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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR DETERMINING TRIAZINE HERBICIDE RESIDUES IN SOIL

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SUMMARY

The extraction of eight triazines from soil and their isolation from the soil extract were simultaneously performed using two mini-columns connected in series, one containing the soil sample and the other filled with a strong acid exchanger. By allowing 3 ml of acetone to flow along the two cartridges in tandem, triazines were removed from the soil particles and then selectively readsorbed via salt formation on the exchanger surface. After disconnecting the two cartridges, the triazines were eluted from the exchanger trap by potassium chloride-saturated methanol. This procedure was also adopted to desorb from the soil surface residual amounts of chemically adsorbed triazines, which acetone is unable to remove. After removal of methanol from the two combined eluates, the triazines were fractionated and quantified by reversed-phase high-performance liquid chromatography with UV detection at 220 nm. Using this procedure, the recoveries of triazines were greater than 90% and independent of the triazine concentration, the type of soil samples and their ageing on storage. The mean limit of detection was about 1 ng/g in soil. The results obtained were compared with those obtained by two techniques of known efficiency (reflux extraction and Soxhlet extraction). Some effects on the adsorption of triazines of the type of soil and its ageing on storage are discussed.

INTRODUCTION

Since their introduction around 1960, triazine derivatives have become widely used in agriculture as selective herbicides and, consequently, they can give rise to residues in soil. Methods for the determination of triazine herbicides in soil are important from both agricultural[1,2] and environmental[3,4] points of view. A wide variety of solvents have been used for the extraction of triazines from soil[5–8], including agitation of the soil and a solvent for various periods of time at various temperatures, sonication and Soxhlet extraction. Clean-up procedures are generally needed for most soil types in order to attain a satisfactory limit of detection, such as 20 ng/g. However, these procedures are invariably tedious, time consuming, not highly selective and require excessive manipulation of the sample.

Triazine derivatives are very weakly basic compounds with pK_b values ranging

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between 10 and 12, depending on the nature of the substituent. It has been shown[9] that, under strictly anydrous conditions, very weakly basic compounds can be adsorbed on a strong acid exchanger via salt formation.

This paper describes an original, simple and rapid procedure for accurately determining trace amounts of eight triazines in soil samples.

The extraction and isolation of triazines were simultaneously performed by connecting two mini-columns in tandem, the upper column being packed with soil and the lower column with a sulphonic acid-type silica-based cation exchanger (SCX). Solvent percolation through a soil column is a technique already performed with success by Mangani *et al.*[10] for the extraction of chlorinated pesticides from soil. Subfractionation and quantitation of triazines were performed by high-performance liquid chromatography (HPLC) with UV detection at 220 nm.

EXPERIMENTAL

Reagents

Authentic triazine derivatives were obtained from Supelco (Bellefonte, PA, U.S.A.) as follows: 2-chloro-4,6-bis-ethylamino-*s*-triazine (simazine), 2,4-bis-ethylamino-6-methylthio-*s*-triazine (simetryn), 2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine (atrazine), 2,4-bis-isopropylamino-6-methoxy-*s*-triazine (prometon), 2-isopropylamino-4-ethylamino-6-methylthio-*s*-triazine (ametryn), 2-chloro-4,6-bis-isopropylamino-*s*-triazine (propazine), 2,4-bis-isopropylamino-6-methylthio-*s*-triazine (prometryn) and 2-ethylamino-4-*tert*.-butylamino-6-methylthio-*s*-triazine (terbutryn). The pK_a values of chlorotriazines are *ca.* 2 and those of the other triazines considered are *ca.* 4[11]. A standard solution was prepared by dissolving 100 mg/l of each herbicide in acetone. This solution was further diluted to obtain a working standard solution of 10 mg/l.

For HPLC, distilled water was further purified by passing it through a Norganic cartridge (Millipore, Bedford, MA, U.S.A.). Acetonitrile was of HPLC grade from Carlo Erba (Milan, Italy). All other solvents were of analytical-reagent grade (Carlo Erba) and were used as supplied.

