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SINGLE-LABORATORY VALIDATION OF EPA METHOD 8150 FOR THE ANALYSIS OF CHLORINATED HERBICIDES IN HAZARDOUS WASTE

by

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FOREWORD

The Resource Conservation and Recovery Act of 1976 (RCRA) and its amendments require the U.S. Environmental Protection Agency (EPA) to regulate hazardous waste activities.[1,2,3] Implementation and enforcement of the RCRA requires analytical procedures that can provide data of known precision and accuracy on analytes in hazardous waste samples. Reliable data collected under prescribed quality assurance/quality control procedures will allow the EPA to identify and delineate waste sites, characterize waste composition, and detect environmental contamination resulting from operations that generate hazardous wastes.

The document[4] Test Methods for Evaluating Solid Waste, Office of Solid Waste Manual SW-846, was published to provide a compilation of state-of-the-art methodology for evaluating RCRA solid wastes for environmental and human health hazards. SW-846 Method 8150 for chlorinated herbicides required validation as part of an ongoing program to evaluate SW-846 methods. Detailed single-laboratory validation guidelines are available as reported by the Association of Official Analytical Chemists (AOAC) Committee on Interlaboratory Studies[5] and the EPA report "Validation of Testing/Measurement Methods"[6] were followed, where feasible, in this study.

ABSTRACT

Method 8150, published in the second edition of Test Methods for Evaluating Solid Waste, Manual SW-846, required optimization, ruggedness testing, linearity determinations, precision tests, bias testing, gas chromatography/mass spectrometric (GC/MS) confirmation, and quality control guidelines in order to validate the protocol in a single laboratory prior to interlaboratory validation. The single-laboratory validation, which is applicable to the determination of the herbicides Dicamba, Silvex, 2,4-D, 2,4-DB, 2,4,5-T, Dinoseb, MCPP, MCPA, and Dichlorprop in hazardous waste extracts, was completed and is described in this report.

The extraction procedure was modified to use methylene chloride and sonication with an acidic buffer. Ruggedness testing indicated that the volume and pH of the buffer were important variables as was the power setting of the sonicator. These variables were optimized using simplex optimization. The amount of methanol added to facilitate ester hydrolysis was found to be the only important condition in the hydrolysis step and was optimized by varying the methanol concentration. The optimized protocol works well for nine of the ten target herbicide analytes. Analysis of Dalapon is excluded from this method.

Methylation was carried out in a mixed solvent in which iso-octane was added as a "keeper" to decrease evaporation losses and methanol was added to increase the reactivity of diazomethane. Capillary column gas chromatography using electron capture detection (GC/EC) allowed the determination of the herbicide analytes as the methyl derivatives in a single, 20-minute GC run. The original Method 8150 procedure required three packed columns for the ten target analytes.

Final ruggedness testing on the optimized procedure gave a mean recovery of 89.3% with a standard deviation of 4.3%. The precision of the method is excellent. Percent relative standard deviations (% RSD's) are less than 10 ($n = 20$, each analyte) over a 10^2 linear range of concentration for MCPP and MCPA and over a 10^3 linear range of concentration for the other target herbicide esters. Detection limits for electron capture detection and mass spectrometric identity confirmation were determined and found to be matrix dependent.

CONTENTS

FIGURES

FIGURES (Continued)

FIGURES (Continued)

TABLES

TABLES (Continued)

TABLES (Continued)

ABBREVIATIONS AND SYMBOLS

ABBREVIATIONS

RCRA	- Resource Conservation and Recovery Act of 1976 and its amendments.
% RSD	- percent relative standard deviation.
2,4-D	- 2,4-dichlorophenoxyacetic acid.
2,4-DB	- 4-(2,4-dichlorophenoxy)butyric acid.
Dalapon	- 2,2-dichloropropanoic acid.
Dicamba	- 3,6-dichloro-2-methoxybenzoic acid.
Dichlorprop	- 2-(2,4-dichlorophenoxy)propionic acid.
Dinoseb	- 2-(1-methylpropyl)-4,6-dinitrophenol.
MCPA	- 2-methyl-4-chlorophenoxyacetic acid.
MCPP	- 2-(4-chloro-2-methylphenoxy)propionic acid.
Silvex	- 2-(2,4,5-trichlorophenoxy)propionic acid.
2,4,5-T	- (2,4,5-trichlorophenoxy)-acetic acid.
GC/MS	- gas chromatograph-mass spectrometer system or analysis.
GC/EC	- gas chromatography-electron capture detection.
ppb	- parts per billion.
Diazald®	- N-methyl-N-nitroso-p-toluenesulfonamide.
K-D	- Kuderna-Danish concentrator.
σ	- standard deviation
KHz	- kilohertz

ACKNOWLEDGMENT

The authors thank Professors M. E. Munk and M. L. Parsons of Arizona State University for helpful suggestions and evaluations of the project work plan. T. L. Vonnahme, P. J. Marsden, and A. R. Bujold of Lockheed Engineering and Management Services Company, Incorporated, provided helpful suggestions and some preliminary work. We also thank Fred C. Hart Associates for carrying out the diazomethane methylations.

SECTION 1

INTRODUCTION

Four related procedures for the analysis of chlorinated herbicides have been proposed by the FDA,[7] ASTM,[8] and the EPA (Method 615[9] and Method 8150[4]). All of these procedures use diazomethane for methylation of the free acid herbicides. This reagent is known to be toxic and explosive[10] and these procedures should only be attempted by an experienced analyst. It is interesting to note that one publication states that Dalapon is not methylated by diazomethane.[11]

Another methylating reagent, the boron trifluoride-methanol complex, is known to give better results for chlorinated herbicides in at least some cases.[12,13] However, 2-methyl-4-chlorophenoxyacetic acid (MCPA), Dinoseb, and Dicamba are not methylated by the boron trifluoride-methanol complex.[8] Because analysis of these analytes is required by Method 8150, boron trifluoride-methanol is not a suitable methylating procedure for this study. As a possible alternate derivatization procedure, pentafluorobenzylation[14] was also studied.

The extraction procedure of Method 8150 (and the ASTM Method) uses large amounts of ether which creates a potential fire hazard. The FDA procedure uses chloroform (a suspected carcinogen) for extraction. Use of methyl t-butyl ether[15] or methylene chloride following the Superfund contract laboratory protocol[16] seemed viable alternatives for extraction, as did the mixed solvent acetone/hexane which would allow adjustment of the solvent polarity to optimum.

Method 8150 uses three different packed column runs for GC of the methyl esters; we hoped that a single capillary gas chromatography run could be used. Generally, capillary columns give better chromatographic resolution and less background detector noise than do packed columns.[17] Thus, first priority was to test a capillary column for analysis of the derivatives. Method 8150 states that microcoulombic detection is preferred to electron capture detection for the methylated herbicides. With the added resolution of capillary chromatography, the more sensitive and widely available electron capture detection was deemed preferable.

Method 615 is of more recent vintage than Method 8150 but much of the same methodology is retained, and safety is discussed in more detail in Method 615 (as a separate section). The methylation procedure has been changed to more closely resemble the ASTM method with the addition of methanol (said to give faster and more complete methylation[18]) and distillation of diazomethane directly into the reaction mixture. Packed column GC/EC is recommended. The validation of Method 8150 required the steps summarized in this report and resulted in the optimized and validated protocol given in Appendix A.

The project work plan consisted of:

1. Examination of Method 8150 and related literature.
2. Revision of the method using preliminary experiments.
3. Ruggedness testing to find important variables.
4. Simplex optimization to refine important variables.
5. Final ruggedness testing to test range of important variables.
6. Determination of linear dynamic range.
7. Precision and accuracy testing.
8. Preparation of optimized protocol.
9. Testing of protocol on real samples.
10. Revisions of the final protocol based on sample analysis.

SECTION 2

CONCLUSIONS

- Method 8150 for analysis of chlorinated herbicides in hazardous waste has been validated at the Environmental Monitoring Systems Laboratory, Las Vegas, Nevada (EMSL-LV), as described in this report.

- The optimized protocol is valid for 9 of the 10 analytes. Dalapon is not recovered using this procedure.

- Ruggedness testing of the optimized protocol gives a mean recovery of 89.3% with a 4.3% RSD for the range of experimental conditions tested.

- The validated protocol uses one capillary column GC run rather than the three packed column runs proposed in the original method. This results in considerable savings in analysis time.

- The validated protocol substitutes the less flammable methylene chloride for ether in the extraction step.

- The estimated GC/EC detection limits are 0.1 to 4 ng/g for 8 of the analytes. MCPP and MCPA have higher detection limits, 66 and 43 ng/g respectively.

- The measured % RSD's of all analyte concentrations are below 10%. Horwitz, et al[19,20] suggests interlaboratory data should have variations less than 16% if a single-laboratory variation of 8 to 11% has been obtained.

- Quality control guidelines and the detailed protocol are presented to support a future interlaboratory validation.

SECTION 3

RECOMMENDATIONS

Method 8150 has been revised as shown in the Appendix A protocol. The revised protocol has been single-laboratory validated and is now ready for internal and external review. An interlaboratory comparison test using the chlorinated herbicide analytes in selected matrices is the next laboratory test of the procedure.

The pentafluorobenzylation procedure is quite promising as an alternative method. The greater sensitivity of the analyte derivatives in comparison to the methyl derivatives has promise in multiresidue screening procedures. Single-laboratory validation of the pentafluorobenzylation method for chlorinated herbicides is recommended. Although this extra sensitivity may not be necessary for typically high level RCRA wastes, it may prove valuable for lower level environmental samples (fish, soil, and sediments).

SECTION 4

PRELIMINARY STUDIES

INTRODUCTION

Experiments were designed to define the scope of the Method 8150 modification. This section describes experiments on sonication extraction with an organic solvent, capillary GC/EC optimization, pentafluorobenzylation, matrix selection, stability of herbicide spikes, and precision and accuracy studies. The methylation and safety requirements are also discussed.

SAFETY

General

Because diazomethane is a known carcinogen and a possible explosion hazard, we decided to prepare and use diazomethane in a containment facility using the following precautions:

1. Diazomethane is an explosion hazard; therefore, all generation reactions were carried out in a hood behind an explosion shield.

2. All glassware used in the generation had Clear-Seal® joints or their equivalent. GROUND-GLASS JOINTS SHOULD NOT BE USED. Glassware used in the ether trap was fire-polished.

3. Wire brushes were not used in cleaning the Clear-Seal® apparatus because they can scratch the inner surface of the glass and, thus, increase the explosion hazard of this procedure.

4. Dioxane and other solvents that may freeze were not used because the sharp edges of the crystals formed may cause an explosion.

5. Diazomethane is a volatile carcinogen. All contact with diazomethane or Diazald® was avoided.

6. Ether is a flammable solvent. A funnel was used to pour the ether carefully into the reaction vessel.

7. Compound data sheets were supplied to containment facility personnel prior to work initiation on a given compound.

8. To avoid contamination, separate glassware and supplies were used for each sample preparation.

9. All sample preparations were localized in a hood demonstrated to be free from volatile contaminants.

10. Contact of solutions with any surface besides clean glass or Teflon was not permitted.

11. An analytical balance capable of accurately weighing to at least ±0.01 mg in the hood was required (Mettler AE 163 or equivalent).

12. All containers (inner and outer) removed from the containment facility were wiped clean with ether before removal.

Generation of Diazomethane

Ethereal solutions of diazomethane were prepared using an Aldrich "Mini Diazald® Apparatus" or equivalent and the following procedures.[21] Most derivatization procedures required 2 mmole of diazomethane (0.5 g Diazald® reagent) and the generation of large amounts of diazomethane was avoided. This procedure is taken from Figure 1; the letters identifying laboratory apparatus are shown in Figure 2.

1. The apparatus shown in Figure 2 was assembled except for the separatory funnel (a). The ice water bath (b) that cools the receiving flask (c) was supported by a lab jack so that the flask was easily removed from the apparatus at the end of the distillation.

2. The warm water bath (d) for the reaction vessel (e) was heated with a hot plate and allowed to come to temperature (65-70°C) before the cold finger trap (f) was filled with dry ice/acetone.

3. The ether trap (g) for any excess diazomethane was made from a test tube half filled with absolute ether, a cooled, fire-polished, disposable pipet, and latex tubing.

4. After the apparatus was assembled, a solution of 2.5g of potassium hydroxide in 4 mL water was added to the reaction vessel (e). Next, a mixture of 14 mL 2-(2-ethoxyethoxy)ethanol and 8 mL absolute ether was added. The temperature of the warm water bath was constantly monitored.

5. The separatory funnel (a) was charged with a solution of 0.5g Diazald® in 45 mL absolute ether (unless specified otherwise).

6. The separatory funnel was placed on the reaction vessel (e) and the stopcock was opened so that rate of addition of Diazald®/ether solution approximated the rate of distillation. (This addition took 20 to 40 minutes.) More dry ice was added to the cold finger trap as necessary.

7. After all of the Diazald® solution was added, 10 mL of ether was slowly added and the distillation continued until the distillate was colorless.

8. The hot plate was turned off and the distillate was checked to verify it as yellow. If there was no color, no diazomethane was collected.

Derivatization Reaction

1. The lab jack was lowered, the ether trap (g) was removed, and then the ice bath (b) was removed.

2. The receiving flask was lowered and was supported on a cork ring.

3. Approximately equal amounts (usually 2 mL) of the ethereal diazomethane were added to the standards or extracts using a cooled, fire-polished, disposable pipet. The samples for derivatization were provided in concentrator tubes. The standards were dissolved in approximately 4mL 9:1 absolute ether/absolute methanol in volumetric flasks unless otherwise specified.

4. The mixture was swirled to ensure complete mixing of the solutions. The color was recorded. If the sample was not highly colored, the derivatization mixture was yellow. THE SAMPLE WAS NOT STOPPERED UNTIL AFTER STEP 6.

5. The color was checked and recorded fifteen minutes after the addition of the ethereal diazomethane.

6. The derivatization mixtures were allowed to stand unstoppered overnight in the hood unless otherwise directed.

7. The next day the vessels were stoppered with foil-wrapped corks of the proper size and placed in a walking can for transport to the lab.

PRODUCT NO Z10,889-8

Technical Information Bulletin
Number AL-121

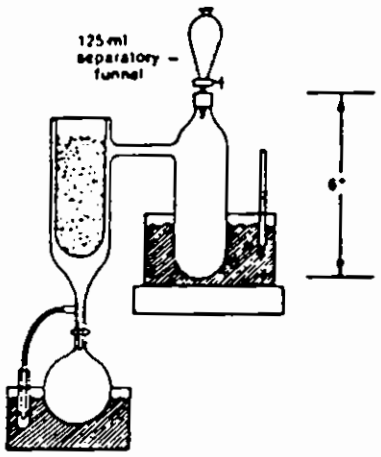
Mini Diazald® Apparatus

The Mini Diazald Apparatus was developed to bridge the gap between the Aldrich MNNG-diazomethane generator (for preparing < 1 mmol of diazomethane) and the Diazald Kit (for preparing ca. 100 mmol of diazomethane). It consists of a reaction vessel and a condenser in one compact unit. The only other glassware needed are an addition funnel and a receiver flask (must be equipped with Clear-Seal® joints, Note 1). Since both of these pieces are included in our Diazald Kit, the Mini Diazald Apparatus makes a perfect addition to the kit.

The major feature of this apparatus is the cold finger, in place of a water-jacketed condenser. When filled with dry ice/acetone slush, the condenser very efficiently prevents dangerous diazomethane/ether vapor from escaping the apparatus. Nevertheless, it is suggested that an ether trap be employed, and that ALL REACTIONS INVOLVING THE PREPARATION AND USE OF DIAZOMETHANE BE CARRIED OUT IN AN EFFICIENT HOOD AND BEHIND A SAFETY SHIELD.

As with all glassware equipped with Clear-Seal joints, this apparatus should be washed very carefully. Wire brushes should not be used since they can scratch the inner surface of the glass.

DIAZOMETHANE PREPARATION



Assemble the equipment as shown. Fill the condenser with dry ice, then add acetone slowly until the cold finger is about one-third full. Add ethanol (95%, 10ml) to a solution of potassium hydroxide (5g) in water (8ml) in the reaction vessel. Attach a 100-ml receiving flask (with Clear-Seal joint) to the condenser and cool receiver in an ice bath. Provide an ice-cooled ether (ca. 2ml) trap at the sidearm (the glass rod must have fire-polished ends).

Place a separatory funnel (with Clear-Seal joint) over the reaction vessel and charge funnel with a solution of Diazald (5.0g, 23mmol) in ether (45ml). Warm the reaction vessel to 65° with a water bath and add the Diazald solution over a period of 20 minutes. The rate of distillation should approximate the rate of addition. Replenish cold finger with dry ice as necessary. When all the Diazald has been used up, slowly add 10ml of ether and continue the distillation until the distillate is colorless. The ethereal distillate will contain about 700mg (16.6mmol) of diazomethane.

If an alcohol-free ethereal (Note 2) solution of diazomethane is required, add 2-(2-ethoxyethoxy)ethanol (14ml) and ether (8ml) to a solution of potassium hydroxide (2.5g) in water (4ml) in the reaction vessel. Distill diazomethane as in above procedure (a similar yield is obtained).

The Mini Diazald Apparatus works well with as little as 500mg of Diazald. It is not necessary to scale-down the alkali solution. This feature, along with the very efficient condenser, makes the apparatus ideally suited for the preparation of deuterated diazomethane. The reagent quantities outlined in the Deutero-Diazald Prep Set instructions can be used in this Mini Apparatus, up to about 15g of Diazald.

Notes:

1. DO NOT USE A SEPARATORY FUNNEL OR A RECEIVER FLASK WITH GROUND-GLASS JOINTS. Glassware without sharp edges or ground-glass joints are recommended to avoid explosions. For the convenience of customers who do not own the Diazald Kit, Aldrich offers the separatory funnel and receivers (three sizes) equipped with Clear-Seal joints.

2. Dioxane and other solvents that may freeze should not be used as the sharp edges of crystals formed may cause an explosion.

Accessories (with ₹19/22 Clear-Seal joints)

Z10,033-1	Round-bottom flask, 50ml, pack of 2
Z10,035-8	Round-bottom flask, 100ml, pack of 2
Z10,036-6	Round-bottom flask, 250ml, pack of 2
Z10,038-2	Separatory funnel with Teflon stopcock, 125ml
Z10,039-0	Teflon stopper, pack of 12
Z10,025-0	Diazald Kit
Z10,851-0	Macro Diazald Set, with ₹24/40 Clear-Seal joints

Please check the current Aldrich Catalog/Handbook for a description of the Diazald Kit and the list of all replacement parts.

☐ See Reverse Side

Figure 1. The Mini Diazald® Apparatus and generation of diazomethane.

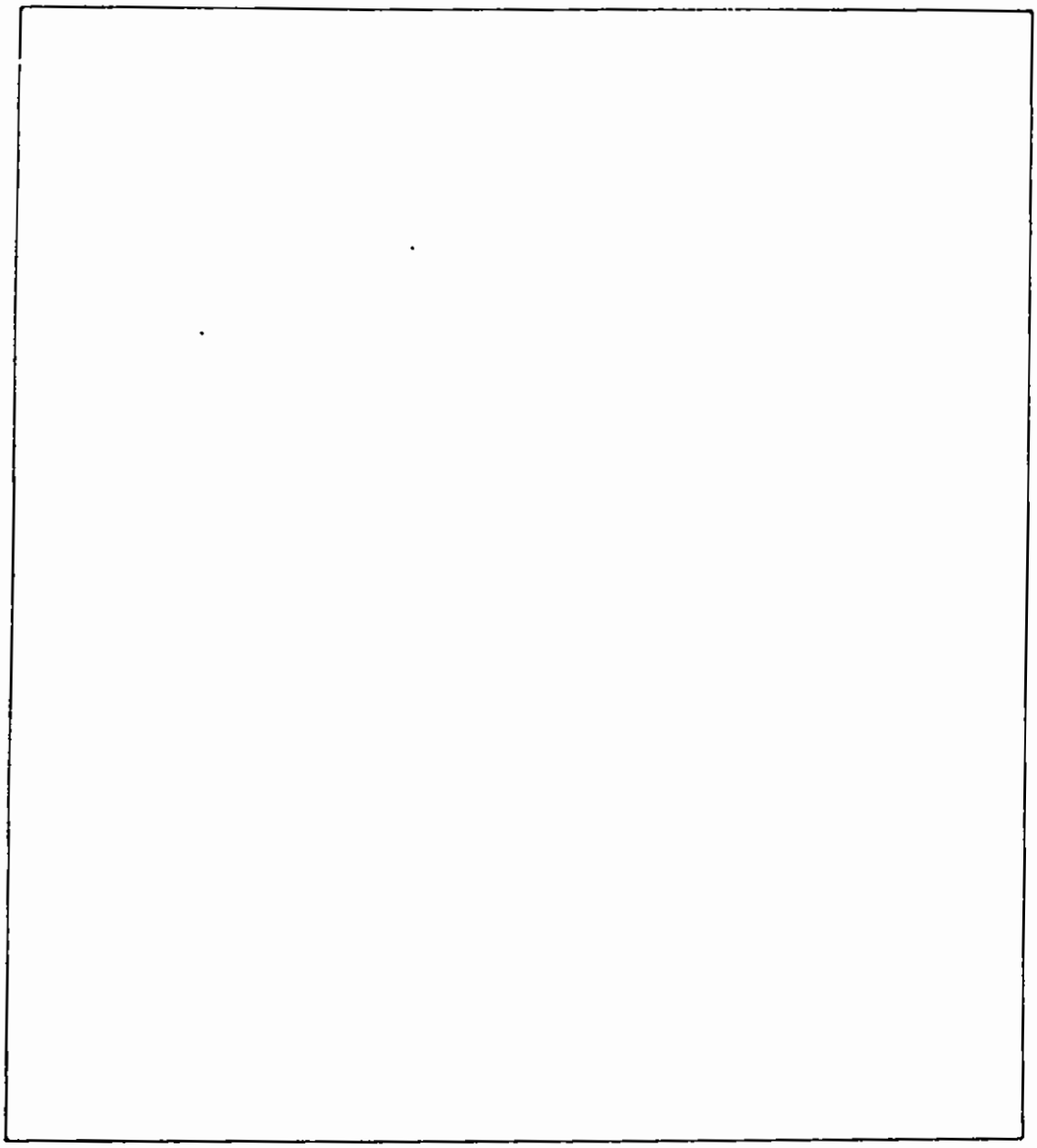


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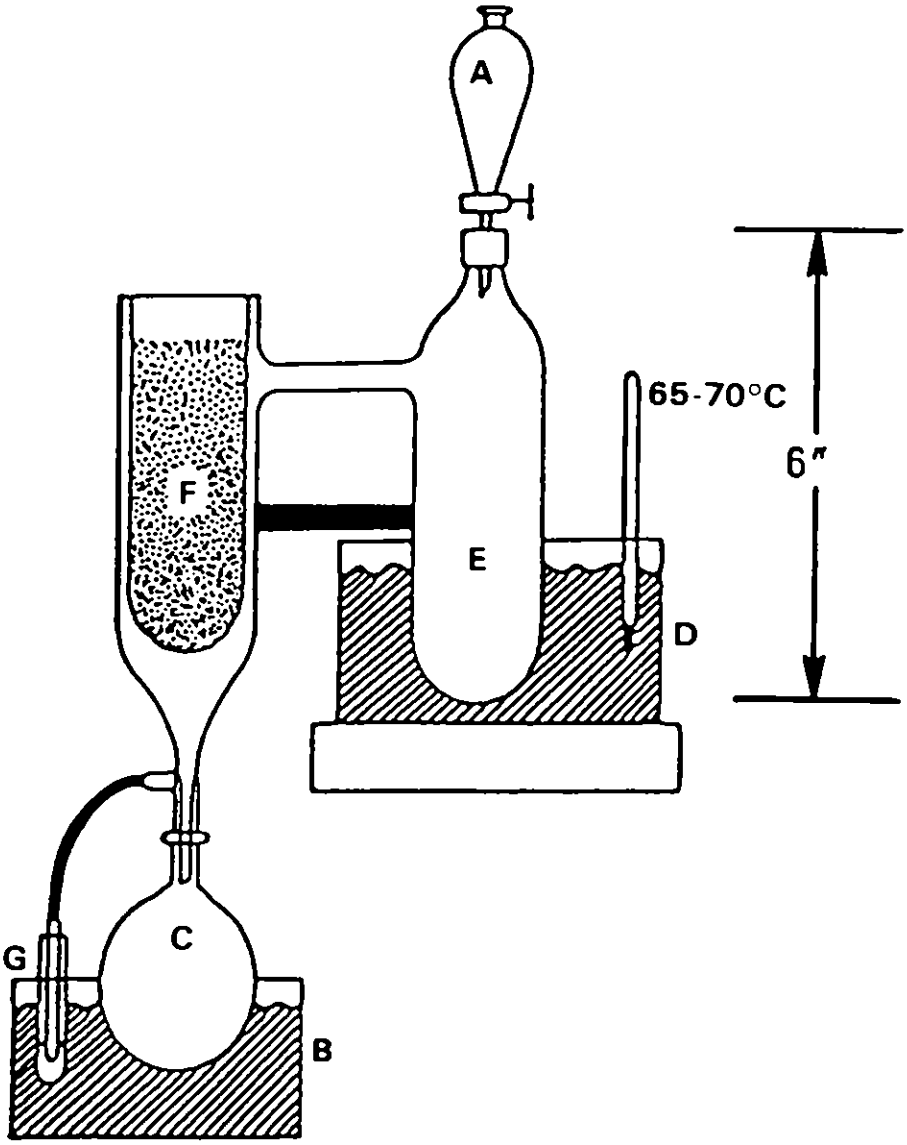
Figure 1. (continued)



A. Separatory Funnel
B. Ice Water Bath
C. Receiving Flask
D. Warm Water Bath
E. Reaction Vessel
F. Cold Finger Trap
G. Ether Trap

Figure 2. The diazomethane generating apparatus.

DATA AND RESULTS

Physico-chemical and toxicity properties of the free acid herbicides selected for Method 8150 are shown in Table 1. The LD_{50}, water solubility, and pKa values were collected from the literature to help in understanding possible problems in the method development.

Because the packed column GC described in Method 8150 was judged to be inadequate for chromatographically separating contaminants from analytes, and because three different columns were required to analyze all analytes, the first laboratory work was to find capillary GC conditions to separate all methylated analytes. This was done on a DB-5 (J & W Scientific) GC column as shown in Figure 3. Precision measurements (% RSD's) ranging from 3.75 to 9.98 were obtained for analysis of a standard mixture as shown in Table 2. Figures 4 and 5 show a sample chromatogram and analysis report from the Tracor 540 GC/EC and the IBM CS9000 data system. An alternative GC column, Supelcowax® (Supelco, Inc.) shows promise for separating the methylated herbicides (see Figure 6).

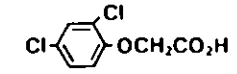
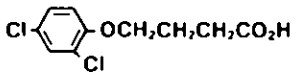
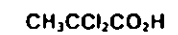
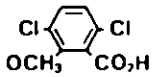
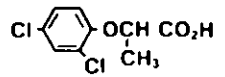
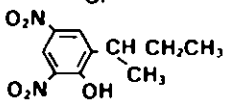
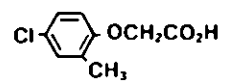
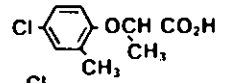
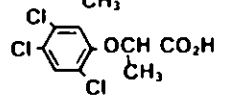
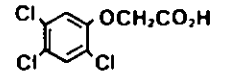
The pentafluorobenzylated herbicides were prepared as a possible alternative to the methyl derivatives of Method 8150. As shown in Figure 7, the gas chromatographic separation of the pentafluorobenzylated (PFB) herbicides on DB-5 is superior to the methylated derivatives. Besides improved separation, increased electron capture sensitivity is noted for all PFB versus methyl derivatives of the analytes.

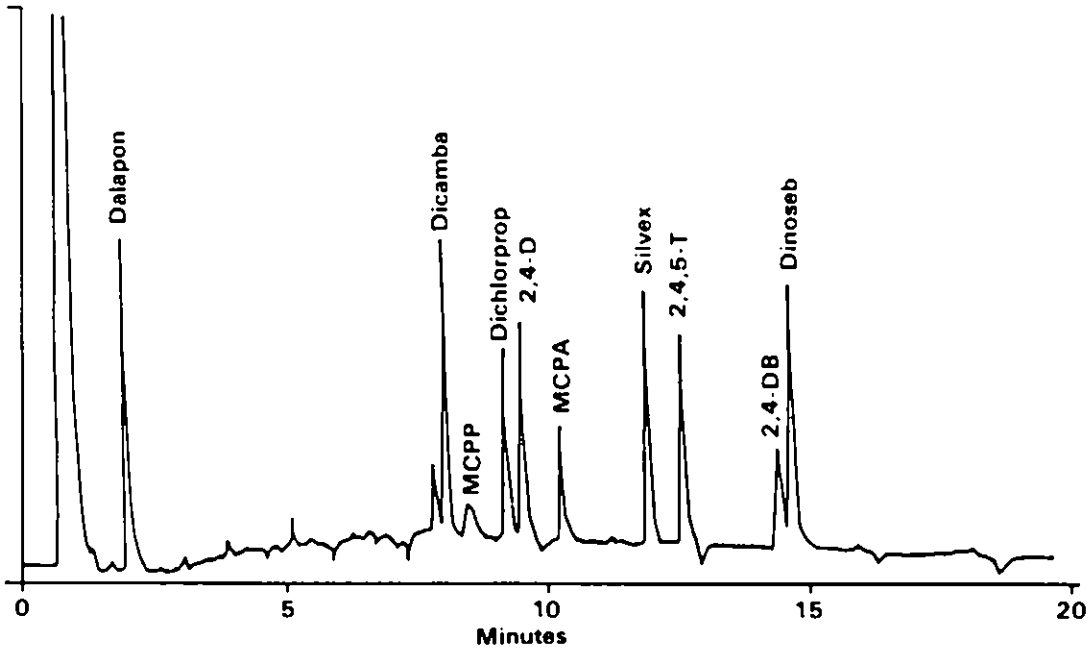
The matrix initially chosen for the optimization was "casting body" clay. This matrix has been used for dioxin organic performance evaluation samples. On analysis of unspiked casting-body clay using Method 8150 with capillary GC/EC analysis, an impressive number of peaks was observed. The manufacturer explained that lignite was a component of casting-body clay. Lignite is decomposed organic material containing many carboxylic acids. Three other matrices, desert soil (a sandy soil collected near Las Vegas, Nevada), Ajax C (type C, kaolin calcined at 2000°C), and Ajax P (type P, pure mineral kaolin), were evaluated as replacements as shown in Table 3. Ajax P (Westwood Ceramic Supply, City of Industry, California) was selected as most suitable for further study.

Kaolin clay (type P) was spiked and analyzed in quadruplicate using Method 8150 as shown in Tables 4 and 5 with peak height and peak area quantitation giving about equal variation and recovery. Three of the analytes were spiked at a higher concentration for a pesticide inter-laboratory comparison study. The analysis of this sample was repeated to verify the integrity of the sample after freezer storage for one week. Because the stored samples gave only slightly higher recoveries, this study serves to show the reproducibility of the method for these analytes at this concentration.

An alternative to the Method 8150 ether extraction procedure is sonication with a suitable organic solvent.[29] The sonication extraction procedure has been successfully tested by Battelle Memorial Institute in a round-robin study[30] and verified by Research Triangle Institute for sonication of solids.[31] Sonications using methylene chloride were done on spiked samples wet with three different buffers: pH 1 to closest approximate Method 8150, pH 3 because the median pKa of the acids is approximately 3 (Table 1) and pH 7 because Dinoseb is reported to be extracted more efficiently at neutral pH.[14,32] The results are reported in Table 8. The recoveries of the phenoxyacid herbicides were lower than with Method 8150, but the best recovery of Dinoseb to that time was obtained at pH 7. The procedure was repeated at pH 7 with several solvents using a larger amount of anhydrous Na_2SO_4 prior to solvent addition; those results are presented in Table 9. Recoveries are shown in Table 10 for the pentafluorobenzylation procedure tried on spiked clay samples extracted by Method 8150 and by sonication with several solvents.

