

LOW COST MONITORING METHOD FOR ORGANOPHOSPHORUS AND CARBAMATE PESTICIDE RESIDUES DETERMINATION

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ABSTRACT

A simplified method for organophosphorus and carbamate pesticides residue determination in some agricultural samples is detailed. Ethyl acetate was used as an extraction solvent eliminating the laborious and costly clean up step. Since ethyl acetate is a polar solvent, the efficiency of the extraction of pesticide residues from samples was significantly increased. The rotary evaporator used in the concentration step was also modified to prevent loss of pesticide residues. The recovery study was performed on various troublesome samples for pesticide analysis such as garlic and tobacco, showing an excellent recovery ranging from 83 to 107%. Compared with classical methods, the described procedure requires less labour, is simple and low in cost.

RESUMEN

Se describe un método simplificado para la determinación de residuos de plaguicidas organofosforados y carbamatos en algunas muestras agrícolas. El acetato de etilo fue utilizado como disolvente de extracción, eliminando el costoso y laborioso paso de purificación. Debido a que el acetato de etilo es un disolvente polar, la eficiencia de extracción de los residuos de los plaguicidas de las muestras se incrementó significativamente. Así mismo, el rotaevaporador empleado durante la concentración fue modificado con el fin de prevenir la pérdida de residuos de plaguicidas. El estudio de recuperación fue realizado en muestras calificadas como problemáticas en el análisis de plaguicidas, tales como ajo y tabaco, mostrando una excelente recuperación que varió de 83 a 107%. Comparado con los métodos clásicos, este procedimiento es simple, menos laborioso y de bajo costo.

INTRODUCTION

Organophosphorus and carbamate pesticides have played a major role in the improvement of agricultural production and in the control of many disease vectors in the area of public health (Iwata *et al.* 1985, Forget 1991, Pimentel 1992). More than 100 organophosphorus and carbamate compounds have been considered pesticides (Worthing and Hance 1991). In México, 46 organophosphorus and 8 carbamate insecticides have been approved for agricultural use and sanitary actions (CICOPLAFEST 1994).

Although pesticides have increased crop yields and provide a considerable benefit in the protection of agriculture

production, their residues may appear in different food products and thereby represent a potential health risk to consumers (Ferrer and Cabral 1991, Torres *et al.* 1996). Pesticide residue monitoring studies are important to determine their environmental pollution level and to evaluate the potential toxicological risk for human health (Hotchkiss 1992). Analytical methods used in pesticide residue monitoring programs should be capable of detecting the residues below the maximum residue limits (MRL) established by several government regulations with reduced analysis time and cost (Chin 1992). The development of rapid and efficient extraction techniques that minimize time required and the use of expendable material, especially adsorbents, could enhance

residue monitoring protocols (Torres *et al.* 1996).

Pesticide residue analytical procedures developed and used during previous decades require several purification and trace enrichment steps in order to quantitatively separate pesticides from coextracted compounds of sample origin. Liquid-liquid partitioning extraction and adsorption column chromatography are generally used for these purposes, however, these techniques are very laborious, costly and present many difficulties in the recovery of polar pesticides (Ferreira and Silva 1980, Specht and Tillkes 1980, Lores *et al.* 1987, Sasaki *et al.* 1987, Tonogai *et al.* 1990, AOAC 1991, Odanaka *et al.* 1991).

This paper describes a simple analytical method for the extraction and screening of organophosphorus and carbamate pesticides used in agriculture by introducing ethyl acetate as an extraction solvent, thereby eliminating the clean-up step in sample preparation before gas chromatographic analysis. This low cost method provides a rapid organophosphorus and carbamate pesticide screen for monitoring studies.

MATERIALS AND METHODS

Experimental

The ethyl acetate used for analysis was fractionally distilled and gas chromatographically tested for the presence of interfering compounds. Anhydrous sodium sulfate was analytical grade and heated overnight at 650°C before use. All analytical equipment was made of glass to prevent contamination by undesirable interfering substances such as plasticizers and phthalate esters. Glassware was washed with a chromic mixture, rinsed with distilled water and finally with distilled acetone.

