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Trace determination of weathered atrazine and terbuthylazine and their degradation products in soil by high-performance liquid chromatography–diode-array detection

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ABSTRACT

It is important to optimize extraction, clean-up and determination of weathered pesticide residues in order to predict their fate in the unsaturated soil zone. A method including hot extraction with acetone, clean-up with solid-phase extraction (cation exchanger) and HPLC–diode-array detection for atrazine and terbuthylazine and their chlorinated and hydroxylated metabolites at relatively low levels, less than 5 μg per kg of soil, is presented.

INTRODUCTION

Application of the pesticide atrazine (2-chloro-4-ethylamino - 6 - isopropylamino - 1,3,5-triazine) can cause pollution of drinking water by the compound in its unmetabolized form or its breakdown product, exceeding the EEC limit of 0.1 μg/l per substance and resulting eventually in an undesirable accumulation of phytotoxic residues in soil. The same potentially applies to terbuthylazine (2-chloro-4-ethylamino-6-butylamino-1,3,5-triazine), an atrazine substitute. It is important to optimize the extraction and clean-up of weathered pesticide residues in order to predict their fate in the environment, especially if one seeks to explain their movement in the unsaturated soil zone. A review of solvent extraction systems for weathered herbicide residues has been reported [1]. The soil extracts are usually subjected to a liquid–liquid partition step before they are cleaned up further by gel permeation chromatography (GPC). The liquid–liquid partition step often results in the formation of emulsions, which render phase separation difficult and cause losses of substance. Frequently, components of soil extracts interfere with compound identification. The application of solid-phase extraction (SPE) offers advantages such as reduced solvent consumption and fewer interferences compared with clean-up with GPC [2].

Gas chromatography (GC) is a common method for the determination of atrazine and its non-polar degradation products with high sensitivity and good separation efficency. A disadvantage of GC is that it is limited to volatile chlorotriazines. The hydroxy derivatives cannot

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be analysed without derivatization. However, in order to determine total atrazine residues in soil, the polar degradation products have to be included. High-performance liquid chromatography (HPLC) is directly applicable to *s*-triazines and their degradation products.

This paper will describe a method for the extraction, clean-up and determination of atrazine and terbuthylazine and their chlorinated and hydroxylated metabolites at relatively low levels, less than 5 μg per kg of soil.

EXPERIMENTAL

Soil sampling

Aiming for representative soil samples, 25 single cores per sampling site were mixed. Characteristic soil parameters of the sampled site were 19% clay, 1.2% organic carbon and pH 6.0. In order to obtain weathered residues, sampling was conducted 1 year after spraying. A hydraulically driven, tractor-mounted drilling device (Fritzmeyer, Grosshelfendorf, Germany) was used for time-efficient and easy sampling down to a depth of 90 cm.

Sample preparation

The complete samples, each around 4 kg of soil, were sieved in the field with a 1-cm screen and transported to the laboratory in aluminium trays. The samples were air dried, homogenized with a mixer and ground with a rotor-disc mill.

Reagents

Atrazine, terbuthylazine and their metabolites were obtained from Ehrenstorfer (Augsburg, Germany). All organic solvents used were of HPLC grade (Labscan, Dublin, Ireland) and used as received.

Extraction

Subsamples of 50 g were extracted with 250 ml of acetone, methanol and a mixture of methanol–water (8:2) in a hot extractor for 2 h, then the volume of the soil extracts was reduced with a Turbovap evaporation workstation (Zymark, Hopkinton, MA, USA) at 40°C water bath temperature. The evaporation was stopped at a volume of around 2 ml.

Clean-up with solid-phase extraction (SPE)

A 0.1-ml aliquot of 10 μg/ml propazine was used as internal standard and acetone was added to a final volume of 5 ml. The clean-up was performed by a sulphonic acid-type silica gel-based cation exchanger (SCX) from Supelco (Bellefonte, PA, USA). The solutions were passed through the 3-ml tubes by using a vacuum manifold (Analytichem, Harbor City, CA, USA). Four samples were cleaned up simultaneously. Before use, the mini-columns were activated with 4 ml of 0.12 *M* hydrochloric acid in methanol at a flow-rate of 0.8 ml/min, followed by 3 ml of methanol and 3 ml of acetone. A 3-ml volume of soil extract was added. Unwanted, weakly retained material was removed by washing the packing with 2 ml of acetone. Atrazine, terbuthylazine and metabolites were eluted with 2.5 ml of potassium chloride-saturated methanol. A 200-μl aliquot of methanolic solution of ammonia (0.1 mol/l) was added to this solution to neutralize the acidity. Methanol was removed with a vacuum rotary evaporator (Heidolph, Kelheim, Germany) at 40°C and the residue was reconstituted with 1 ml of mobile phase acetonitrile–water (1:9) for RP-HPLC.