Table 1. Free Acid Herbicides

Common Name(s)	Systematic Name(s)	Structure	Rats LD_{50}(mg/kg)	Water Solubility (mg/L, 25°C)	pKa
2,4-D	2,4-dichlorophenoxy-acetic acid		375[21]	900[20]	2.64[23]
2,4-DB	4-(2,3-dichloro-phenoxy) butyric acid		700[21]	46[21]	4.8[23]
Dalapon	2,2-dichloropropanoic acid		9330[20,21]	502,000[21]	1.7[24]
Dicamba	3,6-dichloro-2-methoxy-benzoic acid, 3,6-dichloro-o-anisic acid		2900±800[20]	4,500[21]	1.93[25]
Dichlorprop	2-(2, 4-dichlorophenoxy)-propionic acid		800[20]	710[20]	3.28[26]
Dinoseb	2-sec-butyl-4,6-dinitro-phenol, 2-(1-methyl-propyl)-4,6-dinitrophenol		58[20]	52[20]	4.62[22]
MCPA	2-methyl-4-chlorophenoxy-acetic acid, (4-chloro-o-toloxy)acetic acid		700[22]	825[21]	3.07[23]
MCPP, Mecoprop	2-(4-chloro-2-methyl-phenoxy) propionic acid		930[21]	620[21]	3.20[26]
Silvex,2,4,5-TP, Fenoprop	2-(2,4,5-trichlorophenoxy)-propionic acid		650[21,22]	140[21]	2.84[23]
2,4,5-T	(2,4,5-trichlorophenoxy)-acetic acid		500[22]	(30°C)238[20]	2.84[24]



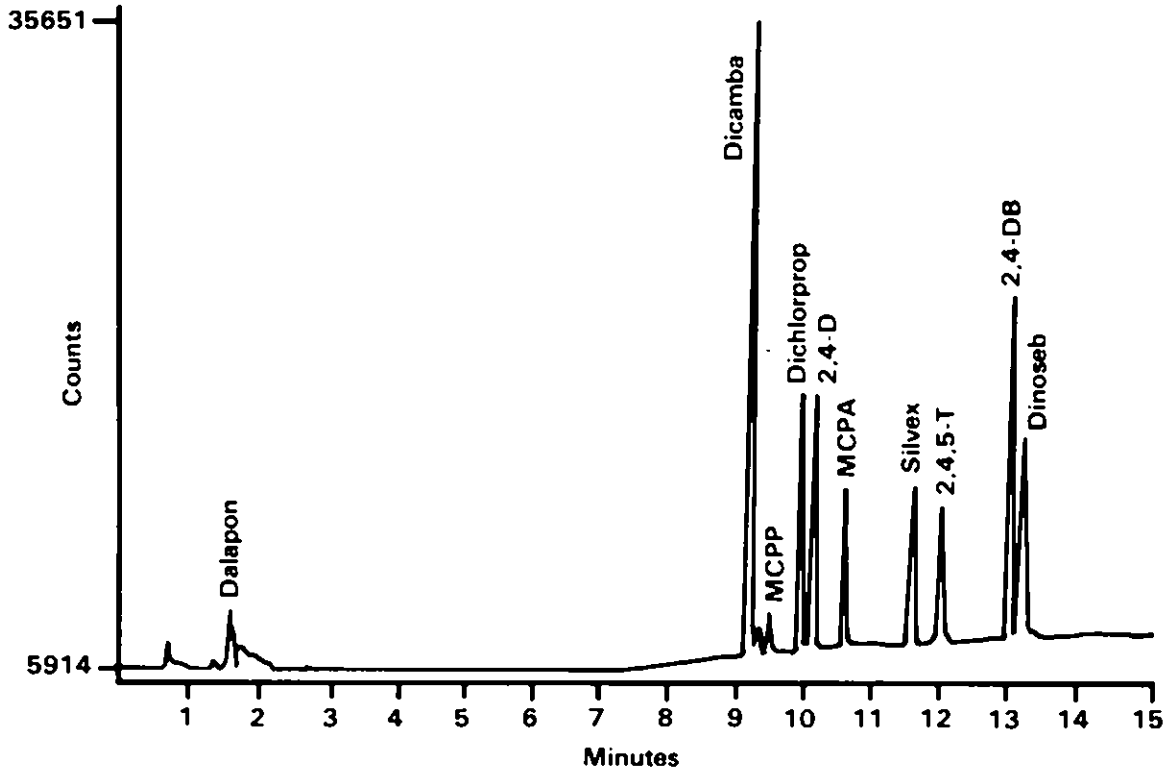
Instrumentation:	Perkin-Elmer, Sigma I E.C. Detector
Column:	DB-5 (J&W Scientific), 1 µm, 0.25mm x 30 meter
Injection:	1µL splitless autosampler
Injection Temp:	220°C
Detection Temp:	350°C
Oven Temp:	110°C for 3 min., 40°/min. to 200°C, hold for 16 min.
Attenuator:	6

Figure 3. Gas chromatogram of methylated herbicides (I).

TABLE 2. METHYL HERBICIDE STANDARD TEST MIXTURE ANALYSIS

COMPOUND	CONCENTRATION ng/µL (as free Acid)	RETENTION TIME (MIN)	% RSD n = 4
Dalapon	1.10	2.46	3.75%
Dicamba	0.83	8.42	5.93%
Dichlorprop	1.15	9.53	5.27%
2,4-D	1.20	9.84	6.77%
MCPA	16.8	10.57	4.03%
Silvex	0.19	12.19	7.32%
2,4,5-T	0.18	12.84	9.98%
2,4-DB	1.66	14.62	4.61%
Dinoseb	0.44	14.88	7.65%

Instrumentation:	Perkin-Elmer, Sigma I E.C. Detector
Column:	DB-5 (J & W Scientific), 1µm, 0.25mm x 30 meter
Injection:	1µL splitless autosampler
Injection Temp:	220°C
Detection Temp:	350°C
Oven Temp:	110°C for 3 min., 40°/min. to 200°C, hold for 16 min.
Attenuator:	6



Instrumentation:	TRACOR 540, E.C. Detector, IBM CS9000
Column:	DB-5 (J & W Scientific), 1μm, 0.25mm x 15 meter
Injection:	5μL splitless autosampler
Injection Temp:	220°C
Detection Temp:	375°C
Oven Temp:	50°C for 1 min., 25°C/min. to 100°C, hold for 1 min., 12°C/min. to 220°C, hold for 12 min.

Figure 4. Gas chromatogram of methylated herbicides (II).

Channel. . . . . .RECALC Time:16:10:51 Date:MON 14 MAY 84
Sample name. . . . . .HERBICIDES
Data file. DEMO:PANHB001
Method name. . . . . .PAN

Author . . . . . .ST PAN
Instrument . . . . . .TRACOR 540
Column 15M DB5
Notes. . . . . .

Run time . . . . . .16.00 min. Delay time. . .0.00 min.
Acq. time. . . . . .15:45:20 Acq. date . . .MON 14 MAY 84
Start PW . . . . . .10.00 sec.. End PW. . . . .10.00 sec.
Slope sens . . . . . .1.00 uv/sec.

Area reject. 500
peaks found. 32

AREA PERCENT REPORT

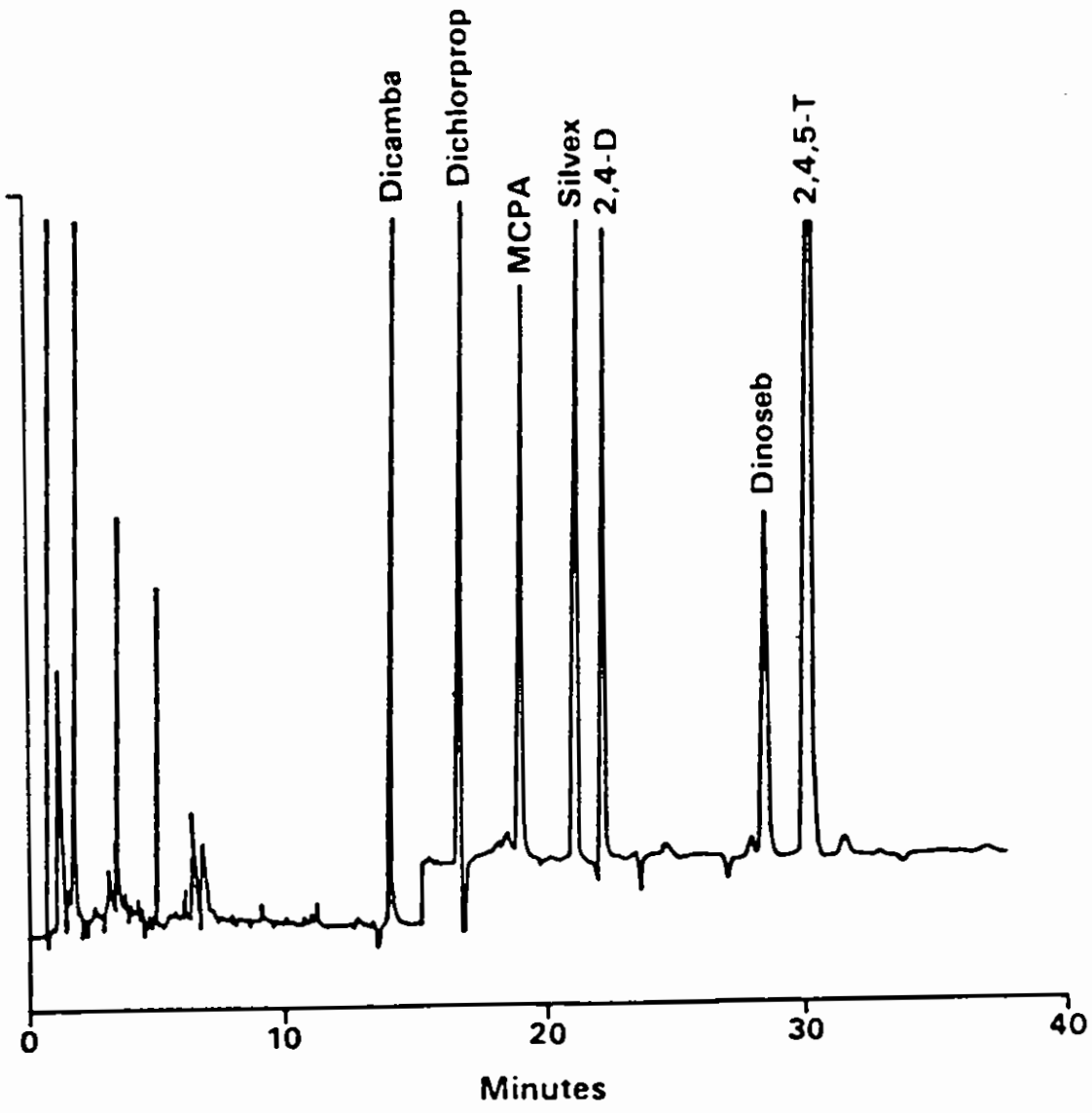
Peak	R.T. (min)	R/S	Peak name	Area %	Area	Peak Ht.	BL
1	0.697			1.907	6972	884	BV
2	0.929			0.878	3210	303	VB
3	1.339			0.438	1601	387	BV
4	1.561		DALAPON	3.282	11999	2424	VV
5	1.710			3.843	14051	808	VE
6	2.602			0.371	1358	299	BB
7	8.862			0.250	913	118	BV
8	9.388		DICAMBA	22.505	82279	25587	VV
9	9.558			1.311	4792	1229	VV
10	9.700		MCPP	1.867	6826	1715	VE
11	10.042			0.334	1221	133	EV
12	10.175		Dichlorprop	8.647	31614	8883	VV
13	10.384		2,4-D	9.214	33686	9455	VV
14	10.855		MCPA	6.312	23076	6523	VV
15	11.247			0.293	1072	155	VV

Figure 5. Analysis report of methylated herbicide gas chromatography (see Figure 4 for conditions).

AREA PERCENT REPORT

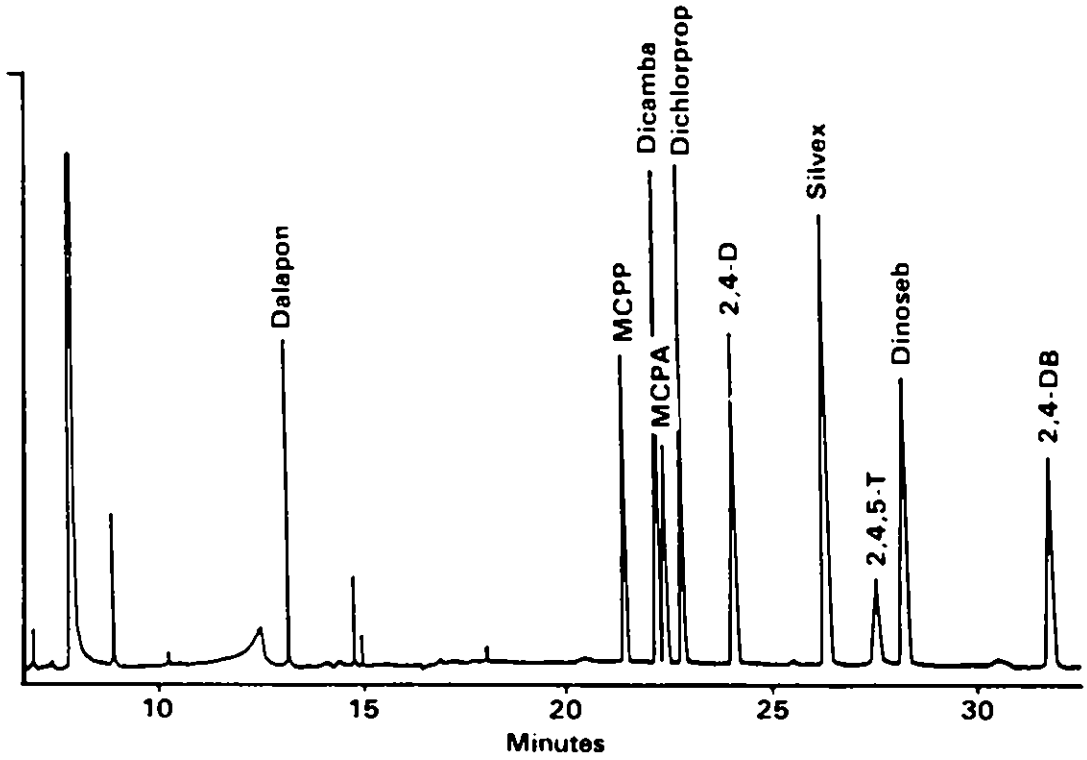
Peak	R.T. (min)	R/S	Peak name	Area %	Area	Peak Ht.	BL
16	11.545			0.248	906	147	VV
17	11.732			0.409	1495	334	VV
18	11.855		SILVEX	6.049	22115	5796	VV
19	12.249		2,4,5-T	5.634	20598	5155	VB
20	13.281		2,4-DB	16.499	60321	13717	BV
21	13.452		DINOSEB	9.403	34377	7713	VB
22	14.534			0.150	547	87	BB
23	15.750			0.159	580	36	VB
TOTALS				100.000	365609		

FIGURE 5. (continued)



Instrumentation:	Perkin-Elmer, Sigma I E.C. Detector
Column:	Supercowax 0.25µL, 0.25mm x 12 meter
Injection:	1µL splitless autosampler
Injection Temp:	220°C
Detection Temp:	350°C
Oven Temp:	60°C for 1 min., 15°/min. to 170°C, 1°/min. to 185°, hold for 15 min.

Figure 6. Gas chromatogram of methylated herbicides (III).



Instrumentation:	Perkin-Elmer, Sigma I E.C. Detector
Column:	DB-5 (J&W Scientific), 1µm 0.25mm, 30 meter
Injection:	1µL splitless autosampler
Injection Temp:	220°C
Detection Temp:	350°C
Oven Temp:	70°C for 1 min., 10°/min. to 240°C, hold for 17 min.
Attenuator:	2

Figure 7. Gas chromatogram of pentafluorobenzylated herbicides.

TABLE 3. RECOVERIES (%) OF HERBICIDES FROM SELECTED MATRICES USING METHOD 8150 AND CAPILLARY GAS CHROMATOGRAPHY WITH A DB-5 COLUMN* (SINGLE DETERMINATION, METHYL DERIVATIVES)

	MATRIX				
Compounds	Solvent Spike	Desert Soil	Kaolin Clay (Type C) (Calcined at 2000°C)	Kaolin Clay (Type P) (Pure Mineral)	Spike (ppb)
Dicamba	103	80+	100	91	164
MCPP	114	**	144**	98	4099
Dichlorprop	109	90	102	102	248
2,4-D	94	90	97	83	252
MCPA	122	80	103	101	3890
Silvex	105	70	121	90	39
2,4,5-T	98	110	97	94	42
2,4-DB	95	100	900	80	774
Dinoseb	0	<5	0	0	99

* Method 8150 with capillary GC/EC as shown in Figure 3. Peak height comparisons.

** Coelution of contaminants.

CLAY (TYPE P)
/ATIVES)

Compounds	Concentration (ppb) 1	2	3	4	Median (ppb)	Mean (ppb) (Range)	Standard Deviation	95% Confidence level	Spike (ppb)	Percent Recovery
Dicamba	154.9	149.8	150.4	156.9	152.7	153.0±1.89%	3.4	5.4	200.8	76.2
MCPP	3603.0	3603.0	3077.1	3902.4	3603.0	3549.4±6.57%	343.3	545.8	4749.6	74.7
Dichlorprop	169.2	169.0	173.3	178.3	171.3	172.4±1.94%	4.4	7.0	214.6	80.4
2,4-D	111.6	106.3	118.8	118.1	114.9	113.7±4.18%	5.9	9.4	182.8	62.2
Silvex	26.06	24.47	26.66	27.06	26.36	26.06±3.07%	1.14	1.8	38.62	67.5
2,4,5-T	22.21	16.89	20.07	21.34	20.71	20.11±8.39%	2.37	3.8	41.18	48.5

TABLE 5. ANALYSIS OF SPIKED HERBICIDES IN KAOLIN CLAY (TYPE P) USING PEAK AREA COMPARISON* (METHYL DERIVATIVES)

Compounds	Concentration (ppb) 1	2	3	4	Median (ppb)	Mean (ppb) (Range)	Standard Deviation	95% Confidence Level	Spike (ppb)	Percent Recovery
Dicamba	153.0	150.9	153.1	155.6	153.1	153.2+0.82%	1.9	3.0	200.8	76.2
MCPP	3796.4	3818.6	4123.8	4067.5	3943.1	3996.4+3.61%	133.3	211.9	4749.6	84.1
Dichlorprop	158.7	159.6	169.8	168.2	163.9	164.1+3.00%	5.7	9.1	214.6	76.4
2,4-D	122.1	112.5	125.6	122.9	122.5	120.8+3.41%	5.7	9.1	182.8	66.1
Silvex	24.66	23.20	25.70	25.77	25.18	24.83+3.63%	1.2	1.9	38.62	63.9
2,4,5-T	20.35	16.94	20.81	20.83	20.58	19.74+7.05%	1.9	3.0	41.48	47.6
2,4-DB	780.1	655.1	765.6	685.4	725.5	721.5+7.11%	60.8	96.7	821.0	87.9
Dinoseb	20.2	13.4	18.7	18.9	18.8	17.8+12.36%	3.0	4.8	103.3	17.3

*Analysis as in Table 4 using peak areas measured by the IBM CS9000.

TABLE 6. ANALYSIS OF FRESHLY SPIKED HERBICIDES IN KAOLIN CLAY (TYPE P)* (METHYL DERIVATIVES)

Compounds	Concentration (ppb) 1	2	3	4	Median (ppb)	Mean (ppb) (Range)	Standard Deviation	95% Confidence Level	Spike (ppb)	Recovery Percent
2,4-D	454.2	451.0	397.4	431.9	441.5	433.6±4.38%	26.1	41.5	601.6	72.1
Silvex	369.6	391.0	379.9	384.1	382.0	381.1±1.68%	9.0	14.3	488.3	78.1
2,4,5-T	359.1	355.8	337.6	346.4	351.1	349.7±2.21%	9.7	15.4	496.5	70.4

*See Table 4 for conditions of Analysis

TABLE 7. ANALYSIS OF SPIKED HERBICIDES IN KAOLIN CLAY (TYPE P) FOLLOWING 7 DAYS STORAGE AT -38°C (METHYL DERIVATIVES)

Compounds	Concentration (ppb) 1	2	3	4	Median (ppb)	Mean (ppb) (Range)	Standard Deviation	95% Confidence Level	Spike (ppb)	Percent Recovery
2,4-D	486.6	434.5	466.5	453.8	460.2	460.3±3.52%	21.9	34.8	601.6	76.5
Silvex	398.8	416.8	404.2	384.8	401.5	401.1±2.33%	13.3	21.1	488.3	82.2
2,4,5-T	410.7	341.9	367.1	360.5	363.8	370.1±5.50%	29.1	46.3	496.5	74.5

*See Table 4 for conditions of analysis.

TABLE 8. RECOVERIES (%) OF SPIKED HERBICIDES FROM KAOLIN CLAY (TYPE P) USING SONICATION WITH METHYLENE CHLORIDE AT SELECTED pH VALUES* (SINGLE DETERMINATION, METHYL DERIVATIVES)

Herbicide Compounds	pH 7	pH 3	pH 1	Spike ppb
Dicamba	15	33	59	201
MCPP	36	60	**	4600
Dichlorprop	14	19	98	215
2,4-D	24	40	30	183
Silvex	35	42	61	39
2,4,5-T	8	15	31	41
2,4-DB	51	58	57	821
Dinoseb	62	46	45	103

* A 5.0g sample + 5.0 mL of phosphate buffer (or dil. HCl for pH=1) plus 10-15g of anhydrous Na_2SO_4, mixed by spatula. Sonicated 3 times with 60 mL of methylene chloride for 3 minutes each. Combined extracts concentrated and exchanged to hexane using a K.D. apparatus with a final reduction to 2.0 mL by dry nitrogen stream. Derivatized by diazomethane and GC/EC analysis using the DB-5 column (see Figure 3 for conditions). Calculations based on area integration using the IBM CS9000.

** Coelution of contaminant.

TABLE 9. RECOVERIES OF SPIKED HERBICIDES FROM KAOLIN CLAY (TYPE P) USING SONICATION AT pH 7 WITH SELECTED SOLVENTS* (SINGLE DETERMINATION, METHYL DERIVATIVES)

Herbicide Compounds	Spike ppb	Solvent		
		CH_2Cl_2	1:1 CH_2Cl_2/hexane	1:1 acetone/hexane
Dicamba	201	76	80	11
MCPP	4750	<10	<10	<10
Dichlorprop	215	79	86	43
2,4-D	183	64	64	<10
MCPA	4758	80	73	46
Silvex	39	84	87	38
2,4,5-T	41	64	69	**
2,4-DB	821	93	103	86
Dinoseb	103	86	80	82

* See Table 8 footnote for description of the method. This study was improved by using a larger amount of anhydrous Na_2SO_4 (25g).

** Coelution of contaminant.

TABLE 10. RECOVERIES OF SPIKED HERBICIDES FROM KAOLIN CLAY (TYPE P) USING SELECTED EXTRACTION TECHNIQUES* AND PENTAFLUOROBENZYLATION** (SINGLE DETERMINATION)

Herbicide Compound	Modified Method 8150 Spike ppb	Modified Method 8150 Recovery(%)	Sonication Sample Spike ppb	Sonication CH_2Cl_2 pH7	Sonication 1:1 CH_2Cl_2/ acetone pH7	Sonication 1:1 hexane/ acetone pH7
Dalapon	170	97	170	92	80	100
Dicamba	161	57	201	86	98	85
MCPP	476	99	4750	91	101	98
Dichlorprop	258	--	215	98	96	99
2,4-D	219	75	183	30	18	10
MCPA	633	78	4758	95	10	103
Silvex	39	--	39	73	95	73
2,4,5-T	41	80	41	--	--	--
2,4-DB	821	--	821	42	91	104
Dinoseb	124	--	103	66	85	102

* Method 8150 was modified as described in Table 4. Sonication conditions were the same as those in the Table 9 experiment.

** Following extraction, the solvent was exchanged to acetone (4mL) using a stream of dry nitrogen. Aqueous 30% K_2CO_3 (30 µL) and 20 µL of pentafluorobenzyl bromide were added. The tube was sealed with Teflon tape and a screw cap and was heated in a water bath at 60°C for 3 hours. The volume of the cooled tube was reduced to about 0.5 mL using a stream of dry nitrogen and 2 mL of hexane was then added. The solution was then reduced just to dryness under a stream of dry nitrogen and redissolved in 2 mL of toluene/hexane (1:9). This solution was chromatographed on 5% water-deactivated silica topped with anhydrous Na_2SO_4. Elution of the analytes was done with 75:25 toluene/hexane. Analysis was done by GC/EC using the Tracor 540 gas chromatograph and IBM CS9000 peak areas with a DB-5 (J & W Scientific) 1 µm, 0.25 mmx 15M column.

CONCLUSIONS

1. Capillary GC/EC on DB-5 (J & W Scientific) provides superior chromatographic resolution and requires less time than the packed column GC/EC described in Method 8150.

2. Kaolin Clay (Type P) is a clean and generally nonretentive matrix suitable for optimization of spiked sample analysis.

3. At the concentrations tested, Method 8150 gives very low (or 0) recovery of Dinoseb. Even a spiked solvent blank gave 0 recovery. This is probably a pH problem. (See Conclusion #6 for a solution to this problem.)

4. Variable results were found for Method 8150 analysis for Dalapon. This acid is highly water soluble, highly acidic, and the methyl ester is quite volatile.

5. Better recoveries were obtained for analytes tested at higher concentrations (2,4-D, 2,4,5-T and Silvex).

   This suggests the recoveries of Method 8150 will be limited by the sample preparation or analyte level and not the GC determination.

6. Extraction with methylene chloride using sonication is promising as a replacement for the ether extraction of Method 8150. The procedure is simpler and uses less solvent. The only acceptable recovery of Dinoseb was obtained using this method.

7. Derivatization with pentafluorobenzyl bromide is a promising alternative to methylation. All analytes can be derivatized. The capillary GC on DB-5 (J & W Scientific) gives superior sensitivity and resolution compared to that of the methyl derivatives. The enhanced sensitivity is particularly important for MCPA and MCPP. The methyl esters of these analytes give only about 1/1000th the response of the other methylated analytes in GC/EC. The pentafluorobenzyl derivatives of all the analytes give similar responses in GC/EC. This method requires more development to verify stability of the derivative and GC separation from possible interferences. The derivatization time of three hours does affect sample through-put.

RECOMMENDATIONS

Sonication extraction with an organic solvent and capillary GC/EC promises to yield an improved method for free acid herbicide analysis. This method would be much easier to optimize for all analytes and promises to give better sensitivity than the involved extraction and packed column GC of Method 8150. The pentafluorobenzylation procedure is an attractive alternative to methylation with probably lower detection limits.

SECTION 5

RUGGEDNESS TESTS

INTRODUCTION

A preliminary ruggedness test prior to simplex optimization is useful to define the variables to be optimized by simplex. Ruggedness testing does not result in optimization but can narrow the choices of conditions.

The ruggedness test design of W. J. Youden[34] was used to test seven variable conditions with only eight determinations by using two levels of each variable (designated by a capital and lower case letter) which are distributed as shown in Table 11. To determine the effect of changing experimental condition 1 from A to a, the average analysis results of samples 5 through 8 are subtracted from the average analysis results for samples 1 through 4 yielding a ruggedness difference which indicates the importance of varying condition 1. The importance of condition 2, changing from B to b, is given by the 1, 2, 5, and 6 average results minus the 3, 4, 7, and 8 average results.

TABLE 11. DESIGN FOR TEST OF EXPERIMENTAL CONDITIONS

Experimental Condition	Values of Conditions in Determination Number							
	1	2	3	4	5	6	7	8
1	A	A	A	A	a	a	a	a
2	B	B	b	b	B	B	b	b
3	C	c	C	c	C	c	C	c
4	D	D	d	d	d	d	D	D
5	E	e	E	e	e	E	e	E
6	F	f	f	F	F	f	f	F
7	G	g	g	G	g	G	G	g

The standard deviation of each test analysis mean subtraction difference per herbicide was calculated as 2/7 times the square root of the sum of the squares of the differences as suggested by Youden.[34]

RUGGEDNESS TESTING OF FREE ACID HERBICIDE EXTRACTION AND ANALYSIS

We decided to perform three ruggedness tests, in each case testing three sets of seven variable experimental conditions. In addition, ruggedness differences were calculated for each analyte per sample and per the mean of the samples. The experimental variables are shown in Table 12 for the general procedure diagrammed in Figure 8.

TABLE 12. EXPERIMENTAL VARIABLES AND ASSIGNED VALUES FOR HERBICIDE METHOD

Condition	No.	Letter	Value for Capital Letter	Value for Lower Case Letter
	Experiment 1			
pH of phosphate buffer added to clay	1	A,a	1.0	3.0
Acetone:hexane ratio in sonication	2	B,b	1:1	2:3
Analyte concentration	3	C,c	1X	3X
Beaker size used in sonication	4	D,d	250 mL	400 mL
Base extraction or acid wash of clay extract	5	E,e	Base Extraction	Acid Wash
Filter for clay extract	6	F,f	Celite Column	Whatman No. 2 filter paper
Solution for methylation	7	G,g	4 mL Hexane and 2 mL Isooctane	6 mL Hexane
	Experiment 2			
Volume of buffer or water added to clay	1	A,a	80 mL	50 mL
pH of buffer or water added to clay	2	B,b	5 (acetate buffer)	<1
Sonicator output setting	3	C,c	6	5
Sonication temperature	4	D,d	25°C	0°C
Solvent volume in sonication	5	E,e	100 mL	150 mL
Base extraction or acid wash of clay extract	6	F,f	+	-
Amount of CH_2N_2 (molar excess)	7	G,g	4X	2X

(continued)

TABLE 12. (continued)

Condition	No.	Letter	Value for Capital Letter	Value for Lower Case Letter
		Experiment 3		
pH of phosphate buffer added to clay	1	A,a	1.0	2.0
Volume of buffer added to clay	2	B,b	80 mL	100 mL
Extraction solvent	3	C,c	CH_2Cl_2	$CH_3OC(CH_3)_3$
Sonicator output setting	4	D,d	4	5
Base extraction or acid wash of clay extract	5	E,e	Base extraction	Acid wash
Methylation solution	6	F,f	10% CH_3OH	0% CH_3OH
Destruction of excess CH_2N_2	7	G,g	Silicic acid	Overnight evaporation

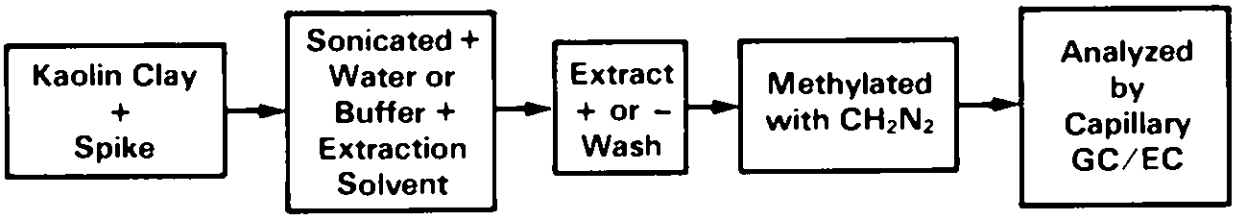


Figure 8. Block diagram of herbicide analysis.

Experimental

Experiment 1

Fifty grams of Ajax Type P clay (Westwood Ceramic Supply, City of Industry, California) was weighed into a beaker and spiked with 1 or 3 mL of the standard solution containing the 10 acid herbicides. The sample was mixed with 20 mL of phosphate buffer. The extraction solvent was added (100 mL) and the sample sonicated for 3 minutes in the pulsed mode at 50 % duty cycle and an output setting of 5. After allowing the clay to settle, the solvent was transferred into a 500-mL centrifuge bottle. The clay was sonicated two more times using the same conditions with 100 mL extraction solvent each time. The extract was combined into the centrifuge bottle, and centrifuged for 10 minutes to settle the fine particles. The extract was filtered into a 500-mL separatory funnel.

Half of the samples were extracted with base and half were washed with acid. For extracting with base, 100 mL of 0.1 N sodium hydroxide was added to the separatory funnel and was shaken for 2 minutes. The aqueous layer was transferred to a beaker and immediately was acidified with HCl to a pH of 1.0. The organic layer was extracted once more with 100 mL of 0.1 N NaOH. The aqueous layer was added to the first extract and the pH was readjusted to $\leq$1.0 if necessary. The organic layer was discarded (or saved for analyses of esters). The acid solution was extracted twice with 100 mL of methylene chloride. The final extract was concentrated to approximately 5 mL in a Kuderna-Danish (K-D) flask on a steam bath. The methylene chloride was evaporated with nitrogen prior to hexane-exchange and methylation.

