Analysis of Sulfonylurea Herbicides by Gas-Liquid Chromatography. 2. Determination of Chlorsulfuron and Metsulfuron-methyl in Soil and Water Samples

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Sulfonylureas are extracted from water samples using solid-phase extraction (SPE) with C18-silica/Teflon disks. Soil samples are extracted with 0.1 M sodium hydrogen carbonate, and the acidified extracts are processed as for water samples. The concentrated eluents are treated with diazomethane in ethyl acetate, which forms the thermally stable *N,N'*-dimethyl derivatives of the herbicides. Residues are screened by capillary GC using effluent splitting to electron capture and nitrogen-phosphorus detectors. Residues are confirmed by GC-MS using selected ion monitoring. Detection limits were below 0.1 μg/L for water and below 1 μg/kg for soil samples. Accuracy and precision at 0.5 and 0.1 μg/L each were, respectively, for chlorsulfuron 95 ± 2% and 110 ± 16% and for metsulfuron-methyl 90 ± 6% and 98 ± 11%. At 5 and 1 μg/kg each in soil the recoveries were, respectively, for chlorsulfuron 78 ± 20% and 69 ± 6% and for metsulfuron-methyl 92 ± 18% and 105 ± 17%. The methods are superior to previous methods for these herbicides based on GC because the intact herbicides are determined rather than thermal degradation products.

INTRODUCTION

The high herbicidal activity and long persistence of sulfonylurea herbicides have led to the need for assays in soil sensitive to the micrograms per kilogram [parts per billion (ppb)] level. Bioassays have been used to achieve the required sensitivity (Rahman, 1989; Guenther et al., 1989). While these methods have been developed to a high degree of sophistication for use in quantitative research trials, they lack the specifity required in diagnostic or regulatory applications. Development of suitable assays based on high-performance liquid chromatography (HPLC) have been limited by the modest sensitivity and lack of selectivity of UV absorption detection. Photoconductivity detection (PCD) has been successfully used in standard HPLC methods for chlorsulfuron (Zahnow, 1982; Slates and Watson, 1988) and metsulfuron-methyl (Hershberger and Brennan, 1988). These methods have proved to be suitable for developing registration data but have disadvantages for more general use due to the unavailability of PCD equipment in most laboratories and the lack of suitable confirmatory techniques such as gas chromatography-mass spectrometry (GC-MS).

Previous methods based on GC have relied on detection of breakdown products due to the thermal instability of sulfonylureas. The method of Long et al. for chlorsulfuron used GC with nitrogen-phosphorus detection (NPD) of the thermal decomposition product 2-amino-4-methoxy-6-methyl-1,3,5-triazine (AMMT) for quantitation (Long et al., 1989, 1990). Ahmad et al. have used diazomethane methylation to produce the *N*-monomethyl derivative of chlorsulfuron (Ahmad, 1987; Ahmad and Crawford, 1990). However, this derivative was also found to be thermally unstable, producing a GC-ECD peak due to the *N*-methylsulfonamide of chlorsulfuron (Klaffenbach et al., 1993). Pentafluorobenzylation has also been used as a basis for GC-ECD determination of chlorsulfuron and metsulfuron-methyl in soil and water (Cotterill, 1992). In this case the derivatization conditions resulted in formation of the bis-PFB derivatives of the sulfonamide hydrolysis products, which were detected with good sensitivity. Although these derivatives were shown to be suitable as the basis for a method for chlorsulfuron and metsulfuron-methyl residues in soil and water, interferences were a problem with plant matrices. While these methods were demonstrated to be capable of determining sulfonylurea herbicides to low levels in soil, they have the disadvantage of a possible lack of specificity as the detected products could also be produced from various similar pesticides or their metabolites.

We have recently shown that the *N,N'*-dimethyl derivatives of chlorsulfuron and metsulfuron-methyl can be produced in high yields using diazomethane in ethyl acetate (Klaffenbach et al., 1993). These derivatives are thermostable and can be detected intact by GC with good sensitivity and peak shape. We now describe procedures for residue determination of these herbicides at low levels in soil and water following solid-phase extraction (SPE).

