Determination of Dicamba and 2,4-D in Water and Soil by Isotope Dilution GC/MS

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An isotope dilution GC/MS technique for the analysis of low part per billion (ppb) concentrations of dicamba and 2,4-D in water and soil is described. Known amounts of stable-labeled isotopes such as dicamba-d_3 and 2,4-D-d_3 are spiked into each sample prior to extraction. Water samples are extracted with methylene choride at pH ≤1; soil samples are extracted with acetone–hexane at pH ≤1. Analysis is performed by high-resolution GC/MS, with the mass spectrometer operated in the selected ion monitoring mode, following derivatization of extracts with pentafluorobenzyl bromide. Accuracy greater than 84% and precision better than 19% were demonstrated with spiked samples. This technique has been used successfully in the analysis of over 300 water and 300 soil samples. Detection limits of 0.1–1.0 ppb for waters and 1–10 ppb for soils were achieved by selected ion monitoring GC/MS.

INTRODUCTION

Stable-isotope dilution analysis is an analytical technique in which a known quantity of a stable-labeled isotope is added to a sample prior to extraction, in order to quantitate a particular compound. The ratio of the naturally abundant and the stable-labeled isotope is a measure of the naturally abundant compound and can be determined only by gas chromatography/mass spectrometry (GC/MS) since the naturally abundant and the stable-labeled isotope cannot be completely resolved by gas chromatography. Parameters such as quantitation ion(s) for isotope ratio measurements, spiking level of the stable-labeled isotope, and data processing have been reported to affect the accuracy and precision of the GC/MS determination (Colby, et al., 1981). Deuterated, ^{18}O- and ^{13}C-labeled isotopes have been commonly used in environmental analysis (EPA, 1984; Ingram et al., 1979; Klein and Klein, 1979). Although the availability of the stable-labeled isotopes is still a problem and the synthesis costs are quite high, the benefits from using stable-labeled isotopes in environmental analysis are quite remarkable. Similarity between the naturally abundant compound and the stable-labeled isotope suggests that the recovery information obtained on the stable-labeled isotope can be used for two purposes: (a) to establish whether the naturally abundant compound has degraded in the sample prior to analysis (the case when the stable-labeled isotope is added to the sample in the field immediately following sample collection) (Pollard and Hern, 1985; Hern et al., 1983; Lopez-Avila et al., 1983); (b) to provide a continuous quality control (the case when the stable-labeled isotope is added to the sample in the laboratory immediately prior to sample extraction) (EPA, 1984). The quantitation technique involving isotope dilution requires that the stable-labeled isotope be recovered, regardless of the value of recovery (EPA, 1984). Thus, method accuracy and precision should not be affected by the sample matrix.

Numerous gas chromatographic methods have been reported for the analysis of dicamba and 2,4-D in water (Olson et al., 1978; Agemian and Chau, 1977; Aly and Faust, 1964; Devine and Zweig, 1969) and soil (Smith, 1972; Renberg, 1974; Kahn, 1975; Gutenmann and Lisk, 1964; Lee and Chau, 1983). Water samples were acidified and extracted with benzene (Olson et al., 1978; Devine and Zweig, 1969), ethyl acetate (Agemian and Chau, 1977), ether–chloroform (1:3) (Aly and Faust, 1964), methylene chloride (Agemian and Chau, 1977), etc. Soil samples were extracted with calcium hydroxide solution (Smith, 1972), 0.2 M NaOH (Renberg, 1974), sulfuric acid–acetone (Kahn, 1974), acetone–phosphoric acid (Gutenmann and Lisk, 1964), and acetone–hexane following acidification (Lee and Chau, 1983). Analysis of dicamba and 2,4-D as pentafluorobenzyl derivatives by gas chromatography with electron-capture detector (GC/EC) was reported by Chau and Terry (1976). Recently, Lee and co-workers (Lee and Chau, 1983) investigated the use of PFBBr for derivatizing dicamba and 2,4-D in soil extracts and reported detection limits of 10 ppb for both compounds. While these methods have been used frequently and were found to work well, in some cases, they are subjected to serious limitations since compound identification is based only on retention time match.

This paper describes an isotope dilution GC/MS technique for detection of low parts per billion concentrations of dicamba and 2,4-D in water and soil. Stable-labeled isotopes such as dicamba-d_3 and 2,4-D-d_3 are spiked into each sample prior to extraction, and the ratio of the unlabeled compound and the stable-labeled isotope is used in quantitating the unlabeled compound. Identification is done by retention time match and mass chromatograms of selected ions specific to the compounds being analyzed. The GC/MS and the isotope dilution technique are being used in this investigation to achieve sensitivities comparable to those reported in the literature for GC/EC.

Table I. SIM Descriptor Used in GC/MS Analysis[a]

begin mass	end mass	dwell time, s set	dwell time, s actual
187.5	188.5	0.08	0.066
202.5	203.5	0.08	0.067
205.5	206.5	0.08	0.066
398.5	401.5	0.17	0.202
401.5	404.5	0.17	0.202

[a] Total acquisition time 0.603 s; total scan time 0.650 s; centroid sampling intensity 0.200 ms.

