Synthesis of Dimethyl Derivatives of Imidazolinone Herbicides: Their Use in Efficient Gas Chromatographic Methods for the Determination of These Herbicides

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The dimethyl derivatives of imazaquin, imazapyr, imazmethapyr, imazethapyr, 2-[4,5 dihydro-1,4-dimethyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5-methoxymethyl-3-pyridine carboxylic acid, 2-[4,5-dihydro-1,4 -dimethyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-4-methyl benzoic acid, and 2-[4,5-dihydro-1,4-dimethyl-4-(1-methyl ethyl)-5-oxo-1*H*-imidazol-2-yl]-5-methyl benzoic acid were prepared and fully characterized. The availability of these derivatives has led to the development of efficient and multiresidue gas chromatographic methods for trace level analysis of imidazolinone herbicides in matrixes such as water, soybean, and soil.

Keywords: *Imidazolinone herbicides; dimethyl derivatives; gas chromatography; mass spectrometry; NMR spectroscopy*

INTRODUCTION

The imidazolinones (Figure 1) belong to a class of herbicides most of which are used to control weeds in legume, cereal crops, and peanuts. Imazapyr (**1**), an important member of this class, has wide applications in the management of brush in forests and in total vegetation control in noncrop areas. This herbicide is also used to counter weed growth in sugar cane, rubber, and palm plantations. Some of the advantages of using these chemicals are their effectiveness as herbicides at very low concentrations (Bhalla and Shehata, 1991) and their low mammalian toxicity (Harris et al., 1991). The mode of action of imidazolinones in weed control is by the inhibition of a plant enzyme: acetohydroxy acid synthase (Shaner et al., 1984, 1985; Anderson and Hibberd, 1985; Scarpani et al., 1995, 1997). The selective herbicidal action is attributed mainly to the differential metabolic rates or pathways and in some cases is due to differences in absorption rates (Shaner and Robson, 1985; Shaner and Mallipudi, 1991). These herbicides persist in soil (Curran et al., 1992; Loux et al., 1989). Therefore, their environmental monitoring is important to avoid injury to rotational crops (Loux et al., 1989; Mills and Witt, 1989; Renner et al., 1988). Because of their wide applications and the selective phytotoxicity at low concentrations, there is a need for a simple, effective, and sensitive method for the deternination of imidazolinones.

A number of residue methods differing in sample type, extraction techniques, and instruments used have been developed over many years for the determination of these chemicals at trace levels. Several methods based on liquid chromatography with ultra violet detection (LC−UV) are available from the manufacturer of these herbicides (Devine, 1991). The extraction techniques of these methods vary depending on the nature of the sample. The extraction and cleanup techniques generally involve many laborious and time-consuming steps. Processing of soil samples, for example, starts with a solvent extraction, followed by a series of precipitation and centrifugation procedures, and then another extraction with a different solvent (Stout et al., 1997). The initial extract subsequently undergoes two solid-phase extractions (SPE) before being suitable for LC−UV analysis. Methods similar to this were used to determine the level of persistence of imidazolinones in soils (Curran et al., 1992; Loux and Reese, 1992). Even after rigorous extraction and cleanup procedures, undesirable interferences from matrix coextractives from soil or plant tissues were experienced (Nejad et al., 1998; Stout et al., 1996d). A LC−UV method for soil samples without the usual cleanup was investigated (Liu et al., 1992). However, the reported detection limit in this method was found to be too high to be of any practical value. To reduce the time for sample extraction, supercritical CO_2 fluid extraction technique (SFE) was applied for the recovery study of imazaquin **(5)** in soil matrix (Reddy and Locke, 1994). Poor recoveries were obtained as determined by LC−UV and a wide variation in extraction efficiency was experienced because of the change of SFE parameters.

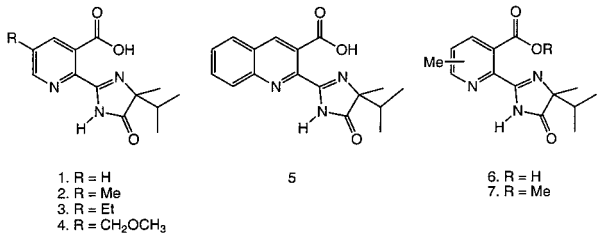

Figure 1. Structures of imidazolinone herbicides: (1) imazapyr, (2) imazmethapyr, (3) imazethapyr, (4) imazamox, (5) imazaquin, (6) imazamethabenz, and (7) imazamethabenz methyl.

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One LC−UV method (Wells and Michael, 1987) used a single solid-phase extraction step to study the recovery of imazapyr (**1**) from water. Although this procedure was found to be less labor intensive, it gave poor recoveries if the pH of the spiked water was not adjusted to a proper value. Another disadvantage of the method is that spurious results were obtained if the extract was not stored overnight in the mobile phase for equilibration. Off-line solid-phase extraction with carbogaph-1 followed by LC−UV determination has been reported to give good results for water samples (Lagana et al., 1998), but with soil samples this cleaning technique gave inconsistent recoveries (Krynitsky et al., 1999).

Analysis of imidazolinones by liquid chromatography/electrospray mass spectrometry was performed for soil (Krytrisky et al., 1999; Stout et al., 1997), water (Stout et al., 1996c), and plant tissues (Stout et al., 1996d). For plant tissues, a microwave-assisted extraction procedure was used, which was followed by filtration, centrifugation, acidification, and the cleanup step with two SPE cartridges. This method gave good recoveries of imazethapyr **(3).**

In addition to various LC techniques, other analytical methods include gas chromatography with nitrogen−phosphorus detection after in situ methylation in the injection vial (Devine, 1991; Loux and Slife, 1989); gas chromatography with nitrogen−phosphorus detection after cyclization (Mortimer and Weber, 1993); gas chromatography/mass spectrometry with electron-capture negative ion chemical ionization after in situ methylation in the injection vial (Stout et al., 1996a,b); and capillary electrophoresis (Nejad et al., 1998; Ohba et al., 1997).

Some of the drawbacks of the existing methods are (a) laborious extraction and cleanup procedures through the use of a series of solid-phase extractions, (b) uncertainty about the extent of chemical transformation during in situ methylation, (c) the necessity of having complex equipment, e.g., LC−mass spectrometer, (d) lack of sensitivity due to interferences from coextractives, or (e) column deterioration due to injection of unprocessed reaction mixtures into the instrument (Stout et al., 1997). Moreover, the applicability of some of the existing methods is generally limited to a single matrix (Curran et al., 1992; Loux and Reese, 1992; Martimer and Weber, 1993; Nejad et al., 1998; Reddy and Locke, 1994; Stout et al., 1996b,c,d, 1997; Wells and Michael, 1987; Kryntsky et al., 1999). To circumvent some of the problems associated with the existing methods, we directed our efforts to developing a simple, multiresidue, and sensitive analytical method for the determination of imidazolinones. Our aim was to utilize common instruments such as a gas chromatograph (GC) equipped with a nitrogen−phosphorus detector (NPD), and a GC coupled with a mass spectrometer, to provide the regulatory requirement for confirmation. This paper describes how this objective was achieved through the preparation of dimethyl derivatives (Figure 2) of the herbicides.

