mobile phase used with the µLC, as well as the electrospray voltages and tuning parameters used in optimizing the ES-ITMS.

1.5 The organotins are classified as hazardous materials, with varying degrees of toxicity. It is incumbent upon the analyst to review the Material Safety Data Sheets (MSDSs) for each of the compounds analyzed. Purified standard material and stock standard solutions should be handled in a hood and personal protective equipment should be used.

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

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

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

2.0 SUMMARY OF METHOD

2.1 This method provides reversed-phase micro-liquid chromatographic (µLC) and electrospray (ES) mass spectrometric (MS) conditions for the detection of the target analytes.

2.1.1 Sample extracts can be analyzed by direct injection into the electrospray (though interference is possible) or through a liquid chromatographic-electrospray interface.

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

2.1.3 Quantitative analysis may be performed by µLC-ES-ITMS, using an external standard approach. µ-LC-ES-ITMS detection is to be performed in the positive ionization mode, with either a ion trap mass spectrometer, or single-quadrupole mass spectrometer.

2.1.4 In some cases, the electrospray interface may introduce variability that leads to less precise quantitation.

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

2.2.1 Two-liter water samples are prepared first acidifying the waters with 12 N hydrochloric acid (HCl) and then the waters are extracted through pre-prepared solid-phase extraction (SPE) discs.

2.2.2 Tissue samples (e.g., fish, brain), are extracted by using a solvent mixture of hexane:acetic acid:tropolone (99:1:0.1 v/v). After sonication is completed, the sample is adjusted to pH 2 with a small quantity of 12N HCl. The acidified sample is then centrifuged for approximately thirty minutes.

2.3 Electrospray ionization is considered a “soft” ionization technique. Consequently, few ions are produced, usually the molecular ion plus some adduct ion from the mobile phase solutions. Based on the fact that ^{120}Sn - tin has ten isotopes electrospray ionization produces a distinctive mass spectral pattern (see fig. 1). However, if further confirmatory analysis is warranted, this can then be done by performing MS/MS experiments (for those analysts using an ion trap mass spectrometer) on those compounds of uncertain identity.

3.0 DEFINITIONS

Refer to the SW-846 chapter of terms and acronyms for potentially applicable definitions.

4.0 INTERFERENCES

4.1 Compounds with high proton affinity may mask the MS response of some of the target analytes. Therefore, except when the electrospray MS/MS system is used for rapid screening of samples (see Sec. 11.9.2), an HPLC must be used to perform the chromatographic separations necessary for quantitative analyses.

4.2 The organotins have an affinity for bonding to glass. In order to make sure of no background contamination glassware needs to be first washed with soapy water, deionized (DI) water, then placed in an HCl acid (< pH 2) bath for 24-hrs. After 24-hrs the glassware is removed rinsed with DI water, methanol, and then placed in a glassware drying oven (60 ^{o}F) until dry.

4.3 Solvents, reagents, glassware, and other sample processing hardware may yield discrete artifacts or elevated baselines, or both, causing misinterpretation of chromatograms or spectra. All of these materials must be demonstrated to be free from interferences under the conditions of the analysis by analyzing method blanks. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be necessary. Refer to each method for specific guidance on quality control procedures and to Chapter Four for guidance on the cleaning of glassware.

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

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

5.0 SAFETY

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

6.0 EQUIPMENT AND SUPPLIES

6.1 The mention of trade names or commercial products in this manual is for illustrative purposes only, and does not constitute an EPA endorsement or exclusive recommendation for use. The products and instrument settings cited in SW-846 methods represent those products and

settings used during method development or subsequently evaluated by the Agency. Glassware, reagents, supplies, equipment, and settings other than those listed in this manual may be employed provided that method performance appropriate for the intended application has been demonstrated and documented.

6.2 μ-LC/ITMS

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

6.2.1 Micro liquid chromatograph (μ-LC) - An analytical system with programmable solvent delivery system and all required accessories, including injection loop (with a minimum 1-μL loop volume), analytical columns, purging gases, etc. At a minimum, the solvent delivery system must be capable of delivering micro flow rates of 3 to 7 μL/min single solvent system. The chromatographic system must be capable of being interfaced with a mass spectrometer (MS).

6.2.2 μ-LC/ITMS interface

6.2.2.1 Interface - Electrospray ionization interface and source that will give acceptable calibration response for each analyte of interest at the concentration required. The source must be capable of generating both positive and negative ions.

6.2.3 Mass spectrometer system

6.2.3.1 An ion trap mass spectrometer capable of scanning from 1 to 2000 amu. For this method the ITMS should be scanned from 150 to 430 amu (full-scan mode) in 3 μscans with an ion injection of 200ms, in the positive ionization mode. In addition, the mass spectrometer must be capable of producing a calibrated mass spectrum for the recommended mass calibration tuning mixture, (see Sec. 7.7) or other compounds used for mass calibration.

