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# Single-step separation of organochlorine pesticide residues from fatty materials by combined use of solid-matrix partition and C<sub>18</sub> cartridges

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## Abstract

A rapid single-step partition step between *n*-hexane and acetonitrile on a two-cartridge system composed of Extrelut-3 with a C<sub>18</sub> cartridge connected downstream has been developed for the separation of organochlorine (OC) pesticide residues from oils and fats. The extract is cleaned up by Florisil minicolumn chromatography before gas chromatography with electron-capture detection. Carry over of fatty material through the partition step is in the order of 4–40 mg for ca. 1.0 g of different oil or fats loaded on the two-cartridge system. Recoveries of 15 OC pesticides from ca. 1 g of soya oil spiked were between 70 and 103% (except HCB=ca. 60%) at spiking levels in the range 0.02–0.1 mg/kg.

**Keywords:** Extraction methods; Vegetable oils; Fats; Food analysis; Pesticides; Organochlorine compounds

## 1. Introduction

The determination of traces of lipophilic compounds, such as organochlorine (OC) pesticide residues in oils, fats and fatty foods have a crucial point where pesticide residues have to be separated from the bulk of fatty material. For this step several techniques are in use including separatory-funnel partition between immiscible solvents [1,2], size-exclusion chromatography (SEC) either with macro-column [3,4] or with mini-column [5,6] or sweep co-distillation [7–11].

Adsorption column chromatography on Florisil macro-column [12–14] or Florisil mini-column [15,16], alumina [17] or silica gel [18] has been used as a further cleanup step before determination by gas

chromatography (GC) with electron-capture detection (ECD). When sufficient sensitivity is available in the determinative step, adsorption cleanup has been applied to a portion of the total amount of fat as the sole cleanup step before GC-ECD [19–21]. But more usually, amounts of fat in the range 0.5–1.0 g are used to attain low determination levels. So, the most widely used technique for removal of fat matrix at present is SEC with columns of 21 or 25 mm I.D., which elute the pesticide fraction in volumes between 60–110 or 100–200 ml, respectively. Further volume of solvent is necessary to recondition the column. Apart from the volume of solvent, with this technique a SEC system composed at least of pump, injector and fraction collector is necessary and has to be checked and maintained in good performance.

Differently from this approach some of us have reported on non-instrumental techniques for sepa-

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ration of pesticide residues from the bulk of lipidic material for the determination of OC [22] and organophosphate (OP) [23] pesticides in fats and oils. Briefly, we have demonstrated that the traditional partition between *n*-hexane and acetonitrile can be carried out advantageously on disposable, ready-to-use Extrelut columns (filled with macroporous diatomaceous earth) for the isolation of OC and OP pesticide residues from fats and oils.

The carry over of fatty matrix after the on-column partition step was in the range of 20–50 mg for an amount of lipid subjected to the partition in the range 1.0–1.8 g, that normally allows the use of adsorption chromatography with mini column. However with certain seed oil such as soya oil and mixed seed oil, the carry over was much higher (126–210 mg from 1.8 g) and it was necessary to split the total extract to allow a suitable amount be loaded onto the adsorption column.

The carry over for soya oil is not reduced even when the amount of soya oil in the partition step is reduced, thus indicating that the carry over is governed mainly by solubility of soya oil in the acetonitrile (the partition solvent).

So, we looked for a remedy to that, and on the basis of our previous experience with fat removal by multiple cartridge system [23], we have investigated the performance of a partition cleanup based on a two-cartridge system, Extrelut-3 connected to a C<sub>18</sub> cartridge.

## 2. Experimental

### 2.1. Reagents

Analytical-reagent-grade chemicals were used. Light petroleum (b.p. 40–60°C), *n*-hexane, benzene, ethyl acetate and acetonitrile (saturated with *n*-hexane) were redistilled from an all-glass apparatus. Water extracted with dichloromethane and helium degassed was used. Sodium sulphate (Carlo Erba, Italy) and sodium chloride (Carlo Erba) were activated at 500°C for 6 h. Florisil PR, 60–100 mesh, cat. No. 2-0280 (Supelco, Bellefonte, PA, USA) was heated at 130°C overnight. This treatment is repeated after 2 days. Extrelut-3 columns (Merck, Darmstadt, Germany, code No. 15372) were used as received.

