Method 282.3 The Determination of Tributyltin Chloride in Marine and Fresh Waters by Liquid-Solid Extraction (LSE) and Gas Chromatography with Electron-Capture Detection (GC/ECD).

Version 1.0

October, 1989

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Otis Evans

Betty Jacobs

Arnold Cohen

Environmental Monitoring Systems Laboratory

Office of Research and Development

U.S. Environmental Protection Agency

Cincinnati, Ohio 45268

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1. Scope and Application

1.1 This method describes liquid-solid extraction (LSE) procedures (1,2) for a particular dissolved organotin species in marine and fresh waters followed by gas chromatography with electron-capture detection (GC/ECD) (1,3-5).

Analyte	Chemical Abstract Services Registry Number (CASRN)
Tributyltin Chloride	1461-22-9

1.2. The analytical range has been verified to be linear from 1 µg/L to 100 µg/L of tributyltin chloride as Sn.

1.3. Quantitative measurements can be obtained by generating an 'external' calibration curve, or preferably by preconcentrating/extracting the aqueous standards in the same manner as the samples.

1.4. This method has been evaluated in a single laboratory and a method detection limit (MDL) has been determined. Observed detection limits will vary with sample types depending on the nature of the interferences in the sample matrix, the particular extraction device and the specific instrumentation used. For this work the MDL was determined to be 6.7 parts-per-trillion of analyte as tributyltin.

1.5. This method should be used by analysts experienced in LSE, and the use of GC and in the interpretation of gas chromatograms.

2. Summary of Method

2.1. Samples containing 1-2% methanol (100 mL - 250 mL) adjusted to pH 4.5 are passed through one milliliter, 100 milligram octadecyl LSE columns or Teflon enmeshed extraction disks at a rate of 5 mL/min. The extraction devices are air dried and subsequently placed in a desiccator for a least one hour, to ensure that all traces of water have been removed from the adsorbent. The analyte is desorbed with acidified ethyl acetate (HCl) into a calibrated GC glass sample vial. The eluent is then adjusted to a final volume of 0.5 mL with ethyl acetate (HCl). The sample extract is refrigerated overnight (4°C) in order to allow solution equilibration. The tin analyte is determined by capillary column GC using electron capture detection (ECD).

3. Definitions

3.1. Field duplicate (FD1 and FD2)--Two separate samples collected at the same time and placed 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.

3.2. Field reagent blank (FRB)--Reagent water placed in a sample container in the laboratory and treated as a sample in all respects, including 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.

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

3.4. Laboratory Fortified Blank (LFB)--An aliquot of reagent water 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 at the required detection limit.

3.5. Laboratory performance check solution (LPC)--A solution of method analytes used to evaluate the performance of the GC instrument system with respect to a defined set of method criteria.

3.6. Laboratory reagent blank (LRB)--An aliquot of reagent water that is treated exactly as a sample. It is exposed to all the glassware, liquid-solid extraction columns and disks, method solvents, and reagents that are used with other samples. The purpose of the LRB is to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.7. 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.

3.8. Primary dilution standard solution--A solution of a single analyte or several analytes prepared in the laboratory from stock standard solutions and diluted as needed to prepare calibration solutions and fortified blanks.

3.9. Stock standard solution--A concentrated solution containing a single certified standard that is a method analyte, or a concentrated solution of a single analyte prepared in the

laboratory with an assayed reference compound. Stock standard solutions are used to prepare primary dilution standards.

3.10. Calibration standard (CAL)--A solution prepared from the primary dilution standard solution and/or stock standard solution. The CAL solutions are used to calibrate the instrument to response with respect to analyte concentration.

3.11. Quality control sample (QCS)--A sample matrix containing method analytes or a solution of method analytes in a water miscible solvent which is used to fortify reagent water or environmental samples. The QCS is obtained from a source external to the laboratory, and is issued to check laboratory performance with externally prepared test materials.

3.12. Speciation--The determination of specific individual physico-chemical forms of an element.

3.13. Organometallic compounds--Compounds in which the carbon atoms of organic groups are bound to metal atoms.

3.14. Liquid Solid Extraction (LSE)--A sample preparation technique based on the separation mechanisms of liquid chromatography (LC). The solubility and functional group interactions of sample, sorbent and solvent are optimized to effect extraction and/or elution. Also, more commonly known as solid-phase extraction (SPE).

Interferences

4.1. Interferences in this method may be caused by contaminants in solvents, reagents, glassware, Teflon and polycarbonate bottles, liquid-solid extraction columns and disks and other sample processing apparatus that lead to artifacts or elevated baselines in gas chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running field and laboratory reagent blanks, including extracts of preconditioned columns.

4.1.1. Glassware, Teflon and polycarbonate bottles must be scrupulously cleaned. All glassware, Teflon and polycarbonate bottles should be soaked in 50% nitric acid and rinsed thoroughly with organic free deionized, distilled water.

4.1.2. The glassware, Teflon and polycarbonate bottles used for organometal solution preparation and storage should be sealed and stored containing deionized, distilled water.

4.1.3. The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

4.1.3.1. The extracting solvent, ethyl acetate, may contain impurities, e.g., preservatives, etc. and/or water which may give rise to extraneous peaks and exacerbate the thermal sensitivity of TBT chloride by enhancing its degradation in the injection port liner. Solvent blanks should be analyzed for each new bottle or solvent before use. An interference free solvent is a solvent containing no peaks yielding data at 0.5 times the MDL and at the retention times of the analytes of interest. Indirect daily checks on the extracting solvent are obtained by monitoring laboratory reagent blanks (3.6) and periodically monitoring the solvent by obtaining solvent blank chromatograms. Whenever an interference is noted in the laboratory reagent blank, either impurity peaks or a depressed analytical signal, the analyst should analyze another solvent blank. Generally, low level interferences can be removed by solvent redistillation. The solvent container should be kept tightly closed to minimize exposure to moisture. Additionally, to insure a "dry" solvent the non-acidified ethyl acetate may be passed through a sodium sulfate drying column, preferably just prior to use.

.1.4. Liquid solid extraction columns and disks may contain interfering impurities (Figures la and lb) which can be extracted by the solvent. Additionally, sample water retained in the column interstices can give rise to a negative interference, i.e., the analyte signal decreases in magnitude over time (Section 4.1.3.1.).

4.1.4.1 Ethyl acetate can extract compounds from the polypropylene housing, polyethylene frit, and the C-18 bonded porous silica of the liquid-solid extraction cartridges. Phthalates, quinones, alkanes, cresols, etc. have been identified in column extracts (b). A representative number of columns in a given batch (lot) should be analyzed before use. An interference free column is a column containing no peaks yielding data at 0.5 times the MDL at the retention time of interest. Variability in background between column lots and within a particular column necessitates careful checking of column performance via analyses of column extracts.

Water must be completely removed from the liquid-solid extraction cartridges and disks.

Water aids in the thermal decomposition of tributyltin (TBT) resulting in decreased analyte response. The extraction devices should be air-dried and placed in a desiccator for an extended period of time (Table 3).

4.1.5. Syringes, and splitless injection port liners must be cleaned carefully, re-silanized (if appropriate), and/or replaced as needed.

4.1.5.1. At the end of each day's analyses it is recommended that a solvent blank be analyzed. The solvent blank should effectively clean the syringe and remove trace amounts of TBT chloride. TBT chloride has been found to be incompatible with some solvents, e.g., methyltert-butyl ether (MTBE). The syringes tend to "freeze" and require frequent cleaning.

