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Comparative determination of multi-pesticide residues in *Pimpinella anisum* using two different AOAC methods

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Abstract

Chlorinated hydrocarbons, pyrethroids, organophosphorus, and other miscellaneous pesticides in dried and powdered fruits of *Pimpinella anisum* were analyzed using two official Association of Official Analytical Chemists methods as a general guidance, and applying Florisil column cleanup. Both methods applied proved to show adequate recovery, repeatability and reproducibility for most of the studied pesticides. But no sole method was able to extract all pesticides residues from real samples. Some pesticides were not recovered sufficiently from the *P. anisum* fortified samples. The applied methods were not suitable for the extraction of tetramethrin, formothion, pyrazophos and primicarb. Tetramethrin, formothion and pyrazophos showed very low recoveries with the mentioned methods while primicarb was not recovered at all.

Keywords: Pesticide residues; *Pimpinella anisum*; Florisil cleanup

1. Introduction

Pesticide residues are found in almost all food items, in grains, fruits, vegetables, milk, oils, eggs and fish (FDA, 2000). Even the so-called ''organically raised crops'' does not necessarily mean ''pesticide free''. Medicinal plants are liable to contain pesticide residues, which accumulate from agricultural practices, such as spraying, treatment of soils during cultivation, and administration of pesticides during storage (De Smet, Keller, Hänsel, & Chandler, 1992). Medicinal plants are commonly used worldwide for a variety of purposes; as food, spice, flavoring agent and/or to prepare herbal teas which are consumed as beverages or in some cases to treat minor ailments. It is therefore recommended that every country producing medicinal plant materials (naturally grown or cultivated) should have at least one control laboratory capable of performing the determination of pesticides in accordance with the guidelines of the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO) (WHO, 1998).

Pimpinella anisum L., Family: Umbelliferae, [Anise] is an annual herb, native to the Mediterranean region and Egypt. Fresh leaves may be used in salads. The dried ripe fruits of anise, commercially called aniseeds, have been highly valued since antiquity. They contain volatile oils (14%), fixed oil, coumarins, flavonoids, choline, carbohydrates (50%) and mucilage (Newall, Anderson, & Phillipson, 1996; Tyler, Speedie, & Robbers, 1996). Aniseeds are used extensively as a spice, the volatile oil is widely used for flavoring breads, candies, cakes desserts, in non-alcoholic beverages and in some liqueurs. It is listed by the council of Europe as a natural source of food flavoring. In the USA, anise is listed as Generally Regarded As Safe (GRAS) (Newall et al., 1996). Anise is stated to possess expectorant, antispasmodic, carminative, and parasiticidal properties and has long been used for relieving colic in children, as well as an intestinal purifier (Tyler et al., 1996). It has been reputed to increase milk secretion,

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Table 1
Groups of the pesticides and the purity of their standards

Pesticide[a]	Purity	Concentration[b] (μg/ml)
1. Chlorinated hydrocarbons		
HCB	99.7%	0.02
α-HCH	97.5%	0.01
β-HCH	98.5%	0.02
γ-HCH	99.4%	0.05
Quintozene	99.8%	0.05
Chlorothalonil	98.5%	0.2
Dicofol	97.5%	0.1
trans-Chlordane	100%	0.03
o,p-DDE	97.5%	0.03
p,p-DDE	99.3%	0.03
Dieldrin	96%	0.03
Endrin	98%	0.03
o,p-DDT	97%	0.2
p,p-DDT	99.3%	0.05
β-Endosulfan	97.5%	0.1
Bromopropylate[c]	99.7%	0.1
Tetradifon	99.1%	0.05
2. Pyrethroids		
Fenpropathrin	92%	0.1
Tetramethrin	100%	0.5
Permethrin	97.5%	0.5
Cypermethrin	94.8%	0.3
Deltamethrin	99.6%	0.4
3. Miscellaneous		
Folpet	99%	0.5
Vinclozolin	97%	0.05
Procymidone	99%	0.1
4. Organophosphorus		
Methacrifos	95.5%	0.2
Formothion	100%	0.5
Chlorpyrifos	99.5%	0.2
Bromophos	99.9%	0.2
Phosalone	98%	0.5
Pyrazophos	99.7%	0.3
5. Carbamates		
Primicarb	98.3%	0.2

[a] The pesticide names used here are as quoted in "The Pesticide Manual" (Worthing, 1991).
[b] Concentration of the prepared pesticide stock solutions.
[c] Bromopropylate is a Br-DDT analogue, in some references it is classified as a miscellaneous pesticide (WHO, 1998).

and facilitate birth (Blumenthal, Goldberg, & Brinckman, 2000; Newall et al., 1996).

The present study deals with the extraction of 33 pesticide residues belonging to five different groups of pesticides: CHs, OP pesticides, pyrethroids, carbamates and the miscellaneous pesticides (Table 1), from *P. anisum* using two different extraction methods followed by Florisil column cleanup. Both methods are multiclass multiresidue methods that are reported in the Pesticide Analytical Manual (PAM) of the FDA, and also in the Official Methods of Analysis of the AOAC.