Sep-Pak C<sub>18</sub> cartridges (Water Associates, Part No. 51910) were washed with 5 ml of acetonitrile; the excess of solvent was removed by aspiration and the cartridges were kept in a closed container. OC reference standards were from the collection in this laboratory and were >99% pure. Compounds in this study have been indicated with their ISO or agreed common names. For unambiguous identification, CAS Reg. No. is also given in Table 1.

### 2.2. Apparatus

The analyses were carried out on a HP 5890 SERIES II Plus gas chromatograph equipped with two on-column injectors and two electron-capture detectors. The main column was a fused-silica capillary column (60 m×0.25 mm I.D., 0.25 μm) DB-1701 (14% CNPrPhMe Siloxane, JW code 12207620), used with a retention gap, 0.9 m×0.53 mm I.D., thin film coated fused-silica (HP code 19095-10050). The second capillary column (60 m×0.25 mm I.D., 0.25 μm) was DB-1 (Crosslinked Methyl Silicone Gum, JW code 12210620), used with a retention gap, 0.9 m×0.53 mm I.D., thin-film coated fused-silica (HP code 19095-10050). The column oven temperature programme was as follows: 90°C (2 min), 20°C/min to 170°C, 1°C/min to 230°C, 230°C (15 min), 2°C/min to 280°C, finally at 280°C (10 min), overall runtime was 116 min. The injector oven temperature was programmed through the oven track program, i.e., column oven +3°C. The detector temperature was set at 300°C. The carrier gas was helium at a flow-rate of 1.12 ml/min (23.3 cm/s average linear velocity at 90°C, supplied in constant flow mode) for both columns.

A rotary evaporator (bath temperature, 40°C; reduced pressure) was used to concentrate solutions.

### 2.3. Procedure

Weigh in a 10 ml graduated glass tube an amount of oil or fat close to 2 g and record the mass as  $P_1$ . Dilute the oil with *n*-hexane up to 5 ml and record the mass of oil+solvent as  $P_{tot}$ . Calculate the amount of oil solution that would contain ca. 1 g oil. Weigh an Extrelut-3 cartridge. Transfer onto the cartridge an amount of the oil solution close to the calculated amount. Weigh by difference the exact amount of oil

Table 1

Composition of standard solution of 15 OC pesticides used for either GC–ECD determination or for spiking soya oil, and the corresponding spiking levels obtained as described under preparation of spiked soya oil

Pesticides	CAS Reg. No	Concentration for ( $\mu\text{g/g}$ )		Spiking levels ( $\mu\text{g/g}$ )		
		GC–ECD determ.	Spiking soya oil	1	2	3
1 HCB	118-74-1	0.040	0.940	0.021	0.040	0.110
2 $\alpha$ -HCH	319-84-6	0.040	0.938	0.021	0.040	0.110
3 $\beta$ -HCH	319-85-7	0.040	0.945	0.021	0.040	0.110
4 $\gamma$ -HCH	58-89-9	0.040	0.939	0.021	0.040	0.110
5 Dieldrin	60-57-1	0.040	0.947	0.021	0.040	0.111
6 Endrin	72-20-8	0.042	0.991	0.022	0.042	0.116
7 <i>cis</i> -Chlordane	5103-71-9	0.038	0.889	0.020	0.038	0.104
8 <i>trans</i> -Chlordane	5103-74-2	0.035	0.813	0.018	0.035	0.095
9 Oxychlordane	27304-13-8	0.045	1.049	0.023	0.045	0.123
10 <i>trans</i> -Nonachlor	39765-80-5	0.039	0.921	0.021	0.039	0.108
11 <i>cis</i> -HEPO	1024-57-3	0.039	0.904	0.020	0.038	0.106
12 <i>p,p'</i> -DDE	72-55-9	0.039	0.914	0.020	0.039	0.107
13 <i>p,p'</i> -TDE	72-54-8	0.040	0.947	0.021	0.040	0.111
14 <i>o,p'</i> -DDT	789-02-6	0.040	0.947	0.021	0.040	0.111
15 <i>p,p'</i> -DDT	50-29-3	0.040	0.935	0.021	0.040	0.109

