Journal of Chromatography, 537 (1991) 385–395
Elsevier Science Publishers B.V., Amsterdam

CHROM. 22 759

Determination of nine acidic herbicides in water and soil by gas chromatography using an electron-capture detector

FUNG NGAN*,[a] and THOMAS IKESAKI
Anlab, 1914 S Street, Sacramento, CA 95814 (U.S.A.)
(First received May 1st, 1990; revised manuscript received August 14th, 1990)

ABSTRACT

Two simple and convenient methods are described for the extraction, analysis and clean-up of nine acidic herbicides in water and soil samples. The extracted acidic herbicides were converted into their methyl esters by a modified method using diazomethane. The methylated herbicides were analyzed by gas chromatography with electron-capture detection using a Restek Rt_x-35 0.53 mm I.D. capillary column and simultaneously confirmed using an Rt_x-5 capillary column. A simple clean-up procedure using a micro disposable Florisil column is also described. The mean recoveries for all herbicides from water were >95% and from soils >86%. The recoveries of herbicides after Florisil column clean-up were greater than 89%. Each sample run required about 25 min, including confirmation. The methods described are suitable as an initial screening procedure in the rapid simultaneous determination of nine acidic herbicides in large numbers of environmental samples at reasonable cost.

INTRODUCTION

Chlorophenoxy acid herbicides and related compounds (Table I) are widely used to control broad-leaved weeds and other vegetation. They are inexpensive and very potent even at low concentrations. These herbicides are formulated in the form of esters, alkaline salts and acids. After application, they may pass into streams, rivers or lakes with the possibility of environmental contamination. Several reports [1–5] have described the effects of ingestion of these herbicides by humans. Generally they cause pyrexia, nausea, hypotonia, confusion, coma, metabolic acidosis, convulsions, cytoskeletal perturbation and renal damage.

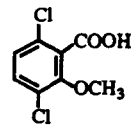
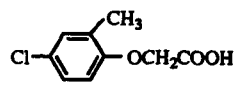
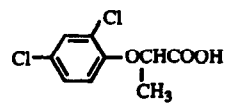
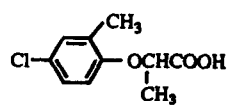
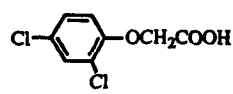
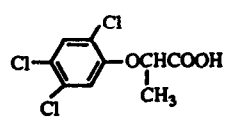
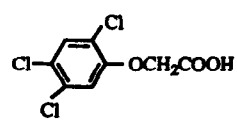
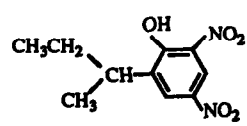
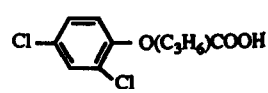
Many methods for the determination of different herbicides have been described [6–11]. At present, there are two Environmental Protection Agency (EPA) methods (615, 8150) [12,13] that have been standardized and are recommended for the determination of these types of herbicides in water and soil, respectively. However, the procedures in these two methods are cumbersome and time consuming. It is impossible to analyse large numbers of samples within a reasonable time and cost by using the current procedures. Recently, new methods [14,15] have been described that

[a] Present address: 1075 Olive Dr. 38, Davis, CA 95616, U.S.A.

0021-9673/91/$03.50

TABLE I

HERBICIDES USED

Systematic name (common name)	Structure
3,6-Dichloro-2-methoxybenzoic acid (dicamba)	
4-Chloro-2-methylphenoxyacetic acid (MCPA)	
2-(2,4-Dichlorophenoxy)propionic acid (dichlorprop)	
2-(4-Chloro-2-methylphenoxy)propionic acid (mecoprop)	
2,4-Dichlorophenoxyacetic acid (2,4-D)	
2-(2,4,5-Trichlorophenoxy)propionic acid (fenoprop)	
2,4,5-Trichlorophenoxyacetic acid (2,4,5-T)	
2-*sec*-Butyl-4,6-dinitrophenol (dinoseb)	
4-(2,4-Dichlorophenoxy)butyric acid (2,4-DB)	

will replace EPA method 8150 [13]. However, the procedures are still long and time consuming. In addition, no clean-up procedures were suggested [15]. Currently there is no alternative efficient method to replace EPA method 615 for the analysis of water samples.

