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Determination of organophosphorus pesticides in fruits by on-line size-exclusion chromatography–liquid chromatography–gas chromatography–flame photometric detection

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ABSTRACT

The determination of organophosphorus pesticides in fruits by size-exclusion chromatography (SEC) on a polystyrene column coupled on-line to a gas chromatography (GC) system was unsatisfactory as a result of interfering peaks in GC. A liquid chromatography step on silica gel was therefore inserted between the SEC and GC steps to filter out polar by-products. Samples of fruit (apples, grapes and kiwi fruits) were extracted, then the extract filtered or centrifuged and injected into an automated on-line SEC–liquid chromatography–GC–flame photometric detection. Recoveries were about 95% and the detection limits about 1 ng/g.

INTRODUCTION

The large number of analyses carried out in laboratories to determine pesticide residues justifies the construction of an automated analyser. However, although considerable time has been devoted to improving analysis speed, resolution and automation in additon to developing and improving instrumentation and detectors for pesticide analysis, sample processing, particulary its automation, has largely been neglected. This is in spite of the fact that an analytical procedure may require only a few minutes, but sample preparation steps, including extraction, clean-up and concentration, are often more time consuming.

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One of the methods widely used for the clean-up of pesticides is gel permeation chromatography (GPC). Pflugmacher and Ebing [1] described a combination of partitioning and GPC for the clean-up of 22 organophosphorus pesticides using the dextran gel Sephadex LH-20 (Pharmacia). GPC clean-up using polystyrene-type gels, such as Bio-Beads SX-2, and SX-6, was introduced by Stalling *et al.* [2] and automated by Tindle and Stalling [3]. Bio-Beads SX-3 has been used with solvents such as ethyl acetate, cyclohexane, toluene, or mixtures of these [4–10]. Lunardini and Passini [11] described a clean-up procedure using a Waters Ultrastyrogel 500 Å column with toluene.

All these GPC techniques, using large columns and low flow-rates, need long analysis times and large amounts of solvents. For these reasons and because the large capacity of such columns is not required for the on-line transfer of complete frac-

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tions, traditional GPC columns are not suitable for on-line coupling to gas chromatography (GC); small columns preferred to reduce the volumes transferred. Ghijs *et al.* [12], Tuinstra *et al.* [13] and Van Rhijn and Tuinstra [14,15] described the packing of small size-exclusion chromatography (SEC) columns. It was noticed that the retention times of the analytes from columns packed with materials such as Bio Beads were unstable due to the compressibility of the packings. This difficulty was overcome by using a harder, cross-linked polystyrol suitable for high-performance liquid chromatography.

Grob and Kälin [16] described on-line SEC–GC for the determination of chlorinated pesticides in fat-containing foods. They used a 250 × 3 mm I.D. column packed with PSS SDV and were able to inject up to 0.3 mg of fat. Reasonable chromatograms were obtained for olive oil and extracts of fish, chicken and lettuce. Detection limits for pesticide in the fat were less than 10 ng/g.

In classical methods, clean-up for GPC is followed by further clean-up with a variety of systems such as solvent partitioning, Florisil, silica, or aluminum oxide liquid chromatography (LC) columns, which separate the extracts by polarity. The combination of two techniques gives a powerful two-dimensional clean-up by molecular size and polarity.

On-line SEC–GC–flame photometric detection applied to the determination of organophosphorus pesticides in fruits gave gas chromatograms with many interfering peaks. Tailing peaks suggested that the interfering components were primarily of high polarity. These were removed by inserting a silica gel column between the SEC and GC steps, which acted as a filter, removing all components more polar than the pesticides of interest.

The proposed method starts with clean-up of the crude dichloromethane extract by SEC on a polystyrene column and is followed by a second clean-up step on a silica column; the fraction containing the pesticides is transferred to a GC system by concurrent solvent evaporation using a loop-type interface. This on-line SEC–LC–GC method was used for the dermination of 22 organophosphorates. One analysis, including the extraction step, took about 50 min.