4.1.5.2. Splitless injection port liners have a finite life and should be checked frequently. Aging of the injection port liner is indicated by diminished peak height and significant peak broadening (7.8). It is recommended that the liner be changed or re-silanized every three days. However, useful liner life may depend on a) frequency of analyses, b) concentration of the organotin analyte, etc.

4.1.6. Interfering contamination (carry over) (see 9.3.1.) may also occur when a sample containing low concentrations of analytes is analyzed immediately following a sample containing relatively high concentrations of analytes. A preventive technique is between-sample rinsing of the syringe. After analysis of a sample containing high concentrations of analytes, one or more laboratory reagent blanks should be analyzed.

4.1.7. Matrix interferences may be caused by contaminants that are present in the sample. The extent of matrix interference will vary considerably from source to source, depending upon the sample type.

Safety

5.1. The toxicity or carcinogenicity of each reagent chemical 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 a current awareness file of OSHA regulations regarding the safe handling of chemicals used in this method. A reference file of material safety data should also be made available to all personnel involved in the chemical analysis. Additional references

(9-11) to laboratory safety should be identified and made available for the information of the personnel using this method.

5.2. Tributyltin (TBT) chloride is an extremely toxic substance (12, 13). Pure standard material (liquid) and stock standard solutions of this compound should be handled with suitable protection to skin, eyes, etc.

5.2.1. In the event of eye exposure - flush with copious amounts of water.

5.2.2. In the event of skin exposure - remove any contaminated clothing and flood skin with large volumes of water.

5.2.3. In the event of accidental ingestion - seek medical attention promptly.

5.3. TBT chloride should be kept away from heat, sparks or open flames. It is a COMBUSTIBLE LIQUID. In contact with acid or acid fumes, highly toxic chloride fumes can be emitted.

5.4. TBT chloride spills should be covered with dry sand or dry vermiculite, mixed well and transferred to specially marked containers.

5.5. Solution pH adjustments should be made in the hood.

5.6. Disposal of waste (solvents, analyte(s), etc.) from the system should be according to local regulations.

6. Apparatus and Equipment (All specifications are suggested.)

6.1. Sample containers-- One liter polycarbonate, Teflon, or amber glass bottles fitted with Teflon-lined or polycarbonate lined screw caps. Bottles in which high purity solvents were received can be used as sample containers. These bottles must be thoroughly cleaned, sealed and stored containing deionized, distilled water prior to use.

6.2. Balances

6.2.1. Analytical, capable of accurately weighing to the nearest 0.1 mg.

6.2.2. General purpose laboratory, metric, suggest automatic calibration, full-range taring, readability to 0.01 g.

6.3. pH meter--Laboratory, capable of measuring to at least 0.01 pH units.

6.4. Filtration Apparatus.

6.4.1. Macro filtration--To filter sample waters. Use 250 mL glass reservoir (connects to 1L bottle or vacuum flask), funnel base and stopper, clamp, SS holder, screen and Teflon gaskets (Figure 2). Recommend using 47 mm filter (Millipore Type HA, 0.45 µm).

6.4.2. Laboratory or aspirator vacuum system. Sufficient capacity to maintain a slight vacuum of 13 cm (5 in) of mercury in the vacuum flask.

6.5. Volumetric flasks, various sizes.

6.6. Beakers, various sizes.

6.7. Liquid-Solid Extraction Apparatus.

6.7.1. LSE column processor-vacuum manifold (stainless steel basin), vacuum hose fitting, cover with luer fittings and gasket), vacuum gauge controller, vacuum manifold luer plugs (Figure 3a) or equivalent.

6.7.1.1. Glass microanalysis (filter) holder, 25 mm (filter size), 2.1 cm^2-filtration area, graduated volume-15 mL, removable stainless-steel mesh support screen and PTFE gasket. Modified to fit column processor (Figure 3b) or used in an arrangement analogous to Figure 2.

6.7.1.2. 304 stainless steel syringe (pressure filter) holder, 25 mm (Figure 4). Should accommodate a syringe with luer fittings, also accepts LSE column/cartridges for easy interfacing to column processor vacuum manifold. (Female LUER-LOK® - inlet and male luer slip outlet). Should have a stainless-steel support screen, PTFE gaskets and o-rings. Can also be used in arrangement analogous to Figure 2 with a No. 18-20 LUER-LOK® syringe needle, to accommodate laboratory/aspirator vacuum system or manual sample loading via metal LUER-LOK® tipped glass syringe.

6.7.1.2.1. Syringe--glass with LUER-LOK® TIP, 50 mL.

6.7.2. Extraction Column Reservoirs, 75 mL.

6.7.3. Extraction Column Adaptors, 1,3,6 mL.

6.8. Digital Automatic Pipettes, variable volumes.

6.8.1. Disposable pipette tips, sizes: 1-100 µl, 10-1000 µl.

6.9. Gas Chromatography System

6.9.1. The GC must be capable of temperature programming and equipped with a linearized electron capture detector, fused silica capillary column, and splitless injector. An auto-sampler/injector is desirable. An on-column injector system may be an alternative to splitless injection because tributyltin compounds are thermally sensitive and may decompose in the injection port liner. Additionally, in the presence of residual water thermal decomposition is enhanced. NOTE: Element selective/specific detection, e.g., atomic absorption spectrometry, induction-coupled plasma spectrometry, induction-coupled plasma/mass spectrometry coupled with ion chromatography (IC) or liquid chromatography (LC) may be acceptable alternatives to electron-capture detection.

6.9.2. GC analytical column

6.9.2.1. Fused silica capillary column. Any capillary column that provides adequate resolution, capacity, accuracy, and precision can be used. A 30 m X 0.32 mm i.d. column with a 0.25 µm (bonded) film thickness is recommended. (J & W DB-1 or equivalent).

6.9.3. GC syringes

6.9.3.1. Micro liter syringe(s) - 10 µl, Hamilton 701N series or equivalent.

Reagents and Consumable Materials

7.1. Helium carrier gas and 5% methane in argon (make-up) gas, as contaminant free as possible.

7.2. Ethyl Acetate (CAS-141-78-6)--Spectrophotometry or Gas Chromatography grade. It may be necessary to redistill the solvent if impurities are observed which co-elute (interfere) with the analyte of interest.

7.2.1. Acidified ethyl acetate: 15 µL of 20% HCl/50 mL solvent.

7.3. Methanol (CAS-67-56-1)--High purity solvent.

7.4. Acetic acid, Glacial (CAS 64-19-7)--Ultrex grade for pH adjustment.

7.5. Ammonium hydroxide (CAS-1336-21-6)--Ultrex grade, 20%, for pH adjustment.

7.6. Hydrochloric acid (CAS-7647-01-0)--Ultrex grade, for preparation of acidified ethyl acetate: 15 µl of 20% HCl/50 mL solvent.

7.7. Deionized, distilled water (CAS-7732-18-5)--Prepared by passing distilled water through mixed bed cation and anion exchange resins. This water was adjusted to pH 4.5 (7.4 and 7.5). In this method, this term will be used interchangeably with reagent water. Water in which an interference is not observed at the method detection limit (MDL) of the compound of interest.

7.8. Stock standard solution (100 µg/mL)--Tributyltin chloride as tin (Sn). Tributyltin chloride, 95 + %, liquid, d. 1.207^{20} (CAS-1461-22-9). An individual solution of analyte is prepared by dissolving 56.8 uL in 250 mL of methanol (7.3). The neat liquid organometal is pipetted into a 250 mL acid cleaned/pre-aged volumetric flask and diluted to volume. Transfer this solution to a 250 mL Teflon bottle and refrigerate (4°C) in the dark. This solution can be stored and used for at least six months.

