Application of Thermospray LC/MS for Residue Analysis of Sulfonylurea Herbicides and Their Degradation Products

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A trace level analytical method was developed for two sulfonylurea herbicides (nicosulfuron and rimriduron) and a major metabolite of each in soil by thermospray LC/MS. This method involves a simple extraction scheme and requires no specific sample cleanup prior to chromatographic analysis. LC/MS demonstrated its applicability, selectivity, and sensitivity in trace level quantitation and qualitative confirmation of thermally labile and highly polar compounds.

With the increasing concern and awareness of the fate and effects of crop protection chemicals in the environment, it is a tremendous challenge to develop sensitive and selective analytical methods that can qualitatively and quantitatively characterize trace levels of residues in the various biological matrices. This challenge is most evident in the detection of water-soluble and polar compounds and their degradation products. Thermospray LC/MS has emerged as a versatile and selective technique that offers chromatographic separation, positive structural confirmation, quantitation, and sensitivity which meet the criteria for multiresidue methods.

Thermospray LC/MS has been used to determine sulfonylurea herbicides (Shalaby and George, 1990) and benomyl residues (Liu et al., 1990). These are predominantly thermally labile and water-soluble compounds which cannot be successfully analyzed by conventional GC/MS or other chromatographic detection techniques without extensive cleanup and derivatization (Shalaby and Reiser, 1990). This paper describes the application of LC/MS for routine residue analysis of nicosulfuron [1, 2-[[[[(4,6-dimethoxypyrimidin-2-yl)amino]carbonyl]amino]sulfonyl]-*N*,*N*-dimethyl-3-pyridinecarboxamide], the active ingredient of Accent herbicide, a nicosulfuron metabolite [4, 2-(aminosulfonyl)-*N*,*N*-dimethyl-3-pyridinecarboxamide], rimriduron (proposed common name) [2, *N*-[[(4,6-dimethoxypyrimidin-2-yl)amino]carbonyl]-3-(ethylsulfonyl)-2-pyridinesulfonamide, the active ingredient of Titus herbicide, and a rimriduron metabolite [3, *N*-(4,6-dimethoxy-2-pyrimidinyl)-*N*-[3-(ethylsulfonyl)-2-pyridinyl]urea] in soil. Nicosulfuron and rimriduron are postemergence, low-use-rate sulfonylurea herbicides. They are registered for use on corn to control many annual and perennial grasses and some broad-leafed weeds.

MATERIALS AND METHODS

Chemicals. Analytical standards (>99% pure) for nicosulfuron, rimriduron, and the two metabolites were synthesized at the Experimental Station (Du Pont Agricultural Products, Wilmington, DE). [*pyrimidine*-2-^{14}C]Rimriduron (51.7 mCi/mg specific activity) and [*pyridine*-2-^{14}C]nicosulfuron (62.9 mCi/mg specific activity) used to determine the extraction efficiencies were synthesized by Du Pont NEN Products (Boston, MA). Chemical structures of test materials are presented in Figure 1.

The solvents were HPLC grade acetonitrile, EM OmniSolv solvent (EM Science, Gibbstown, NJ), and distilled, deionized water obtained from a Milli-Q water purification system (Millipore Corp., Milford, MA). The ammonium acetate used to prepare the 0.5 M solution added postcolumn for thermospray ionization was Baker Analyzed reagent (J. T. Baker, Phillipsburg, NJ). The glacial acetic acid used to prepare the 0.1 M acetic acid mobile phase was ULTREX Baker Analyzed ultrapure reagent (J. T. Baker).

