

Determination of Organophosphorus Pesticides in Fruit Juices by Matrix Solid-Phase Dispersion and Gas Chromatography

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A rapid multiresidue method was developed for the determination of nine organophosphorus pesticides in fruit juices. The analytical procedure is based on the matrix solid-phase dispersion (MSPD) of juice samples on Florisil in small glass columns and subsequent extraction with ethyl acetate assisted by sonication. Residue levels were determined by gas chromatography with nitrogen–phosphorus detection. Spiked blank samples were used as standards to counteract the matrix effect observed in the chromatographic determination. The NPD response for all pesticides was linear in the concentration range studied with determination coefficients >0.999 . Average recoveries obtained for all of the pesticides in the different juices and fortification levels were $>70\%$ with relative standard deviations of $<11\%$. The detection limits ranged from 0.1 to 0.6 $\mu\text{g}/\text{kg}$. The identity of the pesticides was confirmed by gas chromatography with mass spectrometric detection using selected ion monitoring. The proposed MSPD method was applied to determine pesticide residue levels in fruit juices sold in Spanish supermarkets. At least one pesticide was found in most of the samples, although the levels detected were very low, far from the maximum residue levels established for raw fruit.

KEYWORDS: Fruit juices; pesticides; MSPD; gas chromatography

INTRODUCTION

Pesticides used in pre- and postharvest treatments to control diseases of fruits and vegetables may persist over the season and appear in processed products for human consumption, such as fruit juices, which are widely consumed, particularly by children.

Several pesticide groups, fungicides (1–3), pyrethroids (4), and triazine herbicides (5), have been determined in fruit juices. Organophosphorus pesticides have also been studied because some of them are extensively used and have a high solubility in water, the main component of fruit juices. Ethion, chlorpyrifos, phosmet, and azinphos-methyl have been detected in orange juice from Brazil and the United States (6), and methidathion has been detected in other juices (7). Maximum residue levels (MRL), for raw fruit but not for processed products such as juice, have been established by European Union legislation, and $\sim 30\text{--}50\%$ of the fruit and vegetable samples analyzed in Europe contained residue levels, although these were below the MRL in most cases (8).

The methods used to determine organophosphorus pesticides in fruits and vegetables are mainly based in liquid partitioning with organic solvents such as ethyl acetate (9) and dichloromethane (10), usually followed by a solid-phase extraction cleanup step (11, 12). In the past few years, new extraction procedures have been developed, such as solid-phase microextraction (SPME) (13) and supercritical fluid extraction (SFE) (14).

Matrix solid-phase dispersion (MSPD), based on the dispersion of the sample on an adsorbent, such as Florisil, C_{18} , alumina, or silica, is a technique that allows the extraction and cleanup in a single step. For solid samples, the mixture is done in a mortar and then transferred to the extraction columns (15–17), whereas for liquid samples the dispersion of the matrix in the adsorbent is done directly in the extraction columns (18, 19).

Organophosphorus pesticides are usually determined by gas chromatography with flame photometric detection (FPD) (9–11, 13) or nitrogen–phosphorus detection (NPD) (20), although HPLC with diode array detection (21) or coupled with tandem mass spectrometry (7) has also been applied.

The main objective of this work was to develop a rapid and simple method for the determination of nine organophosphorus pesticides (diazinon, parathion-methyl, fenitrothion, malathion, chlorpyrifos, phenthoate, methidathion, profenofos, and ethion) in different fruit juices based on MSPD of samples on Florisil. Residue levels were determined by gas chromatography with nitrogen–phosphorus detection (GC-NPD), and the confirmation of identity was performed by gas chromatography with mass spectrometric detection (GC-MS) using selected ion monitoring (SIM). The developed method was applied to the determination of organophosphorus pesticides in several fruit juices commercialized in Spain.

MATERIALS AND METHODS

Reagents. Residue analysis grade methanol and ethyl acetate were purchased from Scharlau (Barcelona, Spain). Research grade Florisil (60–100 mesh) was supplied by Fluka Chemie (Buchs, Switzerland).

