

METHOD 506

**DETERMINATION OF PHTHALATE AND ADIPATE ESTERS IN DRINKING WATER
BY LIQUID-LIQUID EXTRACTION OR LIQUID-SOLID EXTRACTION AND GAS
CHROMATOGRAPHY WITH PHOTOIONIZATION DETECTION**

Revision 1.1

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DETERMINATION OF PHTHALATE AND ADIPATE ESTERS IN DRINKING WATER BY LIQUID-LIQUID EXTRACTION OR LIQUID-SOLID EXTRACTION AND GAS CHROMATOGRAPHY WITH PHOTOIONIZATION DETECTION

1.0 SCOPE AND APPLICATION

- 1.1 This method describes a procedure for the determination of certain phthalate and adipate esters in drinking water by liquid/liquid or liquid/solid extraction. The following compounds can be determined by this method:

Analyte	Chemical Abstract Services Registry Number
Bis(2-Ethylhexyl) Phthalate	117-81-7
Butyl Benzyl Phthalate	85-68-7
Di-n-butyl Phthalate	84-74-2
Diethyl Phthalate	84-66-2
Dimethyl Phthalate	131-11-3
Bis(2-Ethylhexyl) Adipate	103-23-1
Di-n-octyl Phthalate	117-84-0

- 1.2 This is a capillary column gas chromatographic (GC) method applicable to the determination of the compounds listed above in ground water and finished drinking water. When this method is used to analyze unfamiliar samples for any or all of the compounds listed above, compound identifications should be supported by at least one additional qualitative technique. Method 525.2 provides gas chromatograph/mass spectrometer (GC/MS) conditions appropriate for the qualitative and quantitative confirmation of results for all the analytes listed above, using the extract produced by this method.
- 1.3 This method has been validated in a single laboratory, and method detection limits (MDLs)¹ have been determined for the analytes above (Table 2). Observed detection limits may vary among waters, depending upon the nature of interferences in the sample matrix and the specific instrumentation used.
- 1.4 This method is restricted to use by or under the supervision of analysts experienced in the use of GC, and in the interpretation of gas chromatograms obtained by a computerized system. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 10.0.

2.0 SUMMARY OF METHOD

- 2.1 A measured volume of sample, approximately 1 L, is extracted with methylene chloride followed by hexane using a glass separatory funnel. The solvent extract is isolated, dried and concentrated to a volume of 5 mL or less. The extract is further concentrated by using a gentle stream of nitrogen gas to reduce the sample volume to 1 mL or less.

Alternatively, a measured volume of sample is extracted with a liquid-solid extraction (LSE) cartridge or disk. The LSE media are eluted with acetonitrile followed by methylene chloride (disk extraction) or with methylene chloride only (cartridge extraction). The eluant is concentrated using a gentle stream of nitrogen gas or clean air to reduce the volume to 1 mL or less.

The analytes in the extract are separated by means of capillary gas chromatography using temperature programming. The chromatographically separated phthalate and adipate esters are measured with a photoionization detector, which is operating at 10 eV.

3.0 DEFINITIONS

- 3.1 Laboratory Reagent Blank (LRB) -- An aliquot of reagent water that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.
- 3.2 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 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 method detection limit.
- 3.4 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.

- 3.5 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.
- 3.6 Primary Dilution Standard Solution (PDS) -- A solution of several analytes prepared in the laboratory from stock standard solutions and diluted as needed to prepare calibration solutions and other needed analyte solutions.
- 3.7 Calibration Standard (CAL) -- A solution prepared from the primary dilution standard solution and stock standard solutions of the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.
- 3.8 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 used to check laboratory performance with externally prepared test materials.

4.0 INTERFERENCES

- 4.1 Method interferences may be caused by contaminants in water, solvents, reagents, glassware, and sample processing hardware. These lead to discrete artifacts and/or elevated baselines in gas chromatograms. All of these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks (Section 10.2).
- 4.1.1 Phthalate esters are contaminants in many products found in the laboratory. It is particularly important to avoid the use of plastics because phthalates are commonly used as plasticizers and are easily extracted from plastic materials. Great care must be exercised to prevent contamination. Exhaustive clean up of reagents and glassware must be required to eliminate background phthalate that is not derived from the sample.
- 4.1.2 Glassware must be scrupulously cleaned. Clean all glassware as soon as possible after use by thoroughly rinsing with the last solvent used. Follow by washing with hot water and detergent and thorough rinsing with tap and reagent water. Drain dry and heat in an oven or muffle furnace at 400°C for one hour. Do not heat volumetric glassware. Thorough rinsing with acetone may be substituted for the heating. After cooling, the glassware should be sealed with aluminum foil and stored in a clean environment to prevent accumulation of dust and other contaminants.
- 4.1.3 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in an all glass system may be required.

Warning: When a solvent is purified, stabilizers added by the manufacturer are removed thus potentially making the solvent hazardous. Also, when a solvent is purified, preservatives added by the manufacturer are removed thus potentially reducing the shelf-life.

- 4.2 Matrix interferences may be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences will vary from source to source, dependent upon the nature and diversity of the samples. Clean up procedures can be used to overcome many of these interferences.
- 4.3 It is important that samples and working standards be contained in the same solvent. The solvent for working standards must be the same as the final solvent used in sample preparation. If this is not the case, chromatographic comparability of standards to sample may be affected.

5.0 SAFETY

- 5.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound must be treated as a potential health hazard. Accordingly, exposure to these chemicals must be reduced to the lowest possible level. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material safety data sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified⁵⁻⁷ for the information of the analyst.