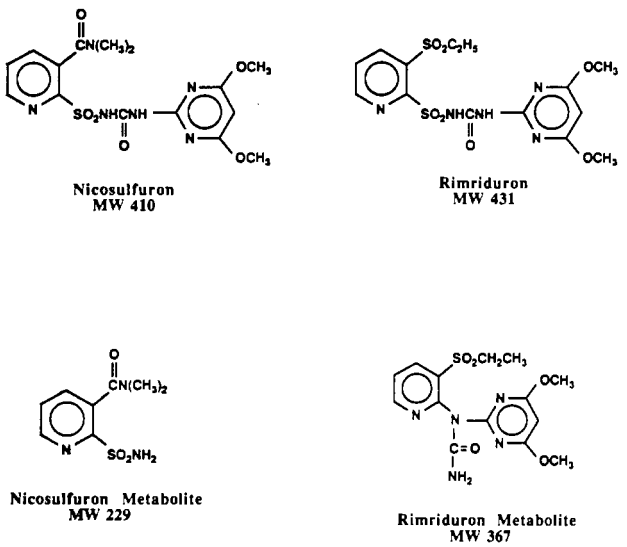

Figure 1. Chemical structures of test substances.

The amount of radiolabeled material in sample extracts was determined by liquid scintillation counting (LSC) in Tru-Count scintillation cocktail (IN/US Service Corp., Fairfield, NJ).

Soil. A sandy loam soil obtained from a corn-growing area near London, ON, Canada, was used to prepare the soil samples for this study.

Equipment. The HPLC system consisted of a Varian Model 5560 liquid chromatograph equipped with a constant-flow pump, a variable-wavelength detector (Varian Instrument Group, Walnut Creek, CA), a Rheodyne injector valve (Rheodyne, Inc., Cotati, CA), and a Whatman Partisil C_8 column, 4.6 mm i.d. × 25 cm (Whatman LabSales, Inc., Hillsboro, OR).

The mass spectrometer was a Finnigan Model 4600 quadrupole instrument with an INCOS Data System (Finnigan MAT, San Jose, CA). The LC/MS interface was a Vestec thermospray interface with discharge electrode and filament ionization (Vestec Corp., Houston, TX).

A Kratos Spectroflow 400 dual-piston pulseless HPLC pump (ABI Analytical Kratos Division, Ramsey, NJ) was used for postcolumn addition of the 0.5 M ammonium acetate solution. A pulseless HPLC pump is necessary with thermospray LC/MS to maintain a stable ion signal.

The LC/MS system is equipped with 2-μm on-line Kel-F A-101X ring filters (Thomson Instrument Co., Newark, DE) located before the injector valve and on the Vestec interface line prior to the mass spectrometer to prevent clogging of the capillary LC/MS interface line.

Samples were extracted using a Thermolyne Maxi-mix Model M16715 vortex mixer (Thermolyne Corp., Dubuque, IA) and a

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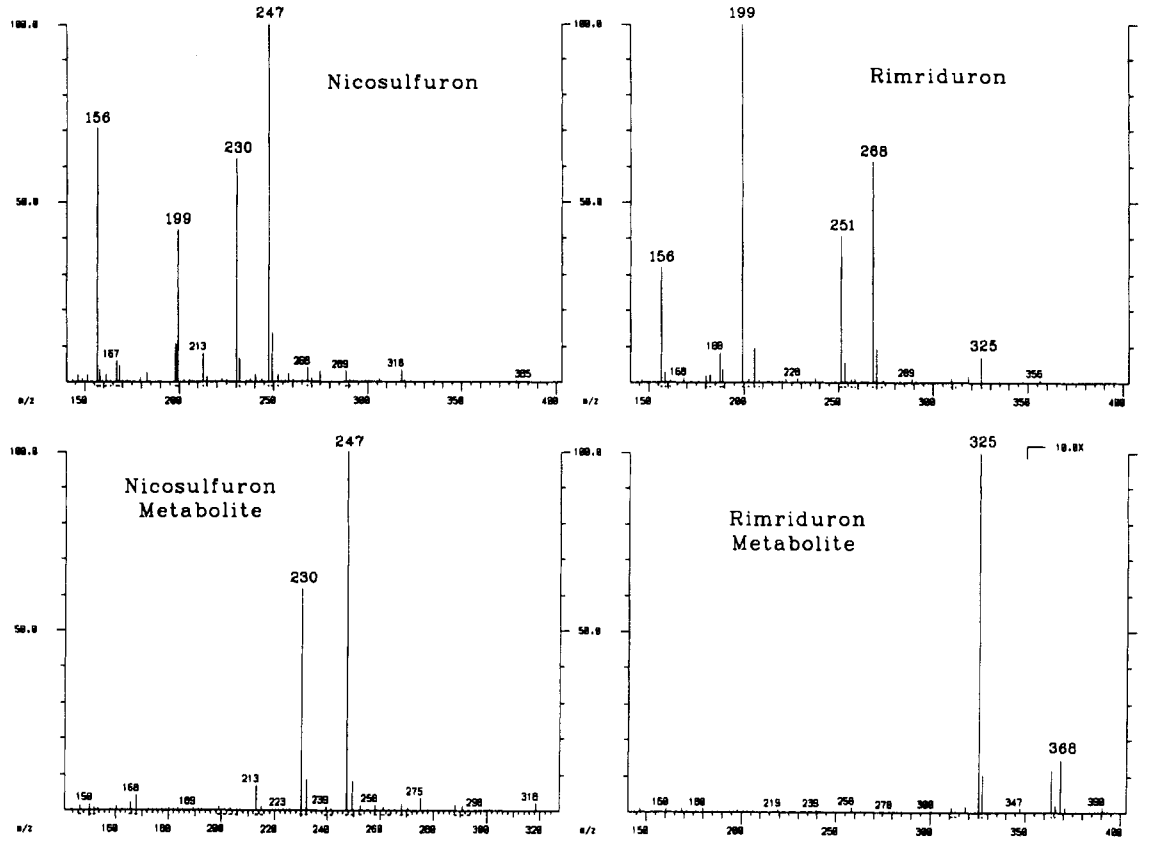


