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Small-scale multi-residue method for the determination of organochlorine and pyrethroid pesticides in vegetables

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ABSTRACT

A simple and inexpensive multi-residue method is described for the determination of organochlorine and pyrethroid pesticides in vegetables. Pesticides in vegetables were extracted with ethanol and partitioned into toluene. A mini-column packed with 0.5 g of Florisil was used for further clean-up prior to gas chromatographic determination. The detection limits were 0.02–0.05 μg/g without concentrating the extract, which are below the maximum residue limits set by the Singapore government. The recoveries of the pesticides from fortified samples were 65–97% at the 0.1 μg/g level and 87–114% at the 0.5 μg/g level. The amounts of the reagents required for analysing one sample are only 100 ml of ethanol, 6 ml of toluene and 0.5 g of Florisil. Among fifteen vegetable samples collected from the Singapore local market and were analysed by this method, five were found to contain detectable amount of organochlorine pesticides. One sample contained 22 μg/g of endosulfan but the residue levels in other four samples were below 1 μg/g.

INTRODUCTION

A multi-residue method is required for a survey of pesticide residues in vegetables produced in Singapore and neighbouring countries. General methods for the determination of pesticide residues in plant samples require several hundred millilitres of organic solvents for one sample [1,2], leading to high analysis costs. Since the early 1980s, several methods that consume much less solvents have been reported. Consumption of solvents is considerably reduced by simplifying the analytical procedures [3] or by miniaturizing the scale of conventional methods [4–8]. Miniaturization is achieved either by replacing liquid–liquid extraction with solid-phase extraction [4] or by replacing the conventional column chromatographic clean-up with small cartridge clean-up [5–8]. As most of the reported small-scale methods for plant samples deal with only three to five pesticides [5–7], modification is required to obtain a small-scale method that can analyse for more pesticides simultaneously. In this work, we evaluated a small-scale multi-residue method for the determination of thirteen organochlorine and pyrethroid pesticides in vegetables. The method uses less toxic solvents and the solvent consumption is considerably decreased by adopting small-scale partitioning and mini-column clean-up. Some vegetable samples collected from the local market were analysed by this method.

EXPERIMENTAL

Materials

Technical-grade toluene and ethanol were redistilled before use. Florisil particle size 0.15–0.25 mm was heated at 400°C for 12 h and then deactivated by mixing with 4% (w/w) distilled

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water. Pesticide standards were of purity above 96% and were dissolved in toluene to prepare standard solutions. The chromatographic mini-column (12 × 0.5 cm I.D.) was of a similar size to a Pasteur pipette. An HP-5890A gas chromatograph (Hewlett-Packard) equipped with an electron-capture detector and a Megabore HP-5 column (12 m × 0.53 mm I.D., 2.65 μm film thickness) was used for the residue analysis.

Methods

A vegetable sample (60 g) was blended for 6 min with ethanol (100 ml) and extract was filtered through a Büchner funnel by suction. An aliquot of the filtrate (6 ml) was mixed with toluene (4 ml) in a 20-ml test-tube by bubbling the liquid for 3 min using a Pasteur pipette. The test-tube was allowed to stand for 20 min to obtain phase separation. The lower, ethanol layer was removed using a Pasteur pipette. The toluene layer was washed with saturated sodium sulphate solution (5 ml) by mixing the two phases using a Pasteur pipette. The upper, toluene layer was transferred to a chromatographic mini-column packed with Florisil (0.5 g) followed by anhydrous sodium sulphate (1 g). The column was eluted with toluene until 5 ml of the eluate had been collected. The eluate was analysed by gas chromatography under following conditions: injection port temperature, 270°C; detector temperature, 280°C; column temperature, programmed from 160°C (held for 10 min) to 235°C at 2°C/min; carrier gas, nitrogen; flow-rate, 13 ml/min.