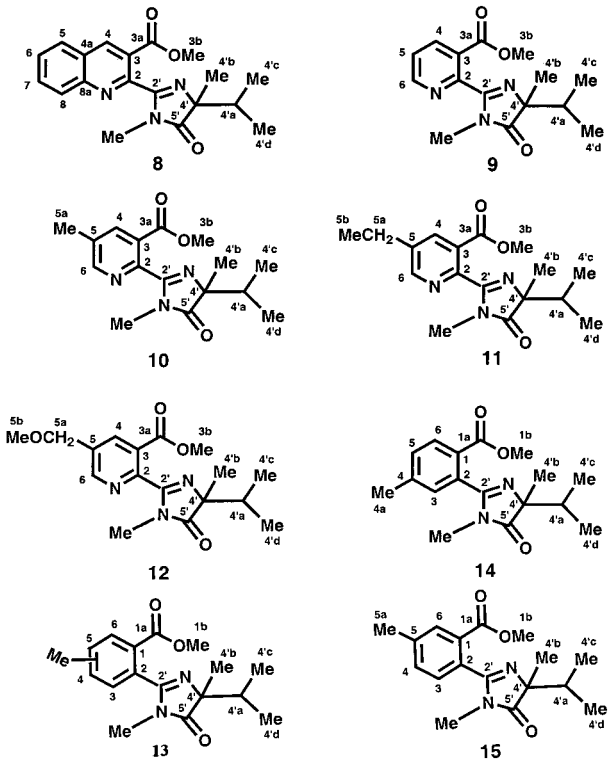


Figure 2. Structure of dimethyl derivatives of imidazoloinone herbicides.

MATERIALS AND METHODS

Reagents. Analytical standards of imazapyr, imazethapyr, imazmethapyr, imazaquin, imazmethabenz acid, imazmethabenz methyl, and imazamox were donated by the American Cyanamid Company, Princeton, NJ. Iodomethane, tetrabutylammonium hydroxide (1.0 M solution in methanol), formic acid, Celite 545, and deuteriochloroform were purchased from Aldrich Chemical Co., Inc., Milwaukee, WI. Silica gel 60 for column chromatography was purchased from Fluka Chemical Corp., Ron Konkoma, NY, and silica gel 60 F254 for thin-layer chromatography (TLC) was purchased from EM Separation Technology, Gibbstown, NJ. The RP-102 resin solid-phase extraction (SPE) cartridge was purchased from Applied Separations, Allentown, PA. Special grade of methanol, acetone, dichloromethane, ethyl ether, petroleum ether (bp 30−60 °C), and *n*-hexane, suitable for pesticide analysis, were obtained from commercial suppliers. As iodomethane (methyl iodide) is a toxic substance, appropriate precautionary measures were taken in handling this chemical.

Matrixes. The matrixes used for recovery studies were deionized water, locally grown soybean seeds, and local loams for water, soybean, and soil samples, respectively.

Experimental. Nuclear magnetic resonance (NMR) measurements were performed in deuteriochloroform on a Bruker AMX 500 spectrometer operating at 500 and 125.7 MHz for ^{1}H and ^{13}C, respectively. Abbreviations were used: s, singlet; d, doublet; q, quartet; and m, multiplet. Gas chromatography (GC) was performed with Hewlett-Packard (HP) 5890 Series II instruments equipped with a nitrogen−phosphorus detector (NPD) and an autosampler. These systems were controlled by HP G2705AA Chem Stations (Revision A.06.03). GC columns for NPD were HP-ultra 2 (25 m × 0.32 mm × 0.52 μm) and JW DB-1 (30 m × 0.53 mm × 1. 5 μm) capillary columns. The conditions for the HP-ultra 2 column were flow of He, 5 mL/min; column temperature, 2 min isothermic at 140 °C, from 140 °C to 220 °C at 30 °C/min, 2 min isothermic at 220 °C, from 220 °C to 260 °C at 10 °C/min, 15 min isothermic at 260 °C; injector temperature, 225 °C; detector temperature, 250 °C; injection volume, 2 mL. The conditions for the DB-1 column were flow of He, 10 mL/min; column temperature, 2 min isothermic at 180 °C, from 180 °C to 220 °C at 15 °C/min, 3 min isothermic at 220 °C, from 220 °C to 260 °C at 10 °C/min, 10 min isothermic at 260 °C; injector temperature, 250 °C; detector temperature, 270 °C; injection volume, 3 μL. Gas chromatography−mass spectrometry (GC−MS) was carried out using a HP 6890 GC coupled with a HP 5973 electron impact mass detector with the conditions: HP1-MS fused silica capillary column (30 m × 0.25 mm × 0.25 μm); 1 mL/min

Table 1. Proton and Carbon Chemical Shifts of Compound 8

proton chemical shifts				carbon chemical shifts			
proton	chemical shift (ppm)	multiplicity	coupling constants (Hz)	carbon no.	chemical shift (ppm)	carbon no.	chemical shift (ppm)
Me (4′c, 4′d)	0.83, 0.94	two ds	$J_{4'a,\,4'c} = J_{4'a,\,4'd} = 6.8$	2	148.11	2′	159.92
Me (4′b)	1.27	s		3	126.61	4′	74.23
Me-N	2.95	s		4	139.19	3b	52.54
Me (3b)	3.78	s		5	128.40	4′a	34.33
4′a	1.97	m		6	128.60	4′b	20.18
4	8.68	s		7	132.10	4′c	16.60
5	7.71	d	$J_{5,\,6} = 8.0$	8	129.62	4′d	17.05
6	7.54	ddd	$J_{6,\,7} = 7.0$; $J_{6,\,8} = 1.0$	4a	124.29	N−CH_3	27.22
7	7.72	ddd	$J_{5,\,7} = 1.4$; $J_{7,\,8} = 8.5$	3a	165.67	5′	185.65
8	8.04	d		8a	147.94		

Table 2. Proton and Carbon Chemical Shifts of Compound 9

proton chemical shifts				carbon chemical shifts			
proton	chemical shift (ppm)	multiplicity	coupling constants (Hz)	carbon no.	chemical shift (ppm)	carbon no.	chemical shift (ppm)
4	8.17	dd	$J_{4,\,5} = 8$; $J = 1.6$	2	149.30	2′	159.79
5	7.43	dd	$J_{5,\,6} = 4.8$	3	127.55	4′	74.06
6	8.72	dd		4	137.84	4′a	34.20
N-Me	2.88	s		5	124.33	4′b	20.11
Me (3b)	3.76	s	$J_{4'a,\,4'c} = J_{4'a,\,4'd} = 6.8$	6	151.74	4′c	16.88
Me (4′b)	1.25	s		2′	159.79	4′d	16.47
Me (4′c, 4′d)	0.81, 0.94	two ds		3a	165.21	5′	185.46
H − 4′a	1.97	m		3b	52.53	N-Me	26.93