EXPERIMENTAL SECTION

Apparatus. A Finnigan 1020 quadrupole mass spectrometer coupled to a Perkin-Elmer Sigma I GC and an Incos 2300 data system were used for all measurements reported here. Column: 6 ft × 2 mm i.d. glass column packed with Ultrabond 20M (Alltech Associates, Inc., Deerfield, IL 60015). Temperature program: initial 150 °C, hold for 1 min; programming rate 15 °C/min; final 240 °C. Operating temperatures: injection port 200 °C; separator 250 °C. Solvent divert time: 150 s. Mass spec-

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trometer operating conditions: electron energy of 70 eV, selected ion monitoring mode (SIM) for ions at m/z 188, 203, 206, and ions in the mass range of 399–404. Total scans acquired: 700. The total acquisition time, total scan time, and the dwell times for the various ions monitored are given in Table I.

Reagents and Standard Compounds. Pesticide-grade solvents were used for preparation of standards and sample extraction.

Analytical reference standards of dicamba and 2,4-D were obtained from the U.S. EPA Pesticides and Industrial Chemicals Repository (MD-8), Research Triangle Park, NC 27711. Stock solutions at 10 mg/mL were prepared in pesticide-grade acetone (Baker Resi-analyzed) and stored at −10 °C.

Stable-labeled isotopes dicamba-d_3 (3,6-dichloro-2-methoxy-d_3-benzoic acid) and 2,4-D-d_3 [(2,4-dichlorophenoxy-3,5,6-d_3)acetic acid] were synthesized by Pathfinder Laboratories (St. Louis, MO 63146). Chemical purity of the stable-labeled isotopes was determined by high-pressure liquid chromatography (HPLC) using concentrated stock solutions (concentration 10 mg/mL). Isotopic purity determinations were performed by GC/MS and direct-probe MS.

Pentafluorobenzyl bromide (PFBBr) reagent was prepared from 5 mL of reagent (Aldrich Chemical Co., Milwaukee, WI 53233) and 95 mL of acetone. This reagent was prepared fresh at 3-week intervals and was stored in dark at 4 °C. *Caution!* Reagent is a strong lacrymator. K_2CO_3 solution was prepared by dissolving 30 g of anhydrous K_2CO_3, ACS grade, in 100 mL of water.

Water Extraction. Standard water samples were prepared by spiking concentrated solutions of dicamba and 2,4-D into organics-free water (pH 6.7) at various concentrations. Stable-labeled isotopes were added at concentrations ranging from 4 to 80 μg/L. The spiking level of the stable-labeled isotope is arbitrary; the level should be comparable to the concentrations of the unlabeled compound expected to be found in the sample. One-liter aliquots of the standard water samples or real samples, adjusted to pH <1, were extracted vigorously with 200 mL of methylene chloride, for 2 min, in a 2-L separatory funnel. Smaller sample aliquots can be extracted if the concentration is in the parts per million range (e.g., 200 mL). Extraction was performed three consecutive times, each time using fresh aliquots of methylene chloride. The extracts were concentrated to approximately 4 mL on a Kuderna-Danish evaporator. Methylene chloride was then exchanged to acetone by addition of 80 mL of acetone and further concentration to 4 mL. The extract was derivatized following a procedure reported in the literature (Lee and Chau, 1983) using derivatization with PFBBr as follows: the acetone extract, contained in a sealed vial, was mixed with 30 μL of 30% K_2CO_3 and 200 μL of PFBBr reagent. The derivatization reaction was performed overnight at room temperature (12–16 h). After reaction, the solution was evaporated almost to dryness. Hexane was added to adjust the volume to 1 mL, and the contents of the vial were sonicated for 2–3 min to ensure complete solubilization of dicamba and 2,4-D derivatives.

Soil Extraction. Standard soil samples were prepared as follows: 50-g aliquots of the fresh sandy loam soil were slurried with 10 mL of organics-free water and were spiked with various amounts of dicamba and 2,4-D. The spike was added in 400 μL of acetone to the wet soil and was allowed to equilibrate with the soil for 1 h. Stable-labeled isotopes were added in 100 μL of acetone and were also allowed to equilibrate with the soil for 1 h. The spiking level is arbitrary; the level should be comparable to the concentration of the unlabeled compound in the sample. In this study the stable-labeled isotopes were spiked at 80 and 100 μg/kg. Following equilibration, the soil slurry was adjusted to pH ≤1 with approximately 10 mL of H_2SO_4 (1 + 1) and was extracted with 100 mL of acetone–hexane (50:50) on an ultrasonic cell disruptor. The extraction with acetone–hexane (50:50) was repeated two additional times. Each time the sonicator probe was activated for 3 min. Following each extraction, the soil was allowed to settle and the combined supernatants were filtered through a column of Celite (5-cm bed height; 1.5-cm diameter). The filtrates from the Celite column were combined. To remove water, the combined filtrates were transferred to a separatory funnel and washed twice with 100 mL of acidified organics-free water. The organic layer contained dicamba and 2,4-D; the aqueous layer contained traces of these compounds. To recover these traces, the aqueous layer was extracted three times with 50 mL of methylene chloride. After the last extraction the aqueous layer was discarded. The methylene chloride extract was combined with the acetone–hexane extract and was concentrated to approximately 4 mL on a Kuderna-Danish evaporator. Further concentration to 0.5 mL was performed with a slow stream of nitrogen. Acetone was then added to adjust the volume of the extract to 4 mL. The extract was derivatized with PFBBr as described for water samples with the exception that the reaction was carried out at 60 °C for 3 h.