EXPERIMENTAL PROCEDURES

Instrumentation. *Gas Chromatography.* A Varian 3500 gas chromatograph equipped with a Model 8100 autosampler, a split/splitless injector, and electron capture (ECD) and nitrogen-phosphorus (NPD) detectors was used. A Hewlett-Packard HP-5 capillary column (25 m × 0.2 mm i.d., 0.33-μm film) was used with a 1 m × 0.53 mm i.d. deactivated FSOT precolumn and postcolumn splitting (1:4) using 0.12 mm i.d. FSOT lines to the ECD and NPD. The column was temperature programmed from 85 °C (1 min) at 40 °C/min to 150 °C (2 min) and at 5 °C/min to 250 °C (20 min). Injections (1 μL) were made in splitless mode at an injector temperature of 220 °C. Both detectors were operated at 300 °C.

GC-MS Confirmation. A Kratos MS80RFA mass spectrometer operated at resolution 1000 (10% valley) and directly interfaced to a Carlo Erba Mega Series gas chromatograph was used. A DB-5 [15 m × 0.32 mm i.d., 1-μm film (J&W Scientific)] was used. The temperature program was 100 °C (1 min) raised at 20 °C/min to 280 °C (10 min). The split/splitless injector and transfer oven were operated at 220 and 250 °C, respectively. Injections (1 μL) were made in splitless mode. Electron impact ionization (EI) used electron energy 30 eV and source temperature 200 °C. Full-scan spectra (50-550 amu) were acquired with 1

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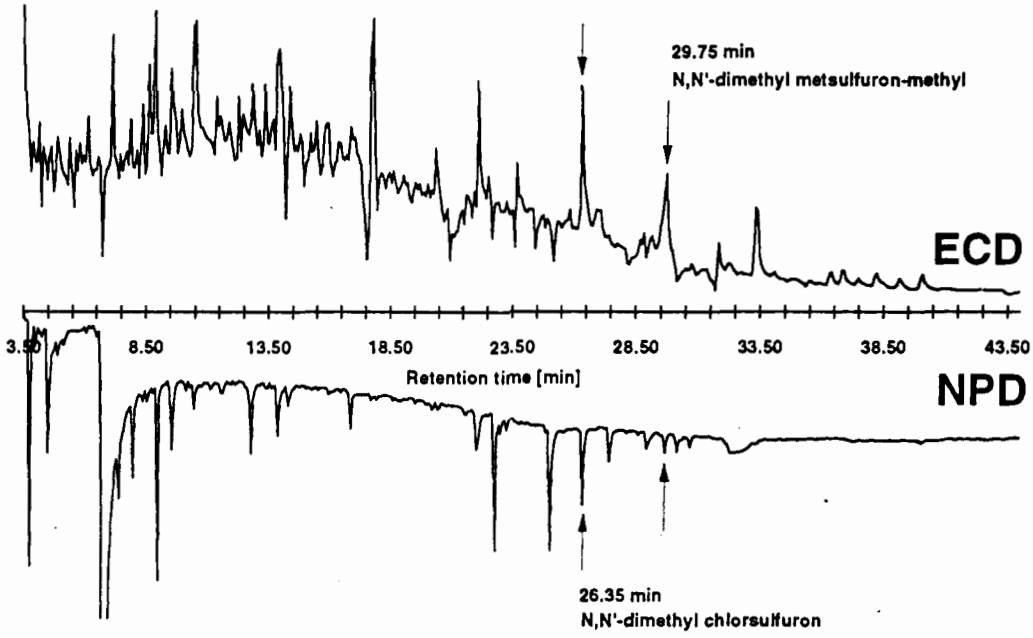

Figure 1. Water sample spiked with 0.1 μg/L of chlorsulfuron and metsulfuron-methyl, screening analysis by GC-ECD/NPD.

