Control No. AM337

METHOD WRITEUP FOR

WATER QUALITY BRANCH

ANALYTICAL METHODS MANUAL

by

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METHOD FOR THE ANALYSIS OF NITROPHENOLS IN NATURAL WATERS

(gas chromatographic)

1. SCOPE AND APPLICATION

1.1 This method is applicable to the qualitative and quantitative gas chromatographic determination of the following phenols in natural waters:

Phenol	NAQUADAT No.
2-nitrophenol	
3-nitrophenol	
4-nitrophenol	
3-trifluoromethyl-4-nitrophenol (TFM)	
2,4-dinitrophenol	
2-methyl-4,6-dinitrophenol	
2-sec-butyl-4,6-dinitrophenol (Dinoseb)	
3-cyano-2,6-dibromophenol (Bromoxynil)	
Pentachlorophenol	

1.2 The practical limits of measurement using electron capture detection range from 0.1 to 100 µg/L. These concentration levels have been validated and are based on using a 1-L water sample and making the final volume of extract for GLC analysis up to 10 mL.

1.3 The method detection limit is 0.025 µg/L for the three dinitrophenols, 0.01 µg/L for the mononitrophenols, TFM and Bromoxynil and 0.001 µg/L for pentachlorophenol (Ref. 13.4).

2. PRINCIPLE AND THEORY

2.1 The water sample is acidified to pH 1 or less and extracted with an organic solvent (dichloromethane).

2.2 The organic layer is base partitioned with a 2% potassium bicarbonate solution to separate the phenols and other acidic compounds from the neutral organic compounds.

2.3 The phenols, now in the aqueous layer, undergo acetylation with acetic anhydride while simultaneously being extracted into petroleum ether.

2.4 The organic extract is dried through anhydrous sodium sulphate and concentrated to less than 3 mL.

2.5 The concentrated sample is applied to a mini Silica Gel column and one fraction, containing all the phenol acetates, is collected for determination by EC-GLC analysis.

2.6 The method presented here can readily be modified for the simultaneous analysis of other phenols as well as other classes of compounds such as PCBs, chlorobenzenes, organochlorinated insecticides, and other neutral pesticides, if required.

3. INTERFERENCES

3.1 Extraneous matter, especially in highly coloured water samples, is a potential interference. The cleanup procedure described in this method will usually eliminate this source of interference.

3.2 All reagents must be thoroughly checked and any interferences from this source eliminated.

3.3 Because the acetylation reaction is specific to phenols, the potential interference from other acidic compounds is greatly reduced.

4. SAMPLING PROCEDURE AND STORAGE

4.1 Water samples should be collected and stored in an all-glass system and acidified immediately to pH 1 or less with dilute sulphuric acid (1+1).

4.2 Teflon-lined bottle caps are recommended to prevent contamination of the water sample from contact with the cap. If Teflon-lined caps are unavailable, the use of solvent-washed aluminum foil beneath the cap is acceptable.

4.3 Samples should be stored in the dark at 4°C and extracted as soon as possible.

5. SAMPLE PREPARATION

5.1 No special preparation is required.

6. APPARATUS

6.1 Capillary GLC analysis of phenol acetates

6.1.1 A gas chromatograph with good sensitivity equipped with a split/splitless capillary column injection port and an electron capture detector (^{63}Ni) such as Hewlett-Packard Model 5880A or equivalent.

6.1.2 Automatic Liquid Sampler such as Hewlett-Packard Model 7671A or equivalent. If this is not available, use a 10-μL Hamilton micro-syringe and inject 2 μL.

6.1.3 Fused Silica Capillary Column (FSCC): 12 m x 0.2 mm i.d. column, coated with cross-linked dimethyl silicone gum (0.33 μm thickness) and surface deactivated by siloxane, such as Hewlett-Packard OV-1 (Part No. 19091-60312) or equivalent.