2. Experimental

2.1. Reagents and equipments

Acetone, acetonitrile, dichloromethane (DCM), hexane and petroleum ether (PE), were all pesticide residue (PR) grade (Scharlau, Barcelona). Pesticide standards were purchased from Ehrenstorfer (Augusburg, Germany). Standards, their purity levels and groups to which they belong are listed in Table 1. Stock solutions of the individual pesticides (1000 μg/ml) were prepared and stored at −20 °C, except *trans*-chlordane which was purchased as a 10 μg/ml standard solution. Individual dilutions were prepared as needed and stored at 4 °C. Seven mixed standard solutions of the chlorinated hydrocarbons (CHs) (hexachlorobenzene (HCB), hexachlorocyclohexane (α, β and γ-HCH), quintozene, chlorothalonil, dicofol, *trans*-chlordane, *o,p*- and *p,p*-dichlorophenyl dichloroethylene (DDE), endrin, dieldrin, *o,p*- and *p,p*-dichlorophenyl trichloroethane (DDT), β-endosulfan, bromopropylate, tetradifon), pyrethroids (fenpropathrin, tetramethrin, permethrin, cypermethrin, deltamethrin) and miscellaneous pesticides (folpet, vinclozolin, penconazole, procymidone), and one mixed solution of the organophosphorus (OP) pesticides (methacrifos, formothion, chlorpyrifos, bromophos, phosalone and pyrazophos), and one carbamate pesticide (primicarb), were prepared with concentrations ranging from 0.01 to 0.5 μg/ml (Table 1), which were selected to suite the sensitivity of the detectors used. An internal standard solution of endrin (0.03 μg/ml) and bromophos (0.2 μg/ml) was also prepared and added to all pesticide standard mixed solutions, and to all extracts in the final step before gas chromatograph (GC) analysis. All pesticide standard solutions and dilutions were prepared in acetone–hexane (10:90, % v/v). Florisil 60–100 mesh (Aldrich, USA) was activated at 675 °C for 6 h in oven (Mod.N7/H, Nr.66341) (Naber, West Germany). Activated Florisil was stored in 500 ml glass flasks with glass stoppers and stored at 130 °C in a Memmert's oven (Schawbach, West Germany). Anhydrous sodium sulfate analytical reagent (AR) grade (Merck, Germany) was heated in oven at 130 °C for 5 h, and then was stored in 500 ml glass jars with glass stoppers in desiccator (Pragati, India). Additionally, sodium chloride AR grade (Nottengham, UK), filter paper Whatman (Cat. No. 1002 110) (Medicell International Ltd., UK) and cellulose extraction thimbles (30×150 mm) (Macherey-Nagel, Germany) were used.

The equipments used included a high-speed blender with a stainless steel jar (Moline, France), a shaking bath (Techne, England), a rotavapor, R110 (Büchi, Switzerland), a cooler circulator (Julabo, Germany), a soxhlet apparatus (Fortuna, Germany), a heating mantle (Electrothermal, England), and chromatographic tubes with Teflon stopcocks and course fritted glass

plate (22 mm i.d. × 300 mm) (Quickfit, England) and syringes (Hamilton Bonadus AG, Switzerland). All glassware were rinsed thoroughly using soap and deionized water, then washed with acetone and dried in oven (100–130 °C) over night.

2.2. Plant materials

Dried *P. anisum* samples (A1–A5) were obtained from five different herbal shops in Jordan and processed as described by Their and Zeumer (1987).

2.3. Chromatographic instrumentation

2.3.1. Determination of CHs, pyrethroids and miscellaneous pesticides

A HP-5890 series II GC equipped with a HP-608 capillary column (30 m, 0.53 i.d., 0.5 μm film thickness) with the stationary phase (50% – phenyl)-methylpolysiloxane, and ^{63}Ni electron capture detector (ECD). GC instrument was operated under the following conditions: injector in the split mode (split ratio 1:17), injector temperature 250 °C, detector temperature 300 °C, argon–methane (5:95, % v/v) as the carrier gas at a flow rate of 1 ml/min, and was also used as the make-up gas at a flow rate of 24 ml/min. Column temperature was initially held at 80 °C for 1 min, then increased by 30 °C/min to 180 °C, followed by 5 °C/min to 200 °C, and 10 °C/min to 280 °C and held for 14 min.

2.3.2. Determination of OP pesticides

A HP-5890 series II GC, equipped with a HP-1 capillary column (25 m, 0.2 mm i.d., 0.5 μm film thickness) with the stationary phase 100% dimethyl polysiloxane, and nitrogen phosphorus detector (NPD). The instrument was operated under the following conditions: injector in the split mode (split ratio 1:10), injector temperature 225 °C, detector temperature 280 °C, helium was the carrier gas with a flow rate of 1 ml/min, detector gas flow rates were 3–3.5 ml/min for hydrogen, and 100 ml/min for air. The column temperature was initially held at 90 °C for 2 min, then increased by 20 °C/min to 150 °C, followed by 6 °C/min to 270 °C and held for 15 min.