EXPERIMENTAL

Chemicals

The analytical standards for the pesticides were obtained from Riedel-de Haën (Selze, Germany) and included phorate, fonofos, diazinon, parathion-methyl, fenchlorphos, fenitrothion, pirimiphos-methyl, malathion, parathion, bromophos-ethyl, tetrachlorvinphos, ditalmifos, isoxathion, ethion, carbophention, pyridaphention, azinphos-methyl, azinphos-ethyl and pyrazophos. Triphenyl phosphate was obtained from Janssen (Geel, Belgium). Dichloromethane and methyl *tert.*-butyl ether (MTBE) were of HPLC grade from Fluka (Buchs, Switzerland).

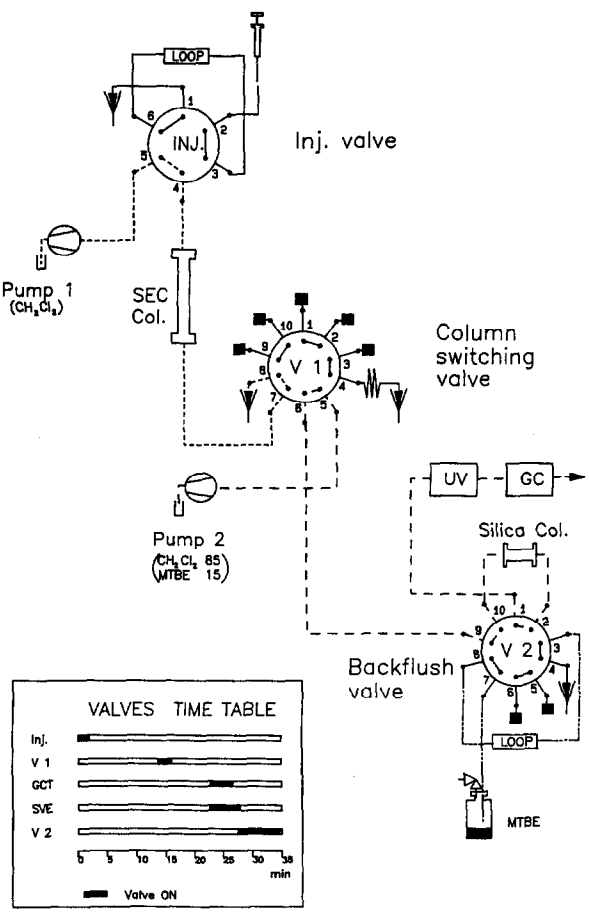

Fig. 1. Schematic diagram of experiment set-up.

Extraction

Chopped fruit (15 g) was extracted with 30 ml of dichloromethane in a 100-ml blender for 30 s. The liquid phase was then centrifuged at 1500 *g* for 30 s to separate solids. The supernatant was filtered through a 0.45-μm disposable syringe filter; a 20-μl volume was injected into the SEC–LC–GC system.

Instrumentation

Analyses were carried out using a Carlo Erba (Milan, Italy) LC-GC Dualchrom 3000 system equipped with two syringe pumps, three multi-port switching valves, a UV detector, an on-column and a loop-type interface, and an automated solvent vapour exit, all under the control of a personal computer. Fig. 1 is a schematic diagram of the experimental layout. The flame photometric detector was a Carlo Erba FPD 500 instrument equipped with a 526 nm interference filter.

Size-exclusion chromatography

SEC was carried out on a 250 × 3 mm I.D. column packed with PSS SDV 100Å, a polystyrene-type material (Polymer Standards Service, Mainz, Germany), with dichloromethane as the mobile phase at a flow-rate of 80 μl/min.

SEC–LC transfer was via the column switching valve (V1) which was actuated 13.5 min after injection and returned 2.5 min later.