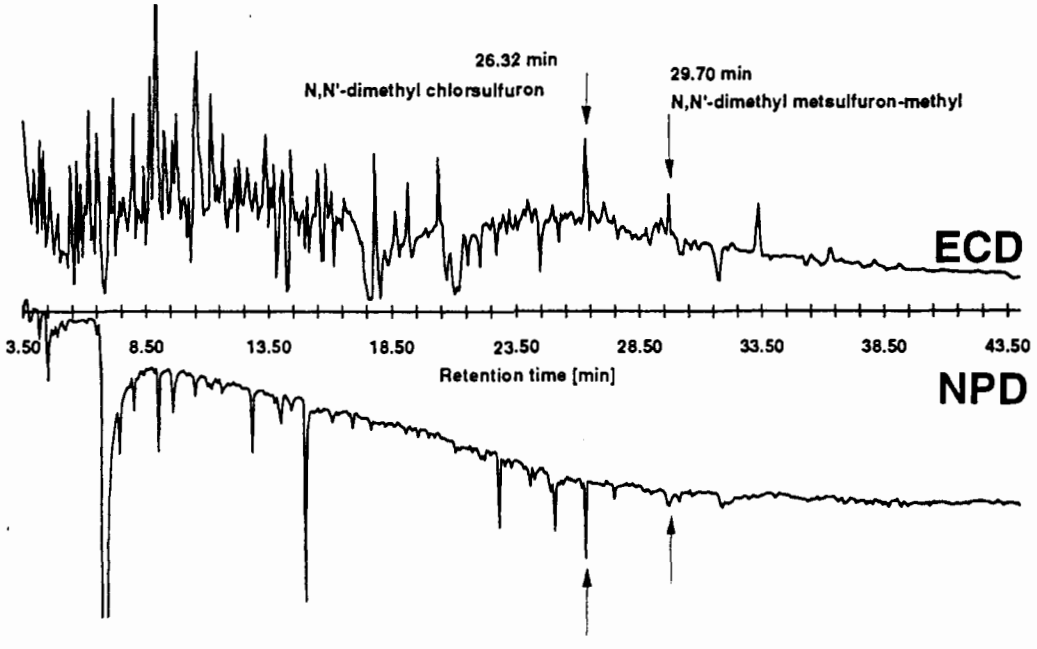

Figure 2. Soil sample spiked with 1 μg/kg of chlorsulfuron and metsulfuron-methyl, screening analysis by GC-ECD/NPD.

scan/s. Selected ion monitoring (SIM) experiments used two masses (181.0726 and 210.0991) with a dwell time of 0.45 s and a cycle time of 1 s. Helium was used as carrier gas in all GC experiments.

Chemicals. All solvents were of pesticide grade purchased from Mallinckrodt, Paris, KY. *N*-Methyl-*N*-nitroso-*p*-toluenesulfonamide (Diazald), used for preparation of diazomethane, was obtained from Sigma Chemical Co., St. Louis, MO. Sodium sulfate (Mallinckrodt) was heated at 600 °C for 6 h. Chlorsulfuron, 1-[(2-chlorophenyl)sulfonyl]-3-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)urea, CAS number 64902-72-3, purity 95.0%, was obtained from Chem Service, West Chester, PA. Metsulfuron-methyl, methyl 2-(4-methoxy-6-methyl-1,3,5-triazin-2-ylcarbamoylsulfamoyl)benzoate, CAS number 74223-64-6, purity 99.3%, was purchased from Dr. Ehrenstorfer, Augsburg, Germany. Empore 47-mm C18 solid-phase extraction disks were obtained from Analytichem International, Harbor City, CA.

Water. Distilled and purified water (MilliQ, Millipore Corp., Milford, MA) was used for recovery experiments.

Soil. A Horotiu sandy loam was used for recovery experiments. The soil characteristics were pH 5.9, organic carbon 6.7%, sand 67%, silt 25%, and clay 8%. The soil was air-dried and sieved (<2 mm).

Diazomethane Generation. A glass and Teflon apparatus was used similar to that of Cohen (1984). Diazald (1 g) was added to a mixture of diethylene glycol monoethyl ether (3 mL) and diethyl ether (2 mL) and the apparatus purged with nitrogen (~1 bubble/s). The gas was passed through ethyl acetate (10 mL) cooled in an ice bath. The diazomethane generation was started by adding 40% aqueous potassium hydroxide solution (2 mL). Transfer of diazomethane into the ethyl acetate was continued until the ether layer in the apparatus was only slightly yellow. The diazomethane solution was stored at −20 °C for a maximum period of 1 week.

