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Note

High-performance liquid chromatographic determination of the herbicide terbutylazine and its dealkylated metabolites in soil

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Terbutylazine (2-*tert*.-butylamino-4-chloro-6-ethylamino-1,3,5-triazine; GS 13529) is a chloro-*s*-triazinic herbicide of the homologous series to which the better known atrazine and simazine belong.

Degradation of *s*-triazines in soil may be either microbiological or physicochemical; the main degradative mechanisms are photodecomposition, volatilization, hydroxylation and dealkylation[1]. The first two are negligible with terbutylazine, whereas the last two have great importance[2]. Within microbiological mechanisms of degradation, dealkylation seems to be the most significant; oxidative dealkylation has been proved to occur not only by microbial enzymatic systems[1] but also by free-radical reactions[3]. In this process three metabolites are formed: two monoalkyl derivatives and one completely dealkylated compound[4] (Fig. 1); two of these compounds

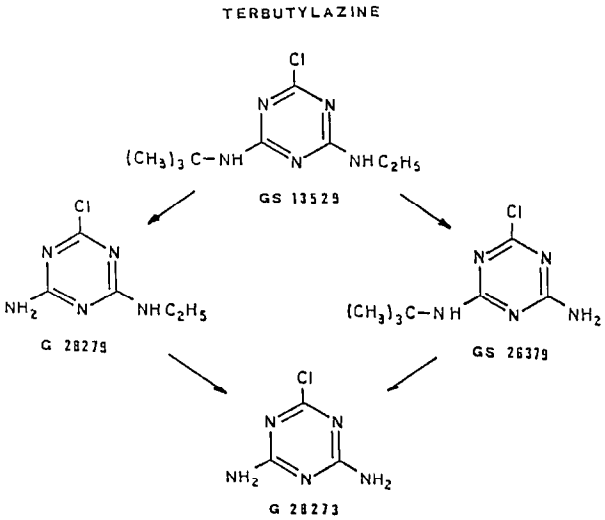

Fig. 1. Metabolic route of terbutylazine in soil.

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(G 28273 and G 28279) originate also from dealkylation of atrazine and simazine. Dealkylation of *s*-triazines has been observed not only in soil but also in higher plants[5,6].

Several methods have been described for the determination of terbutylazine, usually in the presence of other *s*-triazines, including gas chromatography[7–13] and high-performance liquid chromatography (HPLC)[11,13,14–16]; with regard to the study of metabolites, an HPLC method has been reported for the determination of hydroxy-*s*-triazines[17] but, to our knowledge, there is no reference to compounds formed by dealkylation of *s*-triazines.

In this paper, the HPLC separation of terbutylazine from its dealkylated metabolites and their determination in soil are described.

EXPERIMENTAL

Apparatus

A Varian (Palo Alto, CA, U.S.A.) Model 5020 liquid chromatograph was used, fitted with a variable-wavelength UV-100 UV–VIS detector and a Rheodyne injector (50-μl loop). The chromatograph was connected to a Hewlett-Packard 3390 A recorder–integrator.

Chromatography

Merck (Darmstadt, F.R.G.) Hibar RP-8, RP-18 and NH_2 (10 μm) columns (250 × 4.0 mm I.D.) were employed; the mobile phase was water–acetonitrile at various ratios and flow-rates (Table II). The analyses were performed at different wavelengths (Fig. 2), depending on the absorbance maxima previously determined for terbutylazine (221 nm) and its metabolic products G 28273 (205 nm), G 28279 (213 nm) and GS 26379 (213 nm) with a Varian Model DMS 90 UV–VIS spectrophotometer.

Chemicals and materials

Acetonitrile, chloroform, dichloromethane, diethyl ether and methanol were of HPLC grade (Carlo Erba, Milan, Italy); water was distilled twice and filtered through a Milli-Q apparatus (Millipore, Milan, Italy) before use.

Terbutylazine (GS 13529) and its metabolites GS 26379, G 28279 and G 28273 were obtained from Ciba Geigy (Milan, Italy).