Figure 2. Thermospray positive ion mass spectra for test substances.

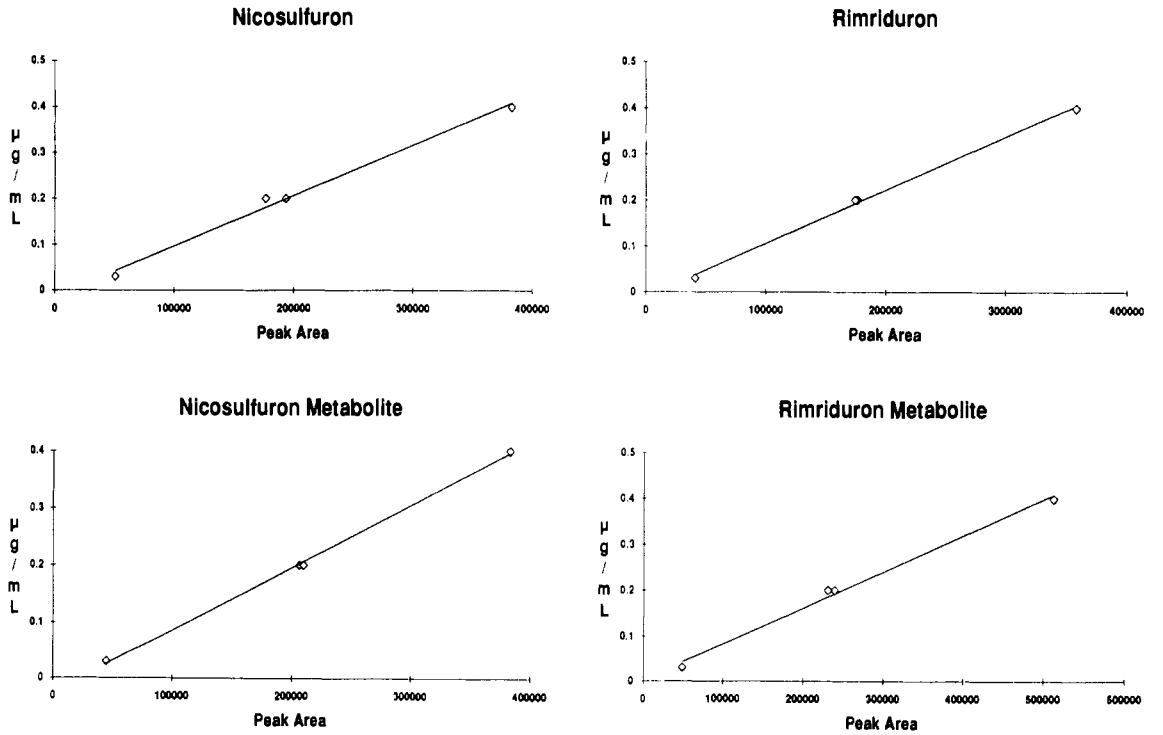


Figure 3. Calibration curves for nicosulfuron, rimriduron, nicosulfuron metabolite, and rimriduron metabolite.

