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Multiresidue analysis of carbamate pesticides in soil by sonication-assisted extraction in small columns and liquid chromatography ☆

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Abstract

A rapid multiresidue method has been developed for the analysis of carbamate insecticides (oxamyl, methomyl, propoxur, carbofuran, carbaryl and methiocarb) in soil. The method is based on the sonication-assisted extraction of soil samples placed in small columns using a low volume of methanol. Residue levels in soil were determined by reversed-phase high-performance liquid chromatography with fluorescence detection after post-column derivatisation. The separation of carbamates is performed on a C_8 column with water–methanol as mobile phase. Recovery studies were carried out at 0.5, 0.1 and 0.01 μg/g fortification levels and average recoveries obtained for carbamates ranged from 82 to 99% with relative standard deviations between 0.4 and 10%. The effect of residue residence time and soil moisture content on the insecticide recovery was also studied. The method is linear over the range assayed, from 0.1 to 1 μg/ml. The detection limit for the carbamates varied from 1.6 to 3.7 μg/kg and the quantification limit obtained was 10 μg/kg. The emission and excitation spectra allowed the confirmation of residues at levels around 0.1 μg/g.

Keywords: Soil; Sonication-assisted extraction; Extraction methods; Environmental analysis; Carbamates; Pesticides

1. Introduction

Carbamates are highly effective insecticides used to protect crops from pests and various compounds of this pesticide group are normally employed in soil or plant treatments.

Direct gas chromatographic (GC) determination of carbamate pesticides is difficult because these compounds are unstable and have a tendency to break down under common GC conditions. Acylation of carbamates with perfluoroacyl anhydrides or acetylation with monochloroacetic aldehyde in presence of pyridine give derivatives with good response in electron-capture detection (ECD) [1]. Carbofuran and its metabolites have been analysed in soil by derivatisation with 1-fluoro-2,4-dinitrobenzene and determination by GC with nitrogen–phosphorus detection (NPD) [2].

Methods based on the derivatisation of carbamates to thermally stable products have several limitations that often reduce their sensitivity. High-performance liquid chromatography (HPLC) has become the preferred choice for the determination of carbamates,

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because in this case the thermal lability problem is obviated. The reversed-phase (RP) mode has been mainly used and a good separation of these compounds was obtained on an octadecylsilyl (ODS) reversed phase [3]. Although carbamates can be determined by HPLC with UV detection, fluorescence detection (Fl) offered at least one order of magnitude improvement in detection sensitivity with respect to UV detection [4]. Therefore, HPLC methods have been developed based on a post-column derivatisation procedure to increase sensitivity. The post-column reaction system in liquid chromatography has interest as a means of converting compounds with unfavourable detection properties into derivatives which show a higher sensitivity and selectivity with specific detectors such as fluorescence. Soil levels of some carbamates have been determined by HPLC–Fl, after post-column derivatisation. *N*-Methyl carbamates were hydrolyzed by NaOH solution at elevated temperature (100 °C) to yield methylamine which subsequently reacts with *o*-phthaldehyde (OPA) and thiofluor at high pH to produce the highly fluorescent isoindole [5–10]. In these methods, the extraction of residues from soil samples are mainly accomplished by conventional methods using mechanical shaking extraction with different organic solvents [11]. Supercritical fluid extraction (SFE) has been successfully applied to soil analysis as a practical alternative to traditional methods [8,12]. In recent years, a low-volume extraction method using sonication has been developed in our laboratory for the GC analysis of herbicides, insecticides and fungicides in soil [13–15].

This paper describes a rapid and sensitive method for the extraction and analysis of carbamate insecticides (oxamyl, methomyl, propoxur, carbofuran, carbaryl and methiocarb) in soil based on the sonication-assisted extraction in small columns (SAESC). Residues were determined by RP-HPLC with fluorescence detection after post-column derivatisation.

