LC/MS Analysis of 4-Methoxy-6-methyl-1,3,5-triazin-2-yl-Containing Sulfonylurea Herbicides in Soil

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A multiresidue method for the determination of chlorsulfuron, metsulfuron-methyl, thifensulfuron-methyl, and triasulfuron in soil by high-performance liquid chromatography/electrospray/mass spectrometry (LC/ES/MS) is reported. A clay loam soil, spiked at 6.0 μg kg^{-1} with all four herbicides, was extracted with 0.1 M ammonium carbonate, and the extract was cleaned up by C_{18}-SPE. The herbicides were quantified using selected ion monitoring. The percent recoveries were as follows: chlorsulfuron, 80.1 ± 7.9%; metsulfuron-methyl, 99.4 ± 8.6%; thifensulfuron-methyl, 95.9 ± 5.9%; and triasulfuron 100.5 ± 24.3%. We have shown that we can analyze and confirm four 4-methoxy-6-methyl-1,3,5-triazin-2-yl-containing sulfonylurea herbicides in soil by LC/ES/MS. This multiresidue method should also be appropriate for other sulfonylurea and polar herbicides.

Keywords: *Chlorsulfuron; metsulfuron-methyl; thifensulfuron-methyl; triasulfuron; LC/MS; electrospray; soil*

INTRODUCTION

Since the early 1980s, there has been a dramatic increase in the number of and the use of sulfonylurea herbicides to control broadleaf weeds and some grasses in cereal crops. Because of the low application rates of these chemicals, <100 g ha^{-1}, analysis at the trace levels (μg kg^{-1}) necessary to monitor environmental fate has been difficult. Plant bioassays and extraction (supercritical fluid or organic solvent) followed by high-performance liquid chromatographic (HPLC), gas chromatographic (GC), supercritical fluid chromatographic (SFC), or capillary electrophoretic (CE) techniques or enzyme immunoassay (EIA) detection have all been used with varying degrees of success (Smith, 1995).

One of the two earliest analytical techniques is plant bioassay (Hsiao and Smith, 1983), and it continues to be used (James et al., 1995). Bioassays can be used to detect residues to <100 μg kg^{-1}, however, there are numerous limitations including that they are limited to soil samples containing only one herbicide. The other early analytical technique, and most commonly used at present, is HPLC with UV or photoconductivity detectors (Zahnow, 1982). The lowest typical detection limit for HPLC UV detection is 0.5−1.0 mg kg^{-1}; however, detection limits of 5 μg kg^{-1} have been reported (Galletti et al., 1995). Photoconductivity detectors, although not commonly used, have been used in methods capable of determining sulfonylurea herbicides as low as 0.2 mg kg^{-1} (Zahnow, 1982, 1985). SFC has also been used with a UV detector (Wheeler and McNally, 1987). After organic solvent (acidic methanol/water) or supercritical fluid (methanol-modified CO_2) extraction and analysis by HPLC UV, recoveries can be 40−90%, depending on soil (i.e., Berdeaux et al., 1994).

Analysis of sulfonylurea herbicides by GC has been difficult because they are nonvolatile and thermally labile. Sulfonylurea herbicides have been analyzed by GC/MS by determining acidic aqueous hydrolysis products of parent compounds after first removing the aryl sulfonamide metabolites from the sample (Thompson and MacDonald, 1992). Formation of *N*,*N*′-dimethyl derivatives using diazomethane allows analysis by GC with specific ion detectors such as NPD, ECD, and MS at levels of 0.4−2 μg kg^{-1} depending on analyte (Klaffenbach and Holland, 1993a,b; Klaffenbach et al., 1993). Recently NPD and ECD have been used with SFC (Berger, 1995) without derivatization of sulfonylurea herbicides extracted from water.

CE has been used for detection and quantification of sulfonylurea herbicides in soil (Dinelli et al., 1995a,b). Recovery (organic solvent extraction) was >80%, and the limit of detection was 10 μg kg^{-1}. CE has also been used in analysis of sulfonylureas in water (Dinelli et al., 1993, 1995) and grain (Krynitsky and Swineford, 1995). EIA has been developed for individual sulfonylurea herbicides (Hall et al., 1990; Schlaeppi, 1994; Brady et al., 1995). Limits of quantification have been reported as low as 0.10 μg kg^{-1} in soil. Limitations of EIA are potential cross-reactivities of analytes, and no commercial multiresidue EIAs are available.

