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Multi-residue matrix solid-phase dispersion method for the determination of six synthetic pyrethroids in vegetables followed by gas chromatography with electron capture detection

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Abstract

An effective multi-residue matrix solid-phase dispersion (MSPD) extraction and gas chromatographic–electron-capture detection method for the determination of six synthetic pyrethroids (fenpropathrin, cyhalothrin, permethrin, cypermethrin, fenvalerate and deltamethrin) in 5 g of vegetables (West Indian gherkin, eggplant, pak-choi, cabbage and garden peas) is described. The method uses a Florisil-based MPSD column for direct in-line clean-up with *n*-hexane–acetone (9:1). Recoveries calculated at 0.1 and 0.5 μg/g fortification levels were between 92 and 113%. The method detection limits were between 5.1 and 91.5 ng/g. The method compared favourably with the traditional method in terms of the sample size, analysis time and overall cost. The method may serve as a screening protocol for the determination of pyrethroids in vegetables.

1. Introduction

Synthetic pyrethroids are increasingly being used for insect control on field crops because of their advantageous environmental properties such as short field life and relatively low mammalian toxicity [1,2]. Nevertheless, pyrethroid residues in vegetables after application to the crops still pose risks to human health and other species. Therefore, monitoring of pyrethroid residue levels in vegetables is of particular concern for human health.

Current pyrethroid residue screening methods usually require extraction with a polar solvent, followed by liquid–liquid partitioning. The sample is concentrated by evaporation, often with a solvent exchange, and subjected to clean-up before the final determination. Extraction solvents such as acetonitrile [3,4], acetone [5,6], *n*-hexane–acetone (9:1) [7,8], acetone–methanol [9], cyclohexane–chloroform (4:1) [10] and ethanol [11] have been used for pyrethroid analysis. The clean-up is generally based on column chromatography using sorbents such as Florisil [3,6,11], a mixture of active carbon, magnesia and diatomaceous earth [5], silica gel [8] and

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active acidic alumina [7,9]. Final determinations are carried out using gas chromatography with electron-capture detection (GC–ECD) [3,5, 9,11,12], liquid chromatography [8,12], thin-layer chromatography [7], differential-pulse polarography [10] and gas chromatography–mass spectrometry (GC–MS) [4,6].

The general drawbacks, such as the use of large amounts of solvent, the occurrence of troublesome emulsions with certain vegetables and slowness associated with liquid–liquid extraction [3,5–9] are avoided by using solid-phase extraction (SPE) cartridges [4,10]. SPE is a simple and rapid technique in comparison with liquid–liquid extraction and offers the advantages of a shorter analysis time, the consumption of smaller volumes of organic solvents and lower cost [13–15]. However, large amounts of solvent are still consumed during the extraction step. Appropriate treatment and activation of the sorbents are also mandatory.

The aim of this work was to develop an SPE-like method to overcome the above shortcomings. The method described in this paper is based on a recently developed multi-residue extraction technique called matrix solid-phase dispersion (MSPD) [16–19] to separate simultaneously six synthetic pyrethroids (fenpropathrin, cyhalothrin, permethrin, cypermethrin, fenvalerate and deltamethrin) in the vegetables West Indian gherkin, eggplant, pak-choi, cabbage and garden peas. These pyrethroids are among the most commonly used pyrethroids for insect control on vegetable fields in Taiwan. MSPD extracts trace organic compounds from homogeneously dispersed solid matrices by adsorbing them on suitable solid adsorbents, followed by desorption with a small amount of organic solvent. In-line clean-up or further purification is feasible by the simultaneous use of a co-column packed with a clean-up sorbent. Existing MSPD methods were mostly developed for biological matrices of tissues [17,18]. To our knowledge, this work represents the first attempt to extend the application fields of MSPD to vegetable samples. The method was applied to some vegetable samples obtained from a local market.

