NWRI CONTRIBUTION 88-67

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METHOD FOR THE ANALYSIS OF ACID AND NEUTRAL HERBICIDES IN SEDIMENTS

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August 1987

MANAGEMENT PERSPECTIVE

In response to a request from Water Quality Branch, Western and Northern Region, a multi-class, multi-residue method for the quantitative analysis of ten acid and six neutral herbicides in sediments was developed. The extensive usage of these herbicides in Western Canada along with the known toxicity and persistence of both the parent compounds and their metabolites, has given the regional laboratories cause for concern. To support their surveillance and monitoring programs, however, large numbers of samples have to be processed. Using the "modular concept" approach to method development, considerable sampling and analysis time would be saved by extracting and analyzing the same sample for both acid-type (phenoxyalkanoic acids) and neutral-type herbicides (carbamates, ureas, and esters). This multi-class, multi-residue extraction is even more effective since it has also been demonstrated elsewhere to be effective in the determination of other classes of compounds such as PCBs, chlorobenzenes, and organochlorinated insecticides.

Dr. J. Lawrence
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PERSPECTIVE GESTION

Pour satisfaire à la demande de la Direction de la qualité des eaux, région ouest et nord, une méthode applicable à des résidus de classes multiples a été mise au point pour doser dix herbicides de type acide et six autres de type neutre dans des sédiments. Comme l'usage de ces pesticides est répandu dans l'ouest du Canada, les laboratoires régionaux s'inquiètent du fait que les composés d'origine et leurs métabolites aient une persistance et une toxicité reconnues. Pour les besoins de leurs programmes de contrôle et de surveillance, ils doivent cependant traiter un grand nombre d'échantillons. Le développement d'une méthode selon le "concept modulaire" ferait gagner beaucoup de temps au stade de l'extraction et de l'analyse en permettant le dosage tant des herbicides de type acide (acides phénoxyalcanoïques) que de type neutre (carbonates, urées et esters) dans un même échantillon. Cette méthode d'extraction de plusieurs résidus de classes différentes est encore plus efficace, car elle s'est aussi avérée applicable à la détermination d'autres classes de composés tels les PCB, les chlorobenzènes et les insecticides organochlorés.

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ABSTRACT

A method for the qualitative and quantitative analysis of ten acid and six neutral herbicides in sediments was developed. After acidification to pH < 1, sediment samples were ultrasonically extracted with an acetone/hexane solvent mixture. By selectively back-extracting the acid herbicides into a 2% $KHCO_3$ solution, the acid and neutral herbicides were effectively separated into aqueous and organic phases, respectively. The aqueous phase was then reacidified to pH < 1 and extracted with dichloromethane. After evaporation to a small volume and replacement of the solvent with acetone, the acid herbicides were derivatized to their corresponding pentafluorobenzyl esters in order to increase their sensitivity to electron capture detection. These extracts were cleaned up and fractionated on a 5% deactivated Silica Gel column. The acid herbicide-PFB esters were then analyzed on a 30 m DB-5 capillary column interfaced to an electron capture detector. The neutral herbicide-containing organic phase was concentrated and applied to a 10% deactivated Florisil column for cleanup. The resultant two fractions were then analyzed on a 3% OV-1 packed column, using electron capture detection. The procedure has been validated with sediment samples fortified from 0.1 to 200 ng/g levels of the acid and neutral herbicides. Recoveries of all herbicides were generally better than 80% at all levels studied. Using a 50 g sample size, the practical limits of detection ranged from 0.1 ng/g to 2.0 ng/g.

RÉSUMÉ

Une méthode a été mise au point qui permet l'analyse qualitative et le dosage de dix herbicides de type acide et de six autres de type neutre dans des sédiments. Après acidification à un pH <1, des échantillons de sédiments sont extraits aux ultrasons avec un mélange acétone-hexane. L'extraction sélective à contre-courant des herbicides acides dans une solution de $KHCO_3$ à 2 % rend possible le partage respectif des herbicides acides et des herbicides neutres entre l'eau et la phase organique. La phase aqueuse est ensuite acidifiée à un pH <1, puis extraite au dichlorométhane. Après évaporation du solvant à un faible volume et remplacement par de l'acétone, les herbicides acides sont transformés en l'ester pentafluorobenzylique correspondant afin de faciliter leur détection par capture d'électron. Les extraits sont purifiés et fractionnés sur une colonne de gel de silice désactivé à 5 %. Les dérivés PFB des herbicides acides sont ensuite analysés sur une colonne capillaire de 30 m garnie de DB-5, avec détection par capture d'électron. La phase organique contenant les herbicides neutres est concentrée, puis purifiée sur colonne de florisil désactivée à 10 %. Les deux fractions résultantes sont ensuite analysées sur une colonne garnie d'OV-1 à 3 %, avec détection par capture d'électron. La méthode a été validée pour des échantillons enrichis de 0,1 à 200 ng/g en herbicides acides et neutres. Les rendements de récupération des herbicides sont généralement supérieurs à 80 % aux teneurs étudiées. Dans un échantillon pesant 50 g, la limite pratique de détection varie de 0,1 à 2,0 ng/g.