Clean-up with gel permeation chromatography (GPC)

The soil extract was evaporated to 0.1 ml and ethyl acetate–cyclohexane (1:1) was added to a final volume of 5 ml. Eluent delivery (ethyl acetate–cyclohexane, 1:1) at 4 ml/min was provided by a Model FR-30 high-pressure pump (Knauer, Homburg, Germany). Sample volumes of 3 ml were injected via a 5-ml sample loop of a Rheodyne valve (Cotati, CA, USA). A glass column (450 mm × 30 mm), packed with Bio-Beads SX-3, mesh size 200–400 (Bio-Rad Labs., Richmond, CA, USA), was used.

HPLC apparatus

A Gynkotek (Germering, Germany) M 480 gradient pump, fitted with a Gilson-Abimed (Langenfeld, Germany) Model 231 sample injector, injection volume 100 μl, and a Hewlett-Packard (Palo Alto, CA, USA) diode-array detector, 1040 M 50 Series II, detection wavelength 220 nm, was employed. A Hypersil ODS

column (250 × 4 mm I.D., 5 μm) together with a precolumn from Grom (Herrenberg, Germany) was used. The gradient programme was from 10 to 60% acetonitrile in 50 min; water was buffered with ammoniuum acetate at pH 6.8 and column temperature was kept at 40°C with a column oven (Gynkotek).

RESULTS AND DISCUSSION

Extraction

The search for a standard extraction method for weathered pesticide residues in soil has been continuous. There is growing evidence that time-dependent non-equilibrium sorption processes can render some analytes more resistant to extraction [3,4]. Therefore recovery values from weathered field samples could be lower than those predicted on the basis of a freshly added spike.

Numerous authors [1,4–6] have compared the efficiency and simplicity of various methods, which are typically shaking, refluxing or sonicating at various temperatures with methanol, acetonitrile, dichlormethane or Soxhlet extractions for 2–24 h. Cotterill [5] reported that shaking with methanol with a water content of 20% is more efficient for the extraction of simazine than other solvent systems based on acetonitrile or chloroform. Mattson *et al.* [4] obtained similar recoveries of triazine herbicides from soil either by using an aqueous methanol (10% water) Soxhlet extraction for 24 h or by refluxing with aqueous acetonitrile (10% water) for 1 h. He concluded that water as one of the components of the extracting system appears to be essential. Huang and Pignatello [6] obtained maximum yields of weathered atrazine soil samples by batch extraction at 75°C for 2–16 h with methanol–water (8:2).

Experiments in our laboratory showed, contrary to the results described above, that hot extraction with methanol–water (8:2) provides the lowest efficiency and the highest coefficient of variation, whereas acetone or methanol yields higher and more reproducible values. With the exception of soil II and the comparison of acetone and methanol, the *t*-test indicated statistically significant differences between the three extraction solvents (Table I). When acetone is used as extraction and clean-up solvent, there is no need to evaporate the soil extract to dryness and therefore one means of losing the analyte is obviated.

Clean-up

We aimed to find a clean-up procedure that results in high recovery values not only for the chlorotriazines but also for the hydroxy derivatives, which are supposed to be important metabolites in soil.

We applied GPC to soil extracts and standards

TABLE I

EFFICIENCY OF HOT EXTRACTION WITH ACETONE, METHANOL AND METHANOL–WATER (8:2)

Values are atrazine concentration (μg/kg) extracted with the indicated extraction solvent and temperature. A = Acetone; M = methanol; M/W = methanol–water.

	Soil I			Soil II			Soil III		
	A, 55°C	M, 63°C	M/W, 66°C	A, 55°C	M, 63°C	M/W, 66°C	A, 55°C	M, 63°C	M/W, 66°C
Extraction 1	12	7	3	68	55	41	148	143	124
Extraction 2	12	5	4	61	64	48	148	147	98
Extraction 3	12	5	4	63	61	28	149	145	138
Mean	12	6	4	64	60	40	148	145	120
S.D.	0.3	1.1	0.6	3.1	3.9	8.6	1.0	3.2	17.1
R.S.D. (%)	3	19	16	5	7	22	1	2	14.3

containing triazines, but this resulted in a complete loss of hydroxy derivatives. We attributed this loss to solubility problems of the hydroxy derivatives in the mobile phase (cyclohexane–ethyl acetate) and/or to a low elution strength of the mobile phase.

Gordon [7] stated that, under strictly anhydrous conditions, very weakly basic compounds are adsorbed on a strong acid exchanger via salt formation. Battista *et al.* [8] reported the successful use of SPE for the isolation of eight triazines from soil extracts. We aimed to use the same type of column, but for the parent compounds and their degradation products. Our experiments confirmed that only 1% water in acetone resulted in a considerable loss of triazines (Table II). Recovery experiments with standard solutions and the use of the internal standard propazine assure the necessary quality of acetone. The analytical recovery of the complete method (extraction with acetone and SPE) was assessed by extracting freshly spiked soil samples (Table III); the hydroxy derivatives in particular showed high recovery values.