solution transferred and record it as  $P_2$ . Calculate and record the oil portion transferred to the Extrelut-3 cartridge by  $P_1 \times P_2 / P_{\text{tot}}$ .

Let the solution drain into the column and wait 10 min to obtain an even distribution onto the filling material. Attach to the end of the Extrelut-3 cartridge a Sep-Pak  $C_{18}$  cartridge with its lower end cut in a diagonal fashion. Elute the system of the combined cartridges with  $3 \times 5$  ml portions of acetonitrile (saturated with *n*-hexane).

Collect the eluates in a 100 ml Erlenmeyer flask with a screw-thread, PTFE-lined cap, containing 100 ml of water, with ca. 5 g sodium chloride dissolved, and 10 ml light petroleum.

Cap the flask, shake, let the phases separate and transfer the light petroleum phase into a 50 ml Erlenmeyer flask passing it through a column of 15 g anhydrous sodium sulphate (glass tube,  $150 \times 15$  mm I.D.). Repeat the extraction with  $2 \times 10$  ml portions of light petroleum and combine the extracts.

Concentrate the light petroleum solution to a small volume (1–2 ml) and then to dryness by manually rotating the flask.

Dissolve the residue in 1 ml *n*-hexane and quantitatively transfer with *n*-hexane washings ( $3 \times 1$  ml) to a Florisil column (2.5 g in a  $300 \text{ mm} \times 10 \text{ mm}$  I.D.

glass column with PTFE stopcock). Elute the column with 25 ml of *n*-hexane–benzene (80:20, v/v) at a flow-rate of 2–3 ml/min, and with 30 ml of *n*-hexane–benzene–ethyl acetate (180:19:1, v/v/v, or, optionally, with a single fraction of 50 ml *n*-hexane–benzene–ethyl acetate 180:19:1, v/v/v, when *p,p'*-DDE and dieldrin can be separated in the same GC run.

Concentrate the fractions to a small volume by rotary evaporator (40°C, reduced pressure) and then to dryness by manually rotating the flask. Dissolve with 1 ml of Standard solution (Methoxychlor 0.831  $\mu\text{g/g}$  in iso-octane) and analyze by GC–ECD.

Quantitation was carried out through peak area comparison with the Internal Standard technique and a single-level calibration.

#### 2.4. Preparation of spiked soya oil

For recovery experiments blank soya oil was mixed mass by mass with different amounts of standard spiking solution to obtain three spiking levels (see Table 1):

- Level 1: 16.0735 g oil + 0.3668 g spiking solution;
- Level 2: 16.1492 g oil + 0.7170 g spiking solution;
- Level 3: 8.2064 g oil + 1.0859 g spiking solution.

### 3. Results and discussion

Extrelut-3 columns are ready-to-use, disposable glass cartridges filled with a macroporous diatomaceous earth with a nominal volume of 3 ml. In this procedure, the cartridges were used as a solid support to carry out the liquid–liquid partition to separate OC pesticide residues from the bulk of lipidic material.

The performance of the solid-matrix partition system has been previously [22] studied with respect to its ability to remove lipidic material and recover a number of the most commonly sought OC pesticide residues.

In the present work a C<sub>18</sub>, directly connected downstream to the Extrelut-3 cartridge, has been introduced to reduce the carry over of lipid material into the acetonitrile eluate.