For samples washed with acid, 100 mL of acidified water was added to the separatory funnel containing the soil extract and was shaken for 2 minutes. This process was then repeated. The organic layer was transferred to a 500-mL K-D flask and the combined aqueous layer was then extracted 3 times with 50 mL of methylene chloride. The methylene chloride was added to the K-D flask and concentrated to approximately 5 mL on a steam bath. The methylene chloride was exchanged with hexane prior to diazomethane methylation.

Experiment 2

One mL of the herbicide standard was added to 50 g of Ajax Type P clay. A given volume of acetate buffer (pH 5) or deionized water (sample pH to <1 by addition of con. HCl) was added and the sample was mixed completely. The sample was extracted 3 times by adding methylene chloride and sonicating in the pulsed mode at 50 % duty cycle, 3 minutes each, at the specified setting. The combined extract was washed with 100 mL of acidified water or with base.

For the base extract, the aqueous layer was acidified to pH <1 with concentrated HCl and was extracted 3 times with 100 mL of methylene chloride. The methylene chloride extracts were then combined. The methylene chloride solution containing the analytes was concentrated to about 5 mL for methylation with diazomethane.

Experiment 3

Fifty grams of Ajax P clay was weighed into a beaker and spiked with 1 mL of a standard solution containing the 10 acid herbicides, Dicamba, MCPP, MCPA, Dichlorprop, 2,4-D, Silvex, 2,4,5-T, 2,4-DB, Dinoseb, and Dalapon. The sample was mixed with either 80 or 100 mL of phosphate buffer at pH 1 or 2. The extraction solvent was added (100 mL) and the sample was sonicated for 3 minutes in the pulsed mode at 50 % duty cycle at an output setting of 4 or 5. After the clay was allowed to settle, the solvent was transferred into a 500 mL centrifuge bottle. The clay was sonicated two more times using the same conditions with 100 mL extraction solvent each time. The extracts were combined into the centrifuge bottle, and were centrifuged for 10 minutes to settle the fine particles. The extract was filtered through Whatman #1 filter paper into a 500-mL separatory funnel.

Half of the solvent extracts were extracted with base, and half were washed with acid. For extracting with base, 100 mL of 0.1 N sodium hydroxide was added to the solvent separatory funnel and was shaken for 2 minutes. The aqueous layer was transferred to a beaker and immediately was acidified with phosphoric acid to a pH of 1.0. The organic layer was extracted once more with 100 mL of 0.1 N NaOH. The aqueous layer was added to the first aqueous extract and the pH was readjusted to $\leq$1.0 if necessary. The organic layer was discarded. The acid solution was extracted twice with 100 mL of extraction solvent. The combined final extract was concentrated to approximately 5 mL in a 500-mL K-D concentrator on a steam bath.

For samples washed with acid, 100 mL of water acidified to pH $\leq$1.0 with H_3PO_4 was added to the separatory funnel containing the clay extract and was shaken for 2 minutes. This procedure was then repeated. The organic layer was transferred to a 500-mL K-D flask and the combined aqueous layer was then extracted 3 times with 50 mL of extraction solvent. This extract was added to the K-D flask and concentrated to approximately 5 mL on a steam bath.

The sample was prepared for methylation by evaporating the sample just to dryness, then reconstituting it with 1 mL of iso-octane, then diluting it to a volume of 5 mL with hexane. In addition, half of the samples required 0.5 mL of methanol to be added before the samples were diluted to 5.0 mL with hexane.

Common Procedures

The spiking solution (Table 13) and methylation procedures were used in all experiments.

TABLE 13. STANDARD SPIKING SOLUTION (ACETONE)

Analyte	Concentration (µg/mL)
Dalapon	10.9
Dicamba	7.33
Dichlorprop	14.0
2,4-D	12.0
MCPA	1,014
MCPP	1,026
Silvex	2.43
2,4,5-T	3.95
2,4-DB	40.6
Dinoseb	12.0

Methylation of the samples using diazomethane was carried out in the containment facility following the procedures described in Section 4. The excess of diazomethane was adjusted by adding the appropriate volume of diazomethane solution. The GC analysis conditions are listed in the Figure 4 caption except that assignments of GC peaks to analytes were confirmed by GC/MS. The correct assignments are shown in Figure 9 for an actual sample (number 4) from the ruggedness test experiment 1.

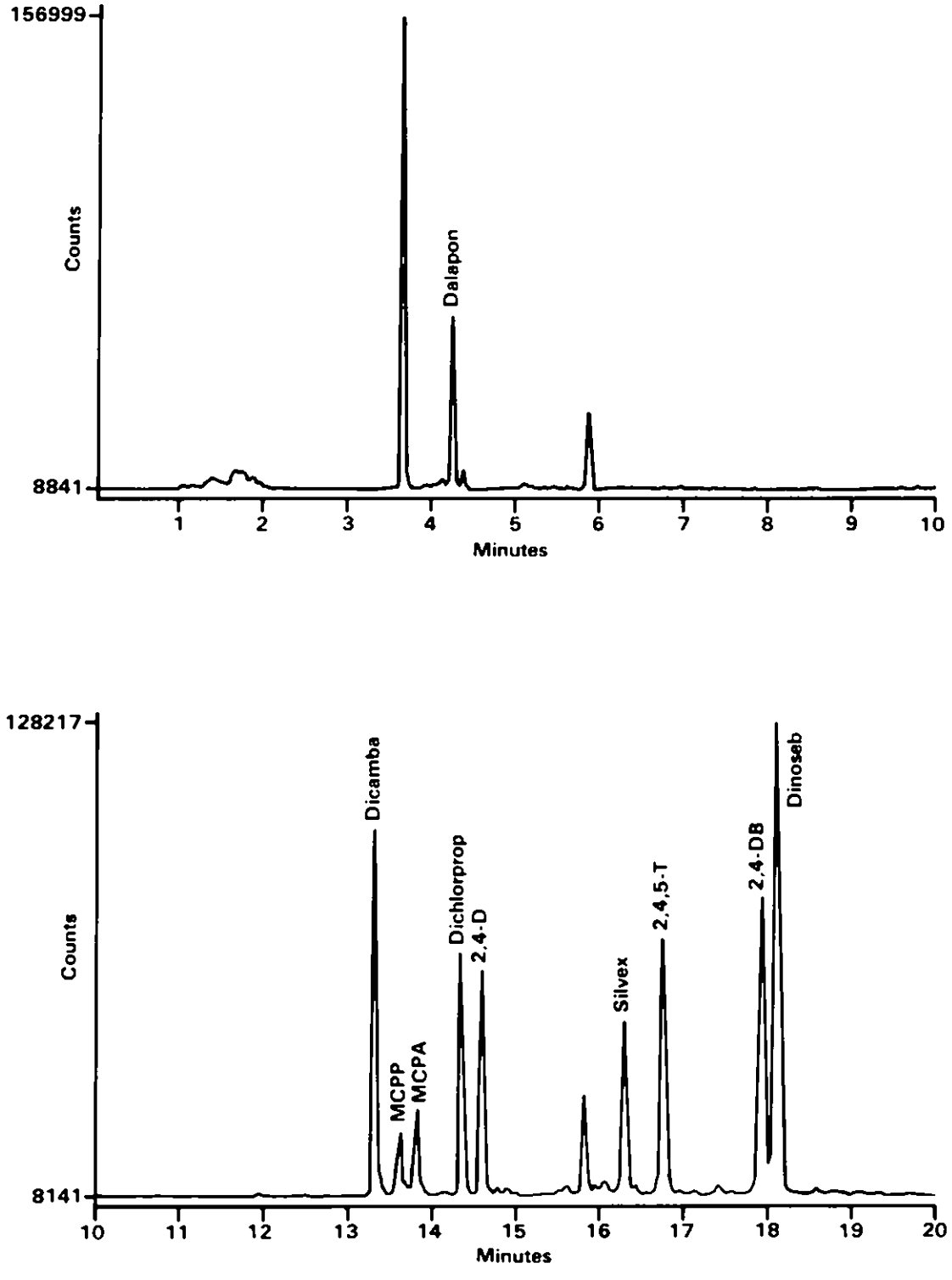


Figure 9. Gas chromatogram of methylated herbicides, sample 4, experiment 1.

Data and Results

The percent recoveries and differences in percent recoveries resulting from changes in the analytical conditions are shown in Tables 14 to 19. Note that the sign of the differences shown in Tables 15, 17, and 19 is important in that the higher (better value) is the condition value represented by the capital letter when the difference is positive and by the lower case letter when the difference is negative (see Table 12 for the condition descriptions).

TABLE 14. PERCENT RECOVERY OF HERBICIDES FOR EXPERIMENT 1 USING CONDITIONS OF TABLE 12

	Recovery (%) of Herbicides - Determination Number							
Herbicide	1	2	3	4	5	6	7	8
Dicamba	64	77	40	102	56	48	63	20
MCPP	75	92	49	122	71	61	84	37
MCPA	73	84	47	105	67	56	73	29
Dichlorprop	71	84	44	109	63	57	73	28
2,4-D	69	82	41	109	64	52	67	20
Silvex	74	88	43	115	74	64	89	28
2,4,5-T	65	84	43	112	64	56	76	21
2,4-DB	88	89	55	111	71	66	87	27
Dinoseb	44	87	40	108	75	40	83	38
Dalapon	0	64	0	84	39	0	32	0

TABLE 15. DIFFERENCES FOR HERBICIDE METHOD, EXPERIMENT 1

Analytical Condition	Dicamba	MCPP	MCPA	Dichlor-prop	2,4-D	Silvex	2,4,5-T	2,4-DB	Dinoseb	Dala-pon	$\bar{X}$(except Dalapon)	$\bar{X}^2$
pH of buffer added	24	22	21	22	24	16	22	23	11	19	21	441
Acetone: hexane ratio	5	2	6	5	8	6	4	9	-5	-3	4	16
Analyte concentration	-5	-8	-4	-7	-6	-4	-5	2	-7	-19	-5	25
Beaker size in sonication	-6	-4	-4	-4	-7	-4	-7	-3	-3	-7	-5	25
Base extraction or acid wash	-32	-36	-31	-33	-35	-40	-38	-31	-47	-55	-36	1296
Filtration method	4	4	4	3	5	2	1	0	3	7	3	9
Solution for methylation	21	24	20	23	22	28	24	27	9	3	22	484

Sum of $\bar{X}^2$ = 2296

2/7 x Sum of $\bar{X}^2$ = 656

Std. Dev. = $(2/7 \times \text{Sum of } \bar{X}^2)^{1/2}$ = 26

TABLE 16. PERCENT RECOVERY OF HERBICIDES FOR EXPERIMENT 2 USING CONDITIONS OF TABLE 12

Herbicide	Recovery (%) of Herbicides - Determination Number							
	1	2	3	4	5	6	7	8
Dicamba	14.0	16.8	97.8	100.3	7.0	16.1	63.0	90.8
MCPP	94.3	83.0	88.0	95.8	43.9	82.2	56.2	77.7
MCPA	84.2	79.6	98.0	103.0	47.8	79.7	68.8	91.3
Dichlorprop	96.8	88.5	111.6	117.6	45.3	78.1	76.7	100.6
2,4-D	72.3	82.2	108.3	116.1	46.4	81.0	72.3	100.9
Silvex	103.3	119.1	124.0	117.5	52.2	86.4	88.2	99.1
2,4,5-T	101.1	108.2	116.3	119.6	59.6	133.9	78.5	107.2
2,4-DB	99.8	57.2	118.5	119.0	63.0	88.1	89.5	98.1
Dinoseb	95.2	68.7	85.8	94.8	58.9	78.3	61.1	75.1

TABLE 17. DIFFERENCES FOR HERBICIDE METHOD, EXPERIMENT 2

Analytical Conditions	Dicamba	MCPP	MCPA	Dichlor-prop	2,4-D	Silvex	2,4,5-T	2,4-DB	Dinoseb	$\bar{X}$	$\bar{X}^2$
Amount of water added	13	25.3	19.3	28.4	19.5	34.5	16.5	13.9	17.7	20.9	437
pH of water	-7.5	-3.6	-17.5	-24.4	-28.9	-16.9	-4.7	-29.3	-3.9	-22.6	511
Sonicator setting	-10.5	-14.0	-13.7	-13.6	-20.3	-13.5	-28.3	2.1	-3.9	-12.9	166
Sonication temperature	-9.1	0.4	-1.1	-2.6	-6.1	7.3	-8.5	-14.1	-4.5	-6.0	36
Base extraction or acid wash	8.9	15.8	19.4	14.8	11.3	8.9	23.1	18.9	12.7	14.9	222
Amount of CH_2N_2	-4.7	8.9	4.7	5.8	0.9	0.3	10.3	14.9	10.3	5.7	32

Sum of $\bar{X}^2$ = 1404

2/7 x Sum of $\bar{X}^2$ = 401

Std. Dev. = $(2/7 \times \text{Sum of } \bar{X}^2)^{1/2}$ = 20

TABLE 18. PERCENT RECOVERY OF HERBICIDES FOR EXPERIMENT 3 USING CONDITIONS OF TABLE 12

	Recovery (%) of Herbicides - Determination Number							
Herbicide	1	2	3	4	5	6	7	8
Dalapon	39	71	21	45	15	61	9	61
Dicamba	89	75	76	42	78	78	73	75
MCPP	95	82	74	71	80	73	75	76
MCPA	88	79	80	66	79	81	78	73
Dichlorprop	94	81	86	64	84	81	84	74
2,4-D	88	80	76	60	74	79	78	73
Silvex	95	94	69	114	86	70	91	68
2,4,5-T	107	82	70	74	76	85	89	82
2,4-DB	124	88	75	121	100	80	84	102
Dinoseb	65	66	75	77	114	65	76	51

TABLE 19. DIFFERENCES FOR HERBICIDE METHOD, EXPERIMENT 3

Analytical Condition	Dicamba	MCPP	MCPA	Dichlor-prop	2,4-D	Silvex	2,4,5-T	2,4-DB	Dinoseb	Dala-pon	$\bar{X}$	$\bar{X}^2$
pH of buffer added	-5	5	0	0	0	14	0	10	-6	7	4.7	22.1
Volume of buffer	13	9	8	8	8	0	9	2	8	13	7.8	60.8
Extraction solvent	11	5	6	12	6	-2	5	-2	18	-39	10.6	112
Sonicator setting	9	7	3	4	8	2	14	6	-18	9	8.0	64
Base extraction or acid wash	13	3	5	6	6	-20	6	-3	-19	11	9.2	84.6
Methylation solution	-5	5	-3	-4	-4	-10	3	30	6	-1	7.1	50.4
Destruction of excess CH_2N_2	-5	1	0	0	0	14	11	11	-6	-3	5.1	26

Sum of $\bar{X}^2$ = 420

2/7 X sum of $\bar{X}^2$ = 120

Std. Dev. = (2/7 X sum of $\bar{X}^2$) 1/2 = 11

Experiment 2 was performed by one analyst and experiments 1 and 3 were performed by a second analyst. The pH of the buffer (acetate or phosphate) affected analyte recovery in the first two tests, with the lower pH yielding higher recoveries. When the pH range was decreased for testing by experiment 3 (pH 1 or 2), the pH effect became less important. Dicamba recovery was severely reduced at higher pH in experiment 2 (Tables 16 and 17).

The base extraction step (useful for separating esters from free acids) or alternative acid wash step had a different result in each test. In experiment 1, a dramatic reduction in the recoveries of all analytes on base extraction was observed with the acetone/hexane solvent (Table 15). In experiment 2 with methylene chloride as the solvent, base extraction improved the recoveries of all analytes (Table 17). In experiment 3, with methylene chloride or methyl t-butyl ether as the solvents, Silvex and Dinoseb had reduced recoveries on base extraction (Table 19). Solvent-dependent competing effects are operating in the base extraction step. This step is very useful if the extracting solvent is methylene chloride.

Addition of methanol to the methylation solution gives enhanced recoveries for most analytes as shown in Table 15. Table 19 shows that this effect can be very important for 2,4-DB.

Recovery of most herbicides is increased by adding iso-octane (a keeper) to the methylation solution as shown by experiment 1 (solution for methylation, Table 15) but it is interesting to note that the most volatile ester, Methyl Dalapon, shows an unexpectedly small effect. In addition, experiment 2 (Table 17) shows two additional sensitive conditions, the amount of water (or buffer) added to the clay and the sonicator setting. A general improvement in recoveries is found with more water and a lower sonicator setting.

The first two ruggedness tests failed for Dalapon. Some condition(s) resulted in zero recovery for at least some of the samples. Under the conditions of experiment 3, Dalapon was recovered in all samples. Dalapon is the most acidic analyte, the most water soluble, the most reactive with base, and the Dalapon methyl ester is the most volatile derivative.

Conclusions

The standard deviations obtained in the three ruggedness tests (26, 20, and 11%) indicate that the optimization was successful and that the final procedure will have an 11% or less standard deviation. This is acceptable for environmental analysis.

Methylene chloride seems to be the best solvent for extraction because recoveries are generally better and the sensitivity to base extraction (versus acid wash) is reduced. Also, the use of iso-octane and methanol in the methylation step is important.

Analytical conditions identified for simplex optimization are;

1. pH of buffer added to clay,

2. volume of buffer added to clay, and

3. sonicator setting.

RUGGEDNESS TESTING FOR HERBICIDE ESTER HYDROLYSIS AND ANALYSIS

A necessary step in the analysis of herbicides present in the ester form is hydrolysis to the free acid. As heating with base is a vigorous procedure, we decided to separate the free acids by base extraction of the methylene chloride extract and then hydrolyze the esters remaining in the methylene chloride.

Experimental, Herbicide Ester Hydrolysis Method

Six available herbicide esters, the iso-octyl esters of MCPP, MCPA, 2,4,5-T, and 2,4-DB, isobutyl ester of 2,4-D and propylene glycol butyl ether esters of Silvex were tested in this experiment. One mL of working standard solution containing various concentrations of the above esters was added to 25 mL of methylene chloride and transferred into a K-D flask. The specified amount of water, 27% KOH solution, and methanol were then added to the flask. The flask was fitted with a Snyder column, heated in a 60°-65°C water bath for the specified time, and then was removed from the water bath. After cooling, the reaction mixture was poured into a 250-mL separator funnel and acidified to pH <2 with either concentrated sulfuric acid or hydrochloric acid. The aqueous solution was extracted 3 or 4 times with 25-mL portions of methylene chloride. The combined extracts were transferred to a K-D flask to reduce the volume on the water bath to about 5 mL. To the extract, 1 mL of iso-octane and 1 mL of methanol were added, the volume was reduced to 4 mL by a stream of nitrogen, and then was methylated following the procedures described in Section 4.

The methylated herbicides mixture was transferred to a 10-mL volumetric flask. One mL of p-dichlorobenzene solution was added as internal standard, and the total volume was brought to 10 mL by hexane. The resulting solution was analyzed by GC/EC.

Standard solution concentrations are shown in Table 20, and the conditions altered and their assigned values are shown in Table 21.

TABLE 20. CONCENTRATION OF STANDARDS USED IN ESTER HYDROLYSIS EXPERIMENT

Compound	Concentration (ng/µL)
MCPP, IOE*	2280
MCPA, IOE	1340
2,4-D, IBE**	15.2
Silvex, PGBEE***	6.8
2,4,5-T, IOE	7.5
2,4-DB, IOE	59.5
p-dichlorobenzene	124

* IOE is the iso-octyl ester.

** IBE is the isobutyl ester.

*** PGBEE is the propylene glycol butyl ether ester.

Data and Results

The percent recoveries from the ruggedness test for herbicide ester hydrolysis and analysis are shown in Table 22. The differences for the experiment are shown in Table 23. Table 23 shows that the concentration of methanol is the only variable that requires optimization.

TABLE 21. CONDITIONS ALTERED AND ASSIGNED VALUES FOR HERBICIDE HYDROLYSIS METHOD

Condition	No.	Letter	Value for Capital Letter	Value for Lower Case Letter
Vol. of water and 37% KOH soln. added for hydrolysis	1	A,a	30 mL + 5 mL	34 mL + 1 mL
Vol. of methanol added	2	B,b	30 mL	10 mL
Reaction time	3	C,c	120 min.	90 min.
K-D flask size	4	D,d	500 mL	250 mL
No. of times extracted	5	E,e	4	3
Boiling chips added during hydrolysis	6	F,f	3	1
Acid used to acidify the soln.	7	G,g	H_2SO_4	HCl

TABLE 22. PERCENT RECOVERY OF HERBICIDES FROM HERBICIDE ESTER HYDROLYSIS METHOD

Herbicide	1	2	3	4	5	6	7	8
MCPP	80	44	8.6	15	60	59	7.7	4.6
MCPA	121	110	77	33	108	121	43	52
2,4-D	83	91	73	33	76	103	20	29
Silvex	71	34	24	7.7	58	62	7.1	8.6
2,4,5-T	76	71	49	16	49	64	19	25
2,4-DB	37	4.9	3.9	2.9	37	29	3.2	2.0

TABLE 23. DIFFERENCES FOR HERBICIDE ESTER HYDROLYSIS METHOD

Analytical Condition	MCPP	MCPA	2,4-D	Silvex	2,4,5-T	2,4-DB	$\bar{X}$
Vol. of H_2O + KOH soln	4.1	4.3	13	0.3	10.7	-5.8	4.4
Vol. of Methanol	40.8	63.7	49.5	44.5	37.7	24	43.3
Reaction Time	7.4	8.3	-1	11.9	4.3	10.6	6.9
K-D flask size	-1.6	-3.3	-16.5	-7.7	3.3	6.4	-3.2
No. of times extracted	6.4	19.3	17	14.7	14.7	6	13
Boiling chips added	10.1	-9.3	-16.5	4.5	9.3	8.4	1.1
H_2SO_4 or HCl	11.1	-17.3	-7.5	6	4.7	6	0.5

RUGGEDNESS TESTING OF OPTIMIZED HERBICIDE EXTRACTION AND ANALYSIS

Introduction

The simplex optimization results (Section 6) and ruggedness tests suggest that a buffer pH range of 1 to 2.5, buffer volume of 80 to 90 mL and sonicator power setting of 5 to 7 should give the best results. These results were tested as described previously with only these variables. The experimental design is shown in Table 24. The design allows duplicate values to be obtained for each condition. For example, the effect of condition 1 is revealed by subtracting the mean results of determinations 3 and 4 from the mean results of determinations 1 and 2. These subtractions are hereafter designated as "differences." The conditions altered are shown in Table 25.

TABLE 24. DESIGN FOR TEST OF EXPERIMENTAL CONDITIONS, EXPERIMENT 4

Experimental Conditions	Values of Conditions in Determination Number			
	1	2	3	4
1	A	A	a	a
2	B	b	B	b
3	C	c	c	C

TABLE 25. EXPERIMENTAL VARIABLES AND ASSIGNED VALUES FOR HERBICIDE METHOD, EXPERIMENT 4

Condition	No.	Letter	Value for Capital Letter	Value for Lower Case Letter
pH of buffer added to sample	1	A,a	1.0	2.5
Sonicator output setting	2	B,b	5	7
Volume of buffer added to sample	3	C,c	80	90

Conditions for Experiment 4

The experimental conditions of experiment 3, Section 5, were used with the conditions listed in Table 25, which were varied as indicated. The solvent for extraction was methylene chloride, the extract was washed with base, the methylation solution contained 10% methanol, and the excess diazomethane was removed by overnight evaporation.

Data and Results for Experiment 4

The percent recoveries from the ruggedness test for the optimized method of chlorinated herbicide analysis are shown in Table 26. The mean of all the recoveries is 89.3% with a 4.3% RSD.

The table of differences is shown in Table 27. No important difference was revealed.

TABLE 26. PERCENT RECOVERY OF HERBICIDES FOR EXPERIMENT 4 USING CONDITIONS OF TABLE 25

	Recovery of Herbicides, Determination Number			
Herbicide	1	2	3	4
Dicamba	88	87	89	96
MCPP	122	97	100	109
MCPA	127	103	107	114
Dichlorprop	88	91	94	102
2,4-D	92	97	97	109
Silvex	60	65	63	71
2,4,5-T	69	76	75	86
2,4-DB	86	94	100	110
Dinoseb	58	72	58	62
Mean	88	87	87	95

TABLE 27. DIFFERENCES FOR HERBICIDE METHOD, EXPERIMENT 4

Condition	Dicamba	MCPP	MCPA	Dichlor-prop	2,4-D	Silvex	2,4,5-T	2,4-DB	Dinoseb	$\bar{X}$*
pH of buffer added	-4	5	4	-8	-8	-5	-8	-15	5	7.0
Sonicator setting	-4	8	9	-6	-9	-7	-9	-9	-9	7.8
Volume of buffer	4	17	16	3	4	2	2	1	-5	6.0

* mean of differences.

CONCLUSIONS

The results of ruggedness testing have shown that the extraction and analysis of free acid herbicides requires the simultaneous optimization of three variables: the pH and the volume of buffer added to the sample, and the power setting of the sonicator. All of these parameters are involved in the extraction process. The ruggedness testing for ester hydrolysis and analysis indicated that the methanol concentration was the only variable requiring optimization. Optimization of ester hydrolysis was carried out with a series of experiments that varied the methanol concentration. Optimization of the extraction and analysis of the free acid herbicides requires simplex optimization so that all the variables can be optimized simultaneously. The final ruggedness test on the optimized experiment, experiment 4, indicated that the procedure is rugged for the range of conditions tested with a mean recovery of 89.3 % and a standard deviation of 4.3%.

SECTION 6

OPTIMIZATION OF ANALYTICAL PROCEDURES

INTRODUCTION

The ruggedness testing of chlorinated herbicide ester hydrolysis and analysis showed that optimization requires only the variation in the amount of methanol added. A series of experiments giving the mean herbicide recovery as a function of the methanol concentration should yield an optimum at maximum mean recovery.

The optimization of the free acid herbicide extraction and analysis using simplex optimization to give the highest mean recovery should give the best values for the important conditions revealed by ruggedness testing, (buffer pH, buffer volume, and sonicator power).

SIMPLEX OPTIMIZATION OF CHLORINATED HERBICIDES

Introduction

Simplex optimization is a statistical process whereby numerous experimental parameters, which are previously identified (for example, by ruggedness testing), are systematically altered to achieve an optimum. An optimum, in this case, is defined as the highest possible mean recovery of analytes. Simplex optimization has been recommended[5,6,35,36] to optimize analysis methods. The work on optimization of the J-Acid Method for determination of formaldehyde[37] and the short review by Dols and Armbrecht[38] on simplex optimization as a step in method development are examples of practical applications of the technique. As these workers suggest, the simplex can rapidly move toward the optimum and should be done early in method evaluation.

Three parameters in the extraction of chlorinated herbicides from clay were optimized simultaneously by simplex optimization using a fixed size simplex. The variables chosen were the pH of the initial clay buffer, the volume of the initial buffer, and the sonicator output setting. An optimum for these three variables was achieved with a high percent recovery of 9 of the 10 herbicide analytes. This information was useful in drafting a new procedure.

Experimental

Initially, four samples were run. The initial sets of experimental values for each sample (vertices) were selected at values that were estimated from consideration of previous studies to give near optimum results. Table 28 displays the range of values that each variable could assume. The calculations involved in simplex optimization require that the value of each variable be expressed as a percentage. The following formula is used for the pH buffer: pH = 14 - (% x 13). Table 29 gives the initial vertices values.

TABLE 28. EXPERIMENTAL VALUE RANGES FOR SIMPLEX OPTIMIZATION

Variable	Low Value (0%)	High Value (100%)
Buffer pH	14	1
Buffer volume (mL)	20	120
Sonicator output	0	7

TABLE 29. INITIAL VERTICES (PERCENT) FOR SIMPLEX OPTIMIZATION

Sample #	pH	Buffer Volume	Sonicator Setting
1	81	50	75
2	100	50	75
3	100	25	75
4	100	50	50

The initial four samples were extracted and analyzed as described in experiment 3, Section 5, (page 35) and the mean percent recovery of all analytes except Dalapon was determined. The recovery of Dalapon was found to be consistently poor (<5%) in all experiments.

The simplex optimization worksheet (Figure 10) was used to calculate a new vertex for each subsequent experiment, with new values then assigned to the variables. The new sample was extracted and analyzed in the same manner as the previous samples, and another simplex optimization worksheet was computed. These results determined the values selected for the next set of variables. Repetition of experiments, with new values used for the variables each time, continued until optimization was achieved. Progress of the simplex optimization was followed by monitoring the mean percent recoveries of the four mean analysis results for each sample as shown in Table 30.

TABLE 30. SIMPLEX OPTIMIZATION PROGRESS

Simplex No.	Mean Recovery (%)	% RSD
1	87.4	5.35
2	88.6	4.71
3	92.4	5.38

Results and Discussion

The results of the first simplex optimization experiment are shown in Table 31. When the first simplex optimization worksheet was prepared, both vertices 3 and 4 yielded, within experimental error, the same recovery. Therefore, the next experiment was performed twice, once with sample 3 considered to be the worst vertex and once with sample 4 considered to be the worst. This created a vertex with two different parameter conditions, vertex 5A and vertex 5B.

A mean recovery of 64.2% was obtained for vertex 5B (vertex 3 defined as the worst), while a preferred mean recovery of 86.8% was obtained for vertex 5A. Therefore, vertex 5B was discarded, and the next experiment was performed with vertex 5A in the worksheet. This experiment resulted in a mean percent recovery of 98.9%. Table 32 shows the mean percent recovery obtained for each experiment of the simplex optimization and the conditions used for each experiment. A 98.9% recovery was deemed adequate and no additional experiments were performed. Also, the four vertices of the last simplex gave a mean recovery of 92.4% with a 5.38% RSD.

Conclusions

The simplex quickly located the optimum recovery value where a buffer pH of 2.5, buffer volume of 86 mL, and sonicator power of 6.3 gave a mean recovery of 98.9% with 7.0% RSD.

	Factor Levels						
Simplex Number	pH of Buffer X_1	Volume of Buffer (mL) X_2	Sonicator Power X_3	Mean Recovery Response	Rank	Vertex Number	Times Retained
Coordinates of Retained Vertices Σ					B - W		
$P = \Sigma/3$							
W					W		
P - W							
$R = P + (P-W)$					R		

where:

Σ = summation of coordinates.

P = centroid of face (the face is that part of a simplex that remains after one of the defining points is removed).

W = worst vertex.

R = reflection vertex.

B = best vertex.

N = next to the worst vertex.

X_n = values of factor n.

Figure 10. Three-factor sequential simplex worksheet for herbicide method optimization.

TABLE 31. RESULTS FOR INITIAL SIMPLEX VERTICES

Herbicide Compound	Vertices (% Recoveries) 1	2	3	4
Dicamba	85.4	49.2	77.4	76.8
MCPP	101	102	92.1	96.9
MCPA	96.1	99.1	89.4	92.4
Dichlorprop	95.2	101	87.4	93.3
2,4-D	91.2	90.3	83.2	80.9
Silvex	85.7	92.8	78.0	82.5
2,4,5-T	80.9	92.3	84.3	74.0
2,4-DB	107	105	89.0	94.3
Dinoseb	89.6	93.5	74.5	48.0
Mean (% RSD)	92.4 (8.9)	91.7 (18.2)	83.6 (7.3)	82.1 (18.6)

TABLE 32. CONDITIONS AND RESULTS FOR EACH VERTEX IN SIMPLEX OPTIMIZATION

Simplex Vertex #	pH of Buffer	Volume of Buffer (mL)	Sonicator Power	Herbicide Mean Percent Recovery (% RSD)
1	1.0	70	5.25	92.4 (8.9)
2	3.5	70	5.25	91.7 (18.2)
3	1.0	70	3.5	83.6 (7.3)
4	1.0	55	5.25	82.1 (18.6)
5A	2.6	75	4.1	86.8 (13.6)
5B	2.6	54	3.5	64.2 (24.5)
6	2.5	86	6.3	98.9 (7.0)

ESTER HYDROLYSIS OPTIMIZATION

The results of ruggedness testing, Section 5, showed that the addition of methanol to the hydrolysis mixture was an important way to increase recoveries. Because methanol was the only experimental variable to be optimized, simplex optimization was not required.