2. Experimental

2.1. Materials

Insecticide standards were obtained from commercial sources: oxamyl and methomyl from DuPont (France), propoxur and methiocarb from Riedel-de Häen (Germany), carbofuran from Mitshubishi Chemical Industries (Japan) and carbaryl from Cequisa (Spain). Methanol, acetonitrile and ethyl acetate, HPLC grade, were from Scharlau (Spain). All the solvents were passed through a 0.45-μm filter from Scharlau (Barcelona, Spain) before use. Ultrapure water was prepared using a Milli-Q water purification system. OPA, dimethyl-2-mercaptoethylamine hydrochloride (Thiofluor) and the hydrolysis reagent (NaOH) were purchased from Pickering Labs. (Mountain View, CA, USA). The OPA solution was prepared by dissolving 100 mg of OPA in a premixed solution of 2 g Thiofluor in 1 l of OPA diluent.

2.2. Preparation of standard solutions

Three stock solutions of the studied compounds were prepared containing 5, 0.5 and 0.1 μg/ml of each insecticide in methanol and used to fortify soil samples.

2.3. Extraction columns

Polypropylene columns (20 ml) purchased from Becton-Dickinson (Spain) with Whatman No. 1 (UK) filter paper circles of 2 cm diameter at the end were used in the extraction step. Durapore membrane filters 0.45 μm HV from Millipore (Ireland) were used in the final filtration step.

2.4. Apparatus

2.4.1. Extraction equipment

An ultrasonic water bath (Raipa, Spain) was used in the extraction procedure. The generator of this apparatus has an output of 150 W and a frequency of 33 kHz.

A 12-port vacuum manifold (Visiprep, Supelco, Spain) was employed for the filtration of the extraction solvent.

2.4.2. HPLC

An Agilent (Wilmington, DE, USA) Model 1100 HPLC system equipped with an autosampler with a 100-μl sample loop and a programmable fluores-

cence detector operated at an excitation wavelength of 330 nm, an emission wavelength of 465 nm and a photomultiplier gain of 11 was used. The mobile phase was delivered by an Agilent Model-1100 LC quaternary pump coupled to a PCX 5200 carbamate post-column reaction system (Pickering Labs.). The HPLC column was maintained at 42 °C. The post-column reaction unit consisted of two reagent pumps (the NaOH solution and the OPA solution were constantly pumped at a flow-rate fixed of 0.3 ml/min during the whole sequential cycle), and two reaction coils. The first reaction coil was heated at 100 °C for NaOH hydrolysis and the second one was kept at ambient temperature for OPA derivatisation.

The analytical column selected for analysis was a C_8 5 μm, 250 mm×4.0 mm I.D. with a C_8 guard column. A HP-ChemStation data system was used for data collection.

The methanol–water mobile phase gradient began with an initial composition of 15% methanol, held for 2 min, then it was increased to 70% over 40 min, and then increased again to 100% methanol over 4.1 min (held 1 min). The 100% methanol was used to provide column clean up before returning to initial conditions (15% methanol) for injection of a subsequent sample. The stop time was fixed at 60 min. The flow-rate was 0.8 ml/min.

The instrument parameters for spectrofluorimetric analysis of carbamate insecticides were: multiexcitation: excitation wavelength (λ_{ex})=270–380 nm, emission wavelength (λ_{em})=465; slit 5 nm, peak width 0.2 min corresponding to a response time of 4 s and a photomultiplier tube (PMT) gain of 11. Multiemission: λ_{em}=400–500 nm, λ_{ex}=330, slit 5 nm, peak width 0.2 min corresponding to a response time of 4 s and a PMT gain of 11.

Carbamates were quantified by comparing the chromatographic peak areas in sample extracts with the corresponding peak areas of standard solutions containing known quantities of the respective substances. The confirmation of carbamate residues was carried out by comparing the excitation and emission spectra at peak maxima of standards with the excitation and emission spectra at peak maxima of samples.

2.5. Soil samples

The main physical–chemical properties (organic matter, pH, texture and field capacity) of soils are given in Table 1. Soil samples (A and B) were collected from the plough layer (0–10 cm) of two experimental plots located in the region of Madrid (Spain). These samples were sieved (2 mm) and stored at room temperature until fortified.