METHOD FOR THE ANALYSIS OF ACID AND NEUTRAL HERBICIDES IN SEDIMENTS

(Gas Chromatographic)

1.0 SCOPE AND APPLICATION

1.1 This method is applicable to the qualitative and quantitative gas chromatographic determination of the following ten acid and six neutral herbicides in sediments:

2-methoxy-3,6-dichlorobenzoic acid	(DICAMBA)
4-chloro-2-methylphenoxyacetic acid	(MCPA)
2-(2,4-dichlorophenoxy)-propionic acid	(2,4-DP)
2,3,6-trichlorobenzoic acid	(2,3,6-TBA)
2,4-dichlorophenoxyacetic acid	(2,4-D)
2-(2,4,5-trichlorophenoxy)-propionic acid	(SILVEX)
2,4,5-trichlorophenoxyacetic acid	(2,4,5-T)
4-(4-chloro-2-methylphenoxy)-butyric acid	(MCPB)
4-(2,4-dichlorophenoxy)-butyric acid	(2,4-DB)
4-amino-3,5,6-trichloropicolinic acid	(PICLORAM)
2,6-dinitro-N,N-dipropyl-4-trifluoromethyl aniline	(TRIFLURALIN)
S-(2,3-dichloroallyl)-N,N-diisopropyl thiocarbamate	(DIALLATE)
S-(2,3,3-trichloroallyl)-N,N-diisopropyl thiocarbamate	(TRIALLATE)
4-chloro-2-butynyl-N-(3-chlorophenyl)-carbamate	(BARBAN)
Methyl 2-[4-(2',4'-dichlorophenoxy)-phenoxy]-propionate	(DICLOFOP-METHYL)
Ethyl N-benzoyl-N-(3,4-dichlorophenyl)-2-aminopropionate	(BENZOYLPROP - ETHYL)

1.2 The practical limits of measurement based on a 50 g sediment sample with a 30% moisture content and using electron capture detection are as follows:

Herbicide	Method Detection Limits* (µg/kg)	NAQUADAT No.
Dicamba	1.0	
MCPA	1.0	
2,4-DP	1.0	
2,3,6-TBA	1.0	
2,4-D	1.0	
Silvex	1.0	
2,4,5-T	2.5	
MCPB	2.5	
2,4-DB	2.5	
Picloram	2.5	
Trifluralin	0.1	
Diallate	2.0	
Triallate	0.2	
Barban	2.0	
Diclofop-Methyl	1.0	
Benzoylprop-Ethyl	0.5	

* Based on a 50 g sediment sample and volume of extracts made up to 10 mL for GLC analysis. For definition of Method Detection Limit, see Ref. 13.7.

2.0 PRINCIPLE AND THEORY

2.1 The sediment sample is acidified to pH 1 or less and ultrasonically extracted with an acetone-hexane solvent mixture.

2.2 The acid herbicides are selectively back-extracted into a 2% potassium bicarbonate solution. Dichloromethane rinsings of this solution are then added to the original organic extract. These steps effectively separate the acid and neutral herbicides into aqueous and organic phases, respectively.

2.3 The aqueous phase is acidified to pH 1 or less and extracted with dichloromethane. The solvent is evaporated and the residue is dissolved in acetone in preparation for derivatization.

2.4 The ten acid herbicides are derivatized to their corresponding pentafluorobenzyl esters in order to increase their sensitivity to electron capture detection. Cleanup and fractionation on a Silica Gel column ready the compounds for determination by EC-GLC analysis.

2.5 The neutral herbicide - containing organic phase (from 2.2) is dried through anhydrous sodium sulphate and concentrated to approximately 3 mL. Cleanup and fractionation on a Florisil column provides two fractions which can then be analyzed by GLC using electron capture detection.

2.6 The method presented here can readily be modified for the analysis of either the acid or neutral herbicides separately, if required.

3.0 INTERFERENCES

3.1 Organic compounds co-extracted from the sediment are potential interferences. The cleanup procedures 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 Other pesticide residues, metabolites or degradation products may interfere in the GLC analysis. As many of these organic compounds have properties similar to those of the compounds of interest, confirmation of identity of the latter is important.

3.4 Elemental sulphur or reactive sulphur-containing compounds may interfere with the analysis of some of the neutral herbicides. These sulphur-compounds must be removed prior to GLC analysis by shaking the extract with clean mercury until HgS no longer precipitates as a black solid.

4.0 SAMPLING PROCEDURE AND STORAGE

4.1 Sediment samples should be collected and frozen immediately in an all-glass system or metal container.

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

4.3 Samples should be kept frozen, in the dark, and extracted as soon as possible.

5.0 SAMPLE PREPARATION

5.1 Before extraction, mix and stir the sample thoroughly. If possible, blend the entire sample in a 5 L stainless steel blender for 10 minutes at medium to high speed, cooling the blender at intervals to maintain the temperature of the

sample at or below room temperature. Subsample 50 g aliquots for extraction.

5.2 Homogenization of wet sediment has been investigated for PCBs (Ref. 13.8) but not extensively studied for other parameters. If debris is present, it should be removed by passing the sample through a coarse sieve (1-2 mm). (Ref. 13.4).

6.0 APPARATUS

6.1 Capillary GLC analysis of acid herbicide PFB esters.

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): SE-54 or DB-5 FSCC (30 m x .25 mm i.d), .25 µm film thickness, available from J&W Scientific Ltd. or equivalent.

