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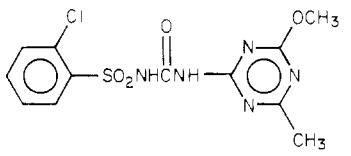
Analysis of the Herbicide Chlorsulfuron in Soil by Liquid Chromatography

Edward W. Zahnow

An analytical method based on the use of a liquid chromatograph and a photoconductivity detector is described for chlorsulfuron, 2-chloro-*N*-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]-benzenesulfonamide, previously known as DPX-W4189, which is the active ingredient in Du Pont Glean Weed Killer. As little as 100 pg can be detected and measured after passage through the chromatographic column. Coupled with extraction, cleanup, and isolation procedures, the method provides a means of determining chlorsulfuron in soil at levels as low as 200 pg/g (0.2 ppb).

Du Pont Glean Weed Killer is effective in controlling a variety of weeds common to cereal grain fields without causing injury to the crop plants themselves. The active ingredient, chlorsulfuron, 2-chloro-*N*-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]benzenesulfonamide, is a sulfonylurea of the structure



chlorsulfuron

The synthesis and toxicology of this compound have been described by Levitt et al. (1981), and these authors also include the results of both pre- and postemergence tests on a wide variety of weeds commonly associated with cereal crops.

Glean is usually applied at very low levels, with a range of 10–40 g/ha being fairly typical on fields of wheat, oats, and barley. Since the half-life in soil of the active ingredient, chlorsulfuron, is approximately 1 month during the growing season, it is apparent that the residues of chlorsulfuron in the soil will be extremely low.

Consequently, a method has been developed that can be used to measure chlorsulfuron in soil with a detection limit of 200 pg/g (0.2 ppb). Derivatization of chlorsulfuron is not required, and the operating conditions are sufficiently mild that decomposition is avoided.

A literature search revealed a number of methods that can be used for the analysis of sulfonylureas. If gas chromatography is to be used for the analysis, the sulfonylureas must be derivatized to more volatile and stable compounds by reacting the polar NH groups with dimethyl sulfate, methyl iodide, or diazomethane. Derivatization with diazomethane has been reported by Braselton et al. (1975, 1976, 1977), Midha et al. (1976), Taylor (1972), and Taylor et al. (1977). Maeda et al. (1981) have demonstrated that sulfonylureas can be determined by methylation with diazomethane followed by acylation with heptafluorobutyric anhydride. The use of dimethyl sulfate is described by Kleber et al. (1977), Prescott and Redman (1972), Sabih (1970), Sabih and Sabih (1976), and Simons et al. (1972). An extractive methylation involving methyl iodide in methylene chloride is given in the paper by Hartvig et al. (1980).

Methods for sulfonylureas based on liquid chromatography have been reported by Beyer (1972), Harzer (1980), Molins et al. (1975), Robertson et al. (1979), Sved et al. (1976), Uihlein and Sistovaris (1982), Waahlin-Boll and Melander (1979), and Weber (1976). Both normal and reverse-phase systems have been used, and it is not necessary to form derivatives since sulfonylureas generally give adequate response with ultraviolet absorbance detectors. Besenfelder (1981) has reported an improvement in sensitivity based on precolumn derivatization and fluorometric detection. However, the sensitivity requirements for chlorsulfuron in soil are much greater than normally encountered, and in addition, extraction procedures used for soil analysis liberate substantial quantities of UV-absorbing substances from soil that interfere with the chlorsulfuron determination.

To obtain adequate sensitivity and also eliminate undesirable responses from coextracted materials, use is made of the photoconductivity detector that is described in detail by Popovich et al. (1979). An application of this detector is discussed by McKinley (1981). The photoconductivity detector is selective for molecules containing sulfur, halogen, nitrogen, and phosphorus atoms. Its sensitivity for chlorsulfuron is 15 times greater than can be achieved with

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the Du Pont 850 absorbance detector at 254 nm and 0.002 AUFS.

EXPERIMENTAL SECTION

Preliminary Treatment. All soil samples were air-dried for 2 or 3 days at room temperature in flat trays capable of holding at least 1-cm thicknesses of soil. If the soils were very damp, they were mixed periodically with a spatula. After being dried, the samples were ball-milled for 15–30 min, depending on consistency, to ensure homogeneity. These were then stored in a freezer until needed.

Extraction Procedure. A 50-g sample was weighed into a 250-mL polypropylene centrifuge bottle, and 100 mL of aqueous 0.1 M Na_2CO_3–0.1 M $NaHCO_3$ (pH 10) was added. The mixture was shaken vigorously by using a wrist-action shaker for 1 h. The resulting slurry was centrifuged at 1500 rpm for 15 min to make a clean separation, and the supernatant liquid was decanted into a 500-mL separatory funnel. The mud cake remaining in the bottle was extracted again in the same manner, and the liquid was combined with that from the first extraction.