Experimental for Herbicide Ester Hydrolysis

Six available herbicide esters, iso-octyl esters of MCPP, MCPA, 2,4,5-T, and 2,4-DB; isobutyl ester of 2,4-D; and propylene glycol butyl ether esters of Silvex were tested in this experiment. The working standard solution (1.0 mL) containing the herbicide concentrations shown in Table 33 was added to 25 mL of methylene chloride and transferred into a 500-mL K-D flask. To the flask, 30 mL of water, 5 mL of 40% NaOH solution, and various amounts of methanol ranging from 10-60 mL were added. The flask was fitted with a Snyder column, was heated in a 60°-65°C water bath for 2 hours, and then was removed from the water bath. After cooling, the reaction mixture was acidified to pH <2 using concentrated phosphoric acid and was transferred into a 500-mL separatory funnel. The aqueous solution was extracted two times with 100-mL portions of methylene chloride. The combined extracts were transferred to a K-D flask and the volume was reduced on a water bath to about 5 mL. The extract was evaporated just to dryness under a stream of nitrogen, then was reconstituted with 1 mL ether and 0.5 mL methanol, then was diluted to 4 mL using iso-octane. The samples were methylated as described in Section 4. The methylated herbicides mixture was transferred to a 10-mL volumetric flask. Dichlorobenzene solution (0.5 mL) was added as internal standard, and the total volume was brought to 10 mL using hexane. The resulting solution was analyzed by GC/EC.

TABLE 33. CONCENTRATION OF HERBICIDE ESTER WORKING STANDARD

MCPP,	Iso-octyl ester	2437	ng/µL
MCPA,	Iso-octyl ester	2613	ng/µL
2,4-D,	Isobutyl ester	15.2	ng/µL
Silvex,	Propylene glycol butyl ether ester	6.8	ng/µL
2,4,5-T,	Iso-octyl ester	7.5	ng/µL
2,4-DB,	Iso-octyl ester	59.5	ng/µL

Data and Results

The recoveries of the herbicide analytes, analyzed as methyl esters, are shown in Table 34. The mean percent recoveries plotted versus the amount of methanol added are shown in Figure 11. The optimum is in the 25-50 mL range; 35 mL is a choice that is obviously rugged.

TABLE 34. OPTIMIZATION OF HERBICIDE ESTER HYDROLYSIS BY METHANOL ADDITION (PERCENT RECOVERIES)

Analytes	Methanol Added							
	10 mL	20 mL	25 mL	30 mL	35 mL	40 mL	50 mL	60 mL
MCPP	39	72.6	99.5	87.8	91.5	91.2	111.3	85.5
MCPA	96.5	105.2	112.8	116.6	112.7	117.1	128.9	108
2,4-D	82.7	83.4	90.0	91.3	91.2	92.4	101.7	81.4
Silvex	49.0	66.8	90.5	64.0	78.4	78.1	84.6	71.9
2,4,5-T	56.4	59.0	61.8	60	61.6	59.5	65.6	52.9
2,4-DB	12.2	38.4	71.9	69.8	70.3	71.7	80.2	60.8
Mean	56	70.9	87.8	81.6	84.2	85	95.5	76.8

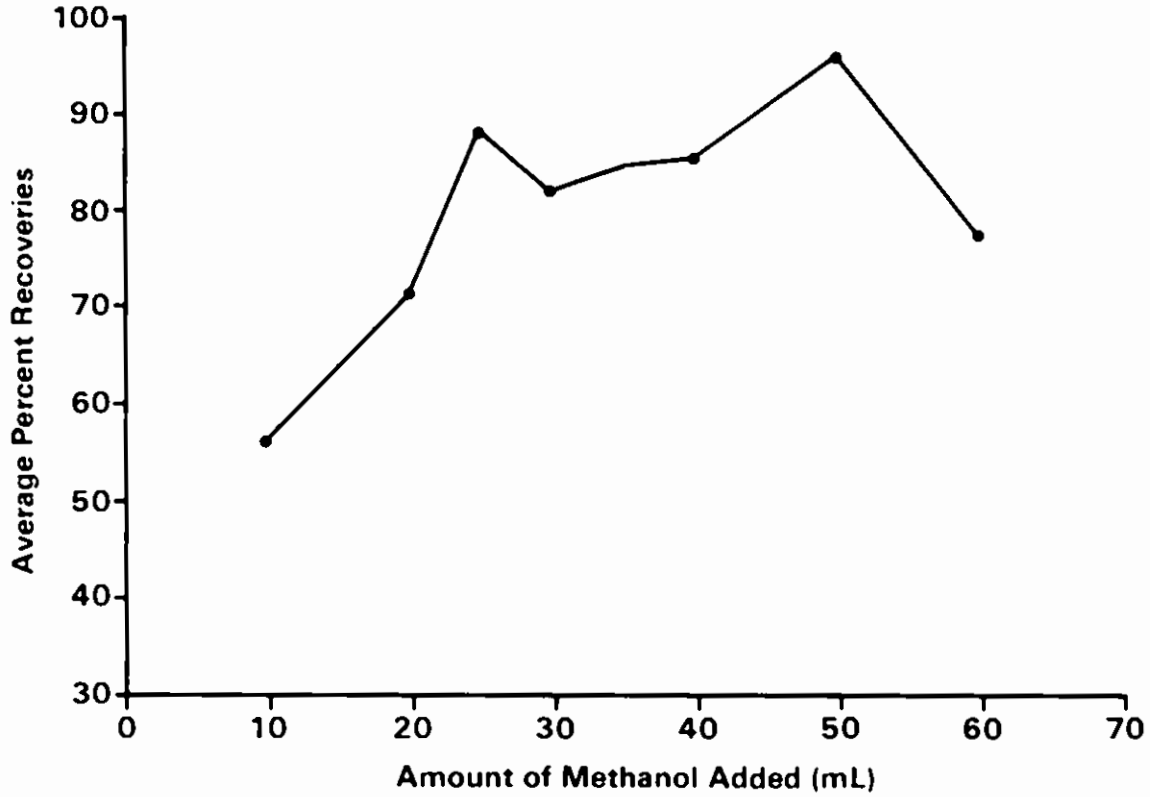


Figure 11. Hydrolysis of acid herbicide esters.

CONCLUSIONS

Variable 6, the ester hydrolysis, was optimized and was shown to be rugged in the only important variable (methanol addition). The free acid herbicide extraction and analysis were quickly optimized by simplex optimization. The ruggedness of the optimum values requires testing using ruggedness procedures as described in Section 5.

SECTION 7

LINEARITY

INTRODUCTION

The linear response range and detection limits were determined for the 10 analytes specified in Method 8150. Standards of known concentrations were prepared, were methylated with diazomethane, and were analyzed by capillary GC/EC.

EXPERIMENTAL

Response factors were estimated from previous in-house analyses of the compounds of interest. Standards that would give an approximately equal GC detector response were prepared. The standards ranged from 0.1 mg of Dicamba to 77.2 mg of MCPA in 25 mL of hexane. These primary standards were diluted to give a concentration range of 10^4 at 13 different concentration levels.

Ten mL aliquots of each standard were methylated with diazomethane as described in Section 4. The linear range of response for each compound was then determined by duplicate injection of the methylated standards into the gas chromatograph. The instrument parameters were as follows:

Instrument:	Tracor 540 gas chromatograph, EC detector
Column:	DB-5, 0.25 µm film thickness, 0.25 µm I.D. X 30M L
Injection:	5 µL Grob-type 30-second splitless injection
Injector Temperature:	220°C
Detector Temperature:	375°C
Temperature Program:	50°C for 1 minute, 25°C/min to 100°C, hold for 1 minute, 12°C to 220°C, hold for 12 min.
Integrator:	IBM CS 9000 Data System

RESULTS AND DISCUSSION

Figures 12 through 21 show plots of analyte concentration versus peak height. Table 35 gives detection limits and linear ranges for the 10 compounds. The responses of both MCPA and MCPP at the lowest concentration were used to estimate the detection limits, even though the responses were outside the linear range. Extrapolation of the linear range of response to zero concentration would have given erroneously low detection limits for these analytes. The MCPA and MCPP curves (Figures 12 and 13) have the greatest deviations from linearity. It is likely that the GC column was overloaded at the highest concentrations. It is also seen that the responses for MCPA and MCPP are nonlinear at low concentrations. Lower GC/EC responses were obtained for MCPP and MCPA than for the other analytes, and, as expected, MCPA and MCPP had much higher limits of detection.

TABLE 35. LINEAR RANGE AND DETECTION LIMITS FOR CHLORINATED HERBICIDES

Compound	Linear Range Tested	Detection Limit (ng/mL)*
Dalapon	7.4 - 736 ng/mL	1.34
Dicamba	2.6 - 520 ng/mL	0.60
MCPP	3.1 - 309 µg/mL	333
MCPA	3.1 - 306 µg/mL	218
Dichlorprop	7.5 - 15000 ng/mL	1.9
2,4-D	6.1 - 12200 ng/mL	1.7
Silvex Acid	2.1 - 4140 ng/mL	0.53
2,4,5-T	2.1 - 4110 ng/mL	0.78
2,4-DB	20.2 - 40300 ng/mL	20.2
Dinoseb	4.1 - 8100 ng/mL	1.4

* Defined as that quantity of compound yielding GC/EC response with S/N $\geq$ 3.

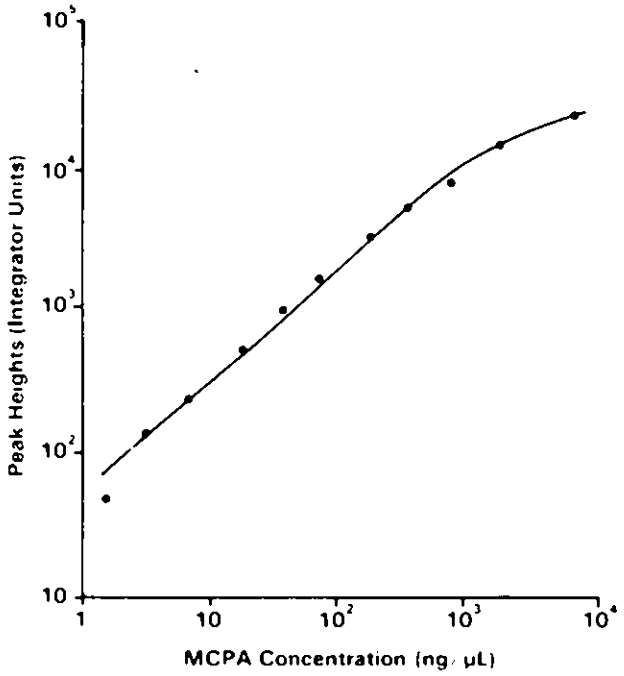


Figure 12. MCPA GC/EC response.

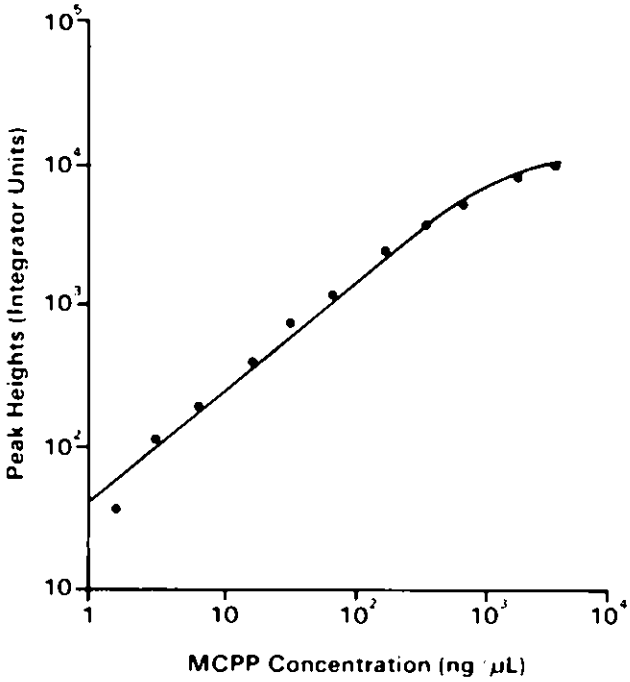


Figure 13. MCPP GC/EC response.

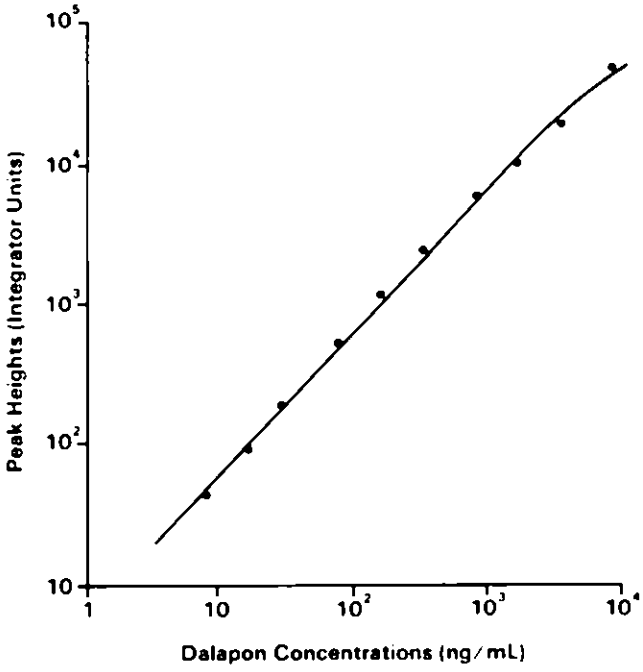


Figure 14. Dalapon GC/EC response.

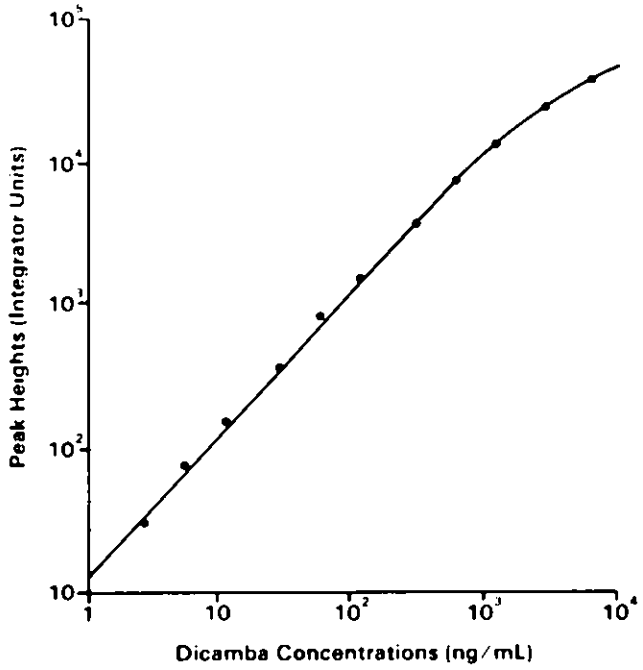


Figure 15. Dicamba GC/EC response.

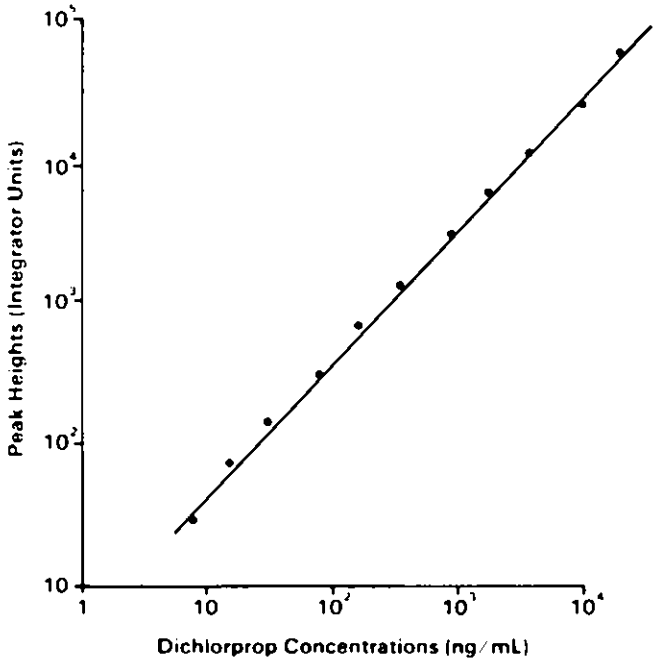


Figure 16. Dichlorprop GC/EC response.

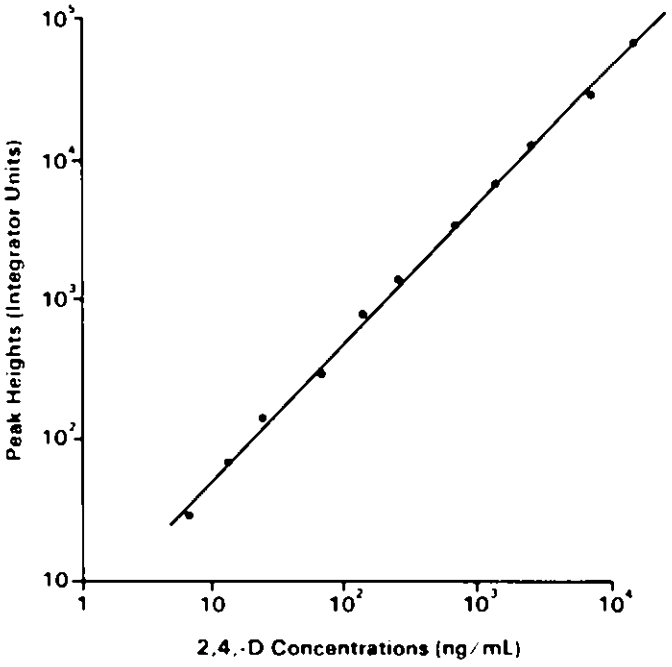


Figure 17. 2,4-D GC/EC response.

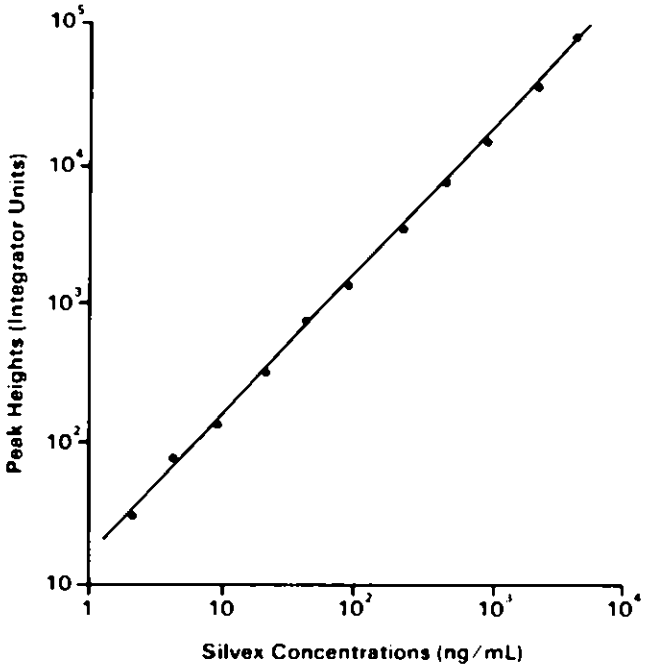


Figure 18. Silvex GC/EC response.

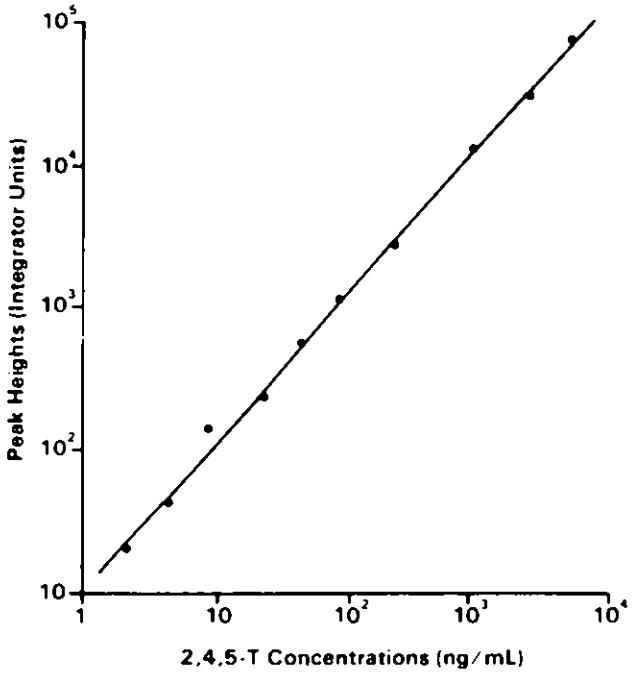


Figure 19. 2,4,5-T GC/EC response.

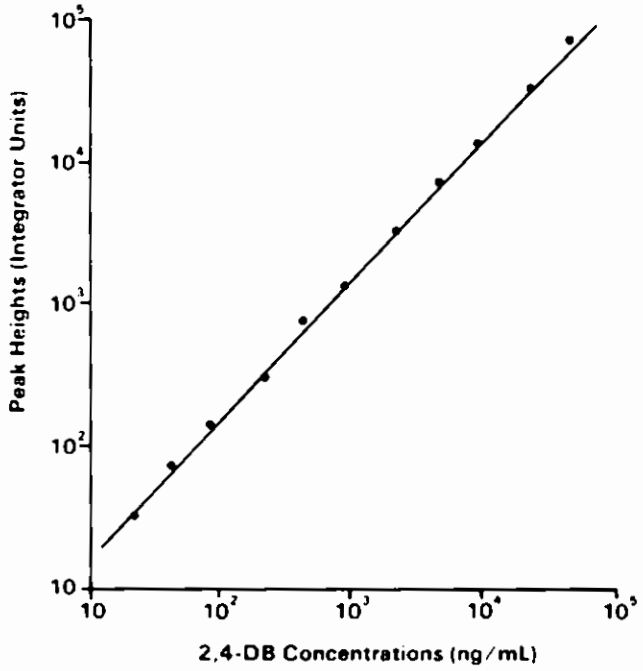


Figure 20. 2,4-DB GC/EC response.

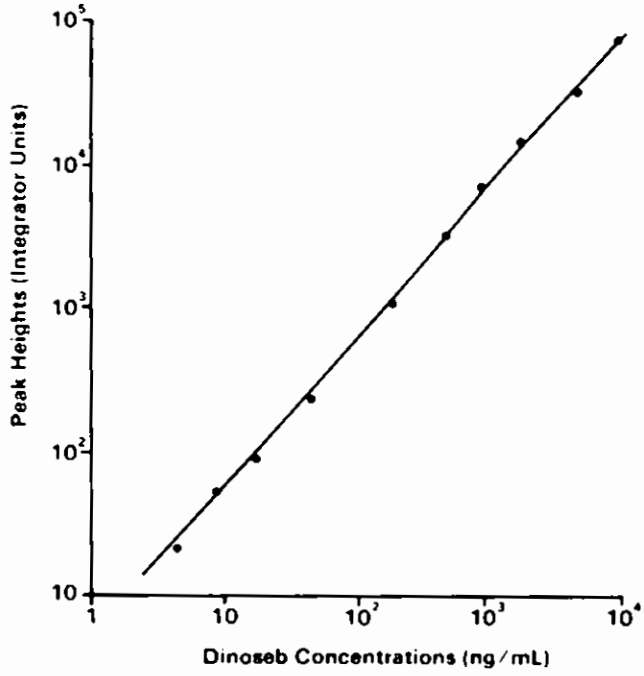


Figure 21. Dinoseb GC/EC response.

CONCLUSIONS

The detection limits and linear range were measured, and all analytes exhibited a linear response over a 10^3 span of concentration.

SECTION 8

PRECISION

INTRODUCTION

The precision for gas chromatographic analyses of the 10 analytes of Method 8150 was determined. Standards of known concentration at the high and low end of the linear range were prepared, were methylated with diazomethane, and were analyzed by gas chromatography. The % RSD was determined for 10 samples at each concentration level. A solvent blank was carried through the analysis to verify that the samples did not contain background interferences.

EXPERIMENTAL

The lower concentration was set at 10 times the detection limit reported in Section 7. The upper concentration was selected at the upper end of the concentration range listed in the linearity report. The samples at each concentration level were then analyzed by the method described in Section 7. Ten injections were made for each compound at both the low and high concentration levels.

RESULTS AND DISCUSSION

The precision determinations are shown in Table 36. Percent RSD values were larger at the low concentration level than at the high concentration level for all analytes except 2,4-D. The mean % RSD was 5.99 and all % RSD values were below 10. These values are considered to represent the experimental error.

The solvent blank and representative chromatograms from both concentration levels are shown in Figures 22-26.

TABLE 36. RESULTS OF PRECISION DETERMINATIONS

Compound	Low Concentration (ng/mL)	Low Concentration % RSD (n=10)*	High Concentration (ng/mL X 10^3)	High Concentration % RSD (n = 10)	Average % RSD (n = 20)
Dalapon	14.7	5.1	0.74	3.1	4.1
Dicamba	6.5	9.4	0.52	5.5	7.5
MCPP	3300	3.7	300.0	3.1	3.4
MCPA	2070	7.3	308.0	3.3	5.3
Dichlorprop	18.8	7.5	15.0	2.4	5.0
2,4-D	15.3	5.0	12.2	5.6	5.3
Silvex	5.2	7.6	4.1	3.8	5.7
2,4,5-T	6.8	9.1	4.1	5.5	7.3
2,4-DB	50.3	9.5	40.3	5.7	7.6
Dinoseb	13.5	9.8	8.1	7.6	8.7

* The letter n indicates the number of determinations.

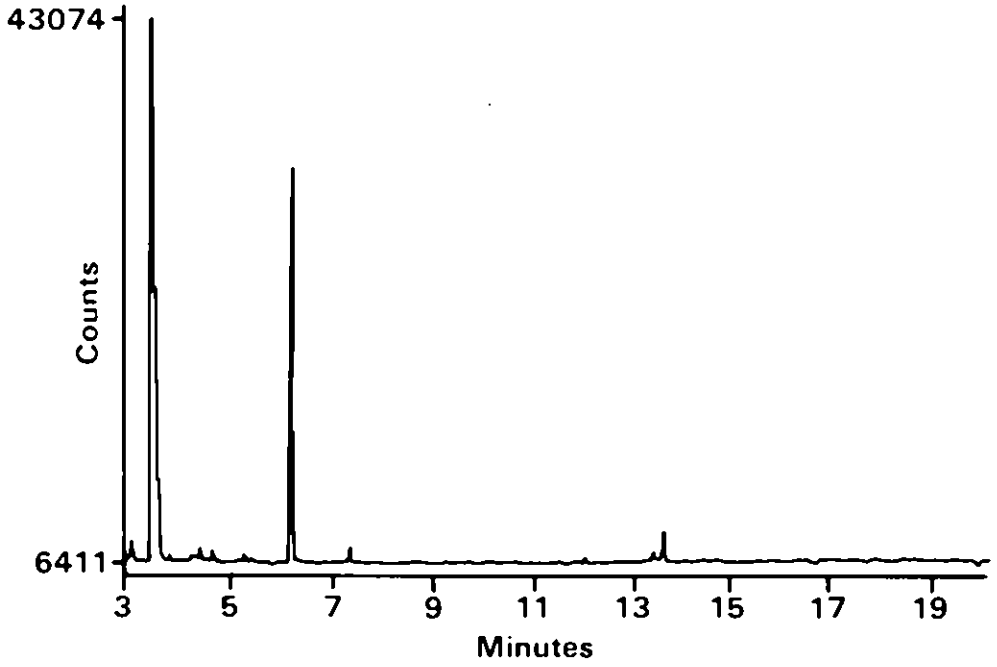


Figure 22. Solvent blank for precision measurements.

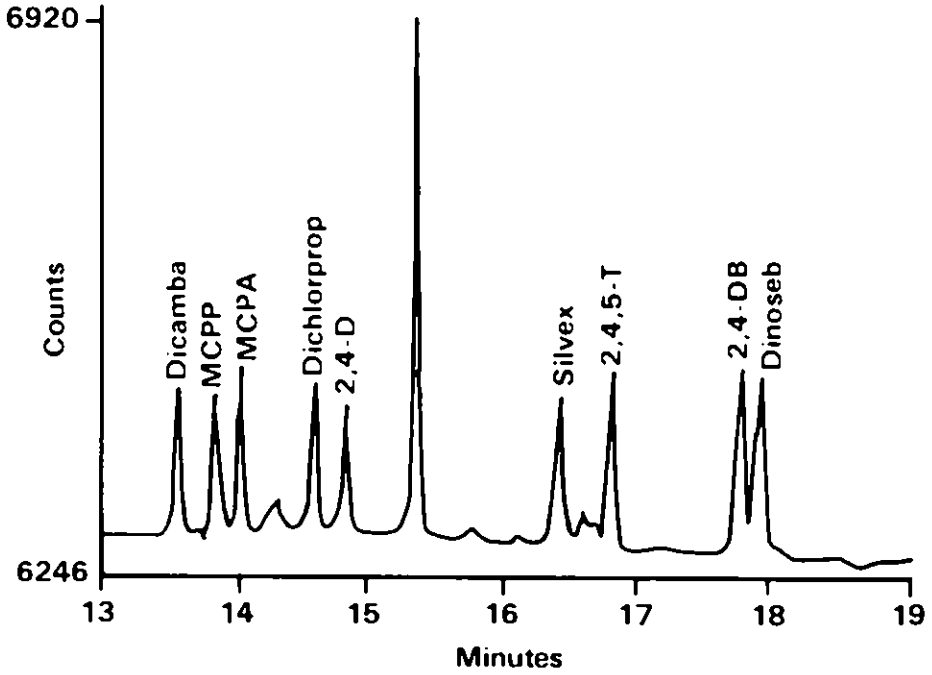


Figure 23. Low concentrations: Dicamba, MCPP, MCPA, Dichlorprop, 2,4-D, Silvex, 2,4,5-T, 2,4-DB, Dinoseb.

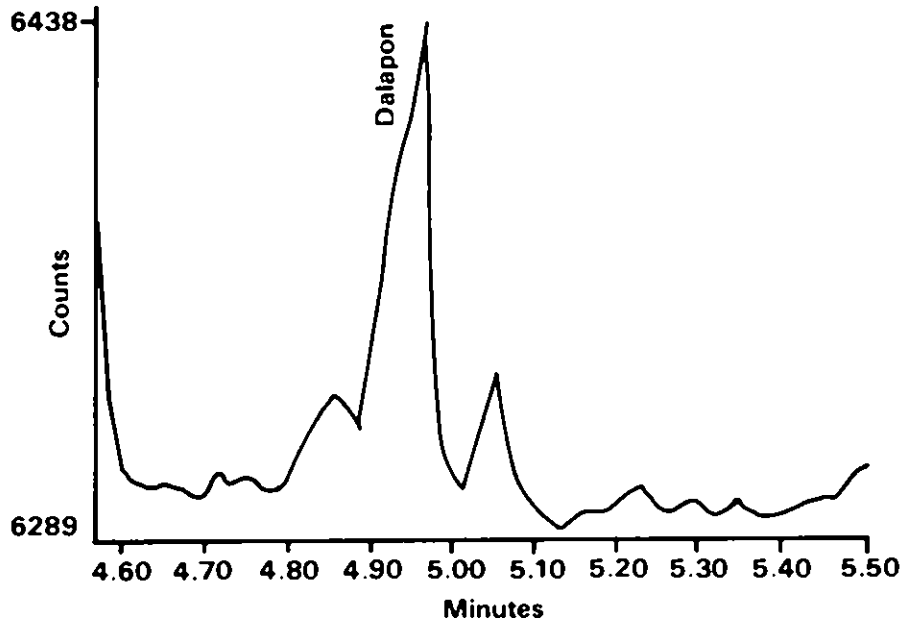


Figure 24. Low concentration: Dalapon.

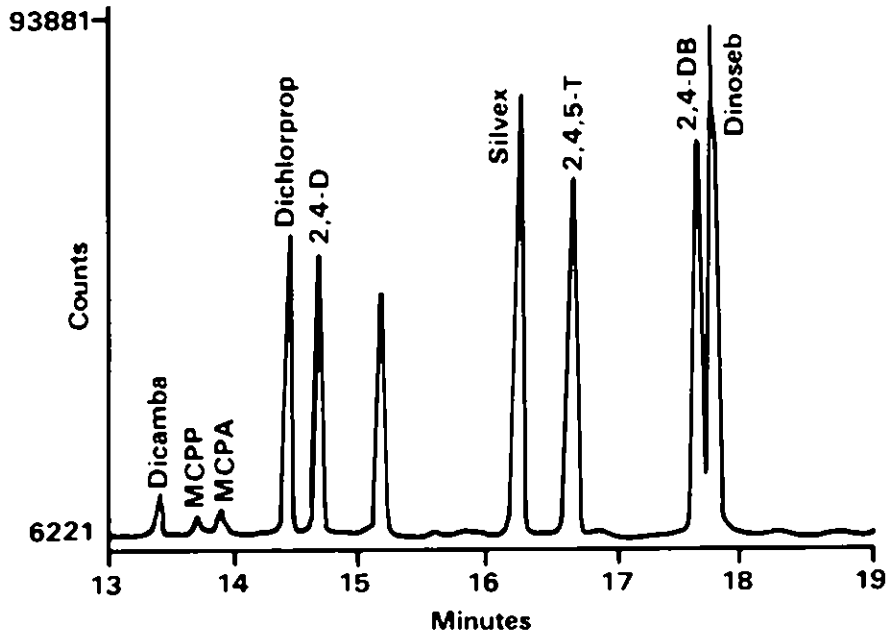


Figure 25. High concentrations: Dicamba, MCPP, MCPA, Dichlorprop, 2,4-D, Silvex, 2,4,5-T, 2,4-DB, Dinoseb.