6.1.4 Chromatographic conditions:

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

Injection Port Temperature: 250°C

Detector: Ni-63 ECD

Detector Temperature: 300°C

Detector Make-Up Gas: Argon/methane (95+5) at 25 mL/min

Carrier Gas: Helium

Column Head Pressure: 20 psi

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

Programming Rate 1: 30°C/min (70°-200°C); hold for 10.0 min

Programming Rate 2: 30°C/min (200°-220°C); hold for 10.0 min

Column Post-Run Final Temperature: 250°C for 15-20 min

6.2 Packed Column GLC Analyses

6.2.1 A gas chromatograph with good sensitivity for packed column analyses equipped with an electron capture detector (^{63}Ni) such as Hewlett-Packard Model 5710A or equivalent.

6.2.2 Automatic Liquid Sampler such as Hewlett-Packard Model 7671A or 7672A or equivalent. If this is not available, use a 10 µL Hamilton micro-syringe for injections.

6.2.3 GLC glass columns (1.8 m x 2 mm i.d.), packed with 3% OV-1 on Gas Chrom Q, 100/120 mesh, available from Chromatographic Specialties Limited.

6.2.4 Chromatographic conditions:

Injection Port Temperature:

(a) 250°C for analysis of Picloram-PFB ester;

(b) 200°C for Neutral Herbicide analyses.

Detector: Ni-63 ECD

Detector Temperature: 300°C

Oven Temperature:

(a) 200°C isothermal for Picloram-PFB ester analysis;

(b) 185°C isothermal for Neutral Herbicide analyses

Carrier Gas: Argon/methane (95+5) at 24 mL/min.

NOTE: Preliminary priming of the column with a 10 ng/μL concentrated Barban solution is recommended to improve the sensitivity to this herbicide.

6.3 Ultrasonic homogenizer such as the Sonicator Cell Disruptor, Model W-350 or equivalent, with a 0.75" solid disruptor horn, available from Heat Systems-Ultrasonic Inc.

6.4 Solvent Evaporator with thermostatted bath such as Buchi Rotavapor Model RE-120 or equivalent, available from Brinkman Instruments.

6.5 Oven, capable of maintaining 200°C.

6.6 Centrifuge tube heater such as the Kontes Tube Heater Block Model K-72002 or equivalent, set at 60°C, combined with a gentle stream of high purity nitrogen gas for controlled evaporation.

6.7 Hamilton micro-syringes (250 µL, 100 µL, 50 µL, 10 µL).

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

6.9 Silanized glass wool.

6.10 Volumetric flasks, "low-actinic" (100 mL, 50 mL, 25 mL).

6.11 Glass beakers (400 mL).

6.12 Separatory funnels with Teflon stop-cocks (500 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 Chromatographic columns (20 mm i.d. x 500 mm) with Teflon stop-cocks.

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

6.17 Test tube mixer such as the Vortex Genie Mixer, Model K-550-G or equivalent, available from Fisher Scientific Co. Ltd.

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.

7.0 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 Acetone.

7.1.4 Benzene.

7.1.5 Methanol.

7.1.6 Iso-octane.

7.1.7 Toluene.

7.1.8 Ethyl Acetate.

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

7.2.1 Celite 545 (not Acid-washed), available from Fisher Scientific Company, Ltd.

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

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

7.2.4 2% Potassium Bicarbonate Solution. Dissolve 20 g anhydrous $KHCO_3$ in purified (organic-free) water and dilute to 1000 mL.

7.2.5 30% Potassium Carbonate Solution. Dissolve 15 g anhydrous K_2CO_3 in purified (organic-free) water and dilute to 50 mL.

7.2.6 5% Pentafluorobenzyl Bromide (PFBBr) Reagent. Dissolve 1 g PFBBr (Aldrich Chemical Co.) in 19 mL dry acetone (< 0.2% water). Keep in the dark at 4°C. Prepare fresh once every two weeks.

CAUTION: Reagent is a strong lachrymator.

7.2.7 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.

7.2.8 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.

7.2.9 10% Deactivated Florisil. Florisil PR, 60-100 mesh (Supelco Inc.) should be calcined at 650°C for 24 hours and then stored at 130°C until needed. Deactivate by adding 10 g purified (organic-free) water to 90 g activated Florisil. Mix well by tumbling for 18 hours in a tightly sealed glass container. Prepare fresh weekly.

7.2.10 Metallic mercury, AnalaR grade, available from BDH chemicals.

7.3 Analytical Standards. Herbicidal acids and neutral herbicides 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 herbicide in "low-actinic" volumetric flasks: (a) Dissolve 100 mg of individual acid herbicides in 100 mL ethyl acetate; and (b) dissolve 100 mg of individual neutral herbicides in 100 mL toluene. Store at 4°C in the dark.

7.3.2 Prepare a mixed Acid Herbicide Solution by combining appropriate aliquots of the ten individual acid herbicide stock solutions and diluting to 100 mL with acetone. To prepare a GLC standard solution for calibration, derivatize

and cleanup 100 µL to 1.0 mL of the mixed solution as per steps 8.2 and 8.3 of the procedure.

7.3.3 Prepare a mixed Neutral Herbicide Standard for GLC calibration by combining appropriate aliquots of the seven individual neutral herbicide stock stolutions and diluting to 100 mL with iso-octane/methanol (99+1). Store at 4°C in the dark.

8.0 PROCEDURE

8.1 Extraction

8.1.1 Weigh a 50.0 g sample of homogeneous sediment into a 400 mL beaker or other suitable container.

8.1.2 At the same time, weigh representative sample aliquants of the sediment into tared containers and heat at 105°C to constant weight for moisture content determination.