The qualitative and quantitative analyses were carried out on a gas chromatograph Varian model 3300 equipped with term-specific detector (TSD) and a 200 cm x 2 mm id. glass column packed with 6% OV-210 on Gas Chrom Q 80-100 mesh. The operating conditions were as follows: temperature of detector base 270°C, injection port 250°C and column 95 to 230°C depending on the volatility of the compounds studied; nitrogen at 30 mL/min as a carrier gas and 1 µL volume of

injection.

For analysis, one hundred grams of sample was weighed in a wide-necked flask, 150 mL of ethyl acetate was then added and left for 30 minutes to macerate. The sample was homogenized with an Ultra-turrax homogenizer and the liquid phase was filtered through a layer of sodium sulfate. The extraction was repeated twice with 100 mL of ethyl acetate. The combined ethyl acetate extracts were evaporated to about 5 mL in a rotary evaporator with a water bath at a temperature below 40°C. The concentrated extract was quantitatively transferred with ethyl acetate into a calibrated tube of 10 mL adjusting the final volume to 10 mL with ethyl acetate.

The sample obtained during the extraction step was rotary evaporated to a small volume. The tube, which passes the air through the condenser to release the vacuum into the round bottomed flask with the extract, was removed from the rotary evaporator. The direct spout of air which causes the loss of pesticides was eliminated, thereby improving the recovery rates as shown in fortification studies.

Statistical analysis

The results were analyzed by Minitab statistical program to determine mean recoveries and standard deviations.

RESULTS AND DISCUSSION

Recovery studies

To determine the quality of the method, the recovery study was performed on ten overspiked blank samples. The recovery study was carried out considering adsorption and binding processes occurred in biological samples. The samples were fortified with 1 mL of standard solution in acetone sprayed on them. The wide-necked flask with the sample was then plugged with an aluminum foil stopper and shaken for one hour in order to promote binding of pesticides to the sample matrix. Thereafter, the extraction procedure was performed. The fortification levels were ten times over the detection limits. The results are summarized in **tables 1** and **2**. Some gas chromatograms of analyses performed are shown as examples

TABLE I. MEAN AND STANDARD DEVIATION (%) OF FORTIFIED SAMPLES WITH DIFFERENT PESTICIDES AT 0.05 mg/kg (n = 10)

SAMPLE	METHYL	ETHYL	DIAZINON	MALATHION	DIMETHOATE	METHAMIDOPHOS
	PARATHION	PARATHION				
	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$
apple	96.2 ± 6.4	95.3 ± 6.1	96.7 ± 4.2	95.8 ± 3.9	97.6 ± 4.4	94.7 ± 5.2
carrot	93.1 ± 7.8	88.9 ± 6.7	92.7 ± 6.1	97.3 ± 4.5	92.8 ± 6.3	90.9 ± 6.7
celery	92.8 ± 4.3	93.6 ± 5.1	91.3 ± 6.4	97.2 ± 3.8	95.4 ± 4.7	90.8 ± 6.3
cauliflower	92.3 ± 4.8	94.3 ± 5.9	92.8 ± 4.7	94.9 ± 3.9	91.6 ± 5.7	91.2 ± 6.7
lettuce	92.5 ± 6.7	98.8 ± 7.3	89.9 ± 7.2	95.3 ± 4.7	97.5 ± 5.9	92.9 ± 7.1
onion	93.7 ± 5.9	92.7 ± 6.2	93.4 ± 5.8	92.8 ± 4.1	96.1 ± 5.3	91.7 ± 5.8
potato	95.4 ± 5.3	93.7 ± 6.9	94.8 ± 5.9	92.7 ± 4.9	96.8 ± 7.2	92.8 ± 5.9
strawberry	94.8 ± 5.1	93.2 ± 5.7	95.9 ± 5.3	97.4 ± 4.2	96.3 ± 6.1	92.1 ± 6.1
tomato	91.9 ± 6.8	95.3 ± 4.9	96.1 ± 4.9	97.1 ± 5.3	94.2 ± 5.6	94.7 ± 5.4

TABLE II. FORTIFICATION LEVELS (mg/kg), MEAN AND STANDARD DEVIATION (%) OF SAMPLES FORTIFIED WITH SEVERAL ORGANOPHOSPHORUS AND CARBAMATE PESTICIDES