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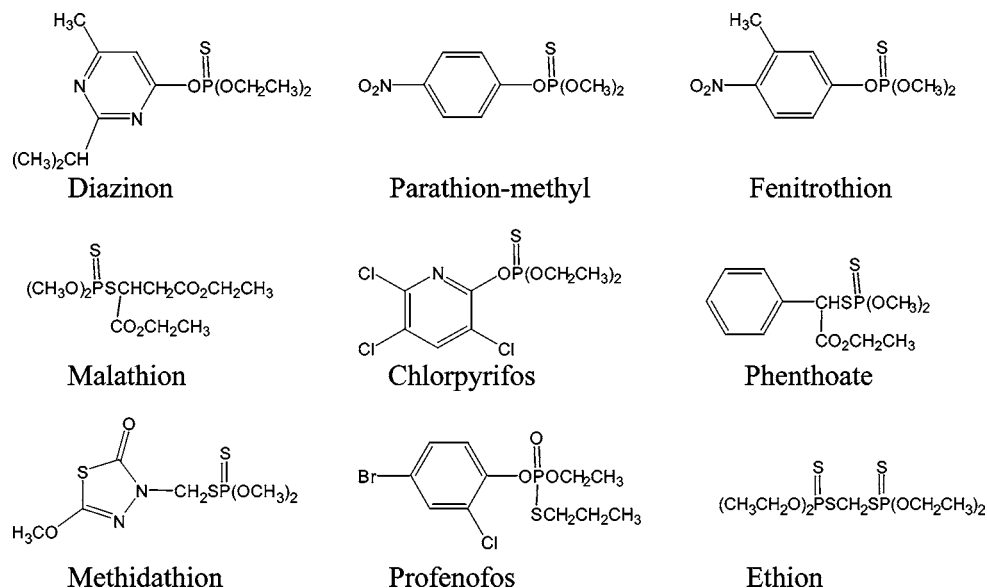


Figure 1. Chemical structures of the organophosphorus pesticides studied.

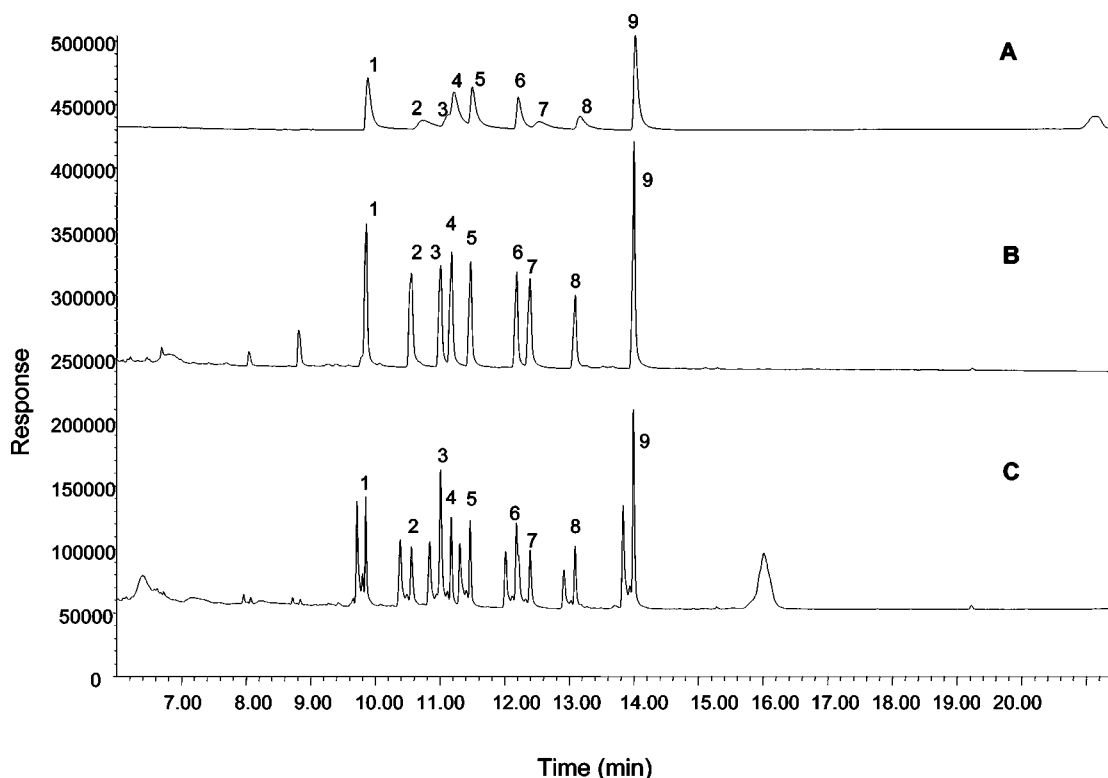


Figure 2. GC-NPD chromatograms of (A) standard mixture solution in ethyl acetate at 0.025 μg/mL, (B) blank apple juice sample fortified at 0.025 μg/g in ethyl acetate, and (C) blank apple juice sample fortified at 0.025 μg/g in methanol. See Table 1 for peak identification.

The adsorbent was heated for 24 h at 140 °C before use. Pesticide standards (99% purity) diazinon, parathion-methyl, fenitrothion, malathion, chlorpyrifos, phenthoate, methidathion, profenofos, and ethion were obtained from Reidel-de Haëns (Seelze, Germany). Figure 1 shows the chemical structures of these compounds. Various commercial fruit juices (apple, peach, orange, pineapple, and grape), made from concentrated juice, were purchased in supermarkets in Madrid.

