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Note

Single-step solid-matrix clean-up of vegetable extracts for organophosphorus pesticide residue determination

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In the analysis of organophosphorus pesticide (OP) residues in fruits and vegetables with multi-residue procedures based on water-miscible solvents (acetone, acetonitrile) as extraction solvents, a major problem is that the purification involves multiple stages (performing separate functions) with successive manual operations[1].

For instance, with acetone as extraction solvent, pesticide residues are separated from crude aqueous acetone extracts by a liquid–liquid partition stage followed by a clean-up stage. The first stage typically employs dilution of the aqueous acetone extract with a salt (sodium chloride, sodium sulphate) solution and several successive separating funnel extractions into dichloromethane. Under these conditions a wide range of both polar and non-polar pesticide residues can be recovered[1–9]. The dichloromethane extract is dried by passage through a column of anhydrous sodium sulphate and subjected to clean-up before the final determination. For the clean-up stage, the most commonly used techniques for OP residues are size-exclusion chromatography[5,8–13], sweep co-distillation[14–17] and column chromatography on Florisil[18] or charcoal and its mixtures[4,19,20]. Taking advantage of phosphorus-selective detectors, such as flame photometric detectors (FPD-P) or the alkali-bead thermionic detector (NPD), the dichloromethane extract has also been used without any clean-up for the determination of OP residues[3,6].

The drawbacks of the above and other similar procedures, however, are the amounts of solvents and reagents required, the washing and preparation of glassware, the occurrence of troublesome emulsions in the aqueous acetone–dichloromethane extraction stage with certain vegetable products, the preparation and maintenance of costly apparatus and, most important, the number of handling operations, which strongly affect the throughput of the residue laboratory.

We have therefore developed a rapid, single-step procedure in which solid-matrix, disposable cartridges are used as a support to carry out the extraction and clean-up of OPs from crude aqueous acetone extracts of vegetable products. The resulting solution is suitable for the direct determination of OP residues by gas chromatography (GC) with FPD-P detection.

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EXPERIMENTAL

Reagents

Analytical-reagent-grade light petroleum (b.p. 40–60°C), dichloromethane and acetone were redistilled in glass.

Ready-to-use Extrelut-20 columns, code No. 11737, were obtained from Merck (Darmstadt, F.R.G.).

Organophosphorus pesticide reference standards were from the collection in this laboratory.

Apparatus

The GC analyses were carried out on a Perkin-Elmer Sigma 4-B gas chromatograph equipped with a flame photometric detector operated in the phosphorus mode (FPD-P). A glass column (1.8 m × 4 mm I.D.) was packed with 5% QF-1 on Chromosorb W HP (100–120 mesh). The temperatures were as follows: oven, 180; and inlet and outlet blocks, 225°C. The carrier gas was helium at a flow-rate of 60 ml/min. The hydrogen and air flow-rates to the detector were set according to the manufacturer's instructions. A source of pure nitrogen, capable of delivering gas at a flow-rate of 2 l/min measured with a rotameter, was used.

Procedure

Prepare aqueous acetone extracts of fruits and vegetables according to ref. 2 or 4. Take a 15-ml aliquot of the extract equivalent to *ca.* 5 g of crop and transfer it on to the top of an Extrelut-20 column. Allow the liquid to drain and wait 10 min to obtain an even distribution on the filling material. Pass through the column, from bottom to top, a nitrogen flow of 2 l/min for 30 min. Disconnect the Extrelut-20 column from the gas line, attach to the column outlet a 0.70 × 32 mm Luer-lock needle (supplied with the column) as a flow regulator and elute the column with four 20-ml portions of light petroleum (b.p. 40–60°C), then with four 20-ml portions of dichloromethane–light petroleum (b.p. 40–60°C) (1:3) to elute dimethoate. Concentrate to a small volume using a rotary evaporator (40°C; reduced pressure), then to dryness by manually rotating the collecting flask. Dilute to a suitable volume with acetone and analyse by GC. Determine the OP concentration in the sample extract by comparison of the peak height with that of an external standard of comparable concentration.