PESTICIDE	SAMPLE	FORTIFICATION	RECOVERY
		LEVELS	$\bar{X} \pm SD$
Aldicarb	soil	0.046	94.8 \pm 6.4
Aldicarb sulfoxide	soil	0.048	90.6 \pm 8.9
Aldicarb sulfone	soil	0.051	92.8 \pm 8.7
Aldicarb	sugar beets	0.046	93.1 \pm 7.2
Aldicarb sulfoxide	sugar beets	0.048	90.3 \pm 8.8
Aldicarb sulfone	sugar beets	0.051	92.0 \pm 7.9
Dichlofuanid	strawberries	0.25	95.8 \pm 3.6
DMSA*	strawberries	0.11	93.1 \pm 5.1
Acephate	tobacco	1.0	96.4 \pm 4.0
Metamidophos	tobacco	0.6	107.4 \pm 3.9
Terbufos	tobacco	0.3	82.7 \pm 4.5
Triazophos	garlic	0.047	90.5 \pm 6.5

* DMSA = Dichlofuanid metabolite: dimethylphenylsulfamid

in figures 1 and 2.

The results of recovery obtained ranged from 90 to 107%, with exception of Terbufos with 82.7%, and standard deviation values were below 10 indicating good reproducibility and precision of this procedure.

Method comparison

Several methods have been recommended for the analysis of organophosphorus and carbamate pesticides. Recoveries

of the analytical method carried out by Torres *et al.* (1996) ranged from 67 to 102% and standard deviations from 2 to 10%. Solvents employed such as acetone, N,N-dimethylformamide and methanol produced large quantities of interferences as evidenced by the number and intensity of peaks recorded by GC-analysis of the extracts. C₁₈ and silica gel were used in the clean-up step. Recoveries of pesticides in agricultural products performed by Sakai *et al.* (1994), ranged from 46.7 to 120% with coefficient of variation of 0.1

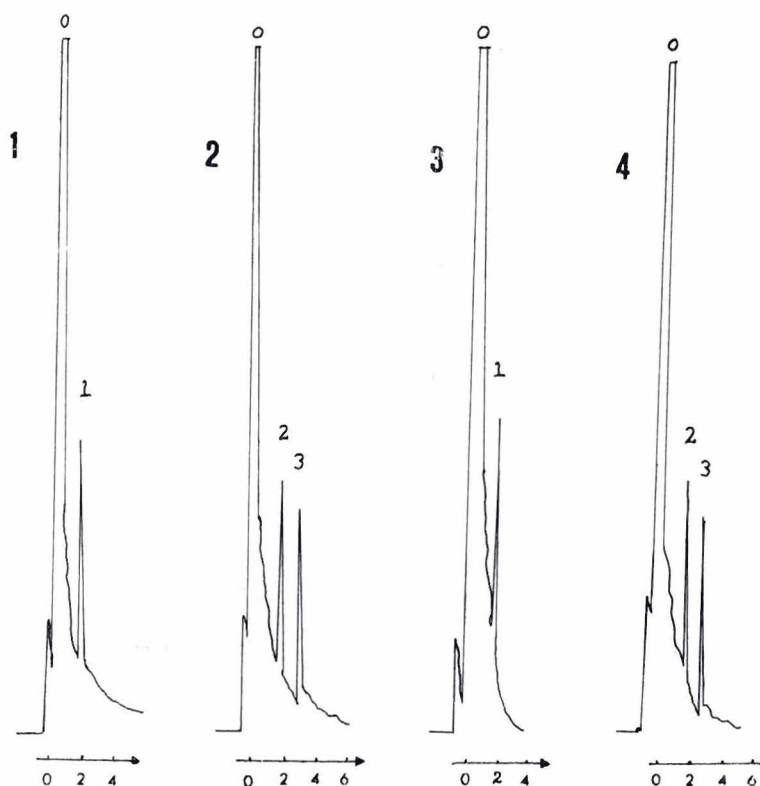


Fig. 1. Gas chromatograms of different samples: 1, soil: 0, solvent; 1, 0.46 ng of Aldicarb. 2, soil: 0 solvent; 2, 4.8 ng of Aldicarb sulfoxide; 3, sugar beet: 0, solvent; 1, 0.46 ng of Aldicarb. 4, sugar beet: 0, solvent, 2, 4.8 ng of Aldicarb sulfoxide; 3, 5.1 ng of Aldicarb sulfone