8.1.3 Wet the sample in the beaker with purified (organic-free) water to an estimated 30% moisture content. Carefully acidify the sediment by adding dilute sulphuric acid (1+1) in drops until the pH is 1 or less. (Use pH paper.)

8.1.4 Add 100 mL of acetone/hexane (1+1) to the acidified sediment.

8.1.5 Immerse the ultrasonic cell disruptor horn 2 cm into the sample suspension. Activate the sonicator in the pulsed mode at 50% duty cycle with the power output control set to 8.0. CAUTION: In order to avoid losses of volatile components, keep the sample temperature below 40°C. (See Remarks 12.3).

8.1.6 After 3 minutes of sonification/extraction, allow the sediment to settle.

8.1.7 Prepare a 5 cm Celite column in a sintered-glass filter funnel connected to a round-bottom flask. Wash the column with 100 mL of acetone/hexane (1+1) and apply a vacuum to remove the excess solvent from the Celite bed. Discard the solvent washing.

8.1.8 Decant the supernatant liquid from the sediment extract onto the Celite column. Apply a vacuum so as to collect the filtrate in a clean 500 mL round-bottom flask.

8.1.9 Repeat the extraction by sonification twice with two 100 mL portions of acetone/hexane (1+1). Filter as described and collect all filtrates in the same round-bottom flask.

8.1.10 After the third filtration, transfer the entire sediment sample from the beaker to the Celite column. Apply a vacuum until the column is nearly dry. Rinse the beaker with two 10 mL portions of the extraction solvent and filter through the Celite column with vacuum suction as before.

8.1.11 Concentrate the combined extract to approximately 50 mL using a rotary evaporator under reduced pressure. (Water bath temperature $\leq$40°C).

8.1.12 Transfer the concentrated extract to a clean 500-mL separatory funnel. Rinse the round-bottom flask with two 5 mL aliquots of hexane and add the rinsings to the sample extract.

8.1.13 Add 100 mL of potassium bicarbonate solution (2%) to the 500-mL separatory funnel containing the sediment extract and shake vigorously for 2 min. Vent often to release the gas. Ensure the pH of the aqueous layer is 8 or greater. (Use pH paper). Allow the layers to separate.

8.1.14 Drain the aqueous (lower) layer back into the original round-bottom flask.

8.1.15 Add 50 mL of potassium bicarbonate solution (2%) to the organic layer remaining in the separatory funnel. Shake vigorously for 2 min. and allow the layers to separate.

8.1.16 Again, drain the aqueous (lower) layer into the round-bottom flask containing the first aqueous partitioning layer. Keep any emulsion that may have formed, with these aqueous extracts.

8.1.17 Drain the organic layer from the separatory funnel 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.

8.1.18 Transfer the combined aqueous layers back into the 500-mL separatory funnel.

8.1.19 Rinse the round-bottom flask with two 50 mL portions of dichloromethane and add the rinsings to the aqueous solution in the separatory funnel. Shake vigorously for 1 min and drain the organic (lower) layer through the sodium sulphate column into the 500-mL round-bottom flask.

8.1.20 Repeat the above extraction with another 50 mL portion of dichloromethane.

8.1.21 Wash the sodium sulphate column with 50 mL methylene chloride and apply a vacuum until the sodium sulphate is dry. Remove the column and add 3 mL iso-octane to the 500-mL round-bottom flask.

8.1.22 Evaporate the combined organic extracts in the round-bottom flask to about 10 mL on a rotary evaporator (water bath temperature at 35°C). Add 50 mL hexane and repeat evaporation to 3 mL. (This extract contains the six neutral herbicides and is ready for the Florisil Column cleanup in Procedure Step 8.4).

8.1.23 Acidify the aqueous layer in the 500-mL separatory funnel (from step 8.1.18 and 8.1.20) with dilute sulphuric acid (1+1) until the pH is 1 or less (use pH paper). Agitate the solution to permit CO_2 to escape.

8.1.24 After evolution of the CO_2, extract the aqueous layer with three portions of 50 mL methylene chloride by shaking for 2 min each time. Transfer the organic layers to a clean 500-mL round-bottom flask. Discard aqueous phase.

8.1.25 Evaporate the methylene chloride to about 20 mL on a rotary evaporator (water bath temperature at 40°C). Add 50 mL benzene and repeat evaporation until just dry. (See Remarks 12.6.2).

8.1.26 Dissolve the residue with several 1 to 2 mL portions of acetone, each time transferring the acetone quantitatively into a 15-mL graduated centrifuge tube, the total volume of acetone being about 4 to 5 mL. (This extract contains the ten acid herbicides and is ready for esterification and column cleanup).

8.2 Esterification of Acid Herbicides by PFBBr

8.2.1 Add 200 µL of pentafluorobenzyl bromide solution (5%) and 30 µL of potassium carbonate solution (30%) to the 15-mL centrifuge tube.

8.2.2 Stopper the tube and mix the contents on a Vortex Genie Mixer for 1 minute. Place the centrifuge tube in a tube heater and let the contents react at 60°C for three hours. Ensure a complete seal is maintained during this reaction time so as to prevent any loss of the acid herbicides.

