Journal of Chromatography, 607 (1992) 37–45
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 298

Sensitive method for the determination of organophosphorus pesticides in fruits and surface waters by high-performance liquid chromatography with ultraviolet detection

R. Carabias Martinez, E. Rodriguez Gonzalo, M. J. Amigo Moran and J. Hernandez Mendez

Departamento de Química Analítica, Nutrición y Bromatología, Universidad de Salamanca, 37008 Salamanca (Spain)

(First received January 17th, 1992; revised manuscript received April 24th, 1992)

ABSTRACT

A sensitive method for the high-performance liquid chromatographic determination of five organophosphorus pesticides (paraoxon, methyl-parathion, ethyl-parathion, guthion and fenitrothion) in fruits and tap and river water samples is described. For the determination of pesticides in fruits a simple and rapid sample preparation procedure was developed that allowed pesticides to be determined at 50–100 μg/kg levels with recoveries ranging from 83 to 118% and relative standard deviations below 6%. The determination of pesticide residues in surface water samples was also successfully accomplished. Concentrations at sub-ppb levels can be measured by using a solid-phase concentration step, the recoveries being over 80%. In analyses of both fruits and surface waters, the sensitivity levels achieved were 2–10 times lower than legal limits admitted in the European Economic Community.

INTRODUCTION

As a result of their relatively rapid degradation and low accumulation in the biological food chain, organophosphorus pesticides are widely applied to a variety of crops, including green vegetables and fruits. However, their widespread use could be expected to leave residues not only on crops but also in surface waters draining the croplands. Hence, the monitoring of pesticide residues in agricultural and food products and in environmental matrices has become a priority field in pesticide research and analysis.

Gas chromatography has undoubtedly been the most common technique for analysing surface waters and vegetable materials for pesticides [1]. High-performance liquid chromatographic (HPLC) methods for pesticide residue analysis were firstly developed for non-volatile or thermally labile compounds such as carbamate insecticides. As HPLC can offer a simpler and/or faster approach to analyses for a wide number of other compounds [2,3], HPLC methods are continually increasing in acceptance and applications [4–6]. Although applications of HPLC to the formulation analysis of organophosphorus pesticides have been reported [7–10], the literature concerning organophosphorus multiresidues in foodstuff is scarce [11–14]. Clark *et al.* [12] reported a method for parathions using HPLC with series UV–amperometric detection. Detection limits of 2–3 ng of injected pesticide and 0.8–0.9 ng were obtained with UV and electrochemical detectors, respectively. Concentrations less than 10 ng/ml in waters and 50 μg/kg in vegetable materials

Correspondence to: Dr. R. Carabias Martinez, Departamento de Química Analítica, Nutrición y Bromatología, Universidad de Salamanca, 37008 Salamanca, Spain.

0021-9673/92/$05.00

were measured using electrochemical detection, but UV detection could not be applied because of the multiple interferences from plant substances.

The number of HPLC methods for pesticide residue analysis is limited, but extraction and clean-up procedures can readily be found for numerous pesticides [15]. Many of these procedures will no doubt produce sufficiently clean extracts for HPLC, but most of them are laborious and time consuming. Pesticide sample preparation is usually achieved by liquid–liquid extraction or by enrichment of trace compounds of interest by solid-phase extraction (SPE). The latter technique is gaining acceptance [16–18] and will probably be increasingly used as a wider variety of solid-phase supports become available.

This paper deals with the determination of five frequently used organophosphorus pesticides in fruits; extraction with benzene and solvent replacement with methanol provides extracts clean enough to avoid any further clean-up step. The analysis of tap and river water samples, including an SPE concentration step, was also accomplished.