6.0 EQUIPMENT AND SUPPLIES (All specifications are suggested, catalog numbers are included for illustration only.)

6.1 Sampling Equipment

- 6.1.1 Grab Sample Bottle -- 1 L or 1 qt amber glass, fitted with a screw cap lined with Teflon. Protect samples from light if amber bottles are not available. The bottle and cap liner must be washed, rinsed with acetone or methylene chloride and dried before use in order to minimize contamination (see Section 4.1.1.).

6.2 Glassware

- 6.2.1 Separatory Funnel -- 2 L with Teflon stopcock.
- 6.2.2 Drying Column -- Chromatographic column-300 mm long x 10 mm ID, with Teflon stopcock and coarse frit filter disc at bottom.
- 6.2.3 Concentrator Tube -- Kuderna-Danish, 10 mL, graduated, calibration must be checked at the volumes employed in the test. Tight ground glass stopper is used to prevent evaporation of extracts.

- 6.2.4 Evaporative Flask -- Kuderna-Danish, 500 mL, attach to concentrator tube with springs.
- 6.2.5 Snyder Column -- Kuderna-Danish, three-ball macro size.
- 6.2.6 Snyder Column -- Kuderna-Danish, two- or three-ball micro size.
- 6.2.7 Vials -- 10-15 mL, amber glass with Teflon-lined screw cap.
- 6.2.8 Boiling Chips -- Approximately 10/40 mesh. Heat to 400°C for 30 minutes or extract with methylene chloride in a Soxhlet apparatus.
- 6.2.9 Flask, Erlenmeyer -- 250 mL.
- 6.2.10 Chromatography column similar to Section 6.2.2.
- 6.2.11 Pasteur Pipets (and Bulb).
- 6.2.12 Autosampler Vials -- Equipped with Teflon-lined septum and threaded or crimp top caps.
- 6.3 Water Bath -- Heated (with concentric ring covers) capable of temperature control ($\pm 2^{\circ}\text{C}$). The water bath should be used in a ventilating hood.
- 6.4 Balance -- Analytical, capable of weighing accurately to nearest 0.0001 gm.
- 6.5 Gas Chromatograph -- An analytical system complete with temperature programmable GC fitted with split-splitless injection mode system, suitable for use with capillary columns and all required accessory syringes, analytical columns, gases, detector and stripchart recorder. A data system for processing chromatographic data is recommended.
 - 6.5.1 Column, Fused Silica Capillary -- DB-5 or equivalent, 30 m long x 0.32 mm ID with a film thickness of 0.25 micron.
 - 6.5.2 The alternate column, Fused Silica Capillary -- 30 m long x 0.32 mm ID with a film thickness of 0.25 micron, DB-1 or equivalent.
 - 6.5.3 Detector -- A high temperature photoionization detector equipped for 10 electron volts (nominal voltage) and capable of operating from 250-350°C is required.
 - 6.5.4 An automatic injector system is suggested, but was not used for the development of this method.
- 6.6 Vacuum source, capable of maintaining a vacuum of 10-15 mm Hg.

7.0 REAGENTS AND STANDARDS

- 7.1 Reagent Water -- Reagent water is defined as water in which an interfering substance is not observed at the MDL of the parameters of interest. Reagent water used to generate data in this method was distilled water obtained from the Millipore L/A-7044 system comprised of prefiltration, organic adsorption, deionization and Millipore filtration columnar units. Any system may be used if it generates acceptable reagent water.
- 7.2 Acetone, Hexane, Methylene Chloride, Ethyl Acetate, Ethyl Ether and Iso-Octane -
- Pesticide quality or equivalent to distillation in glass quality.
- 7.3 Sodium Sulfate, ACS Granular, Anhydrous -- Several levels of purification may be required in order to reduce background phthalate levels towards acceptance: 1) Heat four hours at 400°C in a shallow tray and 2) Soxhlet extract with methylene chloride for 48 hours.
- 7.4 Florisil, PR Grade (60/100 Mesh) -- To prepare for use, place 100 g of Florisil into a 500 mL beaker and heat for approximately 16 hours at 40°C. After heating transfer to a 500 mL reagent bottle. Tightly seal and cool to room temperature. When cool, add 3 mL of reagent water. Mix thoroughly by shaking or rolling for 10 minutes and let it stand for at least two hours. Store in the dark in glass containers with ground glass stoppers or foil-lined screw caps.
- 7.5 Sodium Chloride, ACS Granular -- Heat four hours at 400°C in a shallow tray. When cool, keep in tightly sealed glass (not plastic) bottle. This cleaning step is required to minimize background contamination associated with this reagent.
- 7.6 Ethyl Ether, ACS Reagent Grade.
- 7.7 Sodium Thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$), ACS Reagent Grade.
- 7.8 Alumina, Neutral Activity Super I, W200 Series (ICN Life Sciences Group, No. 404583) -- To prepare for use, place 100 g of alumina into a 500 mL beaker and heat for approximately 16 hours at 400°C. After heating transfer to a 500 mL reagent bottle. Tightly seal and cool to room temperature. When cool, add 3 mL of reagent water. Mix thoroughly by shaking or rolling for 10 minutes and let it stand for at least two hours. Keep the bottle sealed tightly.
- 7.9 Liquid-Solid Extraction (LSE) Cartridges -- Cartridges are inert non-leaching plastic, for example polypropylene, or glass, and must not contain plasticizers, such as phthalate esters or adipates, that leach into methylene chloride. The cartridges are packed with about 1 g of silica, or other inert inorganic support, whose surface is modified by chemically bonded octadecyl (C_{18}) groups. The packing must have a narrow size distribution and must not leach organic compounds into methylene chloride. One L of water should pass through the cartridge in about two hours with the assistance of a slight vacuum of about 13 cm (5 in.) of mercury. The extraction time should not vary unreasonably among LSE cartridges.

