Journal of Chromatography, 549 (1991) 229–236
Elsevier Science Publishers B.V., Amsterdam

CHROM. 23 315

High-performance liquid chromatographic determination of atrazine, deisopropylatrazine and deethylatrazine in soils from corn fields

GEORG KARLAGANIS* and ROLAND VON ARX
Federal Office of Environment, Forests and Landscape, Hallwylstrasse 4, CH-3003 Berne (Switzerland)
HANS ULRICH AMMON
Federal Research Station for Plant Production, Reckenholz, CH-8046 Zurich (Switzerland)
and
RUDOLF CAMENZIND
ILB Laboratories, Birkenweg 6, 3123 Belp (Switzerland)
(First received September 18th, 1990; revised manuscript received March 20th, 1991)

ABSTRACT

A reversed-phase high-performance liquid chromatographic (HPLC) assay of atrazine, deisopropylatrazine and deethylatrazine in soil samples from corn fields (treated with atrazine) is described. Soil (50 g) was homogenized, treated in an ultrasonic bath and extracted with methanol. The extract was purified on an aluminium oxide column, Sep-Pack C_{18} cartridges (atrazine only) and acrodisc filters. HPLC was performed on a LiChrosorb RP-18 5 μm column using a mobile phase of acetonitrile–water (35:65, v/v for atrazine and 20:80, v/v for the metabolites). The method was validated by ultraviolet diode-array spectroscopy and verified by capillary gas–liquid chromatography–mass spectrometry. The method is suitable for monitoring atrazine concentrations in soil from corn fields; it may also be used for routine measurements and for controlling correct atrazine dosing to avoid the misuse of the pesticide.

INTRODUCTION

The intense use of pesticides in agriculture and along roads, railway tracks and in public areas has led to an increasing awareness of the risks of contamination of the environment by xenobiotics. Monitoring programmes in Switzerland indicate that several groundwater and drinking water sources contain pesticide residues [1,2]. Atrazine is among the most commonly found substances. An assessment of the origin of this compound is rather difficult, as the fate of atrazine in soil depends on complex interactions between mass flow, diffusion, hydrodynamic dispersion, routes of water and solutes in the soil, pesticide stability and sorption on soil particles and pesticide stability and sorption on to soil organic matter. Hence measurements of atrazine residues in soils will help to achieve a better understanding of the fate of this compound and to detect possible sources of groundwater contamination. Until now mon-

0021-9673/91/$03.50 © 1991 Elsevier Science Publishers B.V.

itoring progammes have only been based on measurements of residues in groundwater.

Numerous analytical procedures for determining herbicide residues have been described. For the analysis of triazine herbicides the conventional method by gas chromatography (GC) with nitrogen–phosphorus detection [3–6], liquid chromatography [3,7–9] and GC–mass spectrometry (MS) [10–15] have been applied successfully. Recently enzyme-linked immunosorbent assay has also been shown to be a useful method for the determination of triazine herbicide residues [16]. Residues of herbicides in soil samples have been measured [17–19]. The persistence of these substances in soil with respect to possible crop rotation problems [20–23] has been investigated.

This paper describes a high-performance liquid chromatography (HPLC) method for the determination of atrazine residues and metabolites in soil, which may be used for routine monitoring programmes. The sampling and analytical reproducibilities for atrazine have been determined.

MATERIALS AND METHODS

Collection of soil samples

Soil samples were collected at two different sites, Wasterkingen and Reckenholz, from plots treated with five different doses of atrazine (Table I). Each treatment included four plots (repetitions) of either 80 or 10 m^2. The time between the last atrazine application and the sampling data ranged from 109 days to over 3 years, and the doses were between 0 and 1.50 kg of atrazine per ha per year (Table I). From each plot (repetition) 20 subsamples were collected from a depth of 20 cm with a soil sampler (diameter 5 cm) and these were subsequently mixed by hand. The soil samples were stored immediately in a cold room at 2–4°C and then frozen at −20°C until analysis.

Reagents

All solvents were of analytical-reagent grade, with the exception of solvents for

TABLE I

ATRAZINE RESIDUES IN SOIL SAMPLES FROM A CORN FIELD EXPOSED TO DIFFERENT ATRAZINE APPLICATIONS AS DETERMINED BY HPLC

Sampling reproducibility based on four samples per treatment.

