METHOD 548.1

DETERMINATION OF ENDOTHALL IN DRINKING WATER BY ION-EXCHANGE EXTRACTION, ACIDIC METHANOL METHYLATION AND GAS CHROMATOGRAPHY/MASS SPECTROMETRY

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1.0   SCOPE AND APPLICATION

1.1   This method is for the identification and simultaneous measurement of endothall in drinking water sources and finished drinking water. The following analyte can be determined by this method:

Analyte	Chemical Abstract Services Registry Number
Endothall	145-73-3

1.2   This is a gas chromatographic/mass spectrometric (GC/MS) method. However, a flame ionization detector (FID) may be utilized for the determination, but must be supported by an additional analysis using a confirmatory gas chromatographic column.

1.3   The method detection limit[1] (MDL, defined in Section 13.0) for endothall is listed in Table 1 for both GC/MS and FID. The MDL may differ from the listed value depending upon the nature of interferences in the sample matrix. In particular, water sources containing high levels of dissolved calcium, magnesium and sulfate may require sample dilution before extraction to obtain adequate endothall recovery. Guidelines (Sections 4.2 and 11.2.1) are provided on levels of these ions above which dilution is recommended, as well as appropriate dilution factors.

1.4   In this ion exchange liquid-solid extraction procedure, endothall may be esterified directly in the elution solvent, acidic methanol.

1.5   The method performance data provided in this method were obtained using both a GC/MS system and a gas chromatograph with a flame ionization detector (FID). Modern GC/MS instruments have sensitivities at least equivalent to the FID. If the analyst has access to a GC/MS system meeting the specifications described in Section 6.10, it should be as the primary means of identification and measurement.

2.0   SUMMARY OF METHOD

2.1   Liquid-solid extraction (LSE) cartridges containing an intermediate strength, primarily tertiary amine anion exchanger are mounted on a vacuum manifold and conditioned with appropriate solvents. LSE disks may be used instead of

cartridges of all quality control criteria specified in Section 9.0 are met. A 100 mL sample is extracted and the analyte is eluted with 8 mL of acidic methanol. After addition of a small volume of methylene chloride as a co-solvent, the dimethyl ester of endothall is formed within 30 minutes with modest heating (50°C). After addition of salted reagent water, the ester is partitioned into 8-10 mL of methylene chloride. The extract volume is reduced to 1 mL with nitrogen purge for a concentration factor of 100. The extract is analyzed by GC/MS or GC/FID with a megabore capillary column.

3.0 **DEFINITIONS**

3.1 Internal Standard (IS) -- A pure analyte(s) added to a sample, extract, or standard solution in known amount(s) and used to measure the relative responses of other method analytes and surrogates that are components of the same sample or solution. The internal standard must be an analyte that is not a sample component.

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

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

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

3.5 Laboratory Reagent Blank (LRB) -- An aliquot of reagent water or other blank matrix that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, 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.

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

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

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

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

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

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

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

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

4.0   INTERFERENCES

4.1   Method interference may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in the chromatograms. All of these materials must be routinely demonstrated to be free from interferences under the analytical conditions by analyzing laboratory reagent blanks as described in Section 9.2.

4.1.1 Glassware must be scrupulously cleaned[2] as soon as possible after use by rinsing with the last solvent used in it. This should be followed by detergent washing with hot water, and rinses with tap water and distilled water. It should then be drained dry, and heated in a laboratory oven at 400°C for several hours before use. Solvent rinses with methanol may be substituted for the oven heating. After drying and cooling, glassware should be stored in a clean environment to prevent any accumulation of dust or other contaminants.

4.1.2 The use of high purity reagents and solvents is absolutely necessary to minimize interference problems. Purification of solvents by distillation in all-glass systems immediately prior to use may be necessary.

4.2 The major potential interferences in this ion-exchange procedure are other naturally occurring ions in water sources, namely, dissolved calcium, magnesium and sulfate. These are the only ions thus far demonstrated to be interferences when present at concentrations possibly occurring in drinking water sources. For example, the sources identified in Tables 3 and 4 contained elevated concentrations of these ions and reduced recoveries were observed. Sulfate is an effective counter ion, and displaces endothall from the column when present at high concentrations. On the other hand, both calcium and magnesium complex the endothall anion, which then is no longer available in ionic form for ion-exchange extraction. Table 4 illustrates that sample dilution or the addition of ethylenediamine tetraacetic acid for complexing the cations, or a combination of the two, may be used. Figure 1 illustrates quantitatively the separate effects of these ions on recovery.

4.3 The extent of interferences that may be encountered using this method has not been fully assessed. Although the GC conditions described allow for a unique resolution of endothall, other matrix components may interfere. Matrix interferences may be caused by contaminants that are coextracted from the sample. Matrix interferences will vary considerably from source to source, depending on the nature of the matrix being sampled. A distinct advantage of this method is that the anion exchange cartridge provides an effective clean-up mechanism for many potential organic matrix interferences. Many neutral and basic organics retained by the column are removed by the methanol wash step of Section 11.2.3. The most probable matrix interferences are other organic acids or phenols retained by the column. For the cartridge to effectively serve for both sample clean-up and analyte extraction, it is critical that the conditioning steps described in Section 11.2.1 be followed exactly.

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 should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be minimized. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the

chemical specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additionally references to laboratory safety are available[3-5].

6.0 EQUIPMENT AND SUPPLIES

6.1   Sampling Equipment -- For discrete or composite sampling. Amber glass bottles (250 mL or larger) fitted with screw caps lined with Teflon. If amber bottles are not available, protect samples from light. The container must be washed, rinsed with methanol, and dried before use to minimize contamination.

6.2   Separator Funnels -- 125 mL, with Teflon stopcocks, ground glass or Teflon stoppers.

6.3   Screw Cap -- 125 x 13 mm, culture tubes. Screw caps should have Teflon liners.

6.4   Graduated 15 mL centrifuge tubes with #13 ground glass stoppers.

6.5   Pasteur Pipets -- Glass, disposable 5¾" length.

6.6   Balance -- Analytical, capable of weighing to .0001 g.

6.7   Six or 12-position analytical concentrator (Organomation, N-EVAP Model #111/6917 or equivalent).

6.8   pH Meter.

6.9   Gas Chromatograph -- Analytical system complete with GC suitable for flame ionization detection, split/splitless capillary injection temperature programming, and all required accessories including syringes, analytical columns, gases and strip chart recorder. A data system is recommended for measuring peak areas. An auto injector is recommended for improved precision of analysis.