7.9. Primary dilution standard solution (10 µg/mL)--The stock standard solution is diluted further with methanol to prepare a 10 µg/mL solution. Pipet 1 mL into a 10 mL volumetric flask and dilute to volume with methanol.

7.10. Secondary dilution standard solution (1 µg/mL)--Pipet 100 µL of the primary dilution standard solution into a 10 mL volumetric flask and dilute to volume with methanol. Note: Further dilutions as needed should be made to prepare less concentrated standard solutions. Minimize the generation of excess organotin waste by using small volumes; i.e., ≤10 mL.

7.11. Calibration Solutions--A series of calibration solutions (working standards) are prepared by pipetting the appropriate volume and concentration of standard solution and diluting to 10 mL with acidified ethyl acetate (7.6). Prepared external calibration solutions range from 0 to 100 ppb of analyte. These solutions should be refrigerated and stored in the dark until used. These solutions should remain tightly closed to minimize evaporation. HANDLE WITH CARE.

7.12. Extracted external standard solutions--Prepare the calibration standards in water (7.7) to be taken through the liquid solid extraction (LSE) procedure. Assume 100% extraction efficiency and prepare calibration standards to cover the range from 1 ppb to 100 ppb of analyte. For example, a 100 mL sample solution containing 0.1 ng/mL of analyte when extracted, eluted, and brought to a final volume of 0.5 mL in acidified ethyl acetate should yield a signal equivalent to a 20 ng/mL solution.

NOTE: It is recommended that the calibration standards be taken through the LSE procedure for quantitation of the analyte. This corrects for losses in extraction and concentration.

7.13. Liquid-solid extraction cartridges (columns) and disks.

7.13.1. The columns are comprised of polypropylene sample reservoirs, and polyethylene fritted disks. The columns should not contain adipates, quinones, phenols, phthalates, siloxanes, and cresols, etc.that can be extracted from the plastic by the eluting solvent. The columns are prepacked with approximately 100 mg of silica gel bonded phase (C-18) material. The packing should have a narrow size distribution and should not leach any organic compounds into ethyl acetate. One hundred milliliters of water should pass through the column in about 20 minutes with the assistance of a slight vacuum.

7.13.2. Polymer-based extraction columns have the same specifications as their silica based counterparts. The same restrictions also apply for the leaching/extraction of certain organic plasticizers. One hundred milliliters of water should pass through the column in about 2½ hours. This, however, is dependent on the degree of cross-linking.

7.13.3. Teflon enmeshed filter disks feature chemically bonded silica particles enmeshed in an inert PTFE matrix to create a mechanically stable sorbent disk. The 25 mm disk should pass 100 mL of sample in approximately 20 minutes with the assistance of a slight vacuum.

Sample Collection Preservation and Handling

8.1. Sample collection. Samples should be collected in pre-aged polycarbonate or glass containers. All samples should be collected in duplicate. The containers should not be pre-rinsed with sample prior to collection.

8.1.1. When sampling from a water tap, open the tap and allow the system to flush until the water temperature has stabilized. Adjust the flow to about 500 mL/min. and collect duplicate samples from the flowing stream.

8.1.2. When sampling from an open body of water (fresh or sea water), fill the sample container with water from a representative area. Sampling equipment, including automatic samplers, must be free of plastic tubing and other components that may leach interferents into the water. Automatic samples that composite samples over time

should use refrigerated polycarbonate or glass sample containers (14-16).

8.2. Sample preservation. All samples should be iced or refrigerated at 4°C from the time of collection until filtration. The samples should be filtered as soon as possible (Figure 2) upon return to the laboratory.

8.3. Holding time(s). Samples should be analyzed immediately. If immediate sample analysis is not possible, the pH of the sample should be adjusted to 4.5 (optimum pH for analyte extraction), with subsequent refrigeration at 4°C. The maximum sample (aqueous) holding time should be 2 days.

8.3.1. Alternate. It is recommended that sample filtration, pH adjustment and extraction be performed upon immediate return to the laboratory. Laboratory studies confirm the extraction, storage and preservation of tributyltin on column for at least one month. Note: The implications are that LSE of TBT is amenable to field sampling, extraction (preconcentration), storage and preservation.

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.

NOTE: The prevention of contamination and losses are of paramount importance in TBT speciation and analysis. Potential sources of contamination in the laboratory environment are: dust, reagent impurities and sample contact with the laboratory apparatus (resulting in contamination by leaching or surface desorption). Depletion via adsorption (14-16) should also be strongly considered.

9. Calibration and Standardization

9.1. Demonstration and documentation of acceptable initial calibration is required before any samples are analyzed and is required intermittently throughout 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 8 hour period during which analyses are performed. Additional periodic calibration checks are good laboratory practice.

9.2. Establish GC/ECD operating conditions equivalent to those indicated in Tables 1 and 2. Calibrate the GC system using the external standard techniques or use the preferred technique, i.e., carry the aqueous standards through the LSE extraction procedure (Appendix 1), then calibrate with the "extracted" standards.

9.3. Prior to calibration, the GC system must be conditioned. Column conditioning and injection port liner conditioning are a prerequisite for stable and reproducible analytical measurements. System conditioning should be done each day that analyses are to be performed.

9.3.1. The conditioning solution should be at least 100 ppb tributyltin (TBT) as tin. (Analysts may convert this value to reflect the intrinsic entity TBT if desired). Lower concentrations will prolong the length of time needed for conditioning (require more injections). Following conditioning, laboratory reagent blanks should be run to determine whether carry over will be a problem in subsequent sample analyses.

9.4. External Standard Calibration Procedure

9.4.1. An external standard is a known amount of a pure compound that is analyzed with the same procedures and conditions used to analyze samples containing that compound. From measured detector responses to known amounts of the external standard, a concentration of that sample compound can be calculated from measured detector response to that compound in a sample.

9.5. Standards Carried Through the LSE Procedure

9.5.1. Standards carried through the LSE procedure provide an inherent correction for recoveries, because they are preconcentrated in the same manner as the samples.

9.5.1.1. The standards should be prepared in analyte free solutions that resemble the aqueous matrix of the sample as closely as possible.

9.5.1.2. The volume of the standard should be identical to the volume of the sample(s) to be extracted/concentrated.

9.5.1.3. The recoveries of the standards should bracket the expected concentration range of the samples. NOTE: External standards may be used to check the "extracted" standards' curve. (See 9.6).

9.5.1.4. The standards' extract should be diluted to a known, predetermined volume (Table 3).

9.5.1.5. From measured detector responses to known amounts of the standards, the concentration of a sample compound can be calculated from its measured detector response in the sample.

9.6. At least three calibration standards are needed. One should contain the analytes at a concentration near to, but greater than, the method detection limit for the compound; the other two should bracket the concentration range expected in the samples, or define the working range of the detector. For example, if the MDL is 1.0 ng/L, and a sample is expected to contain approximately 5 ng/L, standards should be prepared at concentration of 2.0 ng/L, 5.0 ng/L, and 10.0 ng/L.

9.7. Preparation of Calibration Standards

9.7.1. To prepare an external calibration standard, add an appropriate volume of each secondary dilution standard to acidified ethyl acetate in a 10 mL volumetric flask and fill to the mark. Mix by inverting several times.

9.7.2. To prepare standards to be carried through the LSE procedure, add an appropriate volume of each secondary dilution standard to solutions that closely mimic the sample matrix.

9.8. Inject 2 µL of each calibration standard and tabulate peak heights or area response versus the concentration of the standard. The results are to be used to prepare a calibration curve for each analyte by plotting the peak height or area versus the concentration.