The purpose of this work was to develop a simple, rapid procedure for the determination of these herbicides in environmental soil and water samples. The methods proposed are suitable as an initial screening procedure in the rapid analysis of large numbers of samples.

Generally, the identification of a herbicide in one chromatographic column is not sufficient and should be confirmed by a secondary analysis. The methods described elsewhere [12,13] required three or four chromatographic columns for confirmation, with inconvenient column changes. Gas chromatography–mass spectrometry GC–MS has been suggested [15] for confirmation. However, the price of the instrumentation is high and sample analysis becomes expensive. In this paper, we suggest a dual-column approach for initial confirmation. Herbicides found in one column can be confirmed simultaneously using a different column.

Herbicides present in the environment might be in salt form or as esters such as butyl, isooctyl or butoxyethyl. These different esters show different retention times in gas chromatography (GC) and therefore they must be hydrolysed before any analysis. After hydrolysis, the herbicide slats are acidified. However, the resulting acid forms of the herbicides are insufficiently volatile and sensitive for analysis by gas chromatography (GC), hence they must be converted into a more volatile form. Esterification silylation, alkylation and other derivatization procedures have been used to convert stable compounds into more volatile derivatives before analysis [6,14,16–18]. We have developed a new methyl esterification procedure by modifying previously described methods [12,19,20]. The modified methyl esterification procedure has proved to be safe, rapid and easy to operate without any hazard of explosion.

Some of the clean-up procedures involved lengthy liquid–liquid partition steps. However, loss of analytes might occur during the transfer processes. Increased contamination due to transfer of solvent between glassware might also interfere with sample analysis. However, some samples are clean enough for the clean-up procedure to be eliminated to save time. Here were present a simple procedure using a micro disposable Florisil column to clean up the extracted samples if the background interference is high. Florisil (synthetic magnesium silicate) has been widely used for the clean-up of pesticide extracts [21–23]. However, the choice of an appropriate solvent is critical. The ideal solvent will elute the extracted herbicides without co-elution of the extraneous material.

EXPERIMENTAL

Reagents

The solvents diethyl ether (peroxide-free), hexane and methylene chloride, all of pesticide quality, were obtained from American Burdick and Jackson (Muskegon, MI, U.S.A.). Sodium sulfate (anhydrous), granular, neutral, analytical-reagent grade (also from American Burdick and Jackson), was washed with methylene chloride and diethyl ether and then heated in an oven at 130°C for 13 h.

Florisil (60–100 mesh) (Fisher Scientific, Pittsburgh, PA, U.S.A.; Lot No.

897135) was activated at 130°C for at least 16 h. Silicic acid, 100-mesh powder (analytical-reagent grade) (Aldrich, Milwaukee, WI, U.S.A.) was used.

N-Methyl-N-nitroso-*p*-toluenesulfonamide (Diazald) of high purity and carbitol (diethylene glycol monoethyl ether) were purchased from Aldrich.

All herbicides in the acid form (1000 mg/l) were purchased as certified high-purity solutions from Nanogens (Watsonville, CA, U.S.A.). Each herbicide was converted into the methyl ester by the esterification procedure described below, and stock standard solutions were prepared. Working calibration standard solutions were prepared in *n*-hexane by serial dilution of the stock standard solutions and compared frequently with check standards for signs of degradation and evaporation. The stock standard solutions were stored at 40°C and protected from light.

Apparatus and materials

The apparatus used included an MNNG (1-methyl-3-nitro-1-nitrosoguanidine) diazomethane generation apparatus (Aldrich, Cat. No. Z10,100-1), a Buchi Rotovapor equipped with a temperature-controlled water-bath and a syringe with a narrow gauge (No. 22) needle.

Silane-treated glass-wool was obtained from Alltech (Deerfield, IL, U.S.A.). Boiling chips were high-purity, plain amphoteric alundum granules (Hengar, Philadelphia, PA, U.S.A.), washed with acetone and diethyl ether and stored in an oven at 200°C for at least 2 h before use.

The GC system consisted of a Varian Model 3400 gas chromatograph equipped with ^{63}Ni electron-capture detectors (dual channels), a Varian Model 8040 autosampler and a Spectra-Physics SP4290 integrator with a memory module installed. The operating conditions were as follows: a 5-mm deactivated glass Uniliner inside a Uniliner sleeve adapter (Restek, Bellefonte, PA, U.S.A.) were installed in the injection port at 210°C; detector temperature, 310°C; carrier gas (helium) flow-rate, 7 ml/min in both columns; nitrogen make-up gas flow-rate, 20 ml/min in both detectors; direct injection mode [24]; column temperature program, from 140 to 180°C at 3°C/min and then to 184°C at 0.5°C/min.