Recovery study

Measured amounts of 10 μg/ml pesticide solution were added to 60 g of finely chopped vegetable in the blender jar to final concentrations of 0.1 and 0.5 μg/g (wet mass). The fortified sample was well mixed and blended for 6 min with 100 ml of ethanol. The extract was partitioned, cleaned up and measured gas chromatographically following the above procedures. The moisture content (W_m) of the vegetable was determined by heating 20 g of chopped vegetable at 105°C for 4 h and measuring the mass before and after the heating. The amount of the vegetable equivalent to 1 ml of extract (W_{eq}) was calculated using the equation

$$W_{eq} = 60/(100 + 60W_m)$$

If the moisture content is 90%, 6 ml of extract will be equivalent to 2.3 g of vegetable sample.

Sample analysis

Vegetable samples were purchased from a wholesale centre near the University. Most of the vegetables sold in Singapore are imported and redistributed at this centre. Some of the collected samples, such as lettuce, spring onion, spinach, leaf mustard and celery, were from the Cameron Highlands, West Malaysia, whereas cabbage mustard was from Johor, Malaysia.

The samples were analysed following the proposed procedures. The analysis of each sample was unreplicated. The identification of the chromatographic peaks was based on a comparison of the retention times with those for known compounds. Because a GC–MS system for confirmation was not available, samples with detectable amounts of pesticides were also analysed using an HP Ultra-2 capillary column (25 m × 0.32 mm I.D.). In addition, the samples were also examined using flame ionization detection (FID). The identity of each peak on the chromatogram with electron-capture detection (ECD) was further confirmed by comparing the response change between unknown and known samples where the detection, method was changed from ECD to FID.

RESULTS AND DISCUSSION

Evaluation of the method

Under the GC conditions, all the pesticides studied were well separated from each other on the wide-bore column (Fig. 1). Fenvalerate gave two overlapped peaks because of its geometric isomers. Its quantification was based on the sum of the heights of the two peaks. In addition to the wide-bore HP-5 column, an Ultra-2 capillary column (25 m × 0.32 mm I.D., 0.52 μm film thickness) was also tested. Although the Ultra-2