EXPERIMENTAL

Apparatus

A Spectra-Physics chromatograph equipped with an SP 8800 ternary pump, an SP 8450 UV–VIS detector and an SP 4290 integrator was used. Columns were Spheri-5 RP-8 (5 μm) (220 × 4.6 mm I.D.) and Spheri-5 RP-18 (5 μm) (250 × 4.6 mm I.D.) from Brownlee Labs. A Rheodyne injection valve with a 10μl injection loop was used throughout. All solvents and samples were filtered through 0.45-μm pore-size nylon membrane filters (Millipore).

Apple samples were homogenized in an electric mixer (Moulinex).For pesticide extraction and preconcentration from water samples, Sep-Pak C_{18} bonded-phase silica cartridges (Waters) were used; samples were pumped through them by a Gilson Minipuls 2 HP 4 peristaltic pump with vinyl tubing.

Reagents

Paraoxon (99% purity), guthion (99%), methylparathion (97%), fenitrothion (99%) and ethylparathion (99%) were obtained from Riedel-de Haën (Seelze-Hannover, Germany). Ultra-high-quality water used for the preparation of solutions was obtained with an Elgastat UHQ water-purification system. Methanol, benzene and dichloromethane were of HPLC grade (Carlo Erba, Milan, Italy). All other chemicals were analytical-reagent grade.

Samples and standards

Golden-type apples were obtained in area retail markets. River water samples were taken from different rivers of SE Salamanca (Spain). They were collected directly in 1-l glass containers, stored at 4°C in the dark and analysed within 24 h after collection.

Stock solutions containing the five pesticides were prepared in pure methanol and stored at 4°C; working standard solutions were daily prepared by dilution with methanol.

HPLC operating conditions

Separation was accomplished in the Spheri-5 RP-18 column, the mobile phase being methanol–water (70:30, v/v) containing $2.5 \cdot 10^{-2}$ *M* acetic acid–acetate buffer at a flow-rate of 1.25 ml/min. It was degassed by bubbling 99.998% helium through it. The injection volume was 10 μl in all experiments. Detection was carried out at 260 nm and peak areas were used for quantification.

Determination of pesticides in apples

Two or three apples were sliced and homogenized in an electric mixer. An amount of 3.0 *g* of homogenized sample was spiked with 1.0 ml of a methanolic solution of the five pesticides and allowed to stand for at least 24 h at room temperature. Then 8.0 ml of benzene were added and the mixture was stirred in a magnetic device for 30 min to improve the sample–benzene contact and hence, the extraction process. After centrifugation at 1307 *g* for 20 min, a 3.0-ml aliquot of the organic layer was evaporated to dryness at room temperature by passing an air stream. The dry extract was dissolved in 2.0 ml of methanol using an ultrasonic bath and the sample was then ready for analysis. Samples were prepared in triplicate and 10-μl aliquots of each sample were injected into the chromatograph. Quantification was carried out by using the external standard method by taking the mean peak-area value of three injections.

Determination of pesticides in waters

Tap water samples were used without any further treatment. All river water samples were filtered through sintered glass filters (No. 5) to remove suspended particulate matter before use. Pesticides were added to water samples by placing 1 ml of pesticide mixture solution in a volumetric flask and making up to 1 l with the water sample. Analytes were adsorbed on the Sep-Pak C_{18} cartridge by a single pass through the cartridge at a flow rate of about 5 ml/min. Desorption was effected by elution with 2.0 ml of a solution with the same composition as the mobile phase and 7.0 ml of methanol and the eluate was collected into a 10-ml volumetric flask, diluting to the mark with mobile phase solution. These samples were then analyzed immediately by triplicate injections of 10-μl aliquots. In addition, non-spiked water samples were analysed following the same procedure to check for the presence of pesticides under study.

The cartridges were equilibrated with 5.0 ml of methanol and 5.0 ml of UHQ-purified water before use for pesticide preconcentration.

