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Solid-matrix partition for separation of organochlorine pesticide residues from fatty materials

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ABSTRACT

A fast, single-step and efficient partition between *n*-hexane and acetonitrile on ready-to-use, disposable cartridges of Kieselghur-type material has been developed for the separation of organochlorine (OC) pesticide residues from oils and fats. The extract is cleaned up with Florisil minicolumn chromatography, followed by a solid-matrix sulphuric acid treatment. Carry-over of lipid material through the partition step is lower compared to conventional, separatory-funnel partition. Recovery of eighteen OC pesticides from 1.0 g olive oil was between 72% and 104% at spiking levels between 0.01 and 0.15 mg/kg for the different compounds.

INTRODUCTION

One of the major problems the analyst faces in the determination of organochlorine (OC) pesticide residues in fats and oils is the isolation of pesticide residues from the bulk of lipidic material.

To this end, several techniques are in use including separatory-funnel partition between immiscible solvents [1,2], size-exclusion chromatography [3–6] or sweep co-distillation [7–11]. Adsorption column chromatography on Florisil [12–14], alumina [15] or silica gel [16] has been used as a final clean up step before determination by gas chromatography (GC) with electron-capture detection (ECD).

All the above-mentioned procedures for the isolation of OC pesticide residues from fatty materials involve time-consuming operations, reusable glassware, large amounts of solvents and reagents, maintenance of costly apparatus and skilled operators.

In previous papers [17,18] we have reported 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 organophosphate (OP) pesticide from several vegetable oils.

The purpose of the present paper is to describe the ability of the solid-matrix partition step to isolate several OC pesticide residues from different fatty materials.

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EXPERIMENTAL

Reagents and materials

Analytical-reagent-grade chemicals were used. Light petroleum (40–60°C), *n*-hexane, iso octane, benzene, ethyl acetate, acetonitrile (saturated with *n*-hexane) and methanol were redistilled from an all-glass apparatus. Sulphuric acid (95%, density 1.824 g/ml) was used. Florisil PR, 60–100 mesh (Supelco, Bellefonte, PA, USA), was activated at 130°C overnight. Extrelut-3® and Extrelut-1® columns (E. Merck, Darmstadt, Germany, Cat. Nos. 15372 and 15371, respectively) were used with a disposable needle (E. Merck, Cat. No. 15373) at the column end as a flow restrictor. OC reference standards were from the collection in this laboratory.

Apparatus

The analyses were carried out on a DANI 6800 gas chromatograph equipped with an electron-capture detector. A glass column (1.8 m × 4 mm I.D.) was packed with OV-17 plus QF-1 (1.5% and 1.95%, respectively) on Chromosorb W HP, 100–120 mesh. The temperatures were as follows: oven, 210°C; inlet block, 230°C; outlet block, 250 °C; detector, 250°C. The carrier gas was nitrogen at a flow-rate of 55 ml/min. A rotary evaporator (bath temperature, 40°C; reduced pressure) was used to concentrate solutions.

Procedure

Between 2 and 3 g of lipidic material were placed in a 5-ml volumetric flask, dissolved and diluted to volume with *n*-hexane. Then, 3 ml of the solution was transferred to an Extrelut-3 column which was left to equilibrate for 10 min. The column was then eluted under gravity alone with three 5-ml portions of acetonitrile saturated with *n*-hexane. Eluates were collected in a 50-ml Erlenmeyer flask, 4 ml of methanol added, and the solution was carefully concentrated to dryness by means of rotary evaporator. Any traces of solvent were removed with a gentle stream of nitrogen.

The residue was dissolved in 1 ml *n*-hexane and quantitatively transferred with *n*-hexane washings to a Florisil column [19] (2.5 g in a 300 mm × 10 mm I.D. glass column with PTFE stopcock). The column was then eluted with 50 ml of *n*-hexane–benzene–ethyl acetate (180:19:1) at a flow-rate of 2–3 ml/min. The eluate was carefully concentrated to dryness, dissolved in 2 ml of isooctane and analysed by GC–ECD for the determination of heptachlor epoxide, dieldrin, endrin and metoxychlor, which otherwise would be destroyed by the successive sulphuric acid clean-up, and, if possible, of other compounds. To remove interfering peaks that prevent the determination of some compounds, the extract was quantitatively transferred with light petroleum washings onto an Extrelut-1 column previously loaded with 1 ml concentrated sulphuric acid [20].