In Table 2 the track of removal of fatty material through separate steps of the procedure is reported. It can be seen that the carry over of soya oil through the sole Extrelut-3 partition cleanup is of the same order (163–204 mg for 1.0 g oil) as observed in our previous paper [22] (ca. 211 mg for 1.8 g oil), while the addition of a C<sub>18</sub> is able to substantially reduce the carry over (mean 39 mg from ca. 1 g oil), thus enabling the use of a mini column for the adsorption cleanup with substantial saving of solvents and time. For the sake of comparison, note that the classical separatory-funnel partition leaves in the acetonitrile

phase some 30–37 mg of oil for 1.0 g of oil, but is much more time-consuming. For the other fats and oils tested (poultry fat, maize oil, olive oil) fat removal was even better.

A further change was introduced in the present procedure compared to our previous scheme, that is a miniaturized back extraction of OC pesticides into light petroleum, instead of the direct concentration of the acetonitrile eluate. That was done to minimize possible losses of volatile residues such as HCB. Unfortunately, the scarce recovery of HCB did not improve and is most probably due to its highly lipophilic properties ( $p$ -value=0.89 between *n*-hexane and acetonitrile;  $p$ -value=fraction partitioning into the non-polar phase of an equi-volume two-solvent system). However the back extraction somewhat improves the GC–ECD track with certain oils removing part of negative peaks.

Recovery of OC pesticides has been studied with 15 compounds, including the ones most frequently sought, from soya seed oil spiked at three different levels in the order of 0.02, 0.04 and 0.1 mg/kg (see Table 1). Results of recovery experiments are reported in Table 3. Recoveries are satisfactory for all compounds except for HCB (not greater than 60%) and borderline satisfactory for endrin, 67% at level 1 and 70% at level 3. In any case recovery values are not correlated with the increasing spiking levels in the range studied. Compounds can be recovered in two fractions, i.e., *n*-hexane–benzene followed by a

Table 2  
Track of removal of fat matrix (soya seed oil) through different cleanup steps

Average amount of oil (g)	Range, or average amount (mg) ± S.D. of oil remaining after				
	Separatory-funnel partition <sup>b</sup>	Extrelut-3 partition	Ext-3+C18 partition	Florisil adsorption cleanup	
				1st fraction	2nd fraction
1.00 ( <i>n</i> = 3)	30–37			0.2–0.8	0.2–1.0
1.02 ( <i>n</i> = 3)		163–204		0.1–0.2	0.4–1.4
1.8 <sup>a</sup> ( <i>n</i> = 6)		211 ± 9.8			
1.0 ( <i>n</i> = 14)			39 ± 25.7		
1.0 <sup>c</sup> ( <i>n</i> = 3)			7.5–8.4		
1.0 <sup>d</sup> ( <i>n</i> = 3)			3.5–3.7		
1.0 <sup>e</sup> ( <i>n</i> = 3)			14.9–17.5		

<sup>a</sup> From previous paper [22].

<sup>b</sup> 1 g soya oil in 15 ml light petroleum with 4 × 15 ml.

<sup>c</sup> Maize oil.

<sup>d</sup> Olive oil.

<sup>e</sup> Poultry fat.

Table 3  
Recovery values of 15 OC pesticides from ca. 1 g soya oil spiked at different levels