8.2.3 After the reaction, evaporate the solution to 1 mL with a gentle stream of nitrogen.

8.2.4 Add 2 mL hexane and repeat evaporation to 0.1 mL.

8.2.5 Take up the concentrated residue with 2 mL of a toluene/hexane (10+90) solution.

8.3 Silica Gel Column Cleanup (of Acid Herbicide-PFB Esters)

8.3.1 Prepare micro-columns by plugging clean disposable 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 8.2.5) to the column. Rinse the

centrifuge tube 2 mL at a time with toluene/hexane (10+90). Apply the rinses to the column, never permitting the solution to drain below the sodium sulphate layer.

8.3.5 Collect a total of 8.0 mL of the toluene/hexane (10+90) solution in a centrifuge tube. Discard this Fraction A, containing excess reagent and contaminants.

8.3.6 Elute with 8.0 mL of toluene/hexane (75+25) into a clean 15-mL centrifuge tube. This Fraction B contains the PFB-esters of Dicamba, MCPA, 2,4-DP, 2,3,6-TBA, 2,4-D, Silvex, 2,4,5-T, MCPB and 2,4-DB.

8.3.7 Elute with 8.0 mL of a toluene/methanol (95+5) solution into a clean centrifuge tube. This Fraction C contains the PFB-ester of Picloram.

8.3.8 Analyze for the PFB-esters of the acid herbicides by means of electron capture gas chromatography.

8.4 **Florisil Column Cleanup (of Neutral Herbicides)**

8.4.1 Prepare macro-columns by filling chromatographic columns (500 x 20 mm i.d.) with 20 g of Florisil (10% deactivated).

Tap columns gently to uniformly settle the solid. Add 1 cm anhydrous sodium sulphate on top of the Florisil layer.

8.4.2 Prewet the columns with 100 mL hexane and let the hexane drain just to the top of the sodium sulphate layer. Discard hexane eluant.

8.4.3 Quantitatively transfer the concentrated extract (from step 8.1.22) plus rinsings onto the column with a disposable pipette.

8.4.4 When the extract just enters the sodium sulphate layer, elute the column with 150 mL of a benzene/hexane (25+75) solution into a clean 500-mL round-bottom flask. This Fraction I contains Trifluralin, Diallate and Triallate.

8.4.5 Elute the column with 200 mL of a benzene/methanol (99+1) solution into a clean 500-mL round-bottom flask. This Fraction II contains Barban, Diclofop-Methyl and Benzoylprop-Ethyl.

8.4.6 Add 3 mL iso-octane to each fraction and evaporate the solvent to 3 mL on a rotary evaporator (water bath temperature at 35°C).

8.4.7 Quantitatively transfer the concentrated extracts to 15-mL graduated centrifuge tubes and make up to the required volume with iso-octane/methanol (99+1).

8.4.8 Remove any sulphur-containing compounds in Fraction I by mixing portions of the extract on a Vortex Genie Mixer with successive drops of clean, triple-distilled mercury until HgS no longer precipitates as a black solid and the mercury drop remains shiny.

8.4.9 Analyze the neutral herbicides by means of electron capture gas chromatography.

9.0 CALCULATIONS

9.1 The concentration of each herbicide 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:

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

where X_{sam} = herbicide concentration in original sediment sample (µg/kg);

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} = herbicide concentration in standard solution (pg/µL);

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

W_{sam} = weight of orginal sediment sample extracted (g).

9.2 The determination of moisture content in the sediment samples is as follows:

$$\% \text{ moisture} = \left(\frac{A - B}{A}\right) \times 100$$

where A = weight in grams of a homogeneous sediment subsample before drying, and

B = weight in grams of the same sample after being dried to a constant weight at 105°C.

10.0 PRECISION AND ACCURACY

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

Herbicide	Fortification Level in 50 g sediment (µg/kg)	Mean Recovery (%)	Relative Standard Deviation (%)
Dicamba	20.0	92	4.3
MCPA	20.0	103	5.4
2,4-DP	20.0	112	4.1
2,3,6-TBA	20.0	107	5.3
2,4-D	20.0	103	7.4
Silvex	20.0	108	4.4
2,4,5-T	40.0	97	3.7
MCPB	40.0	94	5.6
2,4-DB	40.0	90	4.0
Picloram	40.0	104	7.7
Trifluralin	1.0	84	13.0
Diallate	20.0	85	7.8
Triallate	2.0	103	9.3
Barban	20.0	100	6.6
Diclofop-Methyl	10.0	94	5.3
Benzoylprop-Ethyl	5.0	95	2.6

Note: (a) Validation data were obtained from fortified 50 g subsamples of a Battle River sediment having <1% moisture content.

(b) No. of replicates = 10.

11.0 CONFIRMATION OF IDENTITY

11.1 The identity of each peak in the sample extracts may be assigned by comparison with the retention time of each compound analyzed individually under identical chromatographic conditions.

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

11.2.1 The best resolution and GLC response for the neutral herbicides was obtained on a 3% OV-1 column. For confirmation purposes, therefore, an Ultrabond 20 M column is recommended because of the reversal of the elution orders of some of the compounds compared to those obtained on the 3% OV-1 column (Ref. 13.3).

11.2.2 For the 10 acid herbicide PFB esters, the best resolution was obtained on a DB-5 or SE-54 capillary column. For confirmation purposes, a CARBOWAX-20M column has been shown to resolve the 9 PFB esters in Fraction B with an elution order different from that of an OV-1, SE-54 or DB-5 column (Ref. 13.1).