9.9. The working calibration curve must be verified on each working day by the measurement of one or more calibration standards (and when/if the injection liner is changed between analyses). If the response for the analyte varies from the response predicted by the calibration curve (9.8.) by more than ± 10%, the test must be repeated using a fresh calibration standard. If the results still do not agree, i.e., the response is off by more than ± 10%, generate a new calibration curve for each analyte. (Assuming that the injection liner has become "activated," the analyst should change it before proceeding further). Usually the liner can be used 3 to 4 days before its response begins to deteriorate.

9.10. Some possible remedial actions.

9.10.1. Check and adjust GC operating conditions.

9.10.2 Clean and/or replace the splitless injection port liner; silanize injection port liner for later use.

9.10.3. Flush the GC column with solvent according to manufacturer's instructions.

9.10.4. Elevate the temperature of the oven and the detector to "bake-off" any residual components.

9.10.5. Break off a short portion (1 meter) of the column from the injector end; or replace GC column. This action will cause a change in retention times.

9.10.6. Check the mechanical action of the GC syringe daily. Clean or replace as necessary. On occasion decreasing peak height may be caused by clogging of the syringe as opposed to a deteriorating injection port liner.

0. Quality Control

10.1. Each laboratory using this method is required to operate a quality control (QC) program. The minimum requirements of this program consist of the following: an initial demonstration of laboratory capability and regular analyses of laboratory reagent blanks (including solvent/eluent blanks), and laboratory QC samples. More specifically, GC/ECD procedural blanks from commercial C-18 bonded silica, and C-18 polymer cartridges and C-18/C-8 Teflon impregnated filter disks should be obtained when new lots of cartridges and/or disks are used. NOTE: The same lot number should be used throughout any study. The laboratory must maintain records to document the quality of the data generated.

10.2. Initial demonstration of low system background and acceptable particle size and packing. Before any samples are analyzed, or upon receiving a new supply of cartridges or disks from a supplier, it must be demonstrated that a laboratory reagent blank (LRB) is reasonably free of contamination that would prevent the accurate determination of the analyte of interest. The analyst should obtain chromatograms of column/disk extracts prior to and after the conditioning phase. From this information the analyst can determine if further conditioning is warranted or if other action should be taken. These experiments can be used to demonstrate that the particle size/packing of the LSE cartridge are acceptable, and if the flow rate is consistent through the LSE column or disk. (Stable flow rates indicate uniform particle distribution and homogenous packing). Cartridges and disks may be placed in series to test for breakthrough.

10.2.1. Liquid solid extraction (LSE) cartridges can be a major source of contamination (LSE disks appear to be a more attractive alternative with fewer interferences, consistent reproducibility from disk to disk, and from lot

to lot, etc.). Cartridges may contain phthalate esters, silicon compounds, plasticizers and other contaminants that could interfere with and prevent the accurate determination of the method analyte (b). The compounds may be leached from the cartridges into acidified ethyl acetate and produce a background of varying magnitude. If the background contamination prevents accurate and precise analyses, the condition must be corrected before proceeding with the analyses. Figure 1a and 1b show unacceptable background contamination from poor quality commercial LSE cartridges. By contrast, Figures 5a, 5b, and 5c show the lower backgrounds obtainable from LSE disks. (It may be necessary for the analyst to evaluate LSE cartridges from several sources before an acceptable supply is identified.)

10.2.2. Additional sources of background contamination are the solvents, reagents, and glassware.

10.2.3. One hundred milliliters of water should pass through the 100 mg silica-based cartridge of 25 mm Teflon impregnated filter disk in about 25 minutes @ ≈ 10 psi. The extraction time should not vary significantly among LSE cartridges (columns) or disks.

10.3. Initial demonstration of laboratory accuracy and precision. Analyze at least seven replicates of a laboratory fortified blank solution (laboratory QC samples) containing the analyte (see regulations and maximum contaminant levels for guidance on approximately concentrations).

10.3.1. Prepare each replicate by adding an appropriate aliquot of the primary/secondary dilution standard solution, or other certified quality control sample, to reagent water. Analyze each replicate according to the procedure described in Table 3 and in Section 11.

10.3.2. For each replicate, calculate the measured concentration of analyte, and the mean accuracy (as mean percentage of true value) and precision (as relative standard deviation, RSD) of the measurements.

10.3.3. For the analyte at 25 µg/L as tin, the mean accuracy, expressed as a percentage of the true value should be 92-108% and the RSD should be ≤ 8%.

10.3.4. Analysts should develop and maintain a system of control charts to plot the precision and accuracy of analyte measurements over time. For the analyte, the mean accuracy, expressed as a percentage of the true value should be 75-125% and the RSD should be ≤ 25%.

This quality control criteria is applicable to LSE cartridges (Sections 7.13.1 and 10.2.1).

10.3.5. It is recommended that the laboratory periodically document and determine its detection limit capabilities for the analyte of interest.

10.4. Laboratory Reagent Blanks (LRB). Before processing any samples, the analyst must demonstrate that all glassware and reagent interferences are under control. Each time a set of reagents are changed, new solvent bottle opened, or the injection port liner replaced or silanized, a LRB must be analyzed. If within the retention time window of the analyte of interest the LRB produces a peak that would prevent the determination of the analyte, determine the source of the contamination and eliminate the interference before processing samples.

10.5. A single laboratory fortified blank, containing the tin analyte must be analyzed with each set of samples, at a concentration as specified in 10.3.

10.6. A field reagent blank should be analyzed with each set of field samples. Data/information from these analyses will be used to help define and determine contamination related to field sampling and transportation activities.

10.7. Each quarter, replicate laboratory fortified blanks must be analyzed to determine the precision of the laboratory measurements. The data will be used in documenting data quality.

10.8. Each quarter, the laboratory must analyze a quality control sample obtained from an external source. A quality control sample should be analyzed each time a new set of standards are used. The entire analytical procedure must be checked, if unacceptable accuracy data is obtained.

10.9. The laboratory must analyze an unknown performance evaluation sample (if available) at least once per year. Results for each analyte must be within established acceptance limits.

10.10. At various points in this document, quality control measures are incorporated to alert the analyst to potential problems.

11. **Procedure**

11.1. Gas Chromatography

11.1.1. Tables 1 and 2 present the recommended operating conditions for splitless mode GC/ECD, i.e., conditioning, calibration and sample analysis.

11.1.2. Gas Chromatographic conditioning.

11.1.2.1. The analyst must condition the analytical column and the injection liner before starting any tributyltin analyses.

11.1.2.2. The recommended conditioning solution is a 100 ng/mL solution of the tributyltin as tin.

11.1.2.3. Make approximately four 2-µL injections of tributyltin in acidified ethyl acetate.

11.1.2.4. Monitor the peak response--either area and/or height. When these parameters stabilize analyses can begin. NOTE: If the injection liner is replaced, conditioning must be performed again. Fewer injections are required during this phase. However, the analyst should verify this in his/her laboratory.

11.2. Liquid-Solid Extraction

11.2.1. The liquid-solid extraction procedure is presented in Appendix 1.

11.2.2. Set up the liquid-solid extraction column processor as shown in Figure 3. Three options are shown, depending upon the type of extraction device chosen. NOTE: This identical arrangement is not required, but it is convenient for handling small volumes of solution (≤ 200 mL).

11.2.2.1. Sample water is added to the reservoir and drains from it through the LSE column or disks into blunt stainless steel syringe needles (LUER-LOK HUB) permanently mounted in the manifold cover. The sample passes into waste collection containers placed in the stainless steel basin (6.7.1.).

