Short Communication

Determination of acephate by liquid chromatography in the presence of aqueous soil extracts

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ABSTRACT

A liquid chromatographic method has been developed for the determination of acephate in aqueous extracts of agriculture soils. Aqueous extracts of spiked soil samples were used. The lower detection limit was 1 μg/ml; the relative standard deviation for repeatability (R.S.D.) based on peak area measurement ranged from 0.2 to 4.5%. Recoveries from spiked samples ranged from 93.6 to 104.3%. The method used a C_{18} reversed-phase column, a mobile phase of 5% (v/v) methanol–water and UV detection at 215 nm. The application of the proposed method to the study of the adsorption of acephate in aqueous medium by a selected group of soils yielded satisfactory results.

INTRODUCTION

Acephate (O,S-dimethyl acetylphosphoramido-thioate) is a systemic insecticide of moderate persistence in soils [1] that is widely used in agriculture. Study of the adsorption of this insecticide by soils and their constituents in aqueous media is of great interest since the high solubility of this compound in water (650 g/l) [1] increases the possible risk of environmental contamination.

Accordingly, we have developed an analytical method using high-performance liquid chromatography (HPLC) with UV detection that will permit the determination of acephate rapidly, simply and with great precision. This method will be used in the systematic study of the adsorption of acephate in aqueous extracts of soils used for agricultural purposes.

Currently, gas chromatography (GC) is the most widely used procedure for the analysis of this insecticide and its residues [2], but there are very few references in the literature concerning the application of liquid chromatography (LC) in the determination of the compound [3,4]. However, the high solubility of acephate in water suggests that reversed-phase HPLC might be a suitable technique for such determination because it permits direct injection of aqueous extracts. This technique of direct injection is more rapid than gas chromatography, which requires extraction with organic solvents and further clean-up procedures. Detection limits of acephate by GC [2] and by LC [4] are 0.02 and 0.05 μg/ml, respectively.

In the present work we report on the analytical characteristics of the proposed LC method and its application to the determination of acephate in the

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presence of aqueous extracts of various soils which differ in the content and plant origin of organic matter. The aim was to determine the applicability of the proposed LC method for the study of the soil–water equilibrium of acephate.

The structural formula of acephate is as follows:

$$CH_3 - \underset{\overset{\|}{O}}{C} - \underset{\overset{|}{H}}{N} - \underset{\underset{SCH_3}{|}}{\overset{\overset{O}{\|}}{P}} - OCH_3$$
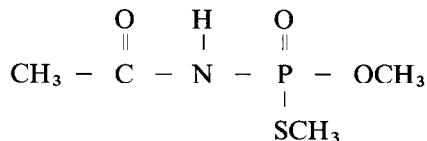
EXPERIMENTAL

Apparatus

The chromatographic system was a Waters chromatograph (Waters Assoc., Chromatography Division, Millipore, Milford MA, USA) equipped with a Waters Model U6K universal liquid chromatography injector) two Model 501 HPLC pumps for solvent delivery attached to a Model 680 automated gradient controller, and a Model 481 LC spectrophotometer. A computerized integrator (Waters 740 data module) was used for area and height measurements of peak. The column was a stainless-steel Nova-Pak C_{18} (Waters Assoc.) column (150 mm × 3.9 m I.D.). Millex-HV$_{13}$ filters (Millipore) used for the samples and Magna nylon membrane filters (MSI) used for solvents had a pore size of 0.45 μm. The Hamilton syringe was of 25 μl, and a 2-ml volume sample loading loop was used.

Reagents

HPLC-grade methanol (Carlo Erba, Milan, Italy) was used in preparing the mobile phase. The water used for the preparations was distilled in glass in the laboratory. The acephate (98% purity) was obtained from Chevron (Richmond, CA, USA). All other chemicals were of analytical-reagent grade.