Cleanup Procedure. The aqueous solution was washed 3 times with 50-mL portions of chloroform by shaking gently for 1 min. Since the pK_A of chlorsulfuron is about 3.8, the compound remained in the aqueous solution in its anionic form. The chloroform layers were discarded. A rotating tumbler unit was useful for this step and was operated at low speed. Care had to be taken with this operation to avoid the formation of an emulsion that was difficult to break. When a persistent emulsion did form, it could usually be broken by centrifuging. When centrifuging was necessary, only glass centrifuge bottles were used. **Note**: Chloroform is known to be a weak animal carcinogen. Polyvinyl alcohol gloves should be worn when handling this liquid, and adequate ventilation should be provided.

The aqueous solution was drained from the separatory funnel into a 400-mL beaker, and the pH was adjusted to 3–4 by adding 10% hydrochloric acid dropwise while measuring with a calibrated pH meter. In this pH range chlorsulfuron exists in the nonionic form and can be extracted into various organic liquids. The pH adjustment had to be performed carefully since a considerable amount of foaming occurred. Also, due to the chemical equilibria involved, the pH changed slowly. If the final pH is too low, there is a danger of chemical decomposition of chlorsulfuron, whereas if it is too high, extraction may be incomplete.

The solution was then transferred back into a 500-mL separatory funnel with 5 mL of distilled water being used to rinse the beaker. It was extracted 3 times with 50-mL portions of toluene by shaking vigorously for 1 min. When a rotating tumbler was used, it was operated at high speed. The toluene layers were separated from the aqueous phase and were then combined in a 250-mL round-bottom flask. Again, when centrifuging was required to break an emulsion, only glass centrifuge bottles were used. The combined extracts were examined to ensure that they were free of water droplets.

To the toluene extract was added 1 mL of glacial acetic acid, and the solution was taken to dryness with a rotary evaporator at about 45 °C by using a water aspirator as the vacuum source.

The residue was dissolved in the mobile phase (see below) by using several small washings that were transferred with a Pasteur capillary pipet to a graduated, 10-mL centrifuge tube. The total volume was not allowed to exceed 2 mL.

This was put through a silica Sep-PAK (Waters Associates) that had been previously washed with 5 mL of mobile phase, and the effluent was collected in a 10-mL centrifuge tube. Then the Sep-PAK was eluted with 5 mL of the mobile phase that was also collected in the 10-mL centrifuge tube. A gentle stream of nitrogen was used to evaporate this combined solution to dryness at room temperature. The residue was taken up in the mobile phase to a final volume of 1 mL. All liquids were put through the silica Sep-PAK by means of a 10-mL hypodermic syringe at a flow rate of ca. 2 mL/min.

Liquid Chromatography. The liquid chromatograph used in this investigation was a Du Pont Model 850. Since the photoconductivity detector must be used at its maximum sensitivity to achieve the desired lower detection level, it was essential that the chromatographic system provided good temperature control of the column and reasonably pulse-free delivery of the mobile phase to minimize base-line fluctuations.

The photoconductivity detector (Tracor Model 965) must be used for this analysis to obtain adequate sensitivity and selectivity. The mercury lamp was used in the detector since it provided much greater sensitivity than the zinc lamp. The detector, including the lamp, was left on at all times to ensure greater stability. The flow of the mobile phase through the reference and analytical loops was balanced to within ±2%. This was accomplished by installing a metering valve (Nupro Model SS-2SA-TFE) in the solvent line that exited from the reference compartment of the conductivity cell. The "T" that brought the two solvent lines from the conductivity cell back together was eliminated from the instrument. Also, the ion-exchange resin tube was not needed to purify the mobile phase and might actually have introduced unwanted materials into the system had it not been removed.

The mobile phase consisted of 750 parts by volume of cyclohexane, 125 parts of 2-propanol, 125 parts of methanol, and 1 part of a solution consisting of 9 parts of glacial acetic acid with 1 part of water. The work of Lawrence and Leduc (1978) showed the importance of acetic acid in the mobile phase in reducing the tailing of both acidic and basic herbicides on silica gel.

The column was a Du Pont Zorbax SIL (25 cm × 4.6 mm) controlled at 35 °C. A new column had to be conditioned by pumping a solution that consisted of 40 parts by volume of 2-propanol, 10 parts of glacial acetic acid, and 1 part of water through it for several hours at 1 mL/min. This treatment was also used to clean columns that had started to lose their efficiency because of contamination from samples. A contaminated column is characterized by broad peaks that tail very badly and by shifting retention times. This conditioning solvent must be thoroughly flushed from the column with the mobile phase. One hour of flushing at 0.5 mL/min was usually sufficient. Both the conditioning solvent and the mobile phase were filtered before use through a 0.5-μm Millipore filter (FHUP 04700) held in a Millipore filter apparatus (XX15 04700).