Stock solutions of individual pesticide standards were prepared in ethyl acetate at 500 μg/mL and stored at 4 °C. Standard working solutions used for the fortification of juice samples were prepared by transferring 0.5 mL of the stock standard solutions to a 250-mL volumetric flask and diluting with methanol to a concentration of 1 μg/mL. The working standard solution was diluted to prepare the 0.02, 0.05, and 0.2 μg/mL standards (transferring 2, 5, and 20 mL of working solution to 100-mL volumetric flasks and diluting with methanol,

respectively). Ethyl acetate was used for the preparation of chromatographic standard solutions.

GC-NPD Analysis. An Agilent 6890 (Waldbronn, Germany) gas chromatograph, equipped with an automatic split-splitless injector model HP 7683 and a nitrogen-phosphorus detector, was employed. A fused silica capillary column (HP-1), cross-linked dimethylpolysiloxane, as nonpolar stationary phase (30 m × 0.25 mm i.d.), and 0.25 μm film thickness, supplied by Agilent (Madrid, Spain), was used. The injection port and detector temperatures were 270 and 300 °C, respectively, with helium as carrier gas at a flow rate of 1 mL/min. The oven temperature was kept at 70 °C for 1 min and then programmed at 20 °C/min to 220 °C, held for 2 min, programmed at 10 °C/min to 280 °C, and held for 5 min (total time = 21.50 min). A 2 μL volume was injected in pulsed splitless mode (pulsed pressure = 310 kPa for

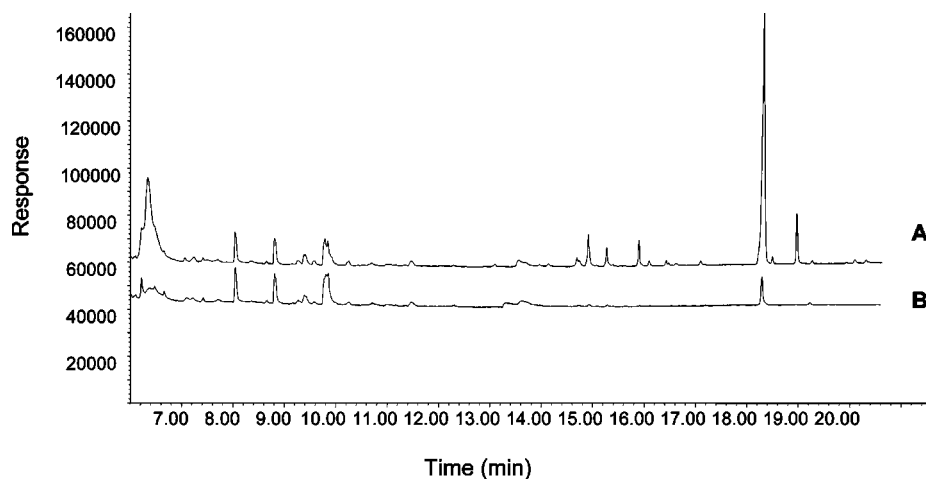


Figure 3. GC-NPD chromatograms of (A) blank apple juice sample extracted in a polypropylene column and (B) blank apple juice sample extracted in a glass column.

1.5 min). The constant flow rates of hydrogen and air were 2.0 and 60.0 mL/min, respectively, with nitrogen as makeup gas (30 mL/min).

GC-MS Confirmation. An Agilent 6890 gas chromatograph, equipped with an automatic injector model HP 7683 and a 5973 series mass selective detector, was used. The mass spectrometer was operated in electron impact ionization mode (ionizing energy = 70 eV) scanning from m/z 80 to 400 at 4.45 scan/s. The ion source and quadrupole temperatures were 230 and 150 °C, respectively. A fused silica capillary column (ZB-5MS), 5% phenyl polysiloxane as nonpolar stationary phase (30 m \times 0.25 mm i.d.) and 0.25 μ m film thickness, supplied by Phenomenex (Torrance, CA), was employed. Operating conditions were as follows: injector port temperature, 270 °C; injection volume, 2 μ L in pulsed splitless mode (pulsed pressure = 310 kPa for 1.5 min); helium as carrier gas at a flow rate of 1.0 mL/min; oven temperature program, 70 °C (1 min), increased at 20 °C/min to 220 °C, held for 2 min, then increased to 280 °C at 10 °C/min, and held for 5 min; solvent delay, 5 min. The total analysis time is 21.50 min, and the equilibration time is 2 min.

Analysis was performed by SIM with the ions indicated in **Table 4** using the following acquisition windows: (1) from 0 to 10.20 min; (2) from 10.20 to 10.90 min; (3) from 10.90 to 11.38 min; (4) from 11.38 to 12.10 min; (5) from 12.10 to 13.00 min; (6) from 13.00 to 13.70 min; (7) from 13.70 to 21.50 min. The dwell time for the ions monitored was 100 ms.

