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Characterization of the thermal decomposition products of the sulfonylurea herbicide chlorsulfuron

AUSTIN R. LONG, BAHRAM CHARKHIAN, LILY C. HSIEH, CHARLES R. SHORT and STEVEN A. BARKER*

Department of Veterinary Physiology, Pharmacology and Toxicology, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803 (U.S.A.)

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SUMMARY

Gas chromatographic injection port thermal decomposition of chlorsulfuron resulted in two volatile decomposition products (2-amino-4-methoxy-6-methyl-1,3,5-triazine and 2-chlorobenzenesulfonamide) which were characterized by gas chromatography and gas chromatography–mass spectrometry. Quantitation of chlorsulfuron as its volatile thermal decomposition product 2-amino-4-methoxy-6-methyl-1,3,5-triazine was accomplished by gas chromatography–nitrogen–phosphorus detection analysis and the linearity of standard curves so obtained was independent of injection volume (0.5–3 μl) and injection port temperature (230–270°C) for the concentration range examined (62.5–1000 ng/ml).

INTRODUCTION

Chlorsulfuron {2-chloro-N-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]-carbonyl]benzenesulfonamide} is the active ingredient in DuPont's pre- and post-emergence herbicide. It is a member of the sulfonylurea class of compounds and finds application in wheat, oat and barley production[1]. The toxicological aspects of chlorsulfuron have been published[1].

Biological extracts containing sulfonylurea residues may be quantitated by analytical techniques such as high-performance liquid chromatography (HPLC)[2,3] utilizing photoconductivity detection or by gas chromatography (GC) of volatile methylated derivatives[4,5]. Chlorsulfuron has been analyzed by both approaches[6–8]. However, reaction conditions required to form volatile methylated chlorsulfuron derivatives necessary for GC analyses must be carefully controlled to avoid the formation of multiple derivative products or hydrolysis[4], both of which can make quantitative determinations difficult. The HPLC analyses of nanogram (ng/ml) levels of chlorsulfuron utilizing ultraviolet (UV) detection in the low UV range (210–230 nm) may be accomplished, provided the sample is free from interfering chromophores[9].

GC can provide greater flexibility than HPLC with UV detection and is capable of achieving sensitivities in the picogram (pg on column) range, provided the

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compound is amenable to GC and responds to a detector capable of attaining such levels of detection. Furthermore, GC coupled with mass spectrometry (MS) can provide structural information which can be difficult to accomplish by HPLC–UV. A combined approach by GC and GC–MS offers the advantages of high-resolution capillary chromatography and sensitive, selective detection and confirmation of compounds of interest.

The purpose of this research was to characterize the thermal decomposition products of chlorsulfuron as a prelude to quantitative determinations of chlorsulfuron present in biological extracts. We report here the characterization of the chlorsulfuron decomposition products. A method for the quantitative determination of the volatile thermal decomposition product 2-amino-4-methoxy-6-methyl-1,3,5-triazine (AMMT) by GC with nitrogen–phosphorus detection (NPD), and its characterization by GC–MS, is also presented.

EXPERIMENTAL

Chemicals and expendable materials

Chlorsulfuron was obtained from DuPont (Wilmington, DE, U.S.A.). Solvents (HPLC grade) were obtained from commercial sources and used without further purification. Stock chlorsulfuron standard solutions (1 mg/ml) were prepared by dissolving pure chlorsulfuron in dichloromethane and then serially diluting with dichloromethane to the desired concentration (62.5, 125, 250, 500 and 1000 ng/ml).