The following GC columns were used: (1) a 0.3–0.5 m × 0.53 mm I.D. capillary guard column (J & W Scientific, Rancho Cordova, CA, U.S.A.) was connected to the injector Uniliner and the other end was connected with a Chromfit low-dead-volume Y-splitter (Western Scientific, Danville, CA, U.S.A.); (2) an Rt_x-35 capillary column with 30 m × 0.53 mm I.D.), film thickness 0.5 μm (Restek), or with DB-608 (J & W Scientific), was connected at one end with the Y-splitter and at the other end with detector A; (3) an Rt_x-5 capillary column (30 m × 0.53 mm I.D.), film thickness 1.5 μm (Restek), or with DB-5 (J & W Scientific), was installed in the same way as the Rt_x-35 column, but connected to detector B.

Analysis of water samples

A reagent water sample (500 ml) was poured into a 1-l round-bottomed flask and NaOH pellets (40 g) were added with a few boiling chips. A reflux condenser was installed immediately on the flask. The water sample was then heated to reflux for *ca.* 25-30 min and then cooled in an ice-bath. The aqueous solution contained salts of free acid herbicides. While the basic aqueous solution was still in the ice-bath, it was acidified with *ca.* 85 ml of concentrated HCl to pH < 1.0. When the acidified solution

has been cooled to room temperature, it was transferred into a 1-l separating funnel and extracted with diethyl ether (4 × 100 ml). Diethyl ether was used to rinse the 1-l round-bottomed flask before performing each extraction. The ether layers were collected, dried by passage through anhydrous sodium sulfate in a stemless funnel plugged with a small amount of glass-wool and evaporated to 1 ml using a rotary evaporator set at 40°C. The concentrated ether solution contained the acid form of the herbicides. The sample was then ready for methylation with diazomethane. For recovery studies, 500 ml of herbicide-free water sample were spiked with herbicide methyl esters (Table II) prepared in *n*-hexane and shaken vigorously to obtain homogeneity before extraction began.

Soil sample analysis

A soil sample (50 g) was thoroughly mixed with *ca.* 10–15 ml of 50% (v/v) HCl in a 500-ml erlenmeyer flask. The pH was < 1. Diethyl ether (100 ml) was added to the acidified soil sample, sealed with a stopcock valve attached to a stopper and shaken manually for 1 min.

After settling, the stopcock was opened slowly and the ether layer was decanted and filtered through Whatman No. 41 (18.5-cm) filter-paper. The above extraction procedure was repeated a further three times. At this point the ether solution contained both the acid and ester parts of the herbicides. The ether layers were collected in a 1-l round-bottomed flask and evaporated to 3–4 ml using a rotary evaporator at 40°C. Distilled water (400 ml) was added to the flask with 40 g of NaOH pellets and the mixture was subjected to base hydrolysis as described above. After hydrolysis, some brown precipitate had formed. It was allowed to settle, cooled in an ice-bath and then passed through filter-paper into a 1-l separating funnel. The filtered basic solution was then acidified with *ca.* 85 ml of concentrated HCl to pH < 1. After cooling to room temperature in an ice-bath, it was extracted with four portions of 100 ml of

TABLE II

SPIKE CONCENTRATION RANGES AND RECOVERIES OF HERBICIDES FROM WATER

Analyte	Spike concentration range (μg/l)[a]	Mean recovery (%)[b]	
		Low concentration	High concentration
Dicamba	0.1–5	101 ± 5.2	109 ± 4.5
MCPA	10–500	98 ± 5.6	118 ± 5.5
Dichlorprop	0.06–3	100 ± 5.2	106 ± 4.8
Mecoprop	1.4–70	97 ± 4.9	117 ± 3.9
2,4-D	0.1–5	96 ± 6.0	99 ± 5.8
Fenoprop	0.05–0.5	101 ± 6.1	105 ± 5.3
2,4,5-T	0.1–5	102 ± 7.2	116 ± 8.5
Dinoseb	0.1–5	95 ± 5.4	111 ± 7.0
2,4-DB	0.1–5	98 ± 4.9	104 ± 5.4
Av. R.S.D.[c]		5.6	5.6

[a] Herbicide methyl ester was used as spike in a 500-ml water sample.

