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Short Communication

High-performance liquid chromatographic determination of dinitroaniline herbicides in soil and water

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ABSTRACT

A high-performance liquid chromatographic method for the simultaneous determination of the dinitroaniline herbicides dinitramine, ethalfluralin, trifluralin, pendimethalin and isopropalin in soil and surface water is reported. The soil was extracted with diethyl ether and analysed without any clean-up. The water was analysed after purification and concentration on a C_{18} cartridge. The average recoveries were in the range 89–104%. The detection limits for the five herbicides were 0.02 mg/kg in dry soil and 0.5 μg/l in surface water.

INTRODUCTION

Dinitramine (**I**), ethalfluralin (**II**), trifluralin (**III**), pendimethalin (**IV**) and isopropalin (**V**) (Fig. 1) are dinitroaniline herbicides used to control most of the annual grasses and broad-leaved weeds in a wide variety of agronomic crops [1]. The behaviour and fate of dinitroaniline herbicides in soil are well known [2]. These compounds are among the least mobile herbicides and therefore the run-off is the principal route, which could lead to the contamination of surface waters.

The traditional method for determining residues of these herbicides in soil involves extraction with an organic solvent, followed by purification. The purified extract is then analysed by gas chromatography (GC) [3–6]. Only one method for the determination of pendimethalin in water has been described [7], and so far no high-performance liquid chromatographic (HPLC) method for the separation and determination of dinitroaniline herbicides in soil and water has been reported.

In this paper, an HPLC method is described which allows the simultaneous determination of dinitramine, ethalfluralin, trifluralin, pendimethalin and isopropalin in soil and surface water.

EXPERIMENTAL

Apparatus

A Model 5020 liquid chromatograph (Varian, Palo Alto, CA, USA) was used, fitted with a UV-100 variable-wavelength UV–VIS detector and a Rheodyne injection valve (50-μl loop), connected to a Model 3390 A reporting integrator (Hewlett-Packard, Avondale, PA, USA).

The extraction of the herbicides from water was performed with a Vac-Elut vacuum system

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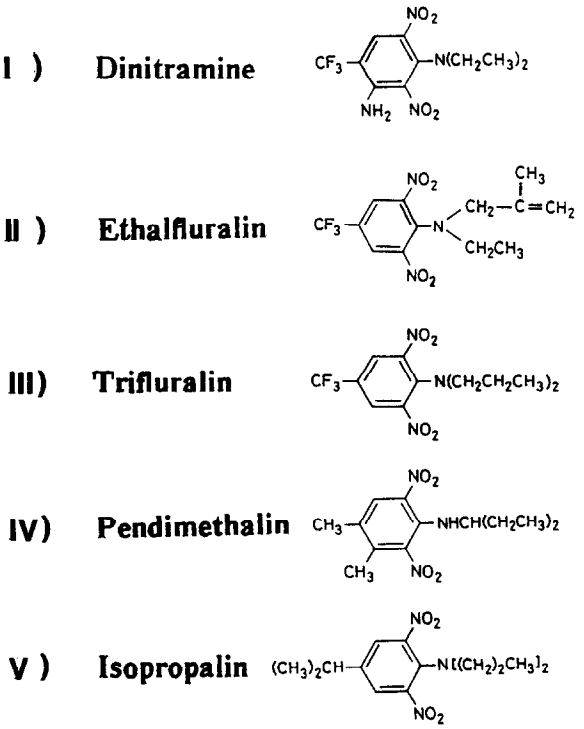

Fig. 1. Structures of dinitroaniline herbicides.

(Analytichem International, Harbor City, CA, USA).

Chromatography

Spherisorb (Waddinxveen, Netherlands) C_1, C_6, C_8, ODS-1 and ODS-2 (10 μm) columns (250 mm × 4.6 mm I.D.) were used; the mobile phase was water–acetonitrile in various ratios at a flow-rate of 1.0 ml/min. The analyses were performed at 220 nm for the simultaneous determination of **I–V** or, for low concentrations, at different wavelengths depending on the previously determined absorbance maxima for dinitramine (220 nm), ethalfluralin (200 nm), trifluralin (200 nm), pendimethalin (240 nm) and isopropalin (200 nm) with a Model DMS 90 UV–VIS spectrophotometer (Varian).

Chemicals and materials

Acetonitrile, methanol and diethyl ether were of HPLC grade (Carlo Erba, Milan, Italy); water was distilled twice and filtered through a Milli-Q apparatus (Millipore, Molsheim, France) before use. Dinitramine, ethalfluralin, pendimethalin, trifluralin and isopropalin were analytical standards purchased from Erhenstorfer (Augsburg, Germany).

Stock standard solutions (*ca.* 100 ppm each) were prepared in acetonitrile; working standard solutions were obtained by dilution with the mobile phase.

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

Soil extraction procedure

A 25-g amount of air-dried soil was weighed in a 250-ml screw-capped flask, 50 ml of diethyl ether were added and the mixture was agitated in a flask shaker (Stuart Scientific) for 30 min. The soil was left to settle and the clear organic layer was transferred into a 20-ml screw-capped tube containing 2 g of anhydrous sodium sulphate. A 2-ml aliquot of the extract was transferred into a 10-ml beaker and evaporated nearly to dryness in a thermo-ventilated stove. The extract was then allowed to evaporate completely in the air; the residue was recovered with 1 ml of mobile phase and injected for HPLC analysis.