GC and GC–MS

GC analysis was conducted on a Varian (Palo Alto, CA, U.S.A.) Vista 6000 (column; 25 m × 0.25 mm I.D. fused silica, 0.25-μm coating, DB-5, J & W Scientific, splitless injection with the purge function being activated at 0.75 min post injection with a temperature program of 90°C for 1 min, increasing 20°C/min to 280°C and holding for 1 min). NPD was accomplished using a detector maintained at 300°C and 8 mV at a sensitivity of $32 \cdot 10^{-12}$. The injection port temperature (230, 240, 250, 260 and 270°C) and injection volume (0.5, 1.0, 2.0 and 3.0 μl) were varied according to the experimental design (Table I). Eluted peaks were integrated utilizing a Varian (Model

TABLE I

QUANTITATIVE RESULTS FOR THE GC–NPD ANALYSIS OF 2-AMINO-4-METHOXY-6-METHYL-1,3,5-TRIAZINE THERMAL DECOMPOSITION PRODUCT OF CHLORSULFURON

Correlation coefficients (r; mean of 5 standard curves ± standard deviation, S.D.) determined for chlorsulfuron at increasing concentrations (62.5, 125, 250, 500 and 1000 ng/ml). IV = Injection volume; IP = injection port temperature; n = number of samples.

	r (mean ± S.D.)		*n*
Interassay variability		11.6 ± 7.5%	25
Intraassay variability		6.2%	5
IV 2 μl, IP 250°C	0.992 ± 0.004		
IV 2 μl, IP = 230, 240, 250, 260 and 270°C	0.997 ± 0.003		
IP 250°C, IV = 0.5, 1, 2 and 3 μl	0.989 ± 0.001		

4290) integrator. Interassay variability was determined by calculating the mean and standard deviation (S.D.) of five replicates for each concentration and specific treatment. The S.D. was devided by its respective mean which gave the coefficient of variation (C.V.). The C.V. values so obtained were averaged and defined as the interassay variability. Intraassay variability was determined by dividing the S.D. by the mean area of an identical sample analyzed five times ($n=5$) under identical conditions.

GC–MS analyses were performed on a Finnigan (Model 9611) gas chromatograph (San Jose, CA, U.S.A.), as described above for GC determinations, linked to a Finnigan triple stage quadrupole mass spectrometer (TSQ-4500), maintaining the transfer line at 300°C. A 70 eV potential with the electron multiplier voltage set at 2000 and a 2 s/mass decade scan were utilized. Data were recorded and processed utilizing a Data General (Nova 4) data processing system.

Preparation of standard AMMT and 2-chlorobenzenesulfonamide (CB)

Standard AMMT and CB were prepared by acid hydrolysis of pure chlorsulfuron. Chlorsulfuron hydrolysis was carried out by acidifying 10 ml of a chlorsulfuron solution (1 mg/ml methanol) with one drop of 1 *M* hydrochloric acid and heating the acidified solution at 50°C for 4 h or until complete hydrolysis of chlorsulfuron had occurred. The formation of hydrolytic products was monitored at 1-h intervals by analyzing a portion of the reaction mixture by HPLC–UV (230 nm for AMMT and 270 nm for CB) until complete hydrolysis was obtained. The HPLC analysis was conducted utilizing a Hewlett-Packard HP1090 HPLC (HP79994A HPLC Chemstation) equipped with a photodiode array detector (UV; bandwidth 20 nm, spectrum range 200–350 nm and reference spectrum of 450 nm) and reversed-phase octadecylsilyl (ODS) derivatized silica column (Varian MCH-10, 10 μm, 30 cm × 4 mm) maintained at 40°C. The HPLC mobile phase was 0.017 *M* aqueous orthophosphoric acid–acetonitrile (35:65, v/v), at an isocratic flow-rate of 1 ml/min.

The identity of the hydrolytic products was confirmed by GC–MS as described under *GC and GC–MS*.

RESULTS AND DISCUSSION

Existing HPLC and GC methods for chlorsulfuron analysis lack sensitiviy in the pg range (HPLC) and/or require inexact derivatization methods to form volatile chlorsulfuron derivatives (GC). The HPLC methods utilizing photoconductivity or UV detection provide sufficient sensitivity at the maximal permissible level (300 ng/g) allowed by law in foods[10] but methods for detecting low levels (pg on column range) of chlorsulfuron are difficult without resorting to residue enrichment procedures such as solvent extractions and/or concentrating samples by column chromatography or solid phase extraction techniques. These procedures can be time and labor intensive. This led us to develop a detection method for a thermal decomposition product of chlorsulfuron which is sensitive, selective and precise enough for quantitative determinations in the low ng/ml (pg on column) range, as well as allowing for the characterization and confirmation of volatile thermal decomposition products of chlorsulfuron by GC–MS.