2.3.3. Confirmation of identity

A HP-5890 Series II GC, equipped with a HP-5 capillary column (30 m, 0.25 mm i.d., 0.25 μm film thickness) with the stationary phase 5% diphenyl and 95% dimethyl polysiloxane, and ^{63}Ni ECD. The instrument was operated under the following conditions: injector was operated in the split mode (split ratio 1:17), injector temperature 280 °C, detector temperature 300 °C, carrier gas was helium with a flow rate of 2 ml/min, make-up gas was argon–methane (5:95, % v/v) with a flow rate of 30 ml/min, column temperature was initially held at 80 °C for 2 min, then followed by 30 °C/min to 175 °C, and at 10 °C/min to 225 °C and held for 2 min, then at 20 °C/min to 280 °C and held for 10 min.

2.4. Sample preparation for GC analysis

2.4.1. Extraction and partitioning

(1) Method I: extraction with water/acetone, liquid–liquid partitioning in PE/DCM, which is known as AOAC method 985.22 and PAM method 302 (AOAC, 1995, Chap. 10; FDA, 1994).

(2) Method II: acetonitrile-PE partitioning known as AOAC acetonitrile partitioning cleanup method and PAM method 304 (AOAC, 1995, Chap. 10; FDA, 1994). This method was used in experiments with the oil extracted from *P. anisum*. The oil itself was extracted using a soxhlet extractor: 15–20 g of the thoroughly ground plant sample was weighed into an extraction thimble and mixed well with 10–20 g anhydrous sodium sulfate, covered with a piece of glass wool, and extracted with 250 ml PE for 3–5 h (40 °C). Evaporation of PE (30–40 °C) in a rotavapor was continued until complete dryness. The flask containing the residue was left in a desiccator for 6 h and weighed to get the lipid weight. Lipid weight was carefully measured to avoid overloading the capacity of the cleanup step.

2.4.2. Florisil column cleanup

Florisil column cleanup was conducted according to the AOAC method (1995, Chap. 10). 250 ml DCM-PE (20:80, % v/v), followed by 150 ml DCM, was used as elution solvent, which was a modification to the AOAC method. The eluate was evaporated, and before reaching complete dryness the solvent was exchanged using a few milliliters of *n*-hexane. The evaporation was continued until only a thin film of solvent was in the flask. The internal standard solution was used to dissolve the residue and to adjust the volume to 5 ml (final volume).

2.5. Determination of the retention times (t_R) and relative retention times

In order to determine the t_R for each individual pesticide, 1 μl of the 1.0 μg/ml pesticide solution was injected into the GC-column. Standard mixtures of the pesticides were prepared as listed in Table 1. One microlitre of each standard mixture was also injected into the GC-column. Pesticides were identified by comparing their t_R and relative retention times (RRTs) (Table 2).

2.6. Limits of detection

Limits of detection (LOD) of the used instruments, equipped with ECD and NPD, were determined for each pesticide by successive dilution of the standard mixed pesticide solution followed by injection into the GC-column several times. Serial dilution experiments

Table 2
Types of detectors, pesticides' retention times, relative retention times, and limits of detection

No.	Pesticide	Detector	t_R[a]	RRT[b]	LOD[c] (pg)
1[d]	Folpet	ECD	11.552	0.490	0.05
2	HCB	ECD	13.552	0.586	0.00086
3	α-HCH	ECD	14.453	0.612	0.0008
4	Quintozene	ECD	15.427	0.654	0.001
5	γ-HCH	ECD	16.029	0.679	0.0025
6	β-HCH	ECD	16.283	0.690	0.0022
7	Vinclozolin	ECD	17.309	0.734	0.003
8	Chlorothalonil	ECD	17.367	0.736	0.01
9	Dicofol	ECD	19.885	0.843	0.003
10	*trans*-Chlordane	ECD	20.896	0.886	0.003
11	Procymidone	ECD	21.128	0.895	0.003
12	*o*,*p*-DDE	ECD	21.434	0.908	0.004
13	*p*,*p*-DDE	ECD	22.090	0.936	0.0056
14	Dieldrin	ECD	22.401	O.949	0.006
15	Endrin	ECD	23.597	1.000	0.006
16	*o*,*p*-DDT	ECD	23.845	1.011	0.01
17	β-Endosulfan	ECD	24.160	1.024	0.01
18	*p*,*p*-DDT	ECD	24.835	1.053	0.01
19	Bromopropylate	ECD	26.331	1.116	0.01
20	Fenpropathrin	ECD	26.447	1.121	0.06
21	Tetramethrin	ECD	27.102	1.149	0.02
22	Tetradifon	ECD	29.029	1.230	0.005
23	Permethrin	ECD	31.708	1.344	0.06
24	Cypermethrin	ECD	34.785	1.474	0.07
			35.094	1.487	
			35.551	1.507	
25	Deltamethrin	ECD	47.622	2.018	0.1
26	Methacrifos	NPD	10.132	0.542	0.0075
27[e]	Formothion	NPD	15.387	0.822	0.019
28	Primicarb	NPD	15.640	0.836	0.01
29	Chlorpyrifos	NPD	18.080	0.966	0.024
30	Bromophos	NPD	18.710	1.000	0.5
31	Phosalone	NPD	26.320	1.407	0.1
		ECD	29.343	1.244	0.14
32	Pyrazophos	NPD	27.655	1.478	0.006