For recovery experiments, add suitable amounts of standards to the chopped vegetables in the homogenization jar. Allow the solvent to evaporate, then proceed with the extraction.

RESULTS AND DISCUSSION

Extrelut-20 columns are ready-to-use, disposable cartridges filled with a macroporous Kieselguhr-type material and have found several applications in the extraction of drugs from body fluids[21] and in pesticide residue analysis[22–24]. Although a column can hold *ca.* 20 ml of liquid, we have applied only 15 ml of aqueous acetone extract (corresponding to *ca.* 5 g of crop) in order to avoid possible mechanical displacement of water into the eluate. The removal of the major part of acetone (*ca.* 90% of the limiting weight loss under the described conditions; see Fig. 1) is necessary

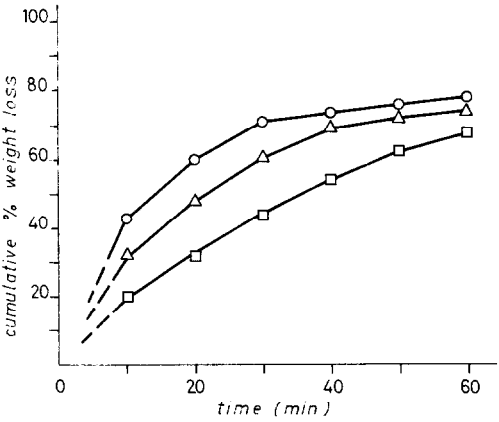

Fig. 1. Rate of removal of acetone from Extrelut-20 columns loaded with 15-ml of sample extract as a function of time and nitrogen flow-rate, expressed as cumulative percentage weight loss. (□) 0.5 ml/min; (△) 1 l/min; (○) 2 l/min.

to prevent the carry-over of the water by the eluting solvent. Further, the small zone of unwetted filling material exerts some adsorptive effect toward the green pigments of vegetables and possibly other coextractives. This effect, combined with the low polarity of the eluent used, leads to a substantial retention of coextractives on the column. In fact, by applying 15 ml of aqueous acetone extracts of different crops, namely lettuce, onion, strawberry, apple, yellow pepper, peach, tomato, broccoli, cauliflower and radish, the amount of coextractives in the eluate ranged from 2 to 10 mg. This weight range is of the same order as that obtained by subjecting the aqueous acetone extracts to the classical, time-consuming sequences of separating funnel partition against dichloromethane[1–9], drying over anhydrous sodium sulphate, solvent exchange and clean-up by Florisil[18] or charcoal column chromatography[4,19,20], size-exclusion chromatography[5,8–13] or sweep co-distillation[14–17]. However, compared with these previous methods, the same performance with our method is obtained in a shorter time (*ca.* 60 min), with very simple operations, and by using only one disposable item and a reduced volume of solvents. With the crops tested, the extracts were almost uncoloured or light yellow.

After this clean-up the extracts are clean enough to be concentrated and analysed by GC without further treatment. Owing to the small amount of coextractives injected, the injection port of the gas chromatograph afforded many injections of sample extracts before cleaning was necessary, and the GC column did not display any loss of performance. Further, owing to the high selectivity of the detector used, the chromatograms of the "blank" crops tested were all free from interfering peaks and were similar to those obtained by injecting solvent alone; the chromatograms of the "spiked" crops, at the levels tested, were indistinguishable from those obtained by injecting the standard compounds. In particular, in contrast to Luke *et al.*'s findings[3], we did not observe interfering peaks in the chromatograms of broccoli, cauliflowers, onions and radishes obtained according to our procedure.