Soil samples

Seven samples from uncultivated soils were used. The clay, organic carbon and nitrogen contents of the soils were determined [5] and the organic matter content (percentage carbon × 1.72) and the carbon–nitrogen relationship (C/N) were calculated (Table II). The C/N relationship is generally considered to be an index of the degree of organic matter

humification [6]. The value decreases with the degree of humification. Before being used, the soils were equilibrated in an atmosphere of 35% relative humidity.

Procedure

HPLC operating conditions. The optimum chromatographic conditions were as follows: eluent, 10% (v/v) methanol–water; flow-rate, 1.0 ml/min; injection volume, 25 μl; wavelength, 215 nm (UV absorption maximum of acephate). Column temperature was ambient.

The solvents were filtered through a 0.45 μm pore nylon membrane filter and degassed daily before use.

Stock solutions of 100 and 500 μg/ml acephate were prepared by dissolving the solid product in distilled water. Working standard solutions were obtained by dilution of suitable aliquots in distilled water which were then filtered through a 0.45-μm Millex-HV$_{13}$ filter. A 25-μl aliquot of each sample was injected into the chromatograph to obtain the calibration curve.

Determinations in aqueous soil extracts. To obtain a solution of extractable matter 1 g of soil was shaken with 10 ml of water at a constant temperature (25.0 ± 0.5°C) over 48 h; this was then centrifuged at 5045 g for 30 min and the aqueous extract was separated.

From 100 and 500 μg/ml stock solutions of acephate, aliquots ranging between 0.05 and 0.5 ml were taken and brought up to a final volume of 5 ml with the aqueous extract of the corresponding soil. The resulting solutions, with concentrations ranging from 1 to 50 μg/ml, were filtered through Millex-HV$_{13}$ filters and injected into the chromatograph. Samples were prepared in triplicate and quantified using the external standard method, measuring the area of the peak eluted as a mean value of three injections made.

Adsorption isotherm. Aliquots of 10 ml of aqueous stock solutions of acephate at a concentration between 10 and 50 μg/ml were added to 1.0 g of soil. After 24 h at 20.0 ± 0.5°C with intermittent periods of shaking, 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 duplicate and the value of the area of the peak obtained was

the mean of three injections carried out for each solution.

RESULTS AND DISCUSSION

The chromatographic behaviour of acephate was examined using methanol–water as the eluent. It was observed that the use of a 10% (v/v) methanol–water mixture as the mobile phase with isocratic elution yielded a suitable separation of the acephate peak from that produced by the non-retained species. An increase in the proportion of methanol in the mobile phase eluted the insecticide too fast and hence provided a poor resolution of the corresponding chromatographic peak. For the same reason, the flow-rate used was 1.0 ml/min. Under these conditions, the retention time of acephate was 3.2 min.

The pH of standard solutions was adjusted to between 6 and 8 (normal pH range in aqueous extracts of agriculture soils) by the addition of hydrochloric acid or 0.01% sodium hydroxide. The addition of buffer solutions is not considered to be necessary because no changes were observed in the chromatograms.

Straight calibration lines were obtained by triplicate injection of aqueous solutions of acephate at concentrations ranging between 1 and 100 μg/ml. The area and height of the peaks were plotted in the calibration. The response was linear throughout the concentration range tested and least-squares linear regression analyses of the data provided excellent correlation coefficients (Fig. 1). As may be seen, the sensitivity of the method was greater when peak areas were evaluated.

The proposed method was used to analyse six identical samples containing 10 μg/ml acephate. The analysis showed that a relative standard deviation of 3.1% was obtained for peak-area calculation and 0.8% for peak-height calculation. The relative standard deviation at a concentration level of 1 μg/ml was 5.2 and 3.8% for areas and heights, respectively.