[a] t_R, Retention time.
[b] RRT, relative retention time = t_R(pesticide)/t_R(internal standard).
[c] LOD, limit of detection.
[d] Pesticides 1–26 are numbered according to sequence of elution from HP-608 GC-column on ECD.
[e] Pesticides 27–32 are numbered according to sequence of elution from HP-5 GC-column on NPD.

provided the necessary information to calculate the detection limits (Boyd-Boland & Pawliszyn, 1995; Lehotay & Valverde-García, 1997).

2.7. Recovery tests

The recovery test was evaluated with all 32 pesticides, for each residue analysis procedure. This was performed by spiking pesticide-free anise samples with a mixed pesticide solution in concentrations ranging from 0.01 to 0.5 μg/ml. The fortified samples were then extracted using either methods I or II, and cleaned up on a Florisil column. In order to evaluate the recoveries of each residue analytical procedure without being affected by interferences from the plant itself, spiking water and extraction thimble instead of plant samples were also performed (blank test). All tests were performed in triplicate. Recovery studies were performed at one concentration level, based on the maximum residue levels of these pesticides (Obana, Akutsu, Okihashi, & Hori, 2001).

2.8. Residue analysis

For residue analysis, the purchased samples (A1–A5) were ground mechanically and sieved through No. 60 mesh sieve. Samples were extracted according to both methods, I and II separately, and cleaned using Florisil column cleanup in both cases. Sample size ranged from 10 g when using method I, to 15–20 g when using method II.

3. Results and discussion

The limits of detection (Table 2) for GC–ECD and GC–NPD were between 0.0008 and 0.05 ppm for the

CHs and the miscellaneous pesticides; 0.02–0.1 ppm for pyrethroids and 0.006–0.5 ppm for OP pesticides.

The mean recoveries of the studied pesticides for both methods (I and II) without plant samples ranged from 72% to 120% for method I combined with Florisil cleanup (Table 3) except for deltamethrin (26%), formothion (14%) and pyrazophos (21%). In the case of method II combined with Florisil cleanup, the mean recoveries ranged from 73% to 115% (Table 4), except for deltamethrin (6%), formothion (18%) and pyrazophos (16%).

From the results above, it is revealed that three pesticides (tetramethrin, formothion and pyrazophos) showed low recovery in methods I and II when Florisil column cleanup was used. Formothion and pyrazophos are OP pesticides, relatively more polar than the CHs and pyrethroids. High polarity is reported to cause separation problems in solid phase extraction (SPE) using a very polar sorbent such as Florisil (Stefani, Buzzi, & Grazzi, 1997). The most common retention mechanisms in SPE is based on Van der Waals forces, hydrogen bonding, and dipole–dipole interaction. All three mentioned pesticides are able to form hydrogen bonding with Florisil, which might be one of the possible causes for the low recoveries of these compounds. Another possible cause for low recoveries could be the instability of these compounds on Florisil column. For example, in case of tetramethrin the low recovery with Florisil, a slightly basic material, might be explained on the basis of instability of this pyrethroid in alkaline conditions (Worthing, 1991).

Primicarb was not recovered at all whenever a Florisil column was used. It is reported that *N*-methyl carbamates are degraded on Florisil column (Fong, Moye, Seiber, & Toth, 1999).

No pesticide showed extreme high recovery (>120%), but some pesticides showed recoveries >100%. However, these recoveries (>100), although exceeding the maximum value stipulated by the European Pharmacopoeia (70–110%), are considered acceptable by other regulatory agencies (FAO/WHO, 1996).

Table 3
The spiked level of each pesticide, mean recovery, RSD, relative errors and total errors for method I and Florisil cleanup