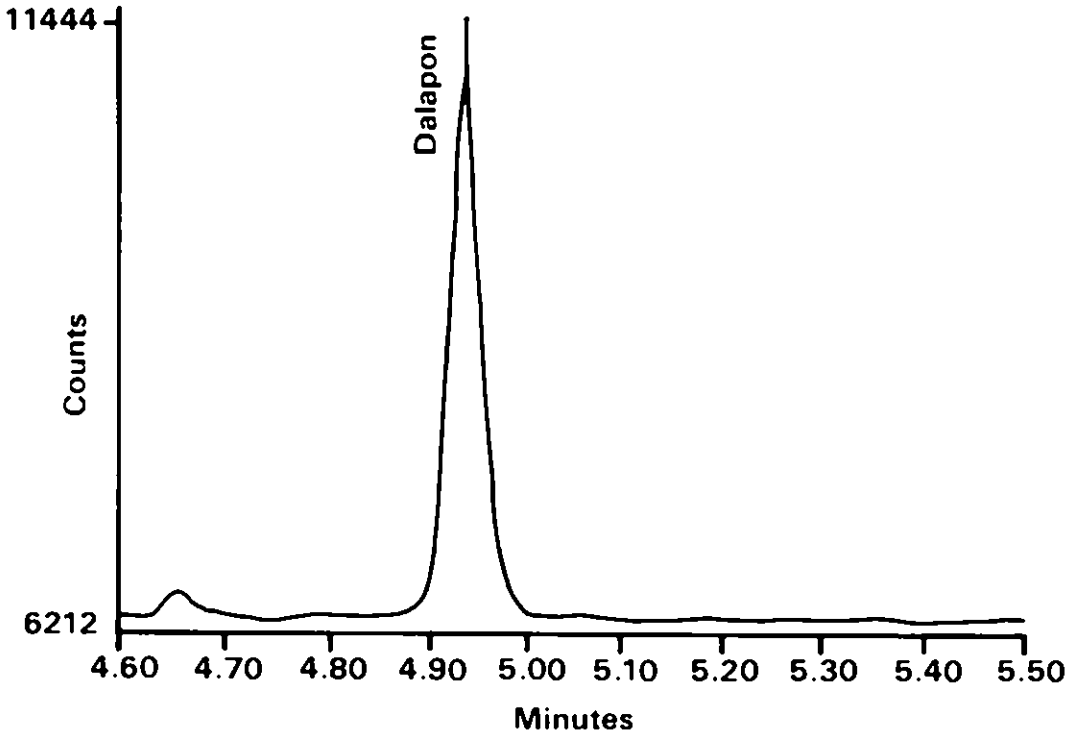


Figure 26. High concentration: Dalapon

CONCLUSIONS

The % RSD's of all analyte concentrations measured were below 10. The precision of this method is excellent for measuring chlorinated herbicide concentration over a 10^2 range for MCPP and MCPA and over a 10^3 concentration for the other analytes. Horwitz, et al[19,20] suggest that analyses at the µg/g level should have a single-laboratory variation of 8-11% to give a reproducibility < 16% in interlaboratory studies. Thus, this method is practical for monitoring residues that are of public health significance.

SECTION 9

BIAS TESTING

INTRODUCTION

Bias or systematic error was tested by determining percent recoveries of analytes at a range of concentrations known to give a linear response and acceptable relative standard deviations as reported in Section 8. For these tests, two matrices were used, kaolin clay (Ajax P, Westwood Ceramic Supply) and kaolin clay spiked with still bottoms from herbicide manufacturing obtained from Dow Chemical, Midland, Michigan, via S-Cubed, San Diego, California.

EXPERIMENTAL

Kaolin clay samples

Fifty grams of kaolin clay were weighed into a 400-mL, thick-wall beaker and spiked with 1 mL of a standard solution containing the 10 acid herbicides (Dicamba, MCPP, MCPA, Dichlorprop, 2,4-D, Silvex, 2,4,5-T, 2,4-DB, Dinoseb, and Dalapon) to give concentrations shown in Table 1. The sample was mixed with 85 mL of phosphate buffer (pH = 2.5). One hundred mL of methylene chloride was added and the sample was sonicated for 3 minutes in the pulsed mode at 50 percent duty cycle at an output of 6.3. After the clay was allowed to settle, the solvent was transferred into a 500-mL centrifuge bottle. The clay was sonicated two more times, using the same conditions, with 100 mL of methylene chloride each time. The extracts were combined into the centrifuge bottle and were centrifuged for 10 minutes to settle the fine particles. The extract was then filtered through Whatman #1 filter paper into a 500-mL separatory funnel. To the extract, 100 mL of 0.1 N NaOH solution was added and the funnel was shaken for two minutes. The aqueous layer was transferred to a beaker and immediately was acidified with phosphoric acid to a pH of 1.0. The organic layer was extracted once more with 100 mL of 0.1 N NaOH solution. The aqueous layer was added to the first aqueous extract and the pH was readjusted to $\leq$1.0 if necessary. The organic layer was discarded. The acid solution was extracted twice with 100 mL of methylene chloride. The combined extract was concentrated to approximately 5 mL in a 500-mL K-D concentrator on a steam bath. Samples analyzed for Dalapon required use of a pH-1 phosphate buffer.

The sample was evaporated just to dryness using a stream of nitrogen, then the sample was reconstituted with 1 mL of iso-octane and 0.5 mL of methanol, was diluted to a volume of 5 mL with ethyl ether, and was methylated as described in Section 4.

A Tracor 540 Gas Chromatograph equipped with an autosampler was used to analyze the methylated samples under the following parameters:

Column	= DB-5, 0.25 µM film thickness, 0.25 µM I.D. X 30 M L
Injection	= 5 µL Grob-type 30-second splitless injection
Injector Temperature	= 220°C
Detector Temperature	= 375°C
Detector	= Electron Capture
Temperature Program	= 50°C for 1 minute, 25°C/min to 100°C, hold for 1 minute, 12°C/min to 220°C, hold for 12 min.
Integrator	= IBM CS 9000 Data system.

Kaolin clay samples plus still bottoms

Still bottom # 42 (0.170 g) was extracted with methylene chloride. This was diluted 1 to 100. This dilution (2 mL) was added to each 50 g kaolin clay sample prior to extraction, derivatization, and analysis as described for the kaolin clay. The five clay/still bottom samples were spiked as described in Table 37.

TABLE 37. FINAL CONCENTRATIONS (ppb) OF ANALYTES ADDED TO CLAY AND CLAY/STILL BOTTOM SAMPLES (A-E)

Analytes	Samples (ppb)				
	A	B	C	D	E
Dalapon	147	29.4	14.7	2.9	1.5
Dicamba	146	29.2	14.6	2.9	1.5
MCPP	30600	6120	3060	612	306
MCPA	30700	6140	3070	614	307
Dichlorprop	3020	604	302	60.4	30.2
2,4-D	2480	496	248	49.6	24.8
Silvex	834	167	83.4	16.7	8.3
2,4,5-T	852	170	85.2	17.0	8.5
2,4-DB	8160	1632	816	163	81.6
Dinoseb	1664	333	166	33.3	16.6

DATA AND RESULTS

Figures 27-35 show the percent recoveries versus concentration for the 10 analytes spiked into the kaolin clay and kaolin clay/still bottoms. The Dalapon result was highly variable and was not included. Tables 38 and 39 give the percent recoveries of the 10 measured analytes in kaolin clay and kaolin clay/still bottom matrices. Sample chromatograms of blank and spiked kaolin clay/still bottom extracts are shown in Figures 37 and 38.

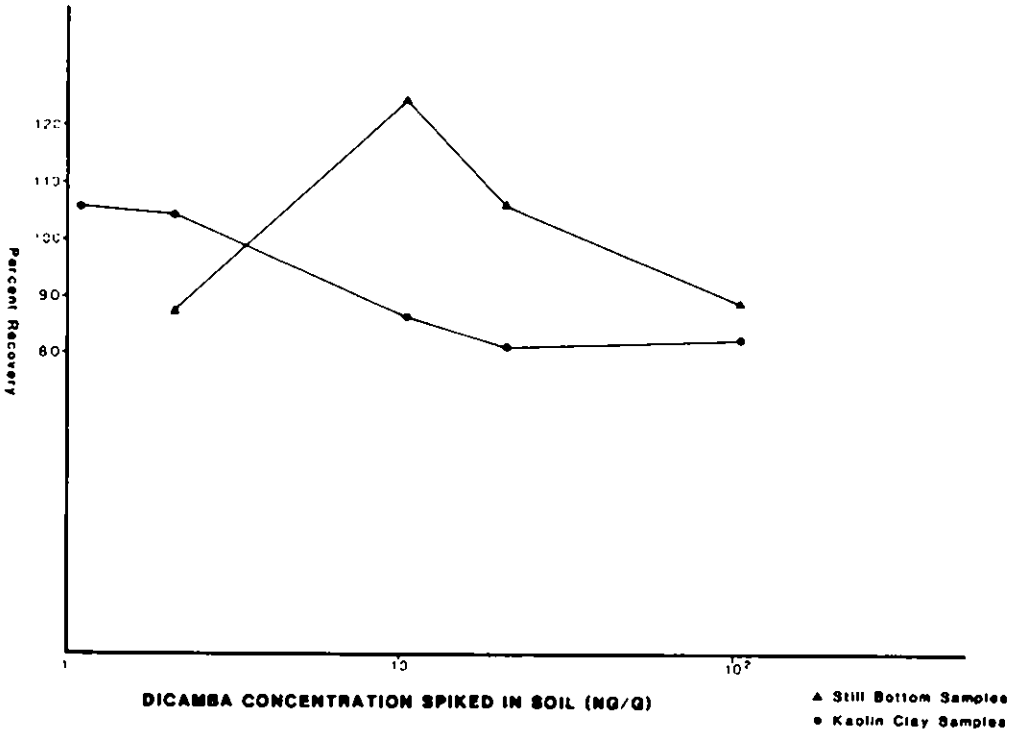


Figure 27. Dicamba concentration bias.

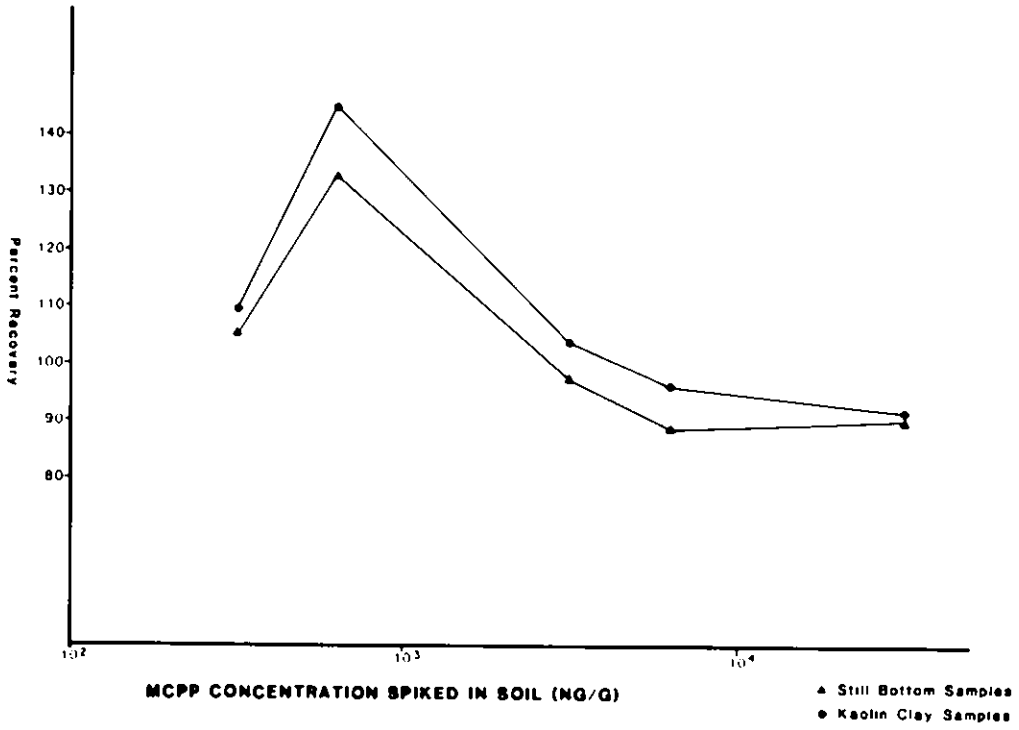


Figure 28. MCPP concentration bias.

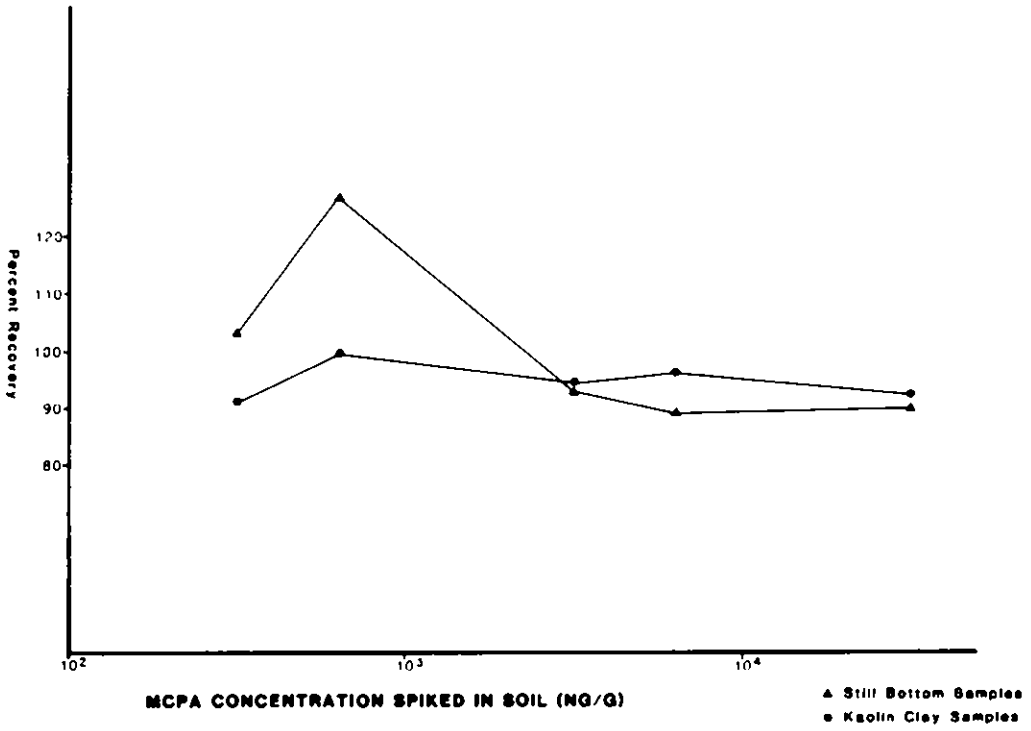


Figure 29. MCPA concentration bias.

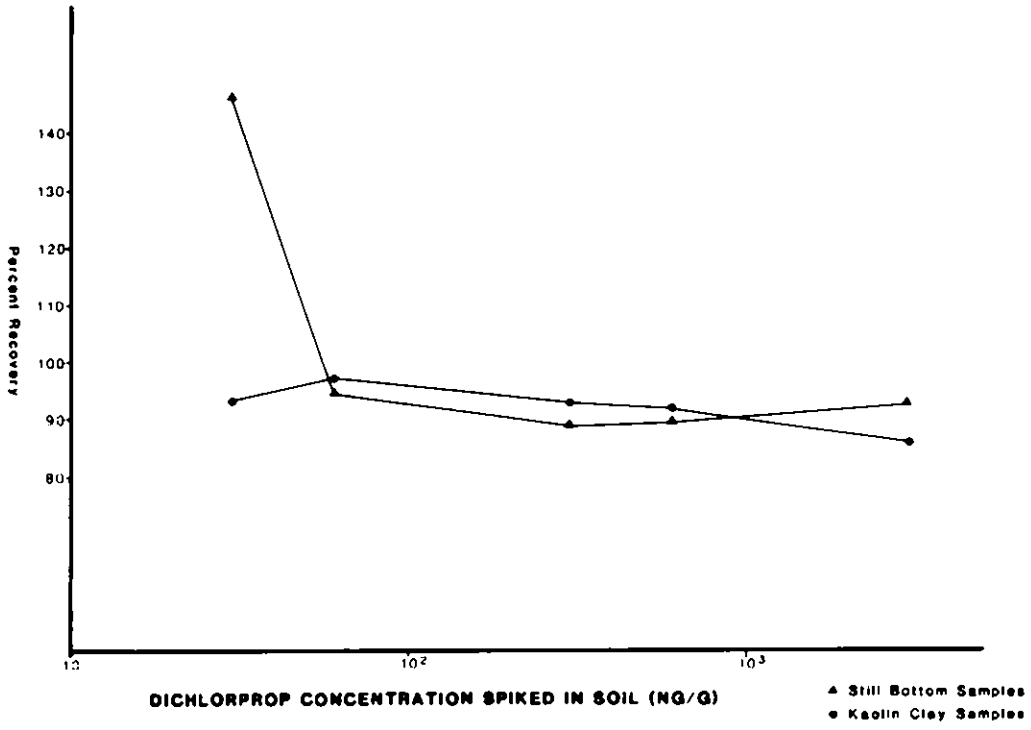


Figure 30. Dichlorprop concentration bias.

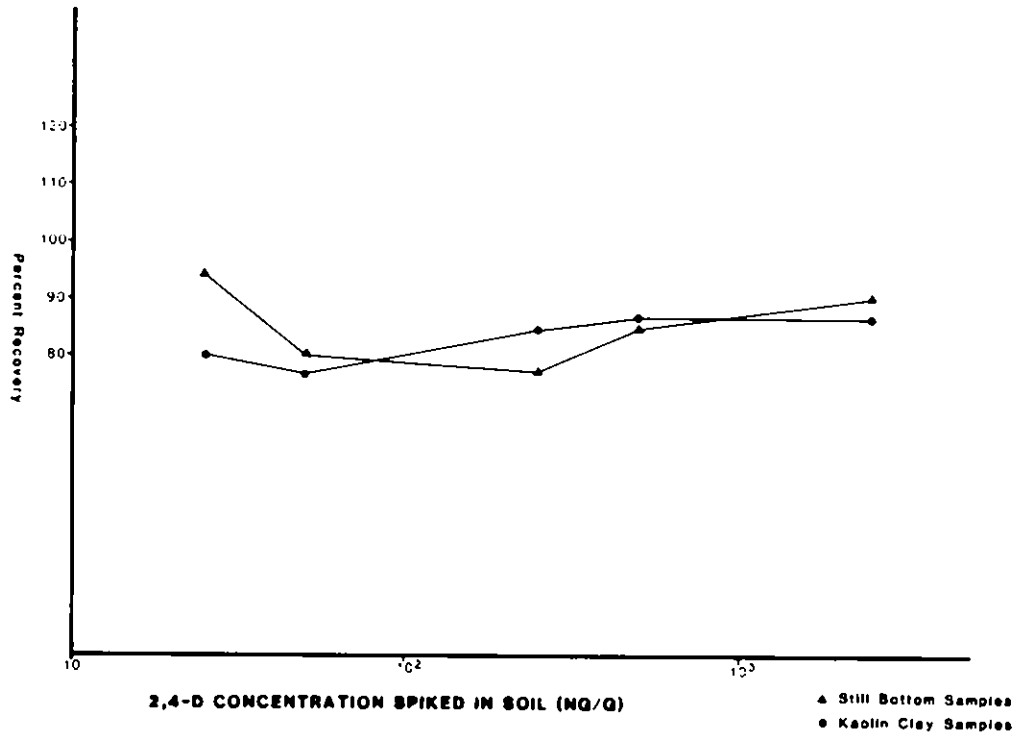


Figure 31. 2,4-D concentration bias.

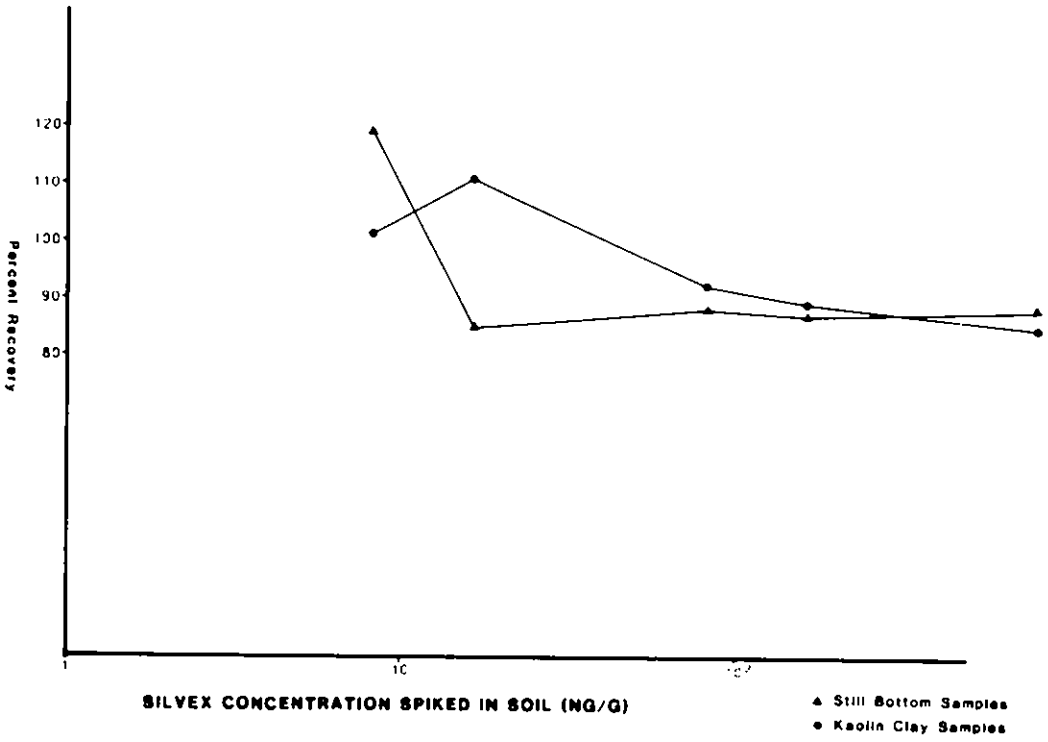


Figure 32. Silvex concentration bias.

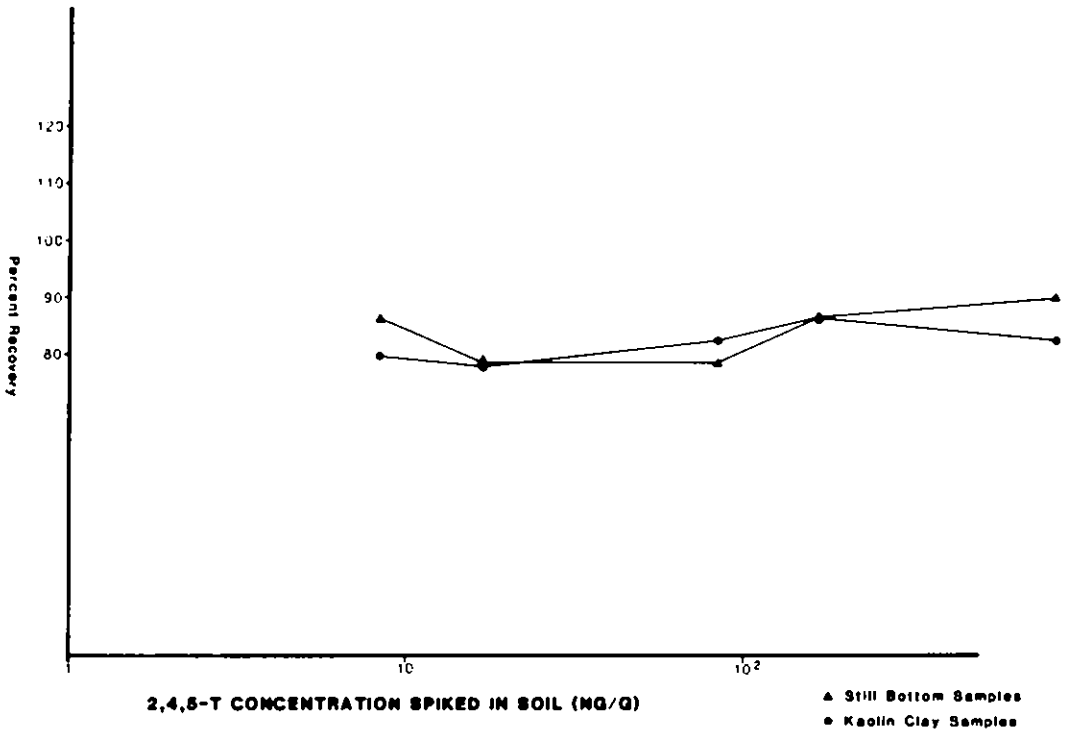


Figure 33. 2,4,5-T concentration bias.

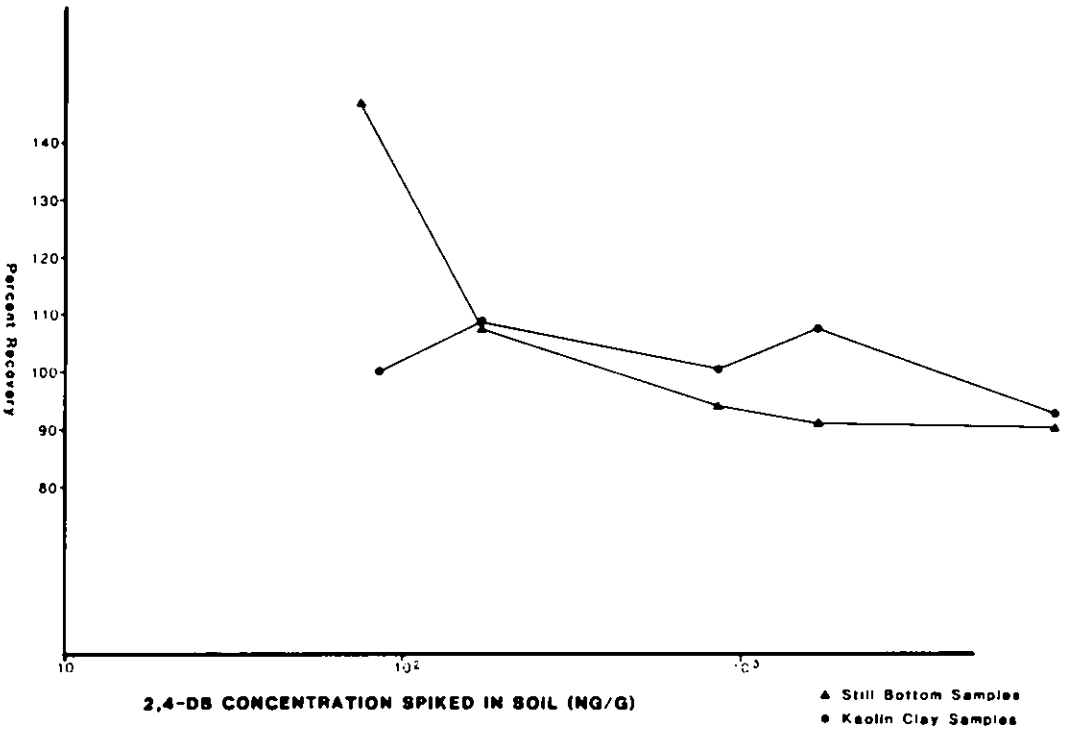


Figure 34. 2,4-DB concentration bias.

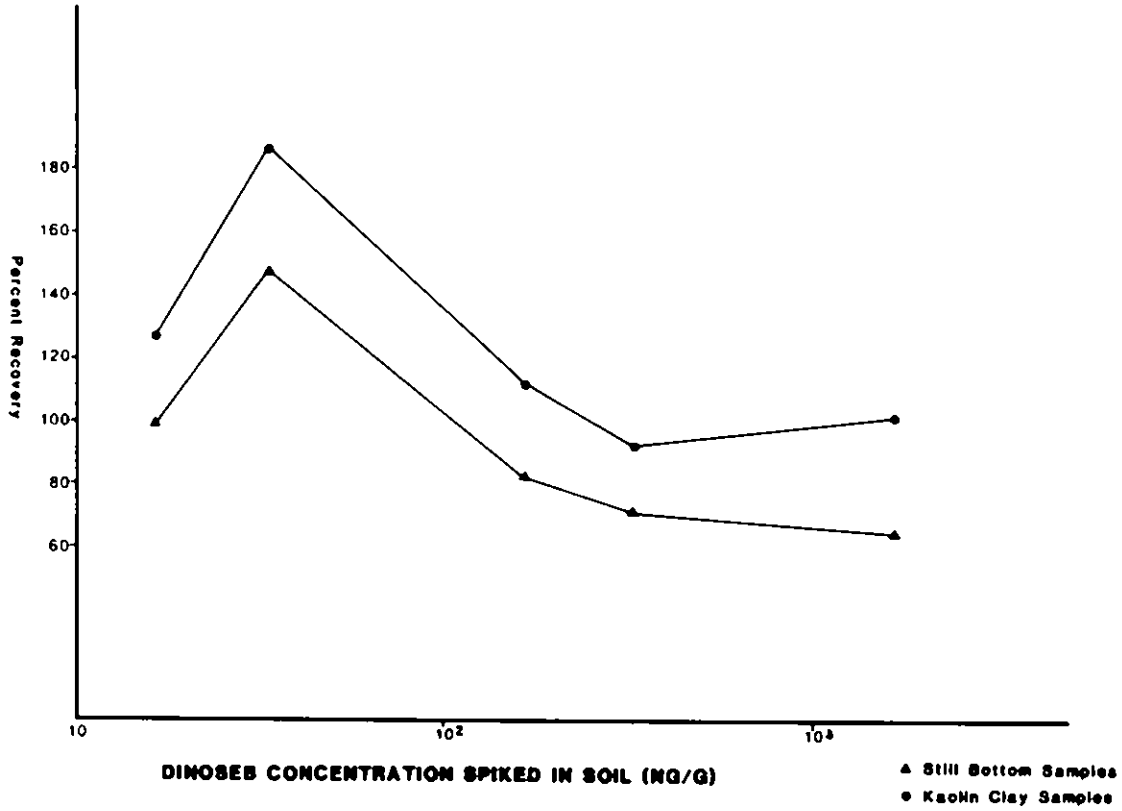


Figure 35. Dinoseb concentration bias.