2. Experimental

2.1. Materials

All solvents used were of HPLC grade from Tedia (Fairfield, OH, USA). Standard pyrethroids with purity >99% were obtained from Dr. S. Ehrenstorfer (Augsburg, Germany). Standard calibration mixtures containing 100 μl of 3.9 ppm 2,4,6-tribromobiphenyl (from UltraScientific, North Kingstown, RI, USA) as an internal standard were prepared by diluting a stock standard solution in acetone. C_{18} (40 μm, Superclean LC-18) and Florisil (60–100 mesh) were obtained from Supelco (Bellefonte, PA, USA). Neutral alumina (70–230 mesh, activity I) was obtained from Merck (Dermstadt, Germany). Vegetable samples (West Indian gherkin, eggplant, pak-choi, cabbage and garden peas) used for blank, fortified and real studies were obtained from a local market. The C_{18} material was prewashed by continuously refluxing it in *n*-hexane–acetone (1:1) for 24 h in a Soxhlet apparatus followed by oven drying at 150°C for 1 h. Florisil and neutral alumina were activated by oven drying at 150°C for 12 h before use.

2.2. Preparation of sample extracts

The percentage of moisture in the vegetables was determined by placing 50 g of homogeneously mixed vegetable sample in an ignited and weighed beaker, drying at 110°C for 24 h and weighing the cooled beaker. The moisture content was calculated using moisture (%) = (g of as-received sample − g of oven-dried sample) /(g of as-received sample) · 100.

A 100-g amount of vegetable sample was chopped in a food chopper and 5 g of chopped and homogeneously mixed sample were weighed into a glass mortar. For the preparation of fortified samples, 50 μl of 50 (or 100) ppm stock standard solution were added to different portions of the sample to produce sample equivalents of 0.1 (or 0.5) μg/g, respectively. An 8-g portion of Florisil was then added. The Florisil–vegetable mixture was gently blended in the

mortar for ca. 2 min using a glass pestle with a clockwise circular motion to yield a semi-dry, homogeneous-appearing material. The homogeneous mixture was placed in a glass column (30 cm × 15 mm I.D.) containing a Pyrex frit, 1 cm of anhydrous sodium sulfate and 0.5 g of Florisil packed at the bottom. The additional 0.5 g of Florisil was used to trap possible co-eluting interfering compounds. The column was lightly tapped to remove air pockets, then tightly compressed to ca. 13 cm using a syringe plunger. Finally, a 0.1–0.2-cm layer of anhydrous sodium sulfate was placed at the top of the column.

A 500-ml round-bottomed flask was positioned below each column to collect the eluate. The column was eluted with 100 ml of eluent by gravitational flow. Eluting efficiencies were studied using ethyl acetate, *n*-hexane–acetone (4:1) and *n*-hexane–acetone (9:1) as eluents. The flow usually ceased after 30 min. The eluate was then transferred into a concentration tube, concentrated using a rotary vacuum evaporator and finally purged with nitrogen to a volume of less than 1.0 ml. A 100-μl volume of 3.9 ppm tribromobiphenyl was added as an internal standard. For the analysis of real samples, the optimum combination of a Florisil co-column and 60 ml of *n*-hexane–acetone (9:1) eluent was used. The final volume of the eluate was adjusted to 1.0 ml and subjected to GC–ECD analysis. Pyrethroid residues detected in real samples were confirmed by GC–MS with selected ion monitoring (SIM).

2.3. Apparatus

GC–ECD analyses were carried out using an HP-5890 Series II gas chromatograph equipped with a J & W DB-5MS capillary column (30 m × 0.25 mm I.D., 0.25-μm film thickness) and a ^{63}Ni electron-capture detector. Samples were introduced into the GC column via an on-column injector system. The injector temperature was programmed similarly to the column, except that it was maintained 3°C higher. The detector was operated at 320°C. Helium and nitrogen were used as the carrier and make-up gas at flow-rates of 1 and 70 ml/min, respectively. The column temperature was initially held at 60°C, then programmed at 30°C/min to 220°C and at 3°C/min to 300°C.

GC–MS analyses were carried out using a Shimadzu QP-1000 EX mass spectrometer equipped with a Shimadzu GC-14A gas chromatograph. The injector was operated in the splitless mode at 250°C. The effluent from the GC column was transferred via a transfer line held at 250°C and fed into a 70 eV electron impact ionization source held at 250°C. Helium was used as the carrier gas at a flow-rate of 1 ml/min. The column type and column temperature used were the same as those used in GC–ECD.

