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Sandwich-type extraction column with on-line sulphuric acid treatment for the determination of organochlorine compounds in vegetable oil or oil seeds by gas chromatography with electron-capture detection

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ABSTRACT

An effective clean-up technique for the determination of hexachlorobenzene (HCB), α-hexachlorocyclohexane (HCH), β-HCH, γ-HCH (lindane), heptachlor, heptachlor epoxide, aldrin, 2,4'-DDE, 4,4'-DDE, 2,4'-DDT, 4,4'-DDT and endosulfan in vegetable oils and oil seeds by gas chromatography with electron-capture detection is described. The separation of these organochlorine pesticides from normally co-extracted fatty material of plant origin was achieved by the use of a new "sandwich"-type extraction column, allowing adsorption of polar matrix interferences on Florisil, Extrelut and sodium sulphate and "on-line" degradation of usually at least partially co-eluting triglycerides with sulphuric acid in a single step. Quantification down to the 1–5 ppb level was performed by external or internal standard calibration using pentachlorobenzene and Mirex as internal standards.

INTRODUCTION

The contamination of vegetable oils with organochlorine pesticides (OCPs) is caused by the uptake of traces of pesticides during the growth period of plants from the surrounding biocompartments such as soil, air and water. Owing to the poor solubility of OCPs in water [1], there is particular concern over the long-lasting adsorption of traces of pesticides onto colloids in the soil and over specific transportation phenomena of highly volatile and sublimable organochlorine compounds in the air [2,3].

Contamination levels can be reduced by refinement of the oil [4], hence also partly decomposing the content of valuable highly unsaturated fatty acids and vitamins, which is provided by cold pressing of the oil seeds such as olives and many other seeds. However, this may have to be paid for by a higher content of OCPs.

The method presented in this paper was specially designed and evaluated for the determination of OCPs in Styrian pumpkin seeds or pumpkin seed oil, but proved also to be a general sample pretreatment technique for various oil seed extracts and vegetable oils.

The Styrian pumpkin, *Cucurbita pepo* var. *styriaca*, is cultivated in some agricultural regions in Styria (Austria), Slovenia and Hungary only and is used for the production (by a special cold pressing procedure) of characteristic dark-coloured, aromatic tasting salad oil distinguished by its high content of unsaturated fatty acids (about 50% linoleic acid [5,6]). Pumpkin seeds have been used in Europe for a long time as a remedy for prostate gland problems due to the content of particular phytostertols [7,8] and have recently becoming popular as snacks (comparable to peanut snacks).

Traces of hexachlorobenzene (HCB) and many other OCPs have been detected in such products and the extracted oil. In most countries the legislative authorities set limits and regulations for OCPs

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in oil seeds (*e.g.*, for Austria 250 ppb (w/w) for HCB, 20 ppb for lindane and 10 ppb for other OCPs in oil seeds [9]), which have to be very low considering the high potential for accumulation of these compounds in all human fatty tissues with particular concern over OCP accumulation in human milk as recently reported in many European countries [10–15].

For monitoring traces of HCB and OCPs in various lipids and oils of plant origin, highly sensitive chromatographic methods have to be employed. For providing reliable results the lipid-soluble contaminants have to be accurately separated from the co-extracted fatty plant material (*e.g.*, long-chain alcohols, fatty acids and esters), which is usually the most painstaking part of the analytical procedure. If not carried out properly, sensitive GC capillaries and detectors can deteriorate and/or false-positive results are obtained owing to various chromatographic interferences.

An effective technique for the separation of analytes from lipids is chemical degradation of the main matrix components by saponification with strong bases or acids as with a potassium hydroxide–alcohol mixture [16–18] or concentrated sulphuric acid [19,20], forming more polar products that can be easily washed out of the organic sample extract with water. The applicability of this method is limited owing to the resistance of the analytes to strong bases and acids, which is, however, fulfilled by many organochlorine compounds.

Clean-up by adsorption chromatography, also termed solid-phase extraction (SPE), is the most common method and has official status in many countries (AOAC [21]). Lipids are retained in an extraction column filled with polar sorbents and the analytes are eluted with organic solvents. Suitable adsorbents are alumina [22], magnesia [17,23,24], Florisil [18,23,25] and plain silica [20].

Gel permeation chromatography (GPC) on Bio-Beads SX-3 (a polystyrene gel) with ethyl acetate–cyclohexane [26–30] utilizes the difference in the molecular size of pesticides (M_r 200–400) and lipids (M_r 600–1500) for the accurate and moderate separation of analytes and matrix interferences [31,32].