Pesticides	Spiking level 1 (mg/kg)	Recovery (%) (n=6)		Spiking level 2 (mg/kg)	Recovery (%) (n=6)		Spiking level 3 (mg/kg)	Recovery (%) (n=4)	
		Mean	S.D.		Mean	S.D.		Mean	S.D.
1 HCB	0.021	59	7	0.040	59	3	0.110	57	4
2 $\alpha$ -HCB	0.021	91	7	0.040	94	5	0.110	95	6
3 $\beta$ -HCH	0.021	92	5	0.040	92	2	0.110	85	4
4 $\gamma$ -HCH	0.021	96	7	0.040	96	5	0.110	91	6
5 Dieldrin	0.021	88	16	0.040	92	4	0.111	89	6
6 Endrin	0.022	67	11	0.042	77	4	0.116	70	4
7 <i>cis</i> -Chlordane	0.020	92	5	0.038	100	3	0.104	89	5
8 <i>trans</i> -Chlordane	0.018	93	7	0.035	95	5	0.095	86	5
9 Oxychlordane	0.023	97	7	0.045	97	4	0.123	84	6
10 <i>trans</i> -Nonachlor	0.021	89	8	0.039	92	3	0.108	84	5
11 <i>cis</i> -HEPO	0.020	94	8	0.038	95	3	0.106	92	5
12 <i>p,p'</i> -DDE	0.020	98	7	0.039	90	9	0.107	81	2
13 <i>p,p'</i> -TDE	0.021	96	4	0.040	94	6	0.111	78	4
14 <i>o,p'</i> -DDT	0.021	89	4	0.040	85	3	0.111	90	3
15 <i>p,p'</i> -DOT	0.021	80	9	0.040	84	7	0.109	103	6

second fraction of *n*-hexane–benzene–ethyl acetate (in which dieldrin, endrin and *cis*-HEPO are recovered), with the first fraction being much more clean, or, optionally, with a single fraction of *n*-hexane–benzene–ethyl acetate when *p,p'*-DDE and dieldrin can be separated in the same GC run.

Typical GC–ECD chromatograms are shown in

Figs. 1–4, for method blank, blank soya oil, soya oil spiked at level 2, standard mixture of OC compounds.

The main scope of the present work was to demonstrate that rapid non-instrumental partition cleanup can be a useful substitute for the conventional techniques used for the separation of OC pesticide

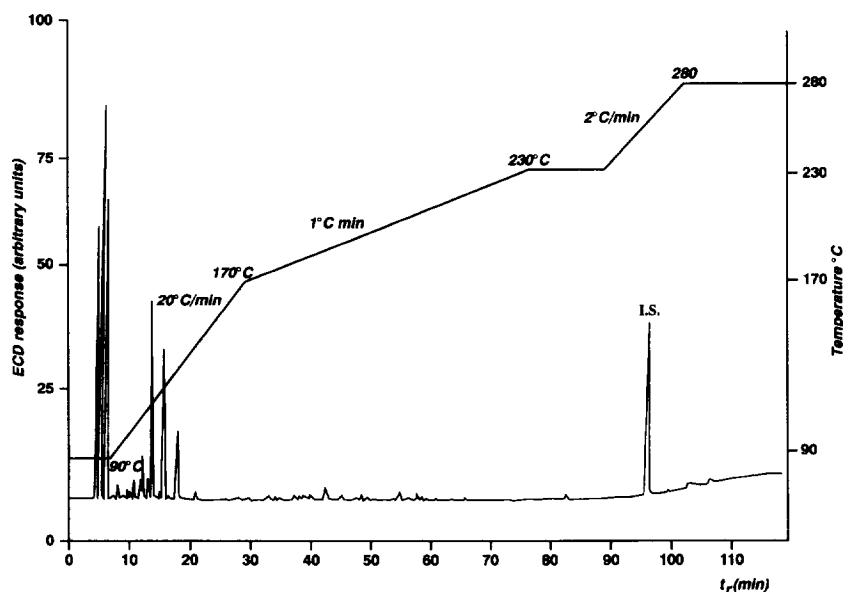


Fig. 1. GC–ECD chromatogram of blank method, 1  $\mu$ l injected. I.S.=Internal standard, methoxychlor.