TABLE 38. RECOVERIES (%) FOR THE CHLORINATED HERBICIDES IN KAOLIN CLAY (CONCENTRATIONS A-E)

Analytes	Recoveries				
	A	B	C	D	E
Dicamba	82.3	80.8	84.3	104.3	105.8
MCPP	91.9	96.5	104.0	143.4	109.7
MCPA	93.1	87.6	94.5	99.7	90.9
Dichlorprop	86.1	92.2	92.9	96.9	93.4
2,4-D	86.8	87.0	84.7	77.3	80.1
Silvex	85.0	89.4	92.2	111.1	101.4
2,4,5-T	83.0	86.8	82.6	78.2	79.8
2,4-DB	93.6	108.1	101.0	108.8	100.3
Dinoseb	101.7	92.2	108.1	182.6	127.1
Dalapon					

TABLE 39. RECOVERIES (%) FOR THE CHLORINATED HERBICIDES IN KAOLIN CLAY/STILL BOTTOM SAMPLES (CONCENTRATIONS A-E)

Analytes	Recoveries				
	A	B	C	D	E
Dicamba	88.7	103.8	124	87.5	-
MCPP	92.3	89.0	97.7	133	105
MCPA	90.2	89.4	93.2	127	103
Dichlorprop	92.5	89.7	89.1	94.6	146
2,4-D	90.3	85.0	77.3	80.1	94.1
Silvex	88.1	87.5	88.2	85.1	117
2,4,5-T	90.2	86.6	78.8	78.5	86.1
2,4-DB	90.9	91.8	94.6	108.0	147
Dinoseb	68.1	71.4	82.3	148	98.9
Dalapon					

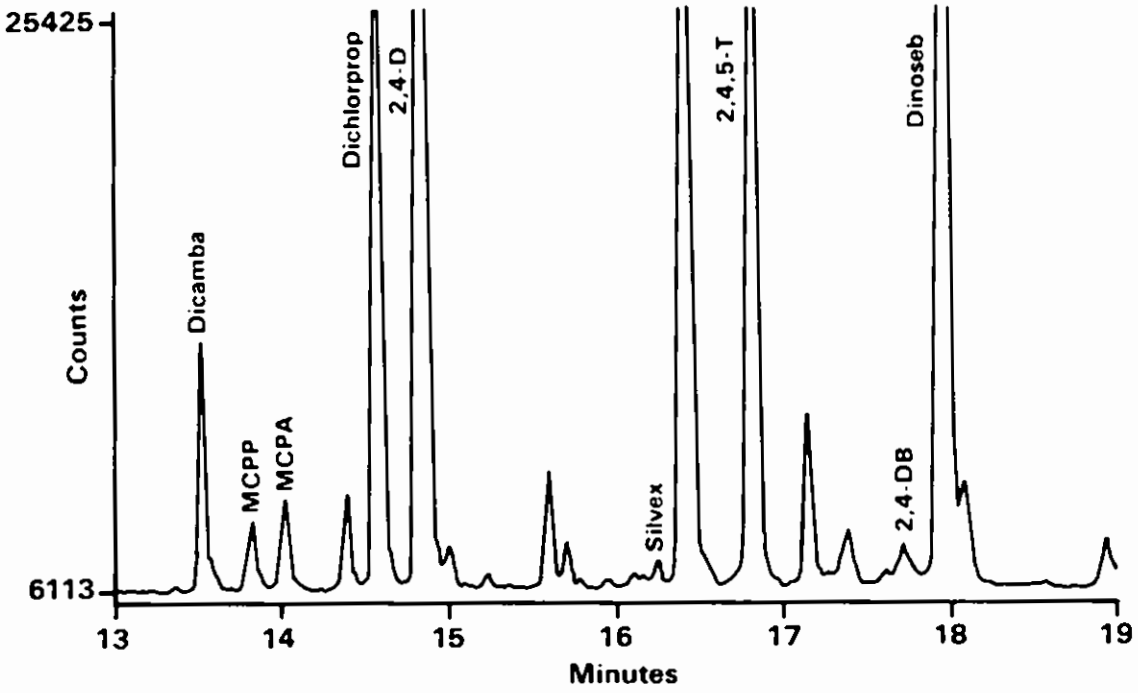


Figure 36. Chromatogram, sample A, (most concentrated spike) from kaolin clay/still bottoms.

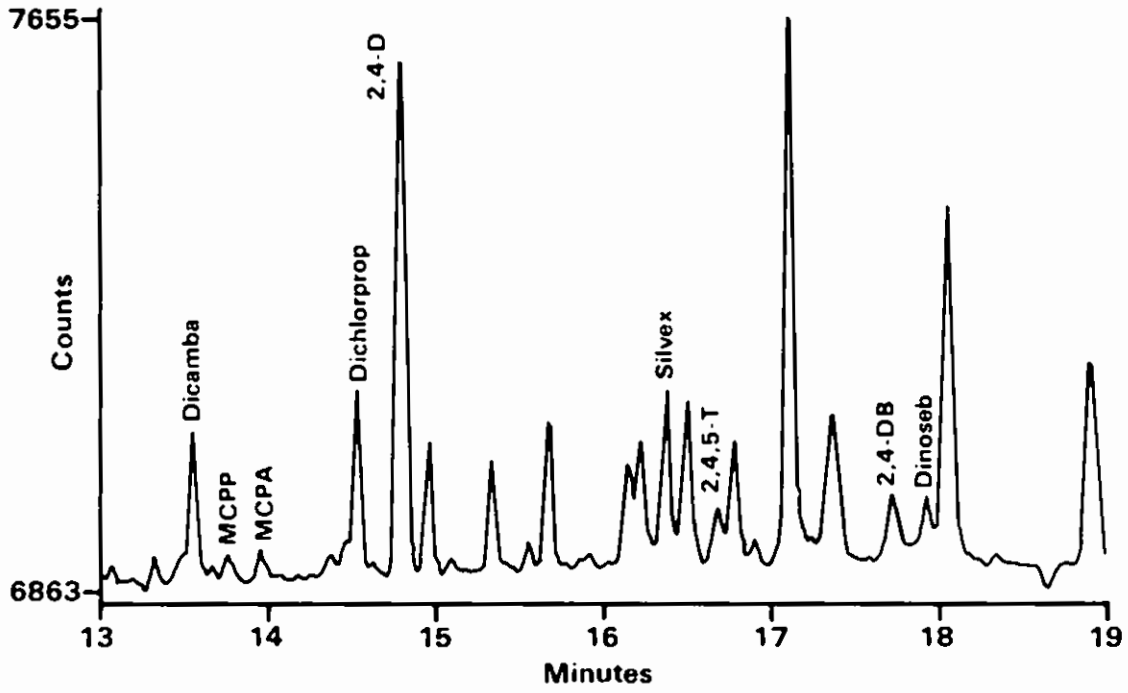


Figure 37. Chromatogram, sample E (least concentrated spike) from kaolin clay/still bottoms.

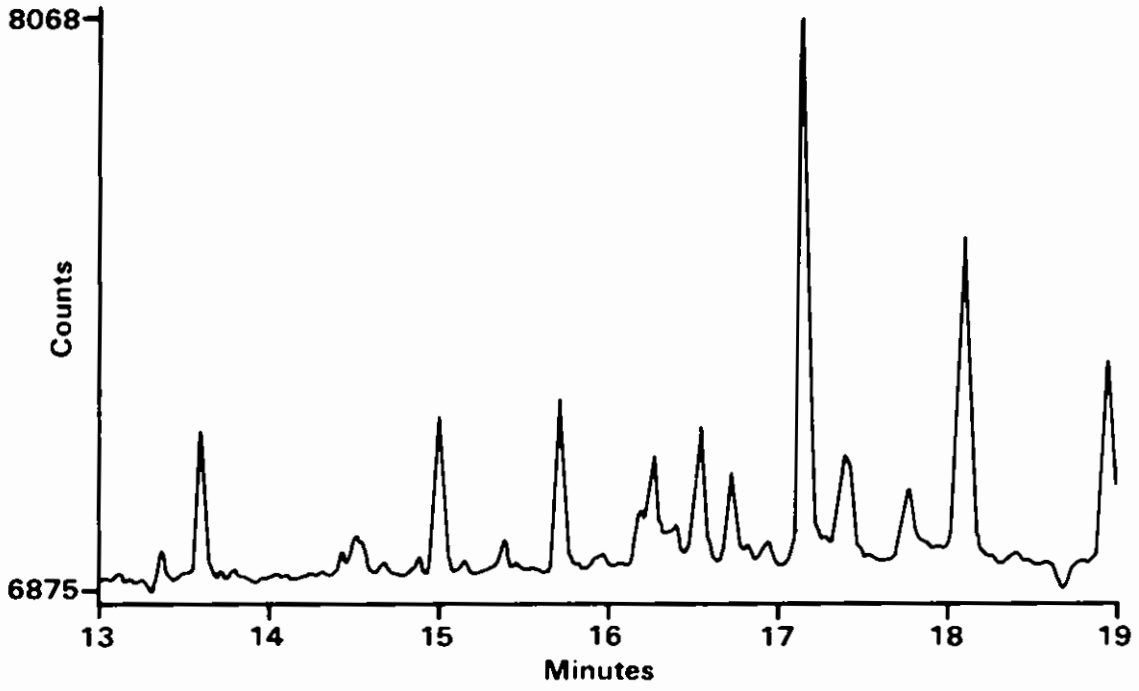


Figure 38. Chromatogram, sample F, blank from kaolin clay/still bottoms.

CONCLUSIONS

There is a general increase in percent recovery with concentration and a general bias toward less than 100% recovery. Dalapon is not recovered using the optimized protocol.

At very low concentrations, the background exhibited by the matrix or by compounds added by the still bottom spike becomes very important and large compared to the signal of the analyte spike. For example, methyl Dicamba coelutes with a major impurity seen in the still bottom spiked clay (compare Figures 38 and 39). The Dinoseb recovery in the kaolin clay samples was excessively high at lower concentrations, yet the kaolin clay/still bottom sample exhibited reasonable recoveries at the same concentrations, which indicates a complex interaction of still bottoms with the clay.

The compounds present in the matrix have an influence on detection limits and bias of this method for these analytes. Coelution and complex matrix interactions are observable with the kaolin clay and still bottom samples.

SECTION 10

GC/MS CONFIRMATION

INTRODUCTION

Compounds present in the matrix can coelute with the herbicide analytes of interest. The compounds eluting cannot be identified by GC/EC analysis alone. GC/MS analysis is required to give full scan spectra of each analyte for comparison with known spectra.

In this section, the mass spectrum of the methyl derivative of each herbicide is reported along with the reconstructed ion chromatogram with the corresponding GC/EC chromatogram. The minimum concentration to obtain a computer Finnigan INCOS "FIT" value of 800 (on special matching to reference spectra taken at 50 ng) was determined for each analyte.

EXPERIMENTAL

Twenty-five milligrams of each of the 10 acid herbicides were weighed out into a 10-mL volumetric flask. The acids were dissolved by adding an adequate amount of acetone and then were brought up to about 4 mL with hexane. The acid solutions were methylated as described in Section 4.

The methylated samples were evaporated to dryness. A series of different solutions of the methylated acid herbicides ranging in concentration from 50 ng/µL to 0.25 ng/µL were prepared for GC/MS confirmation. The instrument parameters were as follows:

Instrument:	Finnigan 9610 GC/Finnigan 4023 Mass spectrometer.
Column:	DB-5, 1.0µM film thickness, 0.32µM ID X 30M L
Injection:	1 µL, Grob-type 30-sec. splitless injection.
Injector Temperature:	220°C.
Temperature Program:	60°C for 2 minutes, 13°C/minute to 220°C, hold for 10 minutes.
Electron Multiplier Voltage:	-1200V.
Scan Range:	45amu - 550amu.
Scan Time:	1 scan/sec.
Data System:	Data General NOVA3 with INCOS.

The mass spectra obtained from the 50 ng/µL methyl esters were used to establish a library. Measurements of the resemblance of the library spectrum to the spectrum of less concentrated samples were done by the computer (the FIT number of library search in INCOS).

DATA AND RESULTS

The reconstructed ion chromatogram is shown in Figure 40 and the corresponding GC/EC chromatogram is shown in Figure 41. The full scan mass spectra of the target herbicide esters are shown in Figures 42 to 50.

The minimum concentrations required to give a FIT of 800 are shown in Table 40. The value of 800 for a good FIT is recommended in the Finnigan INCOS Manual.[39] This number appears to be a valid value because the plots of FIT versus concentration (Figures 52-61) rapidly drop off at concentrations below that which gives a FIT of 800.

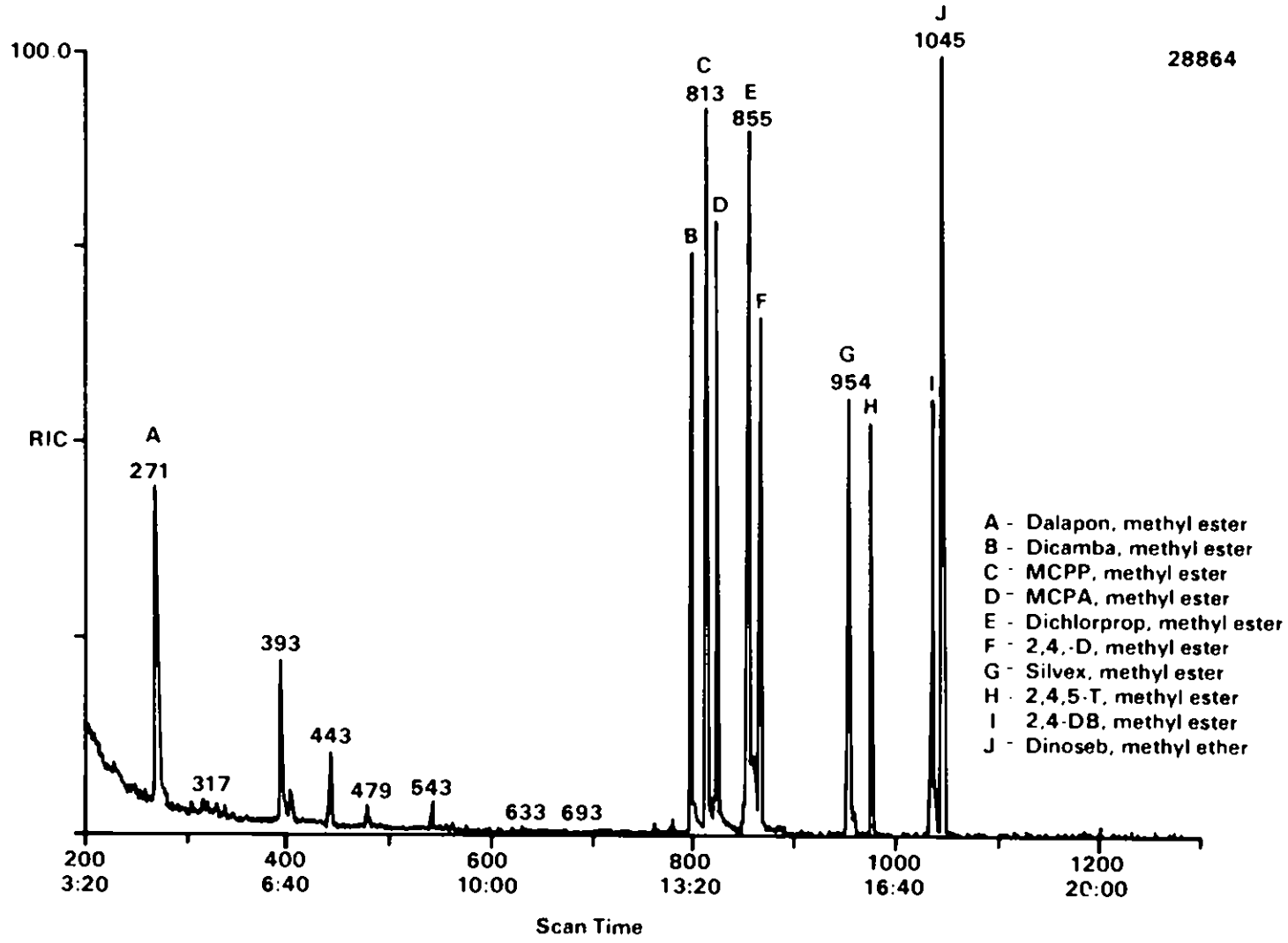


Figure 39. Reconstructed ion chromatogram.

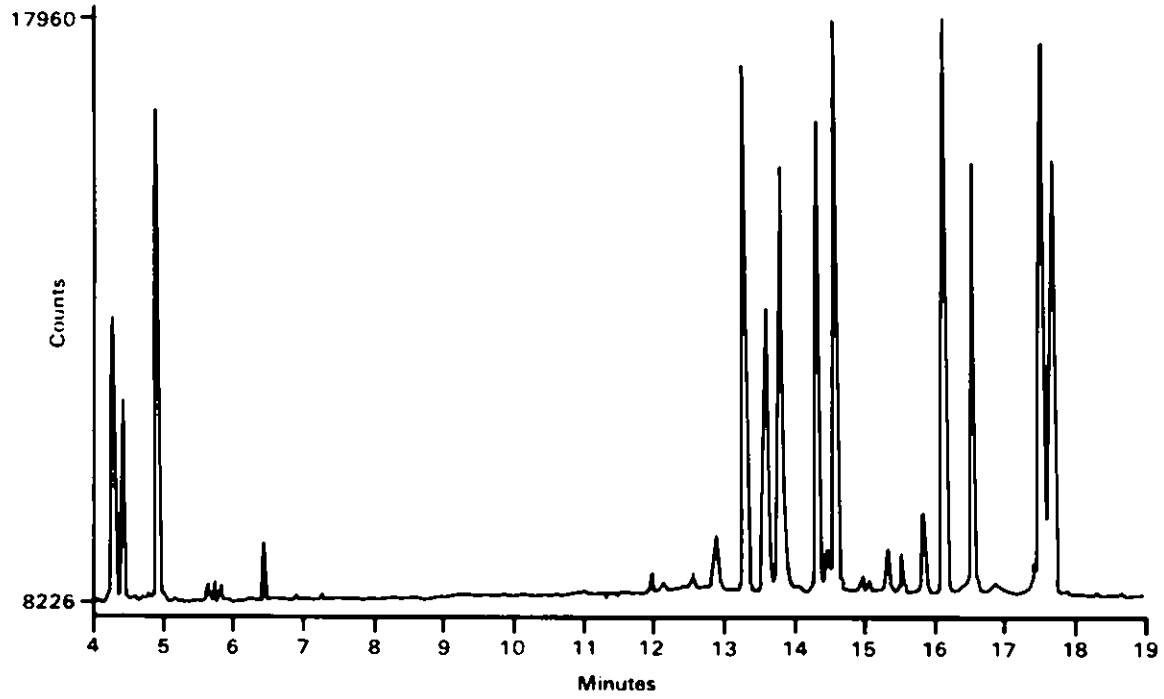


Figure 40. GC/EC chromatogram of target herbicide esters.

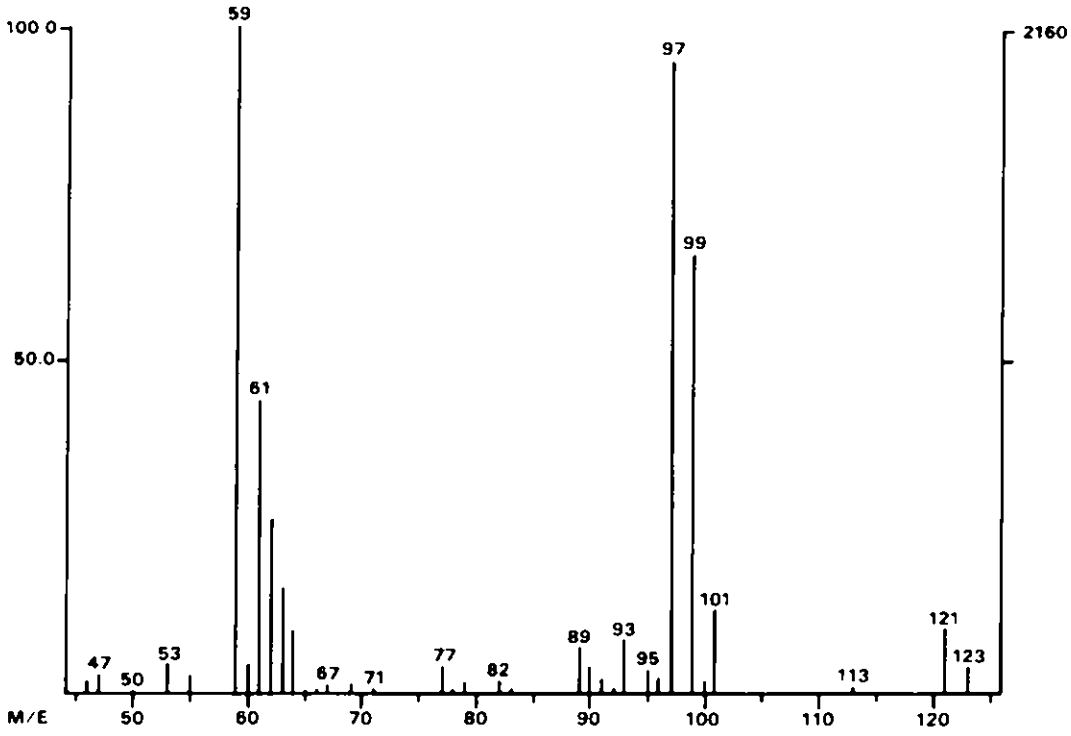


Figure 41. Dalapon electron impact mass spectrum.

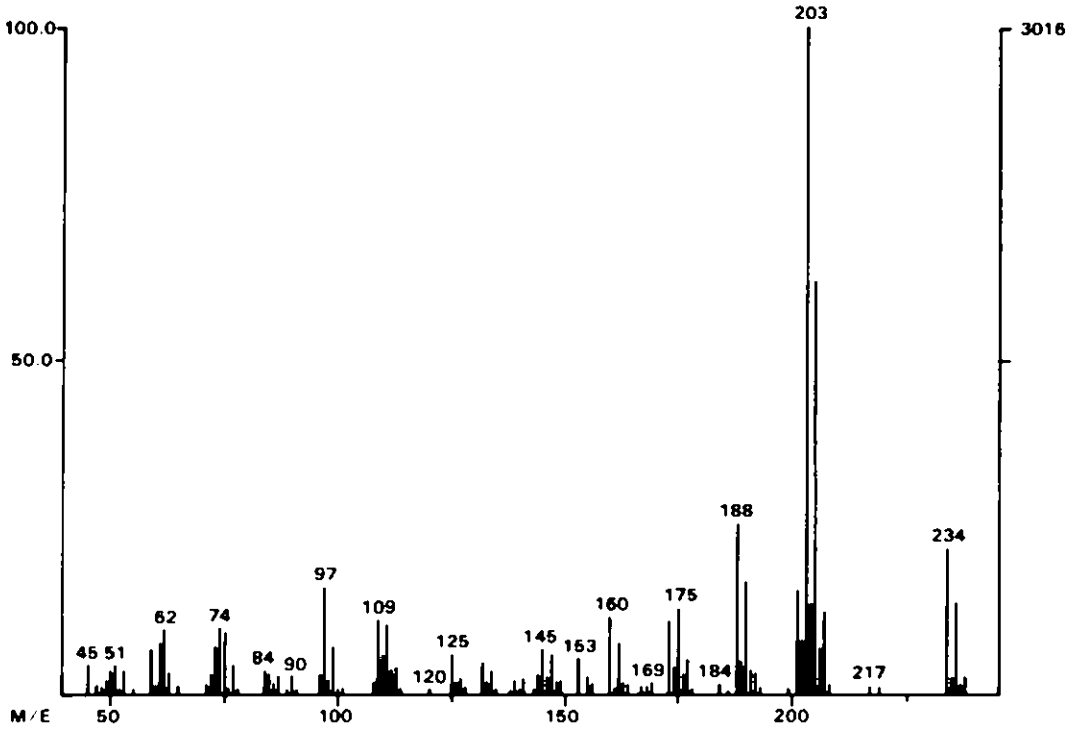


Figure 42. Dicamba electron impact mass spectrum.

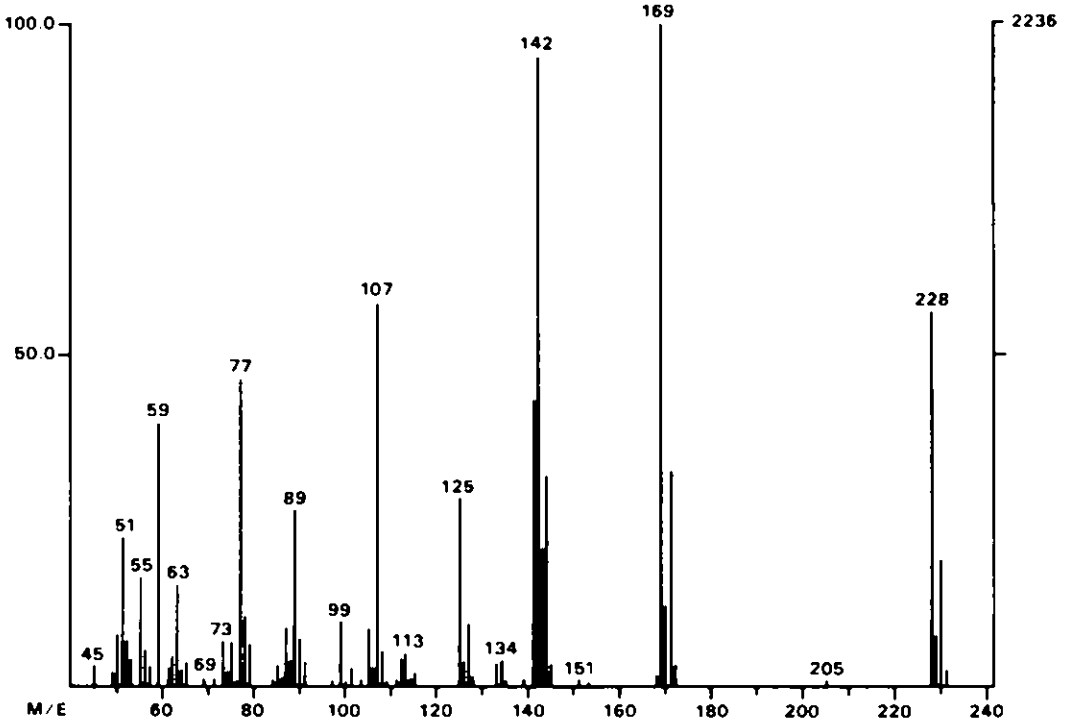


Figure 43. MCPP electron impact mass spectrum.

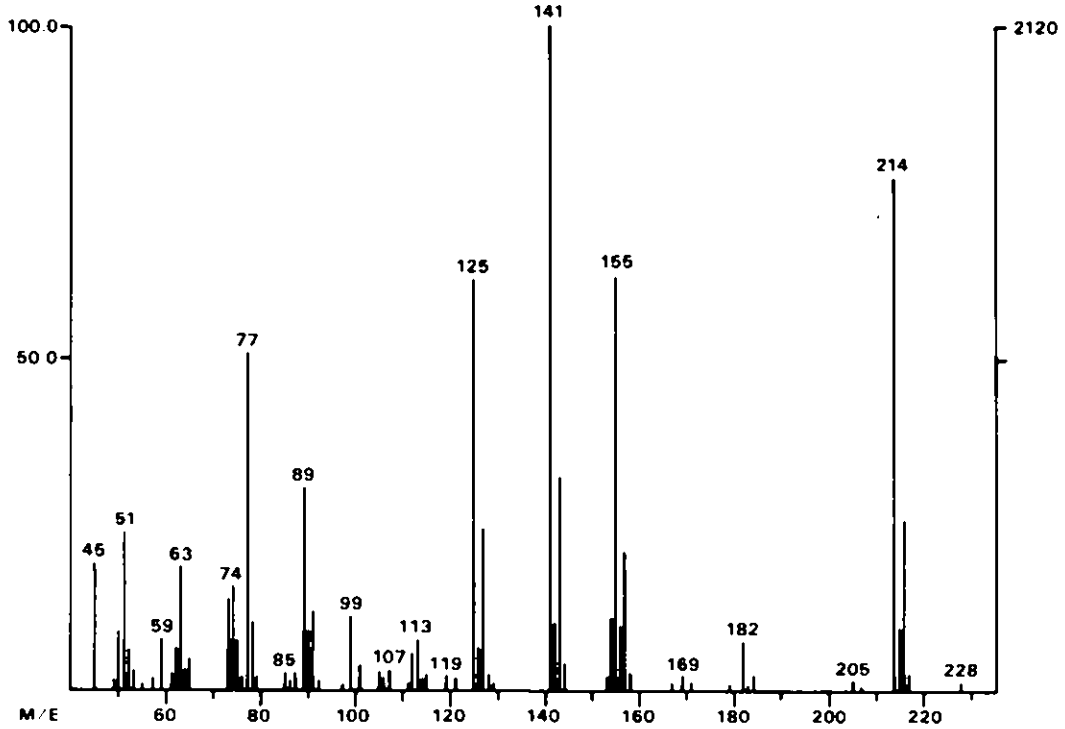


Figure 44. MCPA electron impact mass spectrum.

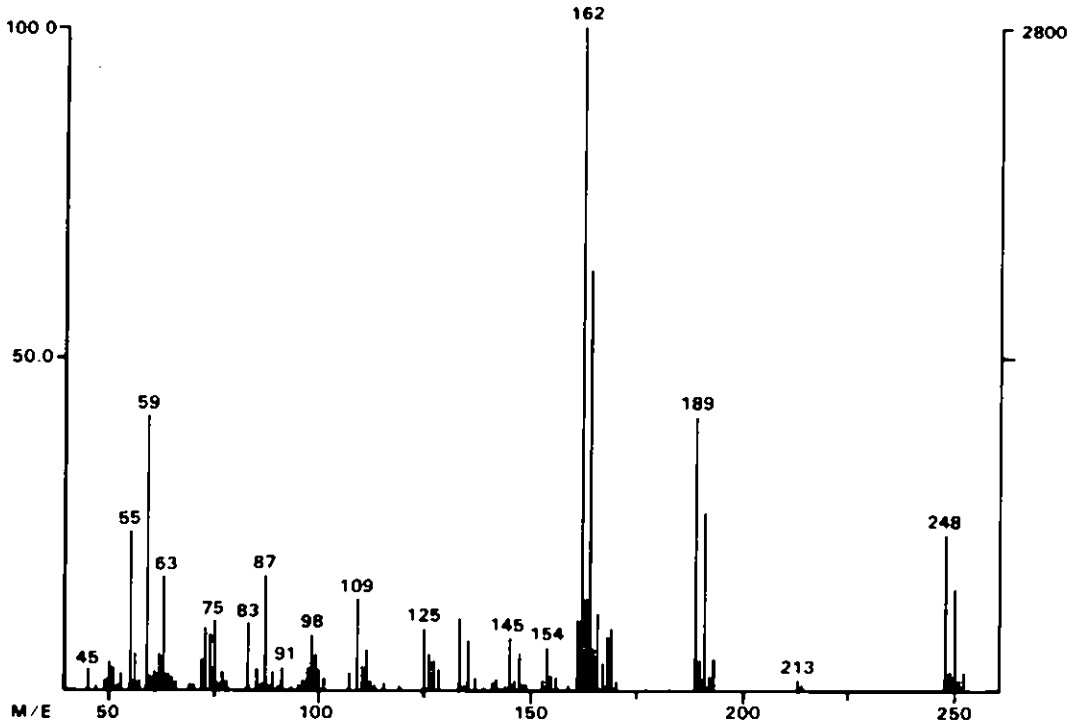


Figure 45. Dichlorprop electron impact mass spectrum.

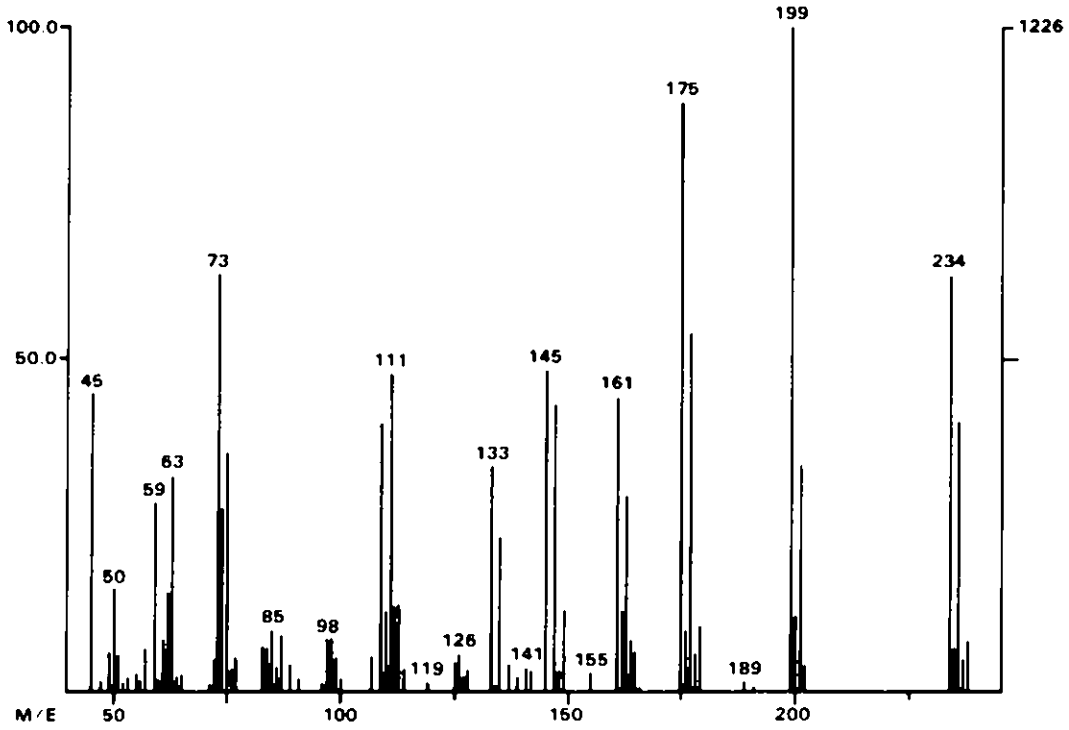


Figure 46. 2,4-D electron impact mass spectrum.