[b] Mean recovery was calculated based on an Rt_x-35 capillary column and five replicate analyses.

[c] Average relative standard deviation (%).

TABLE III

SPIKE CONCENTRATION RANGES AND RECOVERIES OF HERBICIDES FROM SOIL

Analyte	Spike concentration range (μg/kg)[a]	Mean recovery (%)[b]	
		Low concentration	High concentration
Dicamba	0.2–10	90 ± 5.1	98 ± 5.9
MCPA	20–1000	88 ± 5.9	94 ± 6.9
Dichlorprop	1.2–60	91 ± 6.5	101 ± 7.5
Mecoprop	20–1000	89 ± 6.7	99 ± 7.5
2,4-D	2–100	88 ± 5.8	92 ± 6.4
Fenoprop	0.2–10	91 ± 7.9	93 ± 8.3
2,4,5-T	0.4–20	87 ± 5.4	99 ± 6.2
Dinoseb	0.4–20	86 ± 8.8	96 ± 9.6
2,4-DB	2–100	89 ± 7.0	97 ± 8.0
Av. R.S.D.[c]		6.6	7.4

[a] Herbicide methyl ester was used as spike in a 50 g of herbicide-free soil sample.
[b] Mean recovery was calculated based on an Rt_x-35 capillary column and five replicate analyses.
[c] Average relative standard deviation (%).

diethyl ether as described for the analysis of water samples. For recovery studies, 50 g of herbicide-free soil sample were sprayed with herbicide methyl esters prepared in *n*-hexane and homogenized with a glass rod (Table III).

Esterification

The procedure and precautions for using the Aldrich MNNG diazomethane apparatus are described in detail elsewhere [19,25]. A concentrated 1-ml ether sample was transferred from the volumetric flask to the outside tube of the apparatus. The volumetric flask was rinsed with *ca.* 2 ml of diethyl and combined with the diethyl ether in the outside tube of the apparatus. Diethyl ether (1 ml) was added to the inside tube through its screw-cap opening, together with 1 ml of carbitol. Approximately 0-3–0.4 g of Diazald was placed in the inside tube, and then the two parts were assembled with a butyl O-ring and held with a pinch-type clamp. The lower part was immersed in an ice–water bath and about 1.5 ml of 37% KOH was injected dropwise through the silicone-rubber septum via a syringe with a narrow-gauge (No. 22) needle.

The apparatus was shaken gently by hand every 10 min for about 40 min to ensure completion of the reaction. At the beginning, the Diazald might settle at the bottom of the inside tube, so it was necessary to shake well to allow the Diazald to react with the carbitol and the base. After *ca.* 5 minutes, the yellow color of diazomethane should persist in the outside tube of the diethyl ether solution. An additional 0.1–0.2 g of Diazald can be added if the yellow color of the diazomethane–diethyl ether solution does not appear. When the reaction was complete, the yellow diethyl ether solution in the outside tube was evaporated to dryness by shaking in a warm water-bath (60°C) inside a well circulated hood. The diazomethane gas and the diethyl ether should evaporate easily. A gentle stream of nitrogen might also be used to dry the ether solution. *n*-Hexane (5 ml) was added to the tube for water samples. The whole esterification procedure should be carried out inside a fume-hood. The sample

was readily analyzed by GC. For soil samples, 10 ml of *n*-hexane should be added. Any unreacted diazomethane in the inside tube was destroyed by adding 0.1–0.2 g of silicic acid.

Florisil clean-up

Some dirty samples with a lot of background interferences required clean-up. A micro disposable Florisil column was prepared by adding a layer of *ca.* 1 cm of anhydrous sodium sulfate to a 5.75-in. long disposable pipet plugged with a small amount of glass-wool. On top of the sodium sulfate, a layer of 6–7 cm of heat-activated Florisil was added. The micro Florisil column was then rinsed with *n*-hexane to remove any impurities. The esterified sample (1 ml) was pipetted slowly through the Florisil column and the column was then washed with about 5 ml of *n*-hexane. The *n*-hexane layer was discarded. The column was washed with 15 ml of methylene chloride. The herbicide esters were contained in the methylene chloride, which was collected and dried with a gentle stream of nitrogen. *n*-Hexane (1 ml) was added and the sample was ready for GC analysis.