- 7.10 Liquid-Solid Extraction Disks, C-18, 47 mm -- Disks are manufactured with Teflon or other inert support and should contain very little contamination.
- 7.11 Helium Carrier Gas -- As contaminant free as possible.
- 7.12 Stock Standard Solutions (SSS) (1.00 µg/µL) -- Stock standard solutions can be prepared from pure standard materials or purchased as certified solutions.
 - 7.12.1 Prepare stock standard solutions by accurately weighing about 0.0100 g of pure material. Dissolve the material in isooctane and dilute to volume in a 10 mL volumetric flask. Larger volumes can be used at the convenience of the analyst. When compound purity is assayed to be 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards can be used at any concentration if they are certified by the manufacturer or by an independent source.
 - 7.12.2 Transfer the stock standard solutions into Teflon-sealed screw-cap bottles. 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 from them.
 - 7.12.3 Stock standard solutions must be replaced after six months, or sooner if comparison with check standards indicates a problem. Butylbenzyl phthalate is especially vulnerable to autoxidation.

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

- 8.1 Grab samples must be collected in amber glass containers (Section 6.1). Conventional sampling practices should be followed^{8,9}; however, the bottle must not be prerinsed with sample before collection.
- 8.2 Sample Preservation and Storage
 - 8.2.1 For sample dechlorination, add 80 mg sodium thiosulfate to the sample bottle at the sampling site or in the laboratory before shipping to the sampling site.
 - 8.2.2 After the sample is collected, seal the bottle and swirl the sample until the thiosulfate is dissolved.
 - 8.2.3 The samples must be iced or refrigerated at 4°C free from light from the time of collection until extraction. Limited holding studies have indicated that the analytes thus stored are stable up to 14 days or longer. Analyte stability may be affected by the matrix; therefore, the analyst should verify that the preservation technique is applicable to the particular samples under study.

- 8.3 Extract Storage -- Extracts should be stored at 4°C in absence of light. A 14-day maximum extract storage time is recommended. The analyst should verify appropriate extract holding times applicable to the samples under study.

9.0 QUALITY CONTROL

- 9.1 Minimum quality control (QC) requirements are initial demonstration of laboratory capability, analysis of laboratory reagent blanks, laboratory fortified samples, laboratory fortified blanks, and QC samples. A MDL for each analyte must also be determined. Additional quality control practices are recommended.
- 9.2 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 samples is extracted or reagents are changed, a LRB must be analyzed. If within the retention time window of any analyte of interest the LRB produces a peak that would prevent the determination of that analyte using a known standard, determine the source of contamination and eliminate the interference before processing samples.
- 9.3 Initial Demonstration of Capability.
- 9.3.1 Select a representative fortified concentration (about 10 times EDL or at a concentration in the middle of the calibration range established in Section 10.0) for each analyte. Prepare a primary dilution standard (in methanol) containing each analyte at 1000 times selected concentration. With a syringe, add 1 mL of the concentrate to each of four to seven 1 L aliquots of reagent water, and analyze each aliquot according to procedures beginning in Section 11.0.
- 9.3.2 For each analyte the mean recovery value for these samples must fall in the range of $R \pm 30\%$ using the values for R for reagent water in Tables 3 or 4. The precision of these measurements, expressed as RSD, must be 20% or less. For those compounds that meet the acceptance criteria, performance is considered acceptable. For those compounds that fail these criteria, this procedure must be repeated using fresh replicate samples until satisfactory performance has been demonstrated.
- 9.3.3 For each analyte, determine the MDL. Prepare a minimum of seven LFBs at a low concentration. Fortification concentration in Table 2 may be used as a guide, or use calibration data obtained in Section 10.0 to estimate a concentration for each analyte that will produce a peak with a three to five times signal to noise response. Extract and analyze each replicate according to Sections 11.0 and 12.0. It is recommended that these LFBs be prepared and analyzed over a period of several days, so that day to day variations are reflected in precision measurements. Calculate mean recovery and standard deviation for each analyte. Use the standard deviation and the equation given in Section 13.0 to calculate the MDL.

- 9.3.4 The initial demonstration of capability is used primarily to preclude a laboratory from analyzing unknown samples via a new, unfamiliar method prior to obtaining some experience with it. It is expected that as laboratory personnel gain experience with this method the quality of data will improve beyond those required here.
- 9.4 The analyst is permitted to modify GC columns, GC conditions, concentration techniques (i.e., evaporation techniques), internal standards or surrogate compounds. Each time such method modifications are made, the analyst must repeat the procedures in Section 9.3.
- 9.5 Assessing Laboratory Performance -- Laboratory Fortified Blank (LFB)
- 9.5.1 The laboratory must analyze at least one LFB sample with every twenty samples or one per sample set (all samples extracted within a 24-hour period) whichever is greater. Ideally, the fortified concentration of each analyte in the LFB should be the same concentration selected in Section 9.3.1. Calculate accuracy as percent recovery (X_p). If the recovery of any analyte falls outside the control limits (see Section 9.5.2), that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.
- 9.5.2 Until sufficient data become available from within their own laboratory, usually a minimum of results from 20-30 analyses, the laboratory should assess laboratory performance against the control limits in Section 9.3.2 that are derived from the data in Table 2. When sufficient internal performance data becomes available, develop control limits from the mean percent recovery (\bar{X}) and standard deviation (S) of the percent recovery. These data are used to establish upper and lower control limits as follows:
- $$\text{UPPER CONTROL LIMIT} = \bar{X} + 3S$$
- $$\text{LOWER CONTROL LIMIT} = \bar{X} - 3S$$
- After each five to 10 new recovery measurements, new control limits should be calculated using only the most recent 20-30 data points. These calculated control limits should not exceed those established in Section 9.3.2.
- 9.6 Assessing Analyte Recovery -- Laboratory Fortified Sample Matrix
- 9.6.1 The laboratory must fortify each analyte to a minimum of 10% of the routine samples or one fortified sample per set, whichever is greater. The fortified concentration should not be less than the background concentration of the sample selected for fortifying. Ideally, this concentration should be the same as that used for the laboratory fortified blank (Section 9.5). Over time, samples from all routine sample sources should be fortified.

