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Ultrasonic solvent extraction of pesticides from soil

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Abstract

Ultrasonic solvent extraction of the pesticides atrazine, propham, chlorpropham, diflubenzuron, α-cypermethrin and tetramethrin from soil is reported. The extraction procedure was optimized with regard to the amount of solvent, the duration of sonication and the number of extraction steps. Ultrasonic solvent extraction was compared with traditional extraction methods, shake-flask and Soxhlet extraction. The recovery of pesticides was determined by quantitative thin-layer chromatography on RP-18 plates. Ultrasonic extraction using acetone showed satisfactory extraction efficiencies combined with simplicity of use and low solvent consumption.

Keywords: Soil; Ultrasonic solvent extraction; Environmental analysis; Pesticides

1. Introduction

The use of pesticides constitutes an important aspect of modern agriculture. Agrochemicals are used to control pests like insects, plant diseases, worms and rodents. When pesticides are released into the environment they may be broken down, or they may resist degradation and thus remain unchanged in the environment for long periods of time [1].

The increasing number of environmentally significant pesticides requires the development of an analytical method that allows simultaneous determination of different pesticides with minimum extraction and clean-up steps.

Thin-layer chromatography (TLC) is now widely accepted as a rapid and efficient screening technique. It has grown rapidly in recent years because of improvements in the instrumentation for spotting and densitometric evaluation, and in the development of sorbents and modified layers. Although other methods are widely used for pesticide determination, e.g. gas chromatography, supercritical fluid chromatography, spectrometry, enzyme immunoassay and capillary electrophoresis, TLC has retained its status as a valid and simple method for quantitative and qualitative analysis of pesticides and their metabolites [2,3].

Chromatographic analysis usually follows tedious sample preparation to extract compounds to be analyzed from complex matrices, e.g., soil, plant materials and foods. For the isolation of pesticides from soil samples various extraction and clean-up procedures have been proposed. Almost all traditional methods (shake-flask, Soxhlet etc.) are time- and solvent-consuming. Due to the long extraction process, degradation of the components can occur. Typical solvent volumes can range from 50 ml to more than 400 ml per sample. A number of methods such as ultrasonic solvent extraction, solid-phase extraction (SPE), supercritical fluid extraction (SFE), accelerated solvent extraction, microwave extraction, SPME, etc., were proposed to resolve the solvent-consumption problem.

The aim of this work was to optimize the con-

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dition of ultrasonic solvent extraction for six most commonly used pesticides for weed and insect control, from soil samples. Sonication provides a more efficient contact between the solid and solvent than shake-flask method, usually resulting in a greater recovery of analyte [4]. The extraction procedure was optimized with regard to the solvent amount, the duration of sonication and the number of extraction steps. A comparative study on the extraction of α-cypermethrin, tetramethrin, diflubenzuron, chlorpropham, propham and atrazine from soil was conducted employing the ultrasonic technique, Soxhlet extraction and shake-flask method with various solvents. The extracted pesticides were identified and quantified using reversed-phase TLC-densitometry.

2. Experimental

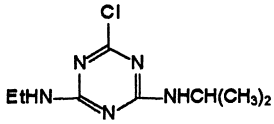
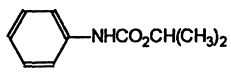
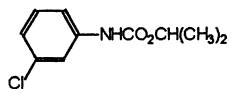
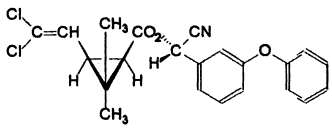
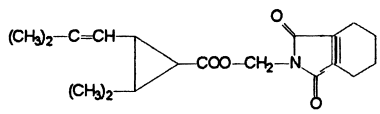
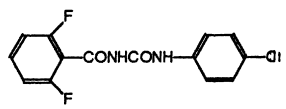
2.1. Materials

All pesticide standards at least 98% purity were furnished by various pesticide manufactures. Pesticide investigated and their characteristics are listed in Table 1. All solvents were of analytical reagent grade supplied by Kemika (Zagreb, Croatia). Soil was collected at hill Medvednica near Zagreb. It was not treated with any agrochemicals for at least 10 years before collection.

