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Determination of linuron in aqueous soil extracts by high-performance liquid chromatography

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Abstract

An analytical method based on high-performance liquid chromatography with photodiode array detection has been developed for the determination of linuron in aqueous soil extracts containing different amounts of organic matter (0.7–11.7%). The detection limit of linuron in the aqueous soil extracts was 0.010 μg/ml (0.2 ng) and the relative standard deviation for repeatability based on peak area measurement ranged between 0.7 and 3.1%. Recoveries from spiked samples ranged from 106.3 to 116.1%. The method uses a C_{18} reversed-phase column, a mobile phase of methanol–water (65:35, v/v) at a flow-rate of 1 ml/min and UV detection at 210 nm. The method was applied to determine the adsorption of linuron by soils.

Keywords: Soil; Environmental analysis; Linuron; Phenylurea pesticides; Pesticides

1. Introduction

Linuron [3-(3,4-dichlorophenyl)-1-methoxy-1-methyl urea] is a selective herbicide belonging to the phenylurea group with pre- and post-emergence activity and is widely used in different types of cultivation. Studies in the literature show that this herbicide is strongly adsorbed by soils [1,2] and that its mobility is low [3]. Both processes contribute to increasing the persistence of this compound in soils [4], above all in soils with high organic matter contents in which the pesticide may constitute an environmental hazard. Accordingly, to prevent the presence of residues it is necessary to continue research into the processes involved in the behaviour of this compound in the soil. For such studies and for the analysis of the herbicide in soils and waters, precise and sensitive methods for the analysis of linuron are required.

In view of this, in the present work an analytical method using high-performance liquid chromatography (HPLC) with photodiode array detection was developed for rapid and sensitive determination of linuron in the presence of aqueous extracts of six soils with different organic matter contents. These extracts contain soluble humic substances extracted from the soils that usually generate the greatest degree of interference in the determination of low concentrations of pesticides [5,6]. The method was also applied to the determination of the equilibrium concentration of linuron in the soil–water system in a study of the adsorption of this herbicide by the same soils.

Like other phenylurea compounds, linuron can be determined by gas chromatography [7,8]. However, this method is tedious since the thermal instability of such compounds demands derivatization before sample introduction into the chromatographic system. HPLC determination with UV detection has been the method chiefly used for the analysis of linuron [9–

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11]. In addition to UV detection, mass spectrometric [12], coulometric [13] and amperometric detection [14] have also been used in the analysis of phenylurea herbicides. In comparative terms, the use of photodiode array detection confers the HPLC method with greater sensitivity for the analysis of pesticides since this combination permits the identification and confirmation of the purity of each peak and the detection of interferences at low concentrations. It also affords quality absorbance spectra with low concentrations of the compound studied.

2. Experimental

2.1. Apparatus

The chromatographic system was a Waters chromatograph (Waters Assoc., Milford, MA, USA) equipped with a Model 600E multisolvent delivery system attached to a Model 717 autosampler, a Model 996 photodiode array detector and a Millenium 2010 chromatography manager data acquisition and processing system. The column was a stainless steel NovaPak C_{18} (Waters Assoc.) column (159 mm×3.9 mm I.D.). Millex-HV_{13} filters (Millipore, Bedford, MA, USA) used for the samples and HV_{47} filters used for solvents had a pore size of 0.45 μm.

2.2. Reagents

HPLC grade methanol (Carlo Erba, Milan, Italy) was used in preparing the mobile phase. Water was obtained from a Millipore Milli-Q purification system (Milli-Q water). The linuron (99.9% purity) was obtained from Promochem (Wesel, Germany).

2.3. Soil samples

Six samples from uncultivated soils were used. The organic carbon and nitrogen contents of the soils were determined [15] and the organic matter content (%C×1.72) and the carbon nitrogen ratio (C/N) were calculated (Table 1). The C/N ratio is generally considered to be an index of the degree of organic matter humification [16], its value decreasing with the degree of humification.

2.4. Procedure

2.4.1. HPLC operating conditions

The optimum chromatographic conditions were as follows: eluent, methanol–water (65:35, v/v); flow-rate, 1 ml/min; injection volume, 20 μl; wavelength, 210 nm (UV absorption maximum of linuron). Column temperature was ambient.