A limitation with all the above methods is the lack of confirmation of the herbicide, which would require a second analytical method. To avoid two methods of analyses, sulfonylurea herbicides have been determined using direct liquid insertion LC/MS (Shalaby, 1985) and HPLC/thermospray/MS (Shalaby et al., 1992; Volmer et al., 1995), CE/electrospray (ES)/MS/MS (Garcia and Henion, 1992), and direct exposure probe in negative-ion desorption chemical ionization MS/MS (Winnick et al., 1995).

A promising multiresidue analytical method is LC/ES/MS. Separation of extracted analytes by HPLC with detection and quantification by mass spectrometry would requre no derivatization or special cleanup steps. Ionization of the analyte could provide a mass spectra with at least three ions necessary for confirmation in

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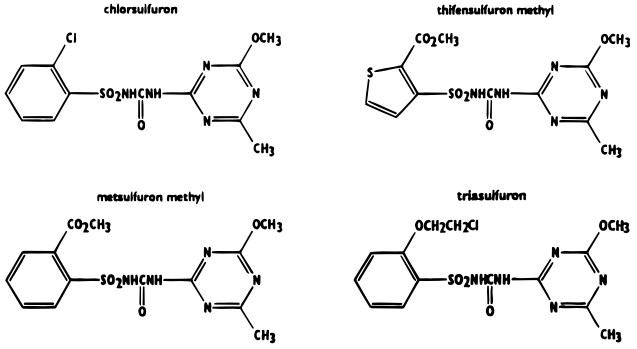

Figure 1. Molecular structures of chlorsulfuron, metsulfuron-methyl, thifensulfuron-methyl, and triasulfuron.

environmental samples. Recently, Volmer et al. (1995) developed a multiresidue method for the determination of trace levels of sulfonylurea herbicides in water. This paper reports the extraction, separation, quantitation, and confirmation of four 4-methoxy-6-methyl-1,3,5-triazin-2-yl-containing sulfonylurea herbicides in soil by LC/ES/MS.

MATERIALS AND METHODS

Reagents. Analytical grade standards (>95% purity) of chlorsulfuron (2-chloro-*N*-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]benzenesulfonamide) (MW 357.8), metsulfuron-methyl (methyl 2-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl]benzoate) (MW 381.4), and thifensulfuron-methyl (methyl 3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl]-2-thiophene carboxylate) (MW 387.4) were donated by DuPont Agricultural Products and triasulfuron (2-(2-chloroethoxy)-*N*-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]benzenesulfonamide) (MW 401.8) was donated by Ciba Geigy. Chemical structures are shown in Figure 1.

Other reagents included ACS/HPLC/GC grade acetonitrile (Mallinckrodt Ultim AR), high-purity solvent methanol and water (Burdick and Jackson), nanograde ethyl acetate (Mallinckrodt), ACS reagent grade ammonium carbonate (J. T. Baker), 88% formic acid (J. T. Baker), 85% phosphoric acid (Mallinkrodt AR), and glacial acetic acid (aldehyde free, Baker Analyzed Reagent) (J. T. Baker). Solid-phase extraction (SPE) cartridges were C-18 Mega Bond-Elut, 1 g/6 mL (Varian).

Liquid Chromatography/Mass Spectrometry. A Hewlett Packard HPLC electrospray mass spectrometer was used for analyses. The HPLC was a HP1090M Series II with binary solvent system, autosampler, heated column compartment, and diode array detector. A Hewlett Packard HP5989B MS engine was connected to the HPLC with a Hewlett Packard electrospray MS interface. The column was an ODS Hypersil 5 μg × 100 × 2.1 mm (Hewlett Packard).