Sample Preparation. Glass columns, with Whatman no. 1 filters (2 cm diameter) placed at the bottom end, were filled with 2 g of Florisil. A 1 mL volume of juice was transferred to the glass column, fortified when required with 0.5 mL of the pesticide mixture in methanol. A 0.5 mL of methanol was added instead to unfortified samples. Methanol was used to allow a better distribution of the sample in the matrix. The columns were placed in a tube rack and closed with one-way stopcocks. Juice samples were extracted twice with ethyl acetate (5 mL) for 15 min in an ultrasonic bath (Raypa, Barcelona, Spain) at room temperature. Water level was adjusted to solvent level inside the columns. After sonication, columns were placed in a vacuum

manifold (Supelco Visiprep, Madrid, Spain) and the extraction solvent was filtered. The combined eluates were diluted to 10 mL with ethyl acetate for the highest fortification level. For the other two fortification levels and for commercial juice samples, the eluates were concentrated with a gentle stream of air to an appropriate volume (4 mL for the intermediate level or 2 mL for the lowest level and real samples) before GC analysis.

Quantification. The concentration of each compound in juice samples was determined by comparing the peak areas obtained in samples with those found in standards. Chromatographic standards were prepared by spiking blank samples with known amounts of pesticides.

RESULTS AND DISCUSSION

Gas Chromatographic Determination. In a first approach, standard mixtures in ethyl acetate were used for the quantification of pesticides in spiked samples by GC-NPD, but due to the low standard response recovery results were far higher than 100%. When standards were prepared by spiking blank juice samples with known amounts of pesticides, higher peak areas were obtained for the same pesticide concentration. Parts A and B of **Figure 2** show the different responses obtained with standard mixtures prepared in ethyl acetate or with a blank juice sample, respectively. There is an evident matrix effect that enhances the chromatographic response of organophosphorus pesticides. This effect has been described in previous works with different food matrices (20, 22). Therefore, the quantification of pesticide residues was carried out with fortified blank samples. The influence of solvent in the injection step of fortified samples was also assayed. **Figure 2C** shows a representative chromatogram of a fortified blank sample in methanol. The peak area of each compound is \sim 50% lower than that obtained with fortified samples in ethyl acetate. Moreover, other peaks were

Table 1. Retention Times (t_R), Calibration Data, and Repeatability of the Pesticides Analyzed by GC-NPD

pesticide	t_R , min	calibration data		repeatability ^a (RSD, %)	
		equation	determination coefficient	t_R	peak area
1. diazinon	9.848	$y = (1.42 \pm 0.02) \times 10^8 x + (4.7 \pm 0.3) \times 10^5$	0.9994	0.03	1.4
2. parathion-methyl	10.548	$y = (1.34 \pm 0.01) \times 10^8 x - (2.7 \pm 0.3) \times 10^5$	0.9998	0.04	2.0
3. fenitrothion	11.004	$y = (1.23 \pm 0.01) \times 10^8 x - (2.3 \pm 0.4) \times 10^5$	0.9997	0.02	1.9
4. malathion	11.717	$y = (1.48 \pm 0.01) \times 10^8 x - (2.9 \pm 0.4) \times 10^5$	0.9997	0.02	1.6
5. chlorpyrifos	11.465	$y = (1.30 \pm 0.02) \times 10^8 x - (2.6 \pm 0.4) \times 10^5$	0.9997	0.02	1.1
6. phenthoate	12.179	$y = (1.13 \pm 0.01) \times 10^8 x - (1.7 \pm 0.4) \times 10^5$	0.9998	0.02	4.2
7. methidathion	12.392	$y = (1.18 \pm 0.01) \times 10^8 x - (2.6 \pm 0.3) \times 10^5$	0.9998	0.03	2.4
8. profenofos	13.089	$y = (8.42 \pm 0.01) \times 10^7 x - (6.0 \pm 1.0) \times 10^4$	0.9997	0.02	3.2
9. ethion	13.996	$y = (2.502 \pm 0.007) \times 10^8 x - (4.1 \pm 0.2) \times 10^5$	0.9999	0.02	1.4

^a Relative standard deviations of retention times and peak areas ($n = 10$).