2.6. Procedure

Two filter paper circles were placed at the end of the polypropylene columns and then 5 g of the sieved soil was placed in the columns. Soil samples were fortified with 0.5 ml of a mixture of the different carbamates in methanol to reach final concentrations of 0.5, 0.1 or 0.01 μg/g and left for 20 min at room temperature for solvent evaporation. To investigate the influence of water on the recoveries, 0.5 ml of water was added to the soils in the columns to increase soil moisture content from the initial 1 to 11%. Half of the samples were extracted after pesticide addition and the other half were capped and stored at 4 °C for 48 h prior to analysis.

Soil samples were extracted with 5 ml of methanol (HPLC grade) for 15 min in an ultrasonic water bath at room temperature. The water level in the bath was adjusted to equal the extraction level inside the columns, which were supported upright in a tube rack and closed with screw-type valves. After the extraction, the columns were placed on the multiport vacuum manifold where the solvent was filtered and

Table 1
Characteristics of the selected soils

Soil	pH	Organic matter (%)	Sand (%)	Silt (%)	Clay (%)	Field capacity (%, at −33 kPa)
A	7.69	0.97	44.34	37.44	18.22	14.76
B	6.70	1.75	64.81	23.65	11.54	13.30

Table 2
Influence of the extraction solvent on pesticide recovery

Compound	Recovery (%) (mean±SD)[a]		
	Ethyl acetate	Methanol	Acetonitrile
Oxamyl	43.9±8.9	79.1±3.3	43.7±3.6
Methomyl	66.0±11.6	88.1±3.0	89.7±4.9
Propoxur	95.5±5.1	92.3±2.5	95.2±5.5
Carbofuran	98.9±5.2	93.1±2.0	95.1±5.5
Carbaryl	97.7±9.6	92.7±2.5	109.0±4.4
Methiocarb	100.4±6.4	92.7±2.2	96.6±3.5

[a] Results are the mean of five replicates±SD.

collected in graduate tubes. Soil samples were extracted again with 4 ml of methanol for 15 min and 1 ml wash. The total extract collected was passed through the Durapore membrane filters, adjusted to 10 ml for the highest fortification levels or to 1 ml for the other sample extracts and stored at 4 °C until analysed by HPLC.

3. Results and discussion

3.1. Optimisation of the extraction procedure

The effect of the extraction solvent was studied using samples fortified at 0.2 μg/g. The recoveries of carbamates obtained with ethyl acetate, methanol and acetonitrile in soil A are shown in Table 2. In general, recoveries obtained with the three solvents were similar except for oxamyl where the best recoveries were obtained with methanol and for methomyl where ethyl acetate showed recoveries lower than the other two solvents. Therefore, methanol was selected as extraction solvent for the carbamate insecticides.

To test the influence of soil water content on the recovery of carbamates, samples of soils A and B were fortified at 0.1 μg/g, the soil moisture content was adjusted at 11% by adding water and carbamates extracted with methanol following the procedure described above. Recoveries higher than 80% were found for all compounds with similar values for soils A and B. Therefore, recovery of carbamates through the proposed method was not affected by the water content of soil.

To study the influence of the residue residence time of carbamates in the two soils studied, samples were fortified at 0.1 μg/g and residue analyses were carried out 48 h after fortification of samples to allow adsorption of pesticides. Good recoveries were obtained after the residence time studied with values around 80% and relative standard deviations (RSDs) lower than 8%.

Untreated soil samples were spiked with 0.5, 0.1 and 0.01 μg/g of the studied insecticides dissolved in methanol and pesticide residues were analysed by HPLC following the procedure described above. Recovery of carbamate insecticides from soil is shown in Table 3. Average recoveries varied from 82 to 99% with RSDs between 0.4 and 10%.