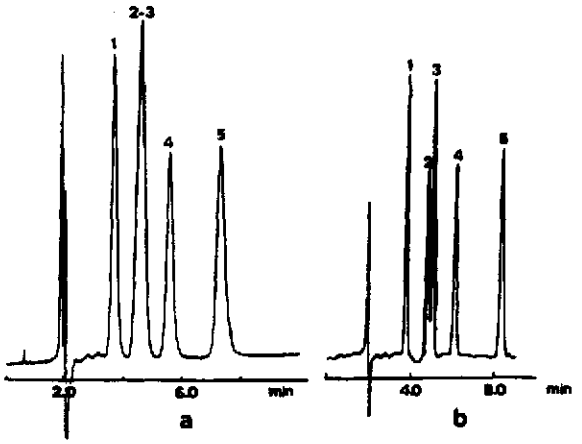

Fig. 1. Representative chromatograms obtained with (a) RP-8 and (b) RP-18 columns. Peaks: 1 = paraoxon; 2 = guthion; 3 = methyl-parathion; 4 = fenitrothion; 5 = ethyl-parathion. Experimental conditions: (a) methanol–water (70:30, v/v) + $2.5 \cdot 10^{-2}$ *M* acetic acid–acetate buffer as mobile phase, flow-rate 1.25 ml/min; (b) methanol–water (75:25, v/v) + $2.5 \cdot 10^{-2}$ *M* acetic acid–acetate buffer as mobile phase, flow-rate 1.0 ml/min.

RESULTS AND DISCUSSION

Two reversed-phase columns (C_8 and C_{18}) were tried for pesticide separation. Using methanol–water (75:25, v/v) as the mobile phase, the C_8 column was unable to resolve guthion and methyl-parathion with retention times of 4.39 and 4.56 min, respectively, when injected alone, the chromatogram showing a single broad peak at 4.45 min (Fig. 1). As further modifications of mobile phase composition were not successful in improving the resolution, the RP-18 column appeared to be more suitable than the RP-8 column for these pesticide separations.

To find suitable conditions for pesticide separation, the HPLC operating conditions, such as mobile phase constituents and flow rate, were optimized. A mobile phase of methanol–water (70:30, v/v) was found to give a good resolution and a reasonable analysis time. Methanol concentrations higher than 80% (v/v) gave poor peak resolution and with concentrations lower than 70% long analysis times were obtained.

The addition of acetate buffer to the mobile phase was found to be an easy way to shorten the analysis time with no decrease in resolution. Buffer concentrations in the range $0.5 \cdot 10^{-2}$–0.1 *M* were tested; concentrations up to $2.5 \cdot 10^{-2}$ *M* produced an earlier elution of the less polar pesticides, such as fenitrothion and ethyl-parathion, the retention times of the other peaks remaining unmodified. For buffer concentrations higher than $2.5 \cdot 10^{-2}$ *M*, no further modifications were found.

Flow-rate was studied in the range 0.5–2.0 ml/min. As expected, shorter analysis times were obtained at higher flow-rates but guthion and methyl-parathion were not well resolved at flow-rates higher than 1.5 ml/min.

Under the optimum conditions described under Experimental, linear relationships were found between peak area or height and pesticide concentration in the studied range, between 2.90 and 210 ng of each pesticide injected. The detection limits, calculated as the ratio between twice the noise and the calibration slope, are given in Table I, together with data from the calibration fittings and standard deviations obtained from ten replicate analyses at a concentration level of 12 ng of each pesticide. A chromatogram corresponding to a standard solution near the detection limit (about 0.30 ng of each pesticide injected) is shown in Fig. 2.

TABLE I

CALIBRATION FITTINGS

Concentration range between *ca.* 2.90 and 210 ng of each pesticide injected.

