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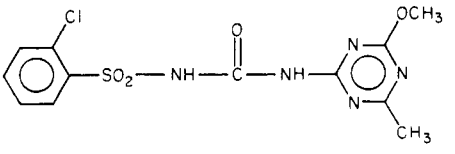
Determination of Chlorsulfuron Residues in Grain, Straw, and Green Plants of Cereals by High-Performance Liquid Chromatography

Robert V. Slates

A high-performance liquid chromatographic method using photoconductivity detection was developed to determine residues in cereal crops of chlorsulfuron, 2-chloro-*N*-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]benzenesulfonamide, the active ingredient in Du Pont Glean weed killer. For grain, straw, and green plants, respectively, detection limits were 0.01, 0.05, and 0.05 ppm and recoveries averaged 84%, 80%, and 87%. No residues were detected in 291 samples of grain nor in 144 samples of straw of wheat, barley, and oats treated postemergence at up to 2240 g of a.i./ha. Residues were, however, detected in green wheat plants following postemergence treatment, and a mathematical model describing residue disappearance with time is given.

Du Pont Glean weed killer is a broad-spectrum herbicide particularly useful for controlling weeds in cereal crops such as wheat, oats, and barley. Preemergence or early postemergence application of Glean at 10–40 g/ha provides effective control of most broadleaf weeds and limited control of grass weeds with no phytotoxicity to the cereal. The active ingredient of Glean is chlorsulfuron, which has the chemical name 2-chloro-*N*-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]benzenesulfonamide. Chlorsulfuron, formerly designated DPX-4189, has the structural formula



chlorsulfuron

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The synthesis, herbicidal efficacy, crop tolerance, and mammalian toxicology of chlorsulfuron were reported by Levitt et al. (1981) and Du Pont (undated). Studies on the mode of action of chlorsulfuron were reported by Ray (1980). Analytical methods for the determination of sulfonylurea compounds in noncrop substrates were reviewed by Zahnow (1982) in an earlier publication from this laboratory.

This paper describes an analytical method which was developed and used to determine chlorsulfuron residues in grain, straw, and green plants of cereals. Chlorsulfuron is isolated from the samples by ethyl acetate extraction and is separated from major interfering components by an aqueous filtration, a size-exclusion chromatographic separation for green plants or particularly oily samples, and solvent partitioning. Chlorsulfuron is then determined in the sample extracts by high-performance liquid chromatography (HPLC) using a photoconductivity detector. The detection limit for the method is 1 ng, which is equivalent to 0.01 ppm in grain and 0.05 ppm in straw and green plants.

No residues of chlorsulfuron have been detected in the grain or straw of wheat, barley, or oats. However, a short-lived metabolite has been detected in green wheat samples when treated postemergence with chlorsulfuron. This metabolite has not been detected in any mature grain or straw samples. A separate residue method for the determination of this metabolite will be published in the near future.

EXPERIMENTAL SECTION

Chemicals and Reagents. The chlorsulfuron reference standard was synthesized, purified, and assayed in the Du Pont Biochemicals Department, Agrichemicals Research Division Laboratories as described by Levitt (1978). All organic solvents used for sample extractions, cleanup, and liquid chromatography were "distilled-in-glass" grade. Other chemicals were "certified" grade.

The working standard of 1 μg/mL chlorsulfuron in methylene chloride was prepared fresh daily for spiking purposes by dilution of a 100 μg/mL stock solution. Standards for HPLC analysis were prepared daily by pipetting the required aliquots of working standard into volumetric flasks, evaporating the methylene chloride with a gentle stream of dry nitrogen, and making each flask to volume with HPLC mobile phase.

The HPLC mobile phase consisted of 750 mL of cyclohexane, 125 mL of isopropyl alcohol, 125 mL of methyl alcohol, and 1 mL of a mixture of 10 mL of glacial acetic acid and 1 mL of water.

The HPLC cleaning solution consisted of 400 mL of isopropyl alcohol, 100 mL of glacial acetic acid, and 10 mL of water.