When standard chlorsulfuron solutions were injected into the GC injection port

a volatile thermal decomposition product was produced which resulted in a characteristic GC chromatogram (Fig. 1) when utilizing NPD. Results (Table I) indicated the detector response for this volatile thermal decomposition product was linear over the concentration range examined (62.5–1000 ng/ml, 2 μl injection volume) with acceptable inter (11.6 $\pm$ 7.5%) and intraassay (6.2%) variabilities, and was independent of injection port temperatures (230–270°C) and injection volumes (0.5–3 μl) tested. Blank sample controls, containing no chlorsulfuron, gave no indication of any peaks which might interfere with such analyses.

Characterization of the volatile thermal decomposition products of chlorsulfuron was conducted by GC–MS. Mass spectral data of the volatile thermal decomposition products indicated a thermal decomposition fragmentation pattern (Fig. 2A) occurred which produced two major volatile compounds which had molecular weights of 140 (Fig. 2B) and 191 (Fig. 2C). The nitrogen–phosphorus detectable fragment had a molecular weight of 140 (AMMT) which represents that part of the molecule which contained four nitrogen atoms (Fig. 2A). The 191 fragment was not a good NPD responder, at the concentrations examined, but the mass spectrum indicated a characteristic chlorine isotope cluster corresponding to a CB fragment.

Apparently the thermal decomposition process involves a unimolecular decomposition with minimal secondary bimolecular reactions, terminating with the formation of a minimal number of volatile fragments as a result of the extrusion of carbon monoxide, independent of the variables examined. There was no indication of intact chlorsulfuron being present when analyzed by GC–NPD or GC–MS, indicating that equilibrium temperature for primary bond fission was achieved under the specified GC operating conditions. The mass spectra generated for these two thermal decomposition products support this hypothesis and were identical to mass spectra of standard AMMT and CB generated by acid hydrolysis of chlorsulfuron. The mass spectrometry fragmentation ions generated for AMMT (Table II) and CB (Table III) are consistent

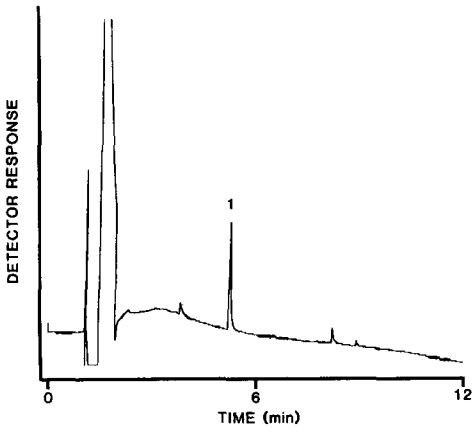

Fig. 1. Representative GC chromatogram of a nitrogen–phosphorus detectable volatile thermal decomposition product (2-amino-4-methoxy-6-methyl-1,3,5-triazine) of chlorsulfuron (peak 1). Peak 1 was not present in a solvent blank.