6.2.3.2 Optional ion trap MS^n mode - capable of generating daughter ion spectra with a collision gas in the ion trap.

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

6.3 μ-LC columns - An analytical column is necessary.

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

6.3.1 Analytical Column - C_{18} reversed-phase column, 10 cm (of packing) x 160 µm ID, 5-µm particle size of ODS-Hypersil; or equivalent (for flow rates of 4 - 6 µL/min, if using the low flow-rate ES head); if using the higher ES flow-rate head, then use a narrow-bore column (2.1 mm I.D.) with a 0.4 mL/min flow through the column with a 40/60 split after the column, but before the ES (so that approx. 160 µL/min flow is entering the ES chamber).

6.4 Extraction equipment for organotin compounds

NOTE: **All glassware** used in the extraction and analysis of organotins **must be acid washed.** Wash glassware first in hot soapy water, rinse with DI water. Prepare an acid bath to pH 2, using 12 N HCl, place glassware in acid bath for 24 hrs. Remove from acid bath, rinse with DI water, rinse with methanol (collect methanol rinsate for proper disposal into hazardous waste bottles), place in oven, set at least 60°F, until dry, about 16 hrs. Remove glassware from oven, let cool, put away.

6.4.1 Water

6.4.1.1 2-L glass volumetric flasks

6.4.1.2 47-mm extraction vacuum manifold, complete with 1-L glass reservoirs, stainless steel screen, glass sample collection vessels (24 x 200 mm) [CPI International (Santa Rosa, CA) Accuprep 7000™ or equivalent]

6.4.1.3 C_{18} solid-phase extraction (SPE) disks, 47-mm [CPI nu-phase™ fiber]

6.4.1.4 Vacuum pump, for manifold

6.4.1.5 Disposable 90-mm glass pasteur pipettes.

6.4.1.6 Nitrogen blow-down apparatus [TurboVap® II (Zymark Corporation, Hopkinton, MA, USA) or equivalent].

6.4.1.7 50-mL glass blow down tubes, 0.5-mL graduated conical tips.

6.4.1.8 Screw-thread vials, 2.0 mL, with PTFE-lined screw-caps.

6.4.2 Tissue

6.4.2.1 Centrifuge

6.4.2.2 40-mL amber glass centrifuge tubes

6.4.2.3 Disposable 90-mm glass pasteur pipettes

6.4.2.4 100-mL glass beakers

6.4.2.5 Nitrogen blow-down apparatus [TurboVap® II (Zymark Corporation, Hopkinton, MA, USA) or equivalent].

6.4.2.6 50-mL glass blow down tubes, 0.5-mL graduated conical tips.

6.4.2.7 Screw-thread vials, 2.0 mL, with PTFE-lined screw-caps.

6.5 Automated pipettors (or equivalent) 1000-µL and 250-µL (Rainin or equivalent)

6.6 Balances - Analytical, 0.0010 g, top-loading, 0.01 g.

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

6.8 Graduated cylinders - 100-mL, 10-mL

7.0 REAGENTS AND STANDARDS

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

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

7.3 Acetic acid, CH_3CO_2H

7.4 Hydrochloric acid (12N), HCl

7.5 Solvents

The choice of solvent will depend on the analytes of interest and no single solvent is universally applicable to all analyte groups. Whatever solvent system is employed *including* those specifically listed in this method, the analyst *must* demonstrate adequate performance for the analytes of interest, at the levels of interest. At a minimum, such a demonstration will encompass the initial demonstration of proficiency described in Method 3500, using a clean reference matrix. Method 8000 describes procedures that may be used to develop performance criteria for such demonstrations as well as for matrix spike and laboratory control sample results.

All solvents should be pesticide quality or equivalent. Solvents may be degassed prior to use.

7.5.1 Methanol, CH_3OH - HPLC quality or equivalent.

7.5.2 Solution for standards and water extraction: Methanol/1% acetic acid, v/v

7.5.2.1 Solution for tissue extractions: Hexane (99%)/Acetic acid (1%)/tropolone (0.1%). Example: For a 250mL extraction solution you would have 247.5 mLs of hexane, 2.5 mLs of acetic acid and 250 mg of tropolone.

7.5.3 Mobile phase solution: 80% methanol/14% water/6% acetic acid/0.1% tropolone, v/v/v/w.

7.5.4 50:50 methanol:water, solution for mass calibration tuning standard.

7.6 Standard materials - pure standard materials or certified solutions of each analyte targeted for analysis.

7.7 HPLC/MS mass calibration tuning standard - Caffeine, UltraMark© 1621, and L-methionyl-arginyl-phenylalanyl-alanine acetate•H_2O (MRFA) are recommended by Thermoquest as tuning standards for their ES-ITMS. However, analysts may use other tuning standards as recommended by their instrument manufacturer or other documented source.