- 9.6.2 Calculate the accuracy as percent recovery, R, for each analyte, corrected for background concentrations measured in the unfortified sample. For each analyte the mean recovery value for these samples must fall in the range of $R \pm 35\%$ using the values for R for reagent water in Tables 3 or 4.
- 9.6.3 If the recovery of any such analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control (Section 9.5), the recovery problem encountered with the dosed sample is judged to be matrix related, not system related. The result for that analyte in the unfortified sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects.
- 9.7 Quality Control Samples (QCS) -- Each quarter, the laboratory should analyze one or more QCS (if available). If criteria provided with the QCS are not met, corrective action should be taken and documented.
- 9.8 The laboratory may adopt additional quality control 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. For example, field or laboratory duplicates may be analyzed to assess the precision of the environmental measurements.

10.0 CALIBRATION AND STANDARDIZATION

- 10.1 Establish gas chromatograph operating conditions equivalent to those given in Table 1. The gas chromatographic system is calibrated using the external standard technique. Calibration standards must be prepared in the same solvent as the final sample extract. This will vary with the extraction option chosen (hexane for LLE, methylene chloride for LSE-cartridge, and acetonitrile for LSE-disk). Section 10.2.1 details the hexane option as an example.
- 10.2 External Standard Calibration Procedure
- 10.2.1 Prepare calibration standards at a minimum of three concentration levels for each analyte of interest by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with n-hexane. Guidance on the number of standards is as follows: A minimum of three calibration standards are required to calibrate a range of a factor of 20 in concentration. For a factor of 50 use at least four standards, and for a factor of 100 at least five standards. One calibration standard should contain each analyte of concern at a concentration 2-10 times greater than the method detection limit for that compound. The other calibration standards should contain each analyte of concern at concentrations that define the range of the sample analyte concentrations or should define the working range of the detector.

- 10.2.2 Using injections of 1-2 μL , analyze each calibration standard according to Section 11.5 and tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each compound. Alternatively, if the ratio of response to amount injected (calibration factor) is a constant over the working range (<20% relative standard deviation, RSD), linearity through the origin can be assumed and the average ratio or calibration factor can be used in place of a calibration curve.
- 10.2.3 The working calibration curve or calibration factor must be verified on each working day by the measurement of a minimum of two calibration check standards, one at the beginning and one at the end of the analysis day. These check standards should be at two different concentration levels to verify the calibration curve. For extended periods of analysis (greater than eight hours), it is strongly recommended that check standards be interspersed with samples at regular intervals during the course of the analyses. If the response for any analyte varies from the predicted response by more than $\pm 20\%$, the test must be repeated using a fresh calibration standard. If the results still do not agree, generate a new calibration curve. For those analytes that failed the calibration verification, results from field samples analyzed since the last passing calibration should be considered suspect. Reanalyze sample extracts for these analytes after acceptable calibration is restored.

11.0 PROCEDURE

11.1 Liquid-Liquid Extraction

- 11.1.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a 2 L separatory funnel containing 50 g of NaCl.
- 11.1.2 Add 60 mL CH_2Cl_2 to the sample bottle. Seal, and shake gently to rinse the inner walls of the bottle. Transfer the solvent to the separatory funnel. Extract the sample by shaking the funnel for two minutes with initial and periodic venting to release excess pressure. Allow the organic layer to separate for a minimum of 10 minutes from the water phase. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Collect the solvent extract in a 250 mL Erlenmeyer flask.
- 11.1.3 Add a second 60 mL volume of methylene chloride to the sample bottle and repeat the extraction procedure a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner.

Then extract with 40 mL of hexane, which extract (top phase) is added to the total.

- 11.1.4 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10 mL concentrator tube to a 500 mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D concentrator, provided the concentration factor attained in Sections 11.1.6 through 11.1.8 is achieved without loss of analytes.
- 11.1.5 Pour the combined extract through a drying column (Section 6.2.2) containing about 10 cm of prerinsed anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20-30 mL of methylene chloride to complete the quantitative transfer.
- 11.1.6 Add one or two clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL of methylene chloride to the top. Place the K-D apparatus on a hot water bath (60-65°C) so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 40 minutes. At the proper rate of distillation the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches approximately 7 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.
- 11.1.7 Increase the temperature of the hot water bath to about 85°C. Remove the Snyder column, rinse the column and the 500 mL evaporative flask with 1-2 mL of hexane. Replace with a micro column and evaporative flask. Concentrate the extract as in Section 11.1.6 to 0.5-1 mL. The elapsed time of concentration should be approximately 15 minutes.
- 11.1.8 Remove the micro Snyder column and rinse the column by flushing with hexane using a 5 mL syringe. Concentrate to a volume of 1 mL by purging the liquid surface with a gentle flow of nitrogen or clean air. If an autosampler is to be used, transfer the extract to an autosampler vial with a Pasteur pipet. Seal the vial with a threaded or crimp top cap. Store in refrigerator if further processing will not be performed. If the sample extract requires no further cleanup, proceed with gas chromatographic analysis (Section 11.5). If the sample requires further cleanup, proceed to Section 11.4.
- 11.1.9 Determine the original sample volume by refilling the sample bottle to the mark and transferring the liquid to a 1000 mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11.2 Liquid-Solid Extraction -- Cartridge Option