Safety Precautions. Diazomethane is explosive and toxic and a suspected carcinogen. It should be prepared and used in a fume hood behind a safety shield. Ground joints and sharp surfaces in the generation apparatus should be avoided. However,

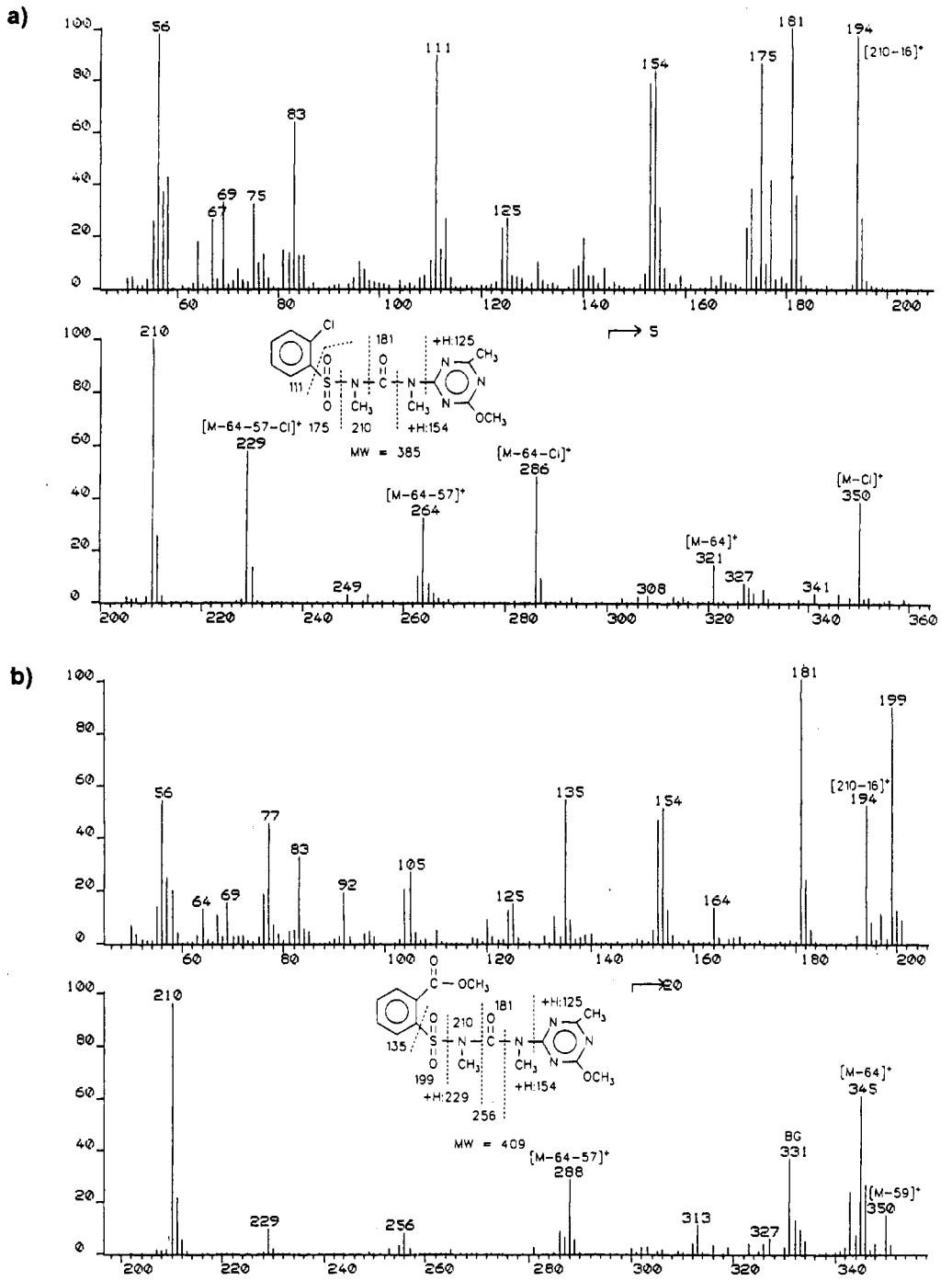

Figure 3. EI mass spectrum (30 eV) of the *N,N'*-dimethyl derivative of (a) chlorsulfuron and (b) metsulfuron-methyl.

the device used for preparation and the small amount of material used minimize the hazards involved.

Derivatization Procedure. Diazomethane solution (0.5 mL) was added to samples dissolved in ethyl acetate (1 mL). The mixture was kept at room temperature for 30 min before removal of solvent and excess reagent with a gentle stream of nitrogen. The residue was redissolved in toluene.