Sample preparation

Soil samples were taken from three different sites located in Italy. The soils were chosen on the basis of differences in physical characteristics (Table I). Moisture was eliminated by heating at 80°C. Soil was ground and sieved to obtain a particle size range between 120 and 270 mesh. Herbicide-fortified soil samples were prepared in a manner similar to that reported elsewhere[10,12]. To a known amount of soil in a flat dish, an appropriate volume of the working, standard solution containing herbicides was added. Additional acetone was added until the solvent completely covered the soil particles. The bulk of the solvent was slowly evaporated at room temperature so that the soil particles became a slush-like mass, which was then stirred thoroughly with a spatula until the material appeared dry. Soil was resieved to maintain the required mesh range and stored in glass containers at room temperature until analysed.

TABLE I

PHYSICAL AND CHEMICAL CHARACTERISTICS OF THE SOILS USED IN THE RECOVERY EXPERIMENTS

Soil No.	*Clay (%)*	*Silt (%)*	*Sand (%)*	*pH*	*Organic matter (%)*
1	26	60	14	8.1	2.1
2	39	38	24	5.9	1.9
3	12	2.1	86	7.5	4.2

Apparatus

Percolation of solvents through the cartridges was carried out using a vacuum manifold with a water pump. Both soil and the cation exchanger were packed in cylindrical polypropylene tubes (6 cm × 0.9 cm I.D.) (Supelco). They were one quarter filled with 1 g of soil, taking care to vibrate the tube in order to avoid loose packing of soil particles. Polyethylene frits (Supelco) were located above and below the soil column to hold minute particles in place and keep the column intact.

The SCX cartridge was prepared in the same way with 1 g of the exchanger material having a particle size range between 200 and 400 mesh (Supelco). Before use, this material was washed with 4 ml of 0.12 mol/l hydrochloric acid in methanol at a flow-rate of about 1 ml/min, followed by 3 ml of methanol and 3 ml of acetone. Washing of the cartridge was stopped when the level of acetone was about 2 cm higher than the SCX bed.

The two cartridges, one containing soil and the other the exchanger material, were then connected in series through an adapter (Supelco) and inserted in the vacuum system. Acetone (3 ml) was allowed to percolate through the soil bed at a flow-rate of 0.8 ml/min to desorb the triazine herbicides, which were readsorbed on the SCX column as the soil extract passed along the second tube. After this, the two cartridges were disconnected and the residual amount of triazine herbicides was extracted from the soil column by passing through it 3 ml of potassium chloride-saturated methanol, which were collected separately. After washing the SCX cartridge with 2 ml of acetone, the herbicides were eluted with 2.5 ml of potassium chloride-saturated methanol at a flow-rate of 0.8 ml/min into the same glass tube containing the methanolic soil extract. Before removal of the solvent, 200 μl of a methanolic solution of ammonia (0.1 mol/l) were added to this solution to neutralize the acidity, which causes some decomposition of chlorotriazines. Methanol was removed at 32°C under a stream of nitrogen and the residue was reconstituted with 100 μl of the mobile phase for HPLC. A 50-μl volume of this solution was injected into the HPLC apparatus.

HPLC apparatus

A Model 5000 liquid chromatograph (Varian, Walnut Creek, CA, U.S.A.) equipped with a Rheodyne Model 7125 injector having a 50-μl loop and with a Model 2050 UV detector (Varian) was used. A 25 cm × 4.6 mm I.D. column filled with 5-μm (average particle size) LC-18-DB reversed-phase packing and a guard column containing Pelliguard (both from Supelco) were used. The mobile phase was acetonitrile–phosphate buffer (10 mmol/l; pH 6.7) (38:62, v/v) and the flow-rate was 1.5 ml/min. Triazines were monitored with the detector set at 220 nm.

The concentrations of the herbicides in soil samples were calculated by comparing the heights of the peaks obtained with the sample and with a standard solution. The latter was prepared by adding an appropriate volume of the herbicide working standard solution to 5 ml of potassium-chloride-saturated methanol, by eliminating the solvent and dissolving the residue in 100 μl of the HPLC mobile phase.

RESULTS AND DISCUSSION

Recovery studies

The abilities of various organic solvents to extract triazine herbicides quantitatively from a soil column were evaluated. These experiments were performed by adding herbicides directly to the top of the soil column and allowing the solvent to be removed by vacuum. In Table II, recovery data for some selected solvents are reported.