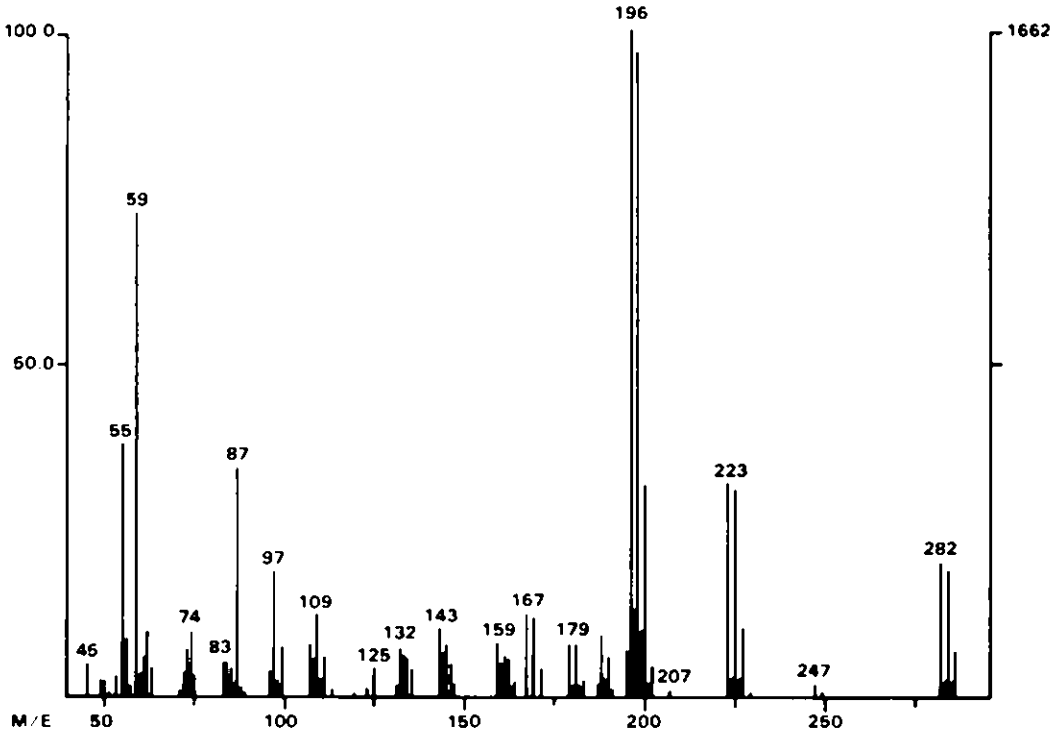


Figure 47. Silvex electron impact mass spectrum.

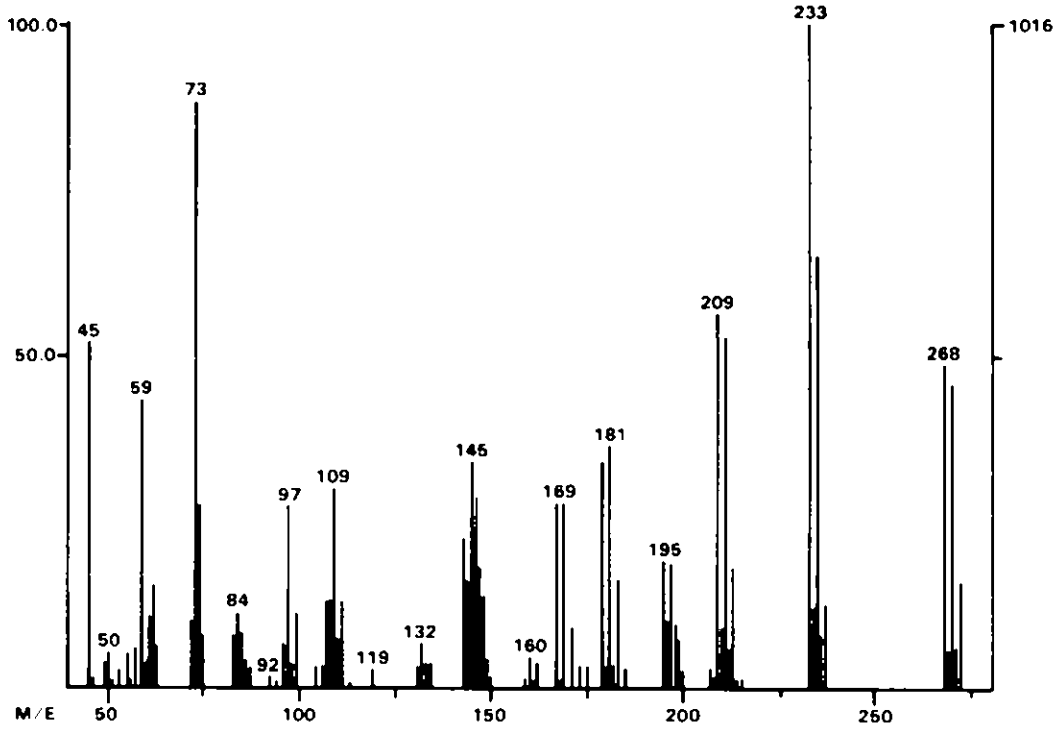


Figure 48. 2,4,5-T electron impact mass spectrum.

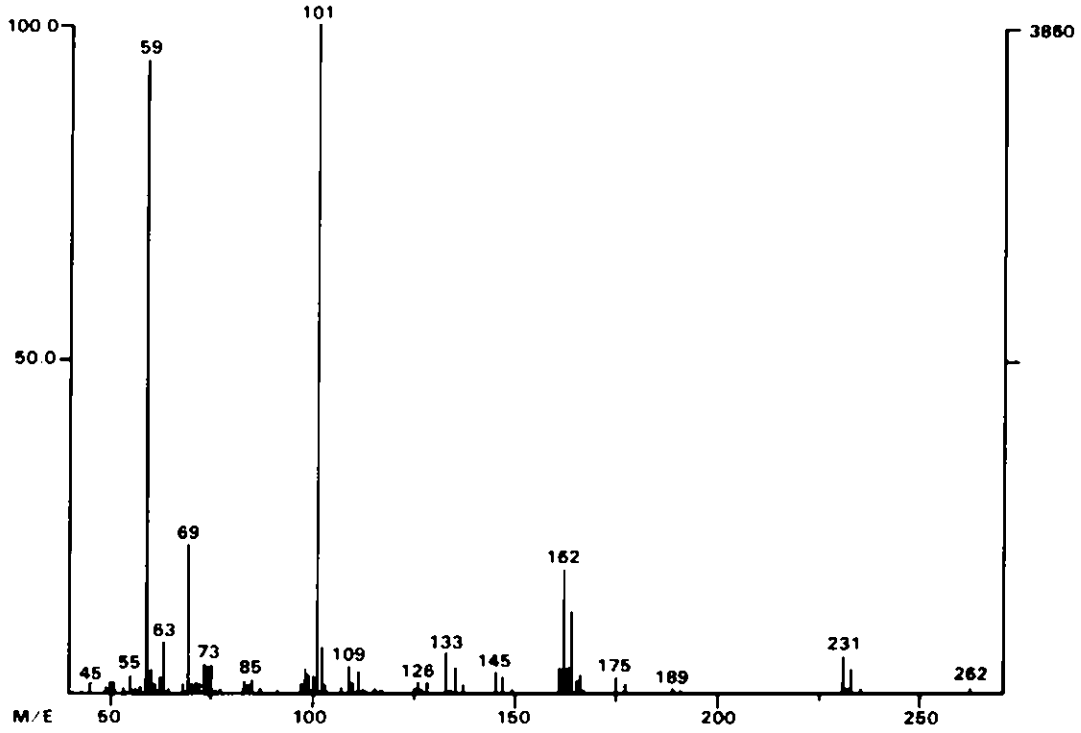


Figure 49. 2,4-DB electron impact mass spectrum.

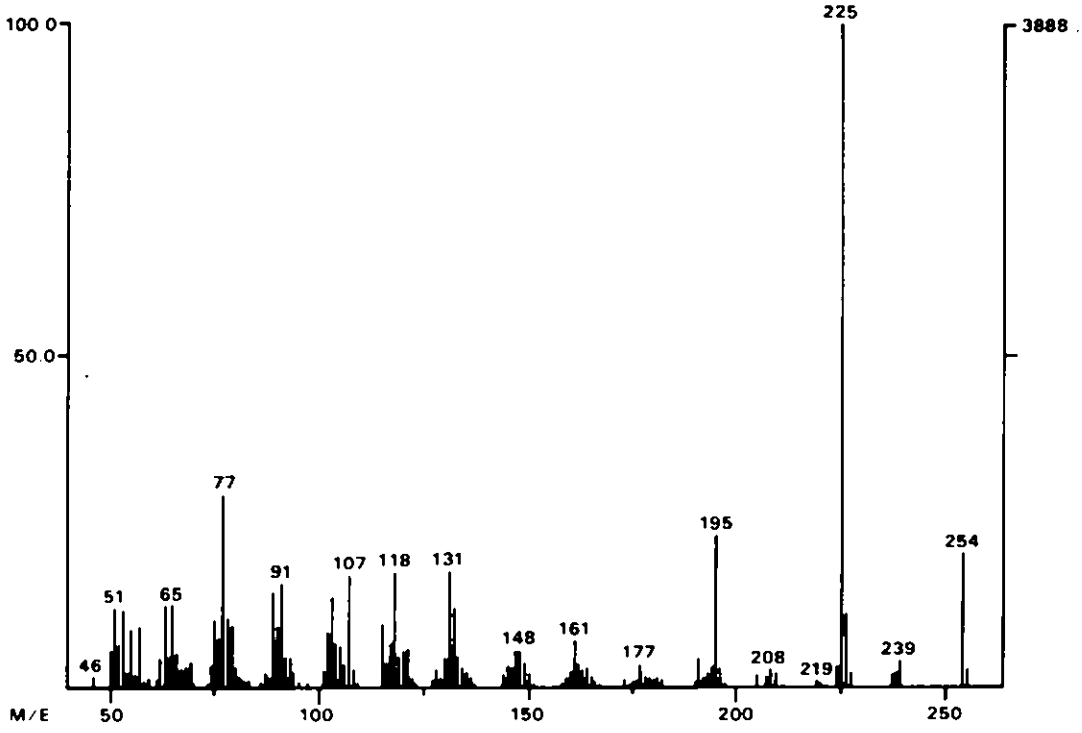


Figure 50. Dinoseb electron impact mass spectrum.

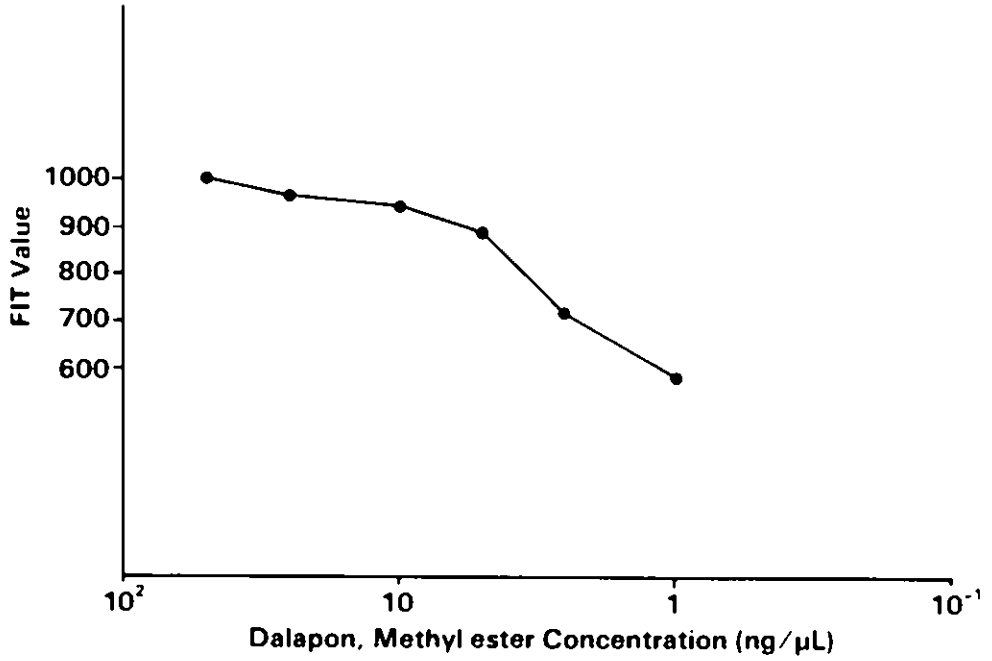


Figure 51. Dalapon Finnigan INCOS FIT value vs. concentration.

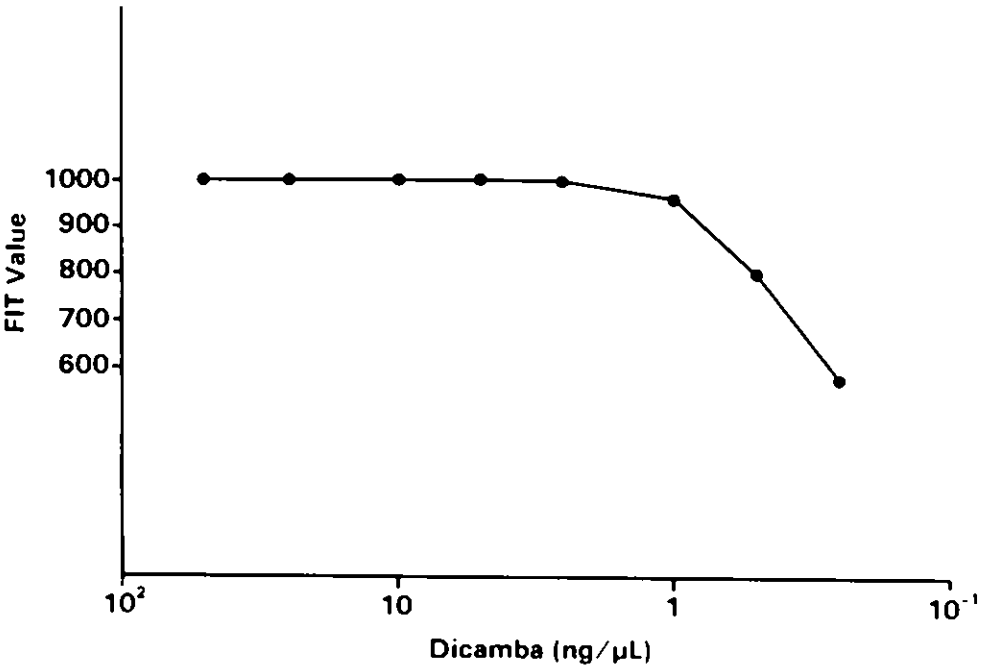


Figure 52. Dicamba Finnigan INCOS FIT value vs. concentration.

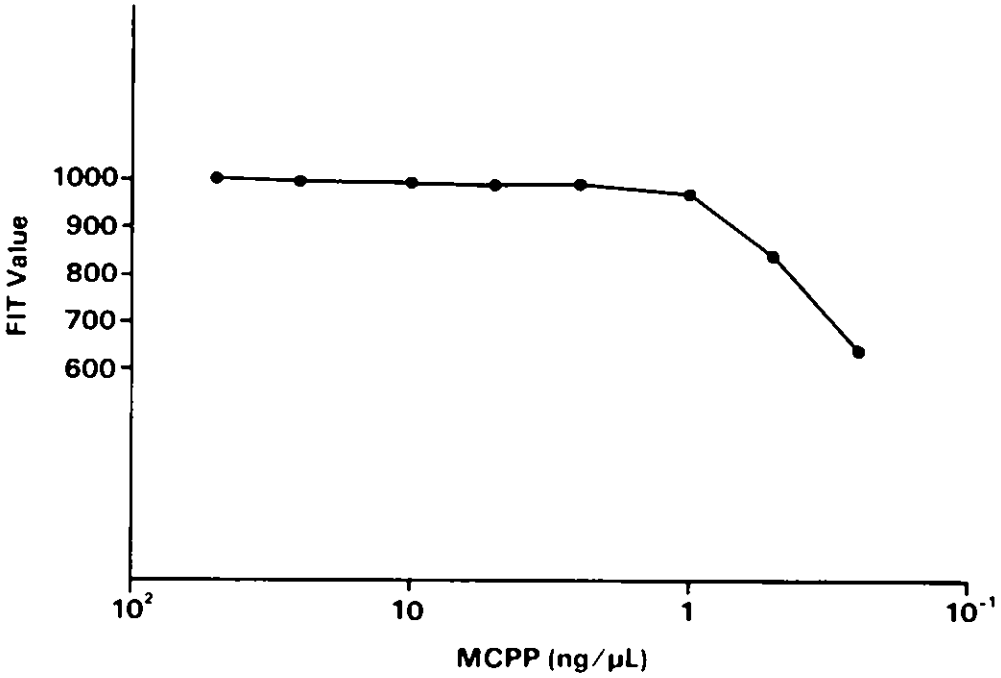


Figure 53. MCPP Finnigan INCOS FIT value vs. concentration.

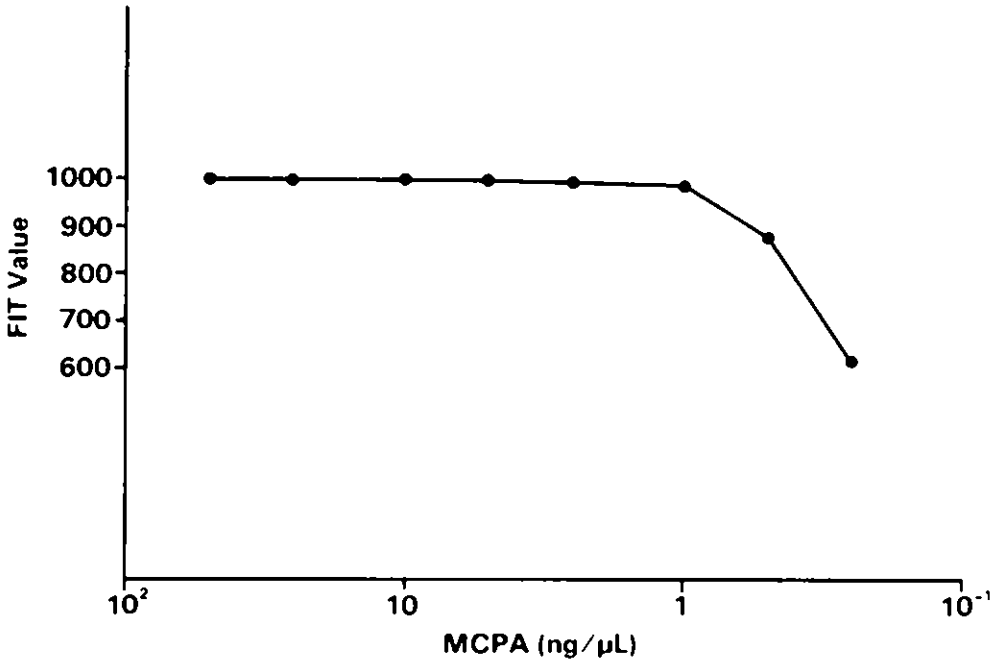


Figure 54. MCPA Finnigan INCOS FIT value vs. concentration.

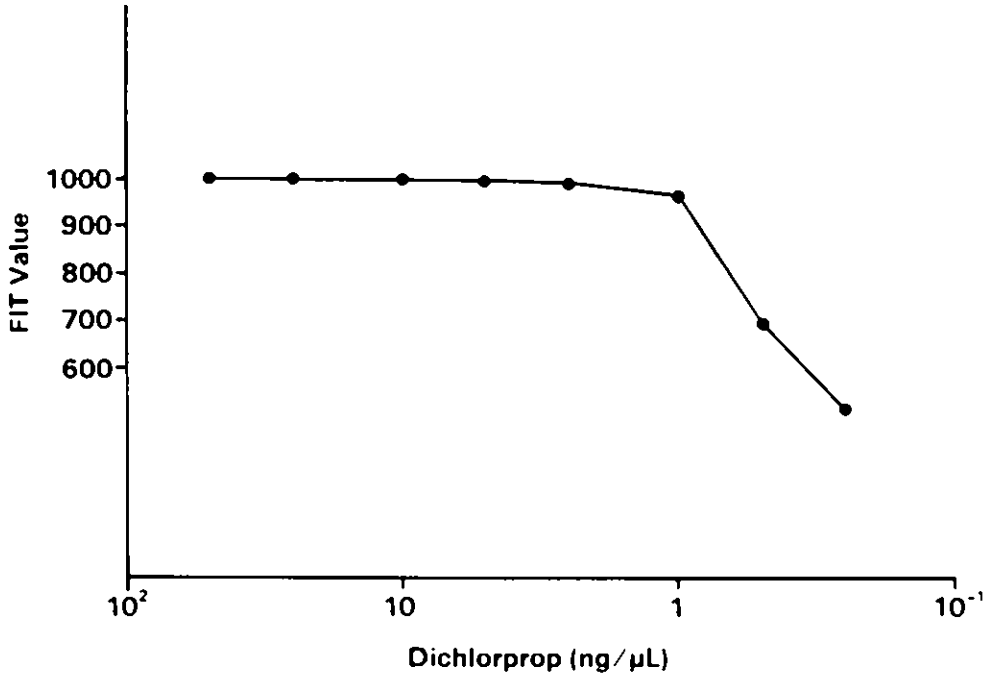


Figure 55. Dichlorprop Finnigan INCOS FIT value vs. concentration.

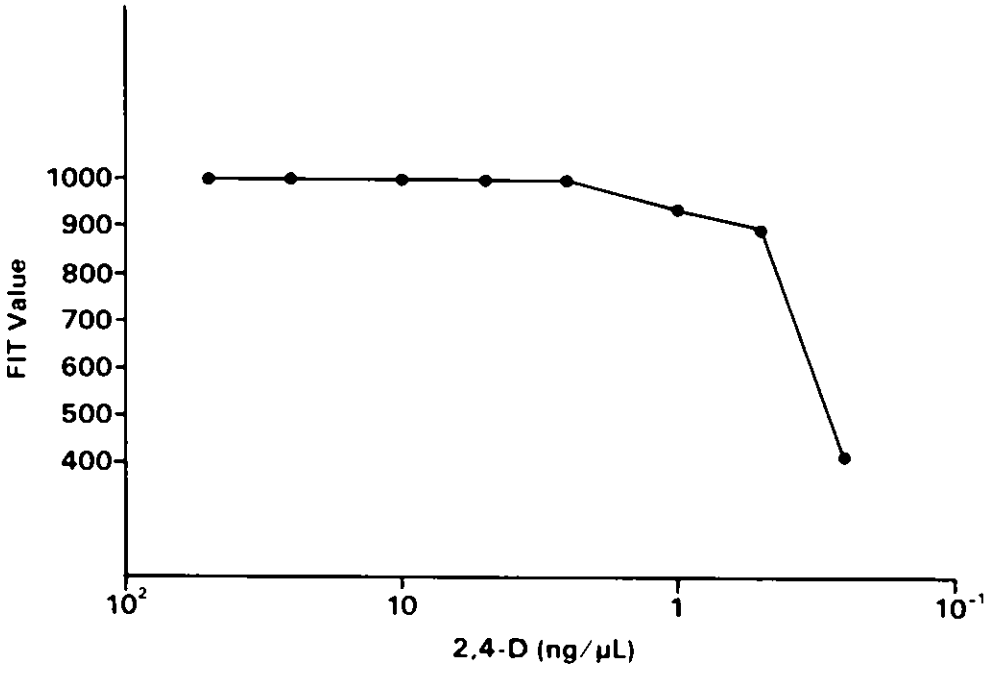


Figure 56. 2,4-D Finnigan INCOS FIT value vs. concentration.

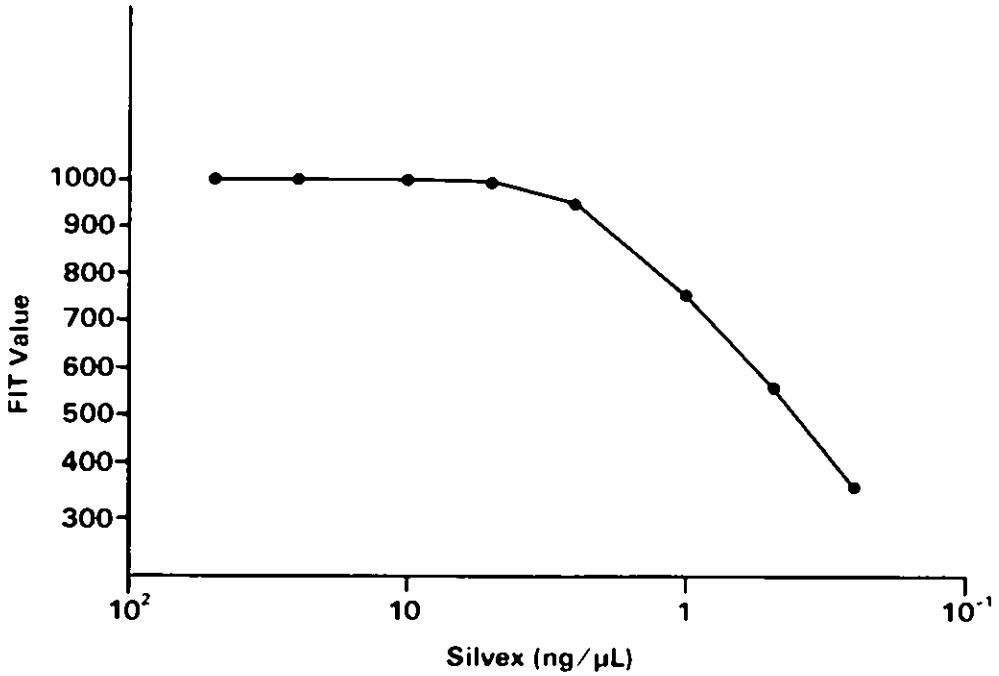


Figure 57. Silvex Finnigan INCOS FIT value vs. concentration.

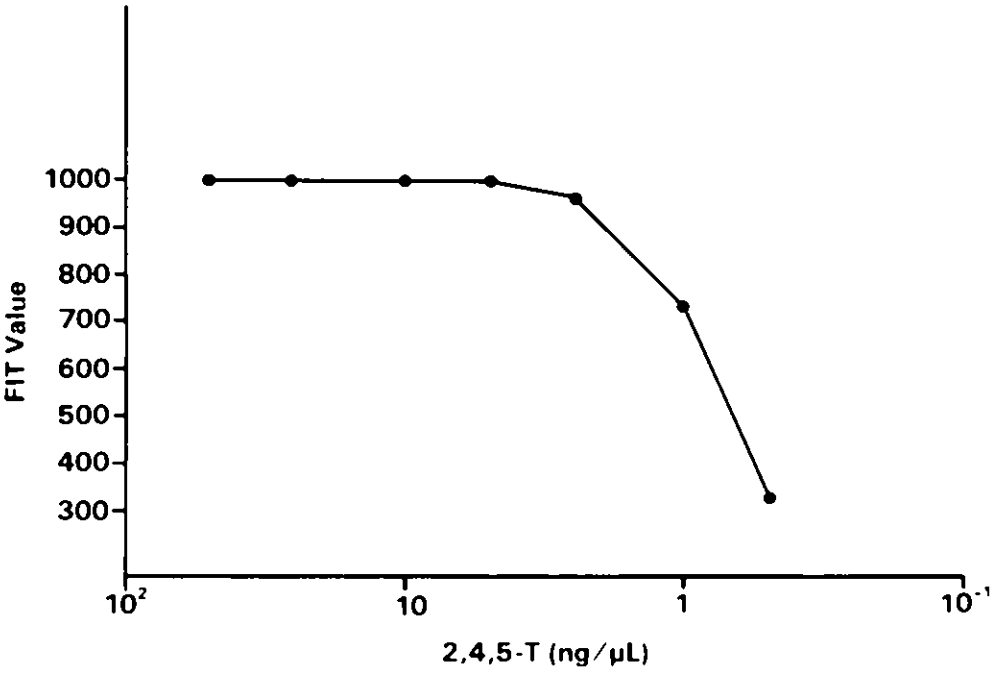


Figure 58. 2,4,5-T Finnigan INCOS FIT value vs. concentration.

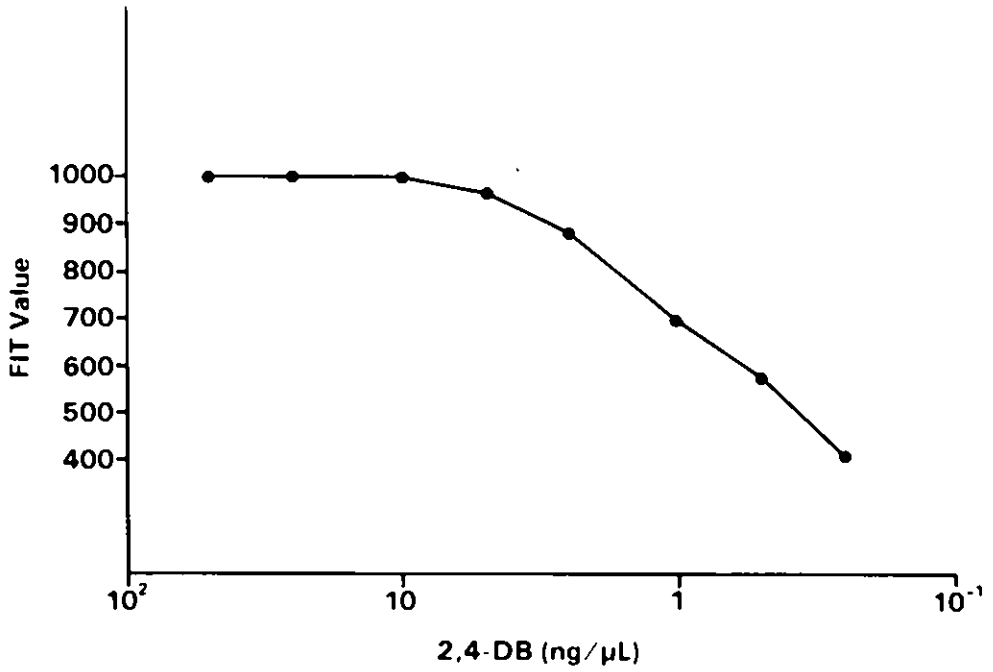


Figure 59. 2,4-DB Finnigan INCOS FIT value vs. concentration.

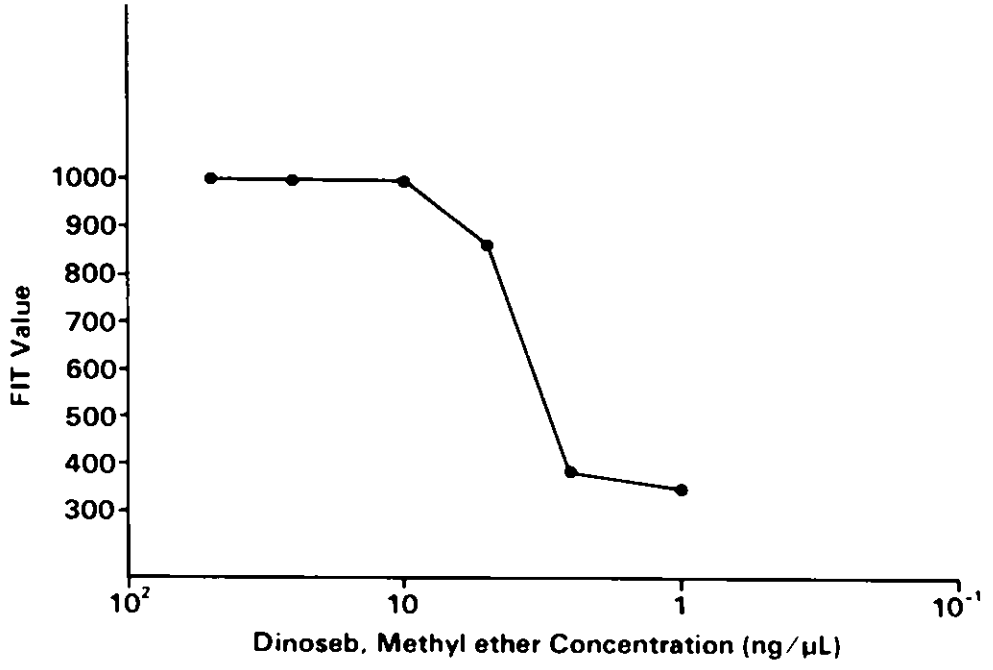


Figure 60. Dinoseb Finnigan INCOS FIT value vs. concentration.