Quantitation. Calibration standards of dicamba, 2,4-D, and their corresponding stable-labeled isotopes were prepared by derivatizing concentrated stock solutions of dicamba–2,4-D and dicamba-d_3–2,4-D-d_3 separately and then compositing the derivatized compounds.

After a preliminary ratio measurement using a four-point calibration, two calibration standards were used subsequently for daily calibration. Concentrations of dicamba and 2,4-D derivatives in the calibration standards were 3.75 and 15 μg/mL. Both calibration standards contained phenanthrene-d_{10} (internal standard) at 2 μg/mL and the stable-labeled isotopes at 1.5 and 6 μg/mL, respectively. Whenever sample concentrations exceeded the concentration of the calibration standards, additional calibration standards were analyzed with the samples. The calibration standards were analyzed at the beginning of the day and after the last sample during a 10- to 12-h period. The concentration of the test compounds was calculated from

$$C = \frac{A}{A_{\text{isotope}}} \frac{W_{\text{isotope}}}{\text{RR}} \frac{1}{W_{\text{sample}}} \quad (1)$$

where C = concentration of test compound in micrograms per liter (water) if W_{sample} is in liters and micrograms per kilogram (soil) if W_{sample} is in kilograms; A = area of the quantitation ion of the test compound; A_{isotope} = area of the quantitation ion of the stable-labeled isotope; W_{isotope} = amount in micrograms of the stable-labeled isotope that was spiked in the sample prior to extraction; and RR = response ratio of the test compound relative to its corresponding stable-labeled isotope (see eq 2 and 3). A_{203}, A_{206},

$$\text{RR}_{\text{dicamba}} = \frac{A_{203}}{A_{206} - A_{203} \times 0.65 \times 0.088} \frac{C_{\text{dicamba-}d_3}}{C_{\text{dicamba}}} \quad (2)$$

$$\text{RR}_{2,4\text{-D}} = \frac{A_{400}}{A_{403} - A_{400} \times 0.65 \times 0.165} \frac{C_{2,4\text{-D-}d_3}}{C_{2,4\text{-D}}} \quad (3)$$

A_{400}, and A_{403} are the areas of the ions at m/z 203, 206, 400, and 403, respectively. Coefficients 0.65, 0.088; 0.65 and 0.165 account for contributions of ions at m/z 203 and