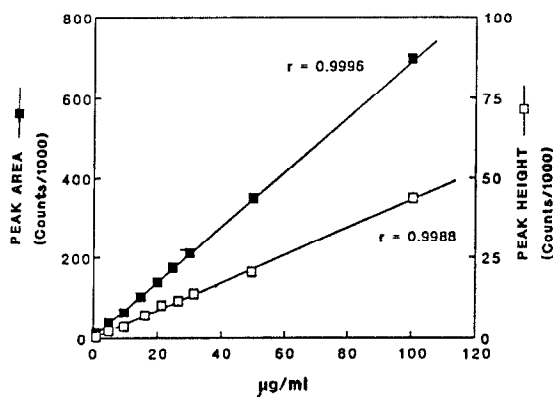Because of these observations it was considered appropiate to use the peak area for the calculation of the results.

Fig. 1. Calibration graphs for acephate. Mobile phase, 10% (v/v) methanol–water; flow-rate, 1.0 ml/min; UV detection, 215 nm; injected volume, 25 μl.

Determination of acephate in the presence of aqueous soil extracts

Before being spiked, none of the aqueous extracts of the soil samples gave any extraneous peaks which might interfere with the determination of the insecticide.

The aqueous extracts were spiked as stated above. Samples were injected in triplicate and bracketed with injections of the standards. The results were calculated based on the average peak area of the sample and the standard.

In all the aqueous extracts of the soils studied, it is possible to determine acephate levels at a concentration equal to or greater than 10 μg/ml with a relative standard deviation of less than 5%. However, the determination of lower concentrations was only possible in soils with low or medium contents of organic matter (Table I).

Although cultivated soils do not have high contents of organic matter, the determination of acephate at concentrations less than 10 μg/ml in those soils in which strong interferences are seen is possible when using a mobile phase of 5% (v/v) methanol–water. It delays the elution of acephate from 3.2 to approximately 5 min, and allows the quantification of even 1 μg/ml with an acceptable degree of precision (Table I). Fig. 2 shows the chromatograms obtained for samples containing 1 μg/ml acephate in the different soils studied when a mobile phase of 5% (v/v) methanol–water was used.

From the studies carried out in aqueous extracts of soils it may be inferred that the minimum

TABLE I

ACEPHATE DETERMINATION IN AQUEOUS EXTRACTS OF SOIL SAMPLE

Soil	Organic matter (%)	C/N	5 ppm[a]		1 ppm[b]	
			Recovery (%)	R.S.D.[c] (%)	Recovery (%)	R.S.D.[c] (%)
1[d]	6.1	22.6	102.8	1.6	104.3	1.1
2[d]	7.3	21.1	95.6	3.5	96.2	3.4
3[d]	10.2	28.3	87.2	3.2	–	–
4[e]	1.1	12.0	100.6	1.4	102.9	4.5
5[e]	1.9	16.8	97.4	0.3	97.5	0.2
6[e]	5.2	20.0	85.8	4.6	93.6	3.8
7[f]	6.3	10.1	86.4	5.8	94.6	1.4

[a] Mobile phase, 10% (v/v) methanol–water.
[b] Mobile phase, 5% (v/v) methanol–water.
[c] Relative standard deviation of three determinations.
[d] Predominant vegetation holm-oak.
[e] Predominant vegetation heather.
[f] Predominant vegetation grassland.