For routine monitoring of OCPs in vegetable oil extracted or pressed from pumpkin seeds, an accurate, simple and rugged sample purification method is particularly needed owing to the exceptionally high content of various matrix compounds of plant origin such as phytosterols, chlorophylls (unique among all other oil seeds [33]) and many others besides the usual triglycerides. For reliable OCP determination we therefore had to combine different clean-up techniques previously reported for other vegetable oils and directed our attention especially to problems concerning the determination of OCPs using external and internal standard calibration.

EXPERIMENTAL

Instrumentation and chromatography

All analyses were performed using a Hewlett-Packard HP 5890 A gas chromatograph equipped with a [63]Ni electron-capture detector and a fused-silica column (30 m × 0.25 mm I.D.) coated with 0.25-μm cross-bonded 65% dimethyl–35% diphenylpolysiloxane (RTX-35; Restec) or alternatively for peak identification a fused-silica column (25 m × 0.25 mm I.D.) coated with 0.25-μm cross-linked 95% dimethyl–5% diphenylpolysiloxane (RTX-5; Restec). The carrier and make-up gases were nitrogen (5.0 quality) (TEGA) equipped with a moisture and oxygen trap at 18 p.s.i. (125 kPa) column head pressure (corresponding to a flow-rate of 55 ml/min at split vent) and a 55 ml/min make-up flow-rate. The septum purge flow-rate was 0.3 ml/min. A 1-μl volume of the sample was injected using an autosampler (HP 7673 A) equipped with a 10-μl Hamilton syringe into a capillary inlet with glass liner and silanized glass-wool in the splitless mode with a split delay of 60 s. The temperatures of the injector and detector were 290 and 350°C, respectively. The oven temperature was held at 60°C for 1 min followed by temperature programming to 220°C at 20°C/min, then to 230°C at 3°C/min and to 290°C at 6°C/min and finally held at 290°C for 2 min. The GC conditions for peak identification on the RTX-5 capillary were 60°C for 1 min, programmed to 290°C at 10°C/min and finally held at 290°C for 5 min. For data storage and integration an HP ChemStation 5895 A was employed.

Standards and reagents

Solvents (acetonitrile, hexane) were of Pestanal® quality from Merck (Darmstadt, Germany). Light petroleum (b.p. 40–60°C) was of Pestanal quality from Riedel-de Haën (Seelze, Germany). Extrelut

(a polar macroporous magnesia–silicate material) was purchased from Merck. Florisil of research grade (0.15–0.25 mm, 60–100 mesh, specific surface area 298 m²/g, 15% MgO, 84% SiO₂, <1% Na₂SO₄) was obtained from Serva (Heidelberg, Germany). H₂SO₄ (95–97%, 36 M), anhydrous Na₂SO₄ and ethanol (96%) were of analytical-reagent grade. Pesticide standards, mix IV [α-HCH, β-HCH, γ-HCH (lindane), HCB, heptachlor, heptachlor epoxide, endosulfan I] and mix V (2,4′-DDD, 2,4′-DDE, 2,4′-DDT, 4,4′-DDD, 4,4′-DDE, 4,4′-DDT, dieldrin, endrin), 1 ng/μl each in cyclohexane, and all single standard compounds (10 ng/ μl each in cyclohexane) were purchased from Dr. Ehrenstorfer (Augsburg, Germany).

Methods

All solvents were monitored for possible OCP traces prior to use by GC with electron-capture detection (ECD). After concentration by a factor of 50 the OCP contamination was found to be below 0.01 ppb. The Florisil purity was tested by extracting a 7-g amount of Florisil with 20 ml of light petroleum and, after concentration to 1 ml, injecting a 1-μl volume into the GC–ECD system. The resulting chromatogram demonstrated the lack of any ECD-sensitive compounds. The same results were obtained for anhydrous sodium sulphate. Similar extraction of Extrelut revealed interferences in the retention window from β-HCH to heptachlor epoxide, which were completely removed by loading the material with concentrated sulphuric acid. Any contact of samples or sample extracts with materials made of paper or plastic was avoided, as these materials contained light petroleum-extractable ECD-sensitive compounds (EOCls, phthalates and others) interfering especially with β-HCH and 4,4′-DDE determinations, often leading to false-positive results. Therefore, only PTFE-lined stoppers (Soxhlet apparatus) and crimp caps (autosampler vials) were used and all Soxhlet extraction cartridges were pre-extracted by Soxhlet extraction for 4 h with 96% ethanol prior to use. Standard solutions could be stored in silylated glassware (preventing adsorption of traces of OCPs on the large glass surface) at − 18°C in a freezer for at least 3 months.

Elution of OCPs from the "sandwich"-type extraction columns described in this paper was performed with a special eluent of constant polarity.