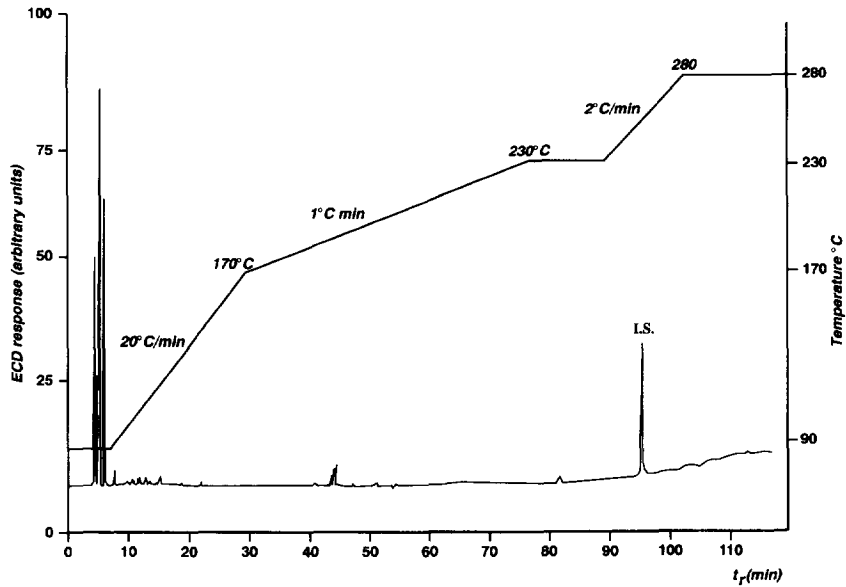


Fig. 2. GC-ECD chromatogram of blank soya oil, 1  $\mu$ l injected. I.S.=Internal standard, methoxychlor.

residues from the bulk of lipidic material. Indeed, compared to the classical separatory-funnel partition [1], the described procedure is rapid, does not require

recovery and preparation of glassware, uses a reduced volume of solvent and is less labor-intensive. Compared to instrumental techniques such as SEC

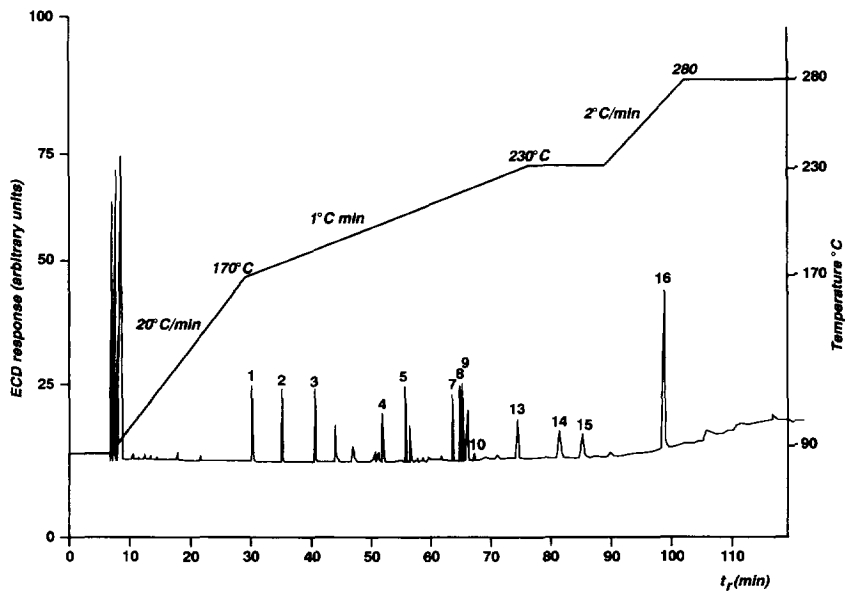


Fig. 3. GC-ECD chromatogram of extract from soya oil spiked at level 2, first fraction of Florisil adsorption cleanup, 1  $\mu$ l injected. 1, HCB; 2,  $\alpha$ -HCH; 3,  $\gamma$ -HCH; 4,  $\beta$ -HCH; 5, oxychlordan; 7, *trans*-chlordan; 8, *cis*-chlordan; 9, *trans*-nonachlor; 10, *p,p'*-DDE; 13, *o,p'*-DDT; 14, *p,p'*-TDE; 15, *p,p'*-DDT; 16, I.S. methoxychlor. For concentrations see Table 1.

