

METHOD 548. DETERMINATION OF ENDOTHALL IN DRINKING WATER BY
AQUEOUS DERIVATIZATION, LIQUID-SOLID EXTRACTION, AND
GAS CHROMATOGRAPHY WITH ELECTRON-CAPTURE DETECTION

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METHOD 548

DETERMINATION OF ENDOTHALL IN DRINKING WATER BY AQUEOUS DERIVATIZATION, LIQUID-SOLID EXTRACTION, AND GAS CHROMATOGRAPHY WITH ELECTRON-CAPTURE DETECTION

1. SCOPE AND APPLICATION

- 1.1 This method covers the determination of endothall in drinking water sources and finished drinking water. The following analyte can be determined by this method:

<u>Analyte</u>	Chemical Abstract Services <u>Registry Number</u>
Endothall	145-73-3

- 1.2 This is a gas chromatographic (GC) method applicable to the determination of the compound listed above. When this method is used to analyze unfamiliar samples, compound identification should be supported by at least one additional qualitative technique. A gas chromatograph/mass spectrometer (GC/MS) may be used for the qualitative confirmation of results for endothall using the extract produced by this method.
- 1.3 The method detection limit¹ (MDL, defined in Section 13) for endothall is listed in Table 1. The MDL for a specific sample may differ from the listed value, depending upon the nature of interferences in the sample matrix and the amount of sample used in the procedure.
- 1.4 The endothall-pentafluorophenylhydrazine derivative employed for chromatographic detection is not available commercially. Thus, this method employs procedural standards, in which endothall calibration solutions (9.2.1) are processed through the analysis procedure (11.2).
- 1.5 This method is restricted to use by or under the supervision of analysts experienced in the use of gas chromatography and in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 11.

2. SUMMARY OF METHOD

- 2.1 A 5.0 mL volume of liquid sample is placed in a Kuderna-Danish tube and the volume is reduced to less than 0.5 mL using a heating block. The tube is charged with glacial acetic acid and sodium acetate, followed by a solution of the derivatization reagent, pentafluorophenylhydrazine (PFPH), in glacial acetic acid. After heating at 150°C for 90 minutes the derivative is extracted by a solid

sorbent from the reaction solution, followed by elution with 5.0 mL of methyl-tert-butyl ether (MTBE). The MTBE extract is analyzed by gas chromatography with electron capture detection (GC/ECD).

3. DEFINITIONS

- 3.1 INTERNAL STANDARD - A pure analyte(s) added to a solution in known amount(s) and used to measure the relative responses of other method analytes and surrogates that are components of the same solution. The internal standard must be analyte that is not a sample component.
- 3.2 SURROGATE ANALYTE - 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 and is measured with the same procedures used to measure other sample components. The purpose of a surrogate analyte is to monitor method performance with each sample.
- 3.3 LABORATORY DUPLICATES (LD1 and LD2) - Two sample aliquots taken in the analytical laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 give a measure of the precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.
- 3.4 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 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) - 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.
- 3.7 LABORATORY PERFORMANCE CHECK SOLUTION (LPC) - A solution of method analytes, surrogate compounds, and internal standards 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 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.
- 3.9 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 - 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.
- 3.11 PRIMARY DILUTION STANDARD SOLUTION - 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 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.
- 3.13 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. 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 conditions of the analysis by running laboratory reagent blanks as described in Section 10.2.

4.1.1 Glassware must be scrupulously clean². Clean all glassware 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 40°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 is highly recommended.

4.2 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the matrix being sampled. If significant interferences occur in subsequent samples, some additional cleanup may be necessary to achieve the MDL listed in Table 1.

4.3 The extent of interferences that may be encountered using gas chromatographic techniques has not been fully assessed. Although the GC conditions described allow for a unique resolution of the specific compound covered by this method, other matrix components may interfere.

5. 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 reduced to the lowest possible level by whatever means available. 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.