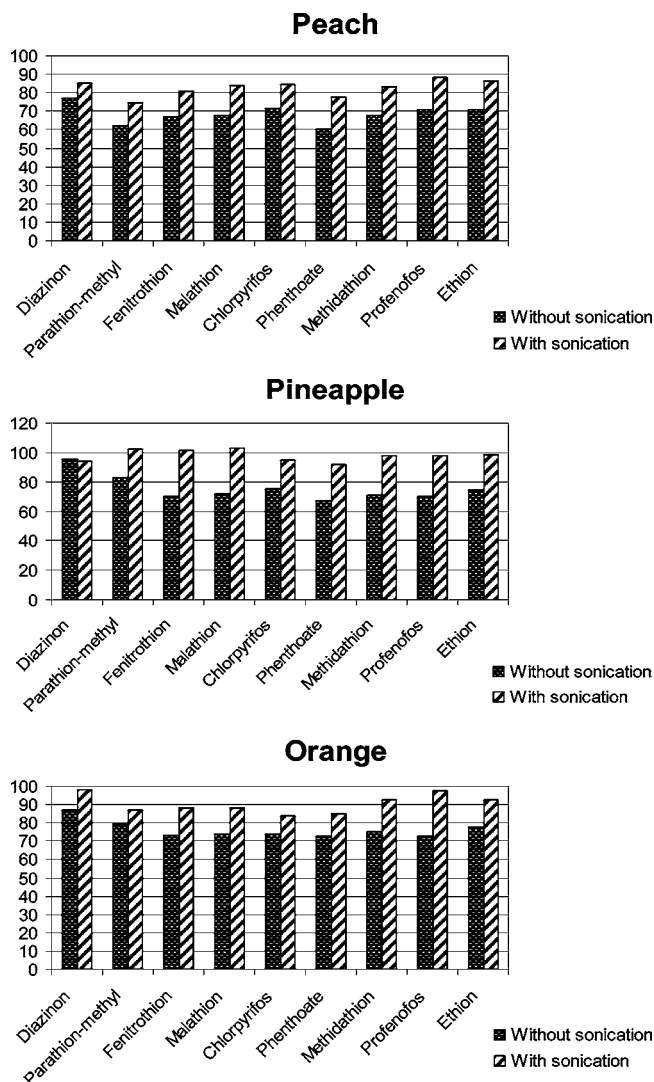


Figure 4. Influence of sonication on pesticide recovery in the extraction procedure. Juice samples were fortified at 0.025 $\mu\text{g/g}$.

observed in the chromatogram that may be explained by degradation of compounds due to the effect of methanol at the high temperatures of the injection port. Hence, ethyl acetate was the solvent selected for the injection of samples and standards.

Optimization of the MSPD Method. The possible effect of column material on the analytical procedure was assayed using polypropylene and glass columns in the sample extraction step. **Figure 3** depicts the chromatograms obtained for blank apple juice samples employing polypropylene or glass columns. The results show that cleaner chromatograms were achieved with glass columns, minimizing the appearance of interfering peaks.

The effect of sonication was also studied in the optimization process of the MSPD method. In general, pesticide recovery from juice samples improved when the extraction was assisted with sonication, particularly in peach, pineapple, and orange juices (**Figure 4**), whereas for the thinner juices, apple and grape, the improvement in recovery was somewhat lower (data not shown). Therefore, the pesticide extraction from juice samples in the proposed method was carried out assisted by sonication.

Method Validation. Linearity. The linearity of all the organophosphorus pesticides was determined using blank apple juice samples fortified at concentration levels of 5, 10, 25, 50, and 100 $\mu\text{g/kg}$. Previously, the influence of the different juices