We used the "upper phase" of a two-layer system obtained by simply mixing light petroleum, acetonitrile and ethanol (100:25:5), which represents a saturated solution of acetonitrile in light petroleum with ethanol as an emulsifier [25]. The exact composition of the "upper phase" was determined by GC with flame ionization detection (FID) and is given in Table I. It should be emphasized that the composition of the "upper phase" was always guaranteed by simply mixing the components in the given proportions (assuming constant room temperature and an appropiate equilibrium time of 12 h). The benefits of this eluent are discussed later.

Sampling

Harvested seeds as sunflower, rape, poppy, soya, thistle, linseed, zulunut (*Cyperus esculentus*) and Styrian pumpkin seeds were dried after harvest at ambient temperature in special rooms on each farm to a humidity of about 10%. For pumpkin seed analysis the entire field crop (500–900 kg/ha) was mixed and after removing of the hulling materials by special machines stored at ambient temperature in metallic containers in a large store. A representative mixed sample of at least 500 g was transferred to the analytical laboratory in paper bags after sampling ten aliquots from different positions in each container.

TABLE I

COMPOSITION OF ELUENT FOR SPE–LPE EXTRACTION OF ORGANOCHLORINE PESTICIDES FROM "SANDWICH"-TYPE EXTRACTION COLUMN

Component	Retention time (min)[a]	Composition (%, v/v)	
		"Upper phase"[b]	"Lower phase"
Light petroleum	5.4–5.8	96.0	23.8
Ethanol	9.7	0.9	10.5
Acetonitrile	12.5	3.1	65.7

[a] GC–FID on a 50 m × 0.32 mm, 0.52 μm film thickness, FFAP capillary. Temperature programmed from 50 to 60°C at 3.5°C/ min, held for 1 min, then increased to 80°C at 3.0°/min.
[b] Eluent for SPE extraction of organochlorine pesticides (mainly light petroleum saturated with acetonitrile and ethanol).

A 50-g aliquot of each pumkin seed sample was finely ground with a grain mill. The degree of fineness was adapted with care to avoid any heating of the mill, otherwise large OCP losses could occur owing to their volatility.

Extraction

In an appropriate Soxhlet extraction cartridge a 10-g aliquot of ground pumpkin seed granulate was mixed with 10 g of anhydrous sodium sulphate and extracted with 180 ml of light petroleum by a 4-h Soxhlet extraction. The extractable content was between 45 and 55% (w/w). After cooling, the extract was concentrated to about 8 ml by rotary evaporation at ambient temperature and reduced pressure. The oleaginous extract was transferred into a calibrated vial, diluted to 10 ml with light petroleum and can be stored at 5°C in a refrigerator for several days.

This procedure worked equally well for all other oil seeds.

Clean-up

A 1-ml aliquot of the above-described (pumpkin) seed extract, which represents a 1:1 dilution of the oil extract with light petroleum) or a 1-ml aliquot of a 1:1 dilution of pumpkin seed oil or any vegetable oil was transferred to the top of a prerinsed (with 15 ml of "upper phase"; for composition see *Methods*) "sandwich-type" extraction column (see Fig. 1) fol-

lowed by penetration into the column. After a delay time of 10 min the column was rinsed with 2 × 10 ml of "upper phase" (method I) or 3 × 10 ml of "upper phase" (method II), whereby only the remaining OCPs were eluted. The total eluate was collected in a conical vial with a 1-ml calibration mark, concentrated by a gentle stream of nitrogen at room temperature to about 0.6–0.8 ml and finally diluted to 1 ml with light petroleum. The final sample extract was transferred to an autosampler vial with PTFE-lined crimp cap and could be stored in a refrigerator at 5°C for at least 2 weeks prior to GC analysis.

RESULTS AND DISCUSSION

Sample pretreatment and clean-up

The off-line treatment of fatty samples and oil seeds with concentrated sulphuric acid [19,20] and the use of SPE clean-up with Florisil as an adsorptive matrix for the determination of OCPs has been reported by several workers [18,23,25]. In this work, a method was developed utilizing the advantages of Florisil and Extrelut as adsorptive materials for sample clean up and the effective removal of triglycerides with concentrated sulphuric acid by an on-line technique using a new "sandwich"-type extraction column.