6. APPARATUS AND MATERIALS

6.1 SAMPLING EQUIPMENT (for discrete or composite sampling).

6.1.1 Grab sample bottle - Amber glass 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 GLASSWARE

6.2.1 Volumetric flasks - 5 mL, 25 mL

6.2.2 Vials - glass, 1 mL, with Teflon-lined caps

6.2.3 Glass syringes, 250 μ L, 500 μ L

6.2.4 Pipets - 1 mL, 4 mL

6.3 BALANCE - analytical, capable of accurately weighing 0.0001 g.

6.4 SOLID SORBENT CARTRIDGES - C-18

6.5 Vacuum manifold for extraction using solid sorbent cartridges - Supelco 5-7030 or equivalent

6.6 Kuderna-Danish (K-D) concentrator tubes - 10 or 25 mL graduated

6.6.1 Snyder column, Kuderna-Danish -2- ball micro

6.7 Tube heater for 25 mL K-D tubes

6.8 Boiling chips - carborundum, #12 granules Heat at 400°C for 30 minutes prior to use. Cool and stored in dessicator.

6.9 Gas chromatographic system capable of temperature programming

6.9.1 Autosampler

6.9.2 Electron capture detector

6.9.3 Column 1: Supelco SPB-5, 0.25 mm x 30 m or equivalent

Column 2: J&W DB-1, 0.32 mm x 30 mm or equivalent

6.9.4 Strip-chart recorder compatible with detector. Use of a data system with printer for measuring and recording peak areas and retention times is recommended.

7. REAGENTS AND SOLUTIONS

7.1 REAGENT WATER - reagent water is defined as a water of very high purity, equivalent to distilled in glass solvents

7.2 PENTAFLUOROPHENYLHYDRAZINE (PFPH) - Aldrich

7.3 SODIUM ACETATE - anhydrous

7.4 SODIUM THIOSULFATE

- 7.5 ACETIC ACID - glacial
- 7.6 METHYL-TERT-BUTYL ETHER (MTBE) - distilled in glass
- 7.7 ENDOTHALL-PFPH DERIVATIVE - See Appendix for synthesis procedure
- 7.8 ENDOSULFAN I
- 7.9 ENDOTHALL, monohydrate
- 7.10 STOCK STANDARD SOLUTIONS
 - 7.10.1 Endothall - 10 $\mu\text{g/mL}$ in reagent water
 - 7.10.2 Endothall - 50 $\mu\text{g/mL}$ in reagent water
 - 7.10.3 Stock standard solutions must be replaced after six months, or sooner, if comparison with check standards indicates a problem.
- 7.11 REACTION SOLUTIONS
 - 7.11.1 PFPH solution - 4 mg/mL in glacial acetic acid
 - 7.11.2 Internal standard stock solution - 10 $\mu\text{g/mL}$ endosulfan I in MTBE

8. SAMPLE COLLECTION, PRESERVATION, AND HANDLING

- 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 in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of Tygon tubing and other potential sources of contamination.
- 8.2 The samples must be iced or refrigerated at 4°C from the time of collection until derivatization. The analyte measured here is not known to be light sensitive, but excessive exposure to light and heat should be avoided.
- 8.3 Some samples are likely to be biologically active and the stability of samples upon storage will be different for each matrix. All samples should be derivatized within 7 days of collection, and analysis completed within 1 day of derivatization. If these criteria are not met, the analyst must demonstrate the stability of the stored sample by performing suitable holding time studies.

9. CALIBRATION

9.1 Establish gas chromatographic operating parameters to produce a retention time equivalent to that indicated in Table 1. The chromatographic system can be calibrated using the internal standard technique (Section 9.2).

9.1.1 Due to the complex nature of the sample chromatogram, the analyst should periodically inject a solution containing only pure endothall-PFPH (See Appendix) to verify the retention time of the derivative.

9.2 INTERNAL STANDARD CALIBRATION PROCEDURE:

9.2.1 Use 250 and 500 μL syringes to add sufficient quantities of 7.10.1 or 7.10.2 stock solutions to reagent water in 25 mL volumetric flasks to produce endothall standard solutions at the following concentrations in $\mu\text{g/L}$: 500 (250 μL of 7.10.2 stock), 200 (100 μL of 7.10.2 stock), 100 (50 μL of 7.10.2 stock) and 50 (125 μL of 7.10.1 stock).