7.7.1 Caffeine - Prepare 1mg/mL stock solution of caffeine in 100% methanol.

7.7.2 Prepare a 1mL of 5 nmol/µL MRFA in 50:50 methanol:water. Obtain L-methionyl-arginyl-phenylalanyl-alanine acetate•H_2O, in this form the MRFA sample has a molecular weight of 607.7 amu. Carefully weigh 3.0 mg of MRFA. Dissolve the MRFA sample in a total volume of 1 mL of 50:50 methanol:water. Mix solution thoroughly.

7.7.3 Prepare 10 mL stock solution of 0.1% Ultramark© 1621 in acetonitrile. Measure out 10 µL of Ultramark© 1621 and dissolve it in 10 mL of acetonitrile. Mix thoroughly.

7.7.4 Prepare 5 mL of mass calibration solution. Pipet 100 µL of the stock solution of caffeine into a clean, dry vial. Pipet 15 µL of the stock solution of MRFA into the vial. Pipet 2.5 mL of the stock solution of Ultramark© 1621 into the vial. Pipet 50 µL of glacial acetic acid into the vial. Pipet 2.34 mL of 50:50 methanol:water into the vial. Mix solution thoroughly. Label vial and store in refrigerator (4°C) until it is needed.

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

NOTE: All organotins are very hazardous/toxic compounds. Good laboratory safety practice must be used when weighing and preparing the stock standard and calibration standard solutions.

7.8.1 Prepare stock standard solutions by accurately weighing 5.000 mg of pure material. Dissolve the material in methanol and dilute to known volume in a 10-mL volumetric flask. Store at 4°C and protect from light. Stock standard solutions should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards

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

7.9 Standard calibration standards - An initial check of the instrumentation should be the analysis of a 5-point calibration curve for each parameter of interest. A minimum of five different concentrations for each parameter of interest should be prepared through dilution of the stock standards with methanol/1%acetic acid. On a more daily basis, at least one of the calibration standards should correspond to a sample concentration at or below that necessary to meet the data quality objectives of the project. The remaining concentrations should correspond to the expected range of concentrations found in real samples, or should define the working range of the HPLC-ES/MS system (see Method 8000). Calibration standards must be replaced after one or two months, or sooner if comparison with check standards indicates a problem.

7.10 Surrogate standards - At this time there are no known surrogate standards to be used

with this method. Instead the analyst should monitor the performance of the extraction, cleanup (when used), and analytical system, along with the effectiveness of the method in dealing with each sample matrix, by including a spiked sample and analytical blank with each “batch” of extractions.

7.11 Internal standards - Currently this method does not use an internal standard, but instead uses the external standard procedure found in Method 8000, Sec 11.4.2. Refer to section 7.9.

7.12 Matrix spiking standards - Consult Method 3500 for information on matrix spiking solutions. Prepare a solution containing the analytes of interest in a suitable solvent, i.e. methanol, for organotins.

NOTE: The form of the compounds used for spiking should be identical to the form of the target analytes.

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 There are no databases indicating the best procedures for sample collection, preservation and handling, regarding organotins. However, best practices, as performed in ref. 8, would indicate the collection of samples (water, soil, sediment, tissue) into glass bottles and jars, fish can be wrapped in plastic. Water samples are collected into 4-L, or 3-L, amber bottles, sealed with parafilm, iced and shipped overnight to the laboratory for storage and subsequent analysis. For additional information, see the introductory material to Chapter Four, Organic Analytes, Sec. 4.1.

8.2 The best practices for preservation during shipment would indicate the immediate cooling of samples (4^{o}C for water, soil, sediment; dry ice for fish) and overnight shipment to the laboratory for processing and analysis. In the laboratory, the water, soil, sediment samples should be kept refrigerated (4^{o}C), and should be extracted and analyzed within a few days, ideally within 24-hrs of receipt. It has been demonstrated that the organotins can bond to glass surfaces. The fish samples should be homogenized into smaller sample portions and can be frozen (< -18^{o}C) indefinitely until extraction and analysis.

9.0 QUALITY CONTROL

9.1 Refer to Chapter One for guidance on quality assurance (QA) and quality control (QC) protocols. Each laboratory should maintain a formal quality assurance program. The laboratory should also maintain records to document the quality of the data generated. All data sheets and quality control data should be maintained for reference or inspection. When inconsistencies exist between QC guidelines, method-specific QC criteria take precedence over both technique-specific criteria and those criteria given in Chapter One, and technique-specific QC criteria take precedence over the criteria in Chapter One.