The HPLC mobile phase was 0.1% formic acid/acetonitrile. For gradient elution, the mobile phase was 10% acetonitrile at 4.0 min, 35% acetonitrile at 10.0 min, 35% acetonitrile at 19 min, 60% acetonitrile at 20 min, 60% acetonitrile at 27.5 min, and 80% acetonitrile at 28.0 min. Mobile phase flow rate was 0.2 mL min^{-1}. Sample injection volume was 250 μL.

MS conditions were as follows: capillary exit voltage was optimized at mass 156.0, 162.0, 326.0, 357.0, 395.8, and 414.7; entrance lens, 100 V; MS quad temperature, 120 °C; mass calibration 5 μg mL^{-1} polypropylene glycol; ionization mode, positive ion electrospray; full-scan spectra, 100−500 amu, acquired at 2 scans s^{-1}.

Soil. A Webster clay loam (fine loamy, mixed, mesic Typic Haplaquoll) was used for extraction recovery experiments. Soil characteristics were organic carbon content = 3.3%, clay content = 32%, silt content = 33%, and pH = 7.7.

Soil Fortification and Extraction. Herbicides were dissolved in acetonitrile to make stock solutions of 1000 μg mL^{-1}. These solutions were used to make standard solutions of 10 μg mL^{-1}. An aliquot of the standard solutions was added to 20 g of soil, and the solvent was allowed to evaporate. The resultant soil herbicide concentration was 6.0 μg kg^{-1}. Triplicate soil samples for each chemical were extracted.

The herbicides were extracted from 20 g of fortified soil by shaking with 120 mL of 0.1 M ammonium carbonate (pH 7) for 20 min. The sample was centrifuged for 20 min, and the clear supernatant was decanted into a beaker. A 100-mL