Water Extraction Procedure. Water samples (1000 mL) were acidified with hydrochloric acid to pH 2, and methanol (5 mL) was added. The sample was then extracted by SPE using the Empore C18 disk system (Kraut-Vass and Thoma, 1991). The disks were prewashed with ethyl acetate (10 mL) in an all-glass filtration apparatus using slow vacuum elution and then dried for at least 5 min. The disks were then conditioned by slow elution with methanol (10 mL) followed by deionized water (2 × 10 mL), leaving some water on the disk. Air contact with the disk was avoided until sample extraction had been completed. The acidified water sample was then passed through the conditioned disk. The vacuum was adjusted to give a flow of approximately 15 mL/min. The sample flask and the extraction funnel were rinsed with water, and the rinsing water was also passed through the disk. The disks were then dried by air suction for 30 min, and a sample tube (25 mL) was placed on the filter outlet. Elution was performed with ethyl acetate (2 × 10 mL). The disk was prewet without vacuum for 1 min before the rest of the solvent was sucked through. The elution procedure was then repeated. The combined eluates were dried over anhydrous sodium sulfate and rotary evaporated to near dryness. The residue was then transferred quantitatively into a sample vial using ethyl acetate and the volume adjusted to 1 mL. This solution was derivatized as described before, and the final toluene solution (100 μL) was analyzed by gas chromatography.

Soil Extraction. Soil samples (100 g) were placed in centrifuge tubes, and 0.1 M sodium hydrogencarbonate (100 mL) was added. The suspension was stirred and sonicated for 3 min. Following centrifugation (10 min, 3000 rpm), the aqueous solution was decanted and the extraction repeated twice. Particles of plant material were removed by filtering the extracts through glass