11.2.2.2. A slight vacuum of ≈ 10 mm Hg is used during the course of sample extraction. The sample flow rate is about 5mL/min. The pressure and flow rate are critical. Variations during operations may result in poor precision. Approximately 20 minutes is required to pass 100 mL of sample solution through the system. This however, is extraction device dependent.

11.2.2.3. Depending on the volume of water extracted, the vacuum may have to be released in order to dump the waste from the collection containers. The

vacuum control valve should be closed slowly followed by gently lifting one of the vacuum manifold luer plugs. Releasing the vacuum too quickly will cause splashing inside the basin and may result in solution getting into the vacuum gauge. The analyst should be aware of the volume of solution that has been added to the column processor to prevent solution overflow inside the vacuum manifold basin.

11.2.2.4. During sample application the solution should never drop below the top edge of the packing in the LSE column. Likewise, the LSE disk should be immersed in solution at all times.

11.2.3. Following sample application the extraction device is air-dried on the vacuum manifold for approximately 20 minutes, then placed in a desiccator.

11.2.4. The analyte is eluted with two 250 µL portions of acidified ethyl acetate into a GC vial mounted inside the vacuum manifold basin.

11.2.5. The final volume of the eluate is adjusted to 0.5 mL with a few drops of acidified ethyl acetate.

11.2.6. The extract is refrigerated overnight (4°C)

11.3. GC-ECD

11.3.1. Inject a 2 µL aliquot with the GC-ECD system under the conditions shown in Tables 1 and 2 and section 9.8.

12. Calculations

12.1. Calculate analyte concentrations (in the sample) by utilizing the calibration curve generated from the relative responses of the standard (analyte) solution.

(a) The calibration curve is generated from the analyte response produced from the "external standard" curves and/or "extracted standard" curve, i.e., analyte standards taken through the LSE procedure.

12.2. Data should be rounded to one decimal place and reported in µg/L or pg/L.

12.3. The data should show which calibration technique is used. The enhancement (or recovery) factor should be reported.

13. Precision and Accuracy

13.1. In a single laboratory the method detection limit (MDL) (17) was determined for the tin analyte. Seven aliquots of the fortified sample are measured and the results used to calculate the MDL at the 99% confidence level. The MDL is calculated using the formula:

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

where:

$t_{[n-1, 1-alpha = 0.99]}$ = Students t value for the 99% confidence level with n-1 degrees of freedom, where n = number of replicates, and S = standard deviation of replicate analyses.

The reported MDL can be lowered substantially by extracting a larger volume of sample and/or concentrating to a lower known/constant volume. NOTE: The analyst should compensate for the increased sample volume or smaller extract volume by lowering the relative concentration of the analyte in the reagent water. Analyte detection at the regulatory level should be achievable.

The MDL was difficult to determine for several reasons: (a) unstable non-reproducible backgrounds which may be attributed to low quality commercial LSE cartridges (Section (10.2). Please see figures 1a and 1b. (b) TBT is thermally unstable and therefore quantitative measurements are difficult. However, an MDL limit of 6.7 parts-per-trillion of the analyte as tributyltin was determined. For the MDL determination, seven replicate measurements were made on solutions fortified with the tin analyte at 0.025 µg/L.

14. References

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TABLE 1. LIQUID-SOLID EXTRACTION

AND GAS CHROMATOGRAPHY

EXPERIMENTAL CONDITIONS

TIN SPECIATION

(TRIBUTYLTIN)

Liquid-Solid Extraction (LSE)

with Gas Chromatography -

Electron Capture Detection (GC/ECD

Liquid-Solid Extraction:

Sample Volume	100 mL (50, 200, 250 mL)
Sample pH	4.5
Extraction Devices:	
Columns/Disks	1 mL, 100 mg silica or polymer-based (C-18); Teflon enmeshed extraction disks (C-18)
Eluting Solvent	Acidified ethyl acetate (HCl)-15µL of 20% HCl/50 mL of solvent

Gas Chromatography:

Column	Fused Silica Capillary (DB-1, 30 mx 0.32 mm i.d., 0.25 µm film)
Detector	Electron Capture
Retention Time	≈14.6 min (TBT)
Injection Volume	2 µL
Injector	Splitless
Carrier Gas	Helium (3.86 $mL\text{-}min^{-1}$)
Make-up Gas	Argon-Methane (30 $mL\text{-}min^{-1}$)
Linear Velocity	20.8 cm/sec @ 160°C (measured isothermally)

TABLE 2. TEMPERATURE PROGRAM

Initial Value = 80°C

Initial Time = 1.00 Min

Level 1

Program Rate = 15.00°C/min.

Final Value = 160°C

Final Time = 10.00 Min

Level 2

Program Rate = 20.00°C/min

Final Value = 230°C

Final Time = 8.00 Min

Injector Temp = 200°C, Oven Temp. = 80°C to 230°C,

Detector Temp = 260°C, Splitless injection with 30s delay

Table 3. Accuracy and Precision Data for Eighteen Determinations of the Method Analyte at 0.025 μg/L (100 uL) with Liquid-Solid Extraction and GC/ECD Using a C-18 Silica-Based Column 100 mg.

Volume of Sample	Conc'n after Extraction (μg/L)				
	Expected	Mean Observed	Std. Dev.	Rel. Std. Dev.	Mean Method Accuracy
(mL)	(μg/L)	(μg/L)	(μg/L)	(%)	(% True Conc'n)
200	10	10.3	0.8	7.7	102.9
250	12.5	12.0	1.4	11.7	96.1

Table 4. Accuracy and Precision Data for Thirteen Determinations of the Method Analyte at 0.1 µg/L (100 mL) with Liquid-Solid-Extraction. Using a C-18 Polymer-Based Column (Polystyrene-100 mg).

Concentration After Extraction (µg/L		Std.	Rel.	Mean
Expected (µg/L)	Mean Observed (µg/L)	Dev. (µg/L)	Std. Dev. (%)	Method Accuracy (% True Conc'n)
20	18.8	1.01	5.4	93.8

200 mL sample volume, standards taken through the LSE procedure.

Table 5. Accuracy and Precision Data for Eight Determinations of the Method Analyte Using a 25 mm C-18 Teflon Enmeshed Extraction (Filter) Disk.

True Conc'n (ng/L)	Mean Observed Conc. (ng/L)	Std. Dev. (ng/L)	Rel. Std. Dev. (%)	Mean Method Accuracy (% True Conc'n)
50	52.7	3.79	7.2	105.

200 mL sample volume, standards taken through the LSE procedure.

APPENDIX 1. LIQUID-SOLID EXTRACTION PROCEDURE

(a) Adjust a typical 100 mL sample volume to pH 4.5, containing 1-2% (V/V) methanol. (Add 1-2 mL of methanol/100 mL of sample).

(b) Use 1 mL, 100 mg silica or polymer-based (C-18) column or Teflon enmeshed extraction disk (C-18, C-8)

(c) Add 3 column volumes* (3 mL) of non-acidified ethyl acetate (do not allow the column to become dry during additions of column conditioners and before the sample is added). Note: After pre-conditioning the Teflon enmeshed filter disk with ethyl acetate, maintain vacuum to pull air through for 5 minutes (air-dry the disk). The disk is not allowed to go dry with subsequent conditioning and sample application.

(d) Add 4 column volumes* (4 mL) of methanol, 2 column volumes (2 mL) of deionized water, and 2 column volumes of pH 4.5 deionized water.

(e) Attach sample reservoir to column. (If the LSE disk is used the reservoir is attached prior to step C).

(f) Add sample solution and adjust the flow rate to approximately 5 mL/min.

(g) Following sample application, the LSE column and/or disk is air-dried (room air) (@ 10 mm Hg) for at least 20 minutes.

