Note

Comparison of sulphuric acid treatment and column chromatographic clean-up procedures for the gas chromatographic determination of organochlorine compounds in some food commodities

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There is a worldwide concern over the presence of residues of organochlorine compounds in various components of the environment[1-3]. As the diet is the major route of exposure of the general public to chemical contaminants[4,5], the determination of these residues in foodstuffs is of major importance. The determination of organochlorine compounds in food samples involves solvent extraction, clean-up to remove extraneous substances, determination of residues by gas chromatography (GC) and confirmation of the nature of the contaminants[6-9]. Adsorption column chromatography, used to clean-up the extracts before GC determination in standard methods of pesticide residue analysis[8,9],is a major factor affecting the reproducibility of the overall analytical procedure[10]. Moreover, this technique is time consuming and requires large amounts of highly purified solvents and costly adsorbents. Hence there is a need for rapid and inexpensive clean-up techniques[11,12].

The treatment of light petroleum extracts of both fatty[13-17] and non-fatty foods[18] with sulphuric acid to remove co-extractives from acid-stable compounds has been suggested as a convenient alternative to column chromatographic clean-up. This paper reports the relative efficacy of these two clean-up procedures for the determination of organochlorine residues in food commodities.

EXPERIMENTAL

Gas chromatograph

A Packard Model 7624 equipped with a tritium source electron-capture detector and a 1.84 m × 2 mm I.D. glass column packed with 1.5% OV-17 + 1.95% OV-210 on 110–120-mesh Gas Chrom Q was used. The injection port temperature was 210°C, the column oven temperature 190°C and the detector temperature 200°C and the carrier gas was nitrogen at a flow-rate of 70 ml min^{-1}.

Reference standards

The α, β, γ and δ isomers of HCH (1,2,3,4,5,6-hexachlorocyclohexane), *p,p'*-DDT [1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane], *p,p'*-DDD [1,1-dichloro-2,2-bis(4-chlorophenyl)ethane] and *p,p'*-DDE [1,1-dichloro-2,2-bis(4-chlorophenyl)-ethene] of greater than 95% purity were obtained from the U.S. Environmental Protection Agency, Research Triangle Park, NC, U.S.A.

Reagents

Acetone, acetonitrile, anhydrous sodium sulphate, benzene, concentated sulphuric acid (sp. gr. 1.84), hexane (boiling range 67–70°C), methanol, silica gel (60–100 mesh) and sodium chloride was used. The suitability of the reagents for residue analysis was ensured by running reagent blanks.

Sample preparation

The amounts of various food items constiuting food groups of the total diet were determined by conducting a dietary survey (Table I). Representative amounts of constituents of each food group were processed according to local practice and their edible pàrts homogenized to obtain composite material.

TABLE I

CONSTITUTION OF FOOD COMPOSITES USED FOR RECOVERY STUDIES

Food composite	Composition*
Cereals	Wheat flour (79), rice (16), bread (5)
Pulses and legumes	French beans (25), black gram (25), green gram (25), soya beans (25)
Root vegetables	Potato (69), onion (23), carrot (8)
Non-root vegetables	Tomato (60), cauliflower (24), coriander leaves (6), gourd (6), green chillies (4)
Fruits	Mango (66), sapota (34)
Meat and eggs	Chicken (45), eggs (55)

* Figures in parentheses are percentage contributions of different food items in a food composite on a raw weight basis.

Fortification studies

Composites equivalent to 50 g fresh weight were fortified with various organochlorine compounds at the concentrations given in Table II. For calculating recoveries, background levels of residues in unfortified samples were subtracted from the values obtained for fortified samples. All analyses were carried out in duplicate.

Extraction and partitioning

Cereals, pulses, legumes, root vegetables and non-root vegetables. Composites equivalent to 50 g fresh weight were extracted twice with 100- and 50-ml portions of acetonitrile by blending for 3 min each time. The combined extracts were transferred into a separating funnel, diluted with 600 ml of 5% aqueous sodium chloride and partitioned with two 100-ml portions of hexane. The aqueous phase was discarded and the hexane phases were pooled, dried over anhydrous sodium sulphate and concentrated to about 10 ml.

Fruits. Composites representing 50 g fresh weight were extracted twice with 100- and 50-ml portions of acetonitrile–water (2:1, v/v) by blending for 3 min each time and processed further as described for cereals, etc.

Meat and eggs. Composites equivalent to 50 g fresh weight were extracted twice with 100- and 50-ml portions of hexane–acetone (2:1, v/v) by blending for 3 min each time. The combined extracts were transferred into a separating funnel and washed twice with 300-ml portions of 5% aqueous sodium chloride. The aqueous phases were discarded and the hexane layer was dried and concentrated as described for cereals, etc.

Clean-up

Sulphuric acid clean-up. To a concentrated hexane extract in a 25-ml separating funnel, sulphuric acid was added dropwise until the hexane phase became clear. The lower layer of acid was discarded and the upper phase was washed with two 10-ml portions of distilled water.