6.1.4 Chromatographic conditions:

Injection Port: splitless mode, splitless valve on for 30 s

Injection Port Temperature: 200°C

Detector: Ni-63 ECD

Detector Temperature: 300°C

Detector Make-Up Gas: Argon/Methane (95+5) at 27 mL/min

Carrier Gas: Helium

Column Head Pressure: 13.0 psi

Column Temperature Initial: 70°C; hold for 0.5 min

Programming Rate 1: 25°C/min (70° to 140°C);

Programming Rate 2: 2°C/min (140° to 180°C); hold for 1.0 min at 180°C

Column Post-Run Final Temperature: 220°C for 15 min

6.2 Magnetic stirrers with 51 mm x 9.5 mm o.d. Teflon-coated spinbars.

6.3 Oven, capable of maintaining 200°C.

6.4 Hamilton micro-syringes (500 µL, 100 µL, 10 µL).

6.5 Heating mantles with power regulators, to accommodate 500-mL round-bottom flasks.

6.6 Three-stage Snyder columns with a tapered ground-glass joint to fit the neck of the 500-mL round-bottom flasks.

6.7 Boiling chips, Soxhlet-cleaned for 24 hours with acetone/hexane (59+41).

6.8 Disposable Pasteur Pipettes (23 cm x 5 mm i.d.).

6.9 Silanized glass wool.

6.10 Volumetric flasks (250 mL) or Wheaton bottles (250 mL).

6.11 Volumetric flasks, "low-actinic" (100 mL).

6.12 Separatory funnels with Teflon stop-cocks (1000 mL, 250 mL).

6.13 Coarse (70-100 µm) sintered-glass filter funnels (100 mm x 40 mm i.d.) with a tapered ground-glass joint and suction side-arm, available from Pegasus Industrial Specialties Ltd.

6.14 Round-bottom flasks (500 mL).

6.15 Volumetric pipettes (5 mL, 1 mL).

6.16 Graduated centrifuge tubes (15 mL) with ground-glass stoppers.

NOTE: All glassware must be thoroughly washed with a hot solution of laboratory detergent followed by rinses with hot tap water, 2-3 rinses of distilled water and a final acetone rinse to remove the water. The glassware (except for volumetric pipettes and syringes) should be dried at 130°C for at least two hours. Thorough rinsing with organic solvent immediately prior to using the glassware is recommended.

7. REAGENTS

7.1 All solvents must be Distilled-in-Glass Pesticide Residue grade and must be checked before use for low blank values.

7.1.1 Dichloromethane (methylene chloride)

7.1.2 Hexane

7.1.3 Iso-octane

7.1.4 Toluene

7.1.5 Methanol

7.1.6 Petroleum Ether (30°-60°C)

7.1.7 Acetone

7.2 All chemicals must be of highest purity and should be washed with solvent and preheated where necessary.

7.2.1 Purified (organic-free) water. Pass distilled water through Milli-Q Water System (Millipore Corp.). Alternatively, extract 1L distilled water three times by stirring with 50 mL dichloromethane for 30 minutes. Discard organic layers.

7.2.2 Dilute Sulphuric Acid, ACS grade or better. Prepare a (1+1) v/v solution with purified (organic-free) water.

7.2.3 2% Potassium Bicarbonate Solution. Dissolve 20 g anhydrous $KHCO_3$ in purified (organic-free) water and dilute to 1000 mL.

7.2.4 Acetic Anhydride. Distill AnalaR grade $(CH_3CO)_2O$ reagent, available from BDH Chemicals, three times and collect the 138°-140°C fraction for acetylation reactions.

CAUTION: Acetic Anhydride is corrosive and moisture-sensitive.

7.2.5 Sodium sulphate (anhydrous, Reagent grade), available from BDH Chemicals. Heat 18 hours at 650°C and store in a clean glass bottle in a dessicator.

7.2.6 5% Deactivated Silica Gel. Activate Silica Gel adsorbent (grade 950 for gas chromatography, 60/200 mesh, Fisher Scientific Co.) by heating for 18 hours at 130°C. Deactivate by adding 5 g purified (organic-free) water to 95 g activated Silica Gel. Mix well by tumbling for 18 hours in a tightly sealed glass container. Prepare fresh weekly.

7.3 Analytical Standards. Phenols should be analytical grade (98+% purity). Obtain from manufacturers or U.S. Environmental Protection Agency and use without further purification.

7.3.1 Prepare 1000 ppm stock solutions of each individual phenol by dissolving 100 mg of pure analytical standard in toluene and diluting to 100.0 mL in "low-actinic" volumetric flasks. Store at 4°C in the dark.

7.3.2 Prepare and Intermediate Mixed Phenol Solution by combining appropriate aliquots of the individual phenol stock solutions and diluting to 100.0 mL with acetone. Prepare Spiking Solutions by diluting further with acetone as required. Store all solutions at 4°C in the dark.