Table 2. Recovery of the Studied Pesticides from Juice Samples^a

pesticide	fortification level, $\mu\text{g/g}$	apple	peach	orange	pineapple	grape
diazinon	0.100	100.6 \pm 4.1	94.5 \pm 2.9	92.6 \pm 2.7	96.6 \pm 5.6	102.3 \pm 1.5
	0.025	96.1 \pm 5.2	84.9 \pm 3.9	98.2 \pm 2.0	93.8 \pm 7.4	88.8 \pm 9.2
	0.010	93.0 \pm 7.8	94.4 \pm 9.1	102.5 \pm 3.7	88.6 \pm 8.1	80.3 \pm 6.2
parathion-methyl	0.100	96.7 \pm 4.5	95.3 \pm 2.5	98.7 \pm 1.9	104.7 \pm 3.0	108.6 \pm 3.8
	0.025	94.3 \pm 7.6	74.5 \pm 2.1	87.1 \pm 8.0	102.1 \pm 2.4	90.7 \pm 10.5
	0.010	73.2 \pm 5.6	86.1 \pm 8.6	87.7 \pm 4.8	93.5 \pm 5.6	82.9 \pm 6.0
fenitrothion	0.100	92.9 \pm 4.8	90.7 \pm 5.3	94.0 \pm 2.5	95.9 \pm 4.7	94.7 \pm 2.5
	0.025	86.3 \pm 7.9	81.0 \pm 8.1	88.4 \pm 7.8	101.1 \pm 1.9	78.1 \pm 8.5
	0.010	70.2 \pm 7.2	90.4 \pm 10.3	99.8 \pm 5.6	80.1 \pm 6.1	79.0 \pm 10.8
malathion	0.100	97.3 \pm 4.7	94.0 \pm 3.9	96.7 \pm 1.4	101.4 \pm 3.4	101.9 \pm 2.0
	0.025	85.7 \pm 7.7	84.0 \pm 3.8	87.9 \pm 7.2	102.7 \pm 1.6	80.2 \pm 6.9
	0.010	85.9 \pm 5.7	92.3 \pm 8.2	102.3 \pm 7.6	88.2 \pm 7.7	86.1 \pm 8.9
chlorpyrifos	0.100	101.9 \pm 9.8	98.3 \pm 7.6	96.2 \pm 1.1	104.0 \pm 2.7	104.8 \pm 2.6
	0.025	93.3 \pm 3.6	84.3 \pm 4.9	84.1 \pm 5.9	94.4 \pm 10.6	81.5 \pm 6.4
	0.010	85.2 \pm 6.1	95.3 \pm 9.6	100.0 \pm 9.6	89.1 \pm 3.7	83.4 \pm 10.5
phenthoate	0.100	93.4 \pm 2.4	90.6 \pm 4.8	93.4 \pm 2.5	99.5 \pm 4.9	97.5 \pm 4.8
	0.025	82.1 \pm 8.7	77.7 \pm 2.9	84.8 \pm 2.3	91.6 \pm 9.9	75.7 \pm 9.8
	0.010	75.8 \pm 5.3	93.5 \pm 9.4	93.0 \pm 7.2	79.4 \pm 4.3	72.4 \pm 8.4
methidathion	0.100	91.3 \pm 1.9	91.6 \pm 3.1	95.4 \pm 2.5	96.7 \pm 5.0	97.0 \pm 4.6
	0.025	80.6 \pm 8.9	83.4 \pm 2.6	92.4 \pm 3.8	97.4 \pm 9.9	75.6 \pm 9.0
	0.010	80.1 \pm 10.9	94.1 \pm 7.7	97.5 \pm 7.0	79.8 \pm 3.3	78.1 \pm 10.9
profenofos	0.100	101.6 \pm 8.5	99.0 \pm 10.7	101.5 \pm 5.1	109.9 \pm 4.3	105.1 \pm 6.2
	0.025	85.6 \pm 8.2	88.3 \pm 1.1	97.3 \pm 2.9	98.0 \pm 10.5	88.0 \pm 7.2
	0.010	71.2 \pm 5.7	91.5 \pm 9.1	98.8 \pm 9.3	78.3 \pm 6.6	79.2 \pm 10.8
ethion	0.100	100.1 \pm 3.7	97.0 \pm 4.0	98.0 \pm 3.1	104.6 \pm 1.5	107.0 \pm 2.2
	0.025	91.2 \pm 6.5	86.3 \pm 1.8	92.5 \pm 2.3	98.1 \pm 10.4	87.0 \pm 5.1
	0.010	91.8 \pm 7.8	92.9 \pm 7.5	95.6 \pm 7.3	89.6 \pm 4.9	87.6 \pm 7.8

^a Recovery, % \pm RSD, % ($n = 4$ at each fortification level for each juice sample).

Table 3. Limits of Detection (Micrograms per Kilogram) and Limits of Quantification (Micrograms per Kilogram) of the Pesticides Analyzed by GC-NPD

	apple		peach		orange		pineapple		grape	
	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ	LOD	LOQ
diazinon	0.5	1.7	0.6	2.0	0.6	2.0	0.6	2.0	0.4	1.3
parathion-methyl	0.1	0.3	0.3	1.0	0.1	0.3	0.1	0.3	0.2	0.6
fenitrothion	0.1	0.3	0.1	0.3	0.1	0.3	0.1	0.3	0.1	0.3
malathion	0.3	1.0	0.3	1.0	0.2	0.6	0.3	1.0	0.1	0.3
chlorpyrifos	0.1	0.3	0.2	0.6	0.3	1.0	0.1	0.3	0.1	0.3
phenthoate	0.3	1.0	0.1	0.3	0.1	0.3	0.1	0.3	0.1	0.3
methidathion	0.1	0.3	0.2	0.6	0.2	0.6	0.1	0.3	0.1	0.3
profenofos	0.1	0.3	0.3	1.0	0.3	1.0	0.1	0.3	0.3	1.0
ethion	0.3	1.0	0.1	0.3	0.1	0.3	0.1	0.3	0.1	0.3

Table 4. Main Ions and Their Relative Abundance of the Studied Pesticides

pesticide	t_R , min	m/z^a (relative abundance, %)
diazinon	9.749	137 (100), 179 (93), 199 (53), 304* (52)
parathion-methyl	10.691	109 (100), 125 (83), 263* (58)
fenitrothion	11.125	109 (95), 125 (98), 260 (55), 277* (100)
malathion	11.254	127 (82), 158 (47), 173 (100)
chlorpyrifos	11.494	197 (95), 199 (100), 258 (44), 286 (28), 314 (73)
phenthoate	12.361	107 (27), 125 (53), 246 (24), 274 (100), 320* (7)
methidathion	12.719	85 (60), 125 (17), 145 (100), 302* (3)
profenofos	13.271	139 (77), 208 (100), 269 (36), 339 (65), 374* (30)
ethion	14.206	231 (100), 233 (14), 384* (15)

^a Molecular ion is indicated by an asterisk.

in the peak areas of the studied pesticides was assayed, and similar peak areas with relative standard deviations of $<5\%$ were obtained for all of the compounds in the different juices.