Liquid chromatography conditions

LC was performed on a 100 × 2 mm I.D. column packed with Spherisorb Si 5 μm (Stragoma, Wallisellen, Switzerland) with dichloromethane–MTBE (85:15, v/v) as the eluent at a flow-rate of 80 μl/min. The column was cleaned by back-flush with 1 ml of MTBE immediately after the transfer of the fraction of interest to the gas chromatograph.

Liquid chromatograph–gas chromatograph transfer

LC–GC transfer was performed by concurrent eluent evaporation using a loop-type interface [17]. The transfer valve was switched on 22 min 40 s after injection into the SEC system. The volume of the transferred fraction was 450 μl. The carrier gas inlet pressure imposed by the pressure regulator was 1.0 bar (hydrogen); the flow regulator delivered a flow-rate of 2 ml/min. The column temperature during transfer was 80°C. The early vapour exit was closed 1 min after the inlet pressure started to decrease (pressure threshold 20 kPa).

Gas chromatography

Gas chromatographic analysis used a column made up of a 3 m × 0.53 mm I.D. uncoated pre-column deactiviated by phenyldimethyl silation (MEGA, Legnano, Italy), a 3 m × 0.32 mm I.D. retaining pre-column taken from the separation column, a vapour exit with a press-fit T-piece, and a 20 m × 0.32 mm I.D. separation column coated with SE-54 of 0.15-μm film thickness (MEGA). After an isothermal period of 5 min at 80°C (transfer), the temperature was increased at 15°C/min to 130°C/min, at 5°C/min to 260°C and then at 15°C/min to 300°C.

Quantitative data were obtained by the internal standard (I.S.) method, adding 10 μl of triphenyl phosphate at 1 ppm as an I.S. to 1 ml of the sample solution before injection.

Determination of SEC–LC and LC–GC transfer times

The pesticide fraction in the SEC column was determined as follows. Ethion and diazinon were the pesticides least retained in the SEC column. The beginning of the fraction was first determined by injection of ethion (1 ppm) and UV detection at 225 nm. It was then confirmed by transferring fractions before the assumed pesticide fraction to the GC system, shifting the fraction window until the first pesticide peaks appeared on the gas chromatogram. The analogous procedure was applied to determine the end of the fraction using azinphos-methyl. The pesticide fraction in the SEC system had a volume of 200 μl. A similar procedure was adopted to determine the beginning and end of the fraction from LC, *i.e.* the LC–GC transfer time and the volume of the transferred fraction.

RESULTS AND DISCUSSION

The method described here can be used for the automated on-line clean-up and determination of at least 22 organophosphorus pesticides in various fruits such as apples, grapes and kiwi fruit. The method simply requires the extraction of the sample in a blender and, after filtration, injection into the SEC–LC–GC system. Table I gives the recoveries

TABLE I

RECOVERIES FROM UNTREATED FRUITS SPIKED WITH 10 ng/g OF PESTICIDES

Results are means of five determinations. R.S.D. = Relative standard deviation.

Compound (abbreviation)	Grapes		Apples		Kiwi fruits	
	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)
Phorate (Pho)	88.5	4.40	86.3	4.78	82.3	5.54
Fonofos (Fon)	89.4	4.76	80.3	4.35	80.3	5.54
Diazinon (Dia)	94.6	2.11	94.2	2.69	96.3	3.03
Parathion methyl (Par-m)	92.4	0.81	88.8	2.03	93.5	2.20
Fenchlorphos (Fcp)	94.8	1.23	94.7	3.43	92.4	3.12
Fenitrothion (Ftt)	90.7	0.34	94.6	2.33	98.6	1.89
Pirimiphos methyl (Pir-m)	94.5	3.30	91.2	3.33	91.4	2.03
Malathion (Mal)	97.1	3.60	92.5	3.67	88.7	3.23
Parathion (Par)	97.2	2.60	89.5	3.95	96.9	3.48
Bromophos methyl (Brp-m)	97.7	2.22	90.6	3.87	90.5	4.27
Quinalphos (Qui)	98.6	2.59	92.6	2.68	88.8	4.04
Methidathion (Met)	94.4	2.21	93.7	4.50	97.4	2.34
Bromophos ethyl (Brp-e)	98.8	3.34	92.6	4.33	92.3	3.25
Tetrachlorvinphos Tcv)	93.3	4.43	90.8	3.56	86.8	2.75
Ditalmifos (Dit)	94.4	3.23	97.5	3.54	95.6	3.53
Isoxathion (Iso)	97.9	3.76	98.2	4.34	97.6	4.01
Ethion (Eth)	98.4	4.22	96.8	5.22	89.3	4.00
Carbophenthion (Car)	96.4	4.47	92.3	4.64	83.5	3.58
Pyridaphenthion (Pyr)	99.3	3.39	95.4	3.43	97.3	2.22
Azinphos methyl (Azp-m)	99.5	4.23	94.7	5.12	96.8	4.60
Azinphos ethyl (Azp-e)	96.6	3.09	88.9	4.04	94.4	3.23
Pyrazophos (Pzp)	97.2	4.12	95.2	4.32	90.3	4.30