Treatment	Location	Atrazine application rates (kg active ingredient/ha/year)		Last atrazine application (days before sampling)	Mean atrazine residue (ppb)	Standard deviation (ppb)
		1984–1986	1987			
A	Wasterkingen	0.625	1.25	161	20.5	5.8
B	Wasterkingen	0.625	0	>360	8.0	5.9
C	Reckenholz	0	1.00	109	21.5	9.5
D	Reckenholz	0	1.50	136	10.4	4.1
E	Reckenholz	0	0	>1140	>2.0	–[a]

[a] Below limit of determination of 2 ppb.

HPLC (HPLC grade). Aluminium oxide (basic, W200, Woelm No. 04571) was dried for 5 h at 650°C. Water (19 ml per 100 g) was added, mixed and equilibrated at room temperature overnight. Sep-Pack C_{18} cartridges (Water Assoc., Milford, MA, USA) were washed with 10 ml of methanol and 10 ml of water before use. No plastic containers were used to avoid contamination of the samples with phthalates.

Isolation of atrazine from soil

The frozen soil samples (about 500 g each) were stored overnight at room temperature on aluminium foil washed with acetone and hexane. Large pieces were disintegrated mechanically with a shovel. The soil sample was pulverized by repeated division and recombination. From each sample 100 g were dried at 105°C overnight for the determination of dry matter. Another 50-g aliquot of the homegenized soil was suspended in 100 ml of methanol and treated for 10 min in an ultrasonic bath. This procedure was repeated once more, and the combined methanol extract was diluted with 250 ml of water and 50 ml of saturated sodium chloride solution and then extracted three times with 50 ml of methylene chloride. The lower phase was separated, dried with sodium sulphate and filtered. The combined methylene chloride extracts were evaporated at 40°C and the residue immediately redissolved with 5 ml of toluene.

The solution was applied to a chromatography column (22 × 1.8 cm) containing 20 g of aluminium oxide and 2 g of a top layer of anhydrous sodium sulphate. This purification step was necessary for the removal of interfering soil components, especially in the case of soil samples with high humus contents. The column was rinsed with 100 ml of hexane, which was then discarded. The atrazine was eluted with 100 ml of a mixture of hexane–diethyl ether (2:1, v/v), the eluate was evaporated to dryness at 35°C and the residue was immediately redissolved in 5 ml of distilled water and treated in an ultrasonic bath. The aqueous solution was passed through an acrodisc filter and a Sep-Pak C_{18} cartridge, both attached to a glass syringe. Atrazine was eluted with 0.5 ml of methanol and 2 ml of methanol–methylene chloride (3:7, v/v). The combined eluates were evaporated to dryness at 45°C, redissolved in 5 ml of acetonitrile–water (35:65, v/v), treated in an ultrasonic bath and filtered through an acrodisc filter (1.2 μm) into a vial of the HPLC autosampler. This extract was stored at 4°C until determination by HPLC.

Isolation of deethylatrazine and deisopropylatrazine

Another 50 g of the homogenized soil sample was suspended in 100 ml of methanol and treated for 10 min in an ultrasonic bath. The suspension was filtered through a paper filter containing a spoonfull of anhydrous sodium sulphate. The sample was extracted a second time with 100 ml of fresh methanol, as described in the preceding section. The filter was then rinsed with 20 ml of methanol. The combined methanol extracts were evaporated to dryness at a temperature not exceeding 45°C. The residue was immediately dissolved in 5 ml of toluene using an ultrasonic bath for a few seconds. The solution was applied to a chromatography column (22 × 1.8 cm) containing 20 g of aluminium oxide. The column was rinsed in portions with a total of 100 ml of hexane, which was then discarded. The two metabolites were eluted with 100 ml of a mixture of diethyl ether–methanol (2:1, v/v). The eluate was evaporated to dryness at 45°C; it was then immediately redissolved in 5 ml of acetonitrile–water