3. Results and discussion

3.1. Sample pretreatment and clean-up

The MSPD method used in this study differs from traditional methods in that the sample is dispersed over a large surface area on Florisil by the mechanical shearing force of blending. Polar materials such as chlorophylls, triglycerides and phytosterols, which are the common components in vegetables [20], are associated with the surface of the Florisil. This is ascribed to the lipid-absorbing capacity of the activated magnesium silicate solid support that might mimic the lipid-solubilizing character of the octadecylsilane liquid phase used in MSPD. The hydrophobic pyrethroids remain weakly bound on the adsorbent surface. These combined effects assist the unfolding and dispersion of the sample matrix. Subsequent elution using a solvent of high pyrethroid solubility could effectively extract the weakly bound pyrethroids from the adsorbent. However, certain co-extracted compounds might interfere. These interferences were minimized in this study via the use of an appropriate solvent system and clean-up adsorbent. Because the analytes and the matrices involved in this study are different from those in the previous studies [16–19], the need for a new

dispersed adsorbent, eluting solvent and clean-up adsorbent was expected and found to be necessary for the best recoveries of the determined pyrethroids.

The moisture content in vegetables is usually higher than that in biological tissues. To facilitate the preparation of a semi-dry and homogeneously appearing sample, the approximate moisture content in the vegetable is preferably known in advance. The moisture contents were found to be 96% in West Indian gherkin, 94% in eggplant, pak-choi and cabbage and 91% in

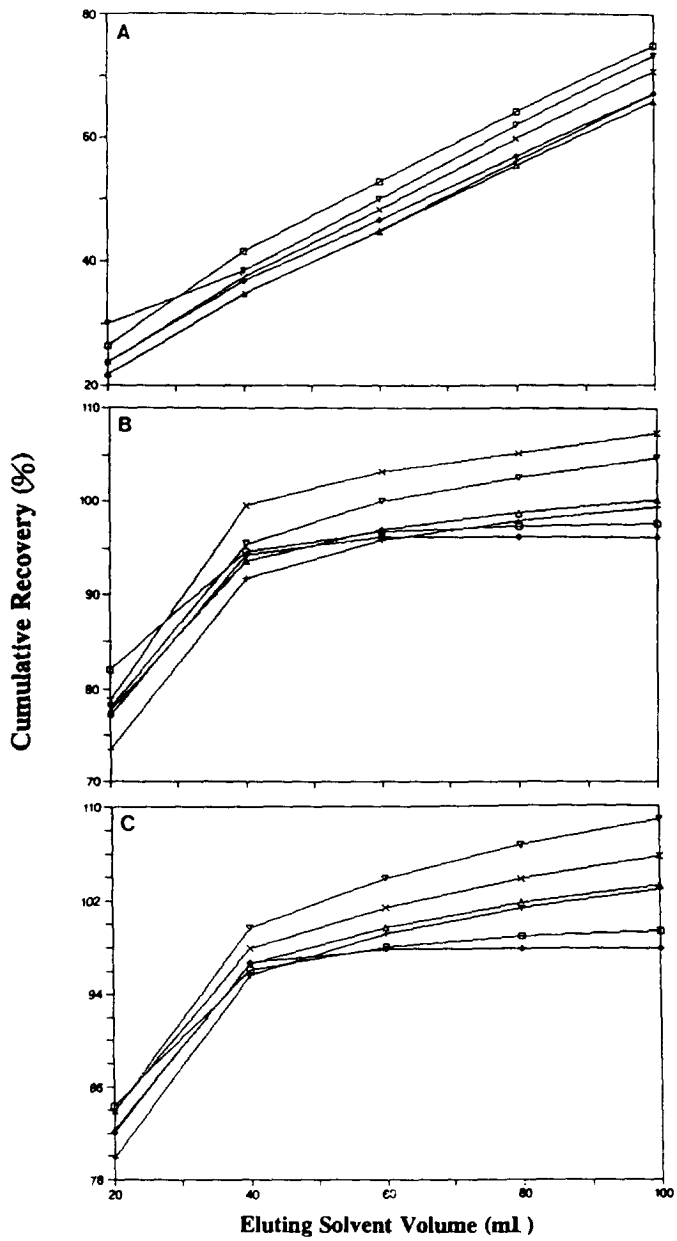

Fig. 1. Cumulative recoveries of the six pyrethroids in West Indian gherkin fortified at 2 μg/g as a function of eluent volume. Eluents: (A) ethyl acetate, (B) *n*-hexane–acetone (4:1) and (C) *n*-hexane–acetone (9:1). ◇ = Permethrin; △ = cypermethrin; × = fenvalerate; □ = fenpropathrin; + = cyhalothrin; ▽ = deltamethrin.