Fig. 1 shows representative chromatograms obtained by HPLC–Fl of a blank soil sample, a soil sample fortified at 0.1 μg/g and another soil sample

Table 3
Carbamate recoveries[a] from soil samples

Compound	Recovery (%) (mean±SD)					
	0.5 μg/g		0.1 μg/g		0.01 μg/g	
	Soil A	Soil B	Soil A	Soil B	Soil A	Soil B
Oxamyl	82.9±6.6	88.5±1.5	83.7±4.9	83.2±2.5	89.4±6.6	85.6±8.8
Methomyl	92.7±9.8	92.5±1.4	90.3±7.4	91.6±2.0	88.4±4.4	87.0±4.6
Propoxur	90.3±7.1	94.0±0.9	89.9±5.3	93.6±2.0	86.1±6.9	89.6±5.5
Carbofuran	93.9±5.8	94.4±0.4	90.6±6.3	88.5±1.2	97.5±8.5	87.6±3.0
Carbaryl	98.9±5.0	94.3±1.0	96.4±7.2	97.7±2.9	88.6±10.0	90.2±7.9
Methiocarb	88.2±3.4	93.2±2.7	88.9±2.5	92.0±4.2	87.0±8.3	91.4±6.2

[a] Results are the mean of five replicates ±SD.