6.10   Gas Chromatograph/Mass Spectrometer/Data System (GC/MS/DS)

6.10.1 The GC must be capable of temperature programming and be equipped for split/splitless or on-column capillary injection. The injection tube liner should be quartz and about 3 mm in diameter. The injection system must not allow the analytes to contact hot stainless steel or other metal surfaces that promote decomposition.

6.10.2 The GC/MS interface should allow the capillary column or transfer line exit to be placed within a few mm of the ion source. Other interfaces, for example, the open split interface, are acceptable as long as the

system has adequate sensitivity (See Section 10.0 for calibration requirements).

6.10.3 The mass spectrometer must be capable of electron ionization at a nominal electron energy of 70 eV and of scanning from 45-450 amu with a complete scan cycle time (including scan overhead) of 1.5 seconds or less. (Scan cycle time = Total MS data acquisition time in sec divided by total number of scans in the chromatogram). The spectrometer must produce a mass spectrum that meets all criteria in Table 5 when 5-10 ng of DFTPP is introduced into the GC. An average spectrum across the DFTPP GC peak may be used to test instrument performance.

6.10.4 An interfaced data system is required to acquire, store, reduce, and output mass spectral data. The computer software must have the capability of processing stored data by recognizing a GC peak within any given retention time window, comparing the mass spectra from the GC peak with spectral data in a user-created data base, and generating a list of tentatively identified compounds with their retention times and scan numbers. The software must also allow integration of the ion abundance of any specific ion between specified time or scan number limits, calculation of response factors as defined in Section 10.3.6 (or construction of a second or third order regression calibration curve), calculation of response factor statistics (mean and standard deviation), and calculation of concentrations of analytes as described in Section 12.0.

6.11 GC Columns

6.11.1 GC/MS -- DB5, 30 m x 0.25 mm, 0.25 μm film thickness.

6.11.2 FID primary -- RTX Volatiles, 30 m x 0.53 mm ID, 2.0 μm film thickness, Restek Catalog No. 10902.

6.11.3 FID confirmation -- DB5, 30 m x 0.32 mm ID, 0.25 μm film thickness.

6.12 Liquid-Solid Extraction Vacuum System -- May be used.

6.13 Liquid-Solid Extraction Cartridges with Frits (8 mL) -- Also available from a number of commercial suppliers. Appropriate liquid-solid extraction disks may also be used in this method if equivalent or better quality assurance data can be demonstrated (See Section 9.0).

6.14 Liquid-Solid Extraction Reservoirs and Adapters (70 mL) -- Baxter Catalog #9442 (adapter catalog # 9430) or equivalent.

7.0 REAGENTS AND CONSUMABLE MATERIALS

7.1   Reagent Water -- Reagent water is defined as water in which an interference is not observed at the endothall method detection.

     7.1.1   A Millipore Super-Q Water System or its equivalent may be used to generate deionized reagent water. Distilled water that has been charcoal filtered may also be suitable.

7.2   Methanol -- Pesticide quality.

7.3   Methylene Chloride -- Pesticide quality or equivalent.

7.4   Sodium Sulfate -- ACS granular. Heat in a shallow tray for four hours at 400°C to remove phthlates and other interfering organic substances or extract with methylene chloride in a Soxhlet apparatus for 48 hours.

7.5   Sulfuric Acid (10%) in Methanol -- Using extreme caution, slowly dissolve reagent grade sulfuric (10% v/v) acid in methanol.

7.6   Sodium Hydroxide (NAOH) 1 N -- Dissolve 4 g ACS grade in reagent water and dilute up to 100 mL in a 100 mL volumetric flask.

7.7   Sodium Sulfate (10%) in Reagent Water -- Dissolve 100 g sodium sulfate in reagent water and dilute to volume in a 1 L volumetric flask.

7.8   Biorex 5 Anion Exchange Resin -- BioRad Laboratories Catalog No. 140-7841.

7.9   Disodium Ethylenediamine Tetraacetate (EDTA) -- Certified ACS Fisher or equivalent.

7.10   Endothall -- Monohydrate, available as neat material from Ultra Scientific, North Kingston, RI or as a concentrated solution from NSI Environmental Solutions, Research Triangle Park, NC.

7.11   Acenapthene-d10 -- Available from MSD Isotopes or Cambridge Chemicals.

7.12   Stock Standard Solutions

     7.12.1 Endothall -- 50 µg/mL in methanol.

     7.12.2 Acenaphthene-d10 -- 500 µg/mL in methanol. Dissolve 25 mg (approximately 32.2 µL) Acenapthnene-d10 in 50 mL methanol. Prepare a working standard at 10 µg/mL by a 1:50 dilution of the stock standard.

     7.12.3 Decafluorotriphenylphosphine (DFTPP) -- 5 µg/mL.

     7.12.4 Stock standard solutions must be replaced after six months, or sooner if comparison with check standards indicates a problem.

8.0   SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1   Grab samples must be collected in glass containers. Conventional sampling practices should be followed, except that the bottle must not be prewashed with sample before collection. Composite samples should be collected in refrigerated glass containers. Automatic sampling equipment must be as free as possible of plastic tubing and other potential sources of contamination.

8.2   Sample Preservation

8.2.1   If residual chlorine is present, add 80 mg of sodium thiosulfate per liter of sample to the sample bottle prior to collecting the sample.

8.2.2   After adding the sample to the bottle containing the sodium thiosulfate, seal the bottle and shake vigorously for one minute.

8.2.3   The samples must be iced or refrigerated at 4°C from the time of collection until extraction and analysis. Endothall is not known to be light sensitive, but excessive exposure to light and heat should be avoided.

8.2.4   A graphical representation of the results of a 14-day holding stability study on endothall in three different water matrices is presented in Figure 2. These matrices were a dechlorinated tap water sample, a filtered river water sample containing considerable biological activity and the same river water biologically preserved at pH 2. These data indicate that the samples may be held for seven days before extraction under the conditions of Section 8.2.3. Endothall appears to be biologically stable over seven days. However, the chemical and biological stability of endothall may be matrix dependent. The analyst may verify analyte stability in the matrix of interest by conducting appropriate holding studies. Samples with unusually high biological activity should be acidified to pH 1.5-2.0 with 1:1 HC1:$H_2$0.

8.3   Extract Storage -- Sample extracts should be stored in the dark at 4°C or less. A maximum extract holding time of 14 days is recommended.