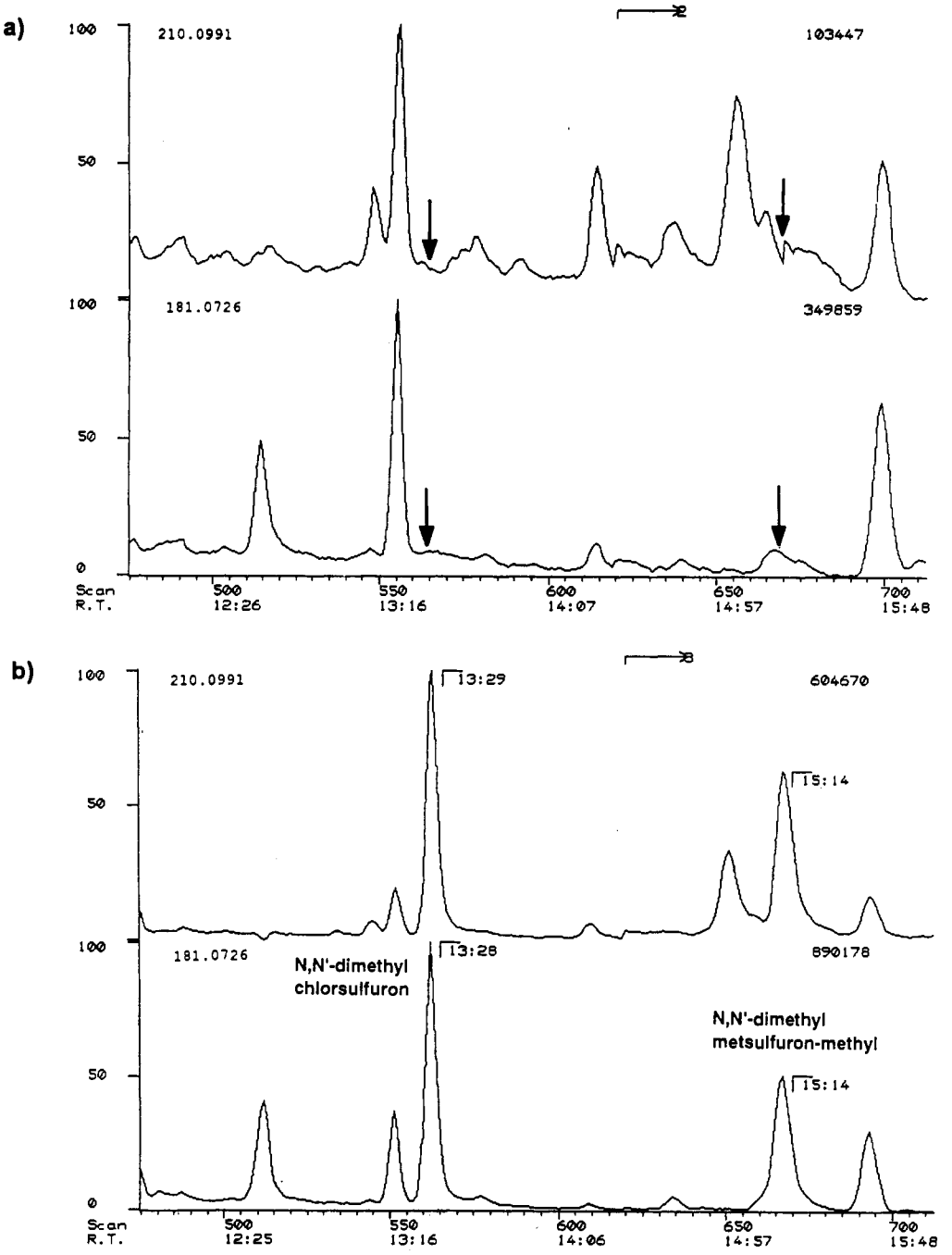

Figure 4. GC–MS-SIM chromatograms of water sample extracts, (a) blank and (b) spiked with 0.1 μg/L of chlorsulfuron and metsulfuron-methyl.

wool with rinsing. The combined soil extract (approximately 300 mL) was acidified using hydrochloric acid to pH 2, and methanol (2 mL) was added. Sulfonylurea herbicides were extracted from this solution with C18 SPE disks. The disk conditioning, sample extraction, and elution procedure were performed as described for water samples except a glass fiber filter (1 μm, Whatman) was used on top of the extraction disk. The eluate was dried, concentrated, and methylated as described above.

Standards and Fortification. (*1*) *Standards.* Chlorsulfuron and metsulfuron-methyl (5 mg each) were dissolved in 50.0 mL of ethyl acetate. This solution was diluted 1:200 and 1:1000 using ethyl acetate.

(2) *Water Samples.* Aliquots (1 mL) of the 1:200 and 1:1000 diluted standard solutions were pipetted into empty flasks (1000 mL). After evaporation of the solvent, deionized water (1000 mL) was added. These fortifications resulted in herbicide concentrations of 0.5 and 0.1 μg/L, respectively. Five replicates at each fortification level were extracted as above and analyzed by GC and GC–MS.

(*3*) *Soil Samples.* One milliliter of the 1:200 and 1:1000 diluted standard solutions was added to soil subsamples (100 g). The solvent was allowed to evaporate over 30 min. These fortifications resulted in herbicide concentrations of 5 and 1 μg/kg, respectively. Five replicates at each fortification level were extracted as above and analyzed by GC and GC–MS.

RESULTS AND DISCUSSION

Methylation of chlorsulfuron and metsulfuron-methyl using diazomethane results in either *N*-monomethyl or *N,N'*-dimethyl derivatives depending on the reaction conditions (Klaffenbach et al., 1993). The highest yields of thermostable dimethyl derivative were achieved using ethyl acetate as solvent for the methylation. The dimethyl derivatives can be analyzed by gas chromatography without decomposition.

Analysis of water samples was straightforward using the SPE method described. Figure 1 shows the ECD and