The Extrelut-1 column was eluted with four 5-ml portions of light petroleum. All the solvent eluates from the column since the transfer of the sample solution were collected, carefully concentrated to dryness and dissolved in 2 ml isooctane.

The final samples were analysed by GC–ECD. For recovery experiments the oil or fat samples were weighed and dissolved, and diluted with the solution of standard compounds.

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 studied with respect to its ability to remove lipidic material and to recover a number of the most commonly sought OC pesticide residues.

In Table I are presented data that show the removal of lipidic material through liquid–liquid partition on Extrelut-3 and Florisil column chromatography.

Five different vegetable oils and one animal fat were used to test the performance of the solid-matrix partition step. The oils were commercial samples from the retail market; the animal fat was extracted from "speck" (a ham typical of a north-eastern region of Italy) with *n*-hexane–acetone (1:1).

Data in Table I indicate that with up to 1.8 g of olive oil, peanut oil and corn oil a fair removal of the bulk of lipid could be obtained with the Extrelut-3 column partition alone. With soya bean oil, mixed seed oil and pork fat, a slightly worse performance was observed. However, even in the worst cases the difference can be explained primarily by the differences in the composition of lipids tested. Also, the fact that the tests were carried out at different times, with different batches of Extrelut-3 columns and acetonitrile could have contributed. The reduced carry-over of lipidic material through the partition step allows the use of a minicolumn of Florisil for clean-up, with substantial saving of solvents and time, compared to classical separatory-funnel partition [1,2].

Under the conditions adopted, the amount of lipids released into the acetonitrile eluate did not vary greatly with the amount applied to the Extrelut-3 column. As to the maximum amount of lipid that can be loaded on the Extrelut-3 columns, it

TABLE I

REMOVAL OF THE LIPIDIC MATERIAL THROUGH THE STEPS OF THE DESCRIBED PROCEDURE

Lipid type	Amount applied to Extrelut-3 column (g)	Amount in the eluate after Extrelut-3 partition ± S.D. (mg)	Amount in the eluate after Florisil chromatography ± S.D. (mg)
Olive oil	1.0	18.9 ± 1.53 ($n=7$)	2.5 ± 0.93 ($n=7$)
	1.8	31.4 ± 3.60 ($n=6$)	–[a]
Peanut oil	1.0	52.8 ± 9.01 ($n=5$)	10.6 ± 6.2 ($n=3$)
	1.8	26.1 ± 1.03 ($n=6$)	–[a]
Corn oil	1.5	37.8 ($n=1$)	7.3 ($n=1$)
		53.9 ($n=1$)	3.5 ($n=1$)
	1.8	34.8 ± 5.76 ($n=6$)	–[a]
Soya bean oil	1.8	210.9 ± 9.80 ($n=6$)	–[a]
Mixed seed oil	1.8	126.4 ± 9.80 ($n=6$)	–[a]
Pork fat extracted from speck	0.4–0.9 ($n=9$)	77.5 ± 28.43 ($n=9$)	6.3 ± 10.9 ($n=9$)

[a] Not determined.

should be noted that a substantial portion of the nominal volume of 3 ml should be left to the *n*-hexane in order that a true *n*-hexane–acetonitrile partition takes place. With applied amounts higher than those in Table I, the sample becomes too viscous and is not readily sorbed into the Extrelut particles. So, a true partition between immiscible solvents does not occur and a tendency for the sample to be mechanically displaced into the acetonitrile eluate was observed.

The extraction *p* values (the fraction of solute partitioning into the non-polar phase of an equivolume two-phase system [21,22]) of the compounds studied can be used as a criterion to estimate the range of applicability of the partition step. As can be seen from the extraction *p* values given in Table II, pesticides having *p* values between *n*-hexane and acetonitrile lower than 0.91 were satisfactorily recovered from 1.0 g of olive oil, indicating a good performance of the method also with respect to highly lipophilic compounds such as mirex ($p = 0.91$). The 15-ml volume of acetonitrile, chosen on the basis of our previous works [17,18], proved to be sufficient to recover the studied compounds. So, no further refinement was deemed necessary.

