Trace Residue Analysis of the Herbicide Chlorsulfuron in Soil by Gas Chromatography–Electron Capture Detection

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A capillary column gas chromatographic method is described for the determination of chlorsulfuron, 2-chloro-*N*-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]benzenesulfonamide, in soil. The soil sample is extracted with aqueous sodium bicarbonate solution by sonic extraction. The aqueous extract is washed with dichloromethane, acidified with hydrochloric acid, and extracted with dichloromethane. The organic extract is dried with anhydrous sodium sulfate and concentrated. The chlorsulfuron in the extract is derivatized with diazomethane to its monomethyl derivative. After Florisil cleanup, the monomethylchlorsulfuron in the sample is analyzed by capillary column gas chromatography–electron capture detection. The recovery of chlorsulfuron from soil samples is greater than 80%. The experimental detection limit of the method is 1 ng/g (1 ppb).

Chlorsulfuron, 2-chloro-*N*-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]benzenesulfonamide, is the active ingredient in Glean weed killer manufactured by Du Pont. It has been licensed in Canada and the United States to control most broadleaf weeds in cereal crops such as wheat, oat, and barley. An important feature of this compound is its very high herbicidal activity at extremely low application rates (10–40 g/ha). Synthesis and toxicology data of chlorsulfuron have been reported by Levitt et al. (1981). The fate of chlorsulfuron in the soil is strongly dependent upon temperature, pH, and moisture content of the soil. The major mechanism for the breakdown of chlorsulfuron is hydrolysis. Campbell (1982) has studied the persistence of chlorsulfuron in Palouse silt loam soils. In an annual cropping system, chlorsulfuron applied to winter wheat during the spring at rates of 17.5 g/ha persisted in the soil for more than 1 year and injured the subsequently planted rotational crops, peas (*Pisum sativum* L.), lentils (*Lens culinaris*, Medik), and sunflower (*Heliantbus annuus* L.). Similar results have been reported by Palm et al. (1980) and Walker and Brown (1982).

Bioassay procedures for determining chlorsulfuron in water and soil at 0.1 ppb level have been reported by Morishita et al. (1985), Hsiao and Smith (1983), and Du Pont (1980). Kelley et al. (1985) have presented an enzyme immunoassay procedure for determining chlorsulfuron in soil extracts at 0.4–1.2 ppb levels. An instrumental procedure to determine chlorsulfuron in soil at the 0.2 ppb level has been reported by Zahnow (1982); the procedure involves high-performance liquid chromatography and photoconductivity detection.

Bioassay and immunoassay procedures are cost-effective. However, these procedures lack selectivity as they may encounter interferences by other herbicidally active compounds that are chemically similar to chlorsulfuron. Furthermore, confirmation of identity may not be possible by these techniques. The photoconductivity detector required for high-performance liquid chromatographic analysis was not available in our and a number of other pesticide residue analysis laboratories. It prompted us to develop a gas chromatographic procedure for measuring chlorsulfuron in soil with a detection level of 1.0 ng/g. The method was required for an interdisciplinary research to study the fate and effect of chlorsulfuron in agriculture/environment. Gas chromatographs are available in majority of the pesticide residue analysis laboratories; therefore, such a procedure can be adapted by these laboratories.

EXPERIMENTAL SECTION

Instrumentation. A Hewlett-Packard (HP) Model 5830 gas chromatograph (GC), equipped with split/splitless injection port, ^{63}Ni electron capture detector (ECD), and a capillary column DB-1, 30 m × 0.25 mm (i.d.), 0.25-μm film (J & W Scientific, Inc., Rancho Cordova, CA), and an HP 18850A GC terminal was used for the analysis. Operating conditions were as follows: injection port temperature, 325 °C; detector temperature, 225 °C; oven temperature, 100 °C for 1 min and then increased to 300 °C at 10 °C/min. Hydrogen was used as a carrier gas (column head pressure, 0.5 kg/cm^2, 2.8 mL/min). ECD makeup gas was argon–methane (90:10); the flow was set at 21.0 mL/min.