9.2 Quality control procedures necessary to evaluate the µ-LC system operation are found in Method 8000 and include evaluation of retention time windows, calibration verification and chromatographic analysis of samples. Check the performance of the entire analytical system daily using data gathered from analyses of blanks, standards, and replicate samples. If any of the chromatographic QC limits are not met, the analyst should examine the LC system for:

- Leaks
- Proper pressure delivery
- A dirty guard column; may need replacing or repacking

- plugged heated capillary tube

Checking any of the above items will necessitate shutting down the µ-LC/ITMS system, making repairs and/or replacements, and then restarting the analyses. A calibration verification standard should be re-analyzed before any sample analyses, as described in Sec. 11.6.

9.3 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. 9.0 for information on how to accomplish this demonstration.

9.4 Before processing any samples, the analyst should demonstrate that all parts of the equipment in contact with the sample and reagents are interference-free. This is accomplished through the analysis of a method blank. Each time samples are extracted, cleaned up, and analyzed, and when there is a change in reagents, a method blank should be prepared and analyzed for the compounds of interest as a safeguard against chronic laboratory contamination.

9.5 Sample quality control for preparation and analysis - The laboratory must also have procedures for documenting the effect of the matrix on method performance (precision, accuracy, and detection limit). At a minimum, this includes the analysis of QC samples including a method blank, matrix spike, a duplicate, and a laboratory control sample (LCS) in each analytical batch. Any method blanks, matrix spike samples, or replicate samples should be subjected to the same analytical procedures (Sec. 11.0) as those used on actual samples.

9.5.1 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.

9.5.2 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.

9.5.3 See Method 8000, Sec. 9.0 for the details on carrying out sample quality control procedures for preparation and analysis.

9.6 Surrogate recoveries - This method currently does not rely upon surrogates.

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

10.0 CALIBRATION AND STANDARDIZATION

See Sec. 11.6 for information on calibration and standardization.

11.0 PROCEDURE

11.1 Water sample preparation

11.1.1 Pour 2 liters of water into a 2-L volumetric flask. Adjust pH to 2.5, using approximately 600 µLs of HCl (12 N). Shake flask to distribute HCl.

11.2 Fish tissue sample preparation

11.2.1 Fish are prepared for extraction by cutting the larger of the fish into large chunks with a saw, subsequent grinding to a fine particulate size using either a blender or Hobart™ grinder, then re-proportioned to 20 g into glass jars and re-frozen until sub-sampled for extraction and analysis. Smaller fish (i.e., minnows, sunfish) can be ground together using the above mentioned methods.

11.2.2 Other tissue samples (plant, mammal) can be prepared as outlined in 11.2.1.

11.3 Water extraction for organotins

11.3.1 Set up extraction manifold per manufacturer's instructions. Close manifold valve to pump. Pour 10 mLs of methanol to cover 47-mm C_{18} solid-phase disc, open manifold valve, pull through approx. 2-mLs of liquid, shut valve and let methanol sit on disc surface for 2 minutes, pull through the rest of liquid till only 5-mm covers disc surface, shut valve. Add 10 mLs of methanol/1% acetic acid solution to disc, open valve, pull through approx. 2-mLs of liquid, shut valve and let methanol/1% acetic acid mixture sit on disc surface for 2 minutes, pull through the rest of liquid till only 5-mm covers disc surface, shut valve. Add 10 mLs DI water to disc, open valve, pull through approx. 2-mLs of liquid, shut valve and let DI water sit on disc surface for 2 minutes, pull through the rest of liquid till only 5-mm covers disc surface, shut valve. Discs are now ready for water samples. **DO NOT** let disc surface become dry, if so steps 1-3 need to be redone.

11.3.2 Add water to 1-L mark of reservoir. Open valve to waste, using gravity extraction let water samples drain through the prepared SPE disks for 30 to 40 minutes, replenishing the water until all 2-L of sample has been poured into the reservoir. Rinse emptied 2-L volumetric flask with 100-mLs of DI water, adding the rinsate water into reservoir. As water level drops to within 10 mLs of disc surface, rinse sides of reservoir with 50 mLs of DI water. Once all of the 2-L of water has been drained through the disc, start vacuum pump, approx. 15 psi, and pull the disc to dryness, approximately 10 minutes. Turn off pump, shut valve.

11.3.3 Place each glass collection tube (24 x 200mm) on manifold. Add 10 mLs of methanol/1% acetic acid solution to each reservoir-disc, rinsing the reservoir sides as solution is added. Open valve to extract, let solution flow through. If hard to start, close valve, start vacuum pump for 20 seconds, turn off pump. Re-open valve. As solution flows out, add another 10 mLs of methanol/1% acetic acid, let flow through, as solution gets low, add a third, and final, 10 mL volume of methanol/1% acetic acid, let flow through till emptied out, turn pump on, and finish extraction until SPE disc is dry. Close valve, turn pump off.