Stock solutions of 1000 and 100 μg/ml linuron were prepared by dissolving the solid product in methanol. Working standard solutions were prepared by dilution of suitable aliquots in Milli-Q water which were then filtered through a 0.45 μm Millex-HV_{13} filter. A 20 μl aliquot of each sample was injected into the chromatograph to obtain the calibration curve.

2.4.2. Determinations in aqueous soil extracts

To obtain a solution of extractable matter, 1 g of soil was shaken with 10 ml of water over 48 h. The suspension was then centrifuged at 5045 *g* for 30

Table 1
Linuron determination in aqueous extracts of soil samples

Soil	Organic matter (%)	C/N	0.010 μg/ml		0.025 μg/ml		0.500 μg/ml	
			Recovery (%)	R.S.D. (%)(n=3)	Recovery (%)	R.S.D. (%)(n=3)	Recovery (%)	R.S.D. (%)(n=3)
1	0.7	8.5	109.2	3.1	85.1	2.2	106.5	0.2
2	2.1	9.6	116.1	2.6	97.2	0.9	112.5	0.6
3	4.2	9.1	109.6	0.7	105.7	0.3	98.7	1.0
4	4.3	15.9	109.4	1.6	92.1	0.5	95.5	1.2
5	6.8	21.6	106.3	1.1	84.2	0.8	104.7	0.8
6	11.7	24.8	110.9	2.3	99.0	1.2	93.3	0.5

min and the aqueous extract was separated. From 100 μg/ml stock solution of linuron, aliquots ranging between 0.01 and 0.5 ml were taken and brought up to a final volume of 100 ml with the aqueous extract of the corresponding soil. The resulting solutions, with concentrations ranging from 0.01 to 0.5 μg/ml, were filtered through Millex-HV_{13} filters and injected into the chromatograph.

Samples were prepared in triplicate and quantified using calibration curves, measuring the area of the peak eluted as a mean value of three injections made.

2.4.3. Adsorption isotherms

A 10 ml volume of aqueous solution of linuron at concentrations between 5 and 25 μg/ml was added to 5.0 g of soil. Samples were equilibrated by shaking for 19 h in a mechanical shaker thermostatted at 20°C. The suspensions were centrifuged at 5045 *g* for 30 min and an aliquot of the supernatant fluid filtered through Millex-HV_{13} filters of 0.45 μm pore size. Samples were prepared in triplicate and the peak area value was obtained for each solution.

3. Results and discussion

Chromatographic analysis of linuron was performed using a methanol–water mixture as eluent. It was seen that methanol–water (65:35, v/v) as the mobile phase with isocratic elution at a flow-rate of 1 ml/min permitted adequate separation of linuron from non-retained species. Under these conditions, the retention time of linuron was 4.2 min.

To obtain calibration curves, triplicate aqueous samples of linuron were injected, the minimum concentration limit being 0.005 μg/ml. The response of the detector as referred to peak areas was linear in the range assayed (0.005–100 μg/ml) (concentrations several orders of magnitude higher) and least-squares linear regression analysis of the data (expressed in log form) provided an excellent correlation (r=0.9995) (Fig. 1). Analysis of six identical samples of linuron carried out to check the precision of the method afforded a relative standard deviation (R.S.D.) of 6.1% for the analysis of samples containing 0.005 μg/ml and a R.S.D. of 0.2% for samples containing 100 μg/ml.

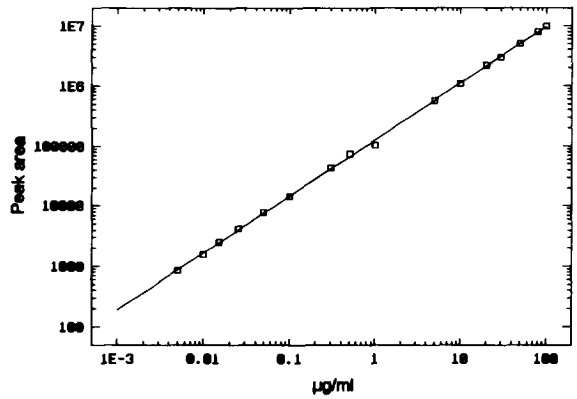

Fig. 1. Calibration graphs for linuron. Mobile phase, methanol–water (65:35, v/v); flow-rate, 1.0 ml/min; UV detection, 210 nm; injection volume, 20 μl

3.1. Determination of linuron in the presence of aqueous soil extracts

Aqueous soil extracts were spiked as described above. Samples were injected in triplicate intercalated with injections of sufficient standards to obtain an appropriate calibration curve. The apparatus was re-equilibrated every six samples. The study performed showed that the separation of linuron with respect to interfering substances is adequate. Additionally, the UV spectrum measured for the chromatographic peak corresponding to linuron confirmed the purity of the peak in all extracts studied.