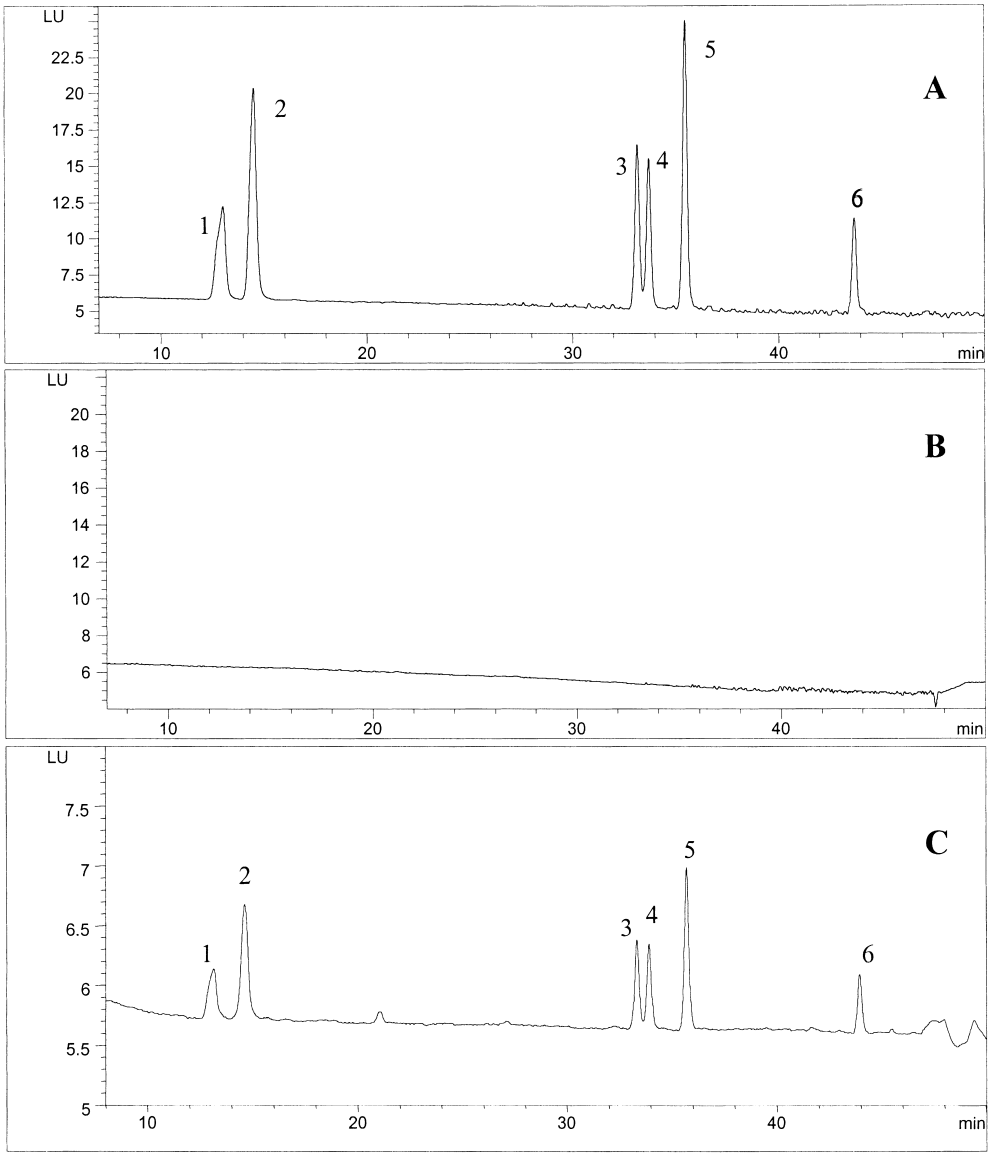

Fig. 1. HPLC–Fl chromatograms of (A) a soil sample fortified at 0.1 μg/g, (B) a blank extract of a soil sample, and (C) a soil sample spiked at the LOQ level (0.01 μg/g). Peak identification: 1=oxamyl, 2=methomyl, 3=propoxur, 4=carbofuran, 5=carbaryl, 6=methiocarb.

spiked at 0.01 μg/g. The chromatogram of the blank sample was very clean, no interference compounds were present in the extract and, therefore, a clean up step was not necessary.

The proposed method for the determination of carbamates in soil by sonication assisted extraction in small columns has the advantages, over the traditional extraction methods, of providing a rapid extraction procedure with a small volume consumption of organic solvent.

3.2. Linearity and detection limit

The detector response was linear in the range of concentrations studied (0.1 to 1 μg/ml) and the correlation coefficients for the carbamates ranged from 0.999 to 1.000 (Table 4).

The method detection limit (MDL) of the proposed method was calculated using the equation reported by Fong et al. [16]: MDL=$t_{99}s$ where MDL is the method detection limit, s is the standard deviation of readings from the identically spiked matrix portions and t_{99} is the confidence interval about the mean, as determined by the Student t value from statistics tables. Under the chromatographic conditions selected and extracting 5 g of soil the limits of detection for the studied pesticides ranged from 1.6 to 3.7 μg/kg (Table 4). These results are in agreement with those obtained by other authors [7,17].

The limit of quantification (LOQ), supported by the recovery data presented, was 10 μg/kg (Fig. 1C). Nevertheless, a lower LOQ could be obtained for some of the insecticides studied.

3.3. Confirmation of carbamates identity

The confirmation of carbamates identity was based on the scanning capabilities of the fluorescence detector. The excitation and emission spectra at peak maxima of a standard and the one corresponding to the sample should match each other. The spectra of the carbamates, after post-column derivatisation, were recorded in the experimental conditions described above. A good correspondence of the spectra was obtained for fortified samples at levels around 0.1 μg/g.

4. Conclusions

Results obtained in this study show that the RP-HPLC–Fl method, with post-column derivatisation, allows the analysis of carbamates in soil at low levels. The proposed procedure is a rapid and sensitive method based on the sonication assisted extraction of soil samples using methanol as extracting solvent. This technique provides good response linearity, high precision and low detection limits. The LOQ for carbamates in soil, supported by the data presented, was 10 μg/kg. The determination of pesticides is carried out in a short time and with a small volume of solvent. The carbamates recovery was not affected by the soil moisture content and the residence time of residues in soil. A good correspondence of the spectra of these compounds in soil extracts with those of standards were obtained at levels around 0.1 μg/g.

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Table 4
Retention times (t_R), method detection limit (MDL[a]) and calibration data of the selected carbamates analysed by HPLC

	Pesticide	t_R (min)	MDL (μg/kg)	Calibration data	
				Correlation coefficient (r)	Equation
1	Oxamyl	12.91	2.4	0.999	$y=9.72\cdot10^5x-1.11\cdot10^4$
2	Methomyl	14.43	1.6	0.999	$y=1.50\cdot10^6x-9.62\cdot10^4$
3	Propoxur	33.13	2.6	1.000	$y=7.37\cdot10^5x-4.67\cdot10^3$
4	Carbofuran	33.67	3.2	1.000	$y=7.10\cdot10^5x-4.67\cdot10^3$
5	Carbaryl	35.44	3.7	1.000	$y=1.26\cdot10^6x-3.96\cdot10^3$
6	Methiocarb	43.58	3.1	1.000	$y=1.08\cdot10^6x+5.60\cdot10^3$

[a] MDLs obtained with the studied soils at the lowest fortification level, 0.01 μg/g.

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