11.3.4 Remove collection tube, qualitatively transfer, by pouring and using disposable glass pipettes, the supernatant to the concentration tubes, rinse sides of collection tube at least once, and transfer to concentration tube.

11.3.5 Micro-concentration by TurboVap® nitrogen evaporation

11.3.5.1 Place the concentrator tube in the TurboVap® in a lukewarm water bath (approximately 30°C) and evaporate the solvent volume to the required level (0.5 mL) using a gentle stream of clean, dry nitrogen, starting with 4 psi (high solvent level) to 9 psi (lower solvent levels).

11.3.5.2 The internal wall of the tube must be rinsed down several times with the final solvent (methanol/1% acetic acid) during the operation. During evaporation, the water level needs to be at proper operating levels (see manufacturer's recommendations). Under normal operating conditions, the extract should not be allowed to become dry. Transfer the extract to a 2 mL glass vial with a PTFE-lined screw-cap or crimp-top and store refrigerated at 4°C. Proceed with µ-LC-ES-ITMS analysis.

11.4 Tissue extraction for organotins

11.4.1 Measure out 0.5 g to 2.0 g of tissue into 40-mL amber glass centrifuge tubes. Add 20 mLs of hexane/tropolone solution (see 7.5.2.1). Place the centrifuge tubes in a rack and place the rack in a water bath sonicator. (Although a rack isn't mandatory, it is best to place the tubes in some type of stable holder for sonication.) Sonicate samples for 45 minutes. Remove samples and adjust pH to approx. pH 2.0 with concentrated HCl (12N).

11.4.2 Place cap on centrifuge tubes. Centrifuge at 4000 rpm for approx. 30 min.

11.4.3 Qualitatively transfer clear supernatant from centrifuge tube (leaving behind fats, solids on bottom) to 50 mL TurboVap® tubes, using glass pipettes.

11.4.4 Micro-concentration by TurboVap® nitrogen evaporation

11.4.4.1 Place the concentrator tube in the TurboVap® in a lukewarm water bath (approximately 30°C) and evaporate the solvent volume to the required level (0.5 mL) using a gentle stream of clean, dry nitrogen, starting with 4 psi (high solvent level) to 9 psi (lower solvent levels).

11.4.4.2 The internal wall of the tube must be rinsed down several times with the final solvent (hexane mix) during the operation. During evaporation, the water level needs to be at proper operating levels (see manufacturer's recommendations). Under normal operating conditions, the extract should not be allowed to become dry. Transfer the extract to a 2 mL glass vial with a PTFE-lined screw-cap or crimp-top and store refrigerated at 4°C. Proceed with µ-LC-ES-ITMS analysis.

11.5 Recommended µ-LC chromatographic conditions

11.5.1 Recommended mobile phase and flow rates for the organotins are shown below. Analysts should also consult the instrument manufacturer's instructions. In the absence of specific recommendations, the following conditions may be a useful starting point:

Flow rate	4 - 6 µL/min if using the low flow-rate ES head and micro-bore column (160 µm I.D.); if using the higher ES flow-rate head, and

narrow-bore column (2.1 mm I.D.) then use 0.4 mL/min flow through the column with a 40/60 split after the column, but before the ES (so that approx. 160 µL/min flow is entering the ES chamber).

isocratic mobile phase	80% methanol/14%water/6% acetic acid/0.1% tropolone (v/v/v/m)

Optimize the HPLC conditions for resolution of the target analytes and sensitivity. The acetic acid and tropolone are necessary to ensure that the organotins remain stable in solution during analysis.

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

11.5 Recommended ES-ITMS operating conditions

Prior to analysis of samples, the analyst should evaluate the relative sensitivity of the target compounds. If the sensitivity has dropped then the analyst should rerun the tuning standard. For the organotins it is recommended that the tri-organo moiety be used. It is possible to tune on the di-organotin and get very good sensitivity, but not be able to recover the tri-organotins. This group of target compounds have much better sensitivity using positive ionization mode. The ion trap mass spectrometer (ITMS) detector that performs real-time mass analyses of liquid chromatograph (LC) eluents over a mass-to-charge ratio range of 50 to 2000.

11.5.1 Positive ionization mode conditions

Mass range	150 to 430 amu full-scan mode
Scan time	3 µscans with an ion injection of 200 ms.
ES needle voltage	4.5 to 5.2 kV.
Capillary temperature	200^{o}C

Other source voltages (i.e., capillary voltage, tube lens offset, octapole lens) to be set through optimization routine of auto tuning mode.

11.5.2 Sample injection volume

For the micro-bore columns (< 160 µm I.D.) an injection volume of 0.5 µL is normally used. The injection loop must be overfilled by, minimally, a factor of four (e.g., 2 -µL sample used to overfill a 0.5 -µL internal injection loop) when manual injections are performed. If solids are present in the extract, allow them to settle or centrifuge the extract and withdraw the injection volume from the clear layer. For the narrow-bore columns (2.1 mm I.D.) an injection volume of 20 µL is normally used.