The sample extracts were cleaned up by Florisil column chromatography as reported by Suzuki *et al.* [19]. This clean-up without any modification proved to be

TABLE II

MEAN RECOVERY VALUES OF EIGHTEEN ORGANOCHLORINE PESTICIDES FROM 1.0 g OF OLIVE OIL THROUGH THE DESCRIBED PROCEDURE

Pesticides[f]	Recovery (mean ± S.D., $n=6$) (%)	Spiking level (mg/kg)	*p* Value[a]	
			This laboratory[b]	Others[c]
HCB	72.4 ± 9.78	0.01	0.89	–
α-HCH	99.5 ± 9.81	0.01	0.03	–
τ-HCH	98.7 ± 9.87	0.01	0.11	0.12
β-HCH	91.6 ± 8.53	0.02	0.05	–
δ-HCH	104.5 ± 9.13	0.01	0.10	–
Aldrin[d]	86.2 ± 8.91	0.01	0.64	0.73
Heptachlor epoxide[d]	82.9 ± 7.99	0.01	0.24	0.29
τ-Chlordane	89.8 ± 7.45	0.03	0.34	0.40
α-Chlordane	89.4 ± 9.55	0.03	0.31	–
p,p'-DDE	89.7 ± 9.39	0.03	0.52	0.56
Dieldrin[d]	95.3 ± 7.23	0.05	0.30	0.33
o,p'-TDE	93.1 ± 8.41	0.05	0.21	–
Endrin[d]	101.2 ± 8.51	0.05	0.34	0.35
o,p'-DDT	94.8 ± 8.51	0.05	0.47	0.47
p,p'-TDE	91.4 ± 7.59	0.05	0.18	0.17
p,p'-DDT	96.9 ± 9.49	0.05	0.34	0.38
Endosulphan sulphate[e]	–	0.06	<0.01	–
Mirex	85.4 ± 9.04	0.11	0.76	0.91
Methoxychlor[d]	101.3 ± 8.72	0.15	0.04	0.07

[a] *p* values between *n*-hexane and acetonitrile.
[b] *p* values by GC–ECD after single distribution between equal volumes of *n*-hexane and acetonitrile.
[c] *p* values from refs. 21 and 22.
[d] Compounds which have been determined after the Florisil clean-up alone.
[e] Not recovered through the Florisil clean-up.
[f] All abbreviations used are according to ref. 23.

generally effective with a lipid load up to about 100 mg. After Florisil column chromatography the amount of lipid left in the eluate is below 10 mg (see Table I). When necessary (for example, soya bean oil and mixed seed oil) the Florisil column chromatography can be conveniently scaled up.

Recovery experiments were carried out by spiking olive oil, chosen as a model lipid, with nineteen OC pesticides at levels ranging, for the different compounds, between 0.01 and 0.15 mg/kg. The compounds selected for the tests include the most commonly sought OC pesticides. In Table II are presented the results of the recovery experiments, which show satisfactory values for the compounds studied, with the exception of endodulfan sulphate. This compound proved to be retained on the Florisil column. However, on the basis of its p value, it is reasonable to assume that it can be recovered through the solid-matrix partition step. Some of the compounds (namely, heptachlor epoxide, dieldrin, endrin and metoxychlor) were determined after Florisil column chromatography and the others after sulphuric acid treatment. Indeed, with certain samples after Florisil chromatography large tailing after the injection and some negative peaks prevented or made it difficult to allocate a good baseline for the determination of some compounds. In these cases an additional clean-up by solid-matrix sulphuric acid treatment [20] was sufficient to remove the interference and to give a clean GC baseline, almost resembling that obtained by injection of the standard mixture. In Fig. 1 are shown typical gas chromatogram obtained by in-