9.2.2 Process each standard as per Section 11.2. The internal standard is added as described in Section 11.2.7. It is recommended that triplicate samples of each standard be processed.

9.2.3 Before analyzing matrix samples, the analyst must process a series of calibration standards to validate elution patterns and the absence of interferences from reagents.

9.2.4 Analyze each calibration standard and tabulate the ratio of the area of the endothall-PFPH derivative peak versus that of the internal standard against endothall concentration. The results may be used to prepare a calibration curve for endothall.

9.2.5 The working calibration curve must be verified on each working day by processing and analyzing one or more calibration standards. If the response varies from the previous response by more than $\pm 20\%$, the test must be repeated using a fresh calibration standard. Should the retest fail, a new calibration curve must be generated.

10. QUALITY CONTROL

10.1 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.

- 10.2 LABORATORY REAGENT BLANKS. 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 method blank must be analyzed. For this method, the method blank is filtered reagent water. If within the retention time window of an analyte of interest, the method blank produces a peak which prevents the measurement of that analyte, determine the source of contamination and eliminate the interference before processing samples.
- 10.3 INITIAL DEMONSTRATION OF CAPABILITY
- 10.3.1 Select a representative fortified concentration (about 10 times MDL) for endothall. Prepare a concentrate (in reagent water) containing the analyte at 10 times the selected concentration. Using a pipet, add 1.00 mL of the concentrate to each of at least four 10 mL aliquots of reagent water and analyze each aliquot according to procedures beginning in Section 11.
- 10.3.2 The recovery value should for at least three out of four consecutively analyzed samples fall in the range of $R \pm 30\%$ (or within $R \pm 3S_R$, if broader) using the values for R and S_R for reagent water (Table 2). If the recovery value meets the acceptance criteria, performance is acceptable and sample analysis may begin. If the recovery value fails these criteria, initial demonstration of capability should be repeated.
- 10.3.3 The initial demonstration of capability is used primarily to preclude a laboratory from analyzing unknown samples by a new, unfamiliar method prior to evidencing a basal level of skill at performing the technique. It is expected that as laboratory personnel gain experience with this method the quality of the data will improve beyond the requirements stated in Section 10.3.2.
- 10.4 The analyst is permitted to modify GC columns, GC conditions, or detectors to improve separations or lower analytical costs. Each time such method modifications are made, the analyst must repeat the procedures in Section 10.3.
- 10.5 Assessing the Internal Standard - In using the IS calibration procedure, the analyst is expected to monitor the IS response (peak area or peak height) of all samples during each analysis day. The IS response for any sample chromatogram should not deviate from the calibration standard IS response by more than 30%.
- 10.5.1 If a deviation of greater than 30% is encountered for a sample, reinject the extract.

- 10.5.1.1 If acceptable IS response is achieved for the re-injected extract, then report the results for that sample.
- 10.5.1.2 If a deviation of greater than 30% is obtained for the reinjected extract, analysis of the sample should be repeated beginning with Section 11, provided the sample is still available. Otherwise, report results obtained from the reinjected extract, but annotate as suspect.
- 10.5.2 If consecutive samples fail the IS response acceptance criterion, immediately analyze a calibration check standard.
 - 10.5.2.1 If the check standard provides a response factor (RF) within 20% of the predicated value, then follow procedures itemized in Section 10.5.1 for each sample failing the IS response criterion.
 - 10.5.2.2 If the check standard provides a response factor (RF) which deviates more than 20% of the predicted value, then the analyst must recalibrate, as specified in Section 9.2.

10.6 ASSESSING LABORATORY PERFORMANCE

- 10.6.1 The laboratory must analyze at least one LFB per sample set (all samples analyzed within a 24 hour period). The fortifying concentration in the LFB should be 10 times the MDL. Calculate accuracy as percent recovery (X_i). If the recovery falls outside the control limits (See Section 10.6.2), the system is judged out of control, and the source of the problem must be identified and resolved before continuing analyses.
- 10.6.2 Until sufficient LFB data become available, usually a minimum of results from 20 to 30 analyses, the laboratory should assess its performance against the control limits described in Section 10.3.2. When sufficient laboratory 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:

$$\begin{aligned}\text{Upper Control Limit} &= \bar{X} + 3S \\ \text{Lower Control Limit} &= \bar{X} - 3S\end{aligned}$$

After each group of five to ten new recovery measurements, control limits should be recalculated using only the most recent 20 to 30 data points.