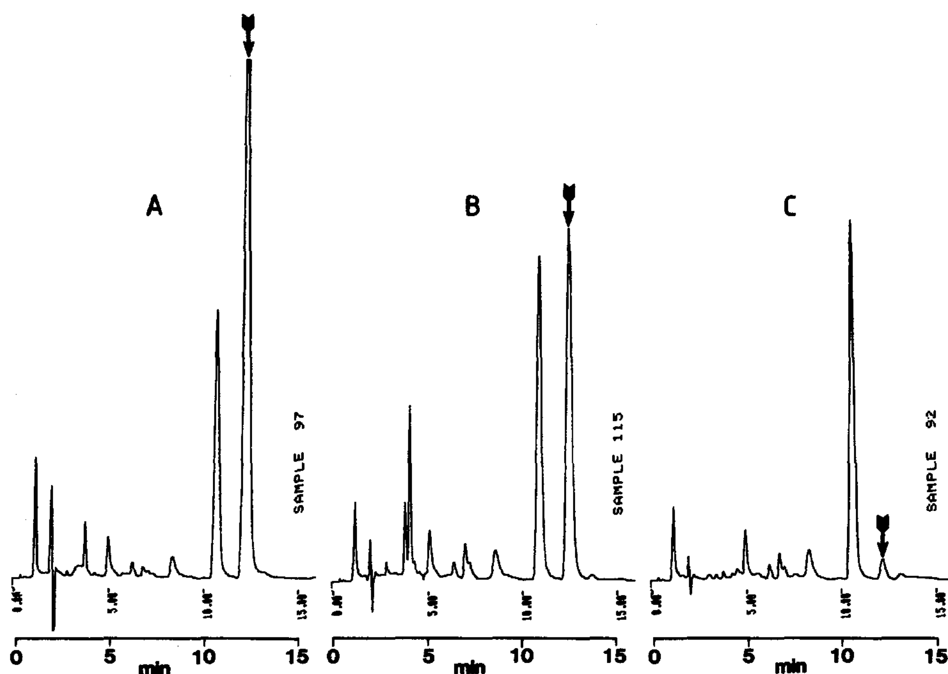

Fig. 1. High-performance liquid chromatogram of atrazine in soil from a corn field. Samples A, B and C correspond to different atrazine concentrations (146, 80 and 5 ppb, respectively). The atrazine peak elutes after 12 min, as indicated by the arrows.

(20:80, v/v) and filtered through an acrodisc filter [chemical resistance (CR), 1.45 μm] into a vial of the HPLC autosampler.

High-performance liquid chromatography

A LiChrosorb RP-18 column (250 × 4 mm, particle size 5 μm; E. Merck, Darmstadt, Germany) was used together with a LiChrosorb RP-18 pre-column (5 μm particle size). The mobile phases were acetonitrile–water (35:65, v/v) for atrazine and acetonitrile–water (20:80, v/v) for the metabolites at a flow-rate of 1.2 ml/min and a pressure of approximately 13 MPa. The injection volume was 20 μl and the solution was pumped with a Waters Model 510 pump. Detection was achieved with a Spar-Holland SPH 125 autosampler (Emmen, Netherlands) and LDC-Milton Roy UV detector (Riviera Beach, FL, USA) at 222 nm, with integration by a Waters 740 delta module integrator. Residues were calculated as ppb[a] wet soil.

RESULTS AND DISCUSSION

Fig. 1 shows three HPLC chromatograms of soil samples from corn fields

[a] Throughout this article, the American billion (10^9) is meant.

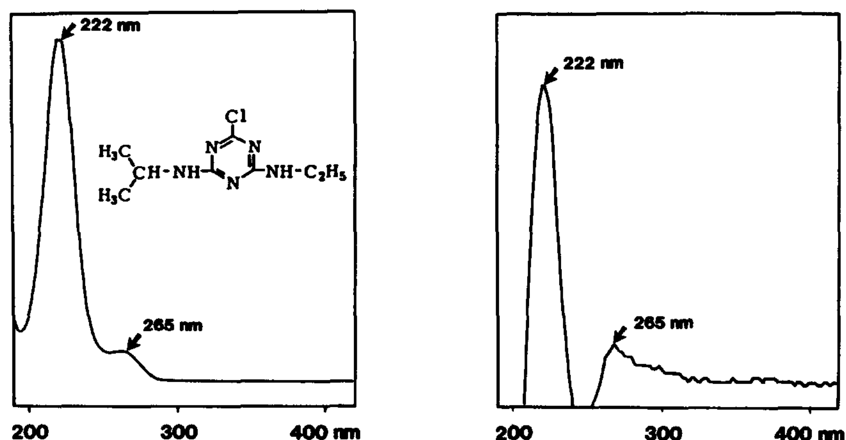

Fig. 2. UV spectra (HPLC–diode array) of atrazine from a standard solution (left) and from an extract of a soil sample (right). Both spectra show the same absorption maxima (222 nm) and shoulders (265 mn).