The mass spectral data were obtained on a Hewlett-Packard (HP) mass spectrometer, Model HP 5987, interfaced to a Hewlett-Packard gas chromatograph, Model HP 5840. Gas chromatographic operating conditions: column, Hewlett-Packard fused silica capillary column, 25 m × 0.2 mm (i.d.); 0.33-μm film (cross-linked methyl silicone); carrier gas, helium; head pressure, 17 psi; linear velocity, 25 cm/s; oven temperature, 100 °C for 1 min and then increased to 290 °C at 10 °C/min; injection port, splitless mode, 290 °C. Mass spectrometer operating conditions: electron ionization (EI); full scan; ion source temperature, 200 °C; analyzer temperature, 200 °C; electron energy, 70 eV; mass range, 41–800; scan rate, 1.3 scan/s, 600 amu/s; transfer line temperature, 300 °C; source tuning, perfluorotributylamine (PFTBA) used to calibrate the mass axis and tune the ion source.

Chemicals. All solvents were pesticide grade (Caledon Laboratories Ltd., Georgetown, Ontario, Canada). Florisil (MC/B), 60–100 mesh, was heated at 600 °C for 6 h, cooled to room temperature, and then deactivated by addition of 3% organic-free water. Anhydrous sodium sulfate (Na_2SO_4) (Fisher Scientific Co., Toronto, Ontario, Canada) was used without further treatment. *N*-Methyl-*N*-nitrosourea (Terochem Laboratories Ltd., Edmonton, Alberta, Canada) was used for diazomethene preparation.

Diazomethane was prepared as follows: To a 250-mL Erlenmeyer flask was added 30 mL of 40% aqueous potassium hydroxide solution and 100 mL of diethyl ether. The mixture was cooled in an ice bath and stirred with a magnetic stirrer. Ten grams of *N*-methyl-*N*-nitrosourea was added in small portions over 20-min periods after which time the reaction mixture was stirred for an additional 15 min. The ether layer containing

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diazomethane was decanted into another Erlenmeyer flask, stoppered, and stored at −20 °C.

A 5-mL portion of above diazomethane solution was diluted 4-fold with diethyl ether and then used in the methylation of chlorsulfuron. Diethyl ether (15 mL) was added to a 20-mL glass test tube (equipped with an aluminum-lined cap), the content was cooled to −20 °C, and then 5 mL of diazomethane solution (prepared above) was added to it. The solution was mixed and stored at −20 °C.

Diazomethane solution was prepared every 1–2 weeks.

Safety Precautions. *N*-Methyl-*N*-nitrosourea should be stored in a refrigerator. It should not be kept above 20 °C for more than a few hours. It is suspected to be carcinogenic. Its contact with eyes and skin should be avoided.

Diazomethane is toxic and should be prepared and used in a well-ventilated fumehood. It should be prepared behind a safety shield in a suitable quantity (sufficient for 1–2 weeks). Contact of ground apparatus with diazomethane, which is explosive, should be avoided.

Preliminary Sample Treatment. Soil samples were air-dried at room temperature, thoroughly mixed to ensure homogeneity, and then stored at −20 °C. The analysis was performed within 6 weeks.

Extraction Procedure. A literature procedure (Zahnow, 1982) was modified. A 50-g portion of air-dried soil sample was weighed into a 250-mL polypropylene centrifuge bottle, and 100 mL of aqueous 0.1 M sodium bicarbonate solution was added in it. The mixture was sonified for 3 min on a sonifier, Model 350 (Branson Sonic Power Co., Danbury, CT). The resulting slurry was centrifuged (IEC B-20A; International Equipment Co., Needham Heights, MA) at 4000 rpm for 25 min or alternative conditions to obtain a clear extract. It was important to obtain a clear extract as turbidity caused emulsion during dichloromethane extraction. The aqueous layer was decanted into a 500-mL separatory funnel. Wood particles (if present) in the aqueous layer could be separated by filtering the solution through a glass funnel plugged with glass wool. The extraction was repeated twice. All the extracts were combined in the separatory funnel.