9.0   QUALITY CONTROL

9.1   Each laboratory that uses this method is required to operate a formal quality control (QC) program. The minimum QC requirements are initial demonstration of laboratory capability, analysis of laboratory reagent blanks, laboratory fortified blanks, laboratory fortified matrix samples and QC check standards.

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 analyzed or reagents are changed, a LRB

must be analyzed. For this method, the blank matrix is filtered reagent water. If within the retention time window of endothall, the reagent blank produces a peak which prevents the measurement of endothall, 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 for endothall. Prepare a methanol solution containing endothall at 1000 times the selected concentration. The concentrate must be prepared independently from the standards used to prepare the calibration curve (Section 10.2). With a syringe, add 100 μL of the concentrate to each of four to seven 100 mL aliquots of reagent water and analyze each aliquot according to procedures in Section 11.0.

9.3.2   Calculate the mean percent recovery (R), the relative standard deviation of the recovery (RSD in Table 2), and the MDL[1]. The mean recovery must fall in the range of R ±20% using the values for R (Recovery) for reagent water (Table 2). The standard deviation should be less than 30%. If these acceptance criteria are met, performance is acceptable and sample analysis may begin. If either of these criteria fails, initial demonstration of capability should be repeated until satisfactory performance has been demonstrated.

9.3.3   The initial demonstration of capability is used primarily to preclude a laboratory from analyzing unknown samples by a new, unfamiliar method prior to demonstrating a basic level of skill at performing the technique. As laboratory personnel gain experience with this method the quality of the data should improve beyond the requirements stated in Section 9.3.2.

9.4   The analyst is permitted to modify GC columns or GC conditions to improve separations or lower analytical costs. Each time such method modifications are made, the analyst must repeat the procedures in Section 9.3.

9.5   Assessing the Internal Standard -- In using the IS calibration procedure, the analyst is expected to monitor the IS response (peak area) of all samples during each analysis day. The IS response for any sample chromatogram should not deviate from the most recent calibration check standard IS response by more than 30%.

9.5.1   If a deviation of greater than 30% is encountered for a sample, reinject the extract.

9.5.1.1 If acceptable IS response is achieved for the reinjected extract, then report the results for that sample.

9.5.1.2 If a deviation of greater than 30% is obtained for the reinjected extract, analysis of the sample should be repeated beginning with Section 11.0, provided the sample is still available. Otherwise, report results obtained from the reinjected extract, but annotate as suspect.

9.5.2 If consecutive samples fail the IS response acceptance criterion, immediately analyze a medium calibration check standard.

9.5.2.1 If the check standard provides a response factor (RF) within 20% of the predicted value, then follow procedures itemized in Section 9.5.1 for each sample failing the IS response criterion.

9.5.2.2 If the check standard provides a response factor (RF) which deviates more than 20% from the predicted value, then the analyst must recalibrate, as specified in Section 10.2.

9.6 Assessing Laboratory Performance

9.6.1 The laboratory must analyze at least one laboratory fortified blank (LFB) per sample set (all samples extracted within a 24-hour period). The fortifying concentration in the LFB should be 10-20 times the MDL. Calculate accuracy as percent recovery (R_i). If the recovery falls outside the control limits (See Section 9.6.2), the system is judged out of control, and the source of the problem must be identified and resolved before continuing analyses.

9.6.2 Until sufficient LFB data become available, usually a minimum of results from 20-30 analyses, the laboratory should assess its performance against the control limits described in Section 9.3.2. When sufficient laboratory performance data become available, develop control limits from the mean percent recovery (R) and standard deviation (S) of the percent recovery. These data are used to establish upper and lower control limits as follows:

UPPER CONTROL LIMIT = R + 3S
LOWER CONTROL LIMIT = R - 3S

After each group of 5-10 new recovery measurements, control limits should be recalculated using only the most recent 20-30 data points.

9.6.3 Each laboratory should periodically determine and document its detection limit capabilities for endothall.

9.6.4 Each quarter the laboratory should analyze quality control samples (if available). If criteria provided with the QCS are not met, corrective action should be taken and documented.

9.7   Assessing Analyte Recovery

    9.7.1   The laboratory must add a known fortified concentration to a minimum of 10% of samples or one fortified matrix sample per set, whichever is greater. The fortified concentration should not be less than the background concentration of the sample selected for fortification. The fortified concentration should be the same as that used for the LFB (Section 9.6). Over time, samples from all routine sample sources should be fortified.

    9.7.2   Calculate the percent recovery for endothall, corrected for background concentrations measured in the unfortified sample, and compare these values to the control limits established in Section 9.6.2 for the analyses of LFBs.

    9.7.3   If the recovery falls outside the designated range and the laboratory performance for that sample set is shown to be in control (Section 9.6), the recovery problem encountered with the fortified sample is judged to be matrix related, not system related. The result in the unfortified sample must be labelled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

10.0   CALIBRATION AND STANDARDIZATION

10.1   Preparation of Calibration Standards

    10.1.1 Calibration standards as dimethyl esters are prepared by addition of aliquots of the endothall stock standard (Section 7.12.1) to the esterification reaction mixture, consisting of 8 mL of 10% H_2SO_4/methanol and 6 mL of methylene chloride in the screw cap culture tubes (Section 6.3). The standards are then esterified and partitioned into the organic phase according to Section 11.4. Prepare endothall acid standards equivalent to aqueous standards at 100, 50, 25, and 5 µg/L by addition of the following aliquots of the stock standard solution (Section 7.12) to the esterification reaction mixture - 200, 100, 50, and 10 µL. By way of illustration, 200 µL of the 50 µg/mL stock contains 10 µg of endothall. When dissolved in 100 mL of water, the aqueous concentration is 100 µg/L.

    10.1.2 Process each standard as described in Sections 11.4.1 and 11.4.2. The internal standard is added as described in Section 11.4.3. Triplicate samples should be prepared at each concentration level.

10.2   Demonstration and documentation of acceptable initial calibration are required before any samples are analyzed and intermittently throughout sample analyses as dictated by results of continuing calibration checks. After initial calibration is successful, a continuing calibration check is required at the

beginning of each eight hour period during which analyses are performed. Additional periodic calibration checks are good laboratory practice.

10.3   Initial Calibration

10.3.1 Calibrate the mass spectrometer with calibration compounds and procedures prescribed by the manufacturer with any modifications necessary to meet the requirements in Section 10.3.2.