No.	Pesticide	Added absolute amount (μg)	Mean recovery[a] (%) ± SD	RSD (%)	Relative error	Total error
1	Folpet	2.5	86 ± 2	3.2	14	20.4
2	HCB	0.1	107 ± 9.0	8.4	7	23.8
3	α-HCH	0.05	93 ± 1.4	1.5	7	10.0
4	Quintozene	0.25	95 ± 4.3	4.5	5	14.0
5	γ-HCH	0.25	100 ± 4.0	4.0	0	8.0
6	β-HCH	0.1	83 ± 1.4	1.7	17	20.4
7	Vinclozoline	0.25	94 ± 3.8	4.0	6	14.0
8	Chlorothalonil	1.0	80 ± 3.4	4.3	20	28.6
9	Dicofol	0.5	118 ± 5.0	4.2	18	26.4
10	*trans*-Chlordane	0.15	91 ± 4.1	4.5	9	18.0
11	Procymidone	0.5	101 ± 6.0	5.9	1	12.8
12	*o,p*-DDE	0.15	92 ± 4.0	4.3	8	16.6
13	*p,p*-DDE	0.15	93 ± 1.4	1.5	7	10.0
14	Deldrin	0.15	92 ± 3.2	3.5	8	15.0
15	Endrin	0.15	96 ± 9.0	9.4	4	22.8
16	*o,p*-DDT	1.0	113 ± 4.0	3.5	13	20.0
17	β-Endosulfan	0.5	95 ± 2.5	2.6	5	10.2
18	*p,p*-DDT	0.25	120 ± 7.0	5.8	20	31.6
19	Bromoprpylate	0.5	89 ± 4.2	4.7	11	20.4
20	Fenpropathrin	0.5	91 ± 9.2	10.1	9	29.2
21	Tetramethrin	2.5	26 ± 7.5	28.8	74	131.6
22	Tetradifon	0.25	88 ± 4.9	5.6	12	23.2
23	Permethrin	2.5	92 ± 3.0	3.3	8	14.6
24	Cypermethrin	1.5	92 ± 2.0	2.2	8	12.4
25	Deltamethrin	2.0	85 ± 2.5	2.9	15	20.8
26	Methacrifos	1.0	93 ± 2.3	2.4	7	11.8
27	Formothion	2.5	14 ± 1.6	11.4	86	108.8
28	Primicarb	1.0	Not recovered			
29	Chlorpyrifos	1.0	103 ± 6.4	6.2	3	15.4
30	Bromophos	1.0	111 ± 3.6	3.2	11	17.4
31	Phosalone	1.0	76 ± 2.0 (NPD)	2.6	24	29.2
			72 ± 1.2 (ECD)	1.7	28	31.4
32	Pyrazophos	1.5	21 ± 2.0	9.5	79	98.0

[a] Recovery % is the mean value of triplicates.

Table 4
The spiked level of each pesticide, mean recovery, RSD, relative errors and total errors for method II and Florisil cleanup

No.	Pesticide	Added absolute amount (μg)	Mean recovery[a] (%) ± SD	RSD (%)	Relative error	Total error
1	Folpet	2.5	79 ± 2.0	2.5	21	26.0
2	HCB	0.1	94 ± 3.6	3.8	6	13.6
3	α-HCH	0.05	101 ± 1.4	1.4	1	3.8
4	Quintozene	0.25	90 ± 5.9	6.5	10	23.0
5	γ-HCH	0.25	100 ± 3.6	3.6	0	7.2
6	β-HCH	0.1	80 ± 3.6	4.5	20	29.0
7	Vinclozoline	0.25	95 ± 4.4	4.6	5	14.2
8	Chlorothalonil	1.0	83 ± 3.4	4.1	17	25.2
9	Dicofol	0.5	108 ± 7.0	6.5	8	21.0
10	*trans*-Chlordane	0.15	91 ± 5.0	5.5	9	20.0
11	Procymidone	0.5	94 ± 9.9	10.5	6	27.0
12	*o*,*p*-DDE	0.15	115 ± 14.0	1.6	15	18.2
13	*p*,*p*-DDE	0.15	102 ± 10.7	10.5	2	23.0
14	Dieldrin	0.15	93 ± 4.8	5.2	7	17.4
15	Endrin	0.15	94 ± 3.5	3.7	6	13.4
16	*o*,*p*-DDT	1.0	113 ± 5.0	4.4	13	21.8
17	β-Endosulfan	0.5	102 ± 18.0	17.6	2	37.2
18	*p*,*p*-DDT	0.25	114 ± 3.5	3.1	14	20.2
19	Bromopropylate	0.5	81 ± 5.6	6.9	19	32.8
20	Fenpropathrin	0.5	86 ± 4.9	5.7	14	25.4
21	Tetramethrin	2.5	6 ± 1.5	26.7	94	147.4
22	Tetradifon	0.25	93 ± 4.2	4.5	7	16.0
23	Permethrin	2.5	92 ± 7.0	7.6	8	23.2
24	Cypermethrin	1.5	95 ± 2.2	2.3	5	9.6
25	Deltamethrin	2.0	85 ± 5.6	6.6	15	28.2
26	Methacrifos	1.0	105 ± 1.0	1.0	5	7.0
27	Formothion	2.5	18 ± 2.2	12.2	82	29.4
28	Primicarb	1.0	Not recovered			
29	Chlorpyrifos	1.0	87 ± 5.6	6.4	13	25.8
30	Bromophos	1.0	97 ± 3.5	3.6	3	10.2
31	Phosalone	1.0	84 ± 4.2 (NPD)	5	16	26.0
			73 ± 1.0 (ECD)	1.4	27	29.8
32	Pyrazophos	1.5	16 ± 5.0	31.3	84	146.6

[a] Recovery % is the mean value of triplicates.