Several mechanisms or combination of mechanisms can be postulated for the adsorption of organic basic compounds by aluminosilicates of the soil particulates. These include physical adsorption, hydrogen bonding, coordination complexes and chemical adsorption. The soil can be considered as a natural "direct phase" whose surface is contaminated to varying extents by chemical groups which may give rise to strong, anomalous adsorption depending on the nature of the adsorbate and the chemical characteristics of the soil. Therefore, it is not surprising that chloroform is unable to desorb relatively polar compounds, such as triazine derivatives, quantitatively from the soil bed. From the results obtained, acetone and methanol appeared to be of comparable value in extracting herbicides from a freshly contaminated soil surface. However, when a soil cartridge was connected in series with that containing the ion exchanger and the extracting solvent flowed down from the first cartridge directly into the second, about a 40 % loss of chlorotriazines occurred when methanol

TABLE II

RECOVERIES OF TRIAZINES USING DIFFERENT EXTRACTION SOLVENTS

Herbicide concentrations: 200 ng/g each.

Triazine	*Recovery (%)**								
	Chloroform			*Acetone*			*Methanol*		
	*I***	*II***	*III***	*I***	*II***	*III***	*I***	*II***	*III***
Simazine	30	23	3	63	27	3	66	26	2
Simetryn	28	25	2	63	28	2	64	27	2
Atrazine	30	25	2	64	26	3	63	27	2
Prometon	27	27	1	65	34	–	67	31	–
Ametryn	26	25	3	63	30	2	64	29	1
Propazine	28	24	2	63	29	1	63	30	2
Prometryn	25	23	2	63	33	1	64	31	1
Terbutryn	25	23	2	64	32	–	63	31	1

* Mean values obtained from duplicate determinations.

** Fractions I–III consisted of 1-ml aliquots of the solvent considered (chloroform, acetone or methanol).

TABLE III

RECOVERIES OF TRIAZINES FROM DIFFERENT SOIL TYPES

Herbicide concentrations: 50 ng/g each.

Triazine	*Recovery (%)**			
	Soil 1: acetone (3 ml)	*Soil 2: acetone (3 ml)*	*Soil 3***	
			Acetone (6 ml)	*Methanol (3 ml)*
Simazine	93	92	75	17 (92)***
Simetryn	93	94	73	16 (89)
Atrazine	92	93	72	19 (91)
Prometon	99	98	84	13 (97)
Ametryn	92	93	83	9 (92)
Propazine	94	93	82	11 (93)
Prometryn	97	96	86	9 (95)
Terbutryn	97	96	85	9 (94)

* Mean values obtained from triplicate measurements.
** Methanol was passed through the soil bed after passage of acetone.
*** Overall recoveries.

was used instead of acetone. Competition of methanol with very weakly basic compounds for binding on exchange sites may be responsible for this loss, as this solvent is able to form a stable hydrogen bond with strong acids.

It has been found that the recovery of atrazine can vary with the soil type[13,14]. With the view of assessing the extent to which the matrix effect could affect the extraction of the eight triazines studied, recovery studies were performed for soil samples with various physical and chemical characteristics (see Table I). Herbicide-fortified soils were prepared as described under Experimental. The results are reported in Table III. A 3-ml volume of acetone failed to elute quantitatively triazines adsorbed on sample soil 3. No further increase in the recovery of herbicides was achieved even by doubling the volume of acetone passing through the soil bed. The residual amount of triazines still remaining adsorbed on soil sample 3 could be removed by methanol.

Various hypotheses have been put forward to explain the recovery of triazines from different soil types. Hayes[15] noted that the clay content of soils could have a considerable influence on the adsorption of triazines in instances where the organic carbon contents of the soils were less than 5%. A high permanent negative charge was considered to be responsible for lower recoveries of hydroxytriazines[16]. With our samples the opposite was true, as anomalies in the adsorption of triazines were found just in the soil type having the lowest clay content and the largest amount of organic matter. Probably, the particular chemical nature of the clay mineral, more than its relative content, influences the mechanism of adsorption of triazine herbicides, as found by Knight and Tomlinson[17] for the adsorption of paraquat on soil. That methanol is able to remove the fraction of triazines remaining adsorbed after extraction with acetone can be then explained assuming that, compared with the other two soil types considered, soil sample 3 has particular and/or easily accessible adsorption sites which are able to establish strong, specific interactions, *e.g.*, hydrogen bonding, with triazines.

The extractable atrazine content of soil has been found to decrease with ageing of samples stored at room temperature[18,19]. This loss was traced to either slow degradation of atrazine or a tighter association of the atrazine with the soil components during storage, so that the extraction system failed to remove it completely from the soil.