The pH 10 buffer solution was prepared by dissolving 1.42 g of anhydrous Na_2HPO_4 and 40.0 g of NaCl in 400 mL of water. Using a pH meter, the pH of the solution was adjusted to 10.0 by adding 1 M NaOH in small increments with stirring.

Apparatus. Bulk samples of straw and green or whole plants were homogenized for analysis by using a Hobart Commercial Food Cutter.

Samples were finely ground during solvent extraction in Waring Commercial Blendors (Waring Products Corp., New York, NY) with 40-oz blender jars. Blenders were operated through variable power transformers to provide speed control.

Size-exclusion chromatographic separations were performed on an AutoPrep Model 1001 instrument (Analytical Biochemistry Laboratories, Inc., Columbia, MO) using a 2.5 cm × 29 cm column of Bio-Beads S-X3 styrenedivinylbenzene copolymer (Bio-Rad Laboratories, Richmond, CA).

Chlorsulfuron analyses were performed on a Du Pont Model 850 HPLC instrument fitted with a 4.6 mm × 25 cm Du Pont Zorbax Sil column, a Tracor Model 965 photoconductivity detector with a mercury lamp, and a Hewlett-Packard Model 3380A integrating recorder. To permit accurate balancing of the mobile-phase flows through the reference and analytical cells of the detector, a metering valve was installed on the reference cell discharge line. The ion-exchange resin tube and the micropump of the detector were not used because deionization of the mobile phase is not necessary and because the resin could actually introduce interfering contaminants into the mobile phase.

The basis of the photoconductivity detector is electrical conductance measurement of ions formed by dissociation of noncharged sample components in the mobile phase following irradiation with ultraviolet light. After the sample components are separated by the chromatographic column, they enter the photoconductivity detector in the mobile-phase stream. The mobile-phase stream is split into two separate streams which have equal flow rates and pass through tubing of equal diameters and lengths to the analytical and reference cells, respectively, where the electrical conductance of each stream is measured. The streams differ in only one respect: Immediately before entering the conductivity cell, the analytical stream passes through a quartz coil and is irradiated by ultraviolet light from a mercury lamp. The reference stream is not irradiated. Energy from the ultraviolet lamp causes a fraction of the chlorsulfuron and a few other ionizable sample components in the analytical stream to ionize. The increased conductance of the analytical stream compared to that of the reference stream is recorded as the detector signal.

Analytical Procedure. Samples of green plants were frozen immediately after collection at the agricultural test site and were maintained in a frozen state until analyzed. Because chlorsulfuron is rapidly metabolized by green cereal plants, freezing of green samples is essential to preserve sample integrity. Samples were frozen at −5 °C until analysis.

Bulk samples of green plants and straw (but not grain) were homogenized before analysis by cutting to lengths of less than 1 cm using a commercial food cutter. Powdered dry ice was added to the sample during this operation to facilitate cutting and to minimize sample decomposition.

All samples were weighed for analysis without drying. However, to permit conversion of green plant residue data to a dry-weight basis for comparison with straw and grain residue data, percent weight loss on drying in air at room temperature was determined on separate portions of all green plant samples.

Each representative sample of green plants (20 g), grain (50 g), or straw (10 g) was blended with 150 mL of ethyl acetate for 5 min at high speed to pulverize the sample and for 5 min at low speed to promote chlorsulfuron extraction. While retaining most of the sample solids in the blender jar, the extract was decanted and vacuum filtered to remove any solids. The blending and extraction procedures were repeated for a total of three extractions on each sample. The combined ethyl acetate extracts were evaporated on a rotary evaporator at 35 °C until all ethyl acetate had been evaporated.

Initial cleanup was accomplished as follows by an aqueous filtration at pH 10. After evaporation of ethyl

acetate from the sample extraction flask, 10 mL of methylene chloride was added and the sample residue redissolved. Twenty-five milliliters of pH 10 buffer solution was added to the flask, and the methylene chloride was removed by rotary evaporation at 35 °C. The sample flask was cooled in an ice–water bath for at least 15 min to promote solidification of oily sample components. The aqueous phase was vacuum filtered while still cold and was retained for further cleanup. Dissolution of the sample residue in methylene chloride prior to addition of the aqueous buffer solution eliminates the possibility that chlorsulfuron could be trapped in the aqueous-insoluble residue and lost from the analysis. Particular care was taken to make certain that all methylene chloride was evaporated before filtration because residual methylene chloride reduces the tendency of aqueous-insoluble components to solidify and thereby reduces the effectiveness of this cleanup step.