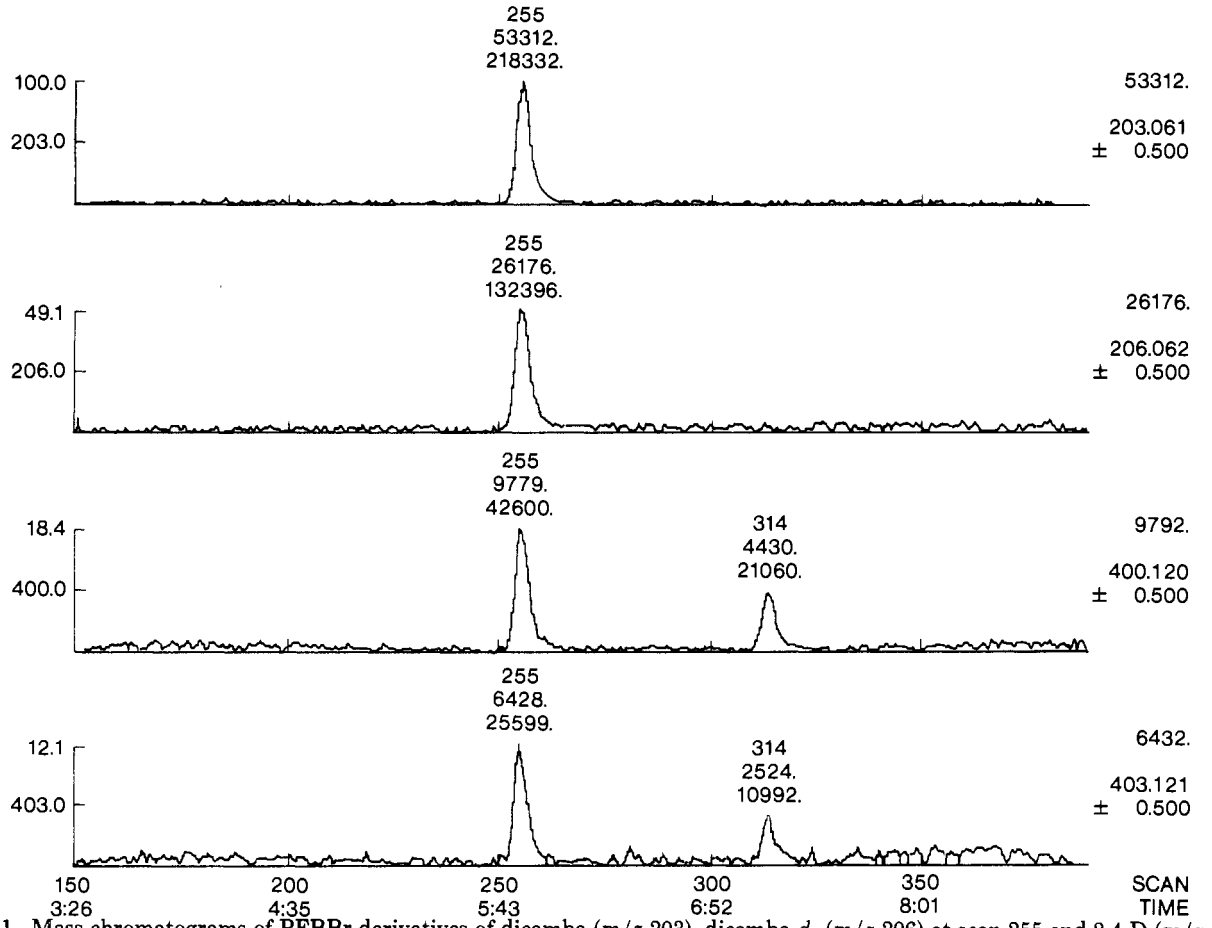

Figure 1. Mass chromatograms of PFBBr derivatives of dicamba (m/z 203), dicamba-d_3 (m/z 206) at scan 255 and 2,4-D (m/z 400), 2,4-D-d_3 (m/z 403) at scan 314.

400 to ions at m/z 206, and 403, respectively (see Quantitation section for an explanation of these values); and W_{sample} = volume of sample (liters) or weight of sample (kilograms).

RESULTS AND DISCUSSION

Chromatography. A recent study (Lee and Chau, 1983) has demonstrated that the Ultrabond 20M column performs well in separating PFBBr derivatives of dicamba, 2,4-D, and other herbicide compounds. Therefore, this column was selected for the GC analysis. Both SIM GC/MS and GC/EC were evaluated.

Chromatographic separation of the PFBBr derivatives of dicamba, 2,4-D, dicamba-d_3, and 2,4-D-d_3 achieved by SIM GC/MS is presented in Figure 1. Each unlabeled compound was present at a concentration of 3.75 μg/mL, while the stable-labeled isotopes were present at 1.5 μg/mL (sample size 4 μL). Total analysis time was 10 min; in addition, a 5-min cooling cycle between analyses was allowed. Under these conditions the reproducibility of retention time was excellent. For example, in 14 repetitive injections of the calibration standards the maximum deviation from the average retention time for any derivative was less than 5 s. It should be noted that there were no other peaks in the chromatogram than those corresponding to dicamba, 2,4-D, and their stable-labeled isotopes.

GC/EC chromatograms of a standard containing dicamba and 2,4-D derivatives and a soil sample also derivatized with PFBBr are shown in Figure 2. It is obvious that GC/EC does not work satisfactorily for soil extracts due to interfering peaks that could have formed from reactions of PFBBr with itself or with acetone under the catalytic influence of K_2CO_3 (Chau and Afghan, 1982). Although the GC/EC analysis was expected to give the best sensitivities in this case, the derivatized extracts could only be concentrated to 5–10 mL for GC/EC analysis; thus, the practical detection limit has been increased by a factor of 5–10 over the reported previously by Lee and co-workers (Lee and Chau, 1983).

Quantitation. The abundances of eight major fragment ions in the mass spectra of the PFBBr derivatives of dicamba, 2,4-D, and their stable-labeled isotopes are summarized in Table II; their mass spectra are shown in Figures 3 and 4. In selecting the quantitation ions for the SIM technique, consideration was given first to the most intense ions in the mass spectra to maximize the instrument response. Both compounds, as well as their stable-labeled isotopes, give strong ions at m/z 181 corresponding to the pentafluorobenzyl fragment. This ion, which would have been the first choice, was not selected because of the overlap between dicamba or 2,4-D derivatives and their corresponding stable-labeled isotopes. The next choice in the case of dicamba are the ions at m/z 203 and 206 for dicamba derivative and dicamba-d_3 derivative, respectively. Both fragments represent approximately 87% of the intensity of the base peak. It should also be noted that the ion at m/z 203 has an isotope at m/z 205 (65 ± 2% of the intensity of ion at m/z 203) that contributes approximately 8.8% from its intensity to the ion at m/z 206. In the case of 2,4-D derivative and its corresponding stable-labeled compound we have chosen the molecular ions at m/z 400 and 403 instead of the fragment ions at m/z 175 and 178, respectively. The ion at m/z 400 has an isotope at m/z 402 (65 ± 7% of the intensity of ion at m/z 400) that contributes approximately 16.5% of its intensity to the ion at m/z 403.