11.6 Calibration

11.6.1 ES-ITMS tuning conditions. It is recommended that a 20 ng/µL solution of tributyltin (methanol/1% acetic acid) be used for a optimization tuning solution.

11.6.1.1 The tributyltin (TBT) solution is placed into a syringe pump and the ES-ITMS source is configured so as to allow a direct flow (flow-injection) into the ES-ITMS at approximately 4 - 6 µL/min flow, while monitoring mass 291. The detection and subsequent spectra of TBT is sensitive to variable electrospray conditions, such as nitrogen flow, capillary tube temperature, and the voltage difference between the tube lens and the capillary tube. Under ion source conditions optimized for tributyltin,

there is only one ion present, 291 *m/z*, due to the loss of one chlorine atom, $(M - Cl)^+$.

11.6.1.2 The 291 ion should be monitored as the variable electrospray parameters are optimized, either through an autotuning program or manually. When the ES-ITMS is optimized for this TBT ion then all other ions due to the other organotins will be optimized. It has been demonstrated, single-laboratory data, that using other ions, especially the diorganotins, will not give optimization for the detection of the triorganotins, and may in fact be deleterious to their detection.

11.6.1.3 At the start of each project prepare five calibration standards (see Sec. 5.12 and Method 8000). Inject each calibration standard onto the HPLC, using the chromatographic conditions outlined in section 7.6. Refer to Sec. 7.0 of Method 8000 for guidance on external calibration options and calibration acceptance criteria. In most cases the $(M - Cl)^+$ ions, and the tropolonium adduct ions $(M - nCl + (n-1)C_7H_5O_2)^+$ are the only ions of significant abundance. Table 1 lists the retention times (micro-bore column) and the major ions (>15%) present in the positive ionization ES-ITMS spectra of five organotins.

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

11.6.1.5 The use of selective reaction monitoring (SRM) is also acceptable when MS^n and enhanced compound identification is needed.

11.6.2 The retention time of the chromatographic peak is an important variable in analyte identification. Therefore, the retention time of the analyte in the daily calibration standard should be monitored for extreme change versus the initial 5-pt calibration standard curve.

11.6.3 Daily Calibration verification. At the beginning of each analytical shift, and at the end of a sample analysis period (not to exceed a 4-hr time period) the response of the instrument system must be verified by the analysis of a single standard at the approximate mid-point of the initial calibration range.

11.6.3.1 An acceptance criteria of ± 20% relative percent difference is acceptable between the area counts, of each individual analyte, of the first standard and the last standard (see RPD equation Method 8000, section 7.10.4). If the RPD is greater than 20% then the standard needs to be rerun, if still unacceptable the samples analyzed since the last acceptable standard need to be re-analyzed. The ES-ITMS system needs to be retuned and then the calibration standards analyzed and samples re-analyzed.

11.6.3.2 An acceptance criteria of ± 30% RPD is acceptable between the average area counts of the individual analytes of the daily standard and their counterparts on the initial calibration curve. If the RPD falls outside this level, then the analyst is encouraged to retune the ITMS and rerun the mid-point daily standard. If the RPD is still $\geq$30% then the analyst should rerun the 5-point calibration curve.

11.7 Sample Analysis. Once the LC system has been calibrated as outlined in Sec. 11.6, it is ready for sample analysis, employing both MS and UV detectors. Depending on the sensitivity necessary for a given project, analyses may be conducted using the MS detector in either the positive or negative ionization modes. The positive ionization mode generally provides greater

sensitivity, and may be more appropriate for samples containing very low concentrations of the analytes of interest. However, analyst are advised that some compounds may be detectable in only the negative ionization mode.

11.7.1 An instrument blank (methanol/1% acetic acid) should be analyzed after the standards, in order to demonstrate that the system is free from contamination.

11.7.2 If performing manual injections, take an appropriate aliquot of the sample as per Sec. 11.5.2. Start the µ-LC gradient elution, load and inject the sample aliquot, and start the mass spectrometer data system analysis.

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

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

11.8 Calculations

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

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

11.9 Optional MS^n confirmation (for ITMS)

With respect to this method, MS^n shall be defined as the collision activated dissociation (CAD) acquisition on a specific ion (parent ion). The ion trap is configured to trap one mass (parent ion), subsequently this mass is excited to a high enough energy that will cause a product ion (daughter ion) spectrum to be produced by collisions with residual helium. This is done by setting % relative collision energies high enough for this CAD to occur. This process can be continued by selecting the appropriate product ion (new parent ion), by trapping them and exciting them, as was done with the original parent ion. Scanning is done in a defined range such that the ions of interest are scanned.