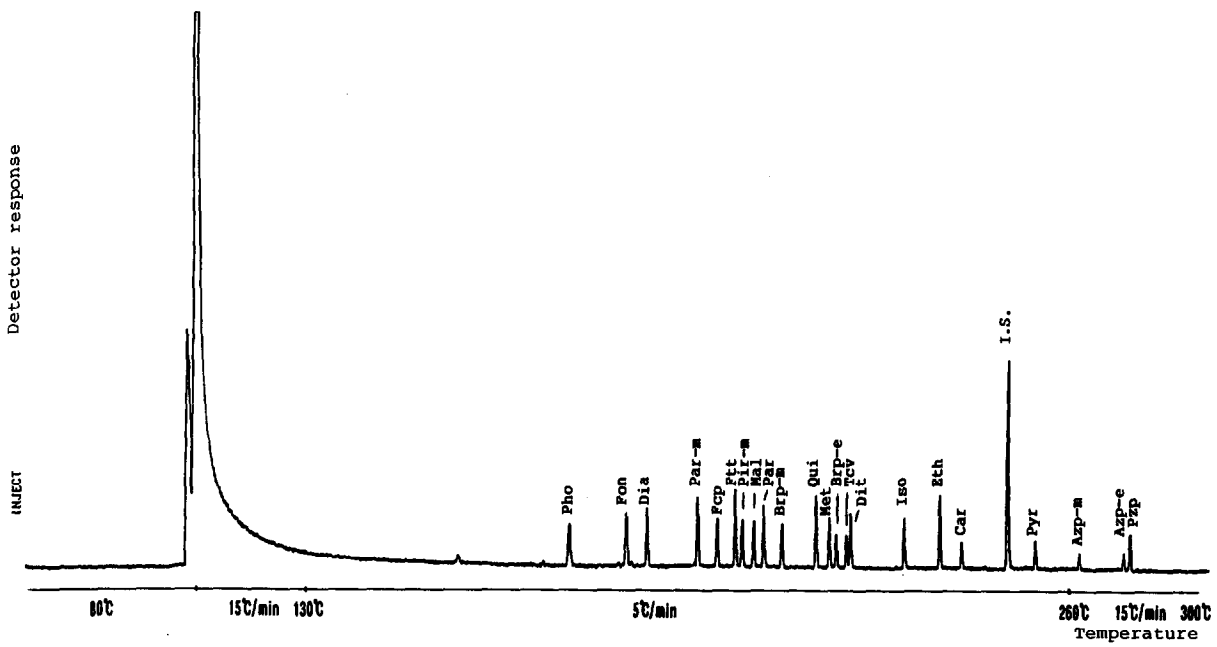

Fig. 2. Chromatogram of pesticides spiked at 5 ng/g in apple samples determined by SEC–LC–GC–flame photometric detection. I.S. = Internal standard (10 ng/ml). For peak identification, see Table I.