2.2. Preparation of standard solution

Stock solution of pesticide mixture was prepared

Table 1
Pesticides studied and their characteristics

Pesticide	Chemical class	Structure	Water solubility	Half-life
Atrazine	Triazine		28 mg/l (20°C)	>60 days
Propham	Carbamate		250 mg/l (20°C)	15 d (16°C), 5 d (29°C)
Chlorpropham	Carbamate		89 mg/l (25°C)	65 d (15°C), 30 d (29°C)
α-Cypermethrin	Synthetic pyrethroid		0.01 mg/l (25°C)	13 weeks in loamy soil
Tetramethrin	Synthetic pyrethroid		–	–
Diflubenzuron	Benzoylurea		0.08 mg/l (pH 5.5, 20°C)	>150 d (pH 5 and 7) 42 d (pH 9)

by dissolving accurate amounts of powdered samples in methanol. Mass concentrations of compounds were 0.12 mg/ml for α-cypermethrin, 0.26 mg/ml for tetramethrin, 0.02 mg/ml for diflubenzuron, 0.05 mg/ml for chlorpropham, 0.04 mg/ml for atrazine and 0.05 mg/ml for propham.

2.3. Preparation of spiked soil samples

Spiked soil samples were prepared by adding 1 ml of standard mixture of pesticides to 10 g of soil. Additional methanol was added until the solvent completely covered the soil particles. The suspension was thoroughly mixed for 1 h with a mechanical shaker. The bulk of the solvent was slowly evaporated at room temperature.

2.4. Extraction

2.4.1. Optimization of ultrasonic extraction

The efficiency of the extraction procedure was checked by recovery experiments. In the first set of experiments the extraction efficiencies of various organic solvents (acetone, diethyl ether, chloroform, hexane, benzene, acetonitrile and dichloromethane) were compared. Accurately weighed spiked soil (10 g) was sonicated 15 min with 20 ml of various solvents in an ultrasonic bath (frequency 25–40 Hz, UZ-20R, Iskra, Kranj, Slovenia). The extracts were filtered through Whatman 40 filter. The filtrates were evaporated on a rotary evaporator (R-114/A, Büchs, Switzerland) at 40°C to dryness and the residues were dissolved in 1 ml of methanol. The amount of extracted pesticides was determined by TLC and the recovery (%) was calculated.

In the second set of experiments the optimum volume of solvent, optimum time of sonication and optimum number of extraction steps were determined. The experiments were performed only with acetone, which gave the highest recovery rate for the pesticides studied. In order to determine optimum volume of acetone 10 g of spiked soil was sonicated 15 min with 10, 15, 20, 25 and 30 ml of acetone. In order to determine optimum time of sonication 10 g of spiked soil was sonicated for 5, 10, 15, 20 and 30 min, with 20 ml of acetone. The extraction of 10 g of spiked soil with 20 ml of acetone for 15 min was repeated up to four times. The amount of extracted pesticides was determined in each extract, and the cumulative recovery was calculated.

2.4.2. Shake-flask extraction

Accurately weighed spiked soil (10 g) was suspended in 20 ml acetone and shaken mechanically in an Erlenmeyer flask for 2 h. Extract was filtered and evaporated to dryness as described previously.

2.4.3. Soxhlet extraction

Spiked soil (10 g) was transferred into an extraction thimble and subjected to Soxhlet extraction for 4 h with 250 ml of acetone.

2.5. Thin-layer chromatography

TLC was performed on 20×20 cm RP-18 F_{254}s plates with a layer thickness of 0.25 mm. Aliquots (10 μl) of standard pesticide solution, of the soil extracts and of a blind extract (from nonspiked soil) were applied 10 mm from the lower edge of the plate as a 1-cm bands using a Hamilton microsyringe. The plates were developed by ascending technique with previous chamber saturation to a distance of 12 cm at room temperature. Methanol–water (8:2, v/v) was used as a mobile phase. After development, the plates were air dried. Spots were detected under 254 nm UV light. Quantitative analysis was performed by measuring absorbance in single beam mode with Camag TLC Scanner II (Camag, Muttenz, Switzerland). The scanning parameters were: deuterium lamp, slit dimension 0.6×8 mm and wavelength 254 nm, scanning speed 20 mm/s. The R_F values of all samples were determined by separate spotting of each individual sample so its identity and corresponding R_F value in the mixture was known.

3. Results and discussion

Quantitative evaluation of chromatograms was performed by measuring the absorbance of the analyte spots at 254 nm. The calibration function of peak area against mass concentration of each pesticide was plotted. The regression lines, correlation

Table 2
Regression lines, correlation coefficients limit of detection and linear functional correlations of pesticides studied

Pesticide	Regression line ($y=ax+b$)	Correlation coefficient	Limit of detection (absolute)	Linear functional correlation
α-Cypermethrin	$y=15251.9x+1984.3$	0.992	0.1 μg	0.5–25 μg
Tetramethrin	$y=7610.5x+1076.5$	0.994	0.2 μg	1–50 μg
Diflubenzuron	$y=110768.4x+24768.4$	0.991	2 ng	0.01–2 μg
Chlorpropham	$y=21681.1x+1635.7$	0.985	0.1 μg	0.25–10 μg
Atrazine	$y=44891.8x+2287.9$	0.990	50 ng	0.2–10 μg
Propham	$y=19081.9x+1472.6$	0.991	50 ng	0.2–10 μg