Branson Model B-22-4 ultrasonic cleaner (Branson Cleaning Equipment Co., Shelton, CT). The soil extracts were centrifuged on an International clinical centrifuge Model CL centrifuge (International Equipment, Co., Needham Heights, MA). Extracts were filtered with Gelman 0.45-μm Acrodisc-CR filters (Gelman Sciences, Ann Arbor, MI). Soil extract aliquots were evaporated on an N-Evap Model 111 analytical evaporator (Organomation Association, South Berlin, MA) in Falcon 2087 15-mL polypropylene centrifuge tubes (Becton Dickinson Labware, Lincoln Park, NJ). Extract concentrates were filtered with 0.45-μm ACRO LC13 filters (Gelman Sciences).

The liquid scintillation counter used for measuring the radioactivity was a TM Analytic Mark 3 Model 6881 (Elk Grove Village, IL).

Preparation of Standards. Separate 100 μg/mL standard stock solutions of nicosulfuron, rimriduron, and the two metabolites were prepared in HPLC grade acetonitrile. A 1.0 μg/mL fortification standard mixture of the four test compounds

Table I. Recovery Results for Multiresidue Analysis of Nicosulfuron, Rimriduron, and Metabolites

test compd	fortification, ppm	% recovery	% SD
nicosulfuron	0.02–0.20	81–96	7–22
nicosulfuron metabolite	0.02–020	83–97	8–14
rimriduron	0.02–0.20	74–88	7–11
rimriduron metabolite	0.02–0.20	80–101	8–22

was prepared by diluting the 100 μg/mL stock solution in HPLC grade acetonitrile in a single volumetric flask. All standard solutions were stable for at least 2 weeks when kept refrigerated.

Calibration solutions were prepared fresh daily from dilutions of the fortification standard to minimize decomposition. Standard concentrations used in LC/MS analyses were 0.03, 0.2, and 0.4 μg/mL. Calibration solutions were prepared to contain less than 10% acetonitrile in an aqueous solution to maintain consistent chromatography, particularly for the early-eluting nicosulfuron metabolite.

Sample Preparation and Fortification. Soil samples were prepared by weighing 10.0 g of soil into tared 50-mL graduated, centrifuge tubes on a top-loading analytical balance. Soil samples were fortified with the four compounds at levels of 0.02, 0.05, 0.1, and 0.2 ppm. The solvent was evaporated from fortified samples under a stream of nitrogen for 5 min. An untreated control sample was prepared for each validation set.

Extraction Procedure. (*1*) Add 10 mL of extraction solvent (80% HPLC grade acetonitrile/20% Milli-Q water) to each 10-g sample.

(*2*) Vortex mix each sample for a few seconds and then ultrasonicate for 10 min, vortex mix, ultrasonicate for 5 min, vortex mix, centrifuge for 15 min at ~1000 rpm, and decant into separate 50-mL graduated cylinders.

(*3*) Repeat extraction steps 1 and 2 twice.

(*4*) Record the total extract volume recovered for each sample.

(*5*) Filter (0.45-μm syringe filter) each sample extract into glass bottles.

(*6*) Transfer a 5-mL aliquot from each sample extract to a 15-mL centrifuge tube for later concentration to approximately 0.5 mL under nitrogen at ambient temperature.

(*7*) Add water to adjust final volume of each concentrate to 1 mL prior to LC/MS analysis.

Radiolabeled Extraction Efficiency Determination. Samples were extracted using the same procedure described in this method for samples fortified with nonradiolabeled material. The recoveries were determined from duplicate 5-mL aliquots removed from each sample extract and analyzed by liquid scintillation counting. The extraction efficiencies were determined by comparing the total recovered radioactivity with the amount originally applied. The recoveries for rimriduron averaged 95% for the day 0 and the 2-week-aged soil extractions. The recoveries for nicosulfuron averaged 85% for the day 0 and 86% for the 2-week-aged soil extractions.