10.3.2 Inject into the GC a 1 µL or 2 µL aliquot of the 5 ng/µL DFTPP solution and acquire a mass spectrum that includes data for m/z = 45-450. Use GC conditions that produce a narrow (at least five scans per peak) symmetrical peak. If the spectrum does not meet all criteria (Table 5), the MS must be retuned to meet all criteria before proceeding with calibration. An average spectrum across the GC peak may be used to evaluate the performance of the system.

10.3.3 Inject a 1 µL aliquot of a medium concentration calibration solution, for example 50 µg/L, and acquire and store data from m/z 45-450 with a total cycle time (including scan overhead time) of 1.5 seconds or less. Cycle time should be adjusted to measure at least five or more spectra during the elution of the GC peak. Figure 3 illustrates a total ion chromatogram and mass spectrum of endothall and the internal standard, acenaphthene-d10, using the prescribed conditions.

10.3.4 If all performance criteria are met, inject a 1 µL aliquot of each of the other calibration solutions using the same GC/MS conditions.

10.3.5 Calculate a response factor (RF) for endothall for each calibration solution by use of the internal standard response as expressed below. This calculation is supported in acceptable GC/MS data system software (Section 6.10.4), and many other software programs. The RF is a unitless number, but units used to express quantities of analyte and internal standard must be equivalent.

$$RF = \frac{(A_x)\,(Q_{is})}{(A_{is})\,(Q_x)}$$

where: A_x = integrated abundance of the quantitation ion of the analyte (m/z 183).

A_{is} = integrated abundance of the quantitation ion internal standard (m/z 164).

Q_x = quantity of analyte injected in ng or concentration units.

Q_{is} = quantity of internal standard injected in ng or concentration units.

10.3.5.1   Calculate the mean RF from the analyses of the calibration solutions. Calculate the standard deviation (SD) and the relative standard deviation (RSD) from each mean: RSD = 100 (SD/M). If the RSD of any analyte or surrogate mean RF exceeds 30%, either analyze additional aliquots of appropriate calibration solutions to obtain an acceptable RSD of RFs over the entire concentration range or take action to improve GC/MS performance. See Section 10.4.5 for possible remedial actions.

10.3.6 As an alternative to calculating mean response factors and applying the RSD test, use the GC/MS data system software or other available software to generate a linear or second order regression calibration curve.

10.4   Continuing calibration check. Verify the MS tune and initial calibration at the beginning of each eight-hour work shift during which analyses are performed using the following procedure.

10.4.1 Inject a 1 µL aliquot of the 5 ng/µL DFTPP solution and acquire a mass spectrum that includes data for m/z 45-450. If the spectrum does not meet all criteria (Table 5), the MS must be retuned to meet all criteria before proceeding with the continuing calibration check.

10.4.2 Inject a 1 µL aliquot of a medium concentration calibration solution and analyze with the same conditions used during the initial calibration.

10.4.3 Determine that the absolute area of the quantitation ion of the internal standard has not decreased by more than 30% from the area measured in the most recent continuing calibration check, or by more than 50% from the area measured during initial calibration. If the area has decreased by more than these amounts, adjustments must be made to restore system sensitivity. These adjustments may require cleaning of the MS ion source, or other maintenance as indicated in Section 10.4.5, and recalibration. Control charts are useful aids in documenting system sensitivity changes.

10.4.4 Calculate the RF for endothall from the data measured in the continuing calibration check. The RF must be within 30% of the mean value measured in the initial calibration. Alternatively, if a linear or second order regression is used, the concentration measured using the calibration curve must be within 30% of the true value of the concentration in the medium calibration solution. If these conditions do not exist, remedial action must be taken which may require repeating the initial calibration.

10.4.5 Some possible remedial actions: major maintenance such as cleaning an ion source, cleaning quadrupole rods, etc. require returning to the initial calibration step.

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

    10.4.5.2   Clean or replace the splitless injection liner; silanize a new injection liner.

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

    10.4.5.4   Break off a short portion (about 1 M) of the column from the end near the injector; or replace GC column. This action will cause a slight change in retention times.

    10.4.5.5   Prepare fresh CAL solutions, and repeat the initial calibration step.

    10.4.5.6   Clean the MS ion source and rods (if a quadrupole).

    10.4.5.7   Replace any components that allow analytes to come into contact with hot metal surfaces.

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

11.0 PROCEDURE

11.1   Preparation of Anion Exchange Cartridges

11.1.1 Prepare a 50% (v/v) slurry of Bio-Rex 5 resin and reagent water.

11.1.2 Attach the required number of 8 mL extraction cartridges (Section 6.13) to the vacuum manifold (Section 6.12), and insert bottom fritted disks into each cartridge.

11.1.3 Fill the cartridges completely with Bio-Rex 5 slurry. Draw off excess water with vacuum. The final wet resin bed height should be 3.5 ±0.1 cm. Adjust the height by adding more slurry and repeating procedure, or add more reagent water to reservoir and remove excess resin slurry.

11.1.4 After the bed heights are adjusted to 3.5 cm and with excess water removed under vacuum, insert a fritted disk on top of the resin bed. The fritted disk should press firmly into the resin and be horizontal to the reservoir to prevent sample channeling around the disk. Fill the

cartridges with reagent water and draw half of the water into the resin. Maintain the resin cartridges in this condition until ready for use.

NOTE: The use of liquid-solid extraction disks instead of cartridges is permissible as long as all the quality control criteria specified in Section 9.0 of this method are met.

11.2   Sample Preparation

11.2.1 As discussed above (Sections 1.3 and 4.2), reduced recoveries will be observed if the sample contains elevated levels of CaII, MgII or sulfate. If facilities are available, measure the concentrations of these ions. Figure 1 graphically presents analyte recovery versus individual ion concentration. Reduced recoveries may be anticipated when the combined CaII + MgII exceeds approximately 100 mg/L or sulfate exceeds approximately 250 mg/L. If measurement of ion concentration is not feasible, determine the actual recovery for a laboratory fortified sample matrix as described in Section 9.7. In the event of anticipated or measured low recoveries, treat the sample as described in Section 11.2.2.

11.2.2 For samples containing moderately high levels of these ions, add 186 mg of EDTA (Section 7.9) per 100 mL sample (0.005 M). The treated ground water characterized in Table 3 is an example of a matrix successfully treated this way. For samples containing very high levels of sulfate, sample dilution may be required in addition to the EDTA. The western surface water characterized in Table 3 (ca. 2000 mg/L sulfate) was successfully analyzed after dilution by a factor of 10 and the addition of 75 mg EDTA per 100 mL of the diluted sample (0.002 M). Samples containing intermediate levels of sulfate can be analyzed with smaller dilution factors. Guidelines on dilution factors and EDTA addition are given below.