7.3.3 To prepare a GLC standard solution for calibration, fortify a 100 mL sample of 2% potassium bicarbonate with 100 µL of an appropriate Spike Solution. Extract/acetylate alongside the sample extracts as per Steps 8.2 and 8.3 of the Procedure.

8. PROCEDURE

8.1 Extraction

8.1.1 Stir a 1-L water sample in a 1.14 L glass bottle or other suitable container, on a magnetic stirrer using a Teflon-coated stirring bar so that the vortex formed at the surface

almost reaches the bottom of the bottle. Carefully add dilute sulphuric acid (1+1) in drops until the pH is 1 or less. (Use pH paper).

8.1.2 Add 50 mL dichloromethane and tightly cover the bottle with an aluminum-lined cap. After stirring for 30 minutes, transfer the contents of the bottle to a 1-L separatory funnel.

8.1.3 Drain the organic layer into a clean 500-mL round-bottom flask.

8.1.4 Return the aqueous layer to the original sample bottle. Rinse the 1-L separatory funnel with 30 and 20 mL aliquots of dichloromethane and transfer to the sample bottle. Tightly cover the bottle with the aluminum-lined cap and stir for 30 minutes. Transfer the contents of the bottle to the 1-L separatory funnel.

8.1.5 Transfer the organic layer to the 500-mL round-bottom flask containing the first 50 mL dichlormethane extract.

8.1.6 Repeat steps 8.1.4 and 8.1.5 with another 50 mL dichloromethane. Discard the aqueous layer.

8.1.7 Add 3 mL iso-octane to the organic extract as a keeper.

8.1.8 Add a few boiling chips to the extract and attach a three-stage Snyder column to the 500-mL round-bottom flask. Wet the Snyder column with 5 mL hexane and clamp securely in a heating mantle.

8.1.9 Gently concentrate the sample to 3-5 mL. Allow the extract to cool and add 50 mL hexane.

8.1.10 Repeat the evaporation in Step 8.1.9 and again allow the extract to cool.

CAUTION: All Snyder column evaporation steps must be performed in a Fume Hood.

8.1.11 Transfer the concentrated sample to a 250-mL separatory funnel. Rinse the Snyder column and the flask with 30 and 20 mL aliquots of hexane and add the rinsings to the sample extract in the separatory funnel.

8.1.12 Add 40 mL potassium bicarbonate (2%) to the same separatory funnel and shake vigorously for 2 minutes. Vent often to release the gas. Ensure the pH of the aqueous layer is 8 or greater. (Use pH paper).

8.1.13 Allow the layers to separate and drain the aqueous (lower) layer into a 250-mL volumetric flask or other suitable container.

8.1.14 Repeat the aqeuous partitioning in Steps 8.1.12 and 8.1.13 with two 30-mL portions of potassium bicarbonate (2%), transferring the aqueous layer each time to the same 250-mL flask. After the final back-extraction, save the organic layer for analysis of other classes of neutral organic compounds, if necessary.

8.2 Acetylation of Phenols

8.2.1 To the 100-mL sample of combined potassium bicarbonate extracts in the 250-mL volumetric flask, add 5 mL acetic anhydride and 25 mL petroleum ether (30°-60°C). Tightly cover the flask with an aluminum-lined or Teflon-lined cap. Stir the sample on a magnetic stirrer using a Teflon-coated stirring bar so that the vortex formed at the surface almost reaches the bottom of the flask.

8.2.2 After stirring for 5 minutes, transfer the contents of the flask to a clean 250-mL separatory funnel and allow the layers to separate.

8.2.3 Drain the aqueous (lower) layer into the original 250-mL flask.

8.2.4 Pass the organic layer through a (vacuum) sintered-glass filter funnel containing 50 mm of anhydrous sodium sulphate. Collect the dried extract in a clean 500-mL round-bottom flask.

8.2.5 Repeat the extractive acetylation procedure twice, adding fresh aliquots of 5 mL acetic anhydride and 25 mL petroleum ether to the aqueous layer in the 250-mL flask each time. Discard the aqueous sample after the third extraction.

8.2.6 Rinse the 250-mL separatory funnel twice with 10 mL petroleum ether and drain the rinsings through the sodium sulphate column into the 500-mL round-bottom flask.