Thermospray Ionization Mass Spectral Analysis. Figure 2 presents the LC/MS thermospray positive ion full-scan (143–650 amu) mass spectra generated for nicosulfuron, rimriduron, and metabolites. The most intense ions were selected to quantitate each compound. These were *m/z* 156, 199, and 325 for rimriduron; *m/z* 325 for rimriduron metabolite; *m/z* 156, 199, 230, and 247 for nicosulfuron; and *m/z* 230 and 247 for nicosulfuron metabolite. Peak integrations were done automatically by the INCOS data system algorithm after each peak was defined using the data system.

The back pressure (35–45 bar) generated from the thermospray evaporation process in the capillary interface line is monitored at the Spectroflow pump used for the postcolumn addition of the ammonium acetate solution. The pressure should be stable (±1 bar) to ensure good reproducibility of the ion signal. An increase in the back pressure could indicate partial clogging of either the in-line filter prior to the mass spectrometer or the thermospray probe tip. The blockage must be eliminated before the analysis is continued. Instability of the high vacuum or excessive noise in the background signal could also indicate a problem with thermospray probe performance. The problem can be easily treated by cleaning the probe tip or replacing the probe insert. Rinsing the interface daily first with water and then with methanol will minimize the clogging problem. The frequency of interface clogging may vary from a few weeks to a few months, depending on how well the system is maintained.

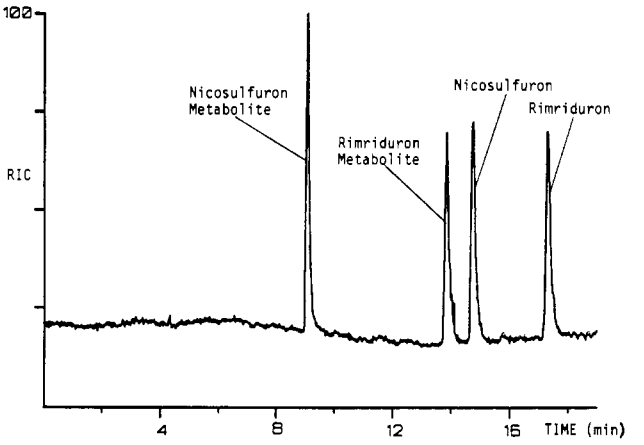


Figure 4. Reconstruction ion chromatogram for 0.2 ppm standard solution containing nicosulfuron, rimriduron, rimriduron metabolite, and nicosulfuron metabolite.

An ammonium acetate solution is required for thermospray ionization. Addition of this buffer on-column could affect the LC retention time, especially for sulfonylurea compounds where an acidic mobile phase is needed for retention on the LC column (Shalaby, 1987). Addition of the ammonium acetate buffer solution postcolumn would eliminate the effect of buffer on retention times.

column	Whatman Partisil C_8 column, 4.6 mm i.d. × 25 cm
LC flow rate	1.0 mL/min on-column
method	0 min, 0% ACN, 100% 0.1 M acetic acid 5 min, 30% ACN, 70% 0.1 M acetic acid 12 min, 45% ACN, 55% 0.1 M acetic acid
postcolumn addition	0.5 M ammonium acetate at 0.3 mL/min
injection volume	200-μL loop
UV detector	254 nm
retention times	nicosulfuron metabolite, 9 min rimriduron metabolite, 14 min nicosulfuron, 15 min rimriduron, 18 min
selected ions monitored	*m/z* 156, 199, 230, 247, 325 nicosulfuron metabolite, *m/z* 230, 247 rimriduron metabolite, *m/z* 325 nicosulfuron, *m/z* 156, 199, 230, 247 rimriduron, *m/z* 156, 199, 325
thermospray probe control temp	153 °C (specific for each probe)
thermospray probe tip temp	200–210 °C
thermospray mass spec source temp	325 °C
ionization mode	thermospray positive ion
mass calibration	poly(propylene glycol) (PPG) with the thermospray LC/MS source (Finnigan)
electron multiplier voltage	1050 V

RESULTS AND DISCUSSION

Extraction efficiencies of nicosulfuron and rimriduron in soil were determined using ^{14}C-labeled test materials and found to be greater than 85%. A simple extraction scheme was used, and filtration prior to chromatographic analysis was the only required cleanup for the soil extract.