TABLE I

PHYSICAL AND CHEMICAL CHARACTERISTICS OF THE SOILS

Characteristic	*Soil*		
	A	*B*	*C*
Sand (%)	82.7	62.5	36.6
Silt (%)	9.0	9.3	27.4
Clay (%)	8.3	28.2	36.0
pH (in water)	6.46	7.67	8.37
Organic matter (%)	1.56	2.10	3.93

Three soils of different physical and chemical characteristics (Table I) were used to set up the extraction procedure.

Extraction procedure

Ten grams of air-dried, finely sieved (< 2 μm) soil were weighed in a 250-ml screw-capped flask, 50 ml of the extraction solvent (chloroform, dichloromethane, diethyl ether or methanol) were added and the mixture was agitated in a flash-shaker (Stuart Scientific) for 30 min. The soil was left to settle and the organic layer was filtered with a PTFE syringe-filter (diameter 25 mm, 0.45 μm) (Alltech, Milan, Italy); a 5-ml aliquot of the filtered extract was transferred into a 20-ml beaker, evaporated to dryness in a thermo-ventilated stove at 50–70°C (depending on the boiling point of the solvent used), the residue was taken up in 1 ml of mobile phase and the solution was injected for HPLC analysis.

RESULTS AND DISCUSSION

In order to separate terbutylazine and its dealkylated metabolites, both normal-phase (NH_2) and reversed-phase (RP-8 and RP-18) columns were employed (Table II). The latter two allowed a good separation of the four compounds with water–acetonitrile (50:50, v/v) as eluent. Under these conditions with the RP-8 column the metabolites were slightly more retained than by the RP-18 column, whereas terbutylazine was less retained. On increasing the water content in the mobile phase, the four compounds were more retained by the column, in the order terbutylazine > GS 26379 > G 28279 > G 28273.

With the normal-phase column (NH_2) and acetonitrile as eluent, the peak elution order was reversed, with the last peak (of the metabolite G 28273) being asym-

TABLE II

RETENTION TIMES OF TERBUTYLAZINE (IV) AND ITS DEALKYLATED METABOLITES (I–III) WITH DIFFERENT COLUMNS AND ELUENTS

Column	*Water–acetonitrile (v/v)*	*Flow-rate (ml/min)*	*Retention time (min)*			
			I	*II*	*III*	*IV*
RP-8	50:50	1.0	2.25	2.86	4.47	8.19
	60:40		2.39	3.30	6.75	16.20
	70:30		2.52	4.06	12.59	–
	80:20		3.09	6.30	–	–
	90:10		4.66	16.45	–	–
	95:5		7.00	–	–	–
RP-18	50:50	1.0	2.01	2.56	4.39	9.68
	60:40		2.07	2.90	6.64	19.58
	70:30		2.48	3.95	14.47	–
	80:20		2.96	5.82	–	–
	90:10		4.10	–	–	–
	95:5		6.85			
NH_2	0:100	0.5	13.83	7.73	6.35	5.40
	1:99		8.45	6.65	5.76	5.26