The aqueous phase was washed with 3 × 60 mL of dichloromethane, and the dichloromethane layers were discarded. The separatory funnel was shaken gently for 5 min with each extraction. Emulsion, if formed during extraction, could be broken in seconds by ultrasonic technique. It involved dipping a portion of the separatory funnel containing emulsion into an ultrasonic bath filled with water. Persistent emulsion could be broken by centrifuging in a glass bottle at 2000 rpm for 15 min.

The aqueous solution was drained from the separatory funnel into a 400-mL breaker, and the pH was adjusted to 2.8 ± 0.2 with a pH meter (Fisher; Model 420 digital pH/ion meter) by adding 1 N hydrochloric acid dropwise. The acidified solution was transferred back to its original separatory funnel. The breaker was rinsed with 2 × 25 mL portions of dichloromethane, and the rinses were added to the separatory funnel. The separatory funnel was shaken gently for 5 min after which time the layers were permitted to separate.

The organic layer (lower) was collected into a 500-mL round-bottom flask through a sintered glass funnel containing approximately 50 g of anhydrous sodium sulfate (prewashed with dichloromethane). The aqueous phase was reextracted with 2 × 60 mL dichloromethane as described above. The combined extract was concentrated to approximately 1 mL on a rotary evaporator (water bath temperature 40 °C) and then quantitatively transferred into a 10-mL Kuderna–Danish (K–D) tube concentrator. The contents of the K–D tube were concentrated to approximately 0.1 mL with Kontes tube heater (40–50 °C) under a gentle stream of nitrogen.

Diazomethane Derivatization. The residue (obtained above) was dissolved in 0.5 mL of toluene, and 50 μL glacial acetic acid was added to it. The mixture was homogenized, kept at room temperature for exactly 12 min, and then evaporated to 0.1 mL with a Kontes tube heater (40–50 °C) under a gentle stream of nitrogen. The residue was redissolved in 0.5 mL of toluene and concentrated again to 0.1 mL. This step was added to ensure removal of acetic acid from the reaction mixture.

The residue was dissolved in 0.5 mL of toluene, and 0.6 mL of diluted diazomethane solution was added to it. The solution was mixed, kept at room temperature for exactly 5 min, and then concentrated to 0.1 mL.

Florisil Cleanup. A minicolumn was prepared. A Pasteur pipet (5 in.) was plugged with silanized glass wool, filled with (1.3 g) 3% deactivated Florisil, tapped to settle Florisil, topped with a 5-mm layer of anhydrous sodium sulfate, and then plugged with silanized glass wool. The column was connected to a custom-made glass reservoir with Teflon sleeve and equilibrated with 20 mL of dichloromethane. The air bubbles were removed from the column by forcing 15 mL of dichloromethane through it with the help of a rubber bulb equipped with a Teflon adaptor. During chromatography, the solvent was always kept above the Florisil top to avoid air contacting the column packing.

The methylated residue was dissolved in 0.5 mL of dichloromethane and transferred to the column. The container was rinsed with 3 × 0.5 mL of dichloromethane, and each rinse was transferred to the column. The column was first eluted with 75 mL of 0.8% (v/v) methanol in dichloromethane, and this fraction was discarded. The column was then eluted with 75 mL of 1.5% (v/v) methanol in dichloromethane. This fraction was collected in a 250-mL round-bottom flask and concentrated to 1 mL on a rotary evaporator. The residue was quantitatively transferred to a 10-mL K–D tube concentrator and concentrated to 0.1 mL. Dichloromethane was replaced with toluene, and the final volume was adjusted to exactly 0.5 mL with toluene. The extract was analyzed by gas chromatography under the conditions described above.