The preseparation of non-volatile oleagenous compounds from OCPs is essential for reliable and
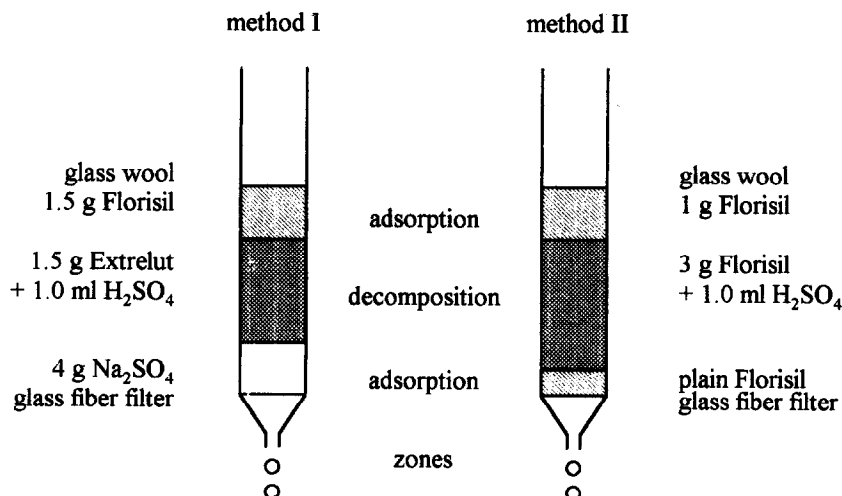

Fig. 1. "Sandwich"-type clean-up columns performing solid-phase extraction (SPE), on-line treatment with concentrated sulphuric acid and liquid-phase extraction (LPE) for the determination of OCPs in vegetable oils. Dimensions of glass column, 200 mm × 9 mm I.D.

rugged capillary GC–ECD of OCPs to prevent uncontrolled adsorption phenomena in the injector inlet or deterioration of the film coating of the GC capillary. However, preloading of the adsorption material in the centre of the specific clean-up column with concentrated sulphuric acid allows complete saponification and decomposition of triglycerides and oxidation of mucilaginous compounds without deterioration of the analytes of interest (with the exception of dieldrin and endrin). The resulting more polar reaction products are essentially not extractable with light petroleum and remain in the sulphuric acid layer and/or are adsorbed on the lower sodium sulphate (method I) and Florisil (method II) layers. The described "sandwich"-type clean-up column offers and combines two different separation techniques: (a) solid-phase extraction (SPE), more correctly termed liquid–solid-phase extraction (LSPE) between the eluent and the Florisil packing material and (b) liquid-phase extraction (LPE) via liquid–liquid partitioning between the non-polar eluent and the polar sulphuric acid layer adsorbed (loaded) on the Extrelut or Florisil surface.

The effectiveness of purification from other interfering matrix components, the recoveries and elution behaviours of several OCPs using this combined SPE–LPE clean-up system using magnesia-silicate materials such as Florisil (method II) and Extrelut (method I) have been studied.

The two "sandwich-type" extraction columns depicted in Fig. 1 revealed similar "clean" extracts with a minimum of ECD-sensitive compounds but not interfering with the OCP analytes. Nevertheless, the higher adsorption strength of Florisil (method II) resulted in high elution volumes and therefore a higher solvent consumption and an increase in sample clean-up time, which makes the column in method I more favourable.

In both instances a top layer of 1.5 g of Florisil was successful in adsorbing first the chlorophyll, revealed by the dark green colour of this zone and further preventing the column bed from plugging, which was particularly convenient when using Extrelut columns.

Elution of OCPs from Florisil or silica gel columns is usually performed with light petroleum, hexane or 6% diethyl ether in hexane [18]. However, the polarity of the adsorbents has to be pread-

justed and standardized for reliable results and constant recoveries for pesticides; this could be achieved by using a polar prerinsing eluent as the "upper phase" of a two-layer system of light petroleum–acetonitrile–ethanol (100:25:5). By this preadjustment of the polarity of the adsorbents, which is otherwise usually carried out by laborious and time-consuming procedures (high temperature treatment of the adsorbents and re-equilibration with polar solvents), the sample work-up procedure is essentially simplified.

Table II presents the elution profiles and total recoveries of OCPs (100 ppb each in pumpkin seed granules) by elution of the adsorbed OCPs from Extrelut (method I) or Florisil (method II) "sandwich"-type extraction columns with light petroleum or "upper phase", revealing better recoveries and less solvent consumption for the Extrelut "upper phase" technique (method I) for most of the investigated OCPs except γ-HCH and endosulfan I.

Fig. 2A shows the profile of OCPs in pumpkin seeds after clean-up according to method I and final GC–ECD analysis. MS detection with electron impact (EI) ionization specific for each pesticide or co-chromatography (Fig. 2B) on a second capillary (RTX-5) was used for peak identification, showing inversion of retention for HCB and α-HCH and for 4,4'-DDT and 4,4'-DDD.