Column chromatographic clean-up. Silica gel washed with acetone–methanol (1:1, v/v) was air dried, activated at 130°C for 1 h and a 20-g portion was packed in a glass column (40 cm × 2 cm I.D.) between 1-cm layers of anhydrous sodium sulphate. The column was pre-washed with 100 ml of hexane and extracts from the extraction and partitioning step were added to it after concentration to about 2 ml. The column was eluted with 100 ml of hexane–benzene (1:1, v/v) and the eluate was concentrated to about 10 ml[19].

Determination of residues

Suitable aliquots of the cleaned-up extracts were injected onto the GC column to obtain peak heights of compounds of interest within the scale. The residues were identified and quantified by comparison of the retention times and peak heights of the sample chromatograms with those of standards run under identical conditions.

RESULTS AND DISCUSSION

The average recoveries for DDT derivatives and HCH isomers following sulphuric acid clean-up of samples belonging to various food groups ranged from 80.6 to 107.0% (Table II) and were satisfactory. The corresponding mean recoveries from the column chromatographic clean-up of cereals, pulses, legumes, root vegetables, non-root vegetables and fruit composite ranged from 71.8 to 112.4%. Considering that a latitude of 20–50% is considered permissible in trace analysis[20], these recoveries values are similar to those observed for acid clean-up. For the column chromatographic clean-up of meat and eggs composite, adequate recoveries (85.0–89.7%) of DDT derivatives were observed. However, low recoveries (47.5–58.7%) for HCH isomers, which eluted late during column chromatography, were obtained. This may be due to a decrease in the resolving power of the adsorbent owing to the lipids present in the extract of this fatty substrate.

Gas chromatograms of samples and reagent blanks cleaned up with sulphuric acid were generally found to be free from extraneous peaks (Fig. 1). For pulse, legume and non-root vegetable composites, a light green colour persisted after acid treatment. It did not produce interfering peaks but, considering that the presence of co-extractives could reduce the lifetime of the GC column and result in a decrease in detector sensitivity, attempts were made to remove it. Centrifugation at 2000 rpm for 20 min or allowing the acid-treated extracts to stand for about 12 h was found to result in sedimentation of the colouring matter.

Reagent blanks and sample extracts cleaned-up by column chromatography frequently gave noisy baselines and early eluting peaks that could interfere in the determination of HCH residues (Fig. 1). This was probably due to the accumulation of impurities from adsorbents and concentration of large volumes of solvents used for column elution. Stringent quality control therefore had to be maintained for the samples being analysed by column chromatographic clean-up to ensure that the solvents and reagents did not produce interferences.

TABLE II

AVERAGE AND RANGE OF RECOVERIES OF VARIOUS ORGANOCHLORINE COMPOUNDS FROM FORTIFIED SAMPLES OF DIFFERENT FOOD COMPOSITES

Compound	Level of fortification ($\mu g\ g^{-1}$)	Recovery (%)					
		Cereals		Pulses		Root vegetables	
		S*	C**	S*	C**	S*	C**
α-HCH	0.016	93.1 ± 2.9	82.5 ± 4.1	96.8 ± 1.8	78.1 ± 1.8	98.7 ± 1.0	82.1 ± 0.8
β-HCH	0.064	92.5 ± 1.8	106.2 ± 8.2	97.5 ± 1.0	85.1 ± 1.6	100.0 ± 0.0	74.2 ± 1.1
γ-HCH	0.016	91.9 ± 0.0	80.6 ± 4.3	80.6 ± 0.0	79.6 ± 2.4	93.1 ± 1.7	71.8 ± 0.5
δ-HCH	0.032	92.0 ± 2.1	100.5 ± 6.2	96.5 ± 1.5	82.1 ± 4.1	98.7 ± 0.2	90.5 ± 0.5
p,p'-DDE	0.02	99.0 ± 2.9	82.4 ± 2.1	99.1 ± 1.0	84.5 ± 1.7	89.4 ± 1.9	89.8 ± 3.2
p,p'-DDD	0.04	94.4 ± 2.4	103.1 ± 3.6	93.2 ± 2.2	80.0 ± 1.9	87.2 ± 0.8	93.2 ± 0.0
p,p'-DDT	0.04	95.6 ± 1.5	112.4 ± 4.3	94.5 ± 1.5	89.6 ± 2.2	95.5 ± 1.5	91.2 ± 2.0