The sample valve was a Valco Model CV-6-UHPa-N60 for manual injection of standards and samples. The loop volume was 10 μL to minimize contamination of the HPLC column and broadening of the chromatographic peak.

During normal operation the mobile phase was pumped through the column at 0.5 mL/min, which was judged to be the minimum practical rate. At this flow rate chlorsulfuron eluted from the column in 15–16 min, depending on the extent of column deactivation. This rate was selected because the detector response increased with decreasing flow rate due to the longer residence time of the

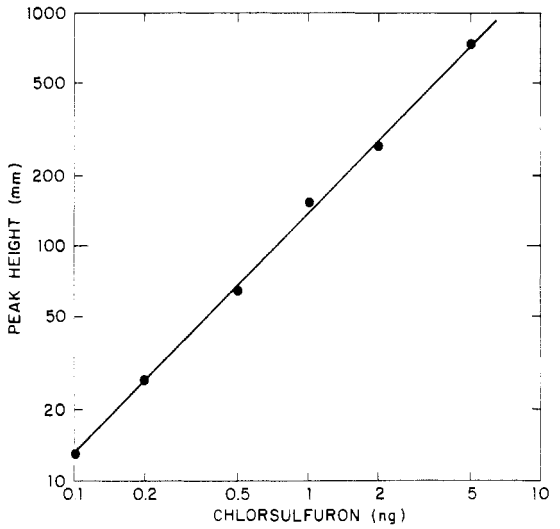

Figure 1. Photoconductivity detector response for chlorsulfuron.

sample in the quartz reactor coil. At 1 mL/min the peak height for chlorsulfuron was about 50% of the height when the mobile phase was pumped at 0.5 mL/min.

Before injection into the chromatograph all samples and standards were filtered through a 5-μm Millipore filter (LSWP 01300) mounted in a Millipore Swinny filter holder (XX3001200) that was, in turn, attached to a 1-mL hypodermic syringe.

Standardization. A standard stock solution of chlorsulfuron was prepared by weighing out 10.0 mg, dissolving it in methylene chloride, and diluting it to 100 mL in a volumetric flask. This solution was quite stable and was used for many months.

The working standards used for liquid chromatography as well as for the spiking of recovery samples were prepared by pipetting 1.0 mL of the stock solution into a clean, dry, 100-mL volumetric flask, evaporating the methylene chloride with a gentle nitrogen stream, dissolving the residue in the mobile phase, and diluting to volume with the mobile phase. Standards with concentrations of 0.50, 0.20, 0.10, 0.05, 0.02, and 0.01 μg/mL were prepared from the 1.0 μg/mL standard by appropriate dilution with the mobile phase. The set of standards prepared in the mobile phase was replaced with a fresh set every month. Over this time period no change in detector response was observed. All standards were stored in a refrigerator when not in use.

RESULTS AND DISCUSSION

A detector response curve is shown in Figure 1. The detector output was linear over this particular weight range of chlorsulfuron. It can also be seen that the minimum detectable quantity of chlorsulfuron was 100 pg put through the chromatography column and that this amount produced a peak 13 mm in height when the detector was operated at maximum sensitivity (1 × 1) using a 1-mV recorder with a chart width of 25 cm as the readout device. Normally, the short-term noise was <1 mm (peak to peak). To achieve this sensitivity, it was necessary to use a pump that produced only small pressure pulses as well as a column of high efficiency. Also, the detector lamp usually needed to be replaced after 500–1000 h of operation, and periodic ultrasonic cleaning of the conductivity cell and electrodes with 0.1% nitric acid was required.

Figure 2 is a chromatogram of chlorsulfuron obtained by injecting 10 μL of the 0.01 μg/mL standard. The detector sensitivity was at its maximum value (1 × 1), and the chromatographic peak displayed represents the detection limit of the method as displayed on a 25-cm chart.

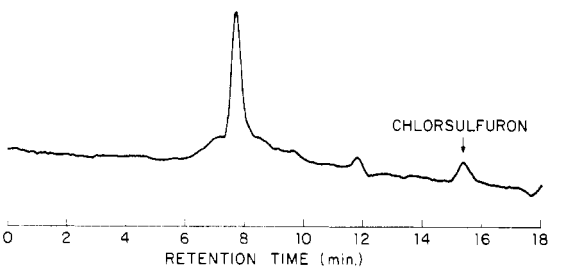

Figure 2. Chromatogram of chlorsulfuron at a detection limit of 100 pg (detector sensitivity 1 × 1).