Table 1 summarizes retention times and calibration data of the

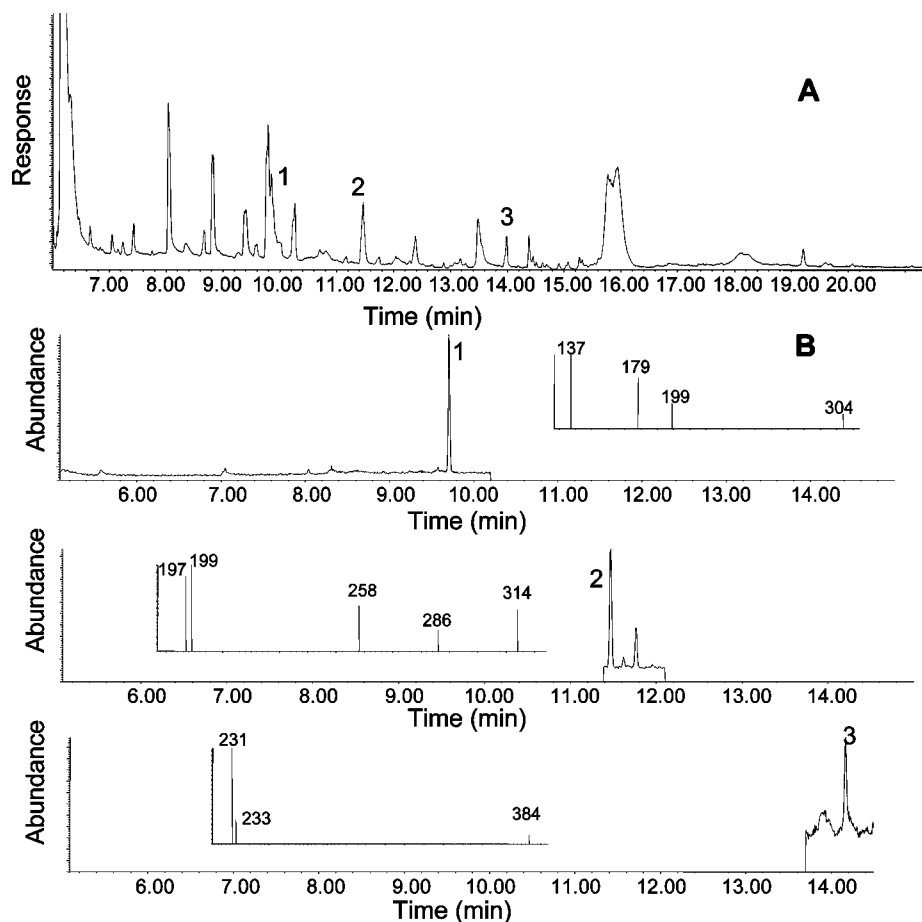


Figure 5. (A) GC-NPD chromatogram of a commercial orange juice sample. Peaks: 1, diazinon; 2, chlorpyrifos; 3, ethion. (B) GC-MS chromatograms with the main ions of the mass spectra of the three pesticides detected.

studied pesticides. The slope and intercept values together with their relative standard deviations were determined by applying regression analyses. Good determination coefficients were obtained for all of the compounds, ranging from 0.9994 to 0.9999.

Repeatability. The repeatability of our chromatographic method was determined by performing the analysis of a sample spiked at 25 $\mu\text{g/L}$. The sample was injected 10 times with an automatic injector, and the relative standard deviation (RSD) values obtained for retention times ranged from 0.02 to 0.04%, whereas for peak areas the values ranged from 1.1 to 4.2% (Table 1). Therefore, the repeatability achieved by automatic injection was very good.

Stability. Stock standard solutions and working solutions were found to be stable for at least 3 months and 1 week, respectively, when stored at 4 $^{\circ}\text{C}$. Moreover, the stability of a fortified blank sample kept in the autosampler for 24 h was assayed, and differences of <3% were obtained.

Specificity. The specificity of the proposed MSPD procedure was assessed by analyzing blank juice samples. The absence of background peaks, above the signal-to-noise ratio of 3, at the retention times of target pesticides, showed that no interferences occurred. A chromatographic peak at 9.70 min was the only peak that partially overlaps with diazinon (9.85 min) (Figure 3).