Table 1
Percentage recoveries and method detection limits for the six pyrethroids in fortified West Indian gherkin, eggplant, pak-choi, cabbage and garden peas

Sample	Concentration (μg/g)	Fenpropathrin	Cyhalothrin	Permethrin	Cypermethrin	Fenvalerate	Deltamethrin
West Indian gherkin	0.1[a]	106 ± 4	102 ± 5	95 ± 5	113 ± 5	101 ± 4	104 ± 5
	0.5[b]	102 ± 2	96 ± 3	99 ± 2	104 ± 2	98 ± 2	99 ± 2
	MDL[c](ng/g)	11.3	14.3	14.8	14.2	12.1	15.2
Eggplant	0.1[a]	104 ± 2	100 ± 2	96 ± 4	100 ± 2	98 ± 2	102 ± 6
	0.5[b]	102 ± 4	97 ± 3	100 ± 4	100 ± 3	96 ± 3	102 ± 3
	MDL[c](ng/g)	5.1	6.5	12.1	7.6	6.0	18.3
Pak-choi	0.1[a]	101 ± 3	102 ± 3	101 ± 6	96 ± 2	97 ± 2	100 ± 3
	0.5[b]	96 ± 2	98 ± 2	101 ± 1	94 ± 2	96 ± 2	94 ± 2
	MDL[c](ng/g)	10.2	8.4	17.4	6.6	7.3	9.2
Cabbage	0.1[a]	104 ± 5	101 ± 2	95 ± 6	94 ± 3	97 ± 2	137 ± 6
	0.5[b]	97 ± 2	97 ± 2	100 ± 2	93 ± 2	95 ± 2	99 ± 4
	MDL[c](ng/g)	16.3	7.6	17.7	9.9	7.0	89.2*
Garden peas	0.1[a]	100 ± 2	98 ± 3	96 ± 3	213 ± 16	97 ± 3	102 ± 3
	0.5[b]	95 ± 3	92 ± 4	95 ± 2	118 ± 4	95 ± 3	98 ± 2
	MDL[c](ng/g)	6.1	8.8	9.9	91.5*	9.3	10.9

[a] $n = 7$.
[b] $n = 4$.
[c] Values without asterisks, 3.14 times the standard deviation of seven replicate analyses of vegetable fortified at the 0.1 μg/g level. Values with asterisks, 4.52 times the standard deviation of four replicate analyses of vegetable fortified at the 0.5 μg/g level.

garden peas. Using 5 g of West Indian gherkin, with the highest moisture content, the amount of dispersed adsorbent needed to prepare a semi-dry sample was 30 g of anhydrous sodium sulfate, 20 g of C_{18}, 8 g of Florisil or 12 g of neutral alumina. In the initial study, it was observed that most of the chlorophylls could be retained on Florisil or neutral alumina, but not on anhydrous sodium sulfate or C_{18}. Because the cost of the Florisil used per run was less than that of neutral alumina, we selected Florisil as the dispersed adsorbent. To ensure the complete removal of the chlorophyll, an additional 0.5 g of Florisil was packed below the Florisil-blended sample in the MSPD column. No visible colour was observed in the eluate. The interfering compounds appeared to be trapped by the Florisil as a green colour developed in the underlying Florisil during elution.

The effect of eluting solvent and its volume was studied by collecting each pyrethroid in every 20-ml fraction during elution using a sample fortified at 2 μg/g. The cumulative recoveries on eluting with 100 ml of ethyl acetate, *n*-hexane–acetone (4:1) and *n*-hexane–acetone (9:1) are shown in Fig. 1. The best recoveries were obtained with *n*-hexane–acetone (4:1) (Fig. 1B) and *n*-hexane–acetone (9:1) (Fig. 1C). The recovery for each pyrethroid increased rapidly to 92% after elution with 40 ml of eluent. The recovery then increased slowly with increasing eluent volume and reached an equilibrium value with 60 ml of eluent. The eluent volume used for subsequent studies was therefore set at 60 ml. Considering the potential of eluting the retained polar chlorophyll and other polar interfering compounds with the more polar *n*-hexane–acetone (4:1), we selected *n*-hexane–acetone (9:1) as the eluent for subsequent studies.