A size-exclusion chromatographic separation was required to eliminate interferences during HPLC analysis of chlorsulfuron in green plant samples and oats grain samples. For other cereal samples, the size-exclusion separation was unnecessary, and final cleanup was accomplished by solvent partitioning. For samples which required size-exclusion separation, the aqueous filtrate was transferred to a separatory funnel and acidified with 1 M HCl to pH 2, and chlorsulfuron was extracted 3 times with 25-mL quantities of chloroform. The combined chloroform extracts were rotary evaporated to dryness at 35 °C, and the extract residue was dissolved and quantitatively transferred in ethyl acetate to a 10-mL volumetric flask. The 10-mL solution was used to load a 5-mL loop of the AutoPrep 1001 instrument for size-exclusion separation. The size-exclusion separation was performed using ethyl acetate mobile phase at a flow rate of 5 mL/min. On the basis of prior calibration of the size-exclusion column, the first 85-mL fraction of eluate was discarded, and the next 75-mL fraction, containing chlorsulfuron, was rotary evaporated to dryness at 35 °C. The residue after evaporation was dissolved in 25 mL of pH 10 buffer and was further cleaned up by solvent partitioning.

Solvent partitioning was the final cleanup step for all samples. The aqueous filtrate for samples which required no size-exclusion separation or the aqueous solution from the size-exclusion separation was quantitatively transferred to a separatory funnel and was washed 3 times with 25-mL quantities of chloroform and 2 times with 25-mL quantities of cyclohexane. After acidification of the aqueous phase to pH 2 with 1 M HCl, the chlorsulfuron was extracted into chloroform by three 25-mL extractions. The combined chloroform extracts were rotary evaporated to dryness at 35 °C, and the residue was redissolved in 5 mL of HPLC mobile phase for analysis.

Chlorsulfuron was determined by HPLC by comparing the chromatographic peak heights for chlorsulfuron in the sample solution with the corresponding peak heights for standard solutions containing known quantities of chlorsulfuron. Chromatographic conditions for chlorsulfuron analysis were as follows: column temperature, 35 °C; mobile-phase flow rate, 1.0 mL/min; injection volume, 10 μL; retention volume, 7 mL. Standard curves were linear with a zero intercept for injections of up to 10 ng of chlorsulfuron. The analytical sensitivity for chlorsulfuron was typically 40 mm/ng with a standard error of estimate of 5.5 mm when the detector was operated at maximum sensitivity and the detector response was recorded on a 1-mV recorder with 167-mm full-scale deflection.

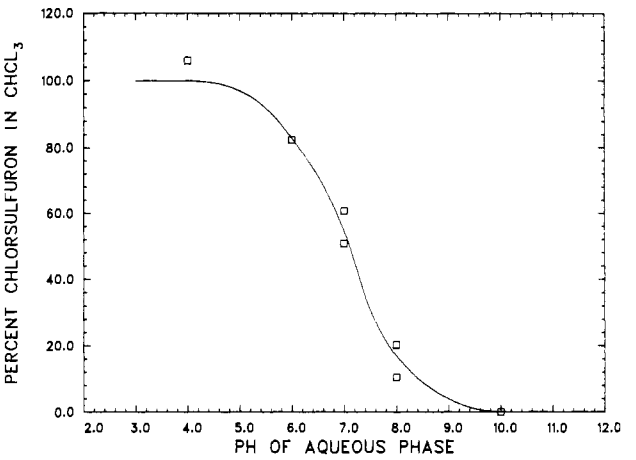

Figure 1. Effect of pH on the distribution of chlorsulfuron between equal volumes of chloroform and buffered aqueous phase.