Table 3. Proton and Carbon Chemical Shifts of Compound 10

proton chemical shifts				carbon chemical shifts			
proton	chemical shift (ppm)	multiplicity	coupling constants (Hz)	carbon no.	chemical shift (ppm)	carbon no.	chemical shift (ppm)
Me (4′c, 4′d)	0.91, 0.78	two ds	$J_{4'a,\,4'c} = J_{4'a,\,4'd} = 6.8$	5′	185.49	4′	73.94
Me-N	2.84	s		3a	165.41	3b	52.41
Me (3b)	3.72	s		2′	159.76	4′a	34.16
Me (4′b)	1.22	s		6	152.13	N-Me	26.90
Me (5a)	2.33	s		2	146.48	4′b	20.10
4′a	1.93	m		4	137.96	5′a	17.96
4	7.95	d	$J_{4,\,6} = 1.5$	5	134.62	4′c	16.86
6	8.51	d		3	127.06	4′d	16.45

Table 4. Proton and Carbon Chemical Shifts of Compound 11

proton chemical shifts				carbon chemical shifts			
proton	chemical shift (ppm)	multiplicity	coupling constants (Hz)	carbon no.	chemical shift (ppm)	carbon no.	chemical shift (ppm)
6	8.52	d	$J_{4,\,6} = 1.9$	6	151.44	4′c	16.43
4	7.94	d		5	140.55	4′d	16.84
Me (3b)	3.71	s		5′	185.47	3	127.22
Me-N	2.84	s		5a	25.51	3a	165.47
Me (5b)	1.16	t	$J_{5a,\,5b} = 7.6$	5b	14.64	3b	52.38
5a	2.63	q		4	136.81	2	146.68
4′a	1.92	m	$J_{4'a,\,4'c} = J_{4'a,\,4'd} = 6.8$	4′	73.91	N-Me	26.68
Me (4′b)	1.20	s		4′a	34.14	2′	159.81
Me (4′c, 4′d)	0.80, 0.90	two ds		4′b	20.10		

constant flow of He; 1 min isothermic at 70 °C, from 70 °C to 150 °C at 10 °C/min, 5 min isothermic at 150 °C, from 150 °C to 280 °C at 10 °C/min, 2 min isothermic 280 °C; injector temperature, 250 °C; transfer line temperature, 280 °C; ion source temperature, 230 °C; ionization energy, 70 eV; injection volume, 1 μL. Full scan mass spectra (mass range of 70−450 amu) were recorded at a rate of 3 spectra per second. Detection of the imidazolinones derivatives was achieved by selecting appropriate ions (SIM) from the full scan spectra using HP Enhanced Chemstation software (B 1701 BA, version B.01.00). Melting points were determined on a Fisher-Johns melting-point apparatus and are uncorrected. Evaporation of solvent from extracts was done under vacuum with the use of a Buchi rotary evaporator at temperature below 35 °C. Elemental analyses were performed by Atlantic Microtab, Inc., Norcross, GA.

General Procedure for Preparation of Dimethyl Derivatives. An imidazolinone (ca 200 mg) in acetone (10 mL) solution, tetrabutylammonium hydroxide (1.6 mL, I M solution in methanol), and methyl iodide (3.2 mL) were heated together at 40 °C in a screw-capped tube for 2 h. The reaction mixture was cooled to 25 °C and the solvent was removed by evaporation. Water (10 mL) was added to the residue and the mixture was extracted with 3 × 20 mL of 30% ethyl ether in petroleum ether. The extract was dried (Na_2SO_4) and evaporated to give crude dimethyl derivatives. The crude derivative was purified by chromatography on a silica-gel column. Elution of the column was performed with either hexane−ethyl acetate (2:1, v/v) or dichloromethane−acetone (4:1; v/v), and the fractions were checked by TLC on silica-gel plates. Fractions containing a pure dimethyl derivative were combined and evaporated to yield the following analytically pure dimethyl derivatives in 70−75% yield.

2-[4,5-Dihydro-1,4-dimethyl-4-(1-methyl ethyl)-5-oxo-1*H*-imidazol-2-yl]-3-quinolinecarboxylic acid methyl ester (8). The compound 8 was obtained as crystalline

Table 5. Proton and Carbon Chemical Shifts of Compound 12

proton chemical shifts				carbon chemical shifts			
proton	chemical shift (ppm)	multiplicity	coupling constants (Hz)	carbon no.	chemical shift (ppm)	carbon no.	chemical shift (ppm)
4	8.20	d		6	150.7	3	127.54
6	8.72	d	$J_{4,6} = 2.0$	5	135.33	3a	165.39
5a	4.54	s		5′	185.65	3b	52.68
Me (5b)	3.42	s		5a	71.02	2	148.53
Me (4′b)	1.32	s		5b	58.69	2′	159.83
Me (4′c, 4′d)	0.90, 1.0	two ds	$J_{4'a,4'c} = J_{4'a,4'd} = 6.8$	4	136.79	N-Me	27.10
4′a	2.03	m		4′	74.25	4′b	20.27
Me-N	2.94	s		4′a	34.36	4′c	16.62
				4′b	20.27	4′d	17.03

Table 6. Proton and Carbon Chemical Shifts of Compound 14

proton chemical shifts				carbon chemical shifts			
proton	chemical shift (ppm)	multiplicity	coupling constants (Hz)	carbon no.	chemical shift (ppm)	carbon no.	chemical shift (ppm)
6	8.00	d	$J_{5,6} = 8.0$	6	130.85	4′c	17.25
5	7.37	d		5	131.06	4′d	16.29
3	7.22	s		5′	185.42	3	130.94
4′a	2.12	m	$J_{4'a,4'c} = J_{4'a,4'd} = 6.8$	4	143.97	2	131.64
Me (1b)	3.82	s		4a	21.40	2′	162.60
Me (4′b)	1.38	s		4′	73.78	1	126.63
Me (4′c, 4′d)	0.93, 1.08	two ds		4′a	34.31	1a	165.63
Me (4a)	2.49	s		4′b	20.73	1b	52.23
Me-N	2.81	s					

Table 7. Proton and Carbon Chemical Shifts of Compound 15

proton chemical shifts				carbon chemical shifts			
proton	chemical shift (ppm)	multiplicity	coupling constants (Hz)	carbon no.	chemical shift (ppm)	carbon no.	chemical shift (ppm)
6	7.84	s		6	130.09	4′c	17.20
4	7.38	d	$J_{3,4} = 8.0$	5	140.61	4′d	16.69
3	7.25	d		5′	185.39	3	130.73
4′a	2.11	m	$J_{4'a,4'c} = J_{4'a,4'd} = 6.8$	5a	21.02	2	128.53
Me (5a)	2.76			4	133.30	2′	162.31
Me (4′b)	1.37	s		4′	73.78	1	129.20
Me (1b)	3.81	s		4′a	34.31	1a	165.44
Me-N	2.80	s		Me (4′b)	20.73	1b	52.10
Me (4′c, 4′d)	0.91, 1.08	two ds		Me-N	26.87		