(h) Place the LSE column and/or disk in a desiccator for at least one hour.

Note: Polymer-based LSE columns must remain in the desiccator overnight to affect removal of all residual water.**

(i) The analyte (TBT) is eluted with two 250 µL portions of ethyl acetate (HCl) into calibrated GC screw cap glass sample vials. (Each portion of ethyl acetate (HCl) remains in contact with the column for at least 30 seconds). Note: TBT is eluted (under vacuum) from polymer-based LSE columns with three 250 µL portions of ethyl acetate (HCl).

(j) The final volume of eluate (column extract) is adjusted to 0.5 mL with a few drops of ethyl acetate (HCl).

(k) The sample vial is refrigerated overnight (4°C) to allow the extract solution to equilibrate. NOTE: We obtained higher recoveries and consistent reproducibility after refrigeration.

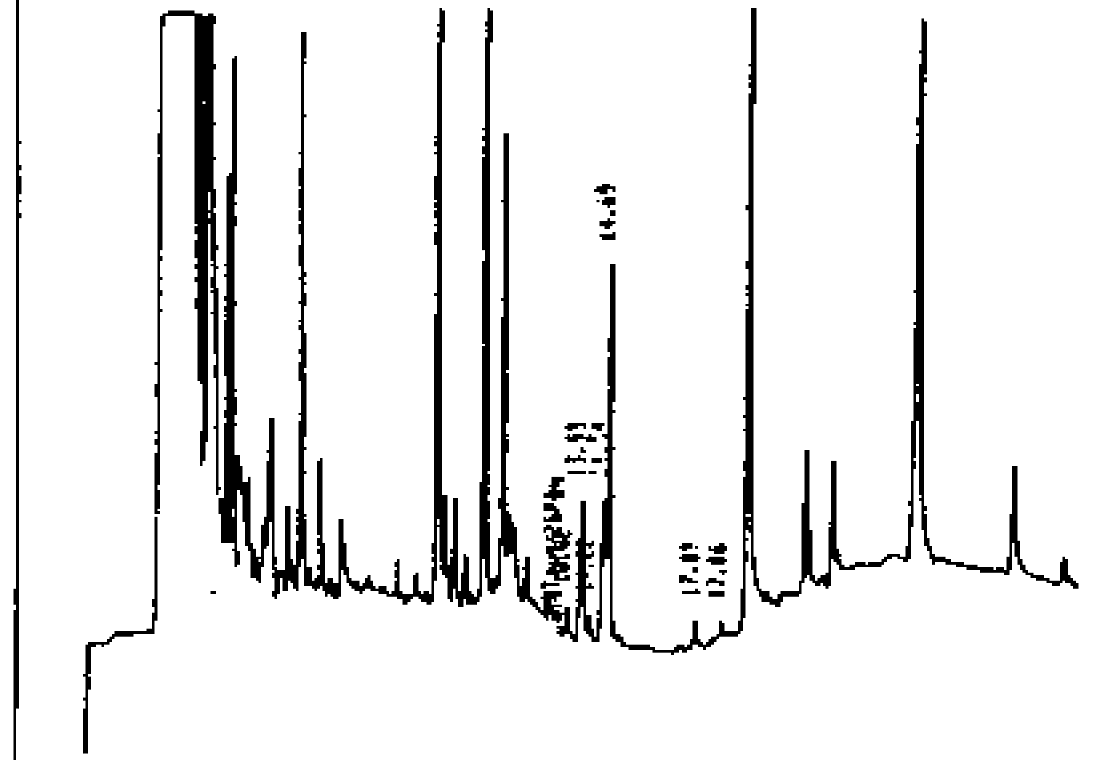
* Column volumes are estimated/approximate. Solutions are dispensed with a squeeze bottle.

** It is essential that all residual water be removed from LSE columns and disks prior to elution of TBT with acidified ethyl acetate. This is an absolute necessity when using splitless injection.

Figure 1. GC/ECD Chromatograms from Commercial C-18 Bonded Porous Silica Columns;

(a),(b) Extracts from Two Pre-Conditioned Columns, 100 mg, 1 mL (Different Manufacturers)

Computer Imaging by Steven Waltrip and James Dryer



DETECTOR RESPONSE

b

0 14 28

TIME (min.)