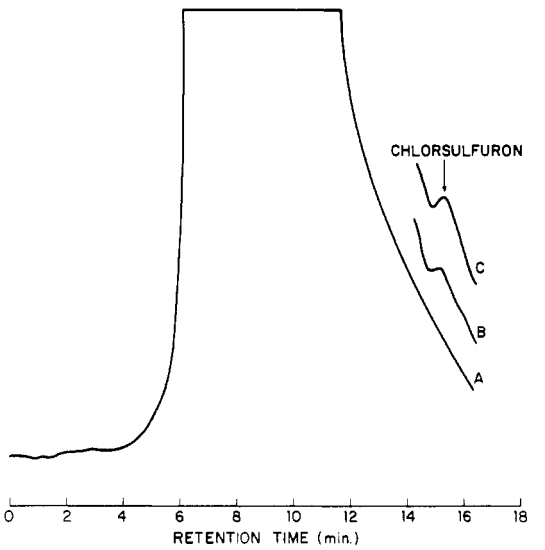

Figure 3. Chromatograms of Flanagan soil extracts: (A) control; (B) 0.2-ppb spike; (C) 0.4-ppb spike (detector sensitivity 1 × 1).

Table I. Soil Properties

	Fallsington	Flanagan
pH	6.0	5.0
sand (USDA), %	46	6
silt (USDA), %	43	77
clay (USDA), %	11	17
cation-exchange capacity, mequiv/100 g	4.4	15.4
organic content, %	0.81	3.6

Confirmation of the elution of chlorsulfuron at the time indicated was obtained by injection of a concentrated standard (100 μg/mL), followed by trapping of the peak and subsequent analysis by mass spectrometry.

For the recovery experiments, two soils of substantially different properties were used primarily. The properties of these soils are given in Table I. Recoveries have been measured on 16 spiked samples over a concentration range of 0.2–2.0 ppb. An average value of 80% was found with a standard deviation of ±16%.

The chromatograms of an extract of untreated Flanagan soil and of extracts of Flanagan soil samples spiked with 0.2 and 0.4 ppb of chlorsulfuron are shown in Figure 3. The lower trace (A) is that of the control extract, the middle trace (B) is that of the extract of the 0.2-ppb spiked sample, and the upper trace (C) is that of the 0.4-ppb spiked sample. The measured recovery at the 0.2-ppb level was 71%, and at the 0.4-ppb level it was 67%.

A bioassay method, based on the growth of corn seedlings, has been developed which also will measure very low levels of chlorsulfuron in soil. This work of Hutchison (1981) has been very useful in demonstrating the effectiveness of the method described in this paper. A set of comparative data obtained on Danish soils treated with

Table II. Danish Soils

plot no.	treatment, g/ha	residue analysis, ppb	bioassay, ppb
2/79	10	0.2	0.2
11/79	10	0.6	0.2
34/79	20	0.2	0.25
5/79	20	0.2	0.1
5/79	40	0.4	0.3
5/79	80	0.7	0.8

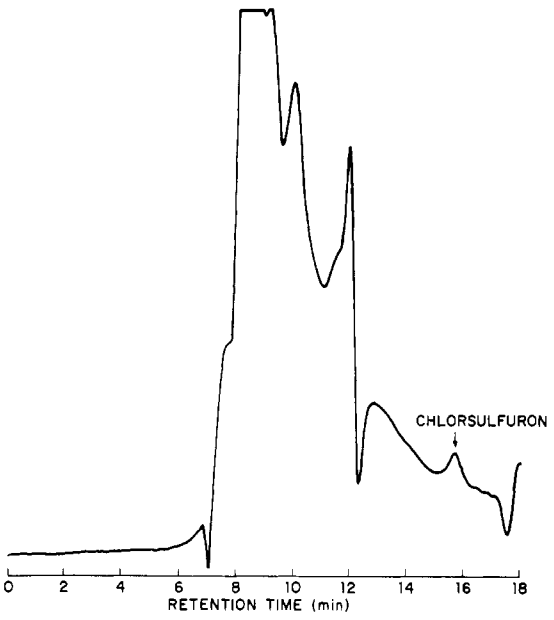

Figure 4. Chromatogram of Danish soil extract: 0.4 ppb of chlorsulfuron.

chlorsulfuron is given in Table II. The samples were taken from the fields 1 year after chlorsulfuron had been applied. These results show a good correlation of the two analytical approaches on field samples treated over an 8-fold concentration range.

Figure 4 is a chromatogram of one of the Danish soil extracts (fifth entry in Table II) showing good resolution of the chlorsulfuron peak from the background.

CONCLUSION

The active ingredient in Glean Weed Killer, chlorsulfuron, can be analyzed in soil samples with a detection limit of 0.2 ppb. The intact molecule was measured in the presence of other extractables by application of a normal-phase HPLC separation coupled with a highly sensitive, selective detector. The reliability of this method has been established by comparison with results obtained by a bioassay method.

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