Validation data generated with soil samples fortified with the four compounds at 0.02, 0.05, 0.10, and 0.20 ppm, plus an untreated control, are presented in Table I. Each sample extract was analyzed twice. The average recovery for all compounds over the four concentration levels was 88.3%, of applied, with an average standard deviation of 14.8% for the data population of 96 data points from 24 sample analyses.

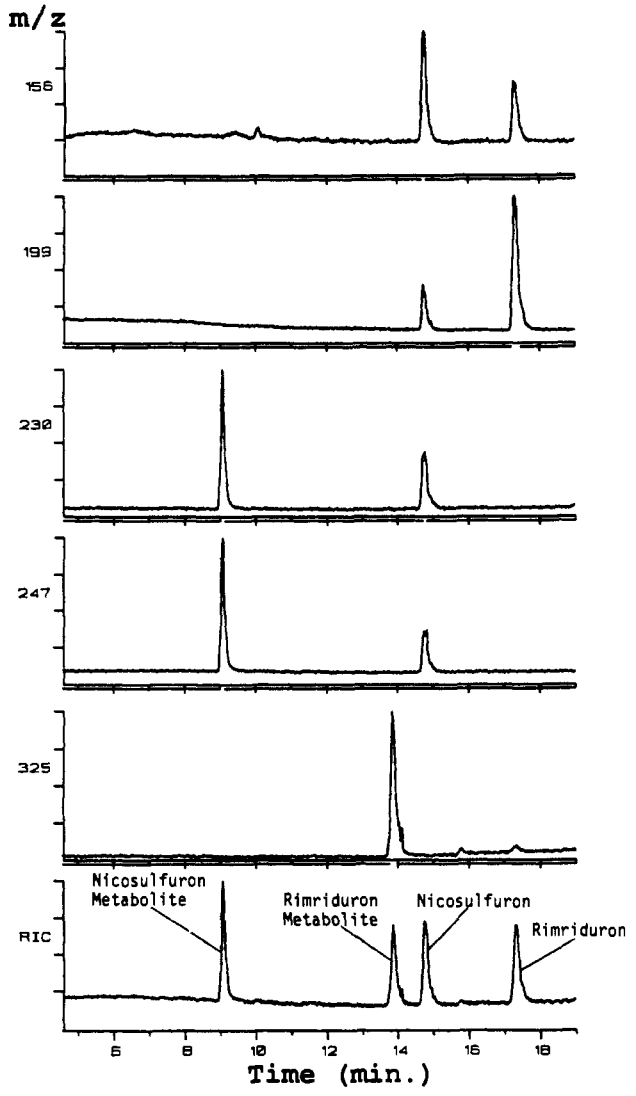
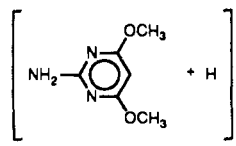
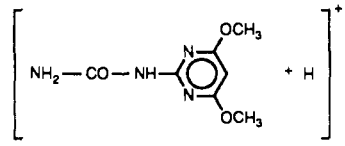
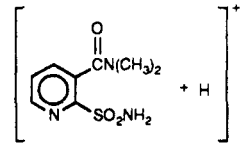
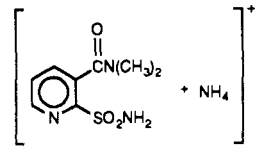
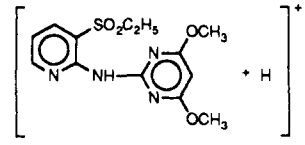


Figure 5. Selected ion chromatograms for each selected mass in the scan descriptor.

A calibration curve was constructed for each test compound on the basis of LC/MS integrated peak areas from standard solutions analyzed with the samples during each single day. Representative calibration curves are shown in Figure 3. The calibration curves demonstrate the linear response of the method over the range 0.03–0.40 μg/mL.