Pesticide	Intercept	Slope (l/mol)	Correlation coefficiënt	RSD[a] (%)	DL[b] (ppb)
Paraoxon	$(1.0 \pm 0.8) \cdot 10^3$	$(8.78 \pm 0.03) \cdot 10^9$	0.997	3.55	26
Guthion	$(6.3 \pm 3.7) \cdot 10^2$	$(6.59 \pm 0.02) \cdot 10^9$	0.999	2.11	53
Methyl-parathion	$(4.5 \pm 2.3) \cdot 10^2$	$(9.55 \pm 0.04) \cdot 10^9$	0.999	2.74	30
Fenitrothion	$(6.4 \pm 7.7) \cdot 10^2$	$(7.76 \pm 0.02) \cdot 10^9$	0.999	2.95	49
Ethyl-parathion	$(1.6 \pm 0.6) \cdot 10^2$	$(9.79 \pm 0.04) \cdot 10^9$	0.999	2.99	57

[a] Relative standard deviation ($n = 10$); amount injected, 12 ng of each pesticide.
[b] Detection limit, $2s/m$, where s is the blank standard deviation and m is the slope of the calibration graph.

Determination of pesticides in fruits

For the development of an appropriate procedure for the determination of pesticide residues in fruits, benzene, methanol and dichloromethane were tested as organic solvents for the extraction of the pesticides; no spiked apple samples were used in studies on the possible interferences from crop components.

Samples spiked at about 10 μg/g were used in comparative recovery experiments (Fig. 3); the pesticides were allowed to equilibrate with the sample matrix for at least 24 h but no longer than 48 h before extraction. Studies on the pesticide–matrix contact time showed that with maceration times longer than 48 h, the pesticide recoveries were dramatically decreased.

The blanks obtained using methanol as extraction solvent showed a large peak early in the chromatogram, whereas dichloromethane and benzene extracts gave only small peaks. Conversely, at high retention times, several peaks were observed in these extracts with benzene and dichloromethane but not in the methanolic extract.

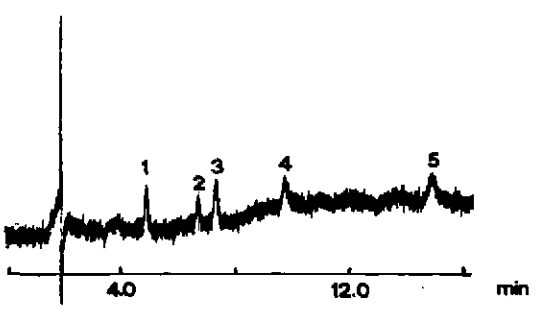

Fig. 2. Chromatogram obtained for a standard solution near the estimated detection limit. Amounts injected: (1) paraoxon, 0.30 ng; (2) guthion, 0.29 ng; (3) methyl-parathion, 0.30 ng; (4) fenitrothion, 0.30 ng; (5) ethyl-parathion, 0.30 ng. Experimental conditions as given under Experimental.

Regarding extraction percentages (Fig. 3), organophosphorus pesticides are not well extracted with methanol (about 70%). If it is considered that for a correct determination of pesticide residues, the recoveries should be within the range 70–110%, with a mean value greater than 80% [19]; adequate recoveries for all five pesticides were obtained with benzene (98–108%) and dichloromethane (86–99%) as extraction solvents, the former giving cleaner chromatograms.

Although both solvents were adequate, benzene was chosen for pesticide extraction from apple samples. Fig. 4 shows a representative chromatogram of a benzene extract where several peaks can be seen; no interferences with relevant analytes were observed. This solvent minimizes co-extractives from the vegetable matrix as vegetables are relatively polar in their matrix profile. The analysis time should be prolonged to allow the elution of less polar matrix compounds, with retention times longer than 25 min.