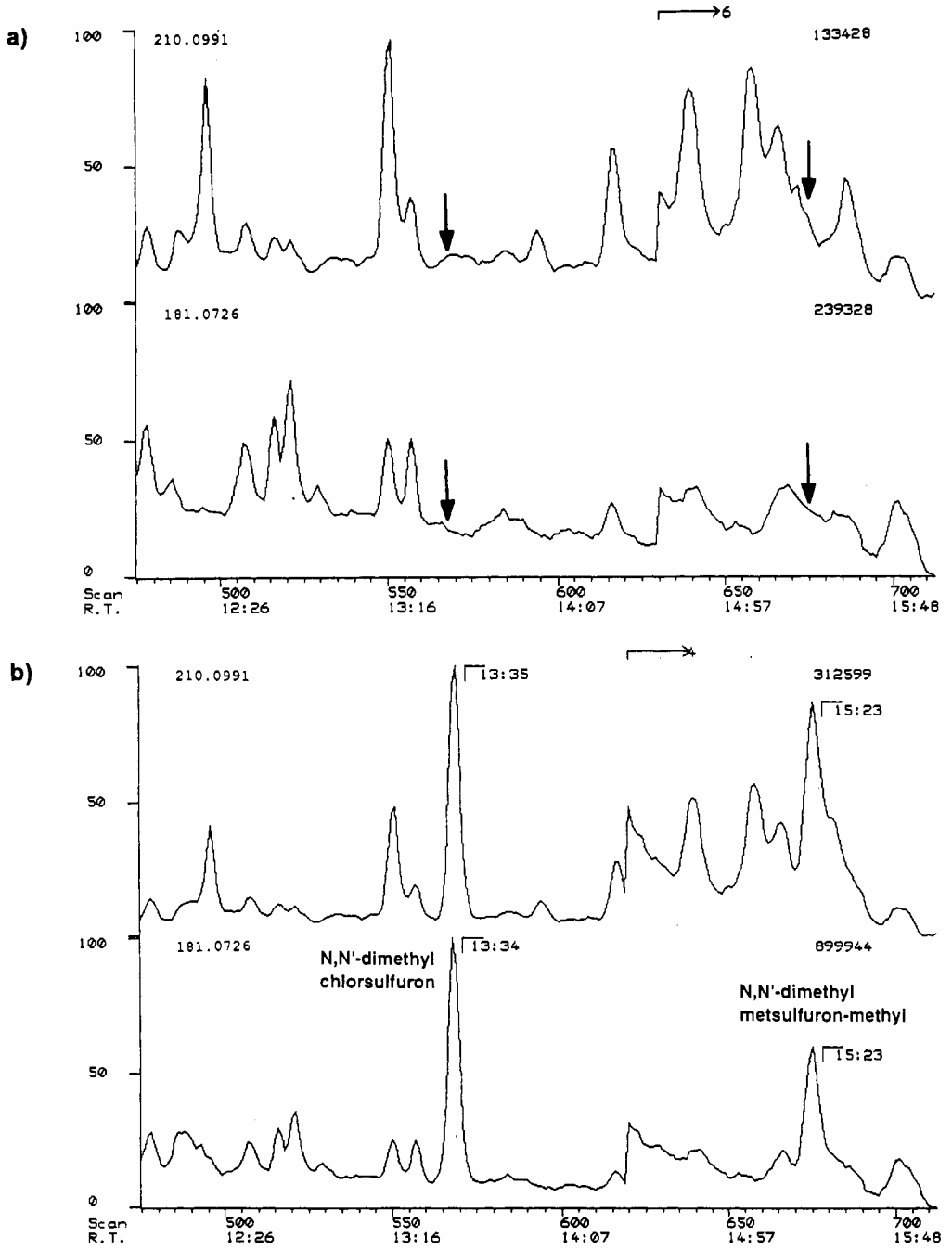

Figure 5. GC–MS-SIM chromatograms of soil sample extracts, (a) blank and (b) spiked with 1 μg/kg of chlorsulfuron and metsulfuron-methyl.

NPD chromatograms of the derivatized extract of a water sample spiked with 0.1 μg/L chlorsulfuron and metsulfuron-methyl. Despite the lack of cleanup, the chromatograms were free of major interfering peaks and the herbicides showed a good signal-to-noise ratio.

The GC-ECD results for chlorsulfuron in water at 0.5 and 0.1 μg/L (five replications) gave mean recoveries of 95% (94–96%, 2% RSD) and 110% (99–120%, 15% RSD), respectively. The corresponding results for metsulfuron-methyl were 90% (84–102%, 7% RSD) and 98% (84–114%, 11% RSD).

This compares favorably with the results of Ahmad (1987), where liquid–liquid extraction of chlorsulfuron in water at 0.5 μg/L fortification level gave a 101% recovery (9% RSD). However, at the 0.1 μg/L level the RSD of the liquid–liquid extraction was 10% compared with 15% when using the SPE method described here. At lower concentration levels SPE disks have been reported to give higher standard deviation values (Wells and Michael, 1987; Kraut-Vass and Thoma, 1991). However, the method still provided good recovery and adequate precision.