8.2.7 Wash the sodium sulphate column with another 25 mL petroleum ether and apply a vacuum until the sodium sulphate is dry. Remove the column and add 2 mL iso-octane to the extract as a keeper.

8.2.8 Add a few boiling chips to the extract and attach a three-stage Snyder column to the 500-mL round-bottom flask. Wet the Snyder column with 5 mL petroleum ether and clamp securely in a heating mantle.

8.2.9 Gently concentrate the sample to 2-3 mL. This extract contains the phenol acetates and is ready for Silica Gel cleanup and analysis by GLC.

8.3 Silica Gel Column Cleanup

8.3.1 Prepare micro-columns by plugging clean, disposable Pasteur pipettes (23 cm x 5 mm i.d.) with a clean piece of silanized glass wool.

8.3.2 Fill the columns with 5 cm of Silica Gel (5% deactivated) and tap them gently with a pencil to uniformly settle the solid. Add 0.5 cm anhydrous sodium sulphate to the top of the column.

8.3.3 Prewet the columns with 5 mL hexane and permit the hexane to drain just to the top of the sodium sulphate layer. Discard hexane eluant.

8.3.4 With a disposable pipette, apply the concentrated sample extract (from Step 8.2.9) to the column. Rinse the 500-mL round-bottom flask, 1 mL at a time, with hexane. Apply the rinsings to the column, never permitting the solution to drain below the sodium sulphate layer.

8.3.5 Collect a total of 5 mL hexane in a 15-mL centrifuge tube. Discard this Fraction A, containing side-products and contaminants.

8.3.6 Elute with 10 mL toluene/methanol (97+3) into a clean 15-mL centrifuge tube. Adjust the volume to precisely 10.00 mL. This Fraction B contains the phenol acetates of interest.

8.3.7 Analyze the sample extract by means of gas chromatography using electron capture detection.

8.3.8 For calibration standards, acetylate a known amount of phenols as per Step 7.3.3.

9. CALCULATIONS

9.1 The concentration of each phenol is determined by comparison of peak height or area of the samples with those of the standards. This can be done by using the following equation:

$$X_{sam} = \left(\frac{H_{sam}}{H_{std}}\right) x \left(\frac{V_{inj\ std}}{V_{inj\ sam}}\right) x \left(X_{std}\right) x \left(\frac{V_{ext}}{V_{sam}}\right)$$

where X_{sam} = phenol concentration in original water sample (µg/L);

H_{sam} = peak height (or area) of sample;

H_{std} = peak height (or area) of standard;

$V_{inj\ std}$ = volume of standard injected (µL);

$V_{inj\ sam}$ = volume of sample injected (µL);

X_{std} = phenol concentration in standard solution (pg/µL);

V_{ext} = final volume of sample extract (mL); and

V_{sam} = volume of original water sample extracted (mL).

9.2 Alternatively, concentration of the nitrophenols may be determined by acetylation of, and comparison to, an internal standard such as pentachlorophenol.

10. PRECISION AND ACCURACY

10.1 Data indicating the single-operator precision and accuracy are summarized in the following table:

Parent Phenol	Mean Recovery (%)	Relative Standard Deviation (%)
2-nitro-	84	6.3
3-nitro-	61	5.1
4-nitro-	53	8.4
3-trifluoromethyl-4-nitro- (TFM)	94	3.3
2,4-dinitro-	102	7.6
2-methyl-4,6-dinitro-	108	8.4
2-sec-butyl-4,6-dinitro- (Dinoseb)	102	2.9
3-cyano-2,6-dibromo- (Bromoxynil)	100	5.0
Pentachloro- (PCP)	102	5.4

NOTE: (a) Samples used for recovery studies were fortified 1-L Lake Ontario water samples.

(b) No. of replicates = 6.

(c) Fortification level for each phenol=1.0 µg/L (except for PCP which was fortified to 0.1 µg/L).

11. CONFIRMATION OF IDENTITY

11.1 The identity of each GC peak in the mixture may be assigned by comparison with the retention time of each phenol acetate obtained by reacting individual phenols under identical experimental conditions.

11.2 The identity of each peak in the sample extracts may be tentatively confirmed on a retention time basis by analyzing the sample and standard on another high resolution capillary column of different polarity.

11.3 Additional confirmation of identity may also be obtained by the formation and analysis of different derivatives.

11.4 Whenever sample concentration permits, confirmation of the identity of the mononitrophenol acetates may readily be obtained by combined GC-MS (EI and/or CI) operating at selected ion monitoring (SIM) or full scan mode. (See 12.12 of Remarks).