Table 8. GC−MS (EI) Data of Dimethyl Derivatives

Compound	M^+	$[M-1]^+$	$[M-2]^+$	$[Ar\overset{C=NMe}{\underset{CO_2Me}{}}]^+$	$[Ar-C=NMe]^+$
Imazapyr	289	247	215	177	118
Imazamethapyr	303	261	229	191	132
Imazaethapyr	317	275	243	205	146
Imazamox	333	291	259	221	162
Imazaquin	339	297	265	227	168
Imazamethabenz	302	260	228	190	131

1 = CMe_2; 2= CO_2Me & Me; Ar = Aromatic ring of imidazolinones

material which had mp 125−126 °C after recrystallization from ethyl ether−hexane. NMR data are shown in Table 1. MS (EI) [*m*/*z*, relative intensity (%)]: 339 ($[M]^+$, 5), 297 ($[M - CMe_2]^+$, 53), 296 ($[M - CHMe_2]^+$, 45), 265 ($[M - CO_2Me + Me]^+$, 11), 227 ($[M-(C_6H_{10}NO)]^+$, 100), 168 ($C_{11}H_8N_2]^+$, 14).

Anal. Calcd for $C_{19}H_{21}N_30_3$: C, 67.24; H, 6.24; N, 12.38. Found: C, 67.39; H, 6.28; N, 12.36.

2-[4,5-Dihydro-1,4-dimethyl-4-(1-methyl ethyl)-5-oxo-1*H*-imidazol-2-yl]-3- pyridinecarboxylic acid methyl ester (9). The methyl ester **9** was obtained as an oil. NMR data are shown in Table 2. MS (EI) [(*m*/*z*, relative intensity (%)]: 289 ($[M]^+$, 3), 247 ($[M - CMe_2]^+$, 69), 246 ($[M-CHMe_2]^+$, 36), 215 ($[M - CO_2Me + Me)]^+$, 13), 177 ($[M-(C_6H_{10}NO)]^+$, 100), 118 ($C_7H_6N_2]^+$, 16).

Anal. Calcd for $C_{15}H_{19}N_30_3$: C, 62.27; H, 6.62; N, 14.52. Found: C, 61.70; H, 6.66; N, 14.15.

2-[4,5-Dihydro-1,4-dimethy-4-(1-methyl ethyl)-5-oxo-1*H*-imidazol-2- yl]-5-methyl-3-pyridinecarboxylic acid methyl ester (10). The compound **10** was obtained as an oil. NMR data are shown in Table 3. MS (EI) [*m*/*z*, relative intensity (%)]: 303 ($[M]^+$, 5), 261 ($[M - CMe_2]^+$, 76), 260 ($[M - CHMe_2]^+$, 59), 229 ($[M - (CO_2Me+Me)]^+$, 14), 191 ($[M - C_6H_{10}NO]^+$, 100), 132 ($[C_8H_8N_2]^+$, 20).

Anal. Calcd for $C_{16}H_{21}N_3O_3$: C, 63.35; H, 6.98; N, 13.85. Found: C, 62.87; H, 7.03; N, 13.58.

2-[4,5-Dihydro-1,4-dimethyl-4-(1-methyl ethyl)-5-oxo-1*H*-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid methyl ester (11). Compound **11** was obtained as a crystalline compound and it had mp 42 °C after recrystallization from hexane. NMR data are shown in Table 4. MS (EI) [*m*/*z*, relative intensity (%)]: 317 ($[M]^+$, 2), 275 ($[M - CMe_2]^+$, 32), 274 ([M

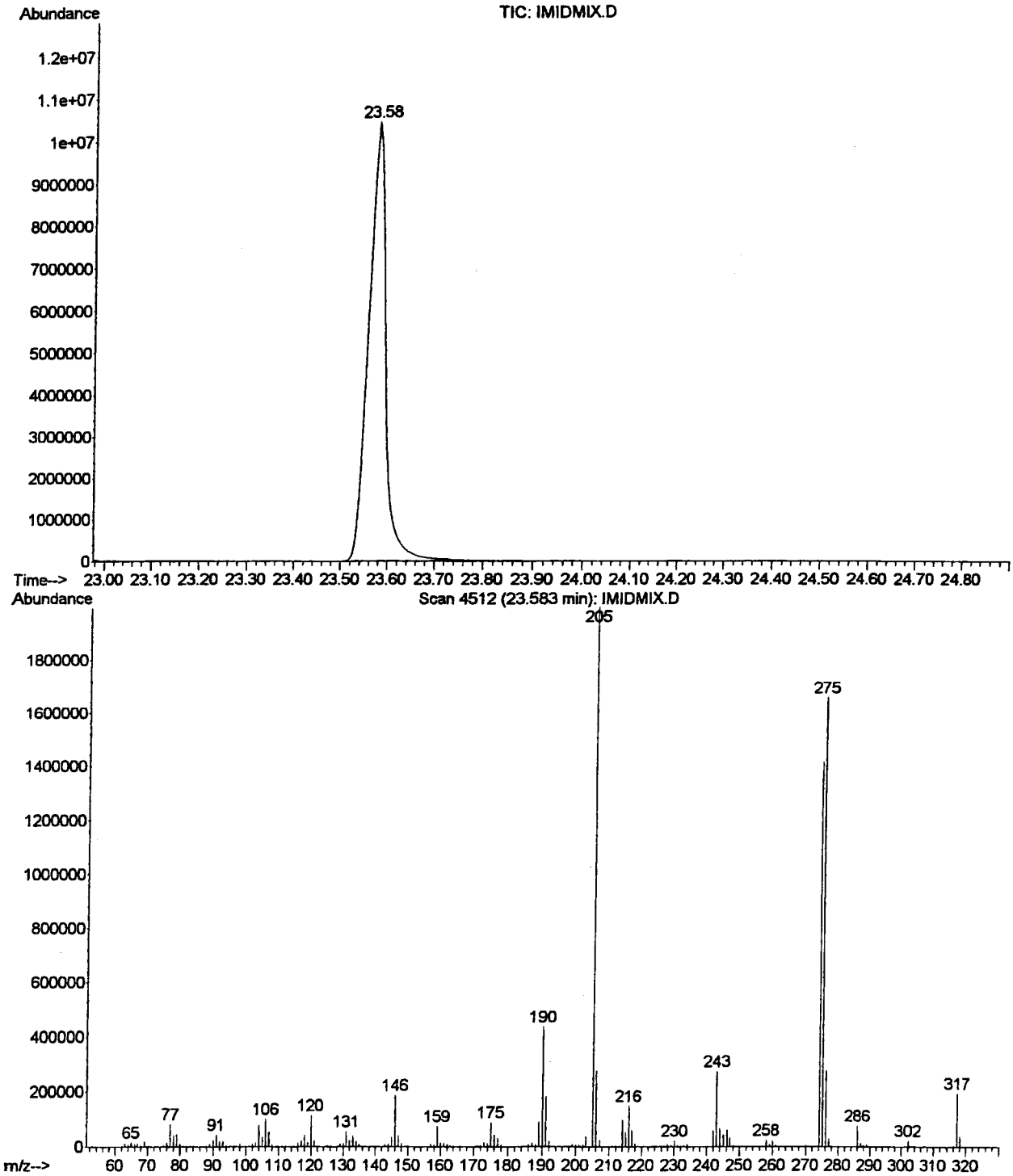


Figure 3. Total ion chromatogram and electron impact mass spectrum of derivative **11** obtained from a HP 6890 GC coupled with a HP 5973 quadropole mass spectrometer (for detailed GC and MS conditions, see Materials and Methods).