Detection

Because of their wide k' range, triazine-containing samples cannot be easily handled by isocratic HPLC methods. Early-eluting peaks are poorly resolved, later peaks are very wide and exhibit tailing and the separation time is excessive. The k' values and therefore the separation of the *s*-triazines depend on the organic solvent content and pH and ionic strength of the mobile phase [9]. Acetonitrile–water was used instead of a methanol–water mobile phase because acetonitrile has advantages over methanol: lower operating pressure and applicability for detection in the very low UV range [10]. A major problem is the separation of desethylatrazine and hydroxyatrazine. *s*-Triazines are weak bases whose pK_a values range from 1.6 for chlorotriazines to about 5 for hydroxy derivatives [9,11]. The chlorotriazines are not protonated at pH values between 3 and 7, and thus their retention time is pH independent. The retention times of hydroxy derivatives increase with increasing pH, the change being largest within the pK_a region. In acidic mobile phases the peaks exhibit tailing, owing to *s*-triazine protonation [9]. A water phase containing 2 mmol/l ammonium acetate, pH 6.8, was selected as optimal as the peak separation is satisfying and does not vary over time. Furthermore, the use of a neutral mobile phase improves the peak symmetry. Vermeulen *et al.* [11] used a neutral but more concentrated (50 mmol/l) ammonium acetate mobile phase.

Fig. 1A shows a triazine standard of 30 μg/l, which corresponds to 1 μg/kg in soil. Usually the peak identification is done by comparing the absolute or relative retention times of the peaks in the sample with those in a standard solution. The diode-array detector records a predetermined spectral range during the analysis, and facilitates positive peak identification and peak

TABLE II

PERCENTAGE LOSS OF A 1 mg/l STANDARD OF DESETHYLATRAZINE (DEA), ATRAZINE (ATR) AND TERBUTHYLAZINE (TERB) DURING SPE, DEPENDING ON THE WATER CONTENT OF ACETONE

The values give the range of results of triplicate determinations at each level of water content.

Water content (%)	DEA (%)	ATR (%)	TERB (%)
<0.03	0	0	0
0.5	0	0–1	0–1
1	0–9	2–12	4–12
2	37–60	47–67	56–72
3	58–79	65–83	68–85

TABLE III

MEAN RECOVERIES OF TRIAZINES IN SPIKED (30 μg/kg) SOIL SAMPLES

Triazine	Recovery (%)[a]	R.S.D. (%)
Atrazine	86	4
Hydoxyatrazine	92	4
Desethylatrazine	87	5
Desisopropylatrazine	78	7
Terbuthylazine	88	4
Hydroxyterbuthylazine	90	4
Desethylterbuthylazine	85	6

[a] Mean values obtained from triplicate determinations.

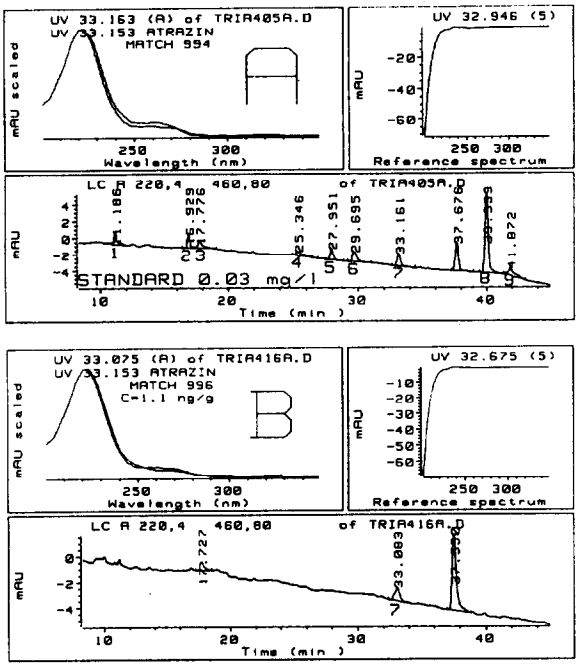

Fig. 1. HPLC chromatograms of (A) a triazine standard corresponding to 30 μg/l, (B) a soil sample with an atrazine concentration of 1.1 μg/kg. 1 = Desisopropylatrazine; 2 = desethylatrazine; 3 = hydroxyatrazine; 4 = hydroxyterbuthylazine; 5 = desethylterbuthylazine; 6 = atratone; 7 = atrazine; 8 = propazine; 9 = terbuthylazine.

purity analysis. The spectra obtained are compared with those stored in a library. The chromatogram of a soil extract after clean-up with an SCX column is shown in Fig. 1B. The atrazine peak corresponds to 1.1 μg per kg of soil.

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