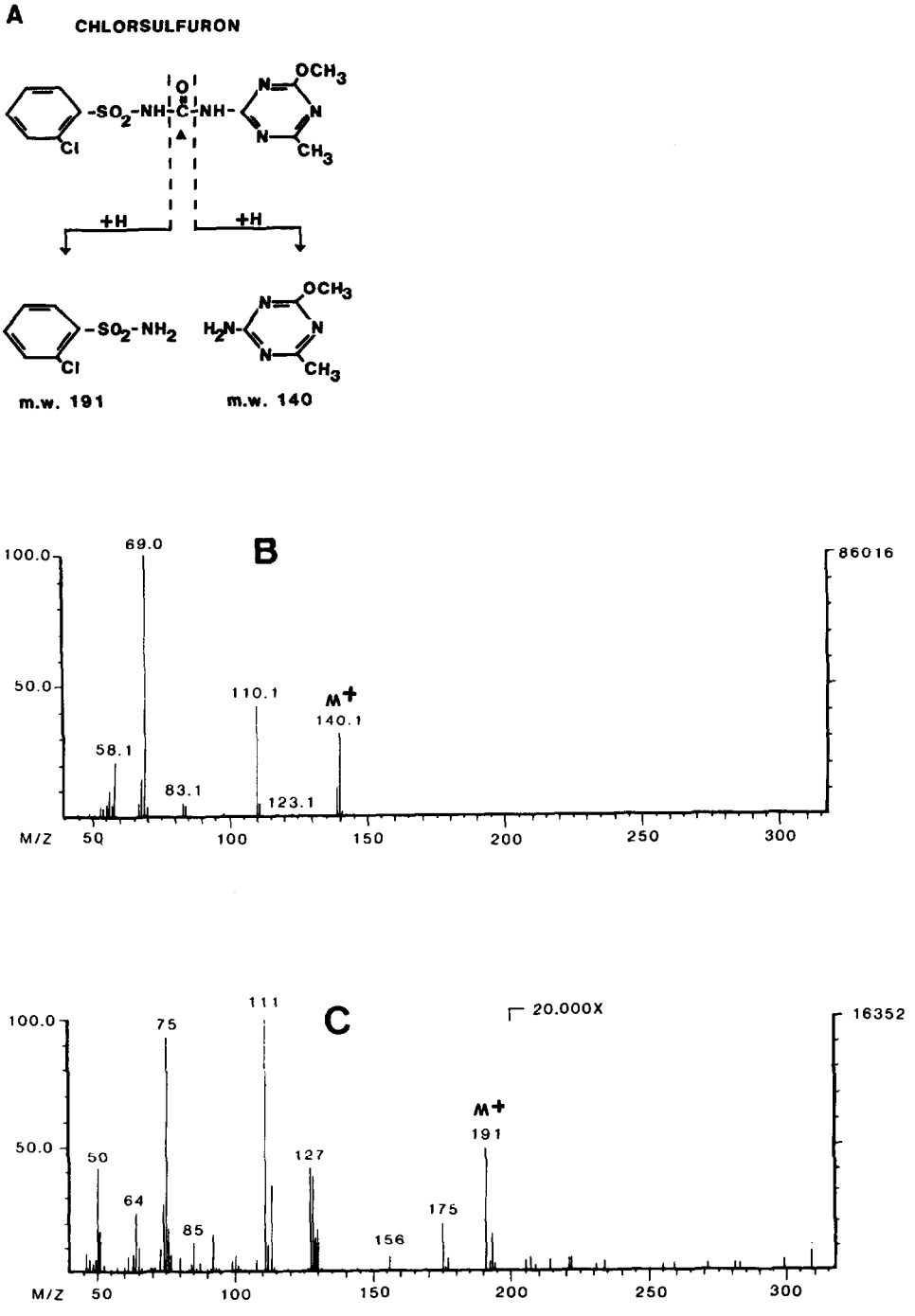

Fig. 2. Proposed thermal decomposition pattern (A) of chlorsulfuron and the positive ion, electron impact mass spectrum of fragments 1 [AMMT (B)] and 2 [CB (C)]. m.w. = Molecular weight.

TABLE II

RELATIVE ABUNDANCE OF MS FRAGMENTATION IONS FOR 2-CHLOROBENZENESULFONAMIDE THERMAL DECOMPOSITION PRODUCT OF CHLORSULFURON

Ion (isotope)	*Fragmentation*	*Relative abundance (%)*
191 (193)	M (molecular ion)	48 (15)
175 (177)	M − 16 (NH_2)	19 (5)
156	M − 35 (Cl)	5
128 (130)	M − 64 ($SONH_2$) + 1 (H)	38 (16)
127 (129)	M − 64 ($SONH_2$)	41 (13)
111 (113)	156 − SO_2NH	100 (33)
93	128 − 35 (Cl)	14
75	C_6H_3 (from benzene)	93
64	SO_2 or H_2NSO	24