3.2. Quantification

The fortification levels used in this study were selected because they covered the ranges of tolerance levels (0.1–0.5 μg/g) set by the ROC Department of Health [21]. The recoveries and detection limits using pyrethroid-fortified vegetables at levels of 0.1 and 0.5 μg/g are summa-

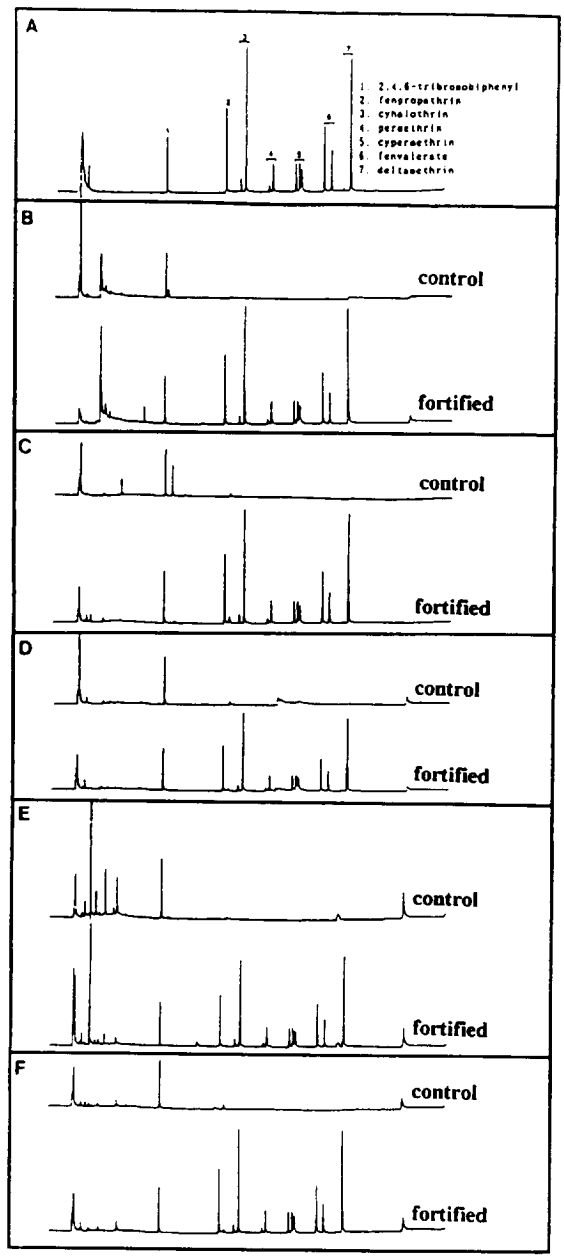

Fig. 2. Representative gas chromatograms from (A) the standard and (B–F) control and fortified vegetable samples of (B) West Indian gherkin, (C) eggplant, (D) garden peas, (E) cabbage and (F) pak-choi.

rized in Table 1. The recoveries were between 92 and 113%. The reproducibility expressed as standard deviation was between 1 and 6%. Representative GC chromatograms from the standard, control and fortified samples of each vegetable are shown in Fig. 2. No attempt has been made to assign isomer identities to the peaks. Several small interfering peaks appeared in the chromatogram of West Indian gherkin, eggplant and pak-choi (Fig. 2B, C and F); these peaks were located outside the retention time window of the analytes and did not jeopardize subsequent quantification work. In the chromatogram of cabbage (Fig. 2E), small interfering peaks appeared in the retention time window of deltamethrin. This might explain the unusually high recovery (137%) of deltamethrin in cabbage sample fortified at the 0.1 μg/g level. This interference became insignificant in samples fortified at the 0.5 μg/g level as the recovery decreased to 99%. The interfering peaks located in the retention time window of cypermethrin in the chromatogram of garden peas (Fig. 2D) were more visible. This might explain the unusually high recoveries (213 and 118%) of cypermethrin sample fortified at both the 0.1 and 0.5 μg/g level. Therefore, the method detection limits (MDLs) for deltamethrin in cabbage and cypermethrin in garden peas were calculated at the 0.5 μg/g level. Other MDLs shown in Table 1 were calculated at the 0.1 μg/g level. The MDLs thus obtained were between 5.1 and 91.5 ng/g and were below the tolerance levels (0.1–0.5 μg/g) set by the ROC Department of Health [21]. The