11.3 Additional confirmation of identity may also be obtained by the formation and analysis of different derivatives. For the acid herbicides, these include the methyl, ethyl, 2-chloroethyl and 2,2,2-trichloroethyl esters.

12.0 REMARKS

12.1 It is strongly recommended that the use and handling of benzene be restricted to a Fume Hood.

12.2 The choice of acetone/hexane (1 + 1) as extraction solvent is based on its wide applicability to the extraction of several other classes of compounds from sediments. (Reference 13.2). The use of a more polar solvent system such as acetone does not yield higher recoveries of the acid herbicides, but may instead increase emulsion problems in the extraction of acidified sediments with high humic and organic contents.

12.3 Intermittent (i.e., pulsed) operation of the Ultrasonic homogenizer during the sediment extraction permits high intensity sonicating while avoiding excessive heat buildup in the processed sample. To maintain a sample temperature of <40°C, it is recommended that an ice-bath be used to cool the beaker containing the sample at one-minute intervals

during the sonification/extraction. (Procedure steps 8.1.5 and 8.1.6).

12.4 In sediment samples with a high humic content, emulsions can form at the solvent/water interface during extraction and/or base-partitioning. If this happens, the emulsion should remain with the aqueous layer until the final organic extraction. For severe cases, extra washes of the aqueous phase with dichloromethane may be required.

12.5 Organic extracts containing acid herbicides should not be passed through anhydrous sodium sulphate for removal of traces of water. It has been shown that the use of such adsorbents, including acidified ones, can cause irreversible adsorption and thus low and erratic recoveries of the herbicides. (Ref. 13.2)

12.6 Extreme care must be exercised by the analyst in the steps in which extracts are concentrated.

12.6.1 Neutral herbicide-containing extracts must never become dry, as these herbicides are quite volatile.

12.6.2 In the final extraction step for acid herbicides, the extracts in benzene must be evaporated just to dryness in

order to release all of the solvent prior to esterification. When a rotary evaporator at $\leq$40°C was used, no losses of these herbicides were observed. However, prolonged evaporation under reduced pressure after the extract goes dry is not recommended. (Ref. 13.1).

12.6.3 Removal of acetone after esterification by a gentle stream of nitrogen should be done in the presence of hexane. While the PFB esters are relatively non-volatile, evaporation to dryness should be avoided. Care must be taken to ensure that the reaction tubes are tightly sealed after the introduction of the PFBBr reagent. Derivatization of the acid herbicides is a very critical step in their analysis. (Ref. 13.1).

12.7 It is recommended that an acid herbicide standard be prepared alongside each set of sediment extracts to account for any minor variations in the derivatization procedure from one set to another.

12.8 The presence of too much water in the acid herbicide extract could inhibit esterification and result in low and erratic recoveries. Under these reaction conditions, water is also known to form dipentafluorobenzyl ether and other side products. (Ref. 13.2).

12.9 Since variations in adsorbent activities may be found among different batches of Florisil, it is recommended that the neutral herbicide elution pattern be checked with standard solutions before using the Florisil for sample cleanup.

12.10 The GLC response to Barban may decrease gradually as the column is used for sample analyses. If this occurs, replacement of the silanized glass wool at the column inlet will restore the signal response and reduce tailing. (Ref. 13.3).

12.11 Addition of 1% methanol (or acetone) to neutral herbicide solutions containing Benzoylprop-ethyl is recommended to increase and stabilize the GLC response to this herbicide. (Ref. 13.3).

12.12 For the determination of just the six neutral herbicides, acidification of the sediment sample prior to extraction is not necessary. Also, as the aqueous phase from the potassium bicarbonate partitioning contains acidic co-extractives, it could be discarded and steps 8.1.14 to 8.1.18 and 8.1.23 to 8.1.26 may be ignored. Finally, the PFBBr derivatization of acidic compounds (step 8.2) and subsequent Silical Gel cleanup (step 8.3) would, of course, also be omitted.

12.13 It has been shown elsewhere (Ref. 13.4) that Atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) is generally poorly recovered from acidified sediment samples. However, recoveries of better than 90% have been achieved for this neutral herbicide using the outlined procedure in this method when the extracted sediment sample has remained unacidified.

12.14 To determine just the ten acid herbicides, steps 8.1.11 to 8.1.23, involving the partitioning with potassium bicarbonate followed by the second extraction sequence, may be omitted entirely. Furthermore, the Florisil Column cleanup (step 8.4) may also be eliminated in this simplified procedure.

12.15 It has been demonstrated elsewhere that the basic analytical scheme is also effective in the determination of other classes of compounds such as PCBs and organochlorinated pesticides.

13.0 REFERENCES

13.1 Lee, Hing-Biu, Yvonne D. Stokker and Alfred S.Y. Chau. 1986. Chemical Derivatization Analysis of Pesticide Residues. X. Analysis of Ten Acid Herbicides in Natural Waters. J. Assoc. Off. Anal. Chem. 69[3]:557-560.

13.2 Lee, Hing-Biu and Alfred S.Y. Chau. 1983. Analysis of Pesticide Residues by Chemical Derivatization. VI. Analysis of Ten Acid Herbicides in Sediment. J. Assoc. Off. Anal. Chem. 66[4]: 1023-1028.