amount of acephate that can be detected depends not only on the content of organic matter in the soil but also on its nature. Thus, in soils with organic matter from the same source (holm-oak or heather) the degree of interference increases as the organic matter content increases and the degree of humification decreases (soils 3 and 6). This finding is logical if one considers that a low degree of humification implies a predominance of fulvic acids over humic acids. The former are the components of the organic matter with a lower molecular weight and a greater polarity, and hence are more easily extractable in aqueous solution. Soils with the same content of organic matter but from different sources cannot be compared to one another.
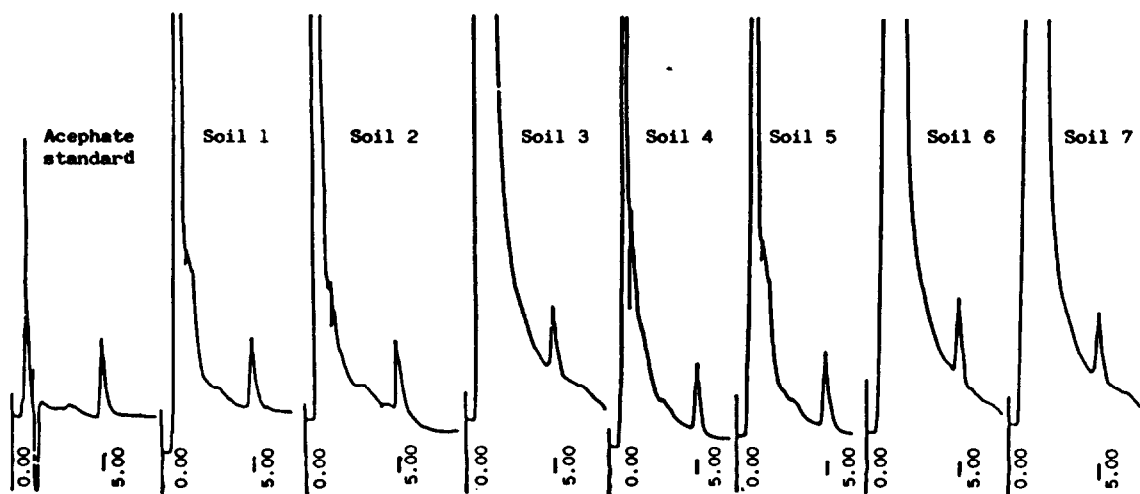

Fig. 2. Chromatograms resulting from the analysis of 1 μg/ml acephate in aqueous extracts of different soil samples. Mobile phase, 5% (v/v) methanol–water; flow-rate, 1.0 ml/min; UV detection, 215 nm; injected volume, 25 μl; attenuation, 4.
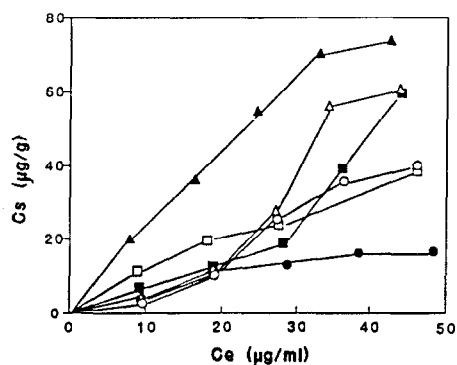
Fig. 3. Adsorption isotherms of acephate by soils. Ce = equilibrium concentration; and Cs = amount spiked − Ce. Equilibrium time, 24 h; temperature, 20°C; soil/solution ratio, 1.0 g: 10.0 ml. Key: ■ = soil 1; □ = soil 2; ○ = soil 4; ▲ = soil 7; △ = soil 8; ● = soil 9.

Application to the study of the adsorption of acephate by soils

The application of the proposed method to the study of the adsorption of acephate by a selected group of soils yielded satisfactory results; the isotherms obtained (Fig. 3) fit the Freundlich equation [7] (with r values $\geqslant 0.097$). The highest values of K, the Freundlich constant, correspond to samples 2 and 7 with a high organic matter content (Table II) and the lowest ones to samples 4 and 8 with a low content in this fraction. This observation suggests that organic matter, as in the case of other organic chemicals [8], is a determinant parameter in the adsorption of acephate. A broader study of the adsorption–desorption process of acephate by cultivated soils is currently under investigation at this laboratory. The statistical study of the results will allow the parameters of the adsorption process to be determined.

TABLE II

CHARACTERISTICS OF THE SOIL SAMPLES AND K ADSORPTION CONSTANTS OF FREUNDLICH

Soil	Organic matter (%)	C/N	Clay (%)	K
1	6.1	22.6	22.0	0.313
2	7.3	21.1	19.3	2.281
4	1.1	12.0	10.6	0.046
7	6.3	10.1	50.9	3.558
8	0.76	5.8	20.7	0.056
9	0.50	3.8	12.1	0.890

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