Although the ions at m/z 175 and 178 would have given better sensitivities (their intensities represent approxi-

Table II. Mass Spectra, Ions Selected for Quantitation by SIM GC/MS Technique, and Relative Responses

compd	mass spec m/z (intens)	ion selected for quantitn, m/z	rel resp[a,b]	no. of determin
dicamba deriv	181 (100), 203 (87), 189 (63), 205 (58), 191 (39), 188 (17), 190 (14), 182 (11), 400 (9.0), 402 (5.4)	203	0.839 ± 0.04	14
dicamba-d_3 deriv	181 (100), 206 (87), 208 (55), 190 (45), 192 (25), 182 (10), 207 (9.0), 210 (8.8), 403 (8.1), 405 (5.2)	206	0.571 ± 0.068	11
2,4-D deriv	181 (100), 175 (57), 177 (36), 145 (23), 111 (22), 147 (21), 161 (12), 109 (12), 400 (9.0), 402 (5.2)	400	1.115 ± 0.052	6
2,4-d_3 deriv	181 (100), 178 (46), 180 (29), 148 (18), 150 (17), 177 (14), 182 (14), 113 (13), 403 (7.8), 405 (4.9)	403	0.063 ± 0.015	7

[a] Average relative response ± SD, determined with standards at 15–300 ng per injection for dicamba and 2,4-D and 6–120 ng per injection for dicamba-d_3 and 2,4-D-d_3; all compounds derivatized with PFBBr. Phenanthrene-d_{10} spiked at 2.0 μg/mL extract. [b] Relative responses of stable-labeled isotopes determined relative to phenanthrene-d_{10}.

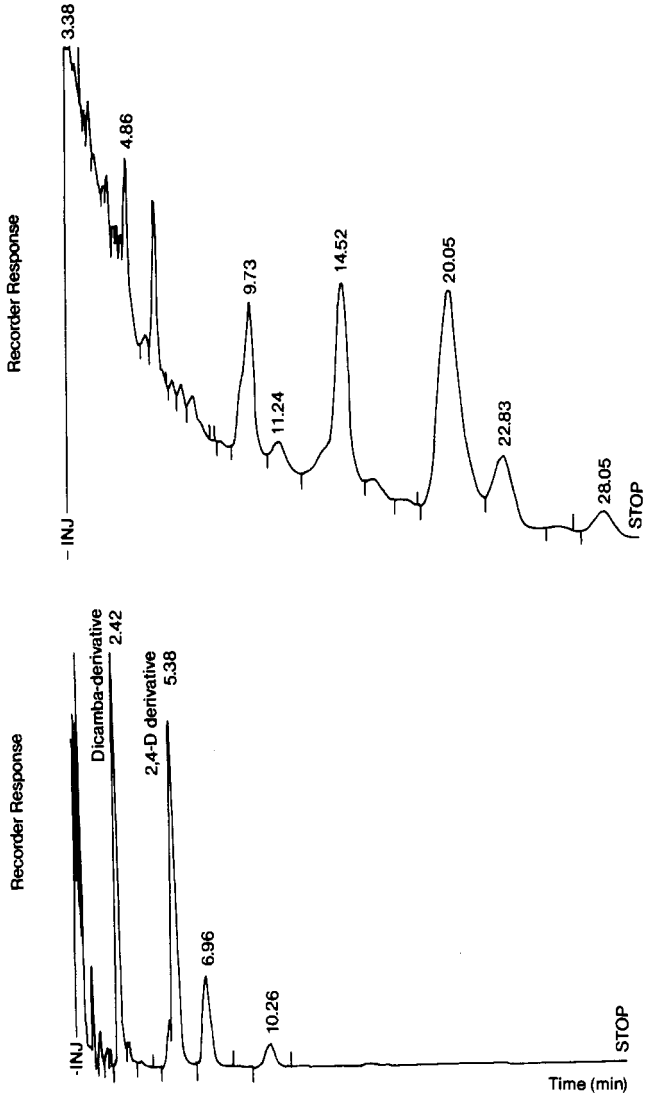

Figure 2. GC/EC chromatograms of soil sample containing dicamba and 2,4-D derivatives at 10 ppb (top) and standard of dicamba and 2,4-D derivatives (bottom).

mately 57% and 48% relative to the parent ion), on the basis of our preliminary experiments we have concluded that we were able to achieve better selectivities by monitoring the ions at m/z 400 and 403 for 2,4-D and 2,4-D-d_3 derivatives.

Under ideal conditions the relative responses of the two naturally abundant compounds relative to their corresponding stable-labeled isotopes would be 1.00. This is not always the case, due to errors introduced in the preparation of standards and in the GC/MS analysis. Thus, the relative responses need to be determined by using standards of known concentration. Relative responses of the stable-labeled isotopes were determined by using phenanthrene-d_{10} as internal standard. They are used in the quantitation of the stable-labeled isotopes, and their values give an indication of the instrument detection limit. For example, dicamba-d_3 derivative gives a relative response of 0.571, which is approximately 10 times higher than that of 2,4-D-d_3 derivative. Consequently, the instrument detection limit for dicamba and dicamba-d_3 derivatives is lower than that of 2,4-D derivative approximately by a factor of 10. Typical instrument detection limits at a signal/noise of 10 and with the instrument operated in the SIM mode are in the range of 10–50 pg for dicamba derivative and 100–500 pg for 2,4-D derivative.