The described SPE–LPE technique with on-line sulphuric acid treatment failed only in the determination of dieldrin and endrin, owing to irreversible deterioration of these compounds by oxidation to undefined products, which could not be detected by GC–ECD. However, the incidence of these pesticides in Austrian oil seeds is rare. Nevertheless, one way of separating dieldrin and endrin from fatty products was to use SPE on Florisil (Fig. 1, method II) with "upper phase" elution but with omission of sulphuric acid treatment. The extracts obtained were relatively "clean" and ready for injection into the GC column, but it turned out that they were not completely free from triglycerides and therefore this method cannot be recommended for long-term routine analysis, owing to the decrease in detector sensitivity and deterioration of the separation efficiency of the GC capillary.

Nevertheless, the combination of decompositional (SPE–LPE) and non-decompositional (SPE) methods can be used as "chemical proof" for the

TABLE II

RECOVERIES AND ELUTION PROFILES OF ORGANOCHLORINE PESTICIDES FROM "SANDWICH"-TYPE SPE–LPE COLUMNS WITH DIFFERENT ELUENTS

A = Method I (Extrelut) with light petroleum; B = method I with "upper phase"; C = method II (Florisil) with light petroleum; D = method II with "upper phase". "Upper phase" = supernatant phase of light petroleum–acetonitrile–ethanol (100:25:5).

Compound	Pesticide recovery (%)																							
	A						B						C						D					
	Total recovery	Fractions[a]					Total recovery	Fractions[a]					Total recovery	Fractions[a]					Total recovery	Fractions[a]				
		1	2	3	4	5		1	2	3	4	5		1	2	3	4	5		1	2	3	4	5
HCB	65	91	5	3	1	—	92	83	16	1	—	—	63	39	46	10	3	2	98	91	9	—	—	—
α-HCH	60	94	3	2	1	—	83	82	15	3	—	—	71	46	52	2	—	—	83	87	13	—	—	—
γ-HCH	63	96	4	-	—	—	82	82	18	—	—	—	72	15	49	26	10	—	88	37	54	9	—	—
β-HCH	97	69	13	8	6	4	97	52	42	6	—	—	58	36	40	17	7	—	66	23	37	32	8	—
Heptachlor	73	98	2	—	—	—	86	88	12	—	—	—	75	84	16	—	—	—	51	100	—	—	—	—
Aldrin	47	100	-	—	—	—	83	93	7	—	—	—	68	85	15	—	—	—	78	96	4	—	—	—
Heptachlor epoxide	44	93	7	—	—	—	81	90	10	—	—	—	51	15	42	21	14	8	81	83	17		—	—
Endosulfan I	11	93	7	—	—	—	69	69	31	—	—	—	40	9	22	28	22	19	70	86	14	—	—	—
2,4'-DDE	75	90	8	2	—	—	88	91	7	2	—	—	51	36	51	9	4	—	56	64	36	—	—	—
4,4'-DDE	94	95	5	—	—	—	86	92	7	1	—	—	59	81	19	—	—	—	77	95	5	—	—	—
Dieldrin	—	—	—	—	—	—	7	—	—	—	—	—	30	—	15	24	30	31	30	77	23	—	—	—
2,4'-DDT	85	92	7	1	—	—	96	86	9	5	—	—	55	70	30	—	—	—	53	55	45	—	—	—
Endrin	—	—	—	—	—	—	6	18	82	—	—	—	32	—	10	28	27	35	73	69	28	2	1	—
4,4'-DDT	85	93	6	1	—	—	97	85	11	4	—	—	53	70	30	—	—	—	19	71	29	—	—	—
4,4'-DDD	87	90	7	2	1	—	96	83	12	3	2	—	52	57	40	2	1	—	71	83	16	1	—	—

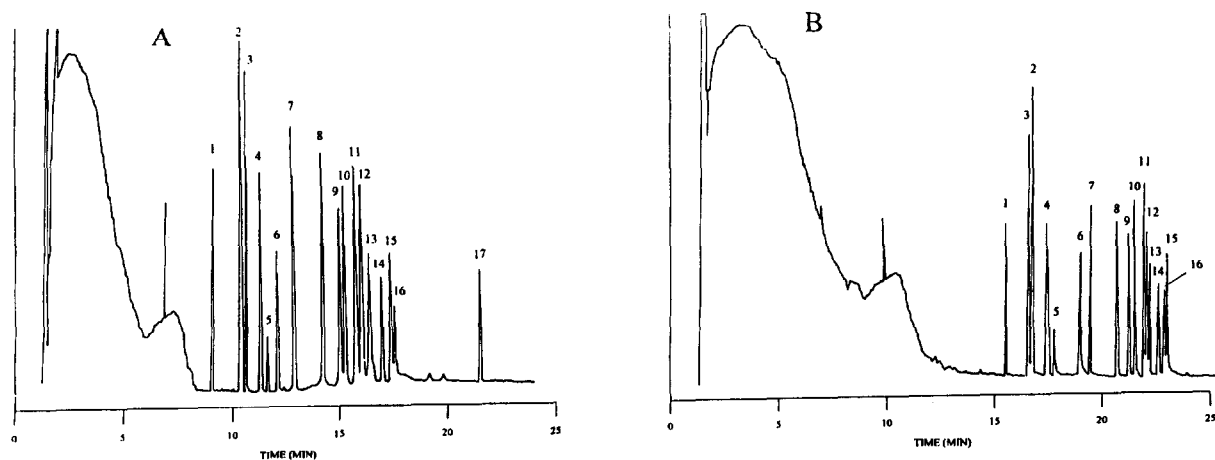[a] Elution with 5 × 10 ml of light petroleum or "upper phase".