Standardization. A standard stock solution of chlorsulfuron (1 μg/μL) was prepared by dissolving 50 mg of analytical-grade chlorsulfuron (99.5% purity) in dichloromethane in a 50-mL volumetric flask and diluting to the 50-mL mark.

The working chlorsulfuron standard (1 ng/μL) used for the spiking of recovery samples and related work was prepared by transferring 10 μL of stock solution (at room temperature) to a 10-mL volumetric flask containing some dichloromethane and diluting to the 10-mL mark with dichloromethane.

Calculation. Monomethylchlorsulfuron standard was not commercially available; therefore, relative recoveries were reported. It involved methylation of chlorsulfuron at an appropriate working level (50–250 ng) followed by Florisil cleanup of monomethylchlorsulfuron to prepare method standard. The recoveries from all the samples were compared with that of method standard. The following equation was used for the external standard method calculation

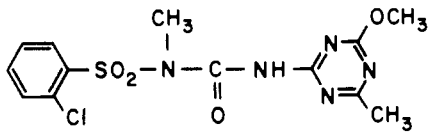
$$\text{chlorsulfuron (ng/g, ppb)} = \frac{W_{std}}{A_{std}} \frac{A_{samp}}{V_{inj}} \frac{V_{ext}}{W_{samp}}$$

where W_{std} = amount of standard injected (ng), A_{std} = area counts of standard peak, V_{inj} = injection volume (μL) to give A_{samp}, A_{samp} = area counts of sample peak, V_{ext} = volume of the final extract (μL), and W_{samp} = weight of sample extracted (g).

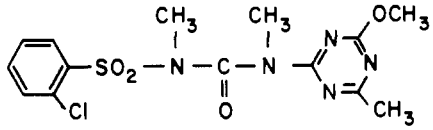
RESULTS AND DISCUSSION

Direct gas chromatographic analysis of chlorsulfuron was difficult. Ten nanograms of chlorsulfuron resulted in a small broad peak on GC columns such as DB-1, DB-5, and OV-1. The problem in gas chromatography of chlorsulfuron could be related to the polar nature of chlorsulfuron, which caused adsorption to the column stationary phase, thermal instability of chlorsulfuron in the injection port/column, and other factors. Chemical modification of chlorsulfuron seemed necessary to achieve the following: increase its hydrophobic character/decrease polarity in order to improve its gas chromatographic properties, increase thermal stability, and improve its response to gas chromatographic detectors.

Various derivatization reactions such as pentafluorobenzylation, alkylation, and alkylation followed by perfluoroacylation of chlorsulfuron were investigated. Alkylation of chlorsulfuron with diazomethane was preferred because of its simplicity and ease of formation. It was



CHLORSULFURON
(Monomethyl Derivative)



CHLORSULFURON
(Dimethyl Derivative)

Figure 1. Methyl derivatives of chlorsulfuron.

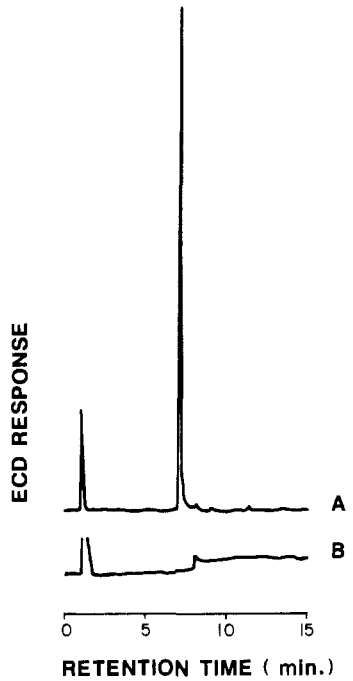


Figure 2. ECD–gas chromatograms of 10 ng of monomethylchlorsulfuron (A) and 10 ng of chlorsulfuron (B). Chromatographic conditions as in text; plot attenuation, 12.