Care was taken to make certain the Zorbax Sil column was properly conditioned and equilibrated with the HPLC mobile phase before analysis. If the column was not properly conditioned, low sensitivity or drifting sensitivity was experienced. The Zorbax Sil column was conditioned by pumping the previously described HPLC cleaning solution through the entire system at 0.5 mL/min for at least 4 h. HPLC mobile phase was then pumped through the system at 0.5 mL/min for at least 4 h to establish equilibrium between the column and the mobile phase.

Throughout the cleanup procedure, the pH of the aqueous phase was checked frequently and readjusted as necessary. The importance of accurate pH control is illustrated in Figure 1 which shows the effect of pH on the extraction of chlorsulfuron from aqueous buffer solution into chloroform. At pH 2, chlorsulfuron extracts nearly quantitatively into chloroform; however, at pH 10, extraction is negligible.

Note: Chloroform is classified as an A2 carcinogen by the American Conference of Governmental Industrial Hygienists. When working with chloroform, adequate ventilation should be provided, and skin contact should be avoided by use of poly(vinyl alcohol) gloves.

RESULTS AND DISCUSSION

Representative chromatograms for the determination of chlorsulfuron in green wheat plants are shown in Figure 2. These chromatograms demonstrate the performance of the photoconductivity detector when operated at maximum sensitivity (attenuation 1), and show that chlorsulfuron is well resolved from sample components. Chromatograms for chlorsulfuron determination in grain and straw samples are similar to those of Figure 2 but generally show lower backgrounds. Some samples were diluted for analysis to keep the chlorsulfuron detector response within the range of the analytical standards. The low backgrounds obtained on cereal samples and the high sensitivity for chlorsulfuron show that the photoconductivity detector is well suited to the determination of chlorsulfuron in plant samples. The chlorsulfuron peaks of Figure 2 would have been totally obscured by full-scale recorder deflection if a 254-nm ultraviolet absorbance detector has been used.

The detection limit for HPLC determination of chlorsulfuron was calculated to be 1 ng at the 95% confidence level by the method of Hubaux and Vos (1970). By this method, confidence limits were calculated for the standard curve, and the detection limit was evaluated from these limits. A detection limit of 1 ng for the HPLC analysis

Table I. Chlorsulfuron Recovery Data for Wheat, Barley, and Oats

sample type	mean recovery, %	SD, %	no. of recoveries	range of recoveries, %
green wheat	88	11	23	65-120
green barley	83	11	4	66-90
green oats	84	7	6	75-94
wheat grain	85	9	12	66-100
barley grain	84	11	9	68-100
oats grain	80	13	6	70-100
wheat straw	81	9	7	65-98
barley straw	76	10	5	65-90
oats straw	84	8	3	76-92

corresponds to a detection limit of 0.01 ppm for 50-g grain samples (0.02 ppm for 50-g samples of oats grain because of the size-exclusion separation) and 0.05 ppm for 10-g straw or 20-g green plant samples.

A control sample and a control sample spiked with a known quantity of chlorsulfuron were analyzed with every set of four field-treated samples to demonstrate the absence of interferences, to provide a check of recovery efficiency, and to confirm that the retention time in the sample matrix was consistent with that of the standards. Standards were analyzed frequently during each series of sample analyses to confirm the stability of the instrument sensitivity and retention time.

Recovery efficiencies were determined on spiked control samples at 1, 2, and 4 times the detection limit for sets of grain, straw, and green plant samples which showed no detectable residues of chlorsulfuron. For sets of green plant samples in which chlorsulfuron was detected, recovery efficiencies were determined at levels comparable to the observed residue levels. Recovery data are summarized in Table I. Average recovery efficiencies in green plants, in grain, and in straw, were respectively 87%, 84%, and 80%.

Chlorsulfuron residues were determined in 291 samples of grain and in 144 samples of straw of wheat, barley, and oats from agricultural test plots which had been postemergence treated with chlorsulfuron at up to 2240 g of a.i./ha. None of the grain or straw samples contained chlorsulfuron residues in excess of the detection limits reported above.