Calibration standards

A minimum of five calibration points for each standard should be prepared by dilution of the stock standard solutions with *n*-hexane. The stock standard solutions were prepared by esterification of 10 ml of the certified pure herbicide acids (1000 mg/l) by the esterification procedure described above. The standards should be checked frequently with the commercially available certified pure herbicide methyl esters for signs of degradation or evaporation. A 1-μl volume of each standard was injected and analysed by gas chromatography.

RESULTS AND DISCUSSION

As shown in Table IV, nine herbicides are well separated in both capillary columns. The run time was about 15 min in channel A and 20 min in channel B. The total run time for both channels to be completed would be about 25 min. With the Y-splitter installed in the gas chromatograph plus a memory module in the integrator, it is easy to confirm the herbicides from the different columns simultaneously. The dual-column approach has two advantages. First, it provides secondary confirmation without the necessity to change columns in the gas chromatograph. Second, the cost of using this approach is much cheaper than GC–MS and yet provides high accuracy. It can initially screen out large amounts of undetected compounds. However, if the concentrations of the herbicides in the sample are high, a GC–MS method should be used for final confirmation. The reproducibility of the dual column system is good and consistent. Both the Rt_x-35 and Rt_x-5 capillary columns gave good resolutions of all nine herbicides. In another experiment, we tested DB-608 and DB-5 capillary columns from J & W Scientific and obtained similar results. Detection limits for nine herbicides in both capillary columns are presented in Table V. They are comparable to or even lower than those for the EPA methods.

A reagent water sample was spiked with nine herbicide methyl esters and extracted with diethyl ether. The spike levels and mean recoveries are given in Table II. The mean recoveries were >95% for all the herbicides with an average relative stan-

TABLE IV

RETENTION TIMES OF NINE METHYL ESTER HERBICIDES SEPARATED ON Rt_x-35 AND Rt_x-5 CAPILLARY COLUMNS

Conditions: 1-μl injection; helium carrier gas, 7 ml/min; nitrogen make-up gas, 20 ml/min; columns temperature programmed from 140 to 180°C at 3°C/min and then to 184°C at 0.5°C/min; direct injection mode.

Analyte	Retention time (min)	
	Rt_x-35	Rt_x-5
Dicamba	6.38	8.87
MCPA	7.26	9.90
Dichlorprop	8.06	11.18
Mecoprop	8.91	12.59
2,4-D	9.14	11.75
Fenoprop	11.39	15.36
2,4,5-T	12.87	16.37
Dinoseb	13.8	19.16
2,4-DB	14.49	18.96

dard deviation of 5.6%. In another experiment, we used different esters of 2,4-D (*e.g.*, butyl, isooctyl or butoxyethyl ester). The mean recoveries were also >95%. The use of a large amount of NaOH (1 mol) is necessary in order to carry out the hydrolysis step efficiently. Also, because it is not clear how high the herbicide concentration will be in the samples, it is safer to use an excess amount of base. When acidified with *ca.* 85 ml of concentrated HCl, a large amount of sodium chloride is formed in the aqueous solution. This salt facilitates the recovery of the herbicides, as the solubility of the acidic herbicides was decreased in the aqueous solution by the salt. Groundwater samples were also tested and similar results were obtained. In addition, similar result to those obtained before (Table II) were obtained when the acid forms of the herbicides were used to spike the samples.

The water extraction and the hydrolysis take about 1 h. The procedure is short

TABLE V

DETECTION LIMITS ON Rt_x-35 AND Rt_x-5 GC COLUMNS

Conditions as in Table IV.

Analyte	Detection limit (μg/l)	
	Rt_x-35	Rt_x-5
Dicamba	0.05	0.03
MCPA	15.0	12.0
Dichlorprop	0.10	0.07
Mecoprop	5.00	3.00
2,4-D	0.20	0.15
Fenoprop	0.06	0.04
2,4,5-T	0.08	0.06
Dinoseb	0.04	0.02
2,4-DB	0.80	0.50

and simple and hence minimizes the chance of loss of analytes and contamination between glassware. Most of time, the water samples are clean enough to be analyzed by GC. If there is a lot of background interference, they can be cleaned by the Florisil clean-up procedure described above.