TABLE III

RELATIVE ABUNDANCE OF MS FRAGMENTATION IONS FOR 2-AMINO-4-METHOXY-6-METHYL-1,3,5-TRIAZINE THERMAL DECOMPOSITION PRODUCT OF CHLORSULFURON

Ion	*Fragmentation*	*Relative abundance (%)*
140	M (molecular ion)	31
139	M − 1 (H)	11
111	M − 30 (formaldehyde) + 1 (H)	5
110	M − 30 (formaldehyde)	42
69	111 − 42 (CH_2N_2)	100
58	83 − 15 (methyl)	21

with the fragmentation pattern of other 1,3,5-triazines[11] and chlorobenzenesulfonamides[12,13].

Standard AMMT and CB were generated by acid hydrolysis of chlorsulfuron. The isolated hydrolytic products were characterized by HPLC–UV, GC–NPD and GC–MS and the mass spectra of isolated AMMT and CB were identical to the mass spectra of the thermal decomposition products of chlorsulfuron. The generation of AMMT and CB from chlorsulfuron by both acid hydrolysis and thermal decomposition, coupled to the almost identical spectra of CB to that of 4-chlorobenzenesulfonamide[13], indicates that the thermal decomposition of chlorsulfuron takes place as described. Additionally, structures of degradative products of chlorsulfuron[14] are consistent with results presented here. The unequivocal mechanistic details of AMMT and CB formation from chlorsulfuron by thermal decomposition will require additional MS characterization of isotopically labelled chlorsulfuron.

The method presented allows for the separation, characterization and quantitative determination of a volatile nitrogen–phosphorus detectable thermal decomposition product (2-amino-4-methoxy-6-methyl-1,3,5-triazine) of chlorsulfuron down to 125 pg on column. This, coupled with GC–MS allows for structural confirmations of chlorsulfuron decomposition products as well. Thus, quantitation and confirmation of chlorsulfuron as AMMT in the low ng/g range can be accomplished by thermal decomposition GC.

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REFERENCES

1 M. B. Levitt, H. L. Ploeg, R. C. Weigel, Jr. and D. J. Fitzgerald, *J. Agric. Food Chem.*, 29 (2) (1981) 416–418.
2 E. Wahlin-Boll and A. Melander, *J. Chromatogr.*, 164 (1979) 541–546.
3 M. Uehlein and N. Sistorasis, *J. Chromatogr.*, 227 (1982) 93–101.
4 P. Hartvig, C. Fagerlund and O. Gyllenhaal, *J. Chromatogr.*, 181 (1980) 17–24.
5 T. Maeda, T. Yamaguchi and M. Hasimoto, *J. Chromatogr.*, 223 (1981) 357–363.
6 E. W. Zahnow, *J. Agric. Food Chem.*, 30 (5) (1982) 854–857.
7 R. V. Slates, *J. Agric. Food Chem.*, 31 (1) (1983) 113–117.
8 I. Ahmad, *J. Assoc. Off. Anal. Chem.*, 70 (4) (1987) 745–748.
9 M. J. M. Wells and J. L. Michael, *J. Chromatogr. Sci.*, 25 (1987) 345–350.
10 *Code of Federal Regulations*, 40CFR (1988) 180.405.
11 Q. N. Porter, in E. C. Taylor and A. Weissbergen (Editors), *Mass Spectrometry of Heterocyclic Compounds*, Wiley, New York, 1985, Ch. 18, pp. 811–825.
12 H. Budzikiewicz, C. Djerassi and D. H. Williams, *Mass Spectrometry of Organic Compounds*, Holden-Day, San Francisco, CA, 1967, Ch. 19, pp. 558–561.
13 *Hewlett-Packard Mass Spectral Library (Part No. 59868A)*, National Bureau of Standards NBS/Wiley registry of mass spectral data, Palo Alto, CA, 1987.
14 M. M. Kelley, E. W. Zahnow, W. C. Petersen and S. T. Toy, *J. Agric. Food Chem.*, 33 (5) (1985) 962–965.