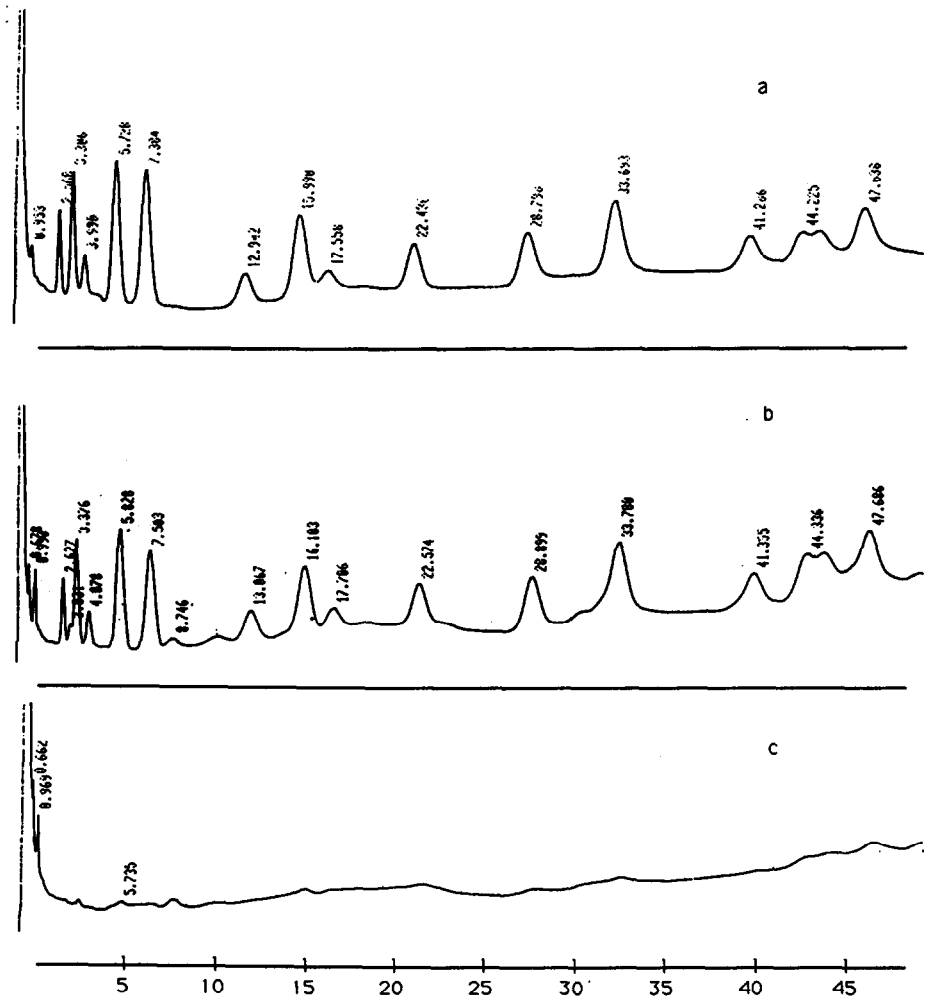

Fig. 1. Chromatograms of (a) standard solution containing α-BHC (2.6 min), γ-BHC (3.3 min), heptachlor (5.7 min), aldrin (7.4), α-endosulfan (12.9 min), DDE (16.0 min), endosulfan (17.6 min), DDT (22.4 min), biphenthrin (28.8 min), cyhalothrin (33.7 min), cypermethrin (41.3 min), fenvalerate (44.2 min) and deltamethrin (47.6 min) (0.1 ng each), (b) cabbage mustard fortified with pesticides at the 0.1 μg/g level and (c) cabbage mustard without adding pesticides. Injection volume: 2 μl. Time scale in min.

column gave better separations and peak shapes for the pesticides, the reproducibility of the analysis was much better when the wide-bore column was used. Therefore, in subsequent analyses the widebore column was used for quantitative analysis and the capillary column was used for confirmation.

The results of the recovery study are given in Tables I and II. The recoveries from Chinese cabbage were 65–95% at the 0.1 μg/g level and 87–113% at the 0.5 μg/g level. For cabbage mustard, the recoveries were 82–164% at the 0.1 μg/g level and 75–92% at the 0.5 μg/g level. The relative standard deviations were generally less than 10%. The recovery of fenvalerate from cabbage mustard at the 0.1 μg/g level was extraordinarily high (164%). A similar phenomenon has been reported with some organophosphorous pesticides [9,10], and the higher recoveries were attributed to matrix-induced re-

TABLE I

RECOVERIES OF PESTICIDES FROM CHINESE WHITE CABBAGE AND THE MAXIMUM RESIDUE LIMITS OF THE PESTICIDES IN LEAFY VEGETABLES SET BY THE SINGAPORE GOVERNMENT

Pesticide	Recovery (%)[a]		MRL[b] (mg kg^{-1})
	0.1 μg/g level	0.5 μg/g level	
α-BHC	79 ± 4	91 ± 9	
γ-BHC	81 ± 3	94 ± 10	0.5
Heptachlor	78 ± 4	89 ± 8	0.05
Aldrin	78 ± 3	91 ± 8	
α-Endosulfan	77 ± 9	92 ± 4	
β-Endosulfan	95 ± 3	112 ± 7	2
DDE	70 ± 3	91 ± 5	
DDT	69 ± 6	87 ± 21	0.2
Biphenthrin	80 ± 8	102 ± 4	
Cyhalothrin	67 ± 4	101 ± 7	
Cypermethrin	68 ± 8	110 ± 18	1
Fenvalerate	95 ± 11	113 ± 26	5
Deltamethrin	65 ± 4	104 ± 23	0.1

[a] Mean ± S.D. for three replicates.
[b] The MRL for DDT includes DDE and the MRL for endosulfan includes the two isomers and the corresponding sulphates.

sponse enhancement, in which the matrix protects the analytes from adsorption and/or decomposition in the hot injector [11]. In the present instance, the high recovery of fenvalerate was obtained only at low residue levels (0.1 μg/g). Therefore, the high recovery is probably due to the effects of some co-extractives with similar retention times. When the residue level of fenvalerate was very low, these effects became very significant.