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

11.9.2 Since the electrospray process often generates only one or two ions per compound, the use of MS^n is a more specific mode of operation yielding molecular structural

information. In this mode, samples can be rapidly screened through direct injection of the sample into the electrospray (e.g., without using the µ-LC to separate the sample components).

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

11.9.3 The MS^n spectra of a calibration standard and the sample should be compared and the ratios of the three major (most intense) ions examined. These ratios should be approximately the same, unless there is an interference (if an interference appears, chromatographic separation must be utilized), this process will then confirm the presence of the "unknown" analyte.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 See Sec. 11.8 for information regarding data analysis and calculations.

12.2 Results must be reported in units commensurate with their intended use and all dilutions must be taken into account when computing final results.

13.0 METHOD PERFORMANCE

13.1 Performance data and related information are provided in SW-846 methods only as examples and guidance. The data do not represent required performance goals for users of the methods. Instead, performance goals should be developed on a project-specific basis, and the laboratory should establish in-house QC performance criteria for the application of this method.

13.2 Single operator accuracy and precision studies have been conducted using spiked water samples. Tables 2 and 3 provide single-laboratory performance data for four organotins in water and fish tissue matrices, respectively.

14.0 POLLUTION PREVENTION

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

14.2 For information about pollution prevention that may be applicable to laboratories and research institutions consult *Less is Better: Laboratory Chemical Management for Waste Reduction* available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th St., N.W. Washington, D.C. 20036, (202) 872-4477.

15.0 WASTE MANAGEMENT

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

16.0 REFERENCES

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17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

The pages to follow contain the tables referenced by this method.

TABLE 1

ELECTROSPRAY MASS SPECTRA OF ORGANOTIN COMPOUNDS

Analyte	MW (Da)[2]	Major ion	Other ions >10% (relative abundance based on 100% relative abundance of major ion)
Tributyltin chloride	326	291 *m/z* $(M - Cl)^+$	
Dibutyltin dichloride	304	355 *m/z* $(M - 2Cl + C_7H_5O_2)^+$	241 *m/z*, $(Sn(II) + C_7H_5O_2)^+$ (15%)‡
Monobutyltin trichloride	282	419 *m/z* $[M - 3Cl + 2(C_7H_5O_2)]^+$	241 *m/z* $(Sn(II) + C_7H_5O_2)^+$ (18%)‡
Triphenyltin chloride	386	351 *m/z* $(M - Cl)^+$	395 m/z, $(M - Cl - C_6H_5 + C_7H_5O_2)^+$ (62%) 369 *m/z* $(M - Cl + H_2O)^+$ (31%) 383 *m/z* $(M - Cl + CH_3OH)^+$ (22%)
Diphenyltin dichloride	344	395 *m/z* $(M - 2Cl + C_7H_5O_2)^+$	

† Daltons, formerly known as atomic mass units (amu).
‡ The presence of Sn(II) ions is not unexpected and has been reported in the literature (Jones and Betowski).

TABLE 2

EXAMPLE OF SINGLE LABORATORY OPERATOR ACCURACY AND PRECISION
FOR LOW CONCENTRATION WATER

Compound	Mean Rec. (%)	%RSD	Spike Conc. µg/L	Recovery Range (%)	# Analyses
Tributyltin chloride	86	36	7 - 18	50 - 127	6
Dibutyltin dichloride	78	30	7 - 15	40 - 113	11
Triphenyltin chloride	77	19	15	56 - 99	7
Diphenyltin dichloride	56	40	9	20 - 81	6

TABLE 3

EXAMPLE OF SINGLE LABORATORY OPERATOR ACCURACY
AND PRECISION FOR FISH

Compound	Mean Rec. (%)	%RSD	Spike Conc. mg/kg	Recovery Range (%)	# Analyses
Dibutyltin dichloride	61	25	1.5	44 - 74	3
Triphenyltin chloride	83	18	5.9	64 - 100	5

TABLE 4

LIMITS-OF-DETECTION

Compound	LOD[a]
Tributyltin chloride	780 pg
Dibutyltin dichloride	970 pg
Monobutyltin trichloride	1 ng
Triphenyltin chloride	920 pg