$-$ $CHMe_2$ $]^+$, 27), 243 ([M $-$ (CO_2Me + Me)]$^+$, 6), 205 ([M $-$ $C_6H_{10}NO]^+$, 100), 146 ([$C_9H_{10}N_2]^+$, 20).

Anal. Calcd for $C_{17}H_{23}N_3O_3$: C, 64.33; H, 7.30; N, 13.24. Found: C, 64.25; H, 7.29; N, 13.19.

2-[4,5-Dihydro-1,4-dimethyl-4-(1-methyl ethyl)-5-oxo-1*H*-imidazol-2-yl]-5 -methoxymethyl-3-pyridinecarboxylic acid methyl ester (12). Compound **12** was obtained as an oil. NMR data are shown in Table 5. MS (EI) [*m/z*, relative intensity (%)]: 333 ([M]$^+$, 7), 291 ([M $-$ CMe_2 $]^+$, 87), 290 ([M $-$ $CHMe_2$)]$^+$, 64), 259 ([M $-$ (CO_2 Me + Me)]$^+$, 15), 221 ([M$-$ $C_6H_{10}NO]^+$, 100), 162 ([$C_9H_{10}N_2O]^+$, 16).

Anal. Calcd for $C_{17}H_{23}N_3O_4$: C, 61.25; H, 6.95; N, 12.60. Found: C, 60.98; H, 6.85; N, 12.47.

2-[4,5-Dihydro-1,4-dimethyl-4-(1-methyl ethyl)-5-oxo-1*H*-imidazol-2-yl]-4 (and 5)-methylbenzoic acid methyl ester (13). The isomeric mixture **13** was obtained from the methylation of either **6** or **7**. Pure isomers **14** and **15** were obtained by subjecting **13** to column chromatography on silica gel using hexanes$-$ethyl acetate (3:1, v/v) as the eluant. The 4-methyl isomer **14** was slightly faster moving than the 5-methyl-isomer **15**.

2-[4,5-Dihydro-1,4-dimethyl-4-(1-methyl ethyl)-5-oxo-1*H*-imidazol-2-yl]-4- methylbenzoic acid methyl ester (14). The isomer **14** was isolated as a crystalline compound from the isomeric mixture **13** by chromatography. It had a mp 78$-$79 °C after crystallization from hexane. NMR data are

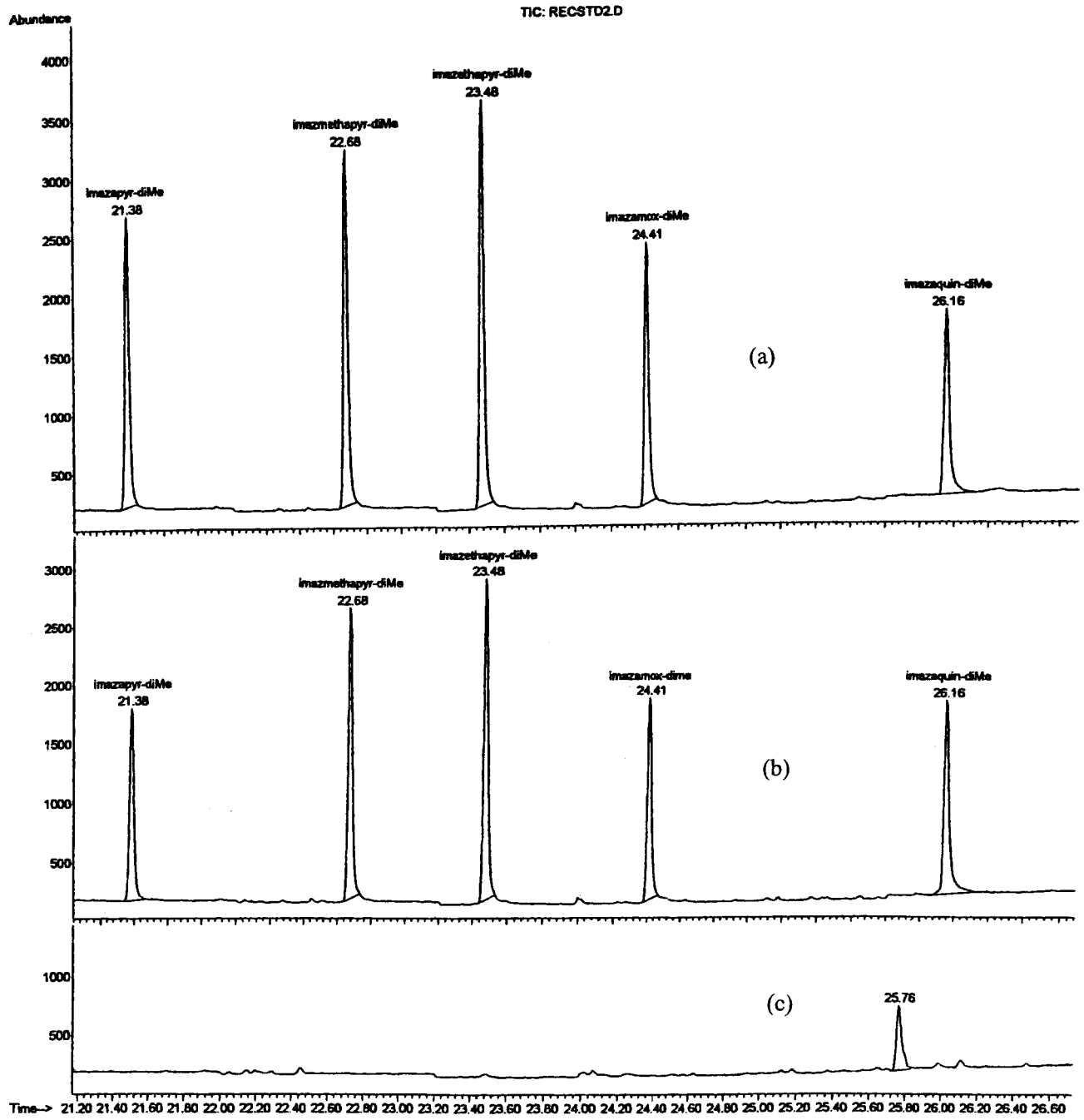


Figure 4. SIM chromatograms of (a) derivatives **8**−**12**, (b) 0.5 ppb fortified water extract, and (c) control water extract obtained from a HP 6890 GC coupled with a HP 5973 quadropole mass spectrometer (for detailed GC and MS conditions, see Materials and Methods).

shown in Table 6. MS (EI) [*m*/*z*, relative intensity (%)]: 302 ([M]$^+$, 6), 260 ([M − CMe_2]$^+$, 35), 259 ([M − $CHMe_2$]$^+$, 29), 228 ([M − (CO_2Me + Me)]$^+$, 4), 190 ([M − $C_6H_{10}NO$]$^+$, 100), 131 ([C_9H_9N] $^+$, 27).