Table III. Accuracy and Precision Data for Dicamba and 2,4-D by Isotope Dilution GC/MS

spike level[a]	matrix	parameter[b]	dicamba	2,4-D
1	water	A ± SD	110 ± 2.6	80; 88[c]
		RSD	2.4	
		n	3	2
5	water	A ± SD	113 ± 13	102 ± 19
		RSD	12	19
		n	3	3
10	water	A ± SD	104 ± 8.3	100 ± 6.8
		RSD	8.0	6.8
		n	3	3
1000	water	A ± SD	102; 109[c]	98; 100[c]
		RSD		
		n	2	2
2500	water	A ± SD	99 ± 1.9	103 ± 8.7
		RSD	1.9	8.5
		n	4	3
100	soil	A ± SD	116 ± 7.6	115 ± 10
		RSD	6.5	8.9
		n	3	3
1000	soil	A ± SD	112 ± 1.5	109 ± 20
		RSD	1.4	18
		n	3	3
10000	soil	A ± SD	99 ± 3	97 ± 3.2
		RSD	3.0	3.3
		n	4	4

[a] Spike level in micrograms per liter for water and micrograms per kilogram for soil. [b] A ± SD = accuracy (average recovery) ± standard deviation; RSD = relative standard deviation; n = number of determinations. [c] Duplicate analysis.

Method Accuracy and Precision. A total of 15 standard water samples and 10 standard soil samples containing dicamba and 2,4-D at known concentrations were analyzed by the isotope dilution GC/MS procedure described above. Compound concentrations ranged from 1 to 2500 μg/L for water samples and 100 to 10000 μg/kg for soil samples. The accuracy and precision data are presented in Table III. These results indicate that the average recoveries (method accuracy) of dicamba and 2,4-D were >84%, and method precision given as relative standard deviation (RSD) is better than 19%. Since in

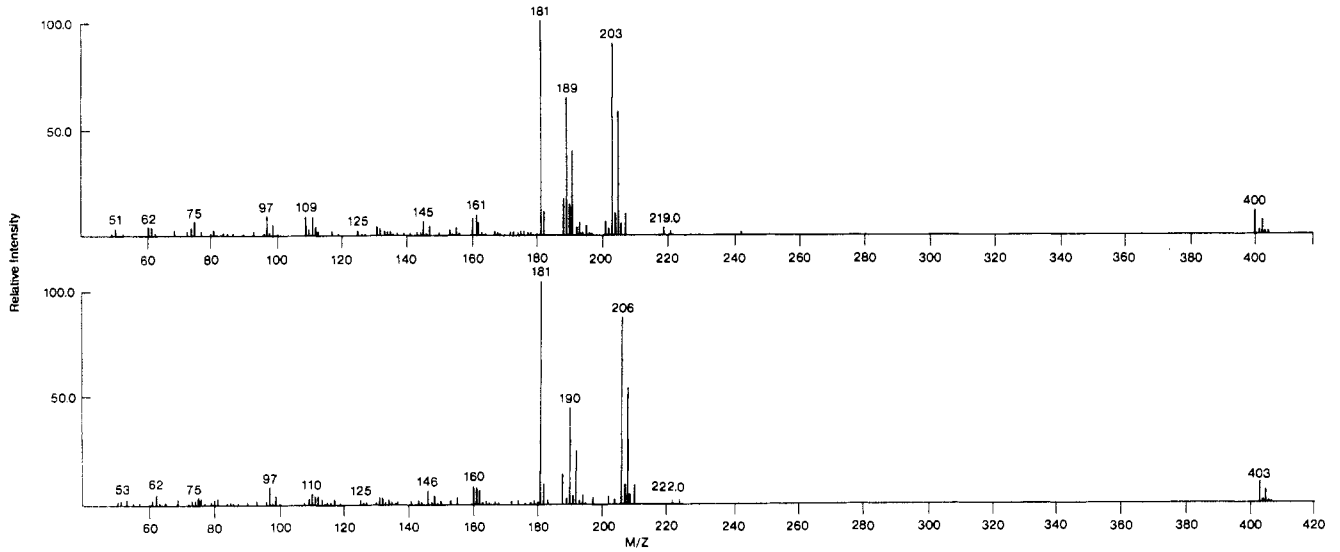

Figure 3. Electron-impact mass spectra of PFBBr derivatives of dicamba (top) and dicamba-d_3 (bottom).