- 11.2.1 This method is applicable to a wide range of organic compounds that are efficiently partitioned from the water sample onto a C₁₈ organic phase chemically bonded to a solid inorganic matrix, and are sufficiently volatile and thermally stable for gas chromatography¹⁰. See Section 11.3 for the disk option procedure. Particulate bound organic matter will not be partitioned, and more than trace levels of particulates in the water may disrupt the partitioning process. Single laboratory accuracy and precision data have been determined at a single concentration for the analytes listed in Section 1.1 fortified into reagent water and raw source water.
- 11.2.2 Set up the extraction apparatus shown in Figure 1A. An automated extraction system may also be used. The reservoir is not required, but recommended for convenient operation. Water drains from the reservoir through the LSE cartridge and into a syringe needle which is inserted through a rubber stopper into the suction flask. A slight vacuum of 13 cm (5 in.) of mercury is used during all operations with the apparatus. With this extraction apparatus, sample elution requires approximately two hours. Acceptable new cartridge and extraction disk technology have recently become available, which allow significantly faster elution rates.
- 11.2.3 Mark the water meniscus on the side of the sample bottle (approximately 1 L) for later determination of sample volume. Pour the water sample into the 2 L separatory funnel with the stopcock closed.
- 11.2.4 Flush each cartridge with two 10 mL aliquots of methylene chloride, followed by two 10 mL aliquots of methanol, letting the cartridge drain dry after each flush. These solvent flushes may be accomplished by adding the solvents directly to the solvent reservoir in Figure 1A. Add 10 mL of reagent water to the solvent reservoir, but before the reagent water level drops below the top edge of the packing in the LSE cartridge, open the stopcock of the separatory funnel and begin adding sample water to the solvent reservoir. Close the stopcock when an adequate amount of sample is in the reservoir.
- 11.2.5 Periodically open the stopcock and drain a portion of the sample water into the solvent reservoir. The water sample will drain into the cartridge, and from the exit into the suction flask. Maintain the packing material in the cartridge immersed in water at all times. After all of the sample has passed through the LSE cartridge, wash the separatory funnel and cartridge with 10 mL of reagent water, and draw air through the cartridge for about 10 minutes.
- 11.2.6 Transfer the 125 mL solvent reservoir and LSE cartridge (from Figure 1A) to the elution apparatus (Figure 1B). The same 125 mL solvent reservoir is used for both apparatus. Wash the 2 L separatory funnel with 5 mL of methylene chloride and collect the washings. Close the stopcock on the 100 mL separatory funnel of the elution apparatus, add the washings to

the reservoir and enough additional methylene chloride to bring the volume back up to 5 mL and elute the LSE cartridge. Elute the LSE cartridge with an additional 5 mL of methylene chloride (10 mL total). A small amount of nitrogen positive pressure may be used to elute the cartridge. Small amounts of residual water from the LSE cartridge will form an immiscible layer with the methylene chloride in the 100 mL separatory funnel. Open the stopcock and allow the methylene chloride to pass through the drying column packed with anhydrous sodium sulfate (1 in.) and into the collection vial. Do not allow the water layer to enter the drying column. Remove the 100 mL separatory funnel and wash the drying column with 2 mL of methylene chloride. Add this to the extract. Concentrate the extract to 1 mL under a gentle stream of nitrogen. The extract is now ready for gas chromatography (Section 11.4) or additional cleanup (Section 11.3).

11.3 Liquid-Solid Extraction -- Disk Option

11.3.1 Preparation of disks

11.3.1.1 Insert the disk into the 47 mm filter apparatus. Wash the disk with 5 mL methylene chloride (MeCl_2) by adding the MeCl_2 to the disk, pulling about half through the disk and allowing it to soak the disk for about a minute, then pulling the remaining MeCl_2 through the disk. With the vacuum on, pull air through the disk for a minute.

11.3.1.2 Pre-wet the disk with 5 mL methanol (MeOH) by adding the MeOH to the disk, pulling about half through the disk and allowing it to soak for about a minute, then pulling most of the remaining MeOH through. A layer of MeOH must be left on the surface of the disk, which shouldn't be allowed to go dry from this point until the end of the sample extraction. **This is a critical step for a uniform flow and good recovery.**

11.3.1.3 Rinse the disk with 5 mL reagent water by adding the water to the disk and pulling most through, again leaving a layer on the surface of the disk.

11.3.2 Add 5 mL MeOH per liter of water sample. Mix well.

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

11.3.4 Remove the filtration top from the vacuum flask, but don't disassemble the reservoir and fritted base. Empty the water from the flask and insert

a suitable sample tube to contain the eluant. The only constraint on the sample tube is that it fit around the drip tip of the fritted base. Reassemble the apparatus.

Add 5 mL of acetonitrile (CH_3CN) to rinse the sample bottle. Allow the CH_3CN to settle to the bottom of the bottle and transfer to the disk with a dispo-pipet, rinsing the sides of the glass filtration reservoir in the process. Pull about half of the CH_3CN through the disk, release the vacuum, and allow the disk to soak for a minute. Pull the remaining CH_3CN through the disk.

Repeat the above step twice, using MeCl_2 instead of CH_3CN . Pour the combined eluates thru a small funnel with filter paper containing 3 grams of anhydrous sodium sulfate. Rinse the test tube and sodium sulfate with two 5 mL portions of MeCl_2 . Collect the filtrate in a concentrator tube.

11.3.5 With the concentrator tube in a 28°C heating block, evaporate the eluate with a stream of N_2 to 0.5 mL.

11.4 Extract Cleanup -- Cleanup procedures may not be necessary for a relatively clean sample matrix, such as most drinking waters. If particular circumstances demand the use of a cleanup procedure, the analyst may use either procedure below or any other appropriate procedure. However, the analyst first must demonstrate that the requirements of Section 9.0 can be met using the method as revised to incorporate the cleanup procedure.

11.4.1 Florisil Column Cleanup for Phthalate Esters

11.4.1.1 Place 10 g of Florisil (see Section 7.4) into a chromatographic column. Tap the column to settle the Florisil and add 1 cm of anhydrous sodium sulfate to the top.