13.3 Lee, Hing-Biu and Alfred S.Y. Chau. 1983. Determination of Trifluralin, Diallate, Triallate, Atrazine, Barban, Diclofop-Methyl and Benzoylprop-Ethyl in Natural Waters at Parts per Trillion Levels. J. Assoc. Off. Anal. Chem. 66[3]: 651-658.

13.4 Lee, Hing-Biu and Alfred S.Y. Chau. 1983. Gas Chromatographic Determination of Trifluralin, Diallate, Triallate, Atrazine, Barban, Diclofop-Methyl and Benzoylprop-Ethyl in Sediments at Parts per Billion Levels. J. Assoc. Off. Anal. Chem. 66[6]: 1322-1326.

13.5 Phenoxyacid Herbicides in Sediments (Gas Chromatographic) Water Quality Branch Analytical Methods Manual, Environment Canada.

13.6 Method for the Analysis of Eight Phenoxy Acid Herbicides in Water by Derivatization with BCl_3/2-Chloroethanol. Water Quality Branch Analytical Methods Manual, Environment Canada.

13.7 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.

13.8 Chau, A.S.Y., Carron, J. and Lee, H.B. 1979. Analytical Reference Materials. II. Preparation and Sample Integrity of Homogeneous Fortified Wet Sediment for Polychlorinated Biphenyl Quality Control Studies. J. Assoc. Off. Anal. Chem. $\underline{62}^{6}$:1312-1314.

13.9 Stokker, Yvonne D. November, 1985. Method for the Analysis of Acid and Neutral Herbicides in Naural Waters. NWRI Contribution No. 85-120.

APPENDIX

APPENDIX

1. Figure A-1. Gas chromatogram of standard mixture of nine Acid Herbicide PFB esters on 30 m DB-5 FSCC. 2.0 µl injected. GLC conditions are outlined in Section 6.1.

	Herbicide (PFB ester)	Amount Injected	Retention Time (min)
1.	Dicamba	20 pg	12.92
2.	MCPA	20 pg	13.23
3.	2,4-DP	20 pg	13.74
4.	2,3,6-TBA	20 pg	13.98
5.	2,4-D	20 pg	15.44
6.	Silvex	20 pg	17.37
7.	2,4,5-T	40 pg	18.91
8.	MCPB	40 pg	19.74
9.	2,4-DB	40 pg	22.02