12. REMARKS

12.1 It is recommended that a phenol standard be prepared alongside each set of water samples to account for any minor variations in the extractive acetylation procedure or column cleanup steps from one set to another.

12.2 In some natural water samples, emulsions can form during extraction with dichloromethane. If this happens, the emulsion should remain with the aqueous layer until the third extraction at which point it is included in the organic phase for base-partitioning.

12.3 Extreme care must be exercised by the analyst in the steps in which extracts are concentrated. Samples must never be allowed to go dry as some phenols are quite volatile.

12.4 All concentration steps using a Snyder column for evaporation of the solvent must be carried out in a Fume Hood.

12.5 To ensure an effective back-extraction of the phenols into potassium bicarbonate (2%), the dichloromethane must be thoroughly and completely exchanged for hexane.

12.6 For complete recovery of the phenols during the base-partitioning steps, it is extremely important that the aqueous phase have a pH of 8 or greater (Steps 8.1.12 to 8.1.14). It may be necessary to increase the strength or quantity of the potassium bicarbonate solution in order to neutralize any co-extracted acid in the sample extracts.

12.7 Special attention should be taken in the handling of acetic anhydride because it is corrosive and moisture-sensitive.

12.8 No difference in yields of the acetates was observed when the reaction steps were increased from 5 to 30 minutes.

12.9 The yields of the nitrophenol acetates were strongly dependent on the proportion of reagent acetic anhydride to the amount and strength of base.

12.10 Due to minor decomposition of some of the nitrophenol acetates on the Silica Gel column, it is strongly recommended that all standard solutions undergo column cleanup alongside the sample extracts.

12.11 To reduce tailing of the phenol acetates on the OV-1 FSCC, it is necessary, after about 100 injections, to replace the splitless insert and to cut off approximately 10-20 cm of the injection port end of the column.

12.12 Confirmation of identity and quantitation of the acetate derivatives by GC-MSD is possible for the mononitrophenols only, which show the acetate moiety at m/z 43, the parent phenol peak at m/z 139 and the molecular ion peak (M+) at m/z 181. However, for the other compounds in the mixture, the acetate peak is present, but both the parent phenol and the molecular ion peaks are absent in the spectrum.

12.13 The recoveries of 3-nitrophenol and 4-nitrophenol were fairly consistent at 60±5% and 55±5%, respectively, over a 100-fold concentration range.

12.14 This method has been shown to be applicable to the co-extraction and analysis of 19 additional chlorinated phenols.

12.13 The determination of other classes of compounds such as PCBs, chlorobenzenes, organochlorinated insecticides and other neutral pesticides can be included by analyzing the organic layer from the base-partitioning step in Procedure Step 8.1.14.

13. REFERENCES

13.1 Lee, Hing-Biu, Li-Da Weng and Alfred S.Y. Chau. 1984. Chemical Derivatization Analysis of Pesticide Residues. VIII. Analysis of 15 Chlorophenols in Natural Water by *in situ* Acetylation. J. Assoc. Off. Anal. Chem. 67[4]: 789-794.

13.2 Lee, Hing-Biu, Yvonne D. Stokker and Alfred S.Y. Chau. 1987. Analysis of Phenols by Chemical Derivatization. V. Determination of Pentachlorophenol and 19 Other Chlorinated Phenols in Sediments. J. Assoc. Off. Anal. Chem. (in press).

13.3 Mathew, John and Alan W. Elzerman. 1981. Gas-Liquid Chromatographic Determination of Some Chloro- and Nitrophenols by Direct Acetylation in Aqueous Solution. Anal. Letters 14[A16] :1351-1361.

13.4 Federal Register, US-EPA 40 CFR Part 136. Guidelines Establishing Test Procedures for the Analysis of Pollutants, October 26, 1984, Vol. 49, No. 209, p. 198.

APPENDIX

1. Figure A1. EC-GLC chromatogram of acetate derivatives of 9 phenols as resolved on a 12m x 0.2 mm i.d. OV-1 fused silica capillary column. GLC conditions are outlined in Section 6.1.