Analogous experiments were performed on soil sample 3, which was fortified with the eight herbicides studied and stored in a screw-capped glass jar at 20°C for 3 months. Recovery data are given in Table IV. As can be seen, the extraction method with acetone followed by methanol, which effectively removed triazines from soil samples after about 1–3 days following their deposition, showed some difficulty in desorbing triazines, particularly simetryn, from a 3-months aged soil sample. The reason for this effect is not clear to us. It seems that the mode of adsorption of triazines turns slowly from physical to chemical adsorption. It may be that basic compounds adsorbed on the silicate surface, even in the absence of an effective means of movement, such as water, migrate slowly from certain adsorption sites to ion-exchange sites where they are chemisorbed via salt formation. This supposition may account for the fact that the extractable triazine content of a soil sample aged on storage increases when an effective organic cation displacer, such as potassium ion, is added to the methanol. If this mechanism of adsorption of triazines takes place even on actually contaminated soils, it follows that the extraction method developed by us is certainly more effective than other, conventional procedures proposed for measuring triazine herbicide content in agricultural samples.

Whatever the mechanism of adsorption, potassium chloride-saturated methanol was able to elute triazines from the soil column. However, using this solvent system alone obviously precluded any possibility of purifying the extract using an ion exchanger. The best compromise was to pass acetone through the soil bed first, in order to remove neutral and acidic compounds together with triazines, followed by potassium chloride-saturated methanol to elute residues of herbicides chemically bound to the soil surface. The former extract also passed through the SCX column,

TABLE IV

RECOVERIES OF TRIAZINES AFTER 3 MONTHS OF SOIL AGEING

Herbicide concentration: 50 ng/g each.

Triazine	*Recovery (%)**	
	Methanol following acetone	*KCl-saturated methanol following acetone*
Simazine	81	93
Simetryn	74	92
Atrazine	83	93
Prometon	87	97
Ametryn	84	92
Propazine	85	94
Prometryn	88	95
Terbutryn	88	95

* Mean values obtained from triplicate measurements.

which was bypassed by the latter. The potassium chloride-saturated methanol fractions from both the soil and SCX columns were then combined and submitted to the solvent removal step.

The effectiveness of the extraction procedure was compared with those obtained by following two other proposed extraction methods. The first procedure involves extraction with water–acetonitrile (10:90, v/v) by refluxing for 1 h[20] and the second procedure was Soxhlet extraction with methanol for 24 h[21]. For these experiments, soil sample 3 was selected and spiked with triazines at individual concentrations of 200 ng/g. At this high contamination level, it was unnecessary to apply clean-up steps. Recovery data are reported in Table V. It can be seen that the water–acetonitrile reflux procedure gave poorer recoveries than those obtained by our method. Moreover, the recovery of atrazine obtained by us using acetonitrile extraction was in good agreement with that obtained by Vermeulen *et al.*[13].

In comparison with data reported by Xu *et al.*[21], unexpectedly low recoveries of chlorotriazines (simazine, atrazine and propazine) were obtained by us using the 24-h Soxhlet extraction from soil sample 3. Reducing the extraction time to 3 h increased the recovery of the chlorotriazines considerably. Moreover, no significant loss of chlorotriazines occurred by Soxhlet extracting them from the other two soil types considered for 24 h. These results suggest that, on 24-h repeated treatment with hot methanol, some strongly adsorbed, unknown components present in particular soil types can be co-extracted and slowly react with chlorotriazines in hot methanol.

Precision

The analytical recovery and the precision of the method at high and low triazine contents of soil samples were assessed by extracting a spiked composite soil sample (Table VI). The recovery of triazines was unaffected by their concentrations in soil, confirming that no procedural loss occurred.

TABLE V

RECOVERIES OF TRIAZINES FROM SOIL BY THE PROPOSED METHOD COMPARED WITH TWO OTHER EXTRACTION METHODS

Herbicide concentrations: 200 ng/g each.

Triazine	*Recovery (%)**		
	19	*20*	*This method*
Simazine	77	46	92
Simetryn	74	76	92
Atrazine	75	43	91
Peometon	84	90	98
Ametryn	76	83	93
Propazine	78	47	93
Prometryn	79	77	96
Terbutryn	80	75	95

* Mean values obtained from triplicate measurements.