Anal. Calcd for $C_{17}H_{22}N_2O_3$: C, 67.53; H, 7.33; N, 9.26. Found: C, 67.29; H, 7.34; N, 9.04.

2-[4,5-Dihydro-1,4-dimethyl-4-(1-methyl ethyl)-5-oxo-1*H*-imidazol-2-yl]-5-methylbenzoic acid methyl ester (15). The isomer **15** was obtained from the isomeric mixture **13** by column chromatography. NMR data are shown in Table 7. MS (EI) [*m*/*z*, relative intensity (%)]: 302 ([M]$^+$, 4), 260 ([M − CMe_2]$^+$, 26), 259 ([M − $CHMe_2$]$^+$, 25), 228 ([M − (CO_2 Me + Me)]$^+$, 3), 190 ([M − $C_6H_{10}NO$]$^+$, 100), 131 ([C_9H_9N]$^+$, 30).

Anal. Calcd for $C_{17}H_{22}N_2O_3$: C, 67.53; H, 7.33; N, 9.26. Found: C, 67.29; H, 7.34; N, 9.09.

Sample Extraction. *Water Samples.* A 500-mg RP-102 resin solid-phase extraction (SPE) cartridge was preconditioned with 3 × 6 mL of methanol followed by 3 × 6 mL of 1% formic acid in water, and a reservoir was attached to the top of the SPE cartridge. A fortified or otherwise water sample (250 mL) premixed with 2.5 mL of 1% formic acid in water was loaded on the cartridge through the reservoir. The flow rate was adjusted to 2−3 drops per s and the sample was allowed to pass through the SPE column completely. The cartridge was dried under vacuum for 10 min and then eluted with 10 mL of methanol adjusting the flow rate to 1 drop per s. Methanol was removed from the elute by evaporation under vacuum followed by addition of 2 mL of acetone and evaporation to dryness. The residue was treated with a few crystals of anhydrous sodium sulfate and quantitatively transferred to a 13 × 100 mm screw-capped tube using 3 mL of acetone. This solution was methylated by the general methylation procedure for sample extracts. This extraction method is applicable to a set of 8 to 10 samples.

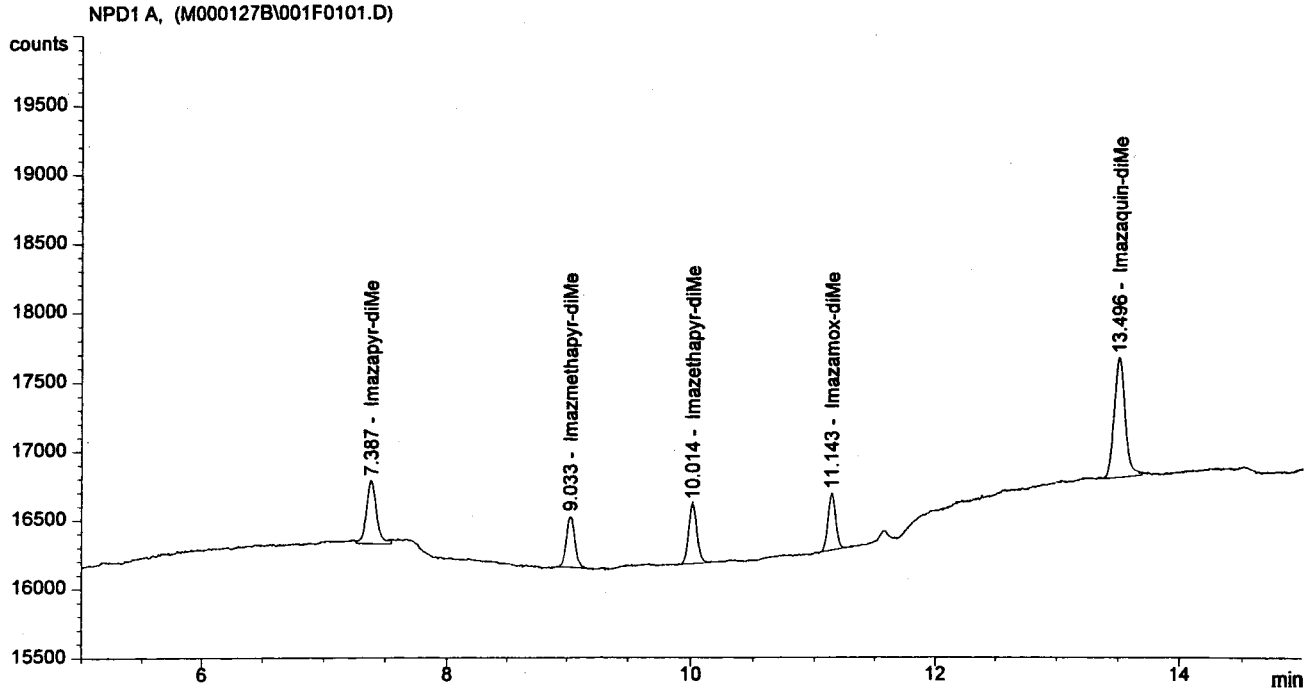
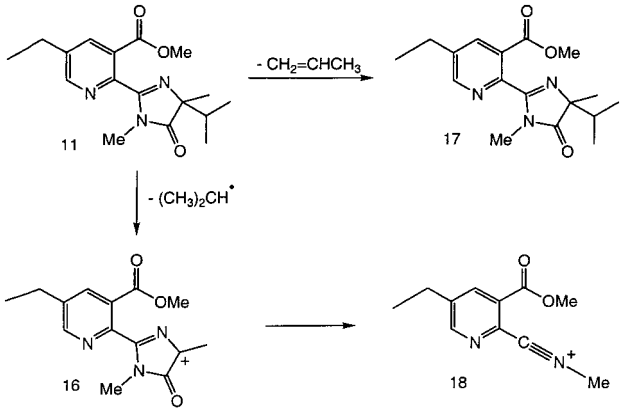


Figure 5. GC−NPD chromatogram of 11 pg of dimethyl derivatives of imidazolinone herbicides obtained from a HP 6890 GC with a DB-1 column (for detailed GC conditions, see Materials and Methods).

Scheme 1. Partial Mass Spectral Fragmentation Pattern for Compound 11.