11.4.1.2 Pre-elute the column with 40 mL of hexane. Discard the eluate and just prior to exposure of the sodium sulfate layer to the air, quantitatively transfer the sample extract (Section 11.1.8 or 11.2.6) onto the column, using an additional 2 mL of hexane to complete the transfer. Just prior to exposure of the sodium sulfate layer to the air, add 40 mL of hexane and continue the elution of the column. Discard this hexane eluate.

11.4.1.3 Next, elute the column with 100 mL of 20% ethyl ether in hexane (V/V) into a 500 mL K-D flask equipped with a 10 mL concentrator tube. Elute the column at a rate of about 2 mL/min. for all fractions. Concentrate the collected fraction as in Section 11.1. No solvent exchange is necessary. Adjust the volume of the cleaned extract to

1 mL in the concentrator tube and analyze by gas chromatography.

11.4.2 Alumina Column Cleanup for Phthalate Esters

11.4.2.1 Place 10 g of alumina into a chromatographic column. Tap the column to settle the alumina and add 1 cm of anhydrous sodium sulfate to the top.

11.4.2.2 Preelute the column with 40 mL of hexane. The rate for all elutions should be about 2 mL/min. Discard the eluate and just prior to exposure of the sodium sulfate layer to the air, quantitatively transfer the sample extract (Section 11.1.8 or 11.2.6) onto the column, using an additional 2 mL of hexane to complete the transfer. Just prior to exposure of the sodium sulfate layer to the air, add 35 mL of hexane and continue the elution of the column. Discard this hexane eluate.

11.4.2.3 Next, elute the column with 140 mL of 20% ethyl ether in hexane (V/V) into a 500 mL K-D flask equipped with a 10 mL concentrator tube. Concentrate the collected fraction as in Section 11.1. No solvent exchange is necessary. Adjust the volume of the cleaned extract to 1 mL in the concentrator tube and analyze by gas chromatography.

11.5 Gas Chromatography

11.5.1 Table 1 summarizes the recommended operating conditions for the gas chromatograph. Included are retention data for the primary and confirmation columns. Other capillary columns, chromatographic conditions may be used if the requirements of Section 9.0 are met.

11.5.2 Calibrate the system daily as described in Section 10.0.

11.5.3 Inject 1-2 μL of the sample extract or standard into the gas chromatograph. Smaller (1.0 μL) volumes may be injected if automatic devices are employed. For optimum reproducibility, an autoinjector is recommended.

11.5.4 Identify the analytes in the sample by comparing the retention times of the peaks in the sample chromatogram with those of the peaks in standard chromatograms. The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

- 11.5.5 If the response for a peak exceeds the working range of the system, dilute the extract and reanalyze.
- 11.5.6 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.
- 11.5.7 The calibration curves should be linear over the range of concentrations in Tables 2-5. Do not extrapolate beyond the calibration range established in Section 10.0. If analyte response is too high, dilute the extract and reanalyze.

12.0 DATA ANALYSIS AND CALCULATIONS

- 12.1 Calculate the amount of material injected from the peak response using the multi-point calibration curve or calibration factor determined in Section 10.2.2. Do not use the daily calibration verification standard to quantitate method analytes in samples. The concentration in the sample can be calculated from the following equation.

$$\text{Concentration } (\mu\text{g/L}) = \frac{(A) (V_t)}{(V_i) (V_s)}$$

where: A = Amount of material injected (ng)
 V_i = Volume of extract injected (μL)
 V_t = Volume of total extract (μL)
 V_s = Volume of water extracted (mL)

- 12.2 Report results in $\mu\text{g/L}$ without correction for recovery data. All QC data obtained should be reported with the sample results.

13.0 METHOD PERFORMANCE

- 13.1 Single laboratory accuracy and precision data were obtained by replicate liquid-liquid extraction analyses of reagent water fortified at two sets of concentrations of method analytes. The data are given in Tables 2 and 3. Accuracy and precision data by liquid-solid extraction of reagent water fortified at a single concentration are given in Table 4. Finally, Method validation data obtained by the analyses of fortified tap water and raw source water are given in Tables 5-7.
- 13.2 Demonstrated MDLs are given in Table 2. To calculate MDLs, the following equations were used:

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

where: $t_{(n-1, 1-\alpha = 0.99)}$ = Student's t value for the 99% confidence level with n-1 degrees of freedom

n = number of replicates
S = standard deviation of replicate analyses

14.0 POLLUTION PREVENTION

- 14.1 One option of this method utilizes the new liquid-solid extraction (LSE) technology to remove the analytes from water. It requires the use of very small volumes of organic solvent and very small quantities of pure analytes, thereby eliminating the potential hazards to both the analyst and the environment. The other option in this method uses significant volumes of organic solvents. It is highly recommended that laboratories use solvent recovery systems to recover used solvent as sample extracts are being concentrated. Recovered solvents should be recycled or properly disposed of.
- 14.2 For information about pollution prevention that may be applicable to laboratory operations, consult "Less Is Better: Laboratory Chemical Management for Waste Reduction" available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036.

15.0 WASTE MANAGEMENT

- 15.1 It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions. The laboratory using this method has the responsibility to protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance is also required with any sewage discharge permits and regulations. For further information on waste management, consult "The Waste Management Manual for Laboratory Personnel", also available from the American Chemical Society at the address in Section 14.2.