10.6.3 It is recommended that the laboratory periodically determine and document its detection limit capabilities for endothall.

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

10.7 ASSESSING ANALYTE RECOVERY

10.7.1 The laboratory must add a known fortified concentration 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 spiking. The fortified concentration should be the same as that used for the LFB (Section 10.6). Over time, samples from all routine sample sources should be fortified.

10.7.2 Calculate the percent recovery (R_i) for endothall, corrected for background concentrations measured in the unfortified sample, and compare these values to the control limits established in Section 10.6.2 for the analyses of LFBs.

10.7.3 If the recovery falls outside the designated range, and the laboratory performance for that sample set is shown to be in control (Section 10.6), the recovery problem encountered with the dosed 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.

11. PROCEDURE

11.1 CLEANUP AND SEPARATION - Cleanup procedures may not be necessary for a relatively clean sample matrix. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must demonstrate that the recovery of endothall is within the limits specified by the method.

11.1.1 If the sample is not clean, or the complexity is unknown, the entire sample should be centrifuged at 2500 rpm for 10 minutes. The supernatant is decanted from the centrifuge bottle and passed through glass fiber filter paper into a container which can be tightly sealed.

11.1.2 Store all samples at 4°C.

11.2 SAMPLE EXTRACTION AND ANALYSIS

11.2.1 Measure out a 5.0 mL aliquot of the sample and place it in a 10 or 25 mL K-D tube. Add boiling chips.

- 11.2.2 Place on tube heater at maximum setting and concentrate sample to less than 0.5 mL.
- 11.2.3 Add 4 mL glacial acetic acid, 200 mg sodium acetate and 1 mL of glacial acetic acid containing 4 mg PFPH. Use glass stirring rod to break-up the sodium acetate solid. Place a Micro Snyder column on each K-D tube.
- 11.2.4 Heat at 150°C for 90 minutes.
- 11.2.5 Dilute the reaction mixture with reagent water and decant into a 50 mL beaker or flask. Wash the K-D tube and residue with aliquots of reagent water and add to the beaker until the total aqueous volume is 40-45 mL.
- 11.2.6 Assemble the vacuum manifold. Rinse the solid sorbent cartridge by passing 5 mL of reagent water through the cartridge. Discard the water. Extract the aqueous sample from 11.2.5 by passing the sample through the solid sorbent cartridge at a rate of 5-6 mL per minute.
- 11.2.7 Wash the cartridge with 5 mL reagent water. Elute the cartridge with two 2 mL aliquots of MTBE. Combine the eluates with .05 mL of the internal standard stock solution (7.11.2) and dilute to 5 mL in a volumetric flask with MTBE.
- 11.2.8 Analyze the eluates by GC/ECD using conditions described in Table 1. This table includes the retention time and MDL that were obtained under these conditions. Sample chromatograms of an endothall standard and a LRB both with internal standard are represented in Figures 1 and 2. Other columns, chromatographic conditions, or detectors may be used if the requirements of Section 10.3 are met.

11.3 IDENTIFICATION OF THE ANALYTE

- 11.3.1 Identify endothall by comparison of its retention time to the retention time of a reference chromatogram. If the retention time of the unknown compound corresponds, within limits, to the retention time of a standard endothall, then identification is considered positive. However, positive identifications should be confirmed by retention time comparisons on the second GC column, or by using GC/MS.
- 11.3.2 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 can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

- 11.3.3 Identification requires expert judgement when sample components are not resolved chromatographically, that is, when GC peaks from interferences are present. Any time doubt exists over the identification of the endothall peak, appropriate techniques such as use of an alternative detector which operates on a chemical/physical principle different from that originally used, e.g., mass spectrometry, or the use of a second chromatography column must be used.
- 11.4 If the peak area exceeds the linear range of the calibration curve, a smaller sample volume should be used. Alternatively, the final solution may be diluted with MTBE and reanalyzed.
- 11.5 If the peak area measurement is prevented by the presence of interferences, further cleanup is required.