anticipated that methylation would occur only at the sulfonamide nitrogen of chlorsulfuron because of its reactivity; instead, the reaction of chlorsulfuron with excess diazomethane lead to two products, tentatively identified by GC/MS to be monomethylchlorsulfuron and dimethylchlorsulfuron (Figure 1). Mass spectra of monomethylchlorsulfuron gave prominent peaks at m/e (percentage relative abundance) 207 (8), 205 (22), 175 (18), 141 (29), 113 (28), 112 (22), 111 (100), and 75 (50). The dimethyl derivative of chlorsulfuron showed m/z 210 (62), 194 (43), 181 (100), and 111 (46). Methylation reaction conditions were modified to obtain preferentially monomethylchlorsulfuron, a procedure reported in an earlier publication (Ahmad, 1987). The methylation of chlorsulfuron was reproducable. The coefficient of variation (within-run, single analyst) for four replicates, each at 50- and 100-ng chlorsulfuron levels, was less than 6%.

The ECD response and peak shape of monomethylchlorsulfuron were considerably better than for chlorsulfuron. Figure 2 shows GC/ECD chromatograms of 10 ng of chlorsulfuron and the monomethyl derivative of

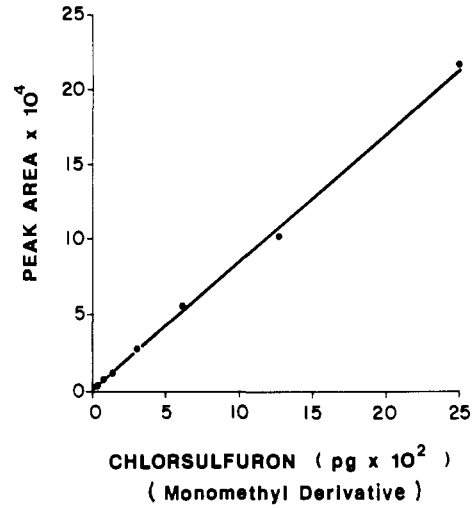


Figure 3. Election capture detector response for chlorsulfuron (monomethyl derivative).

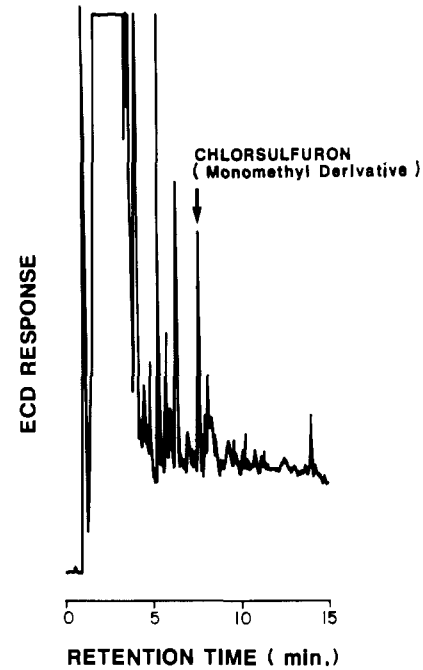


Figure 4. ECD–gas chromatogram of soil sample fortified with chlorsulfuron at the 1 ppb level. Experimental conditions as in text; volume of final extract, 0.5 mL; injection volume, 10 μL; plot attenuation, 10.

Table I. Recovery of Chlorulfuron from Spiked Soil Samples

spike level, μg/g	av rec,[a] %	rel std dev
0.006	84.8 (73.4–95.0)[b]	9.3
0.020	94.8 (75.3–108.6)	11.8

[a] Average recovery of seven recovery measurements. [b] The range of seven determinations is given in parentheses.

chlorsulfuron. Treatment of chlorsulfuron with acetic acid prior to methylation with diazomethane was necessary. It enhanced recovery of monomethylchlorsulfuron by approximately 4-fold, a plausible explanation of which was given in an earlier publication (Ahmad, 1987). Steps were incorporated in the procedure to ensure removal of acetic acid from the reaction mixture before treating it with diazomethane.