TABLE VI

ACCURACY AND PRECISION OF THE METHOD WITH HIGH AND LOW TRIAZINE CONTENTS IN SOIL SAMPLES

Triazine	*Recovery ± S.D.* (%)*	
	400 ng/g	*10 ng/g*
Simazine	92.3 ± 1.6	91.8 ± 3.1
Simetryn	93.2 ± 1.9	91.9 ± 5.0
Atrazine	91.5 ± 1.8	92.6 ± 3.2
Prometon	97.4 ± 1.5	98.1 ± 2.4
Ametryn	94.1 ± 1.8	94.0 ± 2.8
Propazine	92.6 ± 2.0	91.8 ± 3.4
Prometryn	95.3 ± 1.7	94.1 ± 3.9
Terbutryn	96.8 ± 1.9	94.0 ± 3.7

* Standard deviation calculated from six determinations.

Limits of detection

Under the chromatographic conditions selected, the limits of detection (signal-to noise ratio = 3) varied from 0.8 to 3 ng/g in soil, respectively, for simazine and terbutryn, which are the first and last compounds, respectively, to be eluted from the HPLC column. For the more retained herbicides, these limits could be further decreased by using gradient elution for HPLC fractionation. However, isocratic elution was used for simplicity and economy. With this method, the mobile phase could be recycled at least three times without appreciable changes in the retention times of the analytes considered or the sensitivity.

Fig. 1 shows a typical chromatogram obtained by this procedure. It must be pointed out that, except for the compounds under consideration, most of the peaks were for unknown substances released by the plastic tubes used to pack both the soil and the ion exchanger. However, none of these extraneous compounds interfered with the analysis.

Activation and re-usability of the SCX cartridge

When the acetone extract of soil was passed through the untreated exchanger bed, 20% of each chlorotriazine was unaccounted for in the acetone effluent. This effect was traced to the presence of residual water still present in soil samples after drying. Water being removed by acetone was able to compete with chlorotriazines for binding on the exchange sites chemically bonded to the silica surface. This loss of chlorotriazines was eliminated by an acidic washing of the SCX cartridge prior to its use. Probably this washing increased the retention of chlorotriazines by replacing with H^+ ions inorganic cations that are unable to establish sufficiently strong interactions with very weakly basic compounds.

The reusability of the SCX cartridge was investigated by making repeated extractions of herbicides from 1-g aliquots of soil. After each extraction, the exchanger bed was restored by passing 10 ml of 0.12 mol/l hydrochloric acid in methanol, followed by 3 ml of methanol and 3 ml of acetone, at flow-rates of about 1.5 ml/min. Only after ten such extractions was a slight loss of chlorotriazines observed.

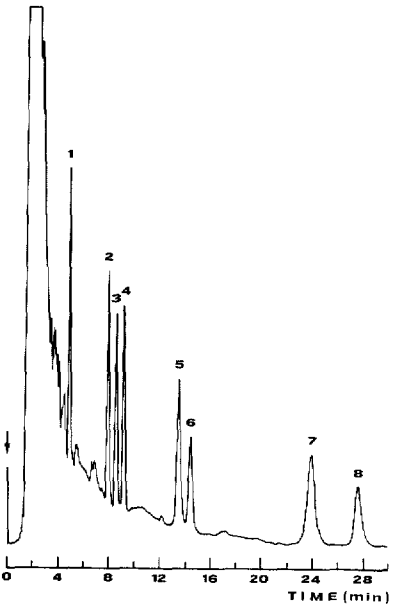

Fig. 1. Chromatogram obtained for 1 g of soil spiked with 10 ng of each herbicide: 1 = simazine; 2 = simetryn; 3 = atrazine; 4 = prometon; 5 = ametryn; 6 = propazine; 7 = prometryn; 8 = terbutryn.

Analytical variables

The influence that the flow-rate at which solvents passing through both the soil and the SCX columns could have on the recovery of triazines was evaluated. Some loss of triazines was observed only when acetone was passed through the two cartridges at flow-rates higher than 1 ml/min. The same effect was observed when the flow-rate of potassium chloride-saturated methanol passing through the soil bed was varied. Less critical was the flow-rate at which the potassium chloride-saturated methanol was passed through the SCX bed to displace triazines. No loss of triazines was observed even at a flow-rate of 1.5 ml/min.

As described under Experimental, the SCX tube was partially filled with acetone before connecting the two cartridges in series. When this precaution was not followed, the initial non-steady-state condition caused the very first drops of acetone flowing from the soil column to be passed too rapidly along the SCX column, which resulted in a loss of chlorotriazines.

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