The recovery of pesticides was investigated for 18 OPs representative of a wide range of polarities and water solubilities (diazinon, etrimfos, chlorpyrifos-methyl,

pirimiphos-methyl, chlorpyrifos, bromophos, bromophos-ethyl, malathion, fenitrothion, methacrifos, fonofos, fenchlorphos, dimethoate, parathion-methyl, parathion, methidathion, carbophenothion and ethion). For recovery experiments, blank vegetables (see Table I) were spiked with nine-component OP mixtures containing those compounds which are separated in a single GC run under the described conditions. Most of the pesticides tested were recovered by light petroleum (b.p. 40–60°C) and dimethoate was recovered by further eluting the column with 80 ml of dichloromethane–light petroleum (1:3). However, especially for screening purposes, all of the pesticides tested may be recovered by a single elution with 80 ml of the latter solvent mixture. Also with this solvent mixture, green pigments are retained on the column.

The results of the recovery experiments are presented in Table I. The recovery of pesticides was determined (in triplicate) at different spiking levels. At the levels tested, ranging for the different OP compounds from *ca.* 0.1 to 1.4 mg/kg, the recoveries were between 75 and 110%. These values are satisfactory for residue analysis and are of the same order as those obtained by using more complicated methodologies.

The main feature of the described procedure is that the column appears to perform in a single step several functions, *viz.*, the removal of water and hydrophilic coextractives, the transfer of pesticide residues into a low-boiling solvent and a low-activity adsorption clean-up, giving a solution amenable to direct GC analysis. In classical schemes, the same functions are carried out through separate, time-consuming and labour- and glassware-intensive operations. Unlike the classical separating funnel partitioning[1–9], with the described procedure the extraction is rapid,

TABLE I

RECOVERY OF ORGANOPHOSPHORUS PESTICIDES FROM VEGETABLES

Blank spaces denotate that the specific pesticide–crop combination concerned was not analysed.

Pesticide	*Spike level (mg/kg)*	*Average recovery (%) (n = 3)*					
		Peach	*Broccoli*	*Radish*	*Onion*	*Tomato*	*Cauliflower*
Methacrifos	0.1	83.2	85.4		79.6	82.5	84.9
Fonofos	0.2	90.9	90.3		85.3	82.2	87.0
Fenchlorphos	0.4	101.1	93.3		87.8	84.5	91.8
Dimethoate	0.6				78.0	75.4	77.9
Parathion-methyl	0.7	97.9	90.0		88.9	88.0	92.8
Parathion	1.0	97.1	92.0		88.8	85.3	90.5
Metidathion	1.2	96.4	88.3		87.9	84.5	87.3
Carbofenothion	1.4	100.0	100.0		89.7	88.5	86.7
Ethion	1.0	97.6	90.5		88.0	88.0	89.7
Diazinon	0.2	95.0		94.6	96.7	106.8	100.0
Etrimfos	0.4	98.7		100.0	102.7	108.4	98.6
Chlorpyrifos-methyl	0.4	101.9		100.0	96.8	79.1	103.7
Pirimiphos-methyl	0.3	96.7		96.4	102.6	107.7	100.0
Chlorpyrifos	0.5	97.9		97.7	98.9	89.8	103.9
Bromophos	0.6	102.5		100.0	101.3	108.1	108.6
Bromophos-ethyl	0.6	102.9		98.5	100.0	108.1	103.0
Malathion	1.0	107.0		91.2	89.7	92.4	102.7
Fenitrothion	0.6	104.4		95.2	101.6	110.0	104.0

emulsions do not occur and addition of salt solution and drying of the extraction solvent with anhydrous sodium sulphate are not necessary. Compared with instrumental clean-up techniques (size-exclusion chromatography, sweep co-distillation) the described procedure is very simple, rapid, inexpensive and does not require the preparation or maintenance of costly apparatus or skilled operators.

In conclusion, the main features of this clean-up system compared with previous methods are the good clean-up and recoveries, the wide range of applicability, the minimum of glassware and reagents required, the lack of emulsions, the reduced time for a single clean-up and the simplicity of the operations involved, which allows parallel handling of several sample extracts.

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