The instrumental detection limit of monomethyl chlorsulfuron by gas chromatography–thermionic specific detec-

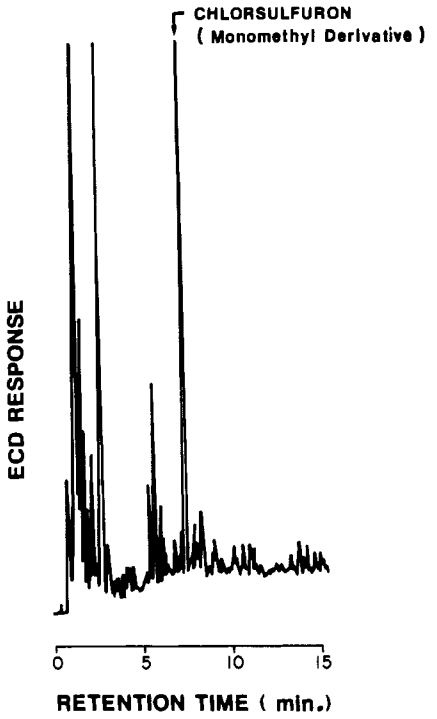


Figure 5. ECD–gas chromatogram of soil sample fortified with chlorsulfuron at the 20 ppb level. Experimental conditions as in text (without Florisil cleanup); volume of final extract, 1.0 mL; injection volume 5 μL; plot attenuation, 12.

tor or flame ionization detector and gas chromatography–mass spectrometry (electron impact, selected ion monitoring mode) was approximately 10 ng. With GC/ECD, the instrumental detection limit was 50 pg of methylchlorsulfuron at a signal to noise ratio of 3; thus, the GC/ECD technique was used in the method.

Extraction of chlorsulfuron from soil was achieved by modifying the procedure reported by Zahnow (1982). Silica or C_{18} Sep-Pak cartridges (Water Associates, Inc., Milford, MA) were tried for cleanup of the extract but were found to be unsuitable for GC/ECD work. Cleanup procedure was established using Florisil.

The ECD response curve for monomethylchlorsulfuron is shown in Figure 3. It was linear over the concentration range of 50–2500 pg of monomethylchlorsulfuron (based on chlorsulfuron).

The characteristics of soil used in the recovery study were as follows: pH 7; conductivity, 0.8 mmho; sand, 49%; silt, 30%; clay, 21%; organic content, 8.8%. The soil was spiked at two levels, 6 ng/g (6 ppb) and 20 ng/g (20 ppb) chlorsulfuron. Seven replicates were made at each fortification level. The results are shown in Table I. Blank samples were also run and showed the absence of chlorsulfuron. Figure 4 is a typical chromatogram of soil sample fortified with chlorsulfuron at the 1 ppb level. At 20 ppb or higher chlorsulfuron levels, the Florisil cleanup was not necessary. Therefore, this step was excluded from the method. Figure 5 is a typical chromatogram (without Florisil cleanup) of soil sample fortified with chlorsulfuron at 20 ppb levels.

With each batch of analysis, quality control checks were performed on the methylation procedure, Florisil cleanup, and gas chromatographic performance. Chlorsulfuron (50 ng) was methylated and Florisil cleaned under the conditions described in the method. Gas chromatographic data for methyl chlorsulfuron were checked. Under the gas chromatographic conditions described in the text the retention time for methylchlorsulfuron was 7.50 min, peak height 7.0 cm, peak width at half-height 4.8 s, and peak area ca. 800 000 counts. If there is no peak at 7.50 min in the chromatogram, reactivity of diazomethane, activity of Florisil, chlorsulfuron standard, and column/GC instrumental problems were checked.

This method has been applied successfully to soils different from the one used in the recovery study. Confirmation of results were done by GC–MS or with a column of different polarity, such as DB-5.

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