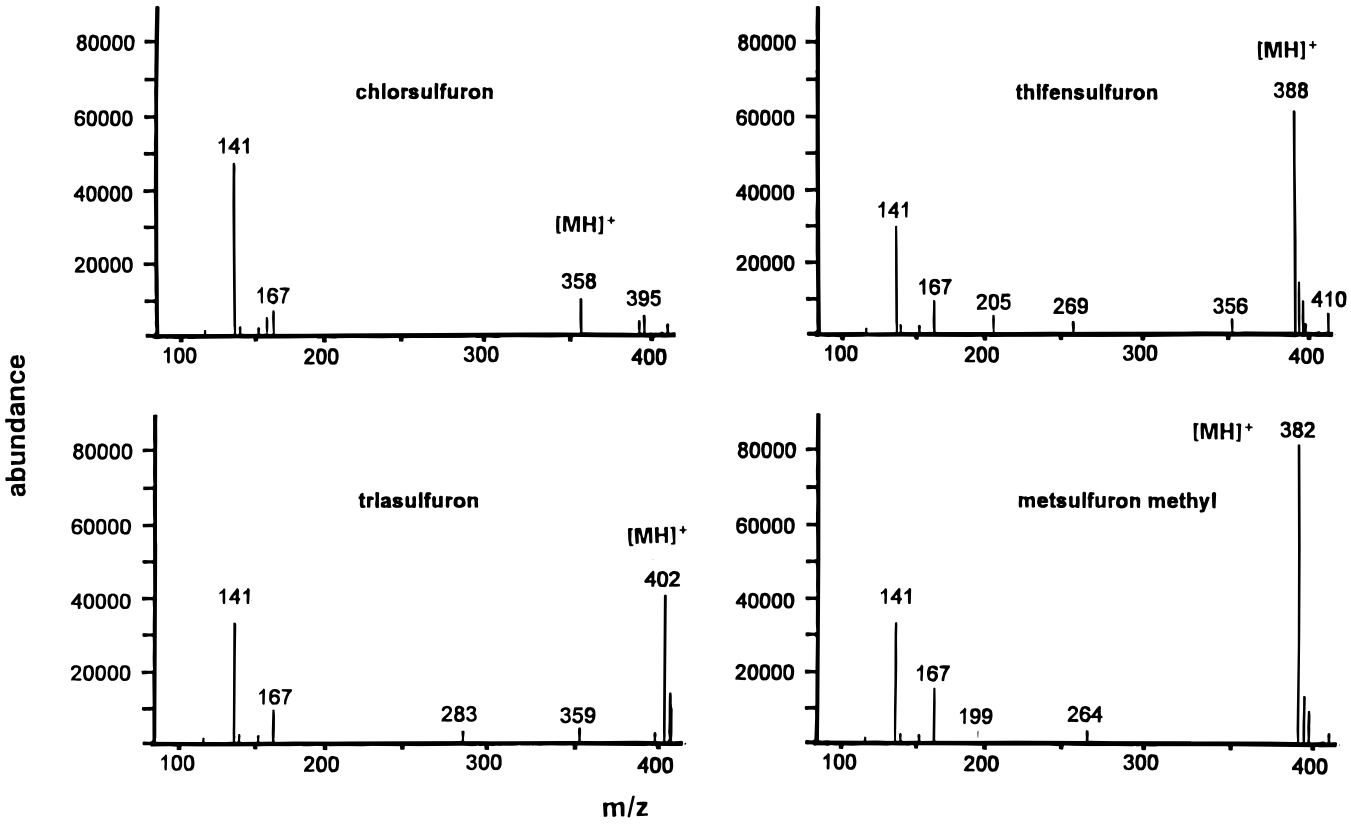

Figure 2. Mass spectra of chlorsulfuron, metsulfuron-methyl, thifensulfuron-methyl, and triasulfuron.

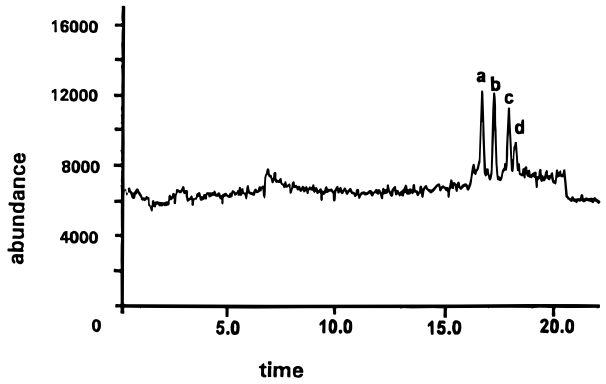

Figure 3. SIM chromatogram resulting from injection of 0.19 ng of thifensulfuron-methyl (a), metsulfuron-methyl (b), triasulfuron (c), and chlorsulfuron (d).

aliquot of supernatant, acidified to pH 3.0−3.5 using dilute phosphoric acid, was passed through a preconditioned C-18 SPE cartridge at a flow rate of 5−10 mL min^{-1}. The cartridge was washed with pure water. The herbicides were eluted from the cartridges using acidic ethyl acetate. Water was removed from the bottom of the test tube by pipet and the sample was evaporated to dryness at <40 °C. After adding 0.5 mL of methanol, agitating using a vortex mixer, sonicating, adding 0.5 mL of water, and agitating again using a vortex mixer, the sample was evaporated to <0.4 mL to remove all methanol. The sample was brought up to 1 mL with 5% acetonitrile in water. The herbicides were quantified by SIM using a four-point external standard curve, 15−170 μg mL^{-1}. To avoid potential analyte decomposition, all samples were extracted and analyzed within an 8-h period. Preliminary studies showed no decomposition within this time period.

RESULTS AND DISCUSSION

ES mass spectra (Figure 2) of chlorsulfuron, metsulfuron-methyl, and thifensulfuron-methyl show the molecular ion $[MH]^+$, a [[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl] fragment (*m*/*z* 167), and a [(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino] fragment (*m*/*z* 141), consistent with the ES spectra of Volmer et al. (1995). The homologous sulfonylurea triasulfuron also has a spectra with the same fragment ions. For selected ion monitoring (SIM), the following masses (*m*/*z*) were monitored: thifensulfuron-methyl, 388, 205, 167, 141; metsulfuron-methyl, 382, 264, 167, 141; triasulfuron, 402, 167, 141; and chlorsulfuron, 358, 167, 141. The SIM chromatogram of the four herbicides (0.19 ng of each herbicide injected) is shown in Figure 3. The retention times are as follows: thifensulfuron-methyl, 16.56 min; metsulfuron-methyl, 17.14 min; triasulfuron, 17.83 min; and chlorsulfuron, 18.12 min. Although triasulfuron and chlorsulfuron are not completely separated on the chromatogram, EnviroQuant software (Hewlett Packard) can quantitate each chemical based on their mass spectra. The combination of retention time and three ions for each herbicide provide the confirmation required for environmental analyses.