16.0 REFERENCES

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TABLE 2. ACCURACY, PRECISION, AND METHOD DETECTION LIMIT DATA FROM SIX LIQUID-LIQUID EXTRACTION ANALYSES OF FORTIFIED REAGENT WATER

Analyte	True Conc. µg/L	Mean Meas. Conc. µg/L	Std. Dev. µg/L	Mean Accuracy % of True Conc.	MDL µg/L
Dimethyl Phthalate	2.02	1.42	0.38	70.3	1.14
Diethyl Phthalate	1.51	1.16	0.28	76.8	0.84
Di-n-butyl Phthalate	2.62	1.78	0.41	67.9	1.23
Butyl Benzyl Phthalate	6.00	3.27	0.89	54.5	2.67
Bis(2-Ethylhexyl) Adipate	6.03	3.94	1.44	65.3	11.82
Bis(2-Ethylhexyl) Phthalate	5.62	2.92	0.75	52.0	2.25
Di-n-octyl Phthalate	17.18	7.96	2.14	46.3	6.42

TABLE 3. ACCURACY AND PRECISION DATA FROM SEVEN LIQUID-LIQUID EXTRACTION ANALYSES OF FORTIFIED REAGENT WATER

Analyte	True Concentration µg/L	Mean Accuracy % of True Concentration	Relative Standard Deviation %
Dimethyl Phthalate	15	73	16
Diethyl Phthalate	15	71	16
Di-n-butyl Phthalate	15	68	15
Butyl Benzyl Phthalate	15	71	15
Bis(2-Ethylhexyl) Adipate	30	69	18
Bis(2-Ethylhexyl) Phthalate	30	67	21
Di-n-octyl Phthalate	30	62	23

TABLE 4. ACCURACY AND PRECISION DATA FROM SIX LIQUID-SOLID EXTRACTION ANALYSES OF FORTIFIED REAGENT WATER

Analyte	True Concentration µg/L	Mean Accuracy % of True Concentration	Relative Standard Deviation %
Dimethyl phthalate	15	74	11
Diethyl Phthalate	15	85	10
Di-n-butyl Phthalate	15	74	11
Butyl Benzyl Phthalate	15	72	14
Bis(2-Ethylhexyl) Adipate	30	84	11
Bis(2-Ethylhexyl) Phthalate	30	101	13
Di-n-octyl Phthalate	30	85	13

TABLE 5. ACCURACY AND PRECISION DATA FROM SIX LIQUID-LIQUID EXTRACTION ANALYSES OF FORTIFIED TAP WATER

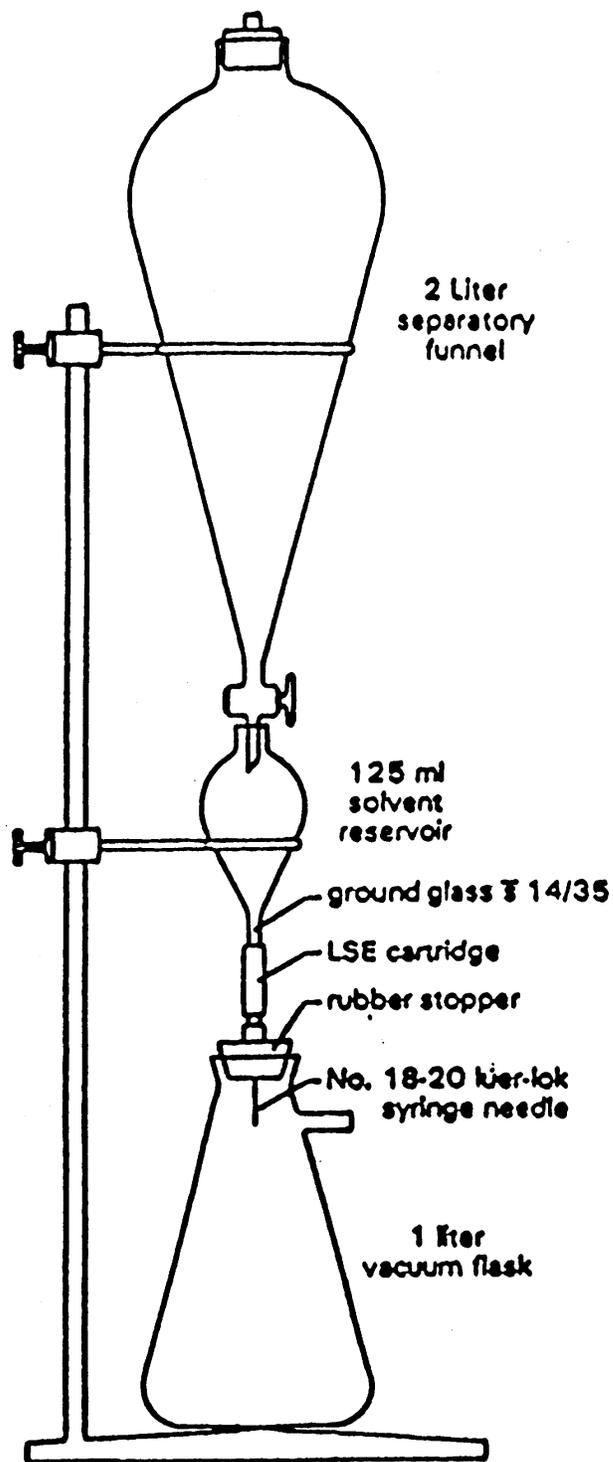
Analyte	True Concentration µg/L	Mean Accuracy % of True Concentration	Relative Standard Deviation µg/L
Dimethyl Phthalate	5	103	10.0
Diethyl Phthalate	5	106	10.0
Di-n-butyl Phthalate	5	94	6.8
Butyl Benzyl Phthalate	5	93	9.1
Bis(2-Ethylhexyl) Adipate	5	87	12.0
Bis(2-Ethylhexyl) Phthalate	5	93	4.9
Di-n-octyl Phthalate	5	72	26.0