Table 3
Recoveries of pesticides obtained by ultrasonic extraction (1 extraction step, 20 ml of solvent, 15 min) with various organic solvents

Solvent	Sample recovery, % ($n=5$)					
	α-Cypermethrin	Tetramethrin	Diflubenzuron	Chlorpropham	Atrazine	Propham
Diethyl ether	75.9±3.2	70.5±2.8	80.5±2.9	67.3±5.3	78.0±1.5	21.4±1.0
Chloroform	83.2±3.5	82.5±4.1	90.2±3.7	62.1±4.9	77.2±1.4	29.4±1.5
Hexane	66.7±2.7	78.0±3.5	63.0±2.6	60.1±4.8	66.9±1.2	36.4±1.8
Benzene	77.0±3.4	42.0±1.8	90.1±3.6	72.2±5.6	64.2±1.0	27.2±1.4
Acetonitrile	69.4±2.9	ND	79.1±3.1	ND	71.2±1.3	42.3±2.1
Dichloromethane	76.8±3.3	78.2±3.7	91.0±4.1	81.3±6.3	89.2±1.8	50.3±2.5
Acetone	97.2±4.4	83.4±4.2	92.8±4.0	93.6±7.9	103.5±2.8	79.7±6.3

ND=not detected due to degradation.

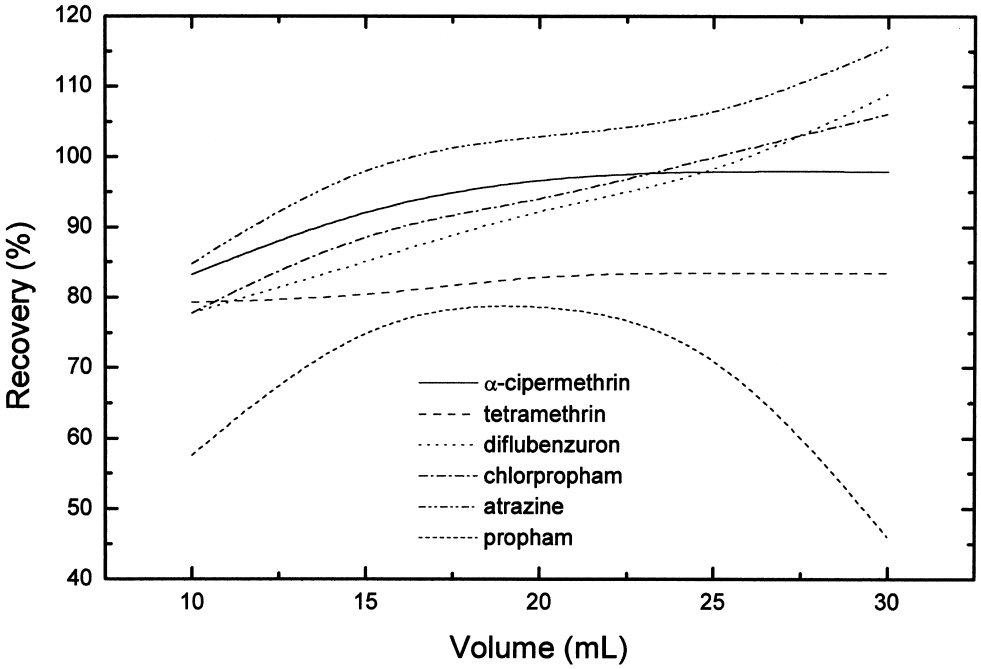


Fig. 1. Recovery of pesticides as a function of the volume of acetone, $t_{sonication}=15$ min.