After extraction, the next step was evaporation to dryness of a 3.0-ml aliquot of the organic extract. The dry residue was then dissolved in 2.0 ml of a solution whose composition was also studied: (a) 2.0 ml of methanol, (b) 2.0 ml of mobile phase and (c) 1.0 ml of methanol + 1.0 ml of mobile phase

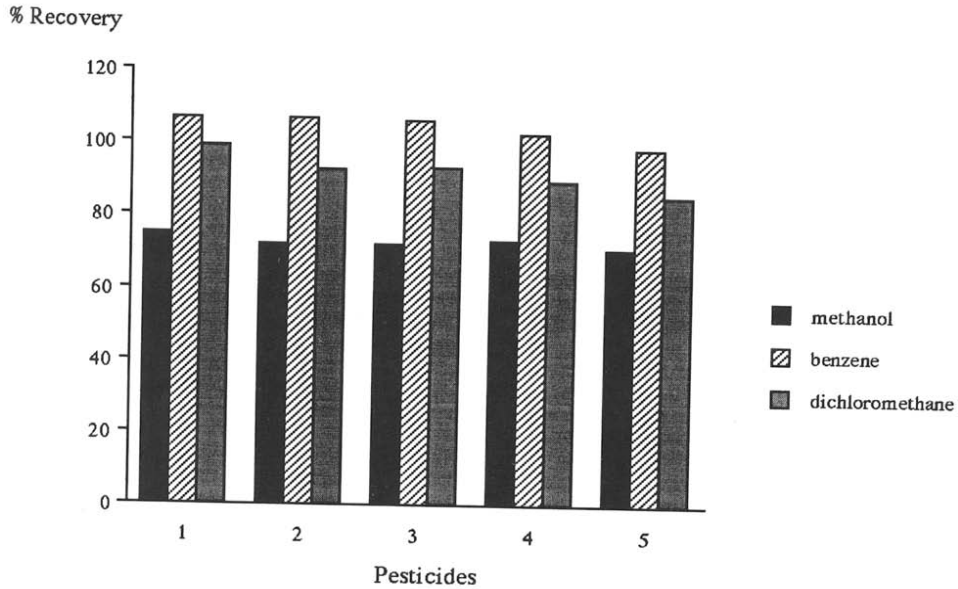

Fig. 3. Influence of the extraction solvent on the percentage extraction of pesticides from apple samples spiked with *ca.* 10 μg/g of each pesticide. Numbers 1–5 as in Fig. 1.

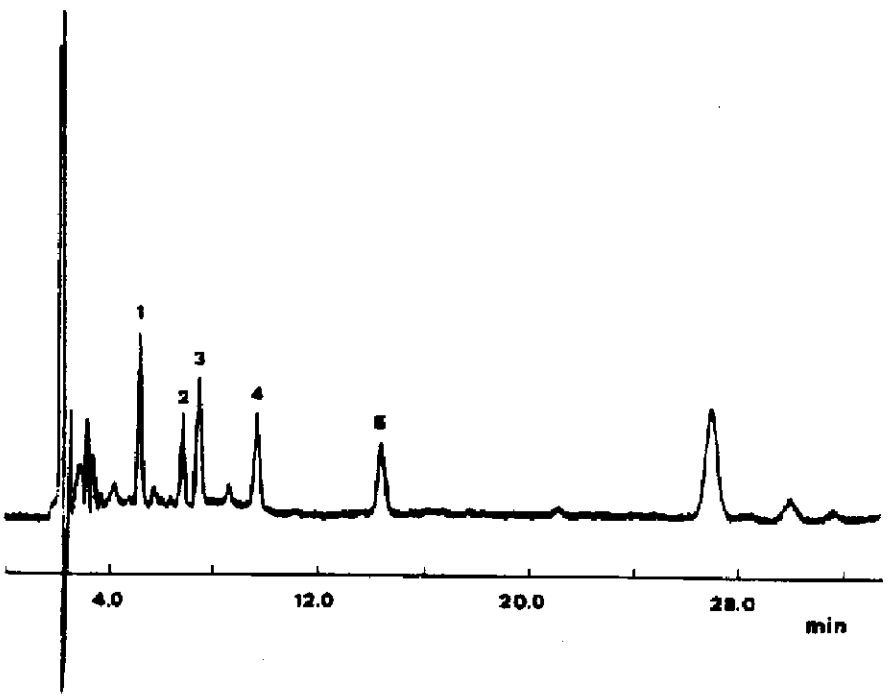

Fig. 4. Representative chromatogram of an apple sample spiked at the 0.46 μg/g level using benzene as solvent for pesticide extraction. Peaks as in Fig. 1.