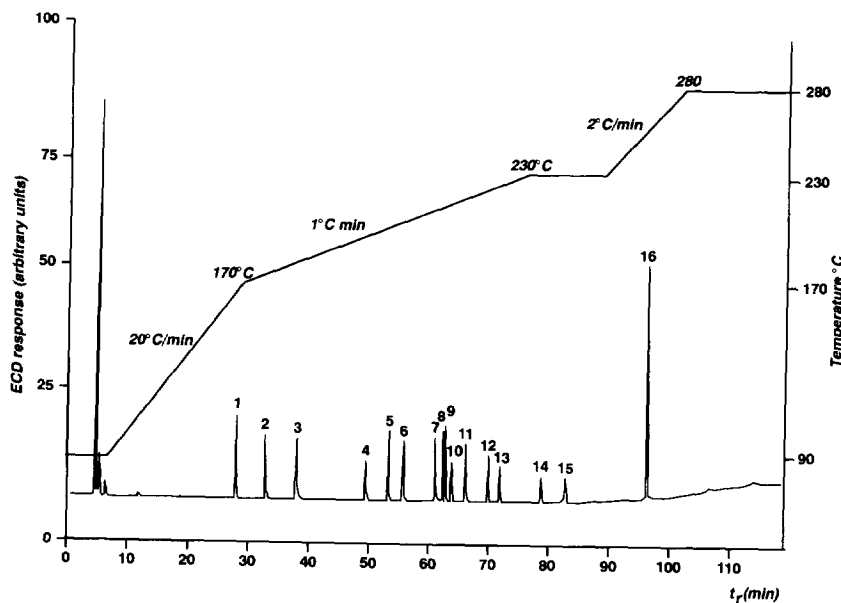


Fig. 4. GC-ECD chromatogram of the standard mixture of OC pesticides, 1  $\mu$ l injected. 1, HCB; 2,  $\alpha$ -HCH; 3,  $\gamma$ -HCH; 4,  $\beta$ -HCH; 5, oxychlordan; 6, *cis*-HEPO; 7, *trans*-chlordan; 8, *cis*-chlordan; 9, *trans*-nonachlor; 10, *p,p'*-DDE; 11, dieldrin; 12, endrin; 13, *o,p'*-DDT; 14, *p,p'*-TDE; 15, *p,p'*-DDT; 16, I.S. methoxychlor. For concentrations see Table 1.

and sweep-codistillation the described procedure does not require skilled operators or maintenance of costly apparatus, and uses less solvent.

Furthermore, the described on-column partition step is based on ready-to-use, disposable items, and requires only simple operations, such as preparation of the oil solution and transferring a portion thereof onto the cartridge. Neither control of flow, nor skilled operators are needed.

Reported methods that use approaches similar to our procedure have some drawbacks. For instance, alumina blending extraction [13] uses 50 g strictly preconditioned alumina (water deactivation between 16 and 19%, w/w), blending of fat–alumina mixture in 350 ml of 20% water in acetonitrile solution, and multiple back extraction into light petroleum, before Florisil macro column cleanup. Matrix solid-phase dispersion extraction (MSPD) [24] of fats by mixing with  $C_{18}$  powder and eluting with acetonitrile, while attractive has been proved by us to give low and erratic recoveries. Also the method reported by Gillespie et al. [25], which incorporates the partition on Extrelut-3 developed by us, uses a much larger amount of  $C_{18}$  (5 g) compared to our procedure (0.36 g), and uses 40 ml of solvent and an off-line

elution of the sole  $C_{18}$  column, before the adsorption cleanup on alumina which is quite difficult to standardize.

In conclusion, the described procedure consists of a partition step on a two-cartridge combined system composed of an Extrelut-3 connected in series to a  $C_{18}$  cartridge, followed by an adsorption cleanup step. It offers a simple, rapid way to determine OC pesticide residues in oils and fats. The solid-matrix partition step offers significant savings of glassware, solvents, reagents and time compared to conventional techniques. Furthermore, the clean-up is based on simple operations which require a minimum of reagents and glassware and do not require skilled operators or costly apparatus, so that several samples can be run in parallel.

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