A reconstructed ion chromatogram of a 0.2 μg/mL calibration solution containing nicosulfuron, rimriduron, and their metabolites is presented in Figure 4. Selected ion chromatograms showing the ion trace for each selected mass in the scan descriptor are presented in Figure 5, and the structure of each ion is given in Table II. The selected ion display illustrates the specific ions for each compound. The figure also shows that some of these ions are common for more than one test compound, which is expected from their structural similarity. Nicosulfuron and rimriduron have the same pyrimidinyl urea moiety, and their spectra contain the ions characteristic of this structural feature at m/z 156 and 199. The mass spectra for nicosulfuron and its metabolite contain ions at m/z 230 and 247 which are characteristic of the sulfonamide moiety. Rimriduron and its metabolite contain a common ion at m/z 325. Figure 6 (top) presents the LC/MS total ion chromatogram of an untreated soil control sample. The chromatogram provides a typical profile of this soil and shows no interferences at the retention times for the four compounds

Table II. Structures of the Monitored Ions for Nicosulfuron, Rimriduron, and Metabolites

selected ion m/z	structure
156 →	
199 →	
230 →	
247 →	
325 →	

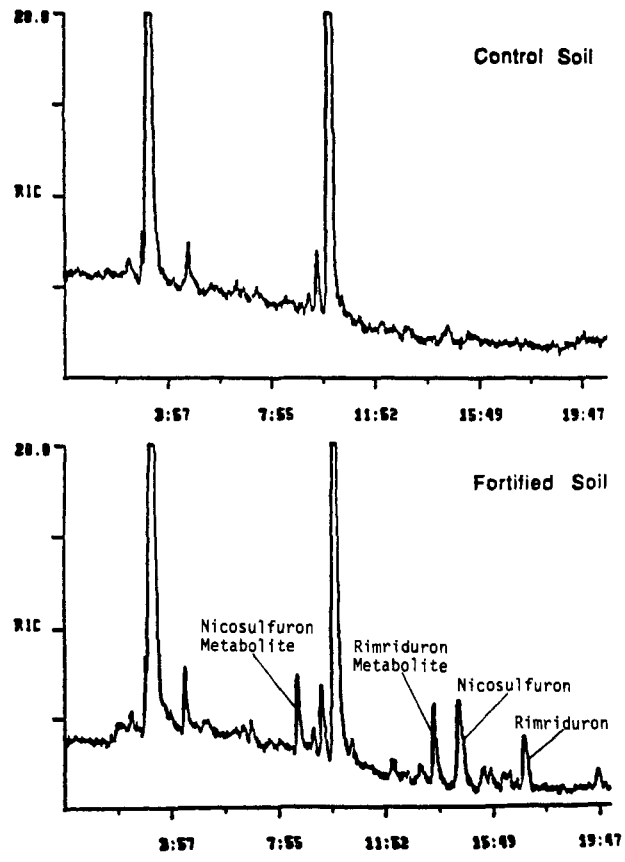


Figure 6. LC/MS total ion chromatograms for (top) untreated control soil and (bottom) soil fortified with 0.02 ppm of each test substance.

of interest. Figure 6 (bottom) displays the LC/MS total ion chromatogram of a 0.02 ppm fortified soil, which is the

lowest fortification level. The chromatogram shows good signal-to-noise ratio for the four test compounds at the method quantitation level.

The ions selected for quantitation were the most intense ions to provide good sensitivity. Interferences at any of the selected ions can be detected by comparing the relative ion abundance to a reference standard. If a soil matrix interference is detected, quantitation can be conducted on ions that do not exhibit interference.

CONCLUSION

LC/MS provides a sensitive and confirmatory method to determine nicosulfuron, nicosulfuron metabolite, rimriduron, and rimriduron metabolite simultaneously in soil at levels down to 0.02 ppm. The application of thermospray LC/MS with selected ion monitoring has been demonstrated for these sulfonylurea herbicides and degradation products. This method offers a simple solvent extraction procedure with minimal sample preparation and the ability to simultaneously analyze four compounds. Filtration is the only cleanup step required prior to LC/MS analysis.

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