TABLE II

PESTICIDE RECOVERIES FROM APPLE SAMPLES

Pesticide	Added (μg/g)	Recovery (%)	RSD[a] (%)	DL[b] (μg/g)
Paraoxon	1.17	95.4	6.0	0.046
	9.88	94.9	2.5	
Guthion	1.15	101.6	3.9	0.094
	9.75	98.8	3.7	
Methyl-parathion	1.16	98.5	4.0	0.053
	10.02	100.6	2.2	
Fenitrothion	1.17	93.3	4.6	0.085
	10.03	100.0	1.4	
Ethyl-parathion	1.16	86.3	3.7	0.099
	10.19	98.3	1.4	

[a] $n = 4$

[b] Detection limit ($2s/m$) calculated with peak-area data from experiment in Fig. 5

solution were tested. Good recoveries, between 97 and 109%, were found for paraoxon, guthion, methyl-parathion and fenitrothion no matter which solution was used for dissolving the dry residue. Ethyl-parathion was quantitatively recovered [19] only when solution (a) was used.

The selectivity of the overall process can be explained as follows: as the non-polar solvent benzene is used to achieve an efficient extraction of organophosphates, most of the polar components of the sample are eliminated in this extraction step. In the next step, benzene is replaced with the polar solvent methanol, evaporating the former and dissolving the dry residue. As methanol is a weak solvent for low polarity compounds, pesticides are dissolved in solution whereas less polar impurities remain mainly in the solid residue. At this point, the sample is relatively clean and no interferences from the fruit

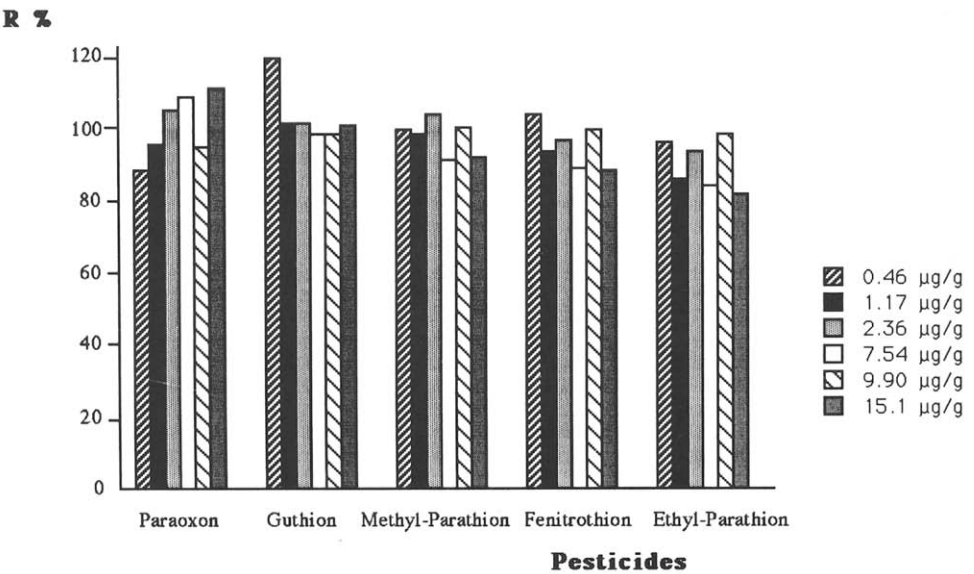

Fig. 5. Recovery from apple samples as a function of pesticide concentration in the original samples.