Encouraged by the good results for disk SPE of water, we modified an extraction method published for the analysis of chlorsulfuron in soil by Ahmad and Crawford (1990), who used 0.1 M sodium hydrogen carbonate as the extractant. Rather than partitioning the aqueous extract with dichloromethane as specified by Ahmad and Crawford (1990), we used the SPE disks to recover the herbicide residues following the method developed for water samples.

Figure 2 shows the ECD and NPD chromatograms of a soil sample spiked with 1 μg/kg chlorsulfuron and metsulfuron-methyl and extracted as described. Both substances, as their *N,N'*-dimethyl derivatives, were well resolved from background peaks. The chromatograms were interference-free, especially in the retention time region after 25 min where the derivatives eluted. The

signal-to-noise ratio was good on both detectors, and the ratio of responses (relative to standards) provides confirmation of identity.

The GC-ECD results for chlorsulfuron in soil at 5 and 1 μg/kg (five replications) gave mean recoveries of 78% (66–104%, 20% RSD) and 69% (59–80%, 8% RSD), respectively. The corresponding results for metsulfuron-methyl were 92% (65–107%, 21% RSD) and 105% (91–121%, 16% RSD). The recovery data obtained for chlorsulfuron were similar to those published for the original method, where liquid–liquid extraction and column chromatographic cleanup gave recoveries of 85% with 9.3% RSD at the 5 μg/kg level (Ahmad and Crawford, 1990).

The dimethyl derivatives proved suitable for GC–MS confirmation of the herbicides in water and soil extracts. Figure 3 gives the EI (30 eV) mass spectra for dimethylchlorsulfuron and dimethylmetsulfuron-methyl. Although the molecular ions are absent, there are a number of diagnostic fragment ions. The prominent ions at 181.0726 and 210.0991, due to cleavage of each side of the *N*-methyl group with charge retention on the triazine fragment, are common to the derivatives of both herbicides and therefore very suitable for selected ion monitoring (SIM) experiments. Figure 4 shows the GC–MS-SIM chromatograms for derivatized extracts of water unspiked and spiked with the herbicides at 0.1 μg/L. Figure 5 shows the corresponding GC–MS-SIM results for soil unspiked and spiked at 1 μg/kg. The unspiked chromatograms show the lack of interferences in the retention regions for the two herbicides, while the extracts of spiked samples show responses in the expected intensity ratios on the two mass channels and with good signal-to-noise ratio. Note that the computer-generated plots have different scale factors for the spike and control chromatograms. The magnetic sector instrument used in these GC–MS experiments was operated at a resolution of 1000 (10% valley). Bench-top quadrupole or ion trap instruments operating at unit mass resolution ($m/\Delta m$ ca. 200 at mass 210) will be significantly less selective in SIM mode, which may lead to more interference than observed here (Holland, 1990).

The instrumental limits of detection of the dimethyl derivatives of chlorsulfuron and metsulfuron-methyl were about 100 pg on the ECD and the MS in SIM mode. Using the extraction and derivatization methods described, both compounds could be detected at low levels in water and soil without cleanup or injection of large volumes of extract into the GC.

Extraction of chlorsulfuron from water samples by using liquid–liquid extraction with dichloromethane was laborious, and an additional column cleanup step was required to achieve clean extracts. The SPE disk system applied in this method generally provided a number of advantages over the cartridges traditionally used for SPE such as enhanced speed, higher recovery values, and lower standard deviations (Markell et al., 1991; Kraut-Vass and Thoma, 1991). Additionally, the disks provide the common advantages of SPE over liquid–liquid extraction of lower solvent use and less background. About 35 mL of ethyl acetate was required to wash the extraction disk, elute the substance, and rinse the glassware compared with more than 300 mL of dichloromethane required for the liquid–liquid extraction method (Ahmad, 1987).

CONCLUSION

Using the extraction and derivatization methods described, chlorsulfuron and metsulfuron-methyl can be analyzed in soil and water samples at very low concentration levels. Confirmation of the results can be carried out using GC–MS. The methods provide good recovery and adequate reproducibility for determination at 0.1 μg/L in water and 1 μg/kg in soil.

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