Sulfate, mg/L	Dilution Factor	Added EDTA, mg/100 mL
<250	1:1	186
250 - 500	1:2	125
500 - 1250	1:5	75
>1250	1:10	75

NOTE: Dilution should not be employed if adequate recovery is attained by the addition of EDTA alone.

11.2.3 The addition of EDTA results in a large reagent peak near the end of the temperature program. Therefore, complete the entire program described in Table 1.

11.2.4 If the ionic nature of the samples being processed is completely unknown, the analyst as an option may routinely dilute all samples by a factor of 10 and add EDTA as above. However, the analyst should be able to demonstrate reagent water MDLs of 2 µg/L or lower. In this event the MDL will be 20 µg/L or less for the diluted sample, still a factor of 5 below the regulated maximum contaminant level.

11.3 Sample Extraction

11.3.1 Attach the 70 mL reservoir to the resin cartridge with the adapter (Section 6.14).

11.3.2 Condition the resin cartridge by drawing the following reagents through the cartridge in the following order:

1. 10 mL methanol
2. 10 mL reagent water
3. 10 mL 10% H_2SO_4 in methanol
4. 10 mL reagent water
5. 20 mL 1 N NaOH
6. 20 mL reagent water

Do not allow the cartridge to become dry between steps. Draw each reagent through the cartridge at a rate of 10 mL/min. Leave a 1 cm layer of reagent water over the resin bed.

11.3.3 Fill the 70 mL reservoir with 60 mL of the sample. Adjust sample flow rate to 3 mL/min. Add the balance of sample when needed to prevent the reservoir from going dry.

11.3.4 After the sample passes through the cartridge, remove the 70 mL reservoir and the adapter. Draw 10 mL of methanol through the resin cartridge. Make sure that any visible water inside the cartridge dissolves in methanol. Next draw room air through the cartridge for five minutes under a vacuum of 10-20 in. Hg. Position the culture tube (Section 6.3) inside the manifold to collect the eluent.

11.3.5 Elute the cartridge with 8 mL of 10% H_2SO_4 in methanol, followed by 6 mL of methylene chloride under vacuum over a one minute period.

11.4 Sample Derivatization, Partition and Analysis

11.4.1 Cap the culture tube and hold at 50°C for one hour in a heating block or water bath. Remove from heat and allow the tube to cool for 10 minutes.

11.4.2 Pour the contents of the culture tube into a 125 mL separatory funnel. Rinse the tube with two x 0.5 mL aliquots of methylene chloride and add the rinsings to the separatory funnel. Add 20 mL of 10% sodium

sulfate in reagent water to the separatory funnel. Shake the funnel three times vigorously, venting with the stopcock, and then shake vigorously for an additional 15 seconds. After the phases have separated, drain the lower organic layer into a 15 mL graduated centrifuge tube (Section 6.4). Repeat the extraction procedure above with two additional 2 mL aliquots of methylene chloride, adding the organic phase to the centrifuge tube each time.

11.4.3 Fortify the extract with 250 µL of the internal standard working solution (Section 7.12.2) and concentrate to a final volume of 1.0 mL, using the N-EVAP (Section 6.7) and dry nitrogen.

11.4.4 Inject 2 µL of the concentrated extract (Section 11.4.3) and analyze by GC/MS using the conditions described in Table 1. This table includes the retention time and MDL that were obtained under these conditions. A sample total ion chromatogram of endothall and d-10 acenaphthene illustrating retention times, and the mass spectrum of the dimethylated endothall are shown in Figure 3. Other columns, chromatographic conditions, or detectors may be used if the requirements of Section 9.3 are met.

11.4.5 If the peak area exceeds the linear range of the calibration curve, a smaller sample volume should be used.

11.5   Identification of the Analyte

11.5.1 Identify endothall by comparison of its mass spectrum (after background subtraction) to a reference spectrum in a user created spectral library. The GC retention time of the sample component should be within 10 seconds of the retention time of endothall in the latest calibration standard. If a FID is used, identifications should be confirmed by retention time comparisons on the second GC column (Table 1).

11.5.2 In general, all ions present above 10% relative abundance in the mass spectrum of the standard should be present in the mass spectrum of the sample component and should agree within absolute 20%. For example, if an ion has a relative abundance of 30% in the standard spectrum, its abundance in the sample spectrum should be in the range of 10-50%. However, the experience of the analyst should weigh heavily in the interpretation of spectra and chromatograms.

11.5.3 Identification requires expert judgement when sample components are not resolved chromatographically, that is, when GC peaks from interferences are present. When endothall coelutes with an interference, indicated by a broad peak or a shoulder on the peak, the identification criteria can usually be met, but the endothall spectrum will contain extraneous ions contributed by the coeluting interfering compound.

12.0   DATA ANALYSIS AND CALCULATIONS

12.1   When using GC/MS, complete chromatographic resolution is not necessary for accurate and precise measurements of analyte concentrations if unique ions with adequate intensities are available for quantitation. However, when using FID, complete resolution is essential.

12.1.1   Calculate endothall concentration

$$C_x = \frac{(A_x)\ (Q_{is})}{(A_{is})\ RF\ V}$$

where: C_x = concentration of endothall in μg/L in the water sample.
A_x = integrated abundance of the quantitation ion of endothall (m/z 183) in the sample.
A_{is} = integrated abundance of the quantitation ion of the internal standard (m/z 164) in the sample.
Q_{is} = total quantity (in micrograms) of internal standard added to the water sample.
V = original water sample volume in liters.
RF = mean response factor endothall from the initial calibration.

12.1.2   Alternatively, use the GC/MS data system software or other available proven software to compute the concentration of the endothall from the linear calibration or the second order regression curves.

12.1.3   Calculations should utilize all available digits of precision, but final reported concentrations should be rounded to an appropriate number of significant figures (one digit of uncertainty). Experience indicates that three significant figures may be used for concentrations above 99 μg/L, two significant figures for concentrations between 1-99 μg/L, and one significant figure for lower concentrations.

13.0   METHOD PERFORMANCE

13.1   Method Detection Limits -- The MDL is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above the background level[1]. The MDLs listed in Table 1 were obtained using reagent water for detection by GC/MS and FID.