The mean recoveries of pesticides from fortified plant samples were within 73–120%, when method I and Florisil cleanup was used (Table 5), with the exception of *p*,*p*-DDT which showed an extra high recovery (>150), which is most probably due to matrix interferences. Formothion (18%) and pyrazophos (30%) showed low recoveries that could be explained on the basis of their high polarity as discussed earlier. The interesting finding here is that the recovery of tetramethrin was 73%. This might be explained once again on the basis of polarity and interactions between analytes and coextractives from the plant with the adsorbent (Florisil), since aniseed is known to contain a variety of constituents (Newall et al., 1996), and the presence of coextractives with polarities higher than that of tetramethrin could have caused more preferential adsorption of these coextractives to Florisil, than tetramethrin, which might caused tetramethrin to elute from Florisil column in higher percentage. It is also possible that some constituents in the *P. anisum* extract might have protected tetramethrin from being degraded. When method II and Florisil cleanup was used with *P. anisum* samples, the mean recoveries were in the range 70–100% (Table 6) including these of *p*,*p*-DDT and tetramethrin, with the exception of formothion (14%) and pyrazophos (10%).

Two parameters were calculated in order to determine the accuracy and precision of the used methods: (1) Relative standard deviation (RSD), a measure of method's precision, RSD = SD/% Recovery × 100, (SD: standard deviation). (2) Relative error (RE), a measure of method's accuracy.

$$\mathrm{RE} = \frac{100 - \mathrm{Recovery} \times 100}{100}$$

Relative errors of 20% or less are considered satisfactory. When the best method available gives less than 80% recovery, it may still be used provided the percent recovery is reproducible. It is useful sometimes to calculate the method's total error where both RSD and RE are included: Total error = RE + 2RSD. Total errors tend to run high in trace analyses. A total error of <50% is considered good, 50–100% acceptable, and occasion-

Table 5
Recovery data in fortified *Pimpinella anisum* samples using method I and Florisil cleanup

No.	Pesticide	Added absolute amount (μg)	Mean recovery[a] (%) ± SD	RSD (%)	Relative error	Total error
1	Folpet	2.5	76 ± 2.8	3.7	24	31.4
2	HCB	0.1	105 ± 9.8	9.3	5	23.6
3	α-HCH	0.05	114 ± 7.1	6.2	14	26.4
4	Quintozene	0.25	97 ± 4.0	4.1	3	11.2
5	γ-HCH	0.25	95 ± 7.8	8.2	5	21.4
6	β-HCH	0.1	77 ± 1.3	1.7	23	26.4
7	Vinclozolin	0.25	92 ± 3.7	4.0	8	12.0
8	Chlorothalonil	1.0	81 ± 5.8	7.2	19	33.4
9	Dicofol	0.5	120 ± 4.3	3.6	20	27.2
10	*trans*-Chlordane	0.15	89 ± 5.9	6.6	11	24.2
11	Procymidone	0.5	90 ± 6.4	7.1	10	24.2
12	*o,p*-DDE	0.15	100 ± 5.7	5.7	0	11.4
13	*p,p*-DDE	0.15	114 ± 6.4	5.6	14	25.2
14	Deldrin	0.15	90 ± 5.4	6.0	10	22.0
15	Endrin	0.15	101 ± 3.2	3.2	1	7.4
16	*o,p*-DDT	1.0	109 ± 11.0	10.1	9	29.2
17	β-Endosulfan	0.5	84 ± 4.2	5.0	16	26.0
18	*p,p*-DDT	0.25	>150			
19	Bromopropylate	0.5	90 ± 5.0	5.5	10	21.0
20	Fenpropathrin	0.5	92 ± 6.1	6.6	8	21.1
21	Tetramethrin	2.5	73 ± 3.6	4.9	27	36.8
22	Tetradifon	0.25	89 ± 5.6	6.3	11	23.6
23	Permethrin	2.5	101 ± 8.9	8.8	1	18.6
24	Cypermethrin	1.5	109 ± 9.9	9.1	9	27.2
25	Deltamethrin	2.0	98 ± 2.8	2.9	2	7.8
26	Methacrifos	1.0	103 ± 4.0	3.9	3	10.8
27	Formothion	2.5	18 ± 1.0	5.5	82	93
28	Primicarb	1.0	Not recovered			
29	Chlorpyrifos	1.0	109 ± 5.0	4.6	9	18.2
30	Bromophos	1.0	107 ± 5.0	4.7	7	16.4
31	Phosalone	1.0	84 ± 7.5 (NPD)	8.9	16	33.8
			85 ± 5.0 (ECD)	5.9	15	26.8
32	Pyrazophos	1.5	30 ± 6.5	22.0	70	114

[a] Recovery % is the mean value of triplicates.

ally methods with >100% total error can still be usable if no better method exists (Fong et al., 1999; McFarren, Liskka, & Parker, 1970). Values of RSD, relative errors and total errors are listed in Table 3.

Pesticide residues present in the real samples were identified tentatively by comparing the RRTs of the sample peaks with RRTs of the injected standards. The identity of the found pesticides was confirmed using GC–ECD equipped with another column of a different polarity, namely HP-5. Pesticide residues were quantitated using the following equation:

$$C_{s\,(mg/kg\ plant)} = (A_s/A_{is} \times C_{st} \times 5\ ml \times F \times R)/(A_{st}/A_{ist} \times weight).$$

C_s, concentration of pesticide residues in sample in mg/kg dry plant material.