FIGURE 2. SCHEMATIC DIAGRAM OF SAMPLE FILTRATION APPARATUS

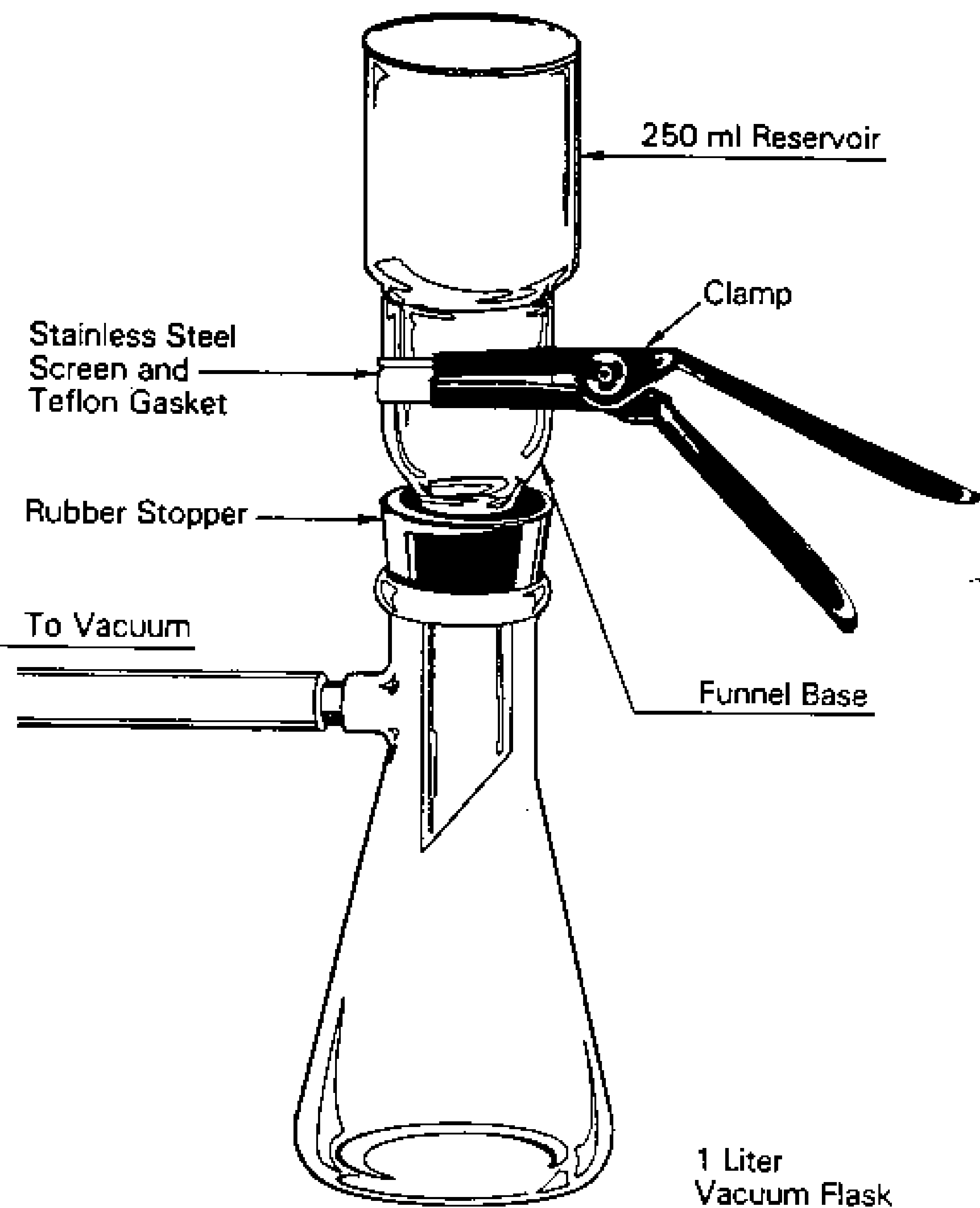


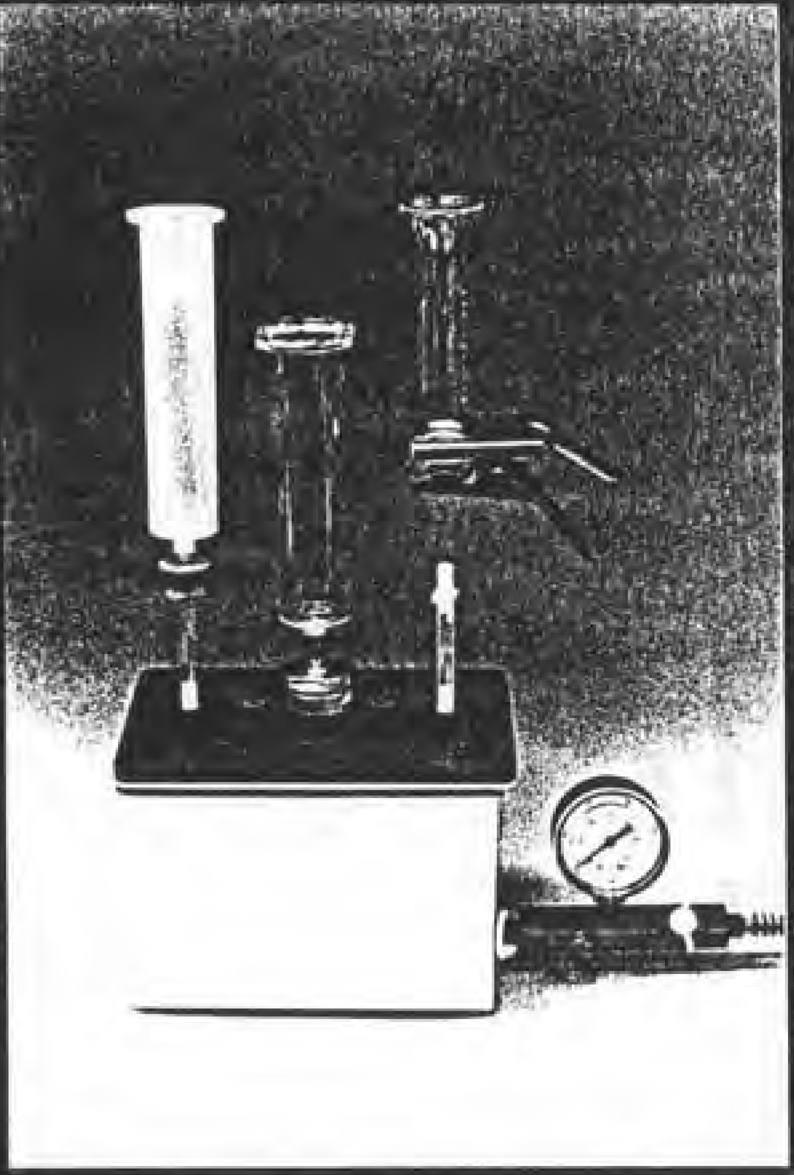
Figure 3. Photographs of LSE Column Processor

(a) Vacuum Manifold Displaying the arrangement for Column (Cartridge) and Disk Extractions

(b) Display of the Component Parts for Disk Extractions Using Glass Filter Holder

Photos by James O'Dell

(a)



(b)

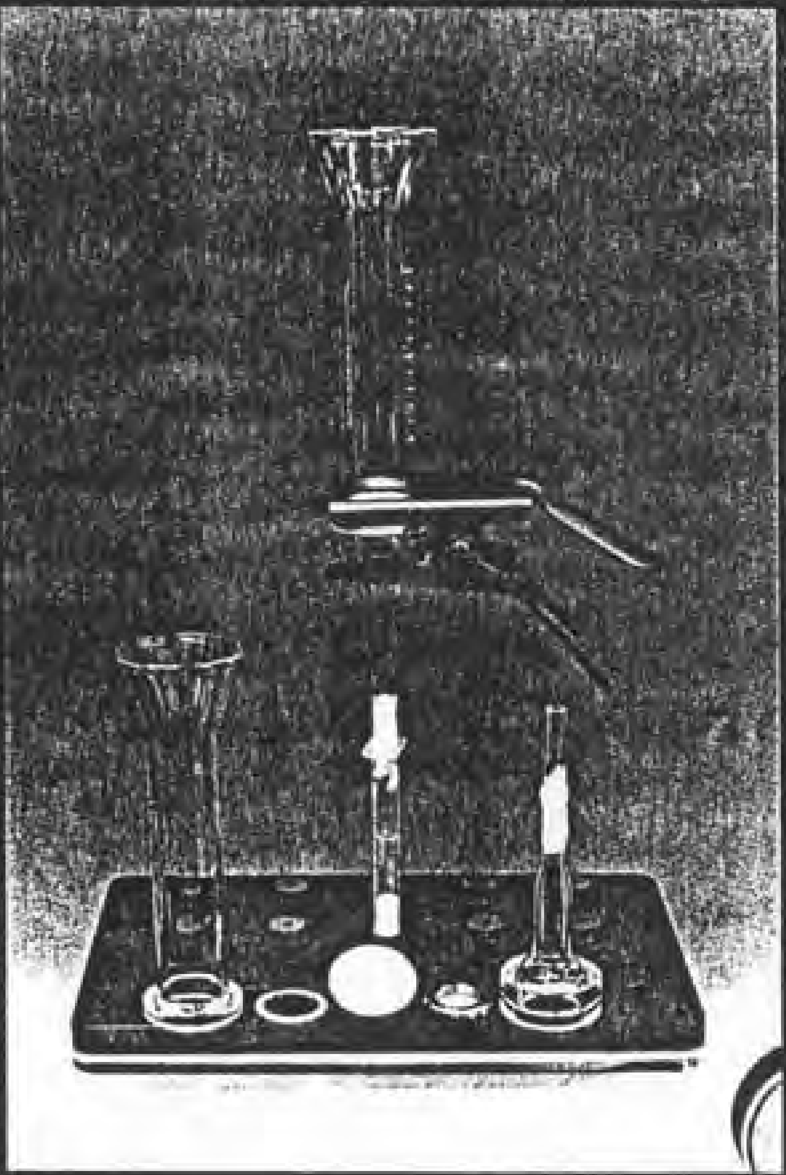


Figure 4. Photograph of Stainless Steel Filter Holder for 25 mm Extraction (Filter) Disks