Chlorsulfuron residues were determined in 112 green plant samples of wheat, barley, and oats. Residues were detected (>0.05 ppm) in several green wheat samples collected during a 2-week period following postemergence treatment with Glean 75 DF. No residues were detected

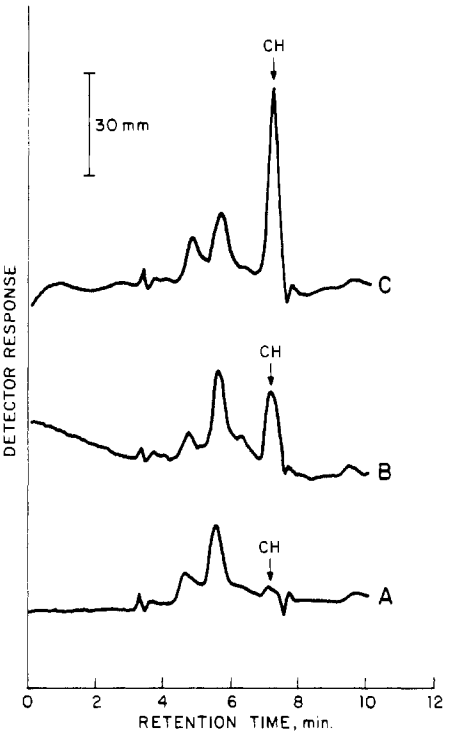

Figure 2. Representative chromatograms for determination of chlorsulfuron in green wheat plants. CH denotes chlorsulfuron. (A) Unspiked control. (B) Control spiked at 0.10 ppm with chlorsulfuron, 94% recovery. (C) Field sample containing 0.26 ppm of chlorsulfuron, sampled 2 days after postemergence treatment with chlorsulfuron at 30 g of a.i./ha.

(<0.05 ppm) in any of the barley or oats greeen plant samples; however, all of these samples were collected more than 2 weeks after treatment. On the basis of the mathematical model discussed below for green wheat data and the known values of T and R, no detectable residues would be expected in these barley and oats green plant samples.

Typical chlorsulfuron residues in green wheat plants are reported in Table II. These data are based on the weight of the fresh sample and are not corrected for recovery. The presence of chlorsulfuron in the green wheat samples was confirmed by gas chromatographic–mass spectrometric analysis of the chlorsulfuron dimethyl derivative using multiple ion detection of the m/e 181 and 210 ions. The dimethyl derivative was prepared by reacting the sample with an excess of diazomethane for 16 h at room temperature in diethyl ether containing 10% methanol.

Table II. Chlorsulfuron Residues in Green Wheat Plants

variety	location of test plot	date planted, M-D-Y	date treated, M-D-Y	date sampled, M-D-Y	treatment level, g of a.i./ha	residue level, ppm	wt loss on air dry, %
Newton	Kansas	10-01-80	4-01-81	4-02-81	70	2.4	78
		10-01-80	4-01-81	4-04-81	70	1.2	81
		10-01-80	4-01-81	4-08-81	70	0.85	79
		10-01-80	4-01-81	4-15-81	70	≤0.05[a]	76
		10-01-80	4-01-81	4-29-81	70	<0.05	73
Gatcher	Queensland, Australia	6-22-81	7-27-81	7-27-81	30	2.6	80
		6-22-81	7-27-81	7-29-81	30	0.26	77
		6-22-81	7-27-81	7-31-81	30	0.09	86
		6-22-81	7-27-81	8-03-81	30	0.05	77
		6-22-81	7-27-81	8-10-81	30	<0.05	78
		6-22-81	7-27-81	7-27-81	60	4.1	80
		6-22-81	7-27-81	7-29-81	60	0.42	76
		6-22-81	7-27-81	7-31-81	60	0.13	82
		6-22-81	7-27-81	8-03-81	60	0.10	77
		6-22-81	7-27-81	8-10-81	60	<0.05	77

[a] One inch of rain prior to sampling on 4-15-81 washed most of the chlorsulfuron from the plants.