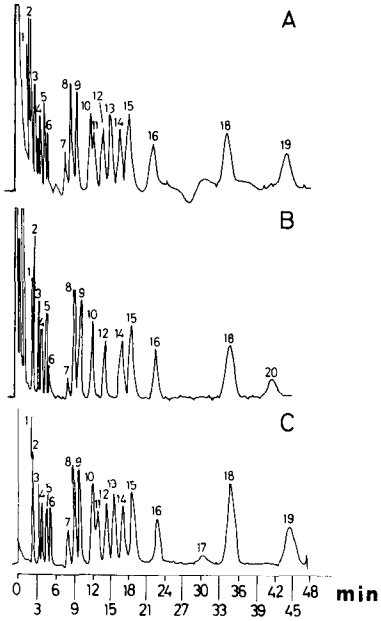

Fig. 1. Typical gas chromatograms of an extract of 1 g olive oil spiked with nineteen OC pesticides at levels shown in Table II after Florisil clean-up (A), the same sample after the additional sulphuric acid treatment (B), and the standard solution of nineteen OC pesticides (C), each of which at concentrations equivalent to the spiking levels. Peaks: 1 = HCB; 2 = α-HCH; 3 = τ-HCH; 4 = β-HCH; 5 = δ-HCH; 6 = aldrin; 7 = heptachlor epoxide; 8 = τ-chlordane; 9 = α-chlordane; 10 = p,p'-DDE; 11 = dieldrin; 12 = o,p'-TDE; 13 = endrin; 14 = o,p'-DDT; 15 = p,p'-TDE; 16 = p,p'-DDT; 17 = endosulfan sulphate; 18 = mirex; 19 = methoxychlor; 20 = δ-keto-Endrin. (For conditions see text.) The abbreviations used are according to ref. 23.

jecting an extract of spiked olive oil after Florisil clean-up (A) and the same sample after the additional clean-up by sulphuric acid (B) in comparison with the gas chromatograms of the standard mixture (C).

The behaviour of the studied compounds during the sulphuric acid treatment on Extrelut-1 has already been reported by us [20]. In particular, endrin and methoxychlor are almost completely lost through sulphuric acid clean-up, while dieldrin is recovered as endrin ketone, which appears as a peak with a retention time longer than that of the parent compound. Although aldrin and heptachlor epoxide were well recovered as pure compounds through sulphuric acid clean-up in our previous work [20], in this procedure they are better determined after Florisil clean-up as they appear to be somewhat retained by the additional sulphuric acid clean-up step. This effect is probably due to the presence of the small amount of charred material that forms on the top of the solid-matrix column loaded with sulphuric acid. Of course, the amount of the charred material depends on the amount of lipid left in the eluate after Florisil and this, in its turn, on the type of fatty material analysed (see Table I).

Thus, to overcome a possible influence of variable amounts of charred material on the recovery of the other compounds also studied, we decided to elute the solid-matrix sulphuric acid column with a total of 20 ml of light petroleum, instead of 10 ml as reported in our previous paper [20]. Indeed, recovery experiments with lipids other than olive oil gave values consistent with those shown in Table II.

After sulphuric acid clean-up, the final eluate contains very low amounts (in the order of 1–2 mg) of lipidic material and for this reason is amenable to both packed and capillary on-column injection systems. The gas chromatograms after sulphuric acid treatment are free from interfering peaks and are almost indistinguishable from those obtained with the standard solution of pure pesticides (see Fig. 1).

Finally, it is reasonable to assume that other chlorinated compounds, such as polychlorinated biphenyls (PCBs) can be recovered through the described procedure, However, the samples used for recovery experiments were "blanks" under the conditions adopted. So, the determination of organochlorine pesticide should not have been impaired by the presence of PCBs, which normally occur at levels well below those used for recovery experiments. Indeed, the main scope of the present work was to demonstrate that a rapid, simple solid-matrix partition can be a substitute for the conventional techniques used for the separation of organochlorine pesticides from the bulk of lipidic material, *i.e.*, separatory-funnel partition between immiscible solvents [1,2], size-exclusion chromatography [3–6] or sweep co-distillation [7–11].

CONCLUSIONS

The described procedure consists of a solid-matrix partition step followed by two clean-up steps, the sulphuric acid treatment step being optional, depending on the compounds being determined and/or on the sample matrix. It offers a simple, rapid way to determine OC pesticides residues in oils and fats. The solid-matrix partition step offers significant savings of glassware, solvents, reagents and time compared to conventional techniques used. 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.

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