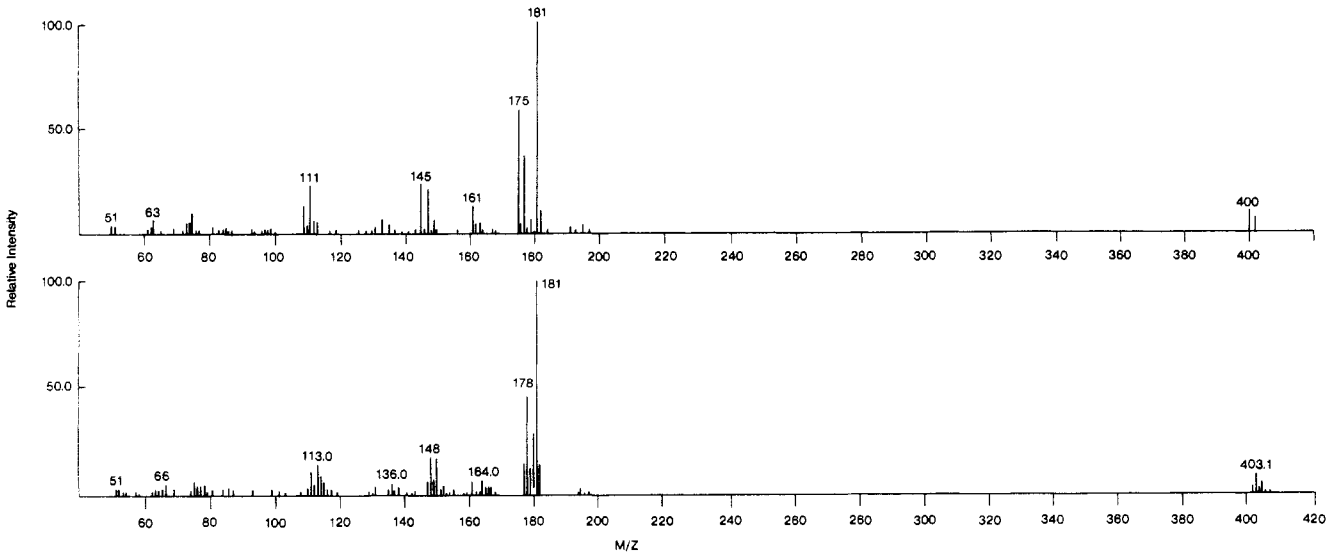

Figure 4. Electron-impact mass spectra of PFBBr derivatives of 2,4-D (top) and 2,4-D-d_3 (bottom).

the isotope dilution GC/MS procedure quantitation is done from the ratio of response of naturally abundant compound to the stable-labeled isotope, the absolute recovery of the stable-labeled isotope is not critical. Typical recoveries using real samples spiked with dicamba-d_3 and 2,4-D-d_3 are presented in Table IV. Results obtained on a set of approximately 170 samples spiked with the stable-labeled isotopes at 4–80 μg/L indicated average recoveries for the stable-labeled isotopes greater than 65% and RSD values between 28% and 52%; results obtained on soil samples indicated lower recoveries and comparable precisions. Nonetheless, these results are typical for those techniques that do not use stable-labeled isotopes for quantitation. The precision data (RSD) given in Table III are better than 9% for 76% of the measurements, which would indicate that method precision of the isotope dilution technique is significantly improved over the method precision of the conventional GC/MS technique.

Although superior in terms of accuracy and precision, the SIM GC/MS technique developed here lacks the specificity of the full-scan mode since only selected ions are monitored. When complex mixtures are analyzed by SIM GC/MS, there is indeed the possibility that other compounds present in the sample might interfere with the ions being monitored. However, if samples should originate from the same source as was in the case of our study (e.g., core samples from soil columns; leachate samples collected over a period of time from the same or similar soil columns), then possible interferences can be identified from the full-scan GC/MS data on selected samples. Furthermore, additional ions could be monitored that would help in the identification process. The fact that

Table IV. Accuracy and Precision Data for the Stable-Labeled Isotopes by GC/MS

spike level[a]	matrix	parameter[b]	dicamba-d_3	2,4-D-d_3
4–50[c]	water	$A \pm$ SD	72 ± 21	86 ± 25
		RSD	29	29
		n	167	173
80	water	$A \pm$ SD	72 ± 21	65 ± 34
		RSD	28	52
		n	65	67
100	soil	$A \pm$ SD	55 ± 13	54 ± 20
		RSD	24	36
		n	38	29
80	soil	$A \pm$ SD	76 ± 22	78 ± 19
		RSD	28	25
		n	26	27

[a] Spike level in micrograms per liter for water and micrograms per kilograms for soil. [b] $A \pm$ SD = accuracy (average recovery) ± standard deviation; RSD = relative standard deviation; n = number of determinations. [c] Spiking level is 4 or 10 μg/L for 1-L sample aliquots and 50 μg/L for 200-mL sample aliquots.

stable-labeled isotopes are being monitored simultaneously with the naturally abundant compounds helps in the identification process since the retention time of the stable-labeled isotope and that of the naturally abundant compound are identical during sample analysis. The lack of specificity of the technique presented here has turned out to be a significant advantage for quantitative work for several reasons: (1) It eliminates the cleanup step and allows concentration of reaction extracts to 0.2 mL even in the absence of silica gel cleanup; thus, the method detection limit achieved by the SIM GC/MS technique presented here is comparable to that reported by Lee and Chau (1983) for GC/EC. (2) Interfering peaks from reactions of PFBBr with itself or with acetone under the catalytic influence of K_2CO_3 are not detected since only certain ions are being monitored.