TABLE II

ANALYTICAL REPRODUCIBILITY (INTRA-DAY) OF ATRAZINE, DEETHYLATRAZINE AND DEISOPROPYLATRAZINE DETERMINATION BY HPLC

Compound	Sample number	Number of determinations (n)	Mean (ppb)	Standard deviation (ppb)	Coefficient of variation (%)
Atrazine	1	6	18.12	1.21	6.66
	2	6	9.48	1.18	12.45
	3	6	5.37	0.55	10.18
Deethylatrazine	4	6	7.37	0.72	9.77
Deisopropylatrazine	5	6	6.75	0.31	4.59

TABLE III

MEAN RECOVERY (STANDARD DEVIATION) OF ATRAZINE DEETHYLATRAZINE AND DEISOPROPYLATRAZINE FROM SPIKED SOIL SAMPLES AS DETERMINED BY HPLC (n = 6)

Compound	Amount added (ppb)	Amount recovered (ppb)	Percentage recovered
Atrazine	10	6.53 ± 0.12	65.3
	100	61.00 ± 3.06	61.0
Deethylatrazine	10	7.37 ± 0.72	73.7
	40	33.17 ± 5.23	82.9
Deisopropylatrazine	10	6.75 ± 0.31	67.5
	40	33.33 ± 5.13	83.3

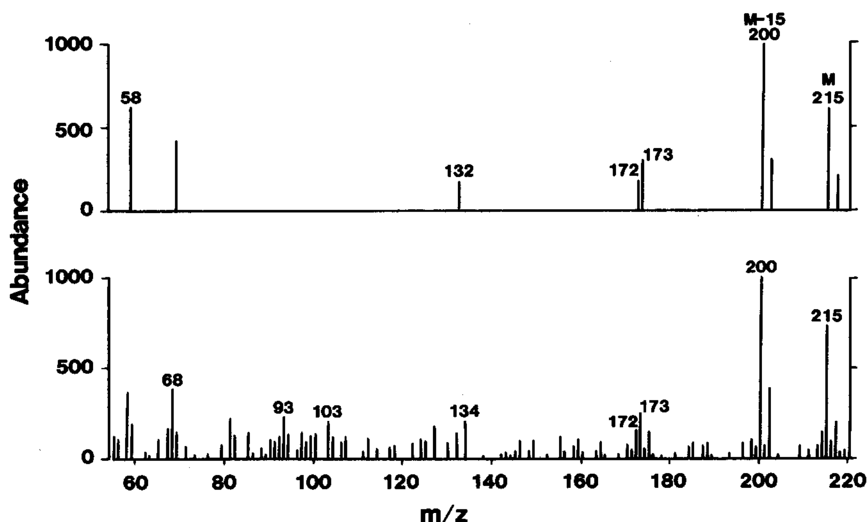

Fig. 3. Mass spectra of atrazine (capillary GC–EI-MS) from a standard solution (top) and from an extract of a soil sample (bottom). A Hewlett-Packard GC–MS system HP-5790A/HP-5970A was used equipped with a 30 m × 0.25 mm I.D. fused-silica capillary column DB-1 (methyl silicone) at 60°C, programmed at 20°C/min to 260°C column temperature.

containing atrazine. Baseline separation was achieved within 15 min. Phthalates may appear as additional peaks, therefore plastic containers should not be used during the extraction procedures. The identity of the peak with a 12 min retention time was confirmed by diode-array UV spectroscopy (Fig. 2).

The variability of atrazine determination in soil is dependent on the analytical method and the heterogeneity of atrazine distribution within the corn field. The intra-day reproducibility of the assay in soil samples with low atrazine concentrations ranged from 6.7 to 12.5% (Table II). The recoveries were 61–65% for atrazine, 74–

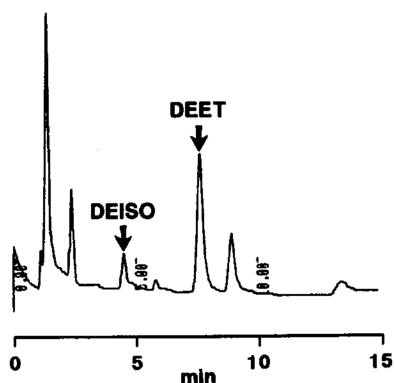

Fig. 4. High-performance liquid chromatogram of atrazine metabolites in soil from a corn field. Deisopropylatrazine (DEISO) elutes after 4.9 min (soil concentration 3 ppb); deethylatrazine (DEET) elutes after 8.5 min (12 ppb).

83% for deethylatrazine and 68–83% for deisopropylatrazine (Table III). Every fifth sample of the assays was a spiked soil sample to check the recovery. The limits of detection were 0.5 ppb for atrazine, 0.5 ppb for deethylatrazine and 0.5 ppb for deisopropylatrazine (signal-to-noise ratio 4:1).

The drying of soil samples before extraction in a heated place should be avoided. Drying of a soil sample for 15 h at 60°C reduces the atrazine content by 24% (n = 6). The HPLC method was validated by capillary GC–electron impact (EI)–MS. The mass spectra from a standard solution and from an extract of a soil sample were comparable (Fig. 3), yielding a molecular ion of m/z 215 and a base peak of m/z 200 (M − 15).
Atrazine metabolites are biologically active and it is therefore important to be able to monitor their concentrations in soil. Fig. 4 shows a chromatogram of the two primary metabolites of atrazine in a soil from a corn field.