13.2   In a single laboratory study on fortified reagent water and ground water matrices, the mean recoveries and relative standard deviations presented in Table 2 were obtained. Table 3 provides the concentrations of CaII, MgII and sulfate for two high ionic strength drinking water sources studied. Table 4 presents mean recovery data for these fortified sources with and without the addition of EDTA and/or sample dilution.

14.0 POLLUTION PREVENTION

14.1 This method utilizes the new liquid-solid extraction technology which requires the use of very little organic solvent thereby eliminating the hazards involved with the use of large volumes of organic solvents in conventional liquid-liquid extractions. It also uses acidic methanol as the derivatizing reagent in place of the highly toxic and explosive diazomethane. These features make this method much safer for the analyst to employ and a great deal less harmful to the environment.

15.0 WASTE MANAGEMENT

15.1 Due to the nature of this method, there is very little need for waste management. No large volumes of solvents or hazardous chemicals are used. The matrices are drinking water or source water, and can be discarded down the sink.

16.0 REFERENCES

1. 40 CFR Part 136, Appendix B.

2. ASTM Annual Book of Standards, Part 31, D3694-78. "Standard Practices for Preparation of Sample Containers and for Preservation of Organic Constituents," American Society for Testing and Materials, Philadelphia, PA.

3. "Carcinogens-Working with Carcinogens," Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Publication No. 77-206, August 1977.

4. "OSHA Safety and Health Standards, General Industry," (29 CFR 1910), Occupational Safety and Health Administration, OSHA 2206, (Revised, January 1976).

5. "Safety in Academic Chemistry Laboratories," American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

TABLE 1. RETENTION TIMES AND METHOD DETECTION LIMITS

Compound	Retention Time (min)			Method Detection Limit	
	Column A	Column B	Column C	GC/MS μg/L[1]	FID
Endothall	16.02	19.85	18.32	1.79	0.7
d10-Acenaphthene	14.69				

[1]Based on seven replicate analyses of a reagent water fortified at 2 μg/L

Column A: DB-5 fused silica capillary for GC/MS, 30 m x 0.25 mm, 0.25 micron film

MS inlet temperature = 200°C
Injector temperature = 200°C

Temperature Program: Hold five minutes at 80°C, increase to 260°C at 10°/min, hold 10 minutes.

Column B: FID primary column, RTX Volatiles, 30 m x 0.53 mm I.D., 2 micron film thickness.

Detector temperature = 280°C
Injector Temperature = 200°C

Carrier gas velocity = 50 cm/sec.

Temperature program: Same as Column A.

Column C: FID confirmation column, DB-5, 30 m x 0.32 mm ID, 0.25 micron film.

Carrier Gas velocity = 27 cm/sec

Same injector, detector and temperature program as Column A.

TABLE 2. ENDOTHALL METHOD DEVELOPMENT DATA

Matrix	Conc. (µg/L)	Recovery[1] (%)	RSD[2] (%)
Reagent Water	2	101	10
Reagent Water	10	86	10
Reagent Water	100	95	3
Ground Water[3]	2	91	25
Ground Water	10	82	14
Ground Water	100	88	6

[1]Based on analysis of seven replicates.
[2]Relative Standard Deviation.
[3]High Humic Content Florida Ground Water.

TABLE 3. MATRIX ANALYSES[1]

Major Ion	Western Surface (mg/L)	Eastern Ground (mg/L)
Ca	330	122
Mg	132	33
Na	400	23
Sulfate	1850	102

[1]Determination by inductively coupled plasma - mass spectrometry for cations and ion chromatography for sulfate.

TABLE 4. ENDOTHALL METHOD VALIDATION DATA

Matrix	Conc. (µg/L)	EDTA[1] (Mole/L)	Recovery[2] (%)	RSD (%)
WS[3]	25	0	9	19
WS-1/10[4]	50	0	66	13
WS-1/10	50	0.002	88	5
EG[5]	25	0	43	17
EG	25	0.005	97	6
EG-1/5	25	0	97	5

[1]Ethylenediamine Tetraacetic Acid.
[2]Based on seven replicates.
[3]WS-treated Western surface water.
[4]Dilution factor in reagent water.
[5]WG-Eastern ground water.

TABLE 5.
ION ABUNDANCE CRITERIA FOR BIS(PERFLUOROPHENYL)PHENYL PHOSPHINE (DECAFLUOROTRIPHENYLPHOSPHINE, DFTPP)

Mass (m/z)	Relative Abundance Criteria	Purpose of Checkpoint[1]
51	10-80% of the base peak	low mass sensitivity
68	<2% of mass 69	low mass resolution
70	<2% of mass 69	low mass resolution
127	10-80% of the base peak	low-mid mass sensitivity
197	<2% of mass 198	mid-mass resolution
198	base peak or >50% of 442	mid-mass resolution and sensitivity
199	5-9% of mass 198	mid-mass resolution and isotope ratio
275	10-60% of the base peak	mid-high mass sensitivity
365	>1% of the base peak	baseline threshold
441	Present and <mass 443	high mass resolution
442	base peak or >50% of 198	high mass resolution and sensitivity
443	15-24% of mass 442	high mass resolution and isotope ratio