C_{st}, concentration of the pesticide in the mixed pesticide standard solution in μg/ml.

A_s, average peak area obtained for the pesticide found in sample.

A_{is}, average peak area obtained for the internal standard injected with the sample.

A_{st}, peak area obtained for the pesticide in the mixed-pesticide standard solution.

A_{ist}, peak area obtained for the internal standard found in the mixed-pesticide standard solution.

R, recovery factor calculated from 100/% recovery.

5, final volume (V_{final}) of the analyzed sample in ml.

F, extraction factor.

Aniseed contains 12% or even more fat, which was suspected to cause extraction problems. Therefore two different extraction procedures were tried with these samples. The findings of this study showed that no sole method was able to extract all pesticides from *P. anisum* samples (Table 7). And although recovery studies indicated that a certain pesticide is recovered by both methods to similar degrees, the analysis in real samples was different. Indicating that spiking is not similarly incorporated or bound, and thus provides no exact measure of extractability (Fong et al., 1999).

Table 6
Recovery data in fortified *Pimpinella anisum* samples using method II and Florisil cleanup

No.	Pesticide	Added absolute amount (μg)	Mean recovery[a] (%) ± SD	RSD (%)	Relative error	Total error
1	Folpet	2.5	78 ± 4.0	5.1	22	32.2
2	HCB	0.1	93 ± 9.8	10.5	7	28.0
3	α-HCH	0.05	75 ± 7.1	9.5	25	33.0
4	Quintozene	0.25	89 ± 6.8	7.6	11	26.2
5	γ-HCH	0.25	81 ± 7.8	9.6	19	38.2
6	β-HCH	0.1	71 ± 1.3	1.8	29	32.6
7	Vinclozolin	0.25	91 ± 5.3	5.8	9	20.6
8	Chlorothalonil	1.0	82 ± 6.2	7.6	18	33.2
9	Dicofol	0.5	70 ± 4.3	6.1	30	42.2
10	*trans*-Chlordane	0.15	91 ± 7.1	7.8	9	24.6
11	Procymidone	0.5	88 ± 9.1	10.3	12	32.6
12	*o*,*p*-DDE	0.15	100 ± 5.7	5.7	0	11.4
13	*p*,*p*-DDE	0.15	91 ± 6.4	7.0	9	23.0
14	Deldrin	0.15	90 ± 5.9	6.5	10	23.0
15	Endrin	0.15	90 ± 5.4	6.0	10	22.0
16	*o*,*p*-DDT	1.0	78 ± 11.0	14.1	22	50.2
17	β-Endosulfan	0.5	97 ± 7.8	8.0	3	19.0
18	*p*,*p*-DDT	0.25	70 ± 5.3	7.6	30	45.2
19	Bromopropylate	0.5	80 ± 7.9	9.9	20	39.8
20	Fenpropathrin	0.5	81 ± 9.1	11.2	19	41.4
21	Tetramethrin	2.5	70 ± 5.7	8.1	30	46.2
22	Tetradifon	0.25	90 ± 5.4	6.0	10	22.0
23	Permethrin	2.5	89 ± 6.3	7.1	11	25.2
24	Cypermethrin	1.5	90 ± 7.1	7.8	10	25.6
25	Deltamethrin	2.0	83 ± 5.1	6.1	17	29.2
26	Methacrifos	1.0	99 ± 6.7	6.8	1	14.6
27	Formothion	2.5	14 ± 7.2	51.4	86	188.8
28	Primicarb	1.0	Not recovered			
29	Chlorpyrifos	1.0	84 ± 8.1	9.6	16	35.2
30	Bromophos	1.0	95 ± 5.0	5.3	5	15.6
31	Phosalone	1.0	76 ± 7.2 (NPD)	9.5	24	43.0
			67 ± 5.3 (ECD)	7.9	33	48.8
32	Pyrazophos	1.5	10 ± 5.0	50	90	190.0

[a] Recovery % is the mean value of triplicates.

Table 7
Pesticide residues in *Pimpinella anisum* samples (A1–A5) and their concentrations

Sample (lipid %)	Method I and Florisil cleanup		Method II and Florisil cleanup	
	Pesticides found	Concentration (mg/kg)	Pesticides found	Concentration (mg/kg)
A1 (7%)	Permethrin	0.542	Vinclozolin	0.011
	α-HCH	0.013		
	Quintozine	0.106		
A2 (12%)	HCB	0.025	None	
	α-HCH	0.011		
	Permethrin	1.981		
A3 (8.4%)	Quintozine	0.011	*p*,*p*-DDT	0.010
			Tetramethrin	0.012
			Tetradifon	0.002
A4 (4%)	Permethrin	1.480	*trans*-Chlordane	0.038
	trans-Chlordane	0.013	Bromopropylate	0.001
	Bromopropylate	0.012		
A5 (7.5%)	None		None	