* Sulphuric acid clean-up.
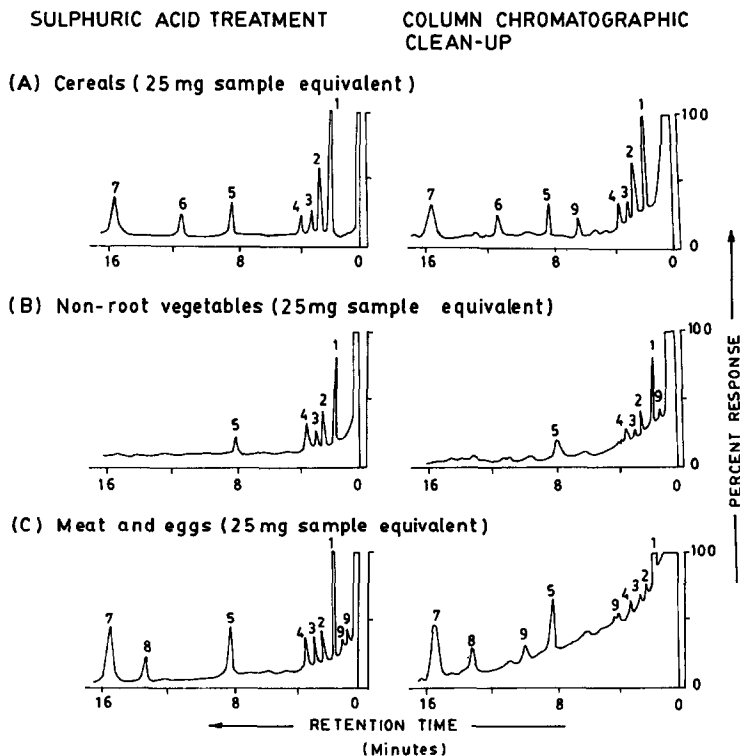** Column chromatographic clean-up using silica gel as adsorbent.

Fig. 1. Gas chromatograms of extracts of some unfortified food composites cleaned up by sulphuric acid treatment or the column chromatographic procedure. Peaks: 1 = α-HCH; 2 = γ-HCH; 3 = β-HCH; 4 = δ-HCH; 5 = p,p'-DDE; 6 = o,p'-DDT; 7 = p,p'-DDT; 8 = p,p'-DDD; 9 = unidentified.

Non-root vegetables		Fruits		Meat and eggs	
S*	C**	S*	C**	S*	C**
92.7 ± 0.1	80.7 ± 0.6	94.0 ± 2.5	83.6 ± 2.4	91.1 ± 5.2	49.7 ± 0.8
91.8 ± 2.8	84.4 ± 0.9	99.0 ± 1.1	82.1 ± 3.2	93.8 ± 4.2	56.7 ± 2.0
89.8 ± 2.1	81.2 ± 0.2	90.7 ± 2.1	87.4 ± 2.1	84.2 ± 0.9	47.5 ± 0.9
92.0 ± 3.5	86.3 ± 0.3	102.9 ± 1.1	81.0 ± 1.6	81.4 ± 2.3	52.6 ± 3.0
90.0 ± 3.2	81.8 ± 0.1	106.0 ± 1.0	82.4 ± 2.7	95.0 ± 3.0	86.4 ± 3.3
84.6 ± 1.6	85.0 ± 2.6	107.0 ± 1.0	84.6 ± 4.1	83.2 ± 3.0	85.7 ± 1.7
86.0 ± 2.8	84.4 ± 2.0	94.3 ± 3.3	88.4 ± 2.8	82.9 ± 1.5	85.0 ± 0.0

When residues are being determined by GC with electron-capture detection, confirmation of the identity of the peaks obtained is considered essential[6,8,9]. The extracts cleaned up with sulphuric acid were generally found suitable for micro-alkali derivatization[21] and thin-layer chromatographic (TLC)[22] confirmatory techniques. However, TLC of meat and eggs composites sometimes produced streaks, even though the GC analysis of such extracts did not show any co-extractives. Such behaviour of fatty foods has also been reported by McGill and Robinson[23], who attributed it to the probability that when an extract is injected into the GC system, less volatile co-extractives such as fats remain at the injection port and do not reach the detector to produce extraneous peaks. However, they interfere with the adsorption mechanism in TLC and cause streaking. Veierov and Aharonson[14] also observed that some lipid carryover or small amounts of undigested fats remain after acid treatment of extracts of fatty substrates.

In addition to the DDT derivatives and HCH isomers determined in this study, sulphuric acid clean-up has been reported to be applicable to other organochlorine residues such as aldrin, Aroclor 1254, α- and γ-chlordane, heptachlor, hexachlorobenzene (HCB) and o,p'-TDE[13]. However, sulphuric acid treatment allows the determination only of acid-stable compounds and column chromatographic clean-up has to be used if the determination of acid-labile compounds is required. For example, dieldrin, endosulfan A, B and sulphate, endrin and heptachlor epoxide are degraded by sulphuric acid and cannot be determined by this technique[13,24].

Recently Hernández et al.[25] evaluated the efficacy of sulphuric acid clean-up for the determination of 24 organochlorine compounds in wastewater samples. Four compounds (dichloran, dieldrin, endrin and trifluralin) were destroyed after treatment with this acid, whereas complete recoveries (90.3–118.6%) were obtained for sixteen organochlorine residues. Fenson and tetradifon were partially degraded by this treatment.

As sulphuric acid treatment clean-up is simple, rapid, efficient and requires less solvents and glassware, it is to be preferred to column chromatographic clean-up for the determination of acid-stable compounds in food products.

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