Soybean Samples. Methanol (100 mL) was added to 20 g of ground soybean (fortified or otherwise) in a 200-mL propylene centrifuge bottle. The mixture was shaken mechanically for 15 min followed by vacuum filtration. The residue was rinsed with 2 × 40 mL of methanol and filtered. The volume of the combined filtrate was made to 200 mL with methanol and half of the filtrate was evaporated under vacuum to an oily residue. Water (100 mL) was added and the mixture was shaken vigorously for one minute. Using a pH meter, the pH was adjusted to 0.75−1.0 with 6 N hydrochloric acid solution. Acidification was followed by stirring with Celite 545 (5 g) for 30 min and vacuum filtration. The filtrate was extracted with 3 × 50 mL of dichloromethane and the extract was dried (Na_2SO_4). The extract was evaporated almost to dryness under vacuum, acetone (25 mL) was added, and the mixture was evaporated to dryness. The residue was treated with a few crystals of anhydrous sodium sulfate and quantitatively transferred with acetone (3 mL) into a 13 × 100 mm screw-cap tube for methylation. This extraction procedure can be adopted to handle 8 to 10 samples at a time.

Soil Samples. To ground soil (10 g) in a 200-mL propylene centrifuge bottle, 0.5 N sodium hydroxide solution (100 mL) was added. The mixture was shaken for 20 min in a mechanical shaker followed by centrifugation for 10 min. The supernatant was decanted into a 500-mL beaker; the insoluble material in the centrifuge bottle was shaken mechanically with 2 × 50 mL of 0.5 N sodium hydroxide solution, centrifuged, and decanted. The combined alkaline extract was adjusted to pH 0.75−1.0 with 6 N hydrochloric acid. Treatment with Celite 545 (3 g), extraction with dichloromethane, and preparation of the acetone solution for methylation were done in the same way as for soybean samples. Like water and soybean, 8 to 10 soil samples can be extracted simultaneously.

General Procedure for Methylation of Sample Extracts. To the acetone solution in the screw-cap tube as obtained from a sample (water, soybean, or soil) extraction, 160 μL of tetrabutylammonium hydroxide solution (1.0 M in methanol) and 320 μL of iodomethane were added. The tube was capped and heated at 40 °C for 1.5 h. The reaction mixture was cooled to room temperature, evaporated to dryness under vacuum, treated with water (10 mL) and extracted with 3 × 30 mL of ethyl ether−hexane (1:2; v/v). The extract was dried (Na_2SO_4) and evaporated to a residue under vacuum. The residue was treated with a few crystals of anhydrous sodium sulfate and dissolved in a known volume of 10% acetone in hexane for analysis by gas chromatographs equipped with nitrogen−phosphorus and mass selective detectors. A series of sample extracts can be methylated and processed simultaneously.

RESULTS AND DISCUSSION

Preparation of Dimethyl Derivatives. This methylation procedure is similar to a published procedure (Hopper, 1987) for phenoxy acid herbicides. The imidazolinone herbicides were treated with methyl iodide in the presence of tetrabutylammonium hydroxide. Isolation of pure derivatives (**8**−**12**) was achieved by the use of column chromatography. Column chromatography also led to the separation of pure *meta* isomer (**14**) and *para* isomer (**15**) from regiomeric mixture (**13**). The derivatives (**8**−**12**, **14**, and **15**) were fully characterized by elemental, NMR, and mass spectral analyses. There were earlier reports (Loux et al., 1989 and Mallipudi et al., 1994) of in situ formation of **8** and **11** at the gas chromatograph's injection port. Previously, however,

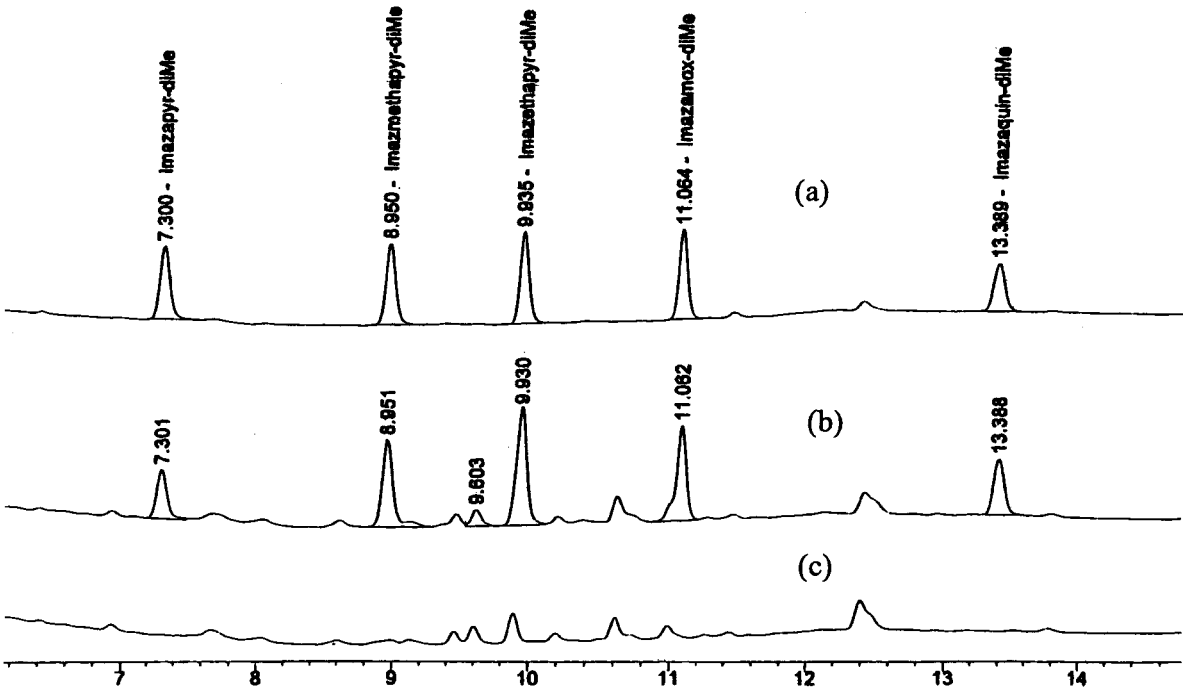


Figure 6. GC−NPD chromatogram of (a) derivatives **8**−**12**, (b) 50 ppb fortified soybean extract, and (c) control soybean extract obtained from a HP 6890 GC with a DB-1 column (for detailed GC conditions, see Materials and Methods).

Table 9. Recoveries of Imidazolinone Herbicides in Water

	1 ppb level						5 ppb level						10 ppb level								
	recovery (%)					%	recovery (%)					%	recovery (%)								%
herbicides[a]	run 1	run 2	run 3	mean	SD	CV	run 1	run 2	run 3	mean	SD	CV	run 1	run 2	run 3	run 4	run 5	run 6	mean	SD	CV
imazapyr	74	90	78	81	8	10	96	102	105	101	5	5	122	113	101	103	112	123	112	9	8
imazmethapyr	71	90	96	86	13	15	102	108	107	106	3	3	126	105	104	111	118	133	116	12	10
imazethapyr	78	93	83	84	8	10	101	101	102	101	1	1	120	96	100	112	113	130	112	13	11
imazamox	81	94	82	86	7	8	113	113	103	110	6	5	98	114	122	-	-	-	111	12	11
imazaquin	96	95	94	95	1	1	105	114	105	108	5	5	111	98	100	103	112	114	106	7	6

[a] Detected in the form of dimethyl derivatives.