12. CALCULATIONS

- 12.1 Determine the peak area ratio for endothall in the injected sample.

12.1.1 Calculate the concentration of endothall injected using the calibration curve in Section 9.2. The concentration in a liquid sample can be calculated from Equation 1:

$$\text{Equation 1} \quad \text{Concentration, } \mu\text{g/L} = \frac{(A)(VF)}{(VS)}$$

where:

A = Concentration of endothall in extract, in $\mu\text{g/L}$
VF = Final volume of MTBE, in mL
VS = Sample volume, in mL

- 12.2 Report results as micrograms per liter. When duplicate and fortified samples are analyzed, report all data obtained with the sample results.
- 12.3 For samples processed as part of a set where the laboratory fortified sample recovery falls outside of the control limits established in Section 10.6, data must be labeled as suspect.

13. 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. The estimated MDL concentration listed in Table 1 was obtained using reagent water. Similar results were achieved using representative matrices.

13.2 This method has not been tested for linearity of recovery from fortified reagent water.

13.3 In a single laboratory using dechlorinated tap and reagent water fortified matrices, the average recoveries presented in Table 2 were obtained. The standard deviation of the percent recovery is also included in Table 2.

14. 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.

TABLE 1. GAS CHROMATOGRAPHY CONDITIONS AND METHOD DETECTION LIMITS

Analyte	Ret. Time (min.)	MDL ($\mu\text{g/L}$)
Endothall	42.3	11.5

GC conditions: 0.25 mm x 30 m SPB-5 column; 2 μL injection; hold one minute at 60°C, program to 300°C at 4°C/minute, hold at 300°C for 15 minutes.

TABLE 2. SINGLE OPERATOR ACCURACY AND PRECISION

Analyte	Matrix Type	Average Percent Recovery	Standard Deviation (percent)	Fortified Conc. ($\mu\text{g/L}$)	Number of Analyses
Endothall	Reagent Water	120	25.3	15	8
		108	15.3	150	8
	Dechlorinated Tap Water	84.0	13.8	15	8
		94.0	13.3	150	8

100 mg/L sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$) added to prior to fortifying with endothall

APPENDIX

Preparation of Endothall-Pentafluorophenylhydrazine

1. Prepare solution A of endothall by dissolving 0.204 g of endothall monohydrate (1.0 mmol) in 14 mL of methylene chloride and 3.6 mL of dry tetrahydrofuran (THF).
2. Prepare solution B of dicyclohexylcarbodiimide (DCC) by dissolving 0.206 g (1.0 mmol) in 3.4 mL of dry THF.
3. Mix solutions A and B and cover with a watchglass. (Note: a white precipitate will form in 3 to 5 minutes).
4. Gently stir the mixture from Step 3 with a magnetic stirrer for 4.5 hours at ambient temperature.
5. Prepare solution C by dissolving 0.206 g of DCC and 0.198 g of pentafluorophenylhydrazine (PFPH) in 18 mL of dry THF.
6. Mix solution C with the mixture from step 4, cover with a watchglass and stir the mixture overnight (16 hours) at ambient temperature.
7. Filter the mixture and dry the filtrate under reduced pressure to yield a beige powder.
8. Recrystallize the beige powder with 20 mL of warm (40°C) methanol: H₂O (8:2 v/v).
9. Filter the solution from Step 8 to remove the insoluble material.
10. Allow the filtrate from Step 9 to cool to room temperature. A precipitate will form immediately upon cooling.
11. Filter and wash the precipitate formed in Step 10 with two 1 mL portions of cold methanol: H₂O (8:2). Save the filtrate.
12. Allow the filtrate from Step 11 to stand overnight covered with a watchglass at ambient temperature. A precipitate will form on standing.
13. Filter and wash the precipitate from Step 12 with two 1 mL portions of cold methanol: H₂O (8:2).
14. Recrystallize the off white precipitate from Step 13 with 20 mL of warm methanol: H₂O (8:2). Filter the warm solution and allow the filtrate to cool, producing a white, crystalline precipitate.
15. Filter the white precipitate from Step 14, wash with two 1 mL portions of cold methanol: H₂O (8:2) and dry under vacuum.