TABLE 40. MINIMUM CONCENTRATIONS REQUIRED TO GIVE FULL SCAN MASS SPECTRA FIT VALUES OF 800 (1-µL INJECTION)

Analyte (as methyl derivative)	Concentration for FIT = 800
Dalapon	3.5 ng/µL
Dicamba	0.5 ng/µL
MCPP	0.43 ng/µL
MCPA	0.3 ng/µL
Dichlorprop	0.65 ng/µL
2,4-D	0.44 ng/µL
Silvex	1.25 ng/µL
2,4,5-T	1.3 ng/µL
2,4-DB	1.7 ng/µL
Dinoseb	4.5 ng/µL

CONCLUSIONS

All analytes, except Methyl Dalapon, gave intense molecular ions and characteristic fragment ions. The minimum amount of analyte required to give a good, full-scan mass spectrum is considerably higher than the detection limits for GC/EC.

SECTION 11

QUALITY CONTROL

INTRODUCTION

Before performing any analyses, the analyst must demonstrate the ability to safely handle toxic and hazardous diazomethane and the ability to generate acceptable accuracy and precision with this method. Acceptable accuracy and precision are described in this section. The minimum requirements of the quality control program consist of an initial demonstration of laboratory capability and regularly performed analysis of spiked samples as a continuing check on performance. Performance records must be maintained to allow comparison with previously recorded accuracy and precision of the method.

PERFORMANCE CRITERIA

Percent relative standard deviation must be <10 for all analytes measured and the mean percent recoveries must be >60 for all analytes measured.

The use of an internal standard for the analysis is strongly recommended. A standard solution of 1,4-dichlorobenzene, 0.2 mg/mL, when diluted 0.5 mL to 10 mL gives a suitable response on GC/EC. Percent recoveries can be calculated using response factors on the following equation:

% Recovery =

$$\frac{\text{analyte peak height in sample}}{\text{analyte peak height in stand.}} \times \frac{\text{1,4-dichlorobenzene peak height in stand.}}{\text{1,4-dichlorobenzene peak height in sample}}$$

The most convenient parameter to use for a quality control chart is the response of the 1,4-dichlorobenzene with an upper control limit of +3 σ from the mean and a lower control limit of -3 σ from the mean. A sample quality control chart is shown in Figure 61.

Each day the analyst must demonstrate through analysis of a method blank that all glassware and reagent interferences are under control.

CONCLUSIONS

The quality control measures recommended should be made part of the quality assurance plan of the laboratory to ensure known accuracy and precision for the analysis of chlorinated herbicides using the validated Method 8150 protocol described in Appendix A.

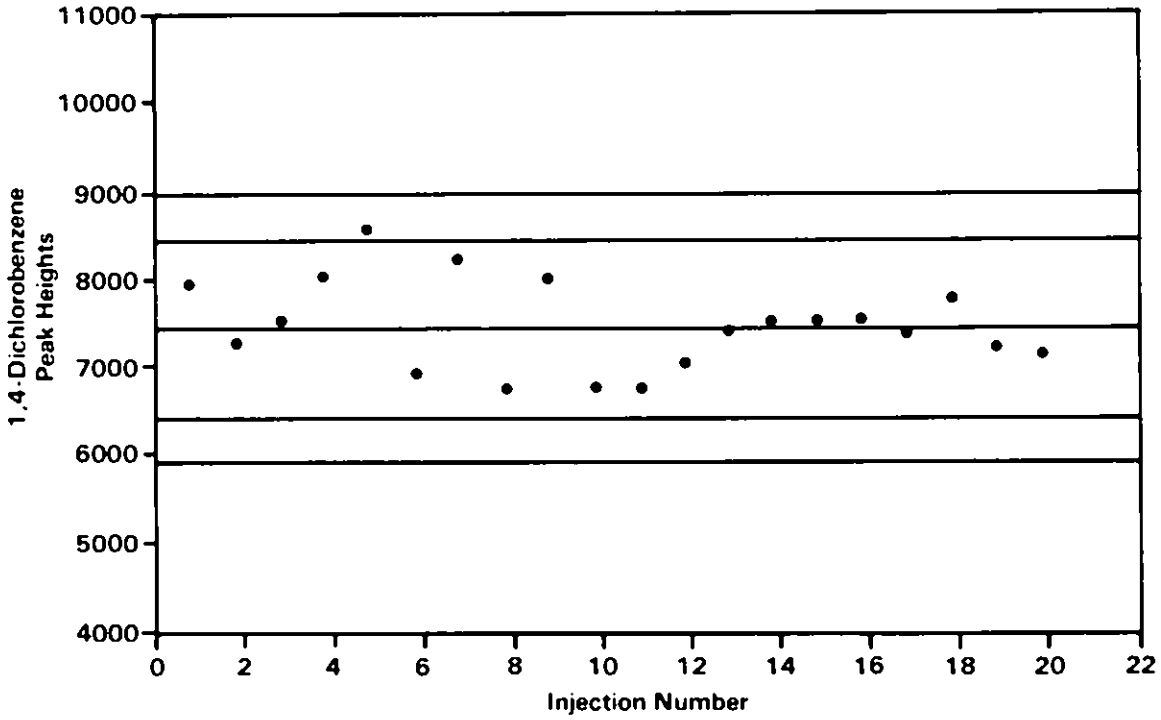


Figure 61. Quality control chart for GC/EC chlorinated herbicide analysis.

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APPENDIX A

Validated Method 8150, Chlorinated Herbicides by Methylation and GC/EC

1.0 Scope and Application

1.1 Method 8150 is a capillary gas chromatographic (GC) method for determining certain chlorinated acid herbicides in solid waste samples. Specifically, Method 8150 may be used to determine the following compounds:

2,4-D	MCPA
2,4-DB	MCPP
Dicamba	Silvex
Dichlorprop	2,4,5-T
Dinoseb	

Because these compounds are produced and used in various forms (i.e., acid, salt, ester, etc.), Method 8150 includes a hydrolysis step to convert the herbicide to the acid form prior to analysis.

1.2 When Method 8150 is used to analyze unfamiliar samples, compound identifications should be supported by at least one additional qualitative technique. Section 8.3 provides gas chromatograph/mass spectrometer (GC/MS) criteria appropriate for the qualitative confirmation of compound identifications.

1.3 The estimated detection limits for each of the compounds in solid waste samples are listed in Table A-1. The detection limits for a specific waste sample may differ from those listed, depending upon the nature of the interferences and the sample matrix.

1.4 CAUTION. Only experienced analysts should be allowed to work with diazomethane due to the potential hazards associated with its use (explosive, carcinogenic). Method 8150 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.

2.0 Summary of Method

2.1 Method 8150 provides extraction, esterification, and gas chromatographic conditions for the analysis of chlorinated acid herbicides in solid waste samples. Extraction is done by sonication of the acidified sample with methylene chloride. The methylene chloride extract is washed with base to remove the free acid herbicides, and the remaining methylene chloride solution of esters is hydrolyzed using potassium hydroxide. Extraneous organic material is removed by a solvent wash. The free acid herbicides and hydrolyzed ester herbicides can be combined to give total herbicides or they can be analyzed separately. After acidification, the acids are extracted with methylene chloride and converted to their methyl esters using diazomethane as the derivatizing agent. After excess reagent is removed, the esters are determined by gas chromatography with an electron capture detector (GC/EC). The results are reported as the acid equivalents.

2.2 The sensitivity of Method 8150 depends on the level of interferences in addition to instrumental limitations. Table A-1 lists the GC/EC and GC/MS limits of detection that can be obtained in solid waste in the absence of interferences. Detection limits for a typical waste sample should be higher.

3.0 Interferences

3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts or elevated baselines in gas chromatograms. All 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 8.1.

3.1.1 Glassware must be scrupulously cleaned. Clean each piece of glassware as soon as possible after use by rinsing it with the last solvent used in it. This should be followed by detergent washing with hot water and rinses with tap water, then with distilled water. Glassware should be solvent-rinsed with acetone and pesticide-quality hexane. After rinsing and drying, glassware should be sealed and stored in a clean environment to prevent any accumulation of dust or other contaminants. Store glassware inverted or capped with aluminum foil. Immediately prior to use, glassware should be rinsed with the next solvent to be used.

3.1.2 The use of high purity reagents and solvents helps minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.2 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from waste to waste, depending upon the nature and diversity of the waste being sampled.

TABLE A-1. CHROMATOGRAPHIC CONDITIONS* AND ESTIMATED DETECTION LIMITS FOR METHOD 8150

Analyte	Retention Time (minutes)	GC/EC Estimated Detection Limit** (ng/g)	GC/MS Estimated Identification Limit*** (ng)
Dicamba	13.47	0.12	0.5
MCPP	13.77	66	0.43
MCPA	13.96	43	0.3
Dichlorprop	14.51	0.38	0.65
2,4-D	14.76	0.34	0.44
Silvex	16.33	0.11	1.25
2,4,5-T	16.72	0.16	1.3
2,4-DB	17.82	4.0	1.7
Dinoseb	18.00	0.28	4.5

* Gas chromatography conditions are:
GC/EC: DB-5 capillary column, 0.25 µm film thickness, 0.25 µm I.D. X 30 M long. Grob-type 30-second splitless injection. Column temperature, programmed: initial 50°C for 1 min., program 25°C/min. to 100°C, hold for 1 min., program 12°C/min. to 220°C, hold for 12 min.
GC/MS: DB-5 capillary column, 1.0 µM film thickness, 0.23 µM I.D. X 30 M long. Grob-type 30-second splitless injection. Column temperature programmed: initial 60°C for 2 min., program 13°C/min. to 220°C, hold for 10 min.

** Detection limits determined from standard solutions corrected back to 50g samples, extracted and concentrated to 10 mL with 5 µL injected.

*** The minimum amount of analyte to give a Finnigan INCOS FIT value of 800 as the methyl derivative vs. the spectrum obtained from 50 ng of the respective free acid herbicide.

3.3 Organic acids, especially chlorinated acids, cause the most direct interference with the determination. Phenols, including chlorophenols, may also interfere with this procedure.

3.4 Alkaline hydrolysis and subsequent extraction of the basic solution removes many chlorinated hydrocarbons and phthalate esters that might otherwise interfere with the electron capture analysis.

3.5 The herbicides, being strong organic acids, react readily with alkaline substances and may be lost during analysis. Therefore, glassware must be acid-rinsed prior to use and then rinsed to constant pH with deionized H_2O.

3.6 Before processing any samples, the analyst should demonstrate daily, through the analysis of an organic-free water or solvent blank, that the entire analytical system is interference-free. Standard quality assurance practices should be used with this method. Field replicates should be collected to validate the precision of the sampling technique. Laboratory replicates should be analyzed to validate the precision of the analysis. Fortified samples should be analyzed to validate the accuracy of the analysis. Where doubt exists over the identification of a peak on the gas chromatogram, confirmatory techniques such as mass spectroscopy should be used. Detection limits for solid waste are given in Table A-1.

3.7 The sonication extraction must be optimized for each type of sample. It is suggested that tar-like samples be mixed with kaolin clay (Type P, Westwood Ceramic Supply, City of Industry, California) to allow efficient extraction. Clay samples are extracted efficiently in a pH range from 1 to 2.5 using 80 to 90 mL of buffer and sonicator power of 5 to 7.

4.0 Apparatus and Materials

4.1 Glassware (all specifications are suggested. Catalog numbers are included for illustration only).

4.1.1 Beaker: 400 mL. Thick wall.

4.1.2 Funnel: 75-mm diameter, 58°.

4.1.3 Separatory funnel: 500 mL, with Teflon stopcock.

4.1.4 Centrifuge bottle: 500 mL (Pyrex 1260 or equivalent).

4.1.5 Concentrator tube, Kuderna-Danish: 10 mL, graduated. Calibration must be checked at the volumes employed in the method. Ground-glass stopper is used to prevent evaporation of extracts.

4.1.6 Volumetric flask: 10 mL, with ground-glass stopper.

4.1.7 Evaporative flask, Kuderna-Danish: 500 mL. Attach to concentrator tube with springs.

4.1.8 Snyder column, Kuderna-Danish: three-ball macro.

4.2 Boiling chips: approximately 10/40 mesh. Heat to 400°C for 30 min. or perform Soxhlet extract with methylene chloride.

4.3 Diazald® Kit: recommended for the generation of diazomethane (available from Aldrich Chemical Co., Cat. No. Z10, 025-0).

4.4 Water bath: Heated, with concentric ring cover, capable of temperature control (± 2°C). The bath should be used in a hood.

4.5 Filter paper: 15-cm diameter (Whatman #1 or equivalent).

4.6 Balance: Analytical, capable of accurately weighing to the nearest 0.0001 g.

4.7 Pipet: Pasteur, glass, disposable (140-mm x 5-mm I.D.).

4.8 Centrifuge (International Equipment Corporation, Model K or equivalent).

4.8.1 Capillary Column: 30 m x 0.32 mm DB-5 (J & W Scientific, Inc., or equivalent): Film thickness: 1 µm.

4.9 Sonicator (Heat Systems Ultrasonics, Inc., Model W375 or equivalent, with 20 KHz Ultrasonic Convertor Model C3 or equivalent).

4.10 Gas chromatograph: Analytical system complete with gas chromatograph suitable for Grob-type injection using capillary columns and all required accessories including syringes, capillary analytical column, gases, detector, and stripchart recorder. A data system is recommended for measuring peak areas or peak heights.

5.0 Reagents

5.1 Reagent water: reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.

5.2 Sodium hydroxide solution (0.1 M): dissolve 4 g NaOH in reagent water and dilute to 1000 mL.

5.3 Potassium hydroxide solution: 37% aqueous solution (w/v). Prepare with reagent grade potassium hydroxide pellets and reagent water.

5.4 Phosphate buffer pH = 2.5 (0.1 M): Dissolve 12 g NaH_2PO_4 in reagent water and dilute to 1000 mL. Add phosphoric acid to adjust to pH = 2.5.

5.5 Methylene chloride, acetone, methanol: pesticide quality or equivalent.

5.6 Carbitol (diethylene glycol monoethyl ether).

5.7 N-methyl (N-nitroso-p-toluenesulfonamide) (Diazald®): high purity, available from Aldrich Chemical Co.

5.8 Silicic acid: 100-mesh powder (analytical reagent).

5.9 Stock standard solutions (500 ng/µL): stock standard solutions may be prepared from pure standard materials or purchased as certified solutions.

5.9.1 Prepare stock standard solutions by accurately weighing about 0.0500g of pure acid. Dissolve the material in pesticide-quality acetone and dilute to volume in a 10-mL volumetric flask. Other volumes may be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight may be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards may be used at any concentration if they are certified by the manufacturer or by an independent source.

5.9.2 Store stock standard solutions at 4°C and protect from light. Stock standard solutions should be checked frequently for signs of degradation or evaporation, especially immediately prior to preparing calibration standards from them.

5.9.3 Stock standard solutions must be replaced immediately if comparison with check standards indicates a problem. Otherwise, stock solutions should be replaced after one week.

6.0 Sample Collection, Preservation, and Handling

6.1 Grab samples must be collected in glass containers. Conventional sampling practices should be followed; however, the bottle must not be prerinsed with the 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 and other potential sources of contamination.

6.2 The samples must be stored at 4°C from the time of collection until extraction.

6.3 All samples must be extracted within 7 days of collection and must be completely analyzed within 30 days of extraction.

7.0 Procedures

7.1 Sample preparation

7.1.1 Thoroughly mix moist solids and weigh an amount of wet sample equivalent to 50 g of dry weight into each of 400-mL, thick-wall beakers.

7.1.2 Acidify solids in each beaker with 85 mL of 0.1M phosphate buffer (pH = 2.5) and thoroughly mix the contents with a glass stirring rod.

7.1.3 Add 100 mL of methylene chloride to each beaker containing the sample. Sonicate the samples for 3 minutes in the pulsed mode at 50 percent duty cycle at an output of 6.3.

7.1.4 Allow the solids to settle. Transfer the organic layer into a 500-mL centrifuge bottle.

7.1.5 Sonicate the sample two more times using the same condition with 100 mL of methylene chloride each time.

7.1.6 Combine the three organic extracts from the sample in the centrifuge bottle and centrifuge 10 minutes to settle the fine particles. Filter the extracts through Whatman #1 filter paper into 500-mL separatory funnels.

7.1.7 Wash the organic extracts two times with 100-mL portions of 0.1 N aqueous sodium hydroxide each time. Combine the aqueous layers containing the salts of the free acid herbicides in a beaker and save. The organic layer contains the herbicide esters, which must be hydrolyzed as follows:

7.1.7.1 Transfer the methylene chloride solution into 500-mL Kuderna-Danish flasks. Add boiling chips to the extracts in the flasks and fit them with three-ball Snyder columns. Evaporate the methylene chloride on the water bath to a volume of approximately 25 mL.

7.1.7.2 Remove the flasks from the water bath. Allow them to cool. Add 5 mL of 37% aqueous potassium hydroxide, 30 mL of distilled water and 40 mL of methanol into the extracts.

7.1.7.3 Add additional boiling chips to the flasks. Reflux the mixtures on a 60°-65°C water bath for 2 hours. Remove the flasks from the water bath and cool to room temperature.

7.1.8 At this point the basic solutions containing the herbicide salts from 7.1.7 can be combined or they can be analyzed separately.

7.1.9 Add phosphoric acid to the basic aqueous extracts to adjust the pH to $\leq$1.

7.1.10 Transfer the acidified aqueous solution into a 500-mL separatory funnel and extract the solution two times with 100 mL of methylene chloride.

7.1.11 Combine the organic extracts in 500 mL Kuderna-Danish flasks. Add boiling chips to the extracts in the flasks and fit them with three-ball Snyder columns.

7.1.12 Evaporate the methylene chloride to approximately 5 mL on a hot water bath (80°-85°C).

7.1.13 Remove the flasks from water bath. Evaporate the extracts just to dryness under a stream of nitrogen.

7.1.14 Reconstitute with 1 mL of iso-octane and 0.5 mL of methanol. Dilute to a volume of 4 mL with ether. The sample is now ready for methylation with diazomethane.

7.2 Esterification

7.2.1 The diazomethane derivatization (1) procedure described below will produce an efficient reaction with all of the chlorinated herbicides described in this method and should be used only by experienced analysts, due to the potential hazards associated with its use. Diazomethane is a carcinogen and can explode under certain conditions. The following precautions should be taken:

- Use a safety screen.
- Use mechanical pipetting aides.
- Do not heat above 90°C - EXPLOSION may result.
- Avoid grinding surfaces, ground-glass joints, sleeve bearings, and glass stirrers - EXPLOSION may result.
- Store away from alkali metals - EXPLOSION may result.
- Solutions of diazomethane decompose rapidly in the presence of solid materials such as copper powder, calcium chloride, and boiling chips.

7.2.2 Instructions for preparing diazomethane are provided with the generator kit.

7.2.3 Add 2 mL of diazomethane solution and let the sample stand for 10 minutes with occasional swirling. The yellow color of diazomethane should be evident and should persist for this period.

7.2.4 Rinse inside wall of ampule with several hundred µL of ethyl ether. Reduce the sample to approximately 2 mL to remove excess diazomethane by allowing solvent to evaporate spontaneously (room temperature). Alternatively, silicic acid, about 10 mg, can be added to destroy the excess diazomethane.

7.2.5 Dilute the sample to 10.0 mL using hexane.

7.3 Gas chromatography conditions

GC/EC: DB-5 capillary column, 0.25 µm film thickness, 0.25 µm I.D. X 30M long. Grob-type 30-second splitless injection. Column temperature, programmed: initial 50°C for 1 min., program 25°C/min. to 100°C, hold for one min., program 12°C/min. to 220°C, hold for 12 min. The retention times of each analyte are shown in Table A-1.

7.4 Calibration

7.4.1 Establish gas chromatographic operating parameters equivalent to those indicated above and in Table 1. The gas chromatographic system can be calibrated using the external standard technique (Section 7.4.2) or the internal standard technique (Section 7.4.3).

7.4.2 External standard calibration procedure

7.4.2.1 For each parameter of interest, prepare working standards at a minimum of three concentration levels by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with diethyl ether. One of the external standards should be at a concentration near, but above, the method detection limit. The other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

7.4.2.2 Prepare calibration standards from the free acids by esterification of the working standards as described under Sample Preparation, Section 7.1.13 and subsequent steps. Using injections of 2 to 5 µL of each esterified working standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each parameter. Alternatively, the ratio of the response to the mass injected, defined as the calibration factor (CF), can be calculated for each parameter at each standard concentration. If the relative standard deviation of the calibration factor is less than 10% over the working range, linearity through the origin can be assumed and the average calibration factor can be used in place of a calibration curve.

7.4.2.3 The working calibration curve or calibration factor must be verified on each working day by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor may be prepared for that parameter.

7.4.3 Internal standard calibration procedure.

To use this approach, the analyst must select one or more internal standards similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. The standard 1,4-dichlorobenzene is suggested as one possibility.

7.4.3.1 Prepare working standards, at a minimum of three concentration levels for each parameter of interest in the acid form, by adding volumes of one or more stock standards to a volumetric flask. Dilute to volume with diethyl ether. One of the standards should be at a concentration near, but above, the method detection limit. The other concentrations should correspond to the expected range of concentrations found in real samples, or should define the working range of the detector.

7.4.3.2 Prepare calibration standards from the free acids by esterification of the working standards as described under Sample Preparation, Section 7.1.13 and subsequent steps.

7.4.3.3 Prior to dilution to final volume for GC analysis, add a known constant amount of one or more internal standards to each calibration standard.

7.4.3.4 Using injections of 2 to 5 µL of each calibration standard, tabulate the peak height of area responses against the concentration for each compound and for each internal standard. Calculate response factors (RF) for each compound as follows:

$$RF = (A_S C_{IS})/(A_{IS} C_S)$$

where:

A_S = Response for the parameter to be measured.

A_{IS} = Response for the internal standard.

C_{IS} = Concentration of the internal standard in µg/L.

C_S = Concentration of the parameter to be measured in µg/L.

If the RF value over the working range is constant, less than 10% relative standard deviation, the RF can be assumed to be invariant and the average RF can be used for calculations. Alternatively, the results can be used to plot a calibration curve of response ratios, A_S/A_{IS} against RF.

7.4.3.5 The working calibration curve or RF must be verified on each working day by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.

7.4.4 The analyst must process a series of standards through the procedure to validate elution patterns and the absence of interferences from the reagents.

7.5 Analysis

7.5.1 Inject 2 to 5 µL of the sample extract using the solvent-flush technique. Smaller (1.0-µL) volumes can be injected if automatic devices are employed. Record the volume injected to the nearest 0.05 µL, and record the resulting peak size in area units.

7.5.2 If the peak area exceeds the linear range of the system, dilute the extract and reanalyze.

7.5.3 A sample chromatogram for methylated chlorophenoxy herbicides is shown in Figure A-1.

7.5.4 Precision and accuracy expected are shown in Table A-2.

8.0 Quality Control

8.1 Before processing any samples, the analyst should demonstrate through the analysis of a distilled water method blank that all glassware and reagents are interference free. Each time a set of samples is extracted or there is a change in reagents, a method blank should be processed as a safeguard against chronic laboratory contamination.

8.2 Standard quality assurance practices should be used with this method. Field replicates should be collected to validate the precision of the sampling technique. Laboratory replicates should be analyzed to validate the precision of the analysis. Fortified waste samples should be analyzed to validate the accuracy of the analysis. Detection limits to be used for samples are indicated in Table A-1. It is suggested that the response of the internal or external standard be plotted daily as a quality control check. Where doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as mass spectrometry should be used (Section 8.3).

8.3 GC/MS Confirmation

8.3.1 GC/MS techniques should be judiciously employed to support qualitative identifications made with this method. The mass spectrometer should be capable of scanning the mass range from 35 amu to a mass 50 amu above the molecular weight of the compound. The instrument must be capable of scanning the mass range at a rate to produce at least 5 scans per peak but not to exceed 3 sec. per scan utilizing 70 V (nominal) electron energy in the electron impact ionization mode. A GC-to-MS interface constructed of all-glass or glass-lined materials is recommended. A computer system that allows the continuous acquisition and storage (on machine-readable media) of all mass spectra obtained throughout the duration of the chromatographic program should be interfaced to the mass spectrometer.

8.3.2 Gas chromatographic columns and conditions:

Instrument:	Finnigan 9610 GC/Finnigan 4023 mass spectrometer.
Column:	DB-5, 1.0 µM film thickness, 0.32 µM ID X 30M L.
Injection:	1 µL, Grob-type 30-sec. splitless injection.
Injector Temperature:	220°C.
Temperature Program:	60°C for 2 minutes, 13°C/minute to 220°C, hold for 10 minutes.
Electron Multiplier Voltage:	-1200V.
Scan Range:	45amu - 550amu.
Scan Time:	1 scan/sec.
Data System:	Data General NOVA 3 with INCOS.

8.3.3 At the beginning of each day that confirmatory analyses are to be performed, the GC/MS system must be checked to see that all DFTPP (decafluorotriphenyl phosphine) performance criteria are achieved, as described in Method 8250 of SW-846.

8.3.4 To confirm an identification of a compound, the background-corrected mass spectrum of the compound must be obtained from the sample extract and compared with a mass spectrum from a stock or calibration standard analyzed under the same chromatographic conditions. The following criteria must be met for qualitative confirmation:

1. The molecular ion and all other ions present above 10% relative abundance in the mass spectrum of the standard must be present in the mass spectrum of the sample with agreement to ±10%. For example, if the relative abundance of an ion is 30% in the mass spectrum of the standard, the allowable limits for the relative abundance of that ion in the mass spectrum for the sample would be 20-40%.

2. The mass spectra obtained from 50 ng of herbicide as the methyl derivative can be used to establish a library. Measurements of the resemblance of the library spectrum to the spectrum of less concentrated samples can be done by the computer (the FIT number of library search in INCOS). A FIT value of 800 or greater is acceptable.

3. The retention time of the compound in the sample must be within 6 sec. of the retention time for the same compound in the standard solution.

4. Compounds that have very similar mass spectra can be explicitly differentiated by GC/MS only on the basis of retention time data.

5. Should these MS procedures fail to provide satisfactory results, additional steps may be taken before reanalysis. These steps may include the use of alternate GC columns or additional cleanup.

9.0 References

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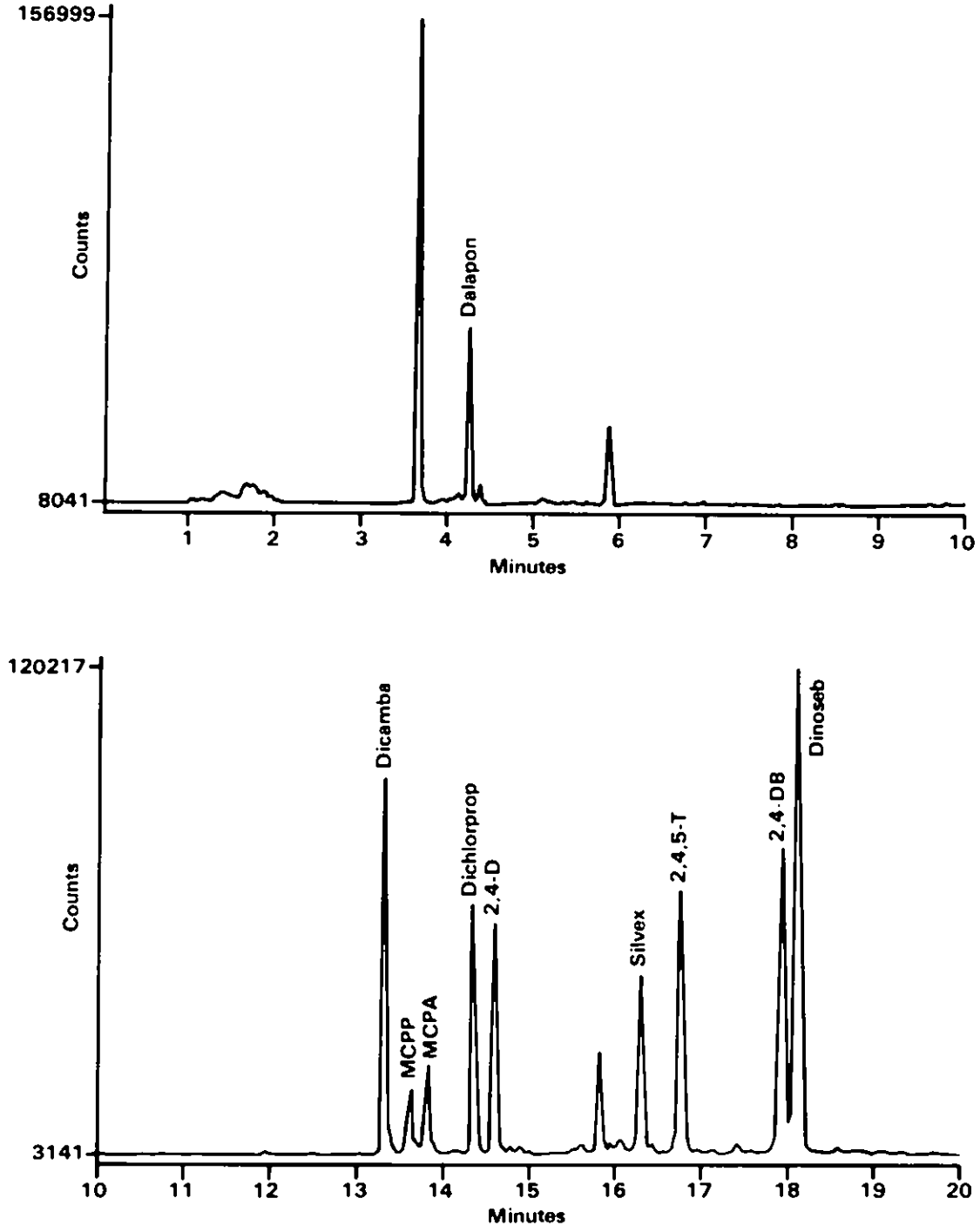

Figure A-1. Gas chromatogram of methylated herbicides.

TABLE A-2. ACCURACY AND PRECISION FOR METHOD 8150

Analyte	Mean* Percent Recovery	Linear** Concentration Range (ng/g)	Percent Relative*** Standard Deviation (n=20)
Dicamba	95.7	0.52- 104	7.5
MCPP	98.3	620 -61,800	3.4
MCPA	96.9	620 -61,200	5.3
Dichlorprop	97.3	1.5 - 3,000	5.0
2,4-D	84.3	1.2 - 2,440	5.3
Silvex	94.5	0.42- 828	5.7
2,4,5-T	83.1	0.42- 828	7.3
2,4-DB	99.7	4.0 - 8,060	7.6
Dinoseb	93.7	0.82- 1,620	8.7

* Mean percent recovery calculated from 10 determinations of spiked clay and clay/still bottom samples over the linear concentration range.

** Linear concentration range was determined on standard solutions and corrected to 50g solid samples.

*** Percent relative standard deviation was calculated on standard solutions, 10 samples high in the linear concentration range, and 10 samples low in the range.

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(Please read Instructions on the reverse before completing)

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16. ABSTRACT

A single laboratory validated analytical protocol is described, which is applicable to the determination of the herbicides Dicamba, Silvex, 2,4-D, 2,4-DB, 2,4,5-T, Dinoseb, MCPP, and MCPA, in hazardous waste extracts. The method consists of herbicide hydrolysis followed by diazomethane esterification and subsequent determination of the herbicide methyl esters by capillary column gas chromatography with electron capture detection (GC/ECD). An electron impact gas chromatography/mass spectrometric (GC/MS) confirmation of the GC/ECD results is included. The protocol validation procedure consisted of ruggedness testing, simplex optimization of key experimental variables, and the determination of extraction recoveries, detection limits, and the GC/ECD linear dynamic range for each herbicide methyl ester. This protocol, which employs a single fused silica capillary column separation for all the target methyl esters, is a significant improvement over earlier gas chromatographic (GC) procedures which utilize three different packed GC columns. The method, however, was inapplicable to Dalapon which eliminates hydrogen chloride during the sample workup.

17. KEY WORDS AND DOCUMENT ANALYSIS

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