TABLE 6. ACCURACY AND PRECISION DATA FROM SIX LIQUID-LIQUID EXTRACTION ANALYSES OF FORTIFIED RAW SOURCE WATER

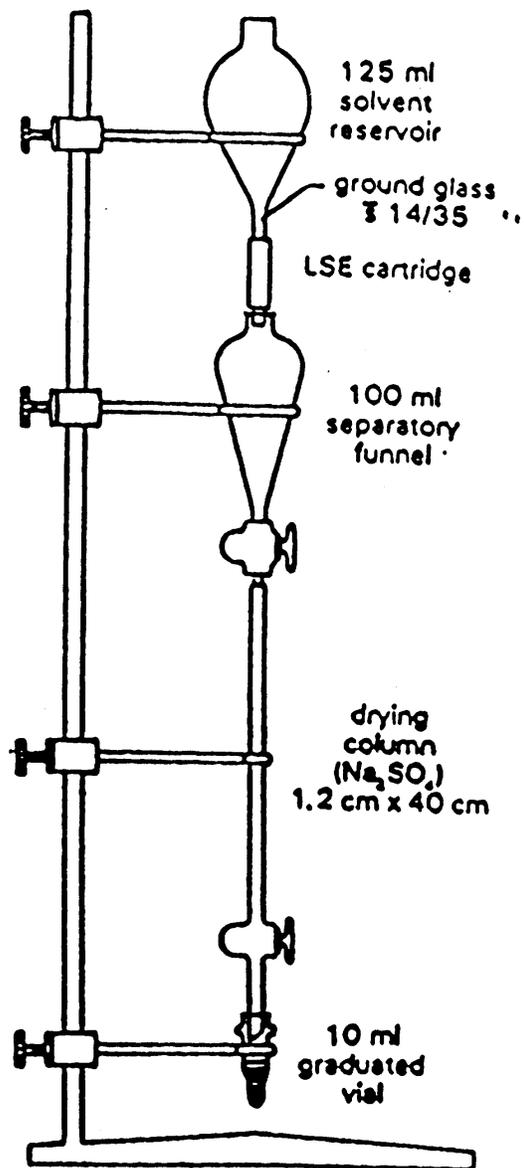
Analyte	True Concentration µg/L	Mean Accuracy % of True Concentration	Relative Standard Deviation %
Dimethyl Phthalate	5	59	51
Diethyl Phthalate	5	78	45
Di-n-butyl Phthalate	5	99	29
Butyl Benzyl Phthalate	5	72	23
Bis(2-Ethylhexyl) Adipate	5	115	32
Bis(2-Ethylhexyl) Phthalate	5	91	35
Di-n-octyl Phthalate	5	54	24

TABLE 7. ACCURACY AND PRECISION DATA FROM SIX LIQUID-SOLID EXTRACTION ANALYSES OF FORTIFIED RAW SOURCE WATER

Analyte	True Concentration µg/L	Mean Accuracy % of True Concentration	Relative Standard Deviation %
Dimethyl Phthalate	5	110	20
Diethyl Phthalate	5	111	32
Di-n-butyl Phthalate	5	95	30
Butyl Benzyl Phthalate	5	82	20
Bis(2-Ethylhexyl) Adipate	5	65	24
Bis(2-Ethylhexyl) Phthalate	5	60	21
Di-n-octyl Phthalate	5	53	15

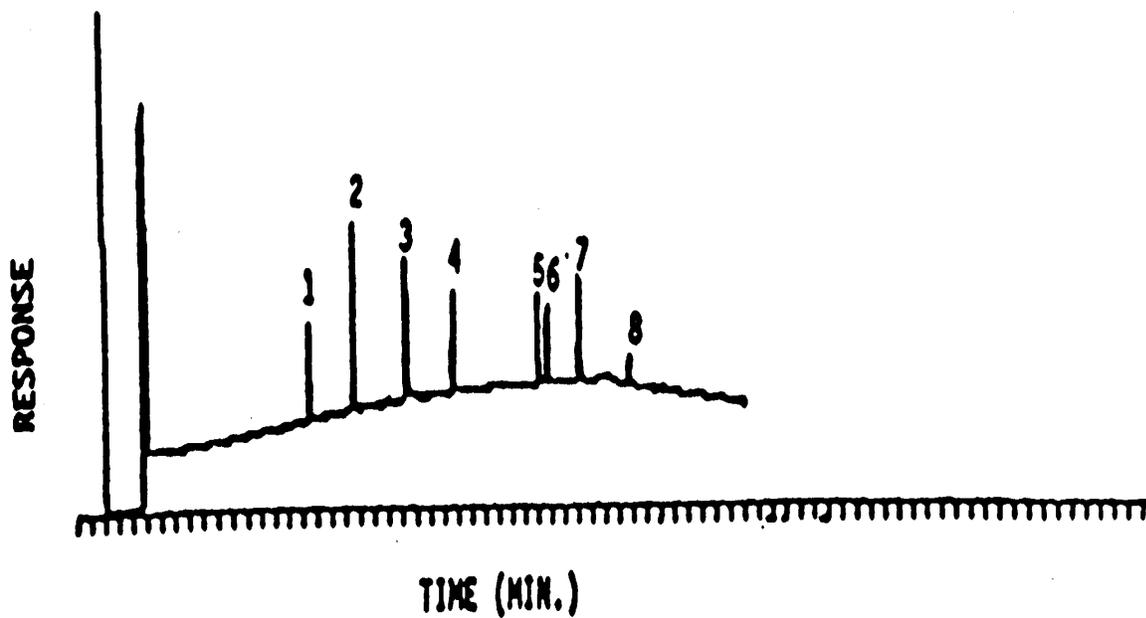


A. Extraction apparatus



B. Elution apparatus

FIGURE 1



Peaks obtained by injecting 5 ng for the 1st, 2nd, 4th and 5th compounds, 10 ng for the 6th, 7th and 8th compounds, and 2.5 ng for the 3rd compound. (Table 1)

FIGURE 2

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