	Parent Phenol	Retention Times (min)	Relative Retention Times
1.	2-nitrophenol	4.97	3.48
2.	TFM	5.41	3.78
3.	3-nitrophenol	5.73	4.01
4.	4-nitrophenol	5.98	4.18
5.	2,4-dinitrophenol	10.09	7.06
6.	Bromoxynil	10.82	7.57
7.	2-methyl-4,6-dinitrophenol	12.34	8.63
8.	Pentachlorophenol	14.30	10.00
9.	Dinoseb	17.98	12.57

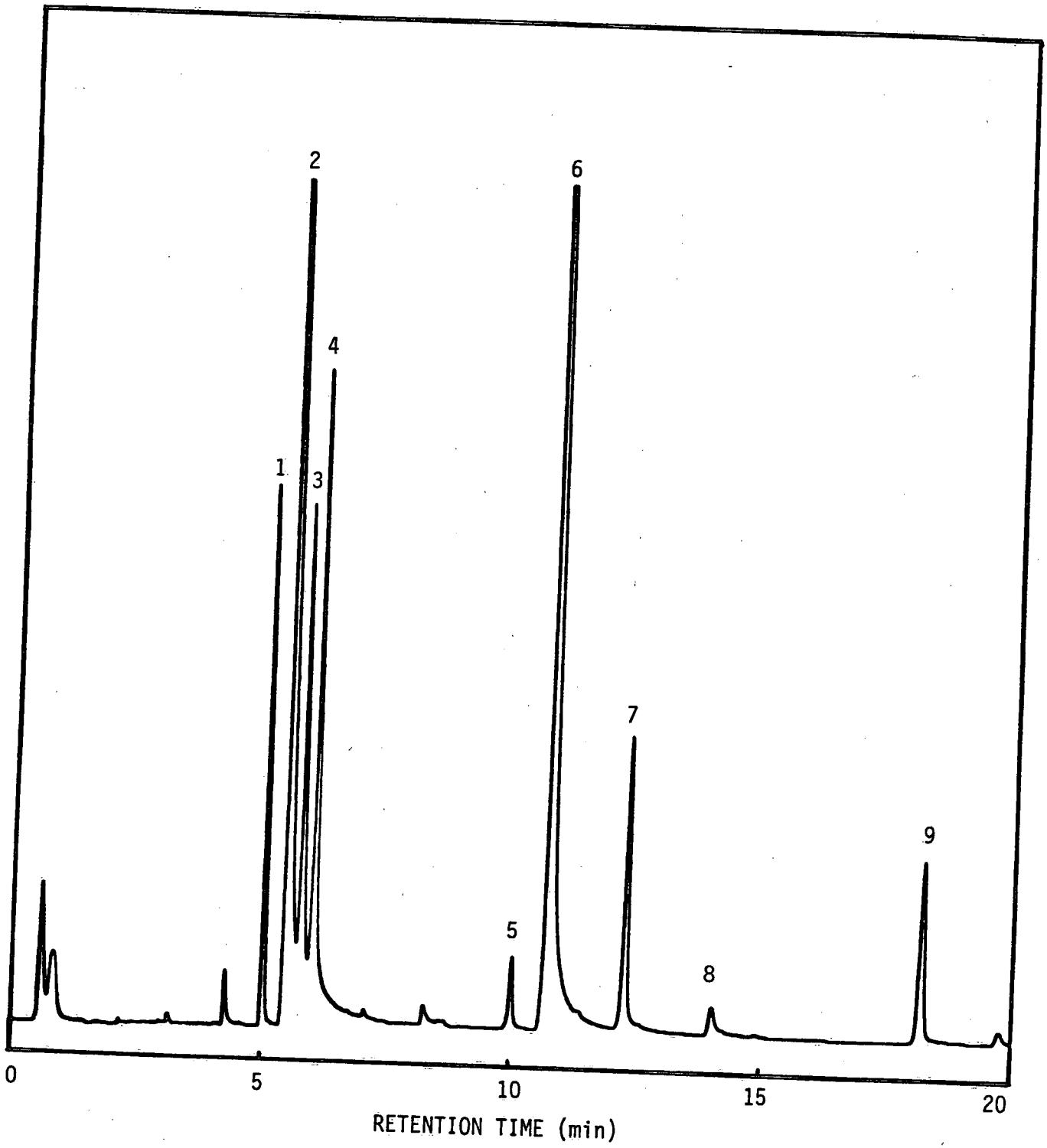

Figure A1. GC-ECD of 9 Phenol Acetates