[a] Calculated as described in ref. 1

TABLE 5

EXAMPLE CONCENTRATIONS OF DBT AND TPT (µg/L) FROM NINE FRESH SURFACE WATER SITES AND ONE WASTEWATER EFFLUENT

Location of State/Site	*n*	DBT[a] (µg/L)	TPT[a] (µg/L)
North Dakota/THRO (Little Missouri River)			
Sample 1, 2	2	nd[c]	2.65 (2.0 - 3.3)
Sample 3 Travel blank	1	nd	nd
DI water spike (DBT/TPT 15 µg/L)	1	15%	114%
Texas/BIBE (Rio Grande River)			
Sample 1, 2	2	nd	nd
Sample 3 Travel Blank	1	nd	nd
DI water spike (DBT/TPT 15 µg/L)	1	66%	85%
Maine/ACAD Seal Cove Pond			
Sample 1, 2	2	nd	nd
Sample 3 Travel Blank	1	nd	nd
DI water spike (DBT/TPT 15 µg/L)	1	104%	79%
North Carolina (Neuse River)			
Sample 1, 2 (year sampled: 1998)	2	1.4 (1.0 - 1.9)	nd
Sample 3 Travel Blank	1	nd	nd
Neuse River water spike (DBT/TPT 25 and 20 µg/L)	1	34%	64%
Sample 1, 2 (year sampled: 1999)	2	nd	nd
Sample 3 Travel Blank	1	nd	nd
Neuse River water spike (DBT/TPT 10 and 19 µg/L)	1	22%	73%
North Carolina (Contentnea Creek)			
Sample 1, 2 (year sampled: 1998)	2	1.6 (0.5 - 2.6)	nd
Sample 1, 2 (year sampled: 1999)	2	nd	nd
Contentnea River water spike (DBT/TPT 10.4 and 18.8 µg/L)	1	22%	68%
North Carolina (Flat River)			
Sample 1,2 (year sampled: 1999)	2	nd	6.0 (4.9 - 7.0)
Sample 3 Travel Blank	1	nd	nd
Flat River water spike (DBT 10 µg/L)	1	85%	na[b]
South Carolina Waste Water Treatment Plant effluent	1	0.5	nd
South Carolina Crystal Lake[e]	2	0.2 (0.1 - 0.4)[d]	nd
South Carolina Durham Pond[f]	1	0.1[d]	nd

[a]Values are the mean with the range in parentheses. [b]Chromatographic interference, unable to quantitate DBT. [c] nd = not detected; [d]analyte concentration is estimated, below limit of detection for DBT = 970 pg. For these samples there was spectral evidence of DBT present, but not at reliable quantitation levels; [e]Tributyltin (TBT) was detected at 2.6 µg/L at this site, due to an industrial discharge of organotins upstream; [f] TBT was detected at 3.7 µg/L at this site, due to an industrial discharge of organotins upstream.

TABLE 6

EXAMPLE CONCENTRATIONS OF DBT AND TPT (ng/g) IN FISH COLLECTED FROM NATIONAL PARKS AND INDUSTRIAL SPILL SITES

State/Site (species)	*n*	DBT (ng/g)[a]	TPT (ng/g)[a]
Maine/ACAD (Seal Cove Pond)			
Sample 1,2 Chain Pickerel (*Esox niger Lesueur*) (SF)	2	nd[b]	nd
Washington/OLYM (Soleduck River)			
Sample 1,2 Rainbow trout (*Oncorhynchus mykiss*)(SF)	2	nd	nd
Sample 3 Sculpin (*Cottus spp.*) (PF)	1	nd	57
Sample 7,8 Sculpin (*Cottus spp.*) (PF)	2	nd	49 (45-53)
California/SEKI			
Sample 1 Rainbow trout (*Oncorhynchus mykiss*) (SF)	1	nd	nd
Sample 3 Rainbow trout (*Oncorhynchus mykiss*) (SF)	1	nd	nd
Sample 4 Rainbow trout (*Oncorhynchus mykiss*) (SF)	1	nd	nd
Sample 5 Spike (DBT 3.7 mg/kg)	1	74%	na
Sample 2 Spike (TPT 2.9 mg/kg)	1	na[c]	72%
Virginia/SHEN(Rose River)			
Sample 1,2 Brook Trout (*Salvelinus fontinalis*) (SF)	2	211 (208-215)	nd
Sample 4,5 Torrent Sucker (*Thorburnia rhothocea*)	2	nd	nd
Sample 3 Spike (TPT 6.2 mg/kg)	1	na	81%
North Dakota/THRO (Little Missouri River)			
Sample 1,2 Shorthead Redhorse *Moxostoma macrolepidotum* (PF)	2	nd	389 (382 - 397)
Sample 5,6 Sauger (*Stizostedian canadense*) (SF)	2	nd	249.5 (0 - 499)
Sample 3 Spike (TPT 5.9 mg/kg)	1	na	59%
South Carolina (Red Bank Creek)			
Redear sunfish (*Lepomis microlophus*)(SF)	1	155	nd
Chain pickerel (*Esox niger Lesueur*) (SF)	1	118	nd
Largemouth bass (*Micropterus salmoides*)(SF)	2	221(166 - 276)	nd
Bluegill sunfish (*Lepomis macrochirus*)(SF)	1	41	nd

[a] Values are the mean with the range in parentheses. [b] nd = not detected. [C] not applicable.