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

ENDOTHALL RECOVERY FROM REAGENT WATER
WITH SO4, MGII AND CAII IONS PRESENT

+ SO4   △ CAII   ○ MGII   + SO4
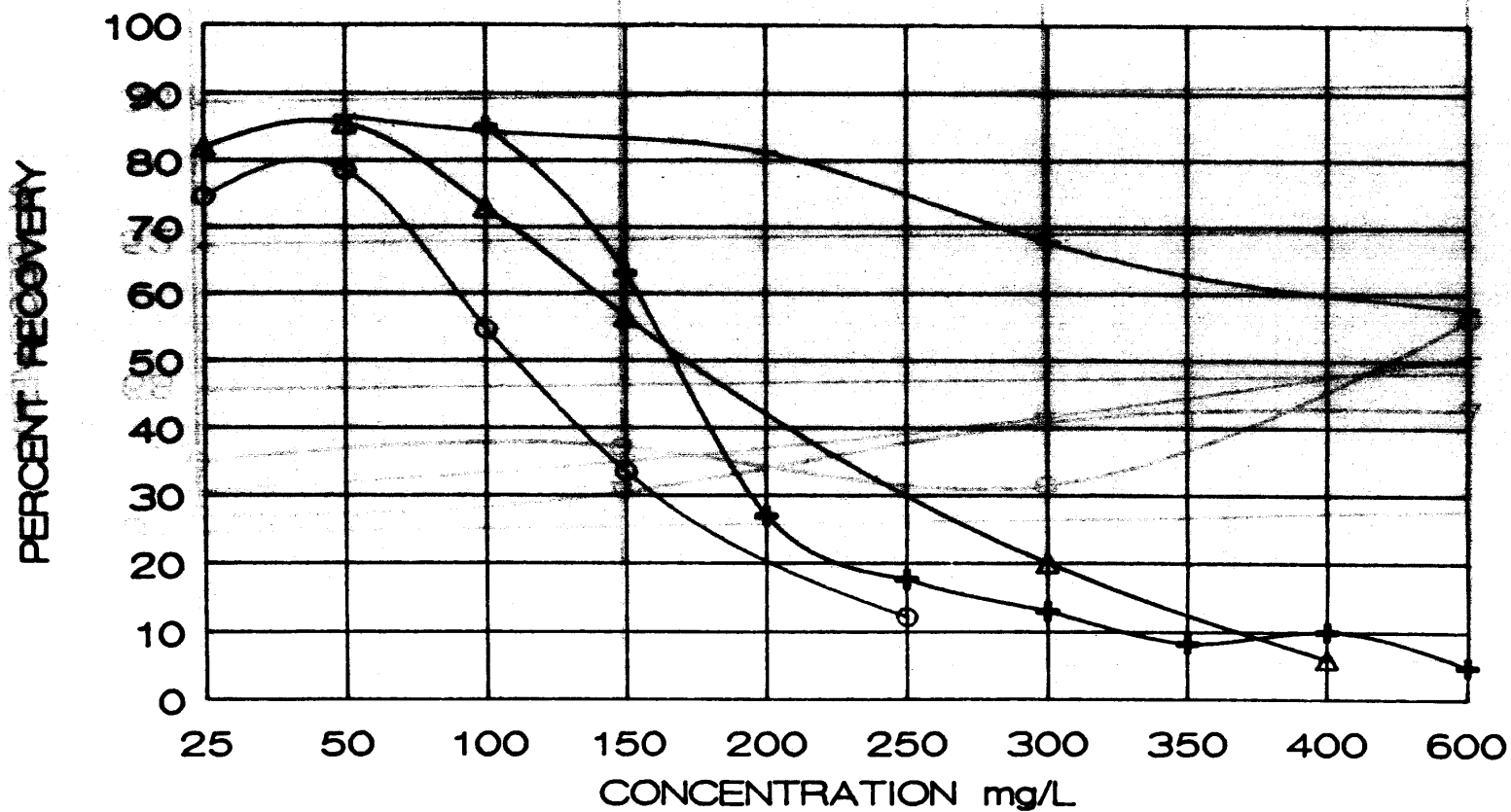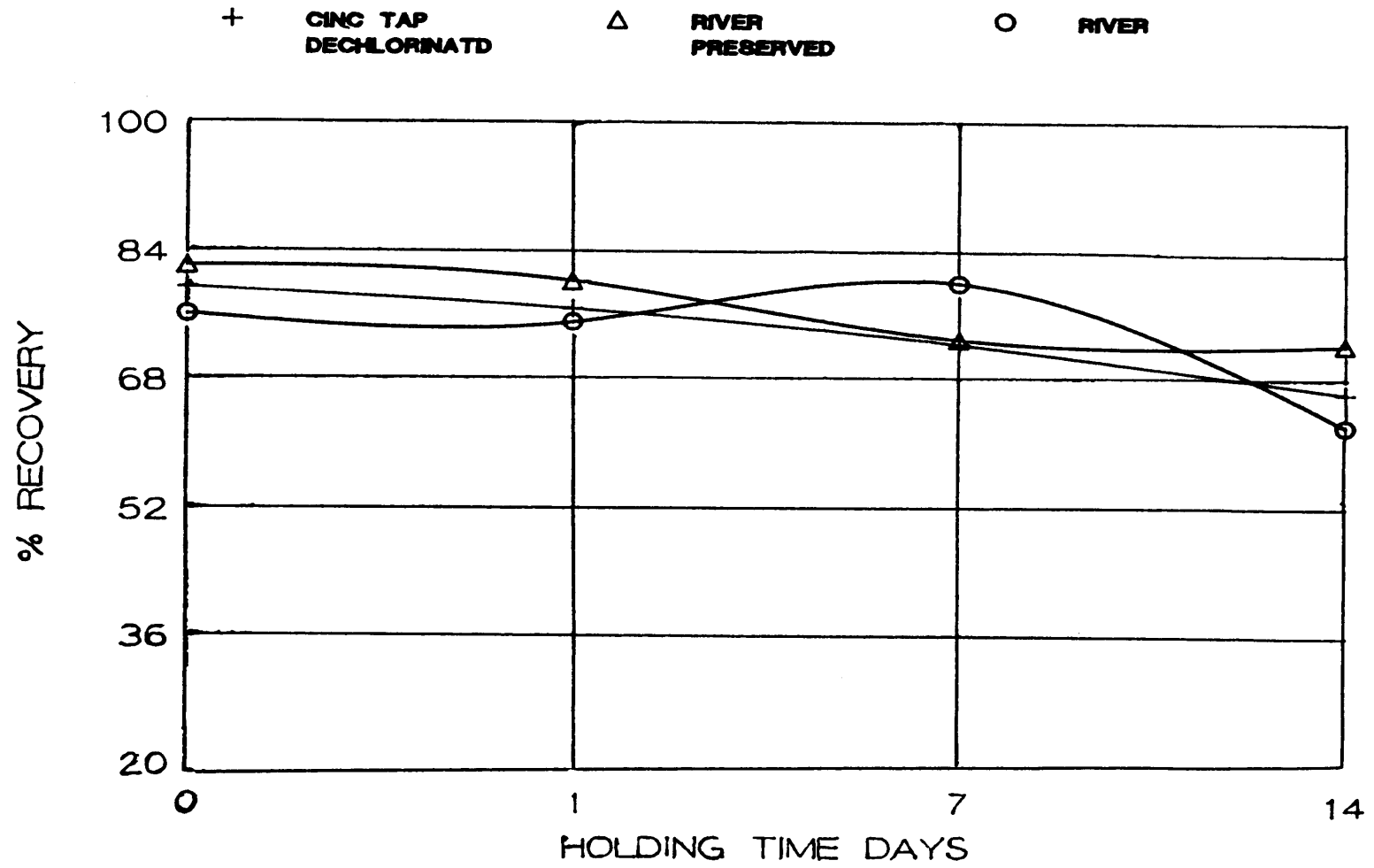
    NA2SO4   CACL2   MGCL2   CASO4