Fig. 2. GC–ECD of OCPs (100 ppb each) in spiked pumpkin seeds after clean-up according to method I, (A) on RTX-35 and (B) on RTX-5 capillary columns. For experimental details, see text. 1 = PCB (pentachlorobenzene); 2 = HCB (hexachlorobenzene); 3 = α-HCH (α-hexachlorocyclohexane); 4 = γ-HCH (lindane); 5 = β-HCH; 6 = heptachlor; 7 = aldrin; 8 = heptachlor epoxide; 9 = 2,4'-DDE; 10 = endosulfan; 11 = 4,4'-DDE; 12 = dieldrin; 13 = 2,4'-DDT; 14 = eldrin; 15 = 4,4'-DDT; 16 = 4,4'-DDD; 17 = Mirex. Pentachlorobenzene and Mirex were added as internal standards.
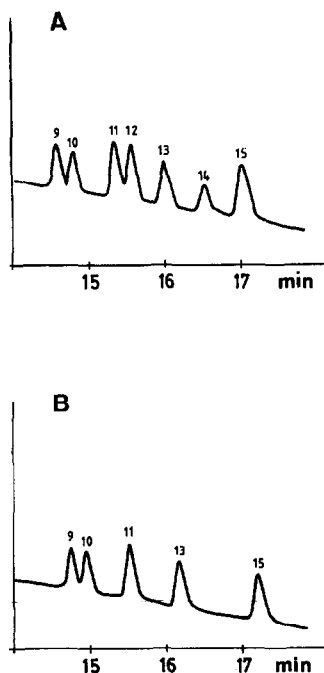
Fig. 3. GC–ECD of OCPs (5 ppb each) in spiked pumpkin seeds showing the retention window of 9 = 2,4'-DDE, 10 = endosulfan, 11 = 4,4'-DDE, 12 = dieldrin, 13 = 2,4'-DDT, 14 = endrin and 15 = 4,4'-DDT after SPE clean-up according to (A) method II without sulphuric acid treatment and (B) method I with sulphuric acid treatment. Note that in (B) peaks 12 and 14 are missing but the other peaks reveal similar peak areas.

presence of dieldrin and endrin in oil samples (Fig. 3).

Very low recoveries of OCPs, especially HCB, were observed after accidentally concentrating the SPE or SPE–LPE extracts to dryness. Addition of high-boiling solvents such as 1-phenyl-2-propanol, 1-phenylethanol and 1-decanol to the light petroleum extract before concentration can prevent evaporation completely to dryness and undesirable volatilization of OCPs. However, this method was not convenient. To avoid additional sources of contamination with organochlorine compounds, we decided to ensure complete OCP recoveries only by accurate concentration to 1 ml by a gentle stream of nitrogen at ambient temperature.

To prevent the formation of emulsions and irreproducible SPE–LPE performance, all extracts have to be completely dry. This could be achieved very simply by mixing the oil seed granules with anhydrous sodium sulphate prior to extraction with light petroleum.

Quantification

Quantification of OCPs in oil seeds was performed using external standard or internal standard calibration methods.

External standard calibration suffers from erratic GC-injections and irregularities in sample pretreatment. As a potential risk we observed an undesirable change in detection sensitivity and retention behaviour with a sequence of multiple injections of oily extracts containing 100 ppb of HCB, although special care was taken with sample pretreatment. However, after injection of at least six "dummies" the accuracy becomes sufficient for external calibration. These effects could also be avoided by prolonging the final heating and equilibration time of the GC column, but this has to be paid for by a longer analysis time. For compensation of deviations during sample pretreatment, calibration standards in the range 1–250 ppb, prepared by spiking oil seeds of very low contamination (< 1 ppb), were treated in exactly the same way as all other samples. All these precautions allowed external standard calibration for accurate determination of the major OCPs.