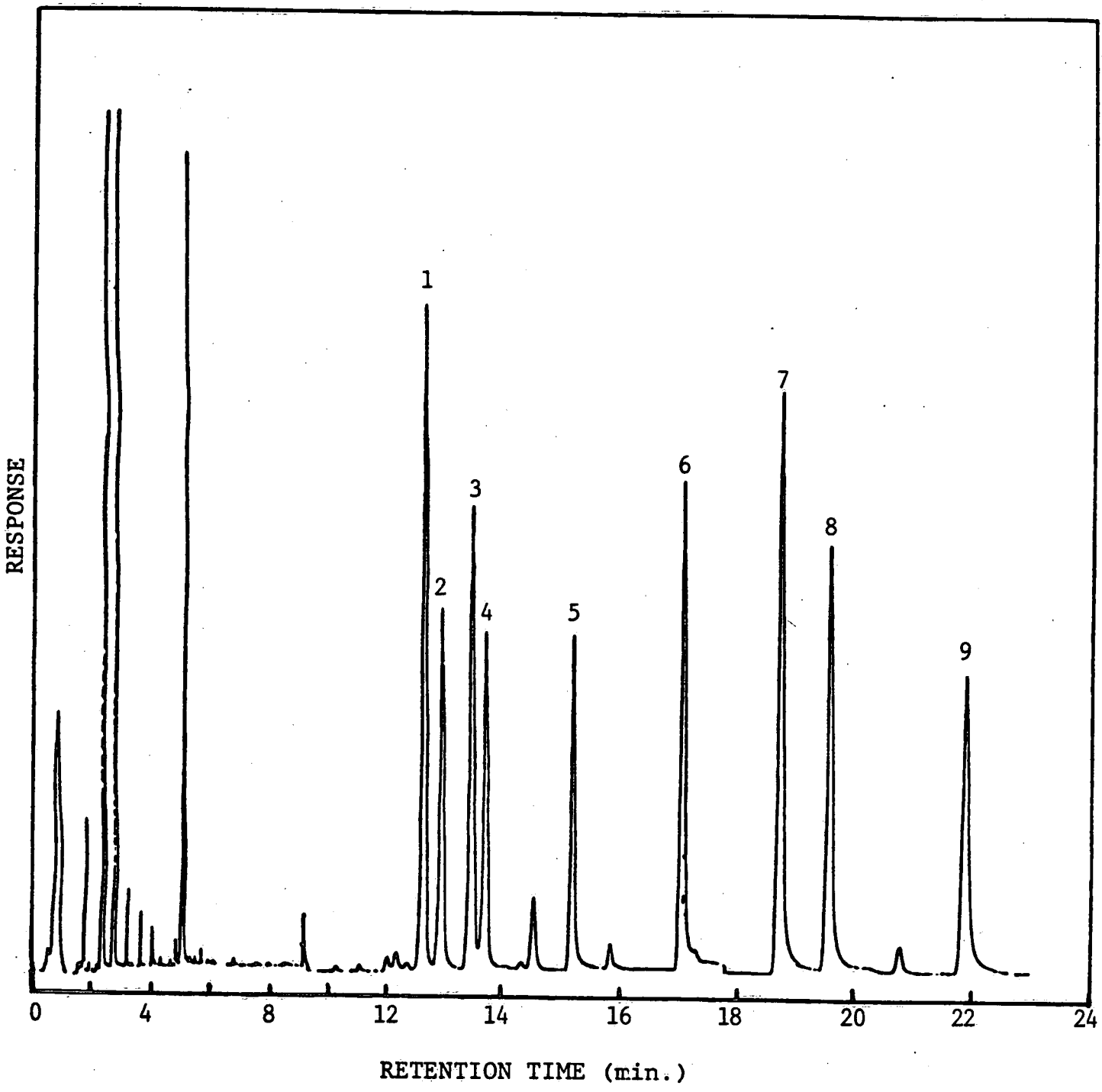

Figure A.1. 9 Acid Herbicide PFB Esters

2. Figure A-2. Gas chromatogram of standard mixture of seven Neutral Herbicides on a 3% OV-1 column (1.8 m x 2 mm i.d.). 10.0 µl injected. GLC conditions are outlined in Section 6.2 .

	Herbicide	Amount Injected	Retention Time (min)
1.	Trifluralin	50 pg	1.96
2.	Diallate	1000 pg	2.18
3.	Atrazine	2000 pg	2.38
4.	Triallate	100 pg	3.32
5.	Barban	1000 pg	11.68
6.	Diclofop-Methyl	500 pg	25.29
7.	Benzoylprop-Ethyl	250 pg	30.10

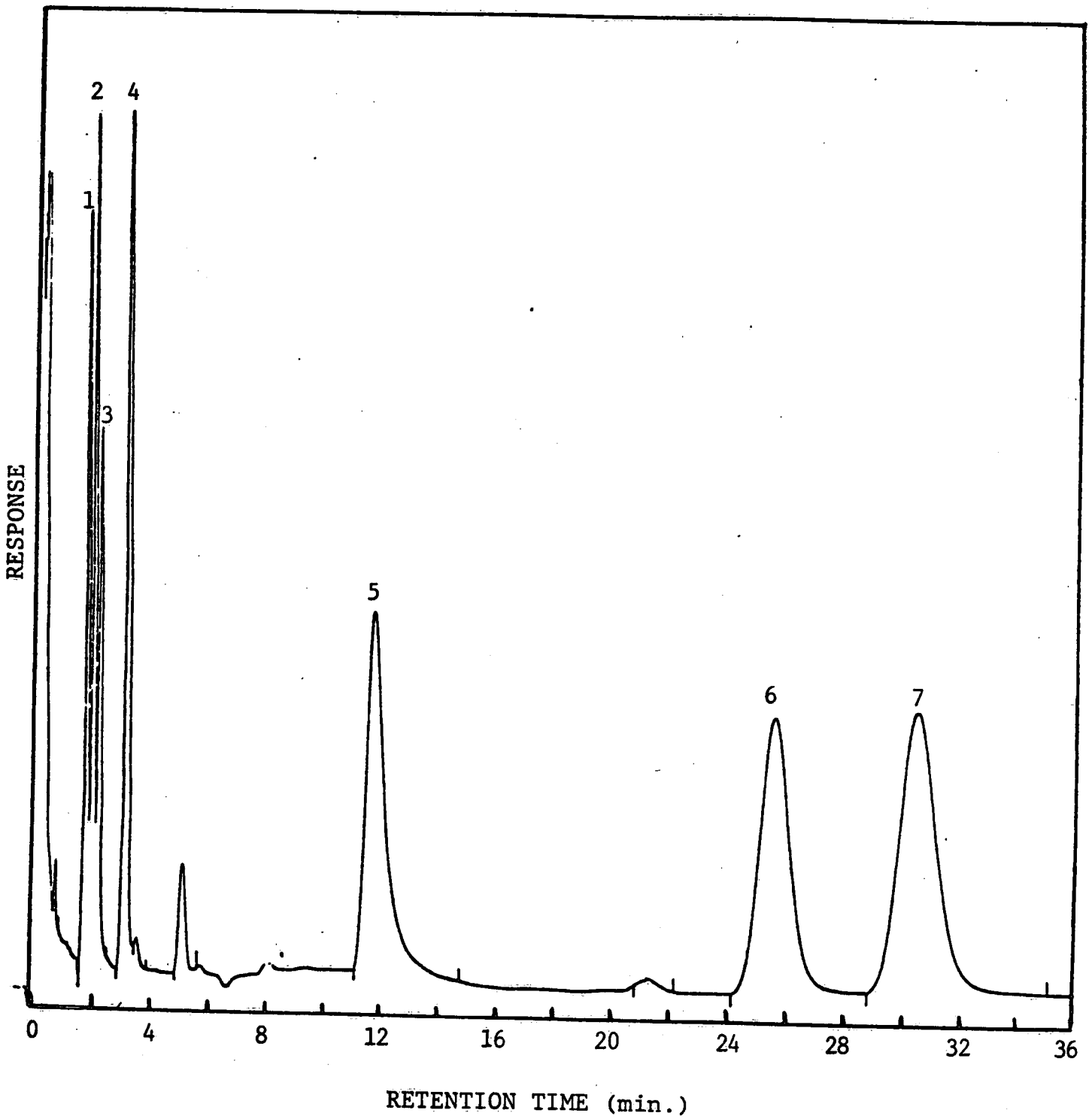

Figure A.2. 7 Neutral Herbicides