Surface water extraction procedure

For sample clean-up, the Vac-Elut system was employed with Bond-Elut C_{18} (500 mg per 2.8 ml)

TABLE I

PHYSICAL AND CHEMICAL CHARACTERISTICS OF THE SOILS

Characteristic	Soil		
	A	B	C
Sand (%)	71	43	71
Silt (%)	20	32	14
Clay (%)	9	25	16
pH (in water)	6.7	8.0	5.5
Organic matter (%)	1.9	3.9	0.6
	Water		
pH	7.9		
Conductance (μS cm^{-1})	745		
Hardness (mg/l $CaCO_3$)	240		
Oxygen (dissolved) (mg/l O_2)	1.9		

cartridges (Analytichem International). The extraction procedure was carried out as follows. The cartridge was treated with 10 ml of methanol, followed by 10 ml of water. The sample (100 ml of surface water) was then added (using a reservoir) and allowed to percolate slowly (1 ml/min). The reservoir was removed and the cartridge washed with 5 ml of methanol–water (50:50, v/v), followed by 5 ml of water. The cartridge was air-dried under vacuum for 2 min and then the pesticides were eluted with 2 ml of diethyl ether and collected in a 2-ml conical tube. The diethyl ether layer was transferred into a 10-ml beaker with a Pasteur pipette and the procedure was carried out as for the soil ethereal extract.

Recovery assays

Untreated soils were air-dried to $< 10\%$ (w/w) water content and sieved through a soil sieve (2-mm mesh). The samples were then fortified by adding 250-μl portions of solutions of the five herbicides in acetonitrile at 0.05 and 1.00 ppm. The solvent was evaporated in a fume-hood (*ca.* 1 h).

Untreated water was fortified with 100-μl portions of a solution of the five herbicides at 0.002 ppm.

The soil and water fortified samples were processed according to the above-described extraction procedure.

RESULTS AND DISCUSSION

In order to achieve the separation of dinitroaniline herbicides, different reversed-phase (C_1, C_6, C_8, ODS-1 and ODS-2) columns were employed (Table II). Each column allowed the separation of the five herbicides with different water–acetonitrile mixtures. The elution order was the same (first and last, respectively) for dinitramine and isopropalin on all the columns tested, whereas ethalfluralin, trifluralin and pendimethalin were eluted with different orders on the different columns.

Calibration graphs for each compound were constructed by plotting concentration *vs.* peak height. Good linearities were achieved in the range 0–1.5 ppm with correlation coefficients between 0.9990 and 0.9998.

For recovery assays of the herbicides, three different soils that had never been treated with any pesticide and one surface water were used. The blanks of the soil extraction solvents did not give any interfering peaks at the retention times of the compounds studied, so making any further clean-up unnecessary. With water samples, different solvents (*n*-hexane, dichloromethane, benzene, acetonitrile and diethyl ether) were tested for the elution of the herbicides, but only diethyl ether allowed satisfactory recoveries without any presence of interfering

TABLE II

RETENTION TIMES OF DINITROANILINE HERBICIDES WITH DIFFERENT COLUMNS AND ELUENTS

Flow-rate, 1.0 ml/min.

Column	Water–acetonitrile ratio	Retention time (min)				
		I	II	III	IV	V
ODS-1	25:75	6.63	8.70	9.82	10.45	13.24
	35:65	10.08	15.41	18.34	18.34	25.68
ODS-2	25:75	6.08	8.92	10.60	11.15	15.87
	35:65	10.18	19.14	23.86	22.81	37.78
C_8	35:65	8.71	13.86	16.26	14.12	21.95
	40:60	11.76	20.68	24.79	20.48	34.56
C_6	25:75	5.44	7.47	8.39	7.95	10.80
	35:65	8.68	14.47	16.96	14.47	23.12
C_1	40:60	7.17	10.12	11.03	9.67	12.70
	45:55	9.52	14.79	16.46	13.60	19.38

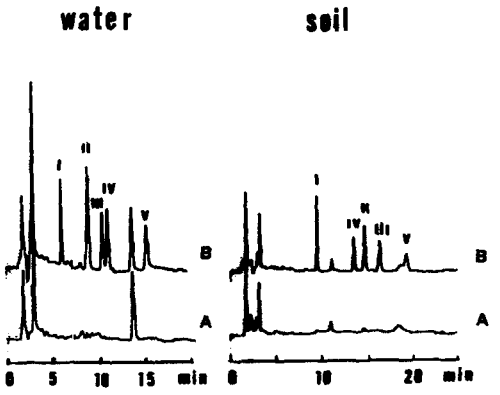

Fig. 2. Left: chromatography of dinitroaniline herbicides in surface water on an ODS-2 column. Mobile phase, water–acetonitrile (25:75, v/v); flow-rate, 1 ml/min; detection, UV at 220 nm. (A) Control; (B) sample fortified with 2 μg/l of each herbicide. Right: chromatography of dinitroaniline herbicides in soil on a C_1 column. Mobile phase, water–acetonitrile (45:55, v/v); flow-rate, 1 ml/min; detection, UV at 220 nm. (A) Control; (B) sample fortified with 50 μg/l of each herbicide.

compounds. The recoveries from soil and water with diethyl ether ranged between 89% and 104% for all the herbicides studied.

Representative chromatograms for the simultaneous determination of dinitroaniline herbicides are shown in Fig. 2. Under the optimum conditions, the detection limit was 0.02 mg/kg in soil and 0.5 μg/l in water for all compounds.

The method described allows the rapid determination of dinitroaniline herbicides in soil and water, and could be employed for routine monitoring of environmental pollution. Further, the possibility of achieving the separation of dinitroaniline herbicides by means of different columns could be useful as a confirmatory assay or to overcome the problem of interfering compounds.

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