Table 10. Recoveries of Imidazolinone Herbicides from Soil

	10 ppb level							50 ppb level						80 ppb level					
	recovery (%)						%	recovery (%)					%	recovery (%)					%
herbicides[a]	run 1	run 2	run 3	run 4	mean	SD	CV	run 1	run 2	run 3	mean	SD	CV	run 1	run 2	run 3	mean	SD	CV
imazapyr	99	110	68	87	91	18	20	88	80	76	81	6	7	60	67	71	66	6	9
imazmethapyr	108	108	80	70	92	19	21	106	114	88	103	13	13	83	81	96	87	8	9
imazethapyr	107	121	80	79	97	21	22	99	92	86	92	7	8	86	88	104	93	10	11
imazamox	102	96	73	77	87	14	16	98	90	83	90	8	9	-	-	-	-	-	-
imazaquin	106	103	103	101	103	2	2	111	111	109	110	1	1	98	102	112	104	7	7

[a] Detected in the form of dimethyl derivatives.

Table 11. Recoveries of Imidazolinone Herbicides from Soybean

	5 ppb level						10 ppb level						50 ppb level					
	recovery (%)						recovery (%)						recovery (%)					
herbicides[a]	run 1	run 2	run 3	mean	SD	% CV	run 1	run 2	run 3	mean	SD	% CV	run 1	run 2	run 3	mean	SD	% CV
imazapyr	79	101	98	93	12	13	69	73	58	67	8	12	72	77	73	74	3	4
imazmethapyr	92	112	100	101	10	10	103	107	97	102	5	5	104	106	105	105	1	1
imazethapyr	112	122	107	113	8	7	102	118	125	115	12	10	105	105	102	104	2	2
imazamox	103	92	102	99	6	6	98	107	89	98	9	9	106	100	102	103	3	3
imazaquin	105	94	113	104	10	10	103	105	104	104	1	1	104	110	114	109	5	5

[a] Detected in the form of dimethyl derivatives.

these derivatives (**8** and **11**) were not isolated in pure forms and no information about their properties was available.

Nuclear Magnetic Resonance (NMR) Spectroscopy. Tables 1−7 provide ^{1}H NMR and ^{13}C NMR information for the dimethyl derivatives (**8**−**12**, **14**, and **15**). As expected, the ^{1}H NMR spectrum of compound **8** showed 21 protons (Table 1) with *N*-methyl and carboxylate methyl protons appearing at 2.95 and 3.78 ppm, respectively.

The heteronuclear ^{1}H−^{13}C correlation spectra of all these compounds (**8**−**12**, **14**, and **15**) were recorded and

were used to assign ^{13}C chemical shift values for many carbons of the dimethyl derivatives. The ^{13}C NMR spectrum of **8** (Table 1) contains nineteen characteristic signals for the 19 carbon atoms with the *N*-methyl carbon and the carboxylate methyl carbon resonances appearing at 27.22 and 52.54 ppm, respectively. The appearance of characteristic signals due to two methyl groups in the ^1H NMR and ^{13}C NMR spectra of compounds **8**−**12**, **14**, and **15**, and other NMR data in Tables 1−7 unambiguously support the structural identity of these compounds.

GC−Mass Spectrometry. Similar to NMR data, the mass spectral properties of the methyl derivatives were consistent with their assigned structures. The GC−MS analyses were performed under electron impact mode which produced characteristic ion chromatograms and the corresponding mass spectra. Electron impact ionization modes are often too strong for molecular ions to be observed in the spectra, but these molecules were sufficiently stable to produce detectable molecular ions. Figure 3 shows the ion chromatogram and mass spectrum of the dimethyl derivatives (**11**) of imazethapyr. The molecular ion, M^+ was observed at *m*/*z* 317 and the ions at *m*/*z* 274, 275, and 205 were due to fragments **16**, **17**, and **18**, respectively (Scheme 1).

The fragmentation patterns of other dimethyl derviatives (**8**−**10**, **12**, **14**, and **15**) were similar to that of **11**, as indicated by mass spectral data in Table 8 and also by the data in the experimental section. The characteristic peaks in the total ion chromatogram (TIC) and the appearance of a number of significant ions in the mass spectra were of considerable analytical importance. These were conveniently exploited for low-level multiresidue detection and confirmation of imidazolinone herbicides in different matrixes. For each of the methylated derivatives, three significant ions corresponding to M-42, M-43, and M-112 (fragment **18** or similar) were selectively monitored to produce extracted chromatograms of excellent quality as illustrated in Figure 4.

The selective ion monitoring (SIM) mode eliminated most of the matrix interferences enabling detection of imidazolinones at very low concentrations in such diverse matrixes as water, soil, and soybean. The limits of detection (LODs) of these compounds were determined from their lowest fortification level that produced characteristic peaks with signal-to-noise ratio of ≥ 3. Using SIM mode, the observed LODs for compounds **8**−**12** in water, soil, and soybean were 0.2, 1, and 3 ppb, respectively.

Gas Chromatography with Nitrogen−Phosphorus Detector. The presence of 2−3 nitrogen atoms in the molecule makes the methyl derivatives (**8**−**12**, **14** and **15**) very sensitive to a nitrogen−phosphorus detector (NPD). Peaks with considerable intensity were observed when a few picograms of these compounds (**8**−**12**) were introduced into a gas chromatography (GC) with nitrogen−phosphorus detector (Figure 5). Thus, similar to GC−MS, GC−NPD has also very low detection limits. For water, soil, and soybean samples, the detection limits have been found to be the same as those of GC−MSD. Excellent linear plots with *R* value ≥ 0.990 were observed over a concentration range of 0.007 to 0.7 ppm. As these derivatives have distinct, well-separated peaks, GC−NPD can be conveniently used for their multiresidue analysis. Figure 6 illustrates how this multiresidue method can generate gas chromatographic information of excellent quality when it is applied to a difficult matrix such as soybean.

RECOVERY STUDY

Data from recovery studies on water, soil and soybean samples fortified with imidazolinone herbicides at various levels are presented in Tables 9−11. These data were from GC−NPD analyses and the values were in good agreement when compared with GC−MSD data. Good recoveries (Tables 9−11) and method precision were observed. The recoveries were generally in the range of 80−116%, covering three matrixes with relative standard deviation mostly in the range of ≤ 10%.

CONCLUSION

The present study has demonstrated that multiresidue analysis of imidazolinone herbicides can be achieved efficiently by the use of common gas chromatographic devices. For complex matrixes such as soil or soybean, these procedures have eliminated the time-consuming cleanup steps involving a series of solid-phase extraction cartridges. The described methods are very sensitive with good fortification recoveries and precision.

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