The results obtained (Table 1) indicate that in the presence of aqueous extracts of soils it is possible to determine linuron concentrations equal to or above 0.500 μg/ml with a R.S.D. lower than 1.2%. The minimum concentration of linuron detected was 0.01 μg/ml (0.2 ng) with a R.S.D. lower than 3.1%. Fig. 2 shows the chromatograms of extracts of a soil without linuron and extracts spiked with concentrations of 0.01, 0.025 and 0.5 μg/ml of linuron.

From the above study, it may be inferred that in the determination of linuron with the proposed method there is no significant interference from compounds present in the aqueous extracts from the organic matter of the soil even though the soils contain very different contents of organic matter (0.7–11.7%) and the composition of the organic matter is also very different according to the C/N ratio of the soils (8.5–24.8). The minimum amount

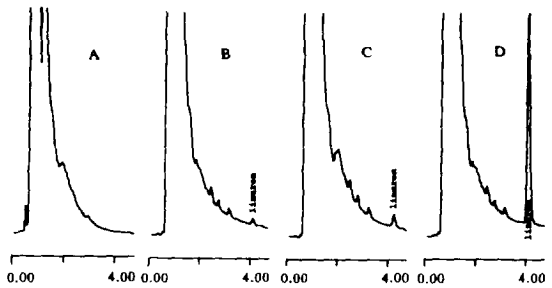

Fig. 2. Chromatograms obtained for extracts of soil 6, without linuron (A) and spiked with concentration of 0.01 (B), 0.025 (C) and 0.5 μg/ml (D) of linuron.

of linuron detected was lower than that obtained with other methods indicated in the literature [9,11].

3.2. Application to the study of the adsorption of linuron by soils

The method described was applied to the study of the determination of linuron adsorption by selected soils in aqueous medium. The isotherms obtained (Fig. 3) fit the Freundlich adsorption equation [17], with r values >0.99. The values of the Freundlich K constant obtained are shown in Table 2. These are very high in comparison with the values reported for the adsorption of other hydrophobic pesticides by soils with organic matter contents similar to those of the soils used in the present study. High values for

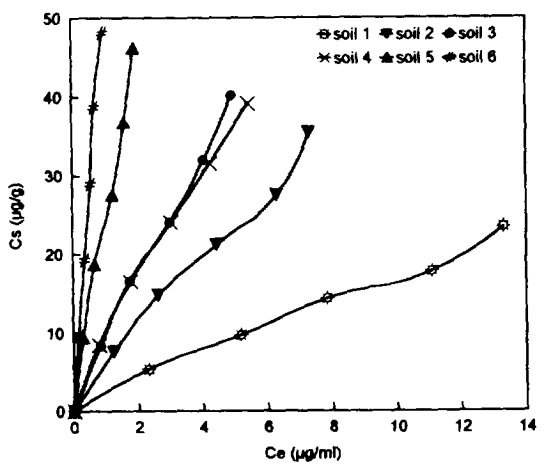

Fig. 3. Adsorption isotherms of linuron by soils. Ce=equilibrium concentration and Cs=amount spiked−Ce. Equilibrium time, 19 h; temperature, 20°C; soil/solution ratio, 5 g:10 ml

Table 2
Organic matter of the soil samples and K adsorption constants of Freundlich

Soil	Organic matter (%)	K
1	0.7	2.59
2	2.1	6.33
3	4.2	9.86
4	4.3	10.34
5	6.8	25.41
6	11.7	51.35

the adsorption of this pesticide by soils with high organic matter contents [1] and also by humic components isolated from soil [18] have also been reported. In agreement with the foregoing, statistical study of the results indicated a highly significant correlation between the K values and the organic matter content of the soils (r=0.98), pointing to the importance of this parameter in the prediction of the possible retention or persistence of linuron in soils, depending on their composition.

According to the results obtained, the proposed analytical method permits rapid and very sensitive determination of linuron, with no interference from the dissolved organic matter which would permit its later use by the authors of this work to study the effect of exogenous organic matter applied to the soil simultaneously with pesticides in agricultural practice on the mobility of linuron.

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