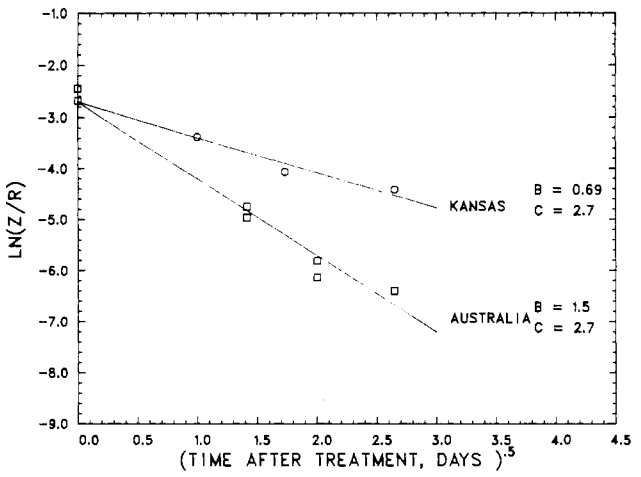

Figure 3. Modeling plots of eq 2 for disappearance of chlorsulfuron from green wheat plants following postemergence treatment with Glean.

Mathematical modeling of the green wheat residue data shows that chlorsulfuron disappeared from the plants according to the empirical equation

$$Z = R \exp(-BT^{1/2} - C) \quad (1)$$

Variables in eq 1 were defined as follows: Z = chlorsulfuron residue level in the plant sample in ppm; R = postemergence treatment rate in grams of active ingredient per hectare; T = time between treatment and sampling in days; B = a parameter which describes the chlorsulfuron disappearance rate; C = a nearly constant parameter which depends on characteristics of the plant at treatment.

Equation 1 can be rearranged to give the linear eq 2

$$\ln (Z/R) = -BT^{1/2} - C \quad (2)$$

Plots of ln (Z/R) vs. $T^{1/2}$ are shown in Figure 3 for the disappearance of chlorsulfuron from green wheat. The linearity of both data plots demonstrates the validity of the mathematical model.

During initial evaluation of the green wheat data, plots of ln (Z/R) vs. T rather than $T^{1/2}$ were made because these plots should be linear if chlorsulfuron disappears according to first-order kinetics. These plots were very clearly nonlinear, indicating that the observed disappearance of chlorsulfuron from green wheat plants involves factors other than or in addition to first-order kinetics.

The common intercept (C = 2.7) for both plots of Figure 3 was expected because the intercept depends only upon the effective leaf area and weight of the plants per unit land area at treatment and is independent of treatment rate. This can be shown as follows: Define WP to be the weight in grams of the green wheat plants on a 1-ha plot at treatment. Define ELA to be the effective leaf area of the plants on the 1-ha plot so that at treatment

ELA = (g of chlorsulfuron retained on plants) × 1 ha/(total g of chlorsulfuron applied to plot)

Then, from the definition of Z

$$Z = R \times \text{ELA} \times 10^6/\text{WP}$$

and at treatment

$$\ln (Z/R) = \ln (\text{ELA} \times 10^6/\text{WP}) = C$$

The intercept C therefore depends upon characteristics of the plants at treatment and not upon treatment rate. As long as the plants are treated at approximately the same stage of growth so that ELA/WP is nearly constant, the intercept will also be nearly constant.

The slopes for the Kansas data plot (B = 0.69) and the Australian data plot (B = 1.5) show that chlorsulfuron disappeared rapidly from the green plants at both locations but the rates of disappearance were clearly different. Half of the chlorsulfuron applied at treatment had disappeared from the Australian plants in 5 h and from the Kansas plants in 24 h.

The mathematical model of eq 1 was tested on the data of Lee and Westcott (1981) for the disappearance of the insecticide dimethoate from green wheat plants after postemergence treatment. Equation 1 accurately described their three sets of residue disappearance data for 17 days after treatment and gave values for B and C which agreed well with those for chlorsulfuron. Their data showed that wheat treated at an early stage of development gave larger values for B (faster disappearance) than wheat treated at later stages of development.

The chlorsulfuron analytical method has proved adequate for all types of cereal samples. Chlorsulfuron residues disappear rapidly from green wheat plants at a rate described by a simple mathematical model. Chlorsulfuron residues have never been detected in grain or straw of mature wheat, barley, or oats.

Registry No. Chlorsulfuron, 64902-72-3.

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