16. Determine the melting point of the precipitate of Step 15. The melting point of the endothall-pentafluorophenylhydrazine derivative is 201.0°C. If the melting point of the precipitate is not within 1.0 C of this melting point, recrystallize again as per Steps 14 - 15.

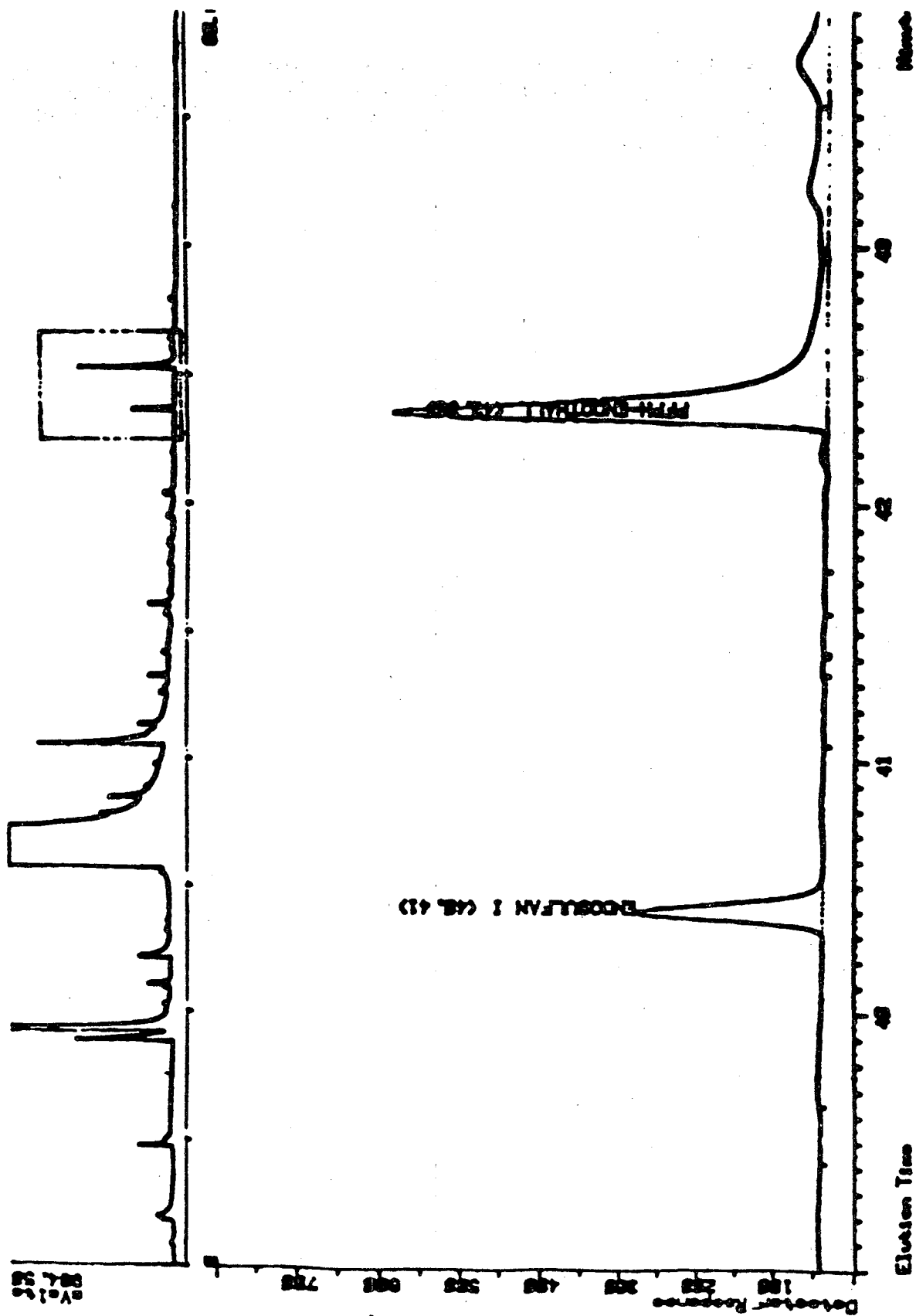


Figure 1. Representative chromatogram from injection of a 200 ug/L endothal-PFPH standard