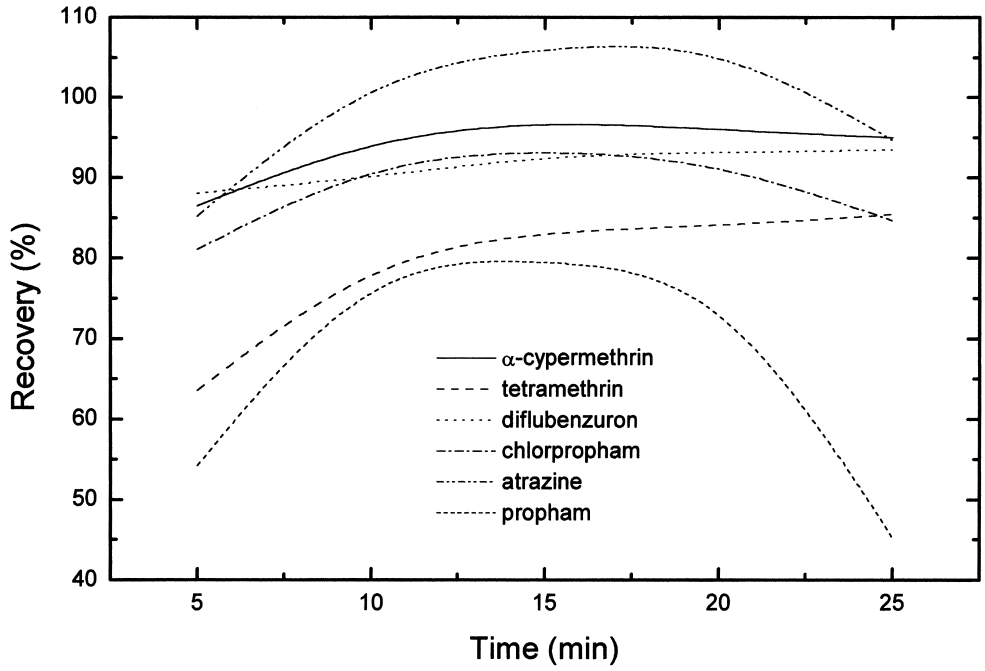


Fig. 2. Recovery of pesticides as a function of the duration of sonication, $V_{acetone}$=20 ml.

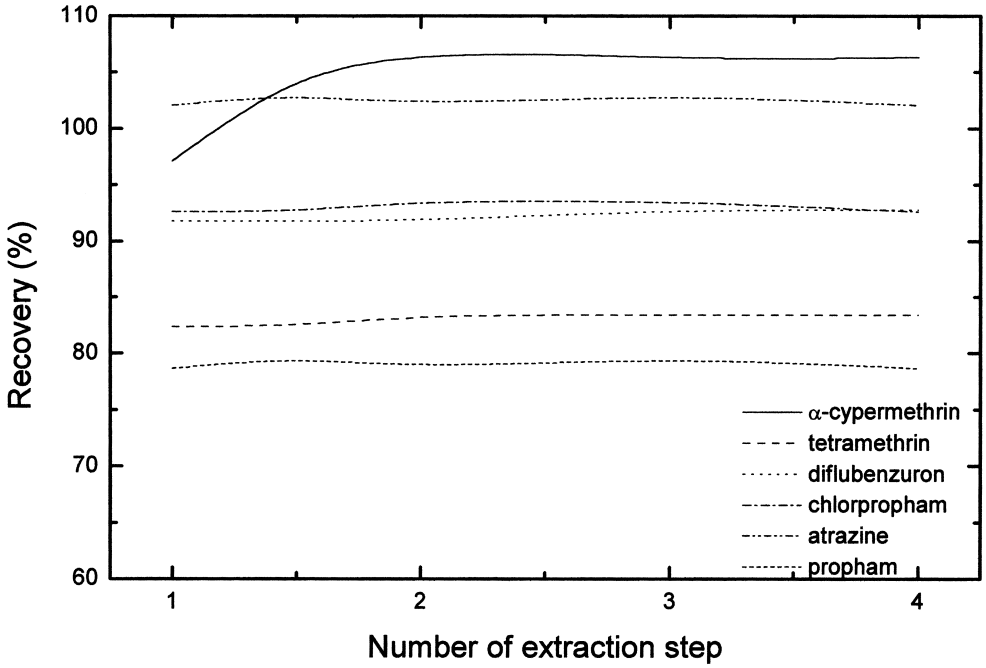


Fig. 3. Recovery of pesticides as a function of the number of extraction steps, $V_{acetone}$=20 ml, $t_{sonication}$=15 min.