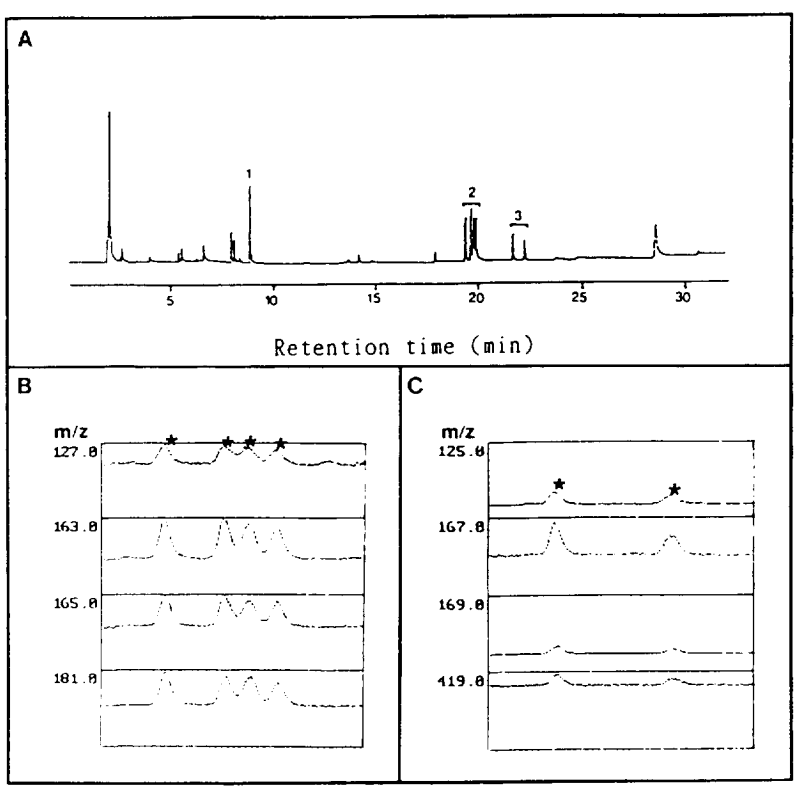

Fig. 3. (A) Gas chromatogram (peaks: 1 = 2,4,6-tribromobiphenyl; 2 = cypermethrin; 3 = fenvalerate) of a cabbage sample with an identifiable level of cypermethrin and fenvalerate, and corresponding mass chromatograms obtained from GC–MS-SIM analysis for (B) cypermethrin and (C) fenvalerate.

results indicate that the proposed MSPD method yields satisfactory extraction and determination of pyrethroids in vegetable samples.

The proposed MSPD method was applied to the determination of pyrethroids in vegetables obtained from a local market. Among the ten vegetable samples, pyrethroid residues were detected in one cabbage sample and confirmed by GC–MS-SIM (Fig. 3). They were 0.58 μg/g of cypermethrin and 0.18 μg/g of fenvalerate, respectively. The characteristic masses [m/z with relative abundance (%) in parentheses] used were 163 (100), 181 (88), 165 (67), and 127 (32) for cypermethrin and 125 (100), 167 (86), 169 (45), and 419 (28) for fenvalerate.

4. Conclusions

The proposed MSPD method can be readily applied to the extraction of six synthetic pyrethroids (fenpropathrin, cyhalothrin, permethrin, cypermethrin, fenvalerate and deltamethrin) in 5 g of vegetables (West Indian gherkin, egg plant, pak-choi, cabbage, and garden peas). The method uses a Florisil-based MSPD column for direct in-line clean up with n-hexane–acetone (9:1). The procedures are simple and rapid and require only small amount of samples and solvents. The method may serve as a screening protocol for the determination of pyrethroids in vegetables.

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