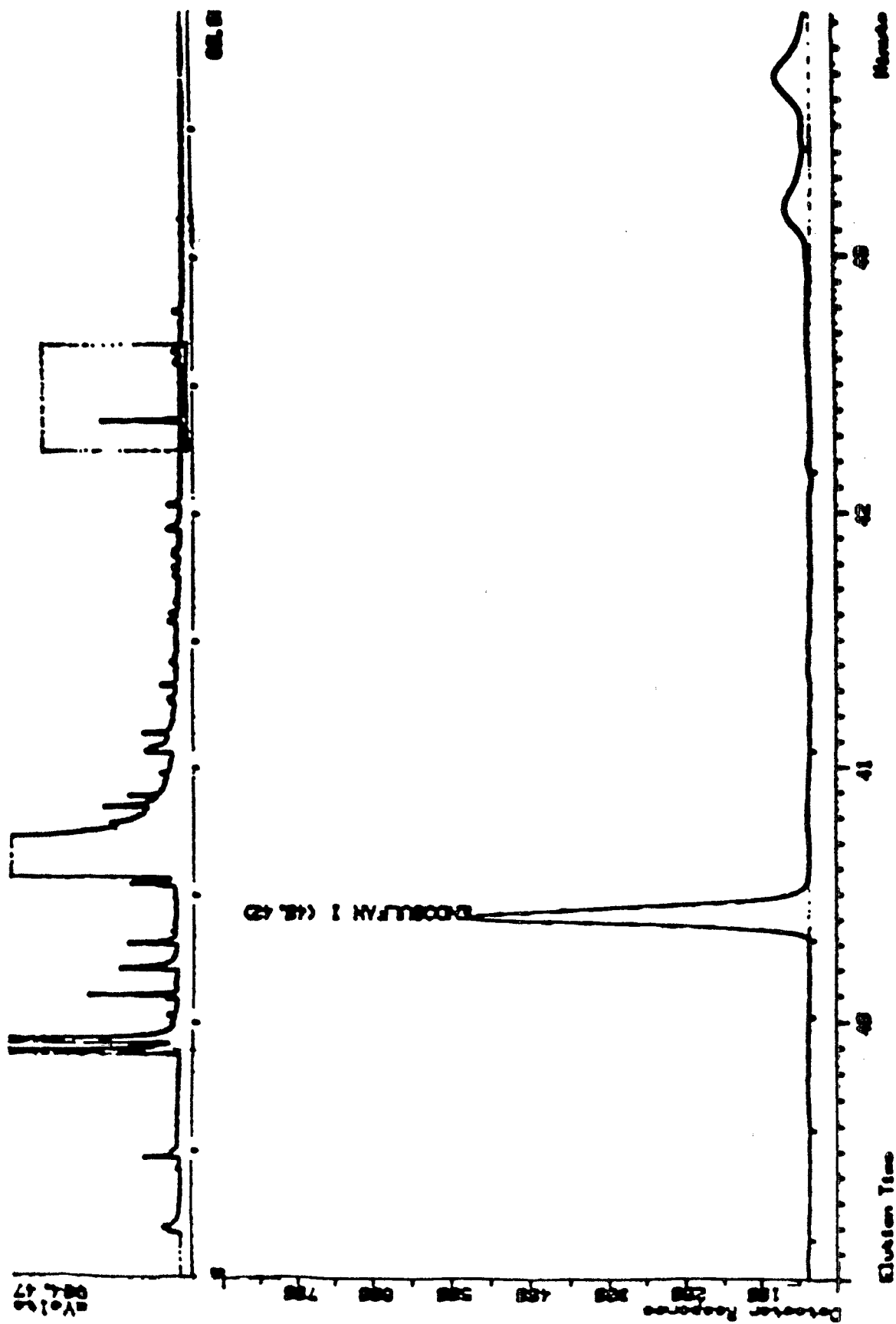


Figure 2. Representative chromatogram of a laboratory reagent blank