Table I gives the sum of sampling and analytical reproducibility for atrazine and shows the atrazine content after different treatments. Four samples per treatment were taken. It is clear that the coefficient of variation is higher (compared to Table II) as a result of the variation of atrazine concentrations in the four soil samples taken within the corn field. However, the soil samples in Table I were taken in November and have low atrazine concentrations of up to 100 ppb (normal dose), or up to 500 ppb (overdose).

This method was developed for controlling atrazine dosing to avoid the misuse of the pesticide. It can be adapted for other similar compounds.

ACKNOWLEDGEMENTS

The authors are grateful to Mr. H. J. Laska and to Mr. A. Litscher for skilfull technical assistance and to Mrs. T. J. Lindt for help with the preparation of the manuscript.

REFERENCES

1 H. R. Kühni *Bericht über das SGCI-Untersuchungsprogramm (Febr. 1987–Febr. 1988), Pflanzenbehandlungsmittel im Grundwasser*, Fachgruppe Agrar der SGCI, Basle (1988) 41 pp.
2 U. Müller *Jahresbericht 1987*, Kantonales Laboratorium, Berne 1988, pp. 123–129.
3 M. Popl, Z. Voznakova, V. Tatar and J. Strnadova, *J. Chromatogr. Sci.*, 21 (1983) 39–42.
4 T. G. Steinheimer and M. G. Brooks, *Int. J. Environ. Anal. Chem.*, 17 (1984) 97–111.
5 T. G. Kreindl, H. Malissa and K. Winsauer, *Mikrochim. Acta*, 1 (1986) 1–13.
6 P. C. Bardalaye and W. B. Wheeler, *Int. J. Environ. Anal. Chem.*, 25 (1986) 105–113.
7 E. Smolkova and V. Pacakova, *Chromatographia*, 11 (1978) 698.
8 A. Di Corcia, M. Marchetti and R. Samperi, *J. Chromatogr.*, 405 (1983) 357–363.
9 I. G. Ferris and B. M. Haigh, *J. Chromatogr. Sci.*, 25 (1987) 170–173.
10 B. A. Karlhuber, W. D. Hörmann and K. A. Ramsteiner, *Anal. Chem.*, 47 (1975) 2450–2452.
11 F. Mangani and F. Bruner, *Chromatographia*, 17 (1983) 337–380.
12 V. Lopez-Avila, P. Hirata, S. Kraska, M. Flanagan and J. H. Taylor, Jr., *Anal. Chem.*, 57 (1985) 2797–2801.
13 E. Davoli, E. Benefati, R. Bagnati and R. Fanelli, *Chemospere*, 16 (1987) 1425–1430.
14 W. E. Pereira, C. E. Rostad and T. J. Leiker, *Anal. Chim. Acta*, 228 (1989) 69–75.
15 L. Q. Huang, *J. Assoc. Off. Anal. Chem.*, 72 (1989) 349–354.
16 E. M. Thurman, M. Meyer, M. Pomes, Ch. A. Perry and P. Schwab, *Anal. Chem.*, 62 (1990) 2043–2048.
17 K. Ramsteiner, W. D. Hörmann and D. O. Eberle, *J. Assoc. Off. Anal. Chem.*, (1974) 192–201.

18 T. H. Byast, E. G. Cotterill and R. J. Hance, *Technical Report*, Vol. 15, Agricultural Research Council Weed Research Organization, Oxford, 2nd ed., 1977.
19 W. D. Hörmann, *Rückstandsanalytik von Pflanzenschutzmitteln, Mitteilung VI der Senatskommission für Pflanzenschutz-, Pflanzenbehandlungs- und Vorratsschutzmittel, Methodensammlung der Arbeitsgruppe "Analytik", 1. Lieferung*, VCH Verlagsgesellschaft, Weinheim, 1985, Nr. 6-A, pp. 1–7; Nr. 6-B, pp. 1–7.
20 L. Stalder and W. Pestemer, *Weed Res.*, 20 (1980) 341.
21 W. Pestemer, L. Stalder and B. Eckert, *Weed Res.*, 20 (1980) 349.
22 W. Pestemer, V. Radulescu, A. Walker and L. Ghinea, *Weed Res.*, 24 (1984) 359.
23 W. Pestemer, *Mitt. Schweiz. Landwirtschaft*, 1/2 (1988) 2.