to 14.9%, by using acetone and dichloromethane. Holstege *et al.* (1994) extracted the pesticides from plant samples with 5% ethanol in ethyl acetate (v/v) and subsequent clean-up steps with gel permeation chromatography and silica gel minicolumns; recoveries ranged from 77 to 113%. The multiresidue method developed by Luke (Luke *et al.* 1981, Luke and Doose 1983, Luke and Masumoto 1987) eliminated the clean-up step and used acetone as solvent extraction, and recoveries ranged from 85 to 109%.

The results of recovery and standard deviation obtained in this study demonstrated that by using ethyl acetate, the clean-up steps of extracts could be eliminated, the extraction efficiency for pesticide residue analysis enhanced and the extraction of interfering compounds are reduced. This procedure is simple, rapid and inexpensive compared to other methods where the clean-up steps on different adsorption materials make them costly and time consuming (Viersino *et al.* 1971, Greve and Grevenstuk 1974, Stijve 1976, Ault *et al.* 1979, Specht and Tillkes 1980, Lores *et al.* 1987, Hopper 1988, AOAC 1991).

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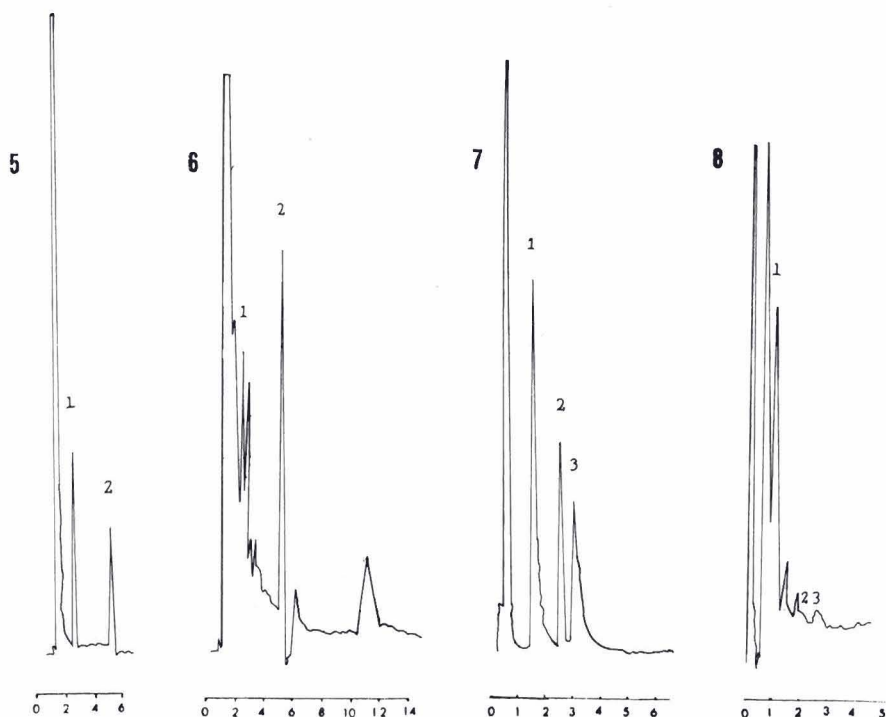


Fig. 2. Gas chromatograms of: 5, standard solution of 10.6 ng of Dimethylphenylsulfamide (DMSA) (1), and 24.8 ng of Dichlofluanid (2). 6, strawberry sample, 1, DMSA 0.09 ppm, 2, Dichlofluanid 0.85 ppm. 7, standard solution, 1, 0.25 ng of Methamidophos, 2, 0.1 ng of Terbufos, 3, 0.8 ng of Acephate. 8, tobacco sample containing 1, 0.21 ppm of Methamidophos, 2, 0.01 ppm of Terbufos and 3, 0.13 ppm of Acephate

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