Day-to-day and within-day reproducibility tests on two "real" samples contaminated with 120 and 2 ppb of HCB, revealed acceptable relative standard deviations for trace analysis (Table III). The calibration points for OCPs in the range 1–250 ppb were sufficiently well correlated (correlation factors between 0.997 and 0.998 were achieved).

Most of the inconveniences in quantification with external standard calibration could be overcome by using calibration with internal standards. An ideal internal standard should have similar chemical and physical properties to the analytes, allowing identical extraction, sample pretreatment, chromatography and detection. In this work, pentachlorobenzene (PCB) and Mirex (MIR) were used as internal standards for the determination of OCPs in pumpkin seeds and vegetable oils, as they best fitted all the requirements listed above. Evaluation of more than 1000 pumpkin seed samples showed that the extracts are essentially free from these compounds, and free from any interferences in the respective retention windows. The use of two internal standards

TABLE III

DAY-TO-DAY AND WITHIN-DAY REPRODUCIBILITY OF DETERMINATIONS OF HCB IN PUMPKIN SEEDS BY EX-
TERNAL STANDARD AND INTERNAL STANDARD CALIBRATION

For experimental details, see text. All samples were pretreated and purified according to method I.

Pumpkin seed spiked with HCB (ppb)	External standard calibration (%)		Internal standard calibration[a] (%)	
	Within-day[b]	Day-to-day[c]	Within-day[b]	Day-to-day[c]
120	8.5	10.6	5.5	7.6
2	10.6	23.1	9.9	19.3

[a] Internal standard = pentachlorobenzene (PCB).
[b] Relative standard deviation of six determinations.
[c] Relative standard deviation of five determinations.

with different volatilities (appearing in the early and late part of the gas chromatogram) allowed better problem solving in cases of observed irregularities. The loss of highly volatile PCB gives a hint of undesirable losses of analytes by evaporation in the course of sample pretreatment, whereas the loss of Mirex indicates erratic aliquoting and dilution procedures or problems in the GC injector line. The loss of both compounds points to irregular SPE–LPE clean-up or aberrant GC injection.

By this procedure, the time-consuming co-determinations of external standard samples could be omitted and all systematic errors and instrumental deviations could be minimized. The reproducibility

of HCB determinations was improved to a relative standard deviation of 3.5% and linearity of calibration was extended to 400 ppb with good correlation factors of 0.998–0.999. The limited range of linearity with electron-capture detection was compensated for by internal standard calibration, leading to an increase in the linearity of the overall method by a factor of 1.5.

Table IV gives the detection limits and limits of determination for all the investigated OCPs in pumpkin seeds achieved by the present clean-up technique (Fig. 1, method I). The described method was especially designed for the highly sensitive determination of HCB, allowing a detection limit of
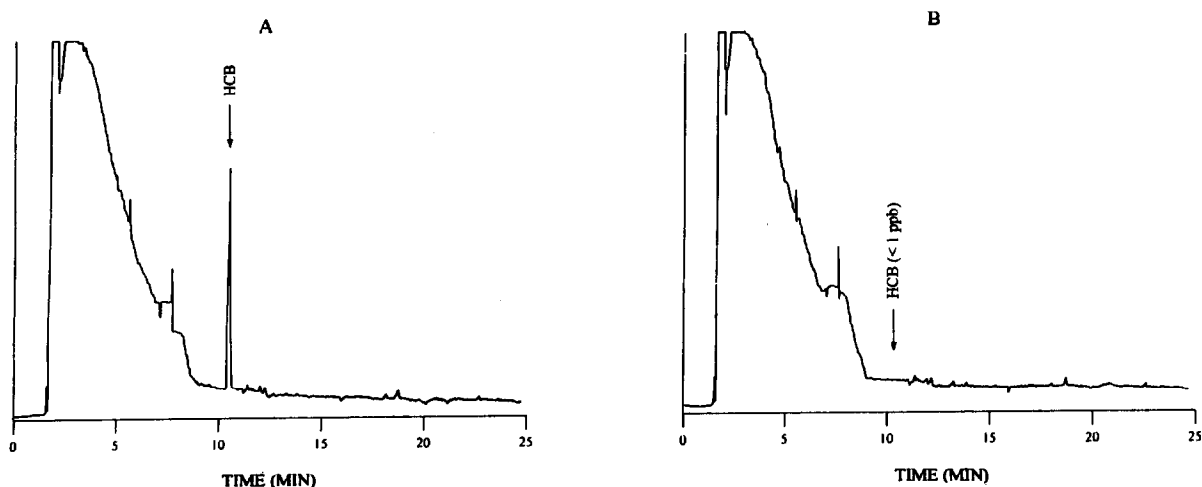

Fig. 4. GC–ECD chromatogram of (A) 70 ppb of HCB in natural contaminated pumpkin seeds and (B) of a non-contaminated "blank" pumpkin seed sample, pretreated according to method I. GC capillary: RTX-35 (for detailed conditions, see Experimental).