The spike levels and mean recoveries for soil samples are presented in Table III. The mean recoveries of nine herbicides were >86% with an average relative standard deviation of 7%. After the hydrolysis, some brown precipitate was formed because some organic material was extracted into the concentrated diethyl ether and became insoluble in the basic solution. The precipitate might be basic or neutral organic compounds. This procedure is a major clean-up step that eliminates many interferences in soil samples. Indeed, it helps to clean the samples as soil usually contains a lot of organic material. This proposed method for soil samples is short, simple and yet accurate in comparison with the current EPA procedures.

These methods do not distinguish between salt, acid or ester forms of the herbicides because after hydrolysis and methylation all the herbicides will be converted to the methyl esters.

The methyl esterification reagent, diazomethane, can be generated from several different precusors by the action of alkali on N-methyl-N-nitrosourea [26], N-methyl-N-nitroso-N'-nitroguanidine [27] or N-methyl-N-nitroso-*p*-toluenesulfonamide (Diazald) [28] in the presence of diethyl ether. Currently, the bubbler method and the Diazald kit method are recommended in EPA methods [12,13,15]. The bubbler method is suggested for samples that have low concentrations of herbicides. However, the method requires a source of nitrogen, test-tubes and several glass delivery tubes, and hence becomes cumbersome when there are many samples that need esterification. The Diazald kit method requires the assembly of a set of distillation glassware for the safe preparation of diazomethane. However, if the temperature is raised above 90°C, it might cause an explosion. In addition, solutions of diazomethane decompose rapidly in the presence of solid materials such as calcium chloride, copper powder and boiling chips. Instead of using the above two methods, we used the MNNG diazomethane generation apparatus [19,25]. However, MNNG is not used in our test because it is toxic, carcinogenic and a potent mutagent and generates only 1 mmol or less of diazomethane [25,29]. Diazald is the preferred reagent because of its large-scale production of diazomethane [29,30] and stability. The time required for the whole reaction is *ca.* 40 min. Diazomethane is a carcinogen and unstable under certain conditions. The precautions required to prepare diazomethane are described in detail in the literature [19,26].

Our modified esterification process is very convenient to operate and we have been using this method for 1 year without any difficulty or accident. However, care should be taken when transferring reagents into the inside tube, as some reagents could leak out of the small hole in the inside tube and thus contaminate the sample in the outside tube. Diazomethane reacts with both carboxylic acids and phenols and also with other compounds that have active hydrogens. Hence it is suitable to convert dinoseb into its methyl ether derivative for analysis. The precusor of diazomethane, Diazald, gave consistent and nearly maximum yields of esters when compared with MNNG. The derivatization yields are 100% when compared to the commercially available esters standard. The diazomethane gas generated from the reaction tube is sufficient to convert all the acidic herbicides to the ester forms, as the concentrations

TABLE VI

MEAN RECOVERIES OF HERBICIDES FROM FLORISIL COLUMN

Analyte	Mean recovery (%)[a]	Analyte	Mean recovery (%)[a]
Dicamba	93	Fenoprop	100
MCPA	89	2,4,5-T	92
Dichlorprop	90	Dinoseb	98
Mecoprop	95	2,4-DB	90
2,4-D	100		

[a] Calculation was based on an Rt_x-35 capillary column and five replicate analyses.

of the herbicides in the environment are usually low. The modified esterification procedure provides several advantages. First, there is no need to distil the diazomethane from its precusors, which might cause an explosion. Second, fresh diazomethane is supplied each time directly from the reaction flask to the samples. Third, the procedure is easy to manage and is rapid, so it can save a lot of work and time. Fourth, Diazald is a safer and more stable compound than MNNG. Fifth, it can generate diazomethane in amounts greater than 1 mmol.

The disposable micro Florisil column is economical and easy to prepare and use. The recovery of the herbicides after the clean-up procedure was >89% (Table VI).

One of the acidic herbicides, dalapon (2,2-dichloropropionic acid), was also tested. However, we found that Dalapon (b.p. 98–99°C) was volatile enough to be analysed by GC without esterification. If it is included in our experiment, losses might occur because dalapon ester is very volatile. Dalapon occurs mainly as the sodium or magnesium salt in the environment. Thus, after acidification, it was ready for analysis by GC.

The proposed methods here are simple and rapid and large numbers of environmental samples can be analyzed within a reasonable time and at reasonable cost in the laboratory.