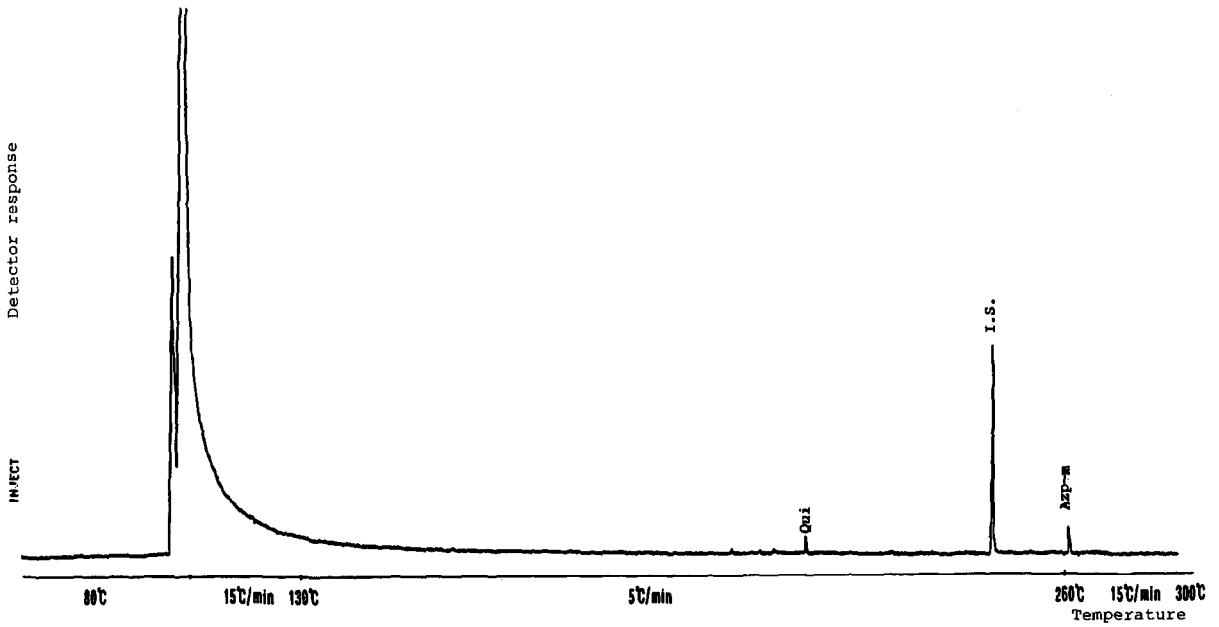

Fig. 3. Chromatogram of pesticides in a real sample containing 1 ppb of quinalphos and 10 ppb of azinophos methyl determined by SEC–LC–GC–flame photometric detection. Note the complete absence of peaks other than the insecticides.

obtained by adding known amounts (10 ng/g) of pesticides to an apple sample; they averaged more than 95%, with a coefficient of variation of about 3%. High recoveries are obtained as losses in on-line systems are largely non-existent. The low coefficients of variation are a consequence of increased accuracy.

Fig. 2 shows a gas chromatogram of an apple spiked with 5 ng/g of the pesticides tested. It shows that the detection limit is about 1 ng/g. Fig. 3 shows the presence of small amounts of quinalphos and azinphos-methyl in apple, approximately 1 and 10 ng/g, respectively, but also the complete absence of interfering peaks. The concentrations of the two insecticides are 100 and 50 times lower than the maximum permitted under Italian law and show the low detection limit obtained. As a result of these low detection limits, maximum residue levels for autorized pesticides can easily be determined. For some pesticides these limits can be determined even with normal analytical methods, although with the method described here it is also possible to monitor some organic products where the illicit use of pesticides is suspected, and the theoretical zero residue can be approached even more closely.

As a result of the efficient clean-up step in this method, a more accurate determination of residues in fatty animal or vegetable samples, such as avocado pears, olives and flours, can be obtained.
No desactivation or loss of efficiency of the analytical column used for GC was noted, even after the analysis of 50 samples.

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