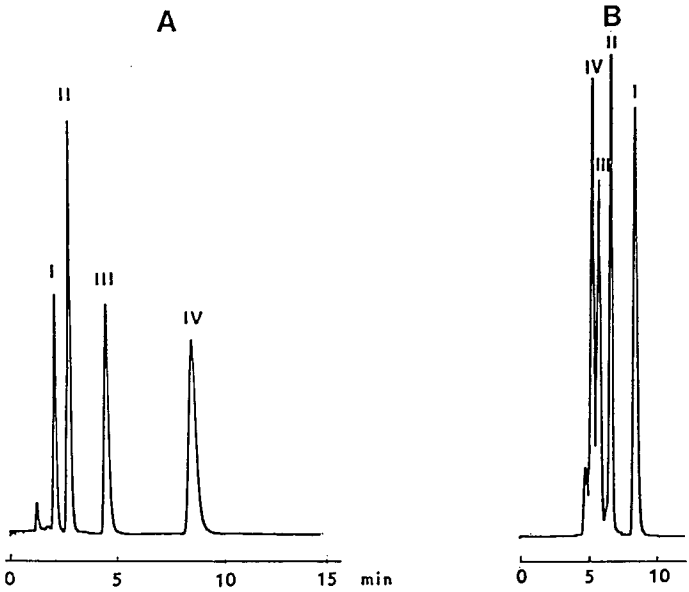

Fig. 2. Chromatography of terbutylazine (IV) and its dealkylated metabolites GS 26379 (III), G 28279 (II) and G 28273 (I). (A) On an RP-8 column: mobile phase, water–acetonitrile (50:50, v/v); flow-rate, 1 ml/min; detection, UV at 210 nm for 6.5 min, then at 220 nm. (B) On an NH_2 column: mobile phase, water–acetonitrile (1:99, v/v); flow-rate, 0.5 ml/min; detection, UV at 215 nm for 7.5 min, then at 205 nm.

metric and not sharp enough. Adding 1% of water to the mobile phase produced a sharp and symmetric peak (Fig. 2), with a considerable decrease in its retention time, whereas a moderate reduction in the retention times of the other three compounds was observed.

TABLE III

RECOVERIES OF TERBUTYLAZINE AND ITS DEALKYLATED METABOLITES WITH DIFFERENT SOLVENTS

Compound	*Fortification level (ppm)*	*Soil*	*Recovery ± R.S.D. (%)*[a]		
			Diethyl ether	*Methanol*	*Dichloro-methane*
G 28273	0.5	A	40.3 ± 1.1	71.6 ± 1.3	6.9 ± 2.4
		B	79.5 ± 3.3	80.8 ± 4.2	25.2 ± 3.6
		C	60.0 ± 1.2	72.8 ± 5.0	12.6 ± 7.1
G 28279	1.0	A	81.4 ± 1.0	80.6 ± 1.3	28.8 ± 5.1
		B	94.0 ± 6.8	84.7 ± 2.4	65.8 ± 2.8
		C	88.1 ± 1.0	77.7 ± 1.7	54.0 ± 3.5
GS 26379	1.0	A	91.1 ± 1.6	69.9 ± 3.6	54.7 ± 6.4
		B	93.8 ± 5.9	60.8 ± 0.1	67.8 ± 4.3
		C	90.7 ± 2.7	63.2 ± 1.4	76.3 ± 4.8
Terbutylazine	1.5	A	94.2 ± 3.0	64.0 ± 2.6	71.1 ± 5.1
		B	91.3 ± 6.1	52.6 ± 0.1	59.0 ± 7.1
		C	86.2 ± 1.7	51.7 ± 1.6	80.9 ± 2.0

[a] Mean values of duplicate analyses from three replicates.

Calibration graphs for each compound were constructed by plotting concentrations against peak areas; good linearities were achieved in the range 0–1.5 ppm, with correlation coefficients between 0.9991 and 0.9997. Under the optimum conditions the detection limit was 0.003 ppm for all compounds.

For recovery assays of terbutylazine and its dealkylkated metabolites three different soils that had never been treated with any pesticide, were used.

The blanks of the extraction solvents (chloroform, dichloromethane, diethyl ether and methanol) did not give any interfering peaks at the retention times of the compounds studied.

With chloroform a very low recovery (< 20%) was achieved for each compound in the three soils. Diethyl ether showed the highest extraction power and, with the exception of the most polar metabolite, G 28273, allowed very good recoveries from the three soils (Table III). In comparison with diethyl ether, methanol gave a poorer extraction of the less polar compounds (terbutylazine and GS 26379) but a better recovery of G 28273. Dichloromethane was less efficient than diethyl ether and methanol and did not allow satisfactory recoveries.

CONCLUSIONS

Considering the extraction percentages, it can be seen that there is no appreciable difference between methanol and diethyl ether as solvents, both giving good recoveries from the three different soils.

The simultaneous separation of terbutylazine and its dealkylated metabolites can be achieved with an RP-8 column and water–acetonitrile (50:50, v/v) as eluent when the concentration of the four compounds in the sample is greater than 0.1 ppm. For lower concentrations, when the presence of interfering compounds is more substantial, it is advisable, mainly for the determination of the metabolites G 28273 and G 28279, to increase the percentage of water in the mobile phase.

The separation on the NH_2 column can be used for the determination of terbutylazine and its metabolites and could be valuable as a confirmatory assay.

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