HCB, α-HCH, quintozene and permethrin were detected in *P. anisum* samples when method I was used (Fig. 1), while none of them was detected when method II was used. On the contrary, vinclozolin, tetramethrin and tetradifon were detected only when method II was used (Fig. 2). One possible reason for such results is the

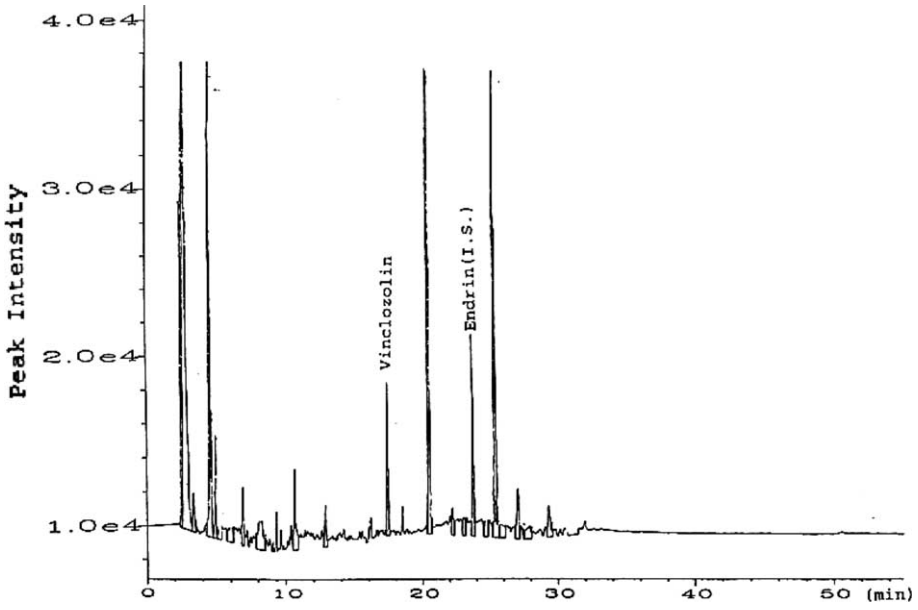

Fig. 1. Representative GC–ECD chromatogram of *Pimpinella anisum* (A2) extract contaminated with HCB, α-HCH and permethrin, using method I and Florisil cleanup.

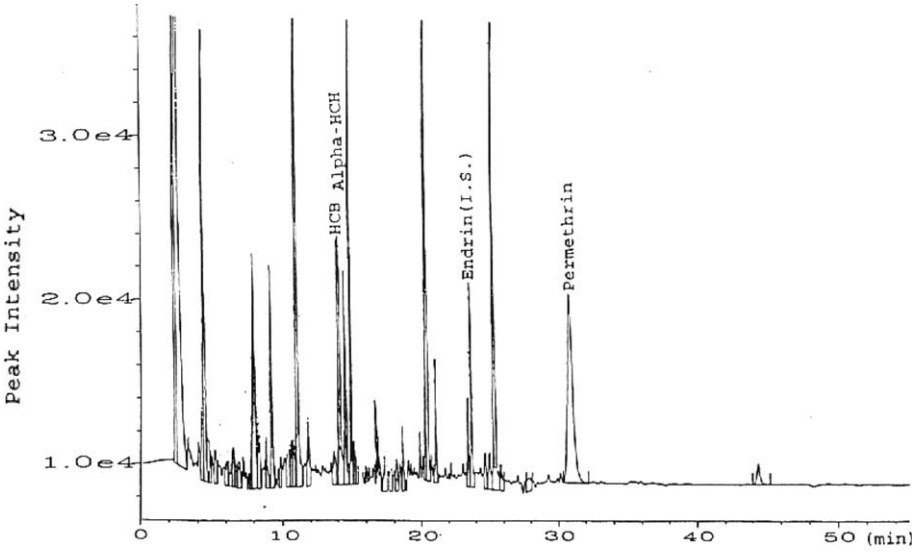

Fig. 2. Representative GC–ECD chromatogram of *Pimpinella anisum* (A1) extract contaminated with vinclozolin, using method II and Florisil cleanup.

nature and the polarity of the chosen solvents. It is also possible that the plant material of *P. anisum*, which contains both volatile and fixed oils in addition to a multitude of other different constituents, would cause extraction problems with some pesticides. The combination pesticide-matrix is very important when selecting an extraction or cleanup method for a certain pesticide. In case of HCB, loss in partitioning from PE to acetonitrile/water is possible especially at low concentrations (FDA, 1994), which might explain its absence in *P. anisum* samples when method II was used.

4. Conclusions

A general multiresidue method cannot be applied to all medicinal plants to extract the residues of interest. It is sometimes necessary to apply more than one extraction procedure to be able to detect greater number of pesticide residues that might be present in the sample of interest. The different methods of extraction applied to *P. anisum* indicated that any change in the extraction conditions affects the ability of the applied procedure to extract certain pesticides. The investigated methods were

not suitable for the extraction of tetramethrin, formothion, pyrazophos and primicarb, while tetramethrin, formothion and pyrazophos showed very low recoveries with the applied methods. Primicarb was not recovered at all.

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