Chromatograms of blank soil extract and extract of soil spiked with all four herbicides at 6 μg kg^{-1} are shown in Figure 4. The percent recoveries were as follows: chlorsulfuron, 80.1 ± 7.9%; metsulfuron-methyl, 99.4 ± 8.6%; thifensulfuron-methyl, 95.9 ± 5.9%; and triasulfuron 100.5 ± 24.3%. Recoveries of chlorsulfuron and metsulfuron-methyl are consistent with previous research. For instance, Berdeaux et al. (1994) recovered >80% of 1 mg kg^{-1} chlorsulfuron and metsulfuron-methyl from 20 g of three soils using SFE and HPLC UV, but only 50% on a fourth soil. In contrast, recovery

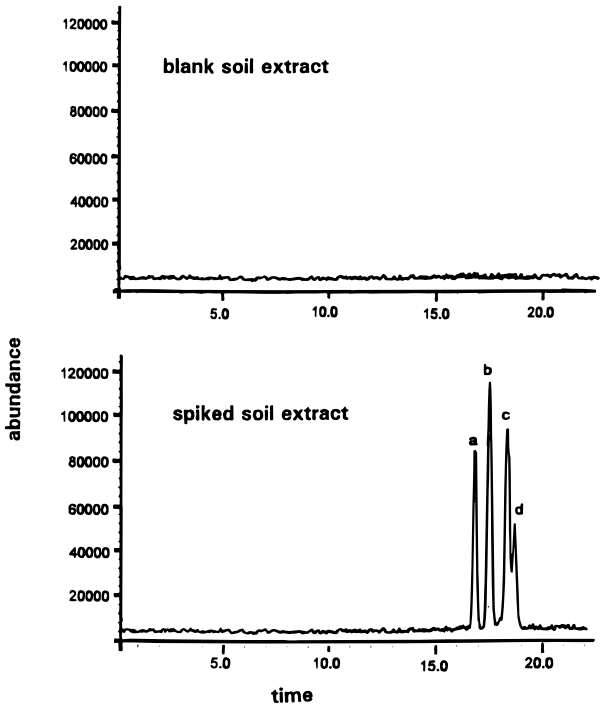

Figure 4. SIM chromatograms of blank soil extract and extract of soil spiked with 6 μg kg^{-1} thifensulfuron-methyl (a), metsulfuron-methyl (b), triasulfuron (c), and chlorsulfuron (d).

was only 47% using acidic acid/methanol. After extraction of chlorsulfuron and metsulfuron-methyl from 100 g of soil with $NaHCO_3$ (James et al., 1995; Klaffenbach and Holland, 1993a,b) or methanol/0.1 M NaOH (Galletti et al., 1995) followed by SPE cleanup of the aqueous solution, recovery ranged from 78 to 88% for chlorsulfuon and from 75 to 96% for metsulfuron when spiked in soil at 5 μg kg^{-1}.

Recoveries of thifensulfuron-methyl and triasulfuron are better than those previously reported. Recoveries of thifensulfuron-methyl have been 71% for 10 μg kg^{-1} in 100 g of soil (Galletti et al., 1995) and 61% for 5 μg kg^{-1} in 100 g of soil (Klaffenbach and Holland, 1993b), and recovery of triasulfuron has been 63% for 5 μg kg^{-1} in 100 g of soil (Klaffenbach and Holland, 1993b). Recoveries of all four herbicides were lower when the aqueous extract was cleaned up with liquid−liquid partitioning using dichloromethane.

In summary, we have shown that we can analyze and confirm four sulfonylurea herbicides in soil by LC/ES/MS. Although the soil spikes were 6 μg kg^{-1}, the ion abundances suggest that this method could easily detect concentrations at <0.1 μg kg^{-1}. This multiresidue method should also be appropriate for other sulfonylureas as well as other polar herbicides.

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