TABLE III

PESTICIDE RECOVERIES FROM DISTILLED WATER SAMPLES

Fortification level (ng/ml)	Recovery (%)				
	Paraoxon	Guthion	Methyl-parathion	Fenitrothion	Ethyl-parathion
1.40	90.5	88.7	84.3	78.6	76.3
2.80	86.3	88.0	85.7	80.8	78.7
27.9	100.7	102.5	101.1	98.2	89.6
46.1	104.4	101.3	97.8	98.1	91.6
69.2	104.3	101.1	101.7	98.1	91.2
DL (ng/ml)	0.26	0.53	0.30	0.49	0.57

matrix were observed in the elution range of interest, so no further residue clean-up step was required. Quantitative recovery results and relative standard deviations were calculated on the average percentage recovery of four replicate samples injected in triplicate and bracketed with injections of the standards (Table II). In the range of fortification levels tested (0.46–15.1 μg/g) the recoveries were found to be independent of concentration and similar for the five pesticides assayed, ranging from 83 to 118% (Fig. 5). By using the peak-area data obtained for the concentration range displayed in Fig. 5, detection limits were calculated (Table II). Values as low as 50–100 μg/kg for only 3.0 g of homogenized sample were obtained. These levels are 5–10 times lower than the legal limits admitted in the European Economic Community (EEC) for fruits and vegetables [20].

Determination of pesticides in tap and river waters

To determine pesticide residues in water samples at levels below the legal limits, trace enrichment on commercially available Sep-Pak C_{18} cartridges was chosen because it facilitates simultaneous extraction and analyte preconcentration.

Pesticide adsorption on the cartridges was carried out as described under Experimental. For pesticide desorption from the cartridge, good recoveries (81–90%) were found on eluting the pesticides with 2.0 ml of mobile phase solution and 7.0 ml of methanol.

The overall procedure was checked for samples at fortification levels between 1.4 and 69.2 ng/ml (Ta-

TABLE IV

PESTICIDE RECOVERIES FROM TAP AND RIVER WATER SAMPLES

Pesticide	Tap water			River water		
	Added (ng/ml)	Recovery (%)	RSD[a] (%)	Added (ng/ml)	Recovery (%)	RSD[a] (%)
Paraoxon	14.1	86.4	0.88	1.5	91.2	8.4
	45.3	100.3	1.12	33.1	94.9	1.6
Guthion	14.0	87.2	0.97	1.7	84.4	3.3
	46.7	97.4	0.71	34.2	91.5	1.7
Methyl-parathion	13.7	88.2	0.77	1.6	86.4	3.9
	45.6	98.1	0.70	33.4	94.0	2.3
Fenitrothion	14.0	83.8	1.25	1.7	86.3	5.9
	46.6	94.8	0.60	34.1	90.6	2.1
Ethyl-parathion	13.9	79.3	1.28	1.6	81.7	7.2
	46.5	88.4	1.28	34.0	82.0	1.6

[a] $n = 4$.

ble III), the recoveries being over 76%. Based on these results and bearing in mind the enrichment factor of 100 achieved in the preconcentration step, it should be possible to determine pesticides in water samples at sub-ppb levels (Table III), which is 2–5 times less than levels admitted in the EEC for surface waters destined for drinking water production [21].

Tap water analysis. None of the blank tap water samples gave peaks that interfered with the determinations of the pesticides of interest. Recovery determinations were made on pesticide-fortified tap water samples (n = 4) at two different concentration levels, 14 and 45 ng/ml (Table IV).

River water analysis. For the analysis of river water samples, six different points in the agricultural area SE of Salamanca (Spain) were sampled. Unspiked aliquots of each sample were analysed to quantify ambient levels of pesticide concentration. Each water sample showed several peaks but none of them corresponded to the studied pesticides except one (Fig. 6a), where a small peak with a retention time (9.54 min) similar to that of fenitrothion was observed. As this peak is below the calculated limit value for fenitrothion, no further conclusions can be drawn. Recovery results for a river water sample spiked at 1.6 and 33 ng/ml are given in Table IV and a chromatogram of a river water at the 1.6 ng/ml fortification level is shown in Fig. 6b.