ACKNOWLEDGEMENT

The authors thank Dr. Dave Cooper of the University of California, Davis, CA, for reviewing the manuscript.

REFERENCES

1 P. Berwick, *J. Am. Med. Assoc.*, 214 (1970) 1114.
2 L. F. Prescott, J. Park and I. Darrien, *Br. J. Clin. Pharmacol.*, 7 (1979) 111.
3 W. D. E. Wells, N. Wright and W. B. Yeoman, *Clin. Toxicol.*, 18 (1981) 273.
4 Y. Li. W. Zhao and I. N. Chou, *J. Toxicol. Environ. Health*, 20 (1987) 11.
5 P. H. Casey and W. R. Collie, *J. Pediatr.*, 104 (1984) 313.
6 G. Yip, *J. Assoc. Off. Agric. Chem.*, 45 (1962) 367.
7 D. C. Abbott, H. Egan, E. W. Hammond and J. Thomson, *Analyst (London)*, 89 (1964) 480.
8 D. F. Goerlitz and W. L. Lamar, *U.S. Geol. Surv. Water Supply Pap.*, (1967) 1817-C.
9 W. P. Cochrane and R. Purkayastha, *Toxicol. Environ. Chem. Rev.*, 1 (1973) 137.
10 A. W. Ahmed, V. N. Mallet and M. J. Bertrand, *J. Assoc. Off. Anal. Chem.*, 72 (1989) 365.

11 T. Tsukioka and T. Murakami, *J. Chromatogr.*, 469 (1989) 351.
12 *Test Methods: Methods for Nonconventional Pesticides. Chemical Analysis of Industrial and Municipal Wastewater, EPA 440/1-83/079C, No. 615*, U.S. Environmental Protection Agency, Washington, DC, 1983.
13 *Test Methods for Evaluating Solid Waste*, SW-846, U.S. Environmental Protection Agency, Washington, DC, 2nd ed., 1982.
14 D. F. Gurka, F. L. Shore and S. T. Pan, *J. Assoc. Off. Anal. Chem.*, 70 (1987) 889.
15 D. F. Gurka, F. L. Shore and S. T. Pan, *J. Assoc. Off. Anal. Chem.*, 69 (1986) 970.
16 C. W. Stanley, *J. Agric. Food Chem.*, 14 (1966) 321.
17 M. J. Bertrand, A. W. Ahmed, B. Sarrasin and V. N. Mallet, *Anal. Chem.*, 59 (1987) 1302.
18 W. P. Cochrane, *J. Chromatogr. Sci.*, 17 (1979) 124.
19 H. M. Fales, T. M. Jaouni and J. F. Babashak, *Anal. Chem.*, 45 (1973) 2302.
20 *Mini Diazald Apparatus* (*Technical Information Bulletin*, No. AL-121), Aldrich, Milwaukee, WI, 1982.
21 P. A. Mills, B. A. Bong, L. R. Kamps and J. A. Burke, *J. Assoc. Off. Anal. Chem.*, 55 (1972) 39.
22 V. Lopez-Avila, N. S. Dodhiwala, J. Milanes and W. F. Beckert, *J. Assoc. Off. Anal. Chem.*, 72 (1989) 93.
23 G. Yip, *J. Assoc. Off. Anal. Chem.*, 54 (1971) 966.
24 *HALFMIL Capillary Columns (0.530 mm ID)*, Restek, Bellefonte, PA, 2nd ed., 1989, p. 38.
25 *MMNG Diazomethane-Generation Apparatus* (*Technical Information Bulletin*, No. AL-132), Aldrich, Milwaukee, WI, 1982.
26 F. Arndt, in A. H. Blatt (Editor), *Organic Syntheses*, Coll. Vol. 2, Wiley, New York, 1950, p. 165.
27 A. F. McKay, *J. Am. Chem. Soc.*, 70 (1948) 1974.
28 Th. J. de Boer and H. J. Backer, in N. Rabjohn (Editor), *Organic Syntheses*, Coll. Vol. 4, Wiley, New York, 1963, p. 250.
29 *Diazald, MNNG and Diazomethane Generator* (*Technical Information Bulletin*, No. AL-180), Aldrich, Milwaukee, WI, 1990.
30 M. Hudlicky, *J. Org. Chem.*, 45 (1980) 5377.