Photo by James O'Dell

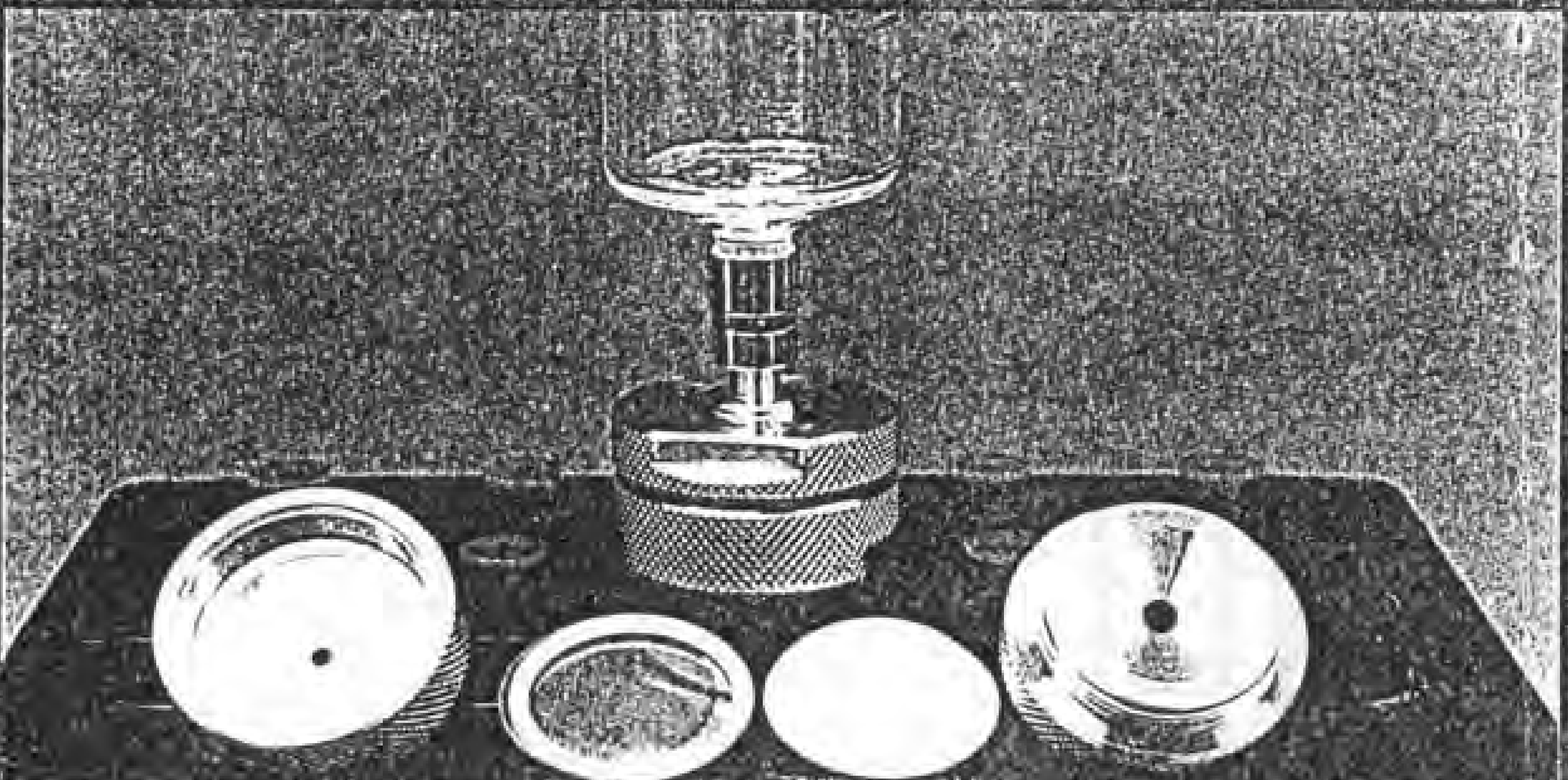


Photo by James O'Dell

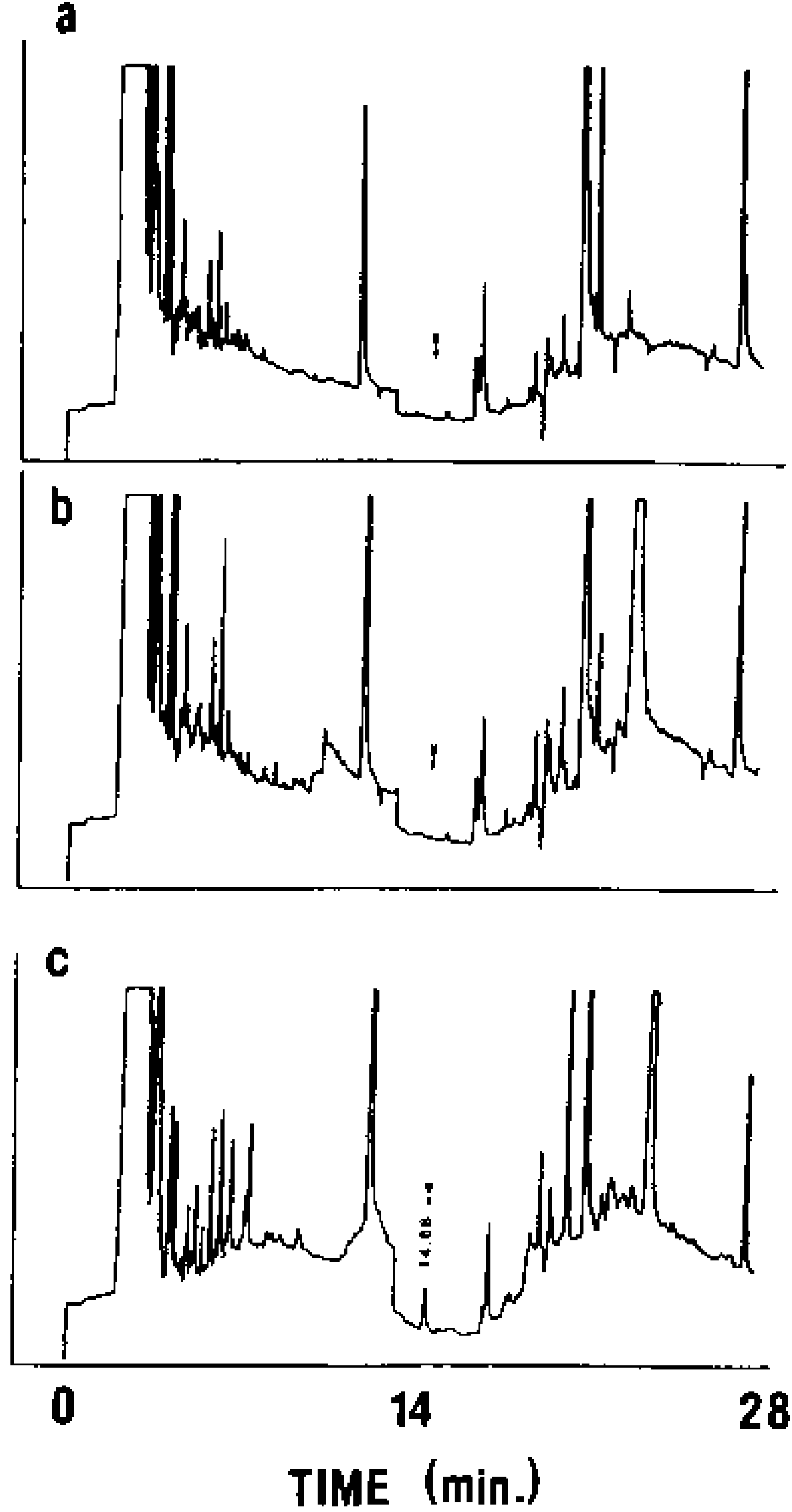
Figure 5. GC/ECD Chromatograms of LSE Solutions from C-18 Disks

(a) Extract from Pre-conditioned Disk

(b) Extract from Laboratory Reagent Blank

(c) Extract from Seawater Containing 0.05 μg/L Tributyltin Chloride

Computer Imaging by Steven Waltrip and James Dryer


a
b
c
DETECTOR RESPONSE
0
14
28
TIME (min.)