CONCLUSIONS

Conditions have been established for the HPLC determination of trace levels of paraoxon, guthion, methyl-parathion, ethyl-parathion and fenitrothion residues in fruits and surface waters. The UV detec-

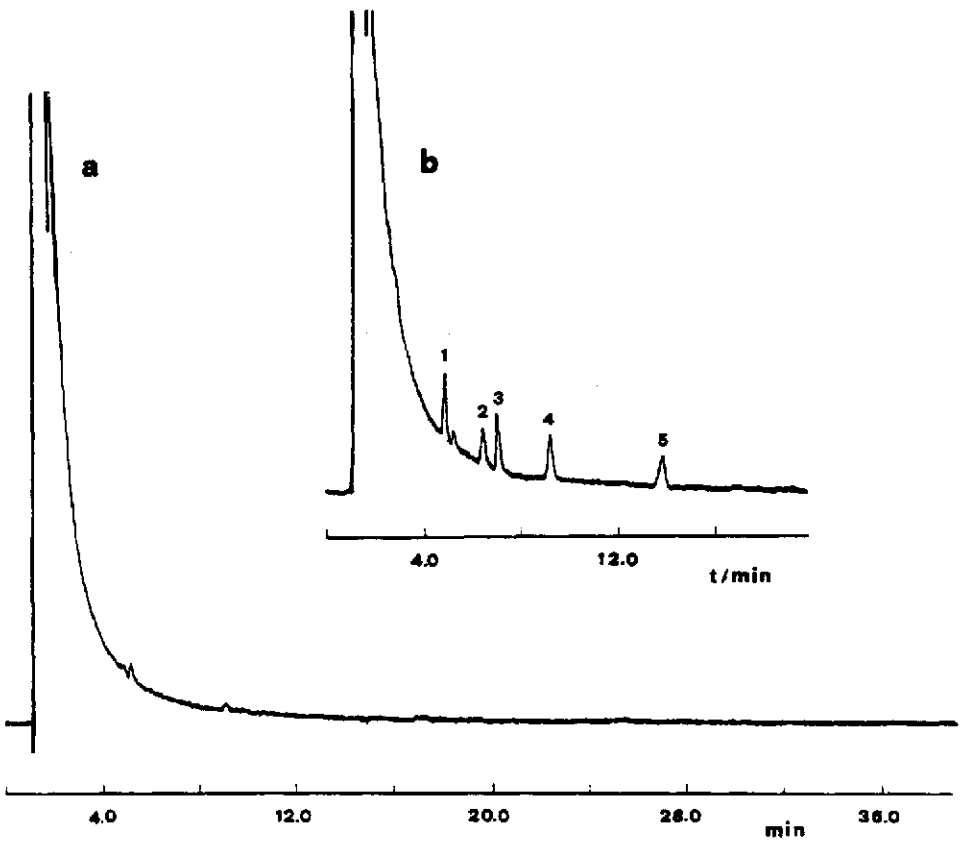

Fig. 6. Chromatogram of a river water sample (Rio Portillo, Salamanca, Spain) (a) before and (b) after being spiked at the 1.6 ng/ml level. Peaks as in Fig. 1.

tion limits are 0.2–0.6 ng of pesticide injected, which compare well with results reported by Clark *et al.* [12] for electrochemical detection and represent some improvement on the results obtained by the same authors with a UV detector.

The sample preparation scheme proposed for fruits is simple and rapid and gives clean extracts without the need for further clean-up steps of the residues, generally required for analyses of real samples. Analysis of river water samples allows pesticide concentrations at sub-ppb levels to be monitored. In both instance the detection limits found are sufficiently low for the method to be applied to crop extracts and river waters at levels 2–10 times lowers than the legal limits admitted in the EEC for these types of samples.

ACKNOWLEDGEMENT

The authors thank the Dirección General de Investigación Científica y Técnica (DGICYT, Spain) for the financial support of this work (project No. PB89-0397)

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