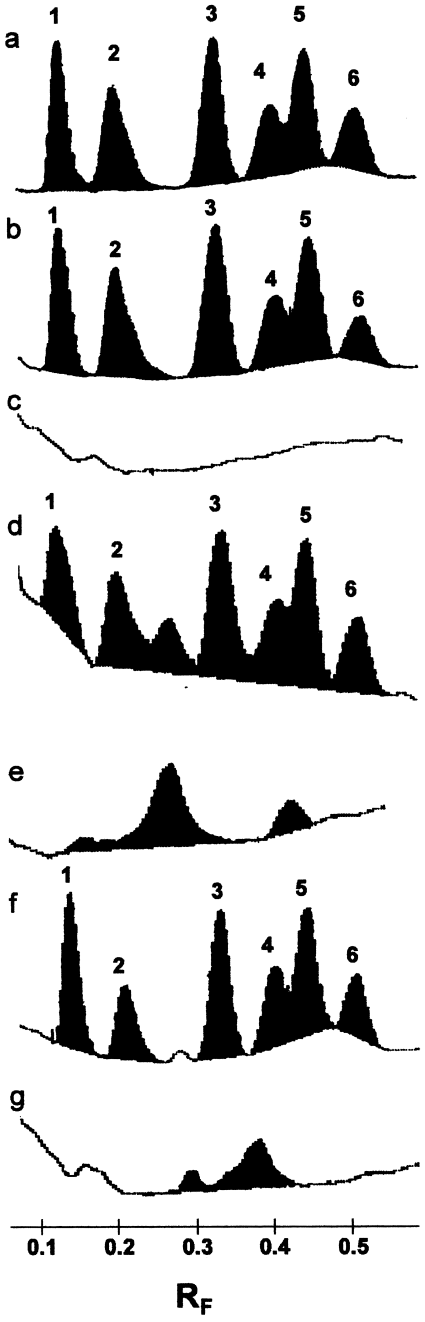


Fig. 4. Chromatogram of pesticide mixture: (a) standard solution, (b) pesticide mixture ultrasonically extracted from spiked soil (20 ml of acetone, 15 min), (c) blind extract from ultrasonic extraction, (d) pesticide mixture extracted from spiked soil by Soxhlet, (e) blind extract from Soxhlet extraction, (f) pesticide mixture extracted from soil by shake-flask extraction, (g) blind extract from shake-flask extraction; Pesticides: 1=α-cypermethrin, 2=tetramethrin, 3=diflubenzuron, 4=chlorpropham, 5=atrazine, 6=propham.

coefficients, limit of detection and linear functional correlations are summarized in Table 2.

In case of the analyte being present in low concentration in complex sample, such as soil or biological plant material, extraction and concentration procedures must usually precede the TLC. In this work the ultrasonic solvent extraction was used as a simple and inexpensive method applicable to the wide range of environmental samples. Pesticides were extracted from soil by various organic solvents with a broad range of polarity. Ultrasonic extraction efficiency of each solvent was checked by recovery experiments. The results are summarized in Table 3. The results show that ultrasonic extraction using acetone gives the highest recovery rates for most pesticides.

The goal of optimization procedure was to improve the extraction efficiency with minimum solvent consumption and minimum time needed for the extraction procedure. This goal was achieved by changing the solvent amount, the duration of sonication and the number of extraction steps (Figs. 1–3).

The best recovery of pesticides from spiked soil samples is obtained with 20 ml of acetone in one extraction step for 15 min. Longer sonication caused a decrease in recovery of pesticides, probably due to the degradation of the compounds.

Recovery of extracted pesticides obtained by ultrasonic solvent extraction was compared with those obtained by shake-flask extraction and Soxhlet extraction (Table 4).

The chromatograms of standard pesticide mixture, samples extracted by ultrasonic solvent extraction, shake-flask and Soxhlet extraction and blind extracts are shown in Fig. 4.

The lowest recoveries were achieved by shake-flask extraction. Extremely high yield and more than six peaks were detected in the extracts from Soxhlet extraction method. Some peaks were detected in the blind extracts of shake-flask and Soxhlet extraction. This suggested that the samples extracted by shake-flask or Soxhlet extraction method cannot be chromatographed without an additional clean-up step.

4. Conclusion

The results obtained indicate that the ultrasonic

Table 4
Comparison of the recoveries ($n=5$) obtained by the traditional and the ultrasonic solvent extraction methods

Pesticide	Recovery (%)		
	Ultrasonic solvent extraction[a]	Soxhlet extraction	Shake-flask extraction
Atrazine	103.5±2.8	201.9±14.6	108.3±6.2
Propham	79.7±6.3	143±18.6	65.1±9.3
Chlorpropham	93.6±7.9	155.6±20.4	88.1±10.0
α-Cypermethrin	97.2±4.4	128.4±16.4	90.1±9.1
Tetramethrin	83.4±4.2	64.3±16.0	52.0±8.3
Diflubenzuron	92.8±4.0	182.5±17.4	98.1±8.9

[a] 20 ml of acetone, 15 min.

solvent extraction method is applicable to extraction of pesticides from soil. The ultrasonic solvent extraction is more rapid than conventional shake-flask or Soxhlet extraction methods, and the solvent consumption is significantly lower. Additionally, the extracts from sonication can be chromatographed without subsequent clean-up step, and the analysis time is considerably reduced.

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