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Registry No. Dicamba, 1918-00-9; 2,4-D, 94-75-7; H_2O, 7732-18-5.

LITERATURE CITED

Agemian, H.; Chau, A. S. Y. *J. Assoc. Off. Anal. Chem.* **1977**, *60*, 1070.
Aly, O. M.; Faust, S. D. *J. Am. Water Works Assoc.* **1964**, *56*, 189.
Chau, A. S. Y.; Afghan, B. K. *Analysis of Pesticides in Water*; CRC Press, Inc.: Boca Raton, FL, 1982; Vol. II.
Chau, A. S. Y.; Terry, K. *J. assoc. Off. Anal. Chem.* **1976**, *59*, 633.
Colby, B. N.; Rosecrance, A. E.; Colby, M. E. *Anal. Chem.* **1981**, *53*, 1907–1911.
Devine, J. M.; Zweig, G. *J. Assoc. Off. Anal. Chem.* **1969**, *52*, 187.
Environmental Protection Agency *Fed. Reg.* **1984**, *49*, 184–198.
Gutenmann, W. H.; Lisk, D. J. *J. Agric. Food Chem.* **1964**, *12*, 322.
Hern, S. C.; Beck, F. P., Jr.; Pollard, J. E. *A Field Test of the EXAMS Model in an Industrial Waste Pond*, EPA 600/X-83-034; U.S. Environmental Protection Agency, ORD, EMSL: Las Vegas, NV, 1983.
Ingram, L. L., Jr.; McGinnis, G. D.; Parlkh, S. V. *Anal. Chem.* **1979**, *51*, 1077–1078.
Khan, S. U. *J. Assoc. Off. Anal. Chem.* **1975**, *58*, 1027.
Klein, E. R.; Klein, P. D. *Biomed. Mass. Spectrom.* **1979**, *6*, 515–545.
Lee, H, B.; Chau, A. S. Y. *J. Assoc. Off. Anal. Chem.* **1983**, *66*, 1023.
Lopez-Avila, V.; Nicoll, G.; Hellier, W.; Taylor, J. H., Jr.; Hern, S. C.; Beck F. P., Jr.; Pollard, J. E. Paper presented before the Divisional of Environmental Chemistry, American Chemical Society, Seattle, WA, April 1983.
Olson, B. A.; Sneath, T. C.; Jain, N. C. *J. Agric. Food Chem.* **1978**, *26*, 640.
Pollard, J. E.; Hern, S. C. *Environ. Toxicol. Chem.* **1985**, *4*, 361–369.
Renberg, L. *Anal. Chem.* **1974**, *46*, 459.
Smith, A. E. *Weed Res.* **1972**, *12*, 364.

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A New Method for the Determination of Glyphosate and (Aminomethyl)phosphonic Acid Residues in Soils

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A new method has been developed and used to analyze soils for the phosphonic acid herbicide glyphosate and its metabolite (aminomethyl)phosphonic acid. The compounds were extracted with aqueous triethylamine solution. The phosphonic acids in the extract were derivatized with 1-fluoro-2,4-dinitrobenzene and quantified with ion-pair HPLC using tetraethylammonium bromide as counterion reagent. *N*-(Phosphonomethyl)-β-alanine was used as internal standard. Minimum detectable quantities were 0.05 $\mu g \cdot g^{-1}$ for glyphosate and 0.1 $\mu g \cdot g^{-1}$ for the metabolite.

Glyphosate, *N*-(phosphonomethyl)glycine (GLYPH), the active ingredient of the commercial herbicide Roundup, is used extensively for controlling many annual and perennial weeds. (Aminomethyl)phosphonic acid (AMPA) has been shown to be the major metabolite of GLYPH in plants and soils (Sprankle et al., 1978).

The literature on the analysis of GLYPH and AMPA residues has recently been fully reviewed (Bardalaye et al., 1984). Residues in soils have been determined quantitatively by gas chromatography (GC) (Pesticide Analytical Manual, 1977) and by high-performance liquid chromatography (HPLC) (Glass, 1983). The former method is very tedious and includes cation- and anion-exchange chromatography and a two-step derivatization procedure prior to the analysis. The HPLC method, which only determines GLYPH, suffers from a high limit of detection.

1-Fluoro-2,4-dinitrobenzene is a well-known reagent for the derivatization of primary and secondary amines in aqueous solutions (Edwards, 1977), and the high molar absorption of the 2,4-dinitrophenyl (DNP) derivatives in the UV–visible region offers a sensitive and selective quantification of the derivatives. In our procedure, GLYPH and AMPA were extracted from three soil types and analyzed as DNP derivatives by reversed-phase ion-pair HPLC. The new compound *N*-(phosphonomethyl)-β-alanine was synthesized for use as internal standard (IS).

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