Recovery. Table 2 shows the pesticide recovery results obtained. Five different fruit juices were fortified at 0.1, 0.025, and 0.010 $\mu\text{g/g}$ before extraction and analyzed by GC-NPD. The recoveries obtained for all pesticides ranged from 70 to 110% with RSDs of <11%. These results show that good

recoveries from juice samples were obtained throughout the proposed method. These values are similar to the recoveries reported by other authors for the MSPD extraction of several pesticides from fruit juices (7).

Detection and Quantification Limits. The limits of detection (LOD) of the proposed method were determined by considering a value 3 times the background noise obtained for blank samples, whereas the limits of quantification (LOQ) were determined considering a value 10 times the background noise. Table 3 summarizes the detection and quantification limits obtained for the individual pesticides in the different juices studied. Diazinon presents the highest limits in all five juices because it is partially overlapped by a peak from the sample. The range of LOD achieved is of the same order as those previously obtained by other authors using new sample preparation techniques, such as MSPD or SPME (7, 13).

GC-MS Confirmation. The identity of the organophosphorus pesticides was confirmed by GC-MS with SIM. Previously, a total ion chromatogram of a standard mixture solution was obtained to determine their main ions and retention times. Table 4 shows the retention times and main ions with their relative abundances of the organophosphorus pesticides. All of the studied compounds can be identified by their main ions, in the PEST library, at levels near 1 ppb per compound.

Determination of Pesticide Levels in Commercial Juices. The developed MSPD method was applied to the determination of organophosphorus pesticides in commercial fruit juices. Table 5 summarizes the pesticide levels found in 21 juices purchased in various supermarkets in Madrid. The pesticides found in the commercial juices analyzed were diazinon, chlorpyrifos, and

Table 5. Pesticide Levels (Micrograms per Kilogram) Found in Commercial Juices^a

sample	diazinon	chlorpyrifos	ethion
Apple			
1	4.7	0.5	ND ^b
2	4.5	ND	ND
3	4.0	1.4	ND
Peach			
1	ND	0.7	ND
2	ND	0.6	ND
3	ND	0.6	ND
4	ND	2.8	ND
5	ND	3.2	ND
Orange			
1	2.1	ND	ND
2	3.7	ND	ND
3	2.5	2.1	0.6
4	2.9	ND	ND
5	NQ ^c	1.2	ND
6	3.0	ND	ND
Pineapple			
1	2.5	ND	ND
2	3.2	ND	ND
3	3.4	ND	ND
4	5.5	ND	ND
5	4.2	1.1	ND
Grape			
1	2.6	ND	ND

^a A total of 21 juice samples, purchased in Spanish supermarkets, were analyzed, and 20 samples (95%) were found to contain at least one of the nine pesticides determined. ^b ND, not detected. ^c NQ, detected but not quantified (lower than LOQ).

ethion. **Figure 5A** shows the NPD chromatogram of a commercial orange juice sample that contained 2.5 $\mu\text{g}/\text{kg}$ diazinon, 2.1 $\mu\text{g}/\text{kg}$ chlorpyrifos, and 0.6 $\mu\text{g}/\text{kg}$ ethion. These pesticide levels were confirmed by GC-MS with SIM, and **Figure 5B** depicts the corresponding chromatograms for these compounds with the main ions of their mass spectra.

Few data on pesticide residues in fruit juices are available in the scientific literature; however, the organophosphorus pesticide levels found in our research are similar to those reported by other authors (6, 7). Despite a high percentage (95%) of the commercial juice samples studied containing residues, the levels detected were very low and clearly lower than the MRL established by the EU for the content in fruit of the pesticides analyzed.

Conclusion. A rapid method, based on MSPD, has been developed for the simultaneous determination of nine organophosphorus pesticides in different fruit juices by GC-NPD. With the proposed analytical procedure, the extraction and cleanup can be performed in a single step requiring a low volume of organic solvent. The good reproducibility and the low detection and quantification limits achieved with this method allow its application in the determination of pesticide levels in commercial fruit juices, below the MRL established for raw fruits. The developed MSPD method has been applied to determine organophosphorus pesticides in fruit juices sold in Spanish supermarkets, and diazinon and chlorpyrifos have been detected in many of the juices analyzed, whereas ethion was found in only one sample. The identities of the detected pesticides have been confirmed by GC-MS with SIM.

LITERATURE CITED

- Sannino, A.; Bandini, M.; Bolzoni, L. Multiresidue determination of 19 fungicides in processed fruits and vegetables by capillary gas chromatography after gel permeation chromatography. *J. AOAC Int.* **1999**, *82*, 1229–1238.
- Frank, R.; Braun, H. E.; Ripley, B. D.; Pitblado, R. Residues of nine insecticides and two fungicides in raw and processed tomatoes. *J. Food Prot