TABLE II

RECOVERIES OF PESTICIDES FROM CABBAGE MUSTARD

Pesticide	Recovery (%)[a]	
	0.1 μg/g level	0.5 μg/g level
α-BHC	84 ± 4	81 ± 7
γ-BHC	85 ± 3	84 ± 6
Heptachlor	96 ± 1	83 ± 8
Aldrin	82 ± 2	85 ± 5
α-Endosulfan	112 ± 5	87 ± 8
β-Endosulfan	145 ± 4	92 ± 17
DDE	93 ± 2	79 ± 6
DDT	91 ± 5	76 ± 10
Biphenthrin	121 ± 3	85 ± 4
Cyhalothrin	115 ± 3	79 ± 7
Cypermethrin	115 ± 15	75 ± 11
Fenvalerate	164 ± 13	80 ± 11
Deltamethrin	123 ± 8	74 ± 9

[a] Mean ± S.D. for three replicates.

Typical chromatograms of fortified vegetable samples and blank vegetable samples are shown in Fig. 1. The results show that the clean-up procedure can remove most of the co-extractives affecting the GC determination. The detection limits calculated from the chromatograms of fortified samples were 0.02–0.05 μg/g, without concentrating the eluates. These detection limits are below the maximum residue limits (MRL) of the pesticides set by the Singapore government (Table I).

Compared with existing small-scale methods for plant samples, the present procedures are more similar to those reported by Joia *et al.* [6], except that the concentration step is omitted in the present method and the eluent benzene is replaced with the less toxic toluene. Joia's *et al.* method and the present method use Florisil mini-columns for clean-up, whereas other small-

TABLE III

ANALYTICAL RESULTS FOR COMMERCIAL VEGETABLE SAMPLES

Sample No.	Vegetable type	Pesticide detected	Residue level[a] (μg/g)
1	Chili (*Capsicum annum* var. *longum*)	α-Endosulfan	0.13
		β-Endosulfan	0.10
		DDE	0.29
2	Chinese boxthorn (*Lysium chinense*)		N.D.
3	Chinese celery (*Apium graveolens*)	α-BHC	0.12
		γ-BHC	0.82
		α-Endosulfan	0.57
		β-Endosulfan	0.16
		DDT	0.09
4	Chinese celery		N.D.
5	Chinese white cabbage (*Brassica chinensis*)		N.D.
6	Chinese white cabbage		N.D.
7	Chinese mustard (*Brassica juncea* var. *rugosa*)	α-Endosulfan	0.03
		β-Endosulfan	0.05
8	Choi-sam (*Brassica chinensis* var. *parachinensis*)	α-BHC	0.06
		γ-BHC	0.51
		α-Endosulfan	17.4
		β-Endosulfan	5.2
		DDT	0.09
9	Choi-sam		N.D.
10	Cowpea (*Vigna sinensis*)		N.D.
11	Cabbage mustard (*Brassica alboglabra*)		N.D.
12	Cabbage mustard		N.D.
13	Lettuce (*Lactuca sativa*)		N.D.
14	Spinach (*Spinacea oleracea*)		N.D.
15	Spring onion (*Allium fistulosum*)	α-Endosulfan	0.73
		β-Endosulfan	0.23

[a] N.D. = Residue level is below the detection limit (0.02–0.05 μg/g).

scale methods use cartridges prepacked with chemically bonded adsorbents. As this kind of cartridge is sometimes not affordable or not readily available in many Asian countries, a method that uses Florisil mini-columns is more practicable.

Analysis of vegetable samples

Among the fifteen vegetable samples collected and analysed, five were found to contain organochlorine pesticides (Table III). The pesticides found were endosulfan, benzene hexachloride (BHC), DDT and DDE. The residue levels were below 1.0 μg/g, except for one sample, which contained 22 μg/g of endosulfan. Such a high residue level could have been due to the short interval between spraying and harvest. No pyrethroid pesticides were found in any of the samples. It should be pointed out that the method used in this work is not sufficient for positive confirmation, although it can give reliable results for negative confirmation. Other methods, such as GC–MS, are required to produce more reliable results for the pesticides detected in the five samples.

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