TABLE IV

SENSITIVITY OF GC–ECD AND LIMITS OF DETERMINATION OF SEVERAL OCP COMPOUNDS IN VEGETABLE OILS AND OIL SEEDS AFTER CLEAN-UP WITH "SANDWICH"-TYPE SPE–LPE EXTRACTION COLUMNS

OCP	Detection limit (ppb)[a]	Limit of determination in pumpkin seeds (ppb)[b]	
		Method I	Method II
HCB	0.5	1	2
α-HCH	1	2	5
β-HCH	2	5	5
γ-HCH (lindane)	1	2	5
Heptachlor	2	5	5
Aldrin	1	2	5
Heptachlor epoxide	1	5	5
2,4′-DDE	1	2	5
Endosulfan I	4	5	10
4,4′-DDE	1	2	2
2,4′-DDT	3	5	10
4,4′-DDT	3	5	10

[a] Signal-to-noise ratio = 3:1.
[b] Samples are rated as positive for peak areas six times higher than the standard deviation of peak areas from a blank "sample".

500 ppt for standard solutions and a limit of determination of 1 ppb in pumpkin seeds (the ratio of the signal to the standard deviation of the background peaks is 6:1).

The overall recovery with the described method was 80–100% (see Table II) for all major OCP compounds (except dieldrin and endrin), combined with sufficient reproducibility for reliable monitoring of OCP contamination in vegetable oils, particularly pumpkin seed oil.

It was found that 99% of all pumpkin seed samples investigated from the 1990 crop showed HCB contamination in the range 1–400 ppb (median = 22 ppb). The Austrian limit of 250 ppb was exceeded by only 1.6% of the samples, but nearly 75% exceeded the limit of 10 ppb set by some countries in the European Community (EC). Fig. 4 shows a GC–ECD trace of HCB in naturally contaminated pumpkin seeds.

Table V gives a survey of OCP contamination of Styrian pumpkin seeds harvested in 1990 including all the investigated compounds.

Other oil seeds such as soya, sunflower, rape, poppy, linseed, thistle and zulunut, also grown on HCB-contaminated sites, revealed no HCB contamination. However, HCB was occasionally detected in commercially avaiable oils of these seeds in the low ppb range.

TABLE V

OCP CONTAMINATION OF STYRIAN PUMKIN SEEDS OF THE 1990 CROP

A collection of 110 samples harvested in 1990 were investigated for determination of OCPs after sample purification according to method I including OCP elution with the special "upper phase". For additional experimental details, see text.

Contaminant	Positives (%)	Range (ppb)	Mean (ppb)	Median (ppb)	Limit (ppb)[a]
HCB	99	0–400	51.1	25	250
α-HCH	1	0–35	–[b]	–[b]	100[c]
β-HCH	15	0–98	20.8	<5 100[c]	
γ-HCH (lindane)	2	0–24	–[b]	–[b]	150
Aldrin	30	0–16	7.4	<5 10	
ΣDDT[d]	18	0–30	13.2	<2 100	
Endosulfan I	4	0–30	–[b]	–[b]	200

[a] From ref. 9.
[b] Number of positives too low.
[c] ΣHCH = 100 ppb.
[d] ΣDDT (all isomers of DDE, DDD and DDT).

In an interlaboratory test programme, this method using the "sandwich"-type SPE–LPE clean up column (method I) proved to be similar in accuracy to the commonly used SPE and gel permeation chromatographic (GPC) methods but was faster. Compared with these other clean-up techniques, the lifetime of the analytical GC capillary was exceptionally high and even after 3000 sample injections the GC column showed good performance without the need for washing, which was essential for GC columns after GPC clean-up [26].

CONCLUSIONS

A simple and highly reproducible sample pretreatment method for the GC–ECD determination of various OCPs in vegetable oils and oil seeds such as pumpkin, soya, sunflower, rape, poppy, linseed, thistle and zulunut has been developed.

The described method (preferably method I) is reasonably rugged, allowing the routine determination of several OCPs in different vegetable oils and oil seed extracts, and will be applied in further studies of the environmental fate of HCB and the parameters that influence the uptake of HCB in oil seeds, which might help in elucidating some of the human health-relevant aspects of OCP contamination.

We see also a particular advantage of this rugged sample preparation technique for laboratories that occasionally have only a small series of oil samples to analyse for HCB and other OCPs; its installation is simple and inexpensive.

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