FIGURE 1. ENDOTHALL RECOVERY VERSUS ION CONCENTRATIONS

ENDOTHALL SAMPLE HOLDING STUDY

+   CINC TAP   △   RIVER   O   RIVER
    DECHLORINATD   PRESERVED

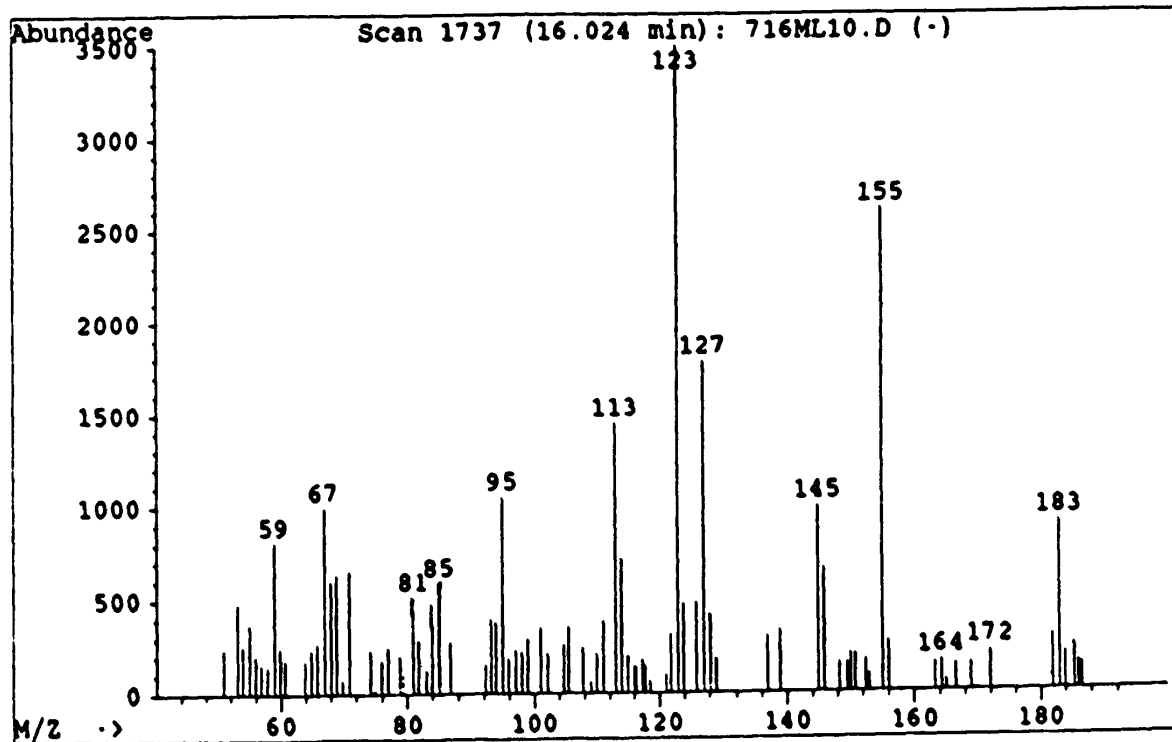
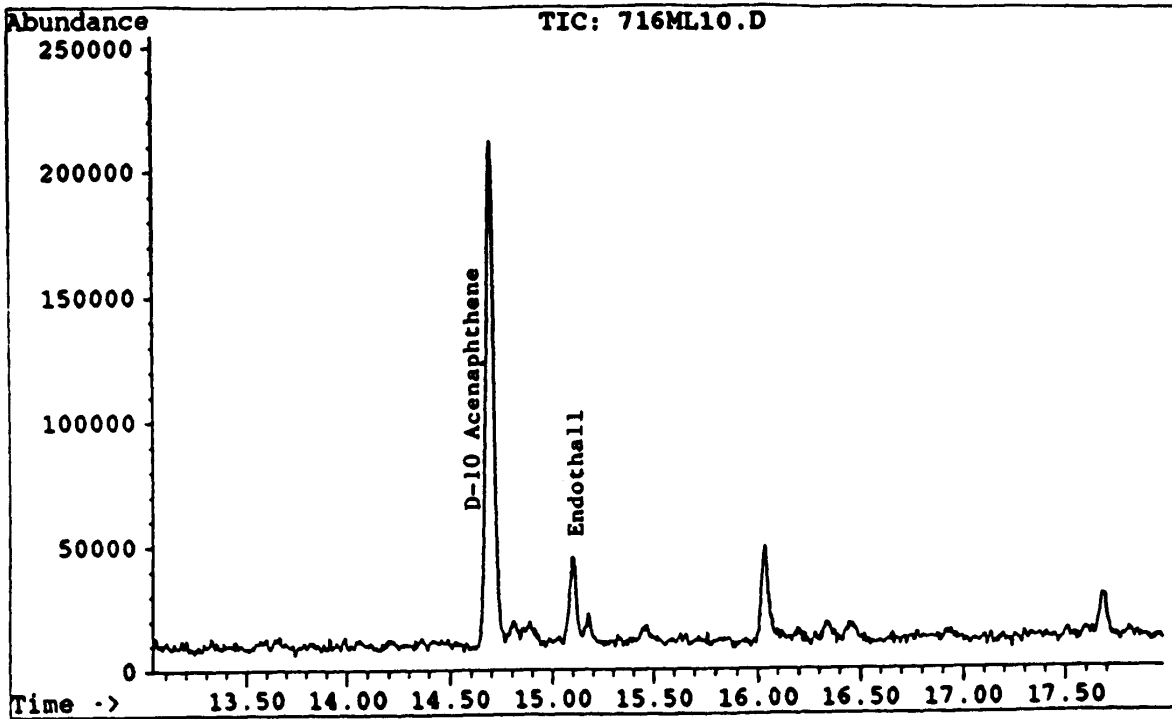FIGURE 2.   ENDOTHALL HOLDING STUDY

FIGURE 3. ENDOTHALL GC/MS

UPPER: TOTAL ION CHROMATOGRAPHY ENDOTHALL: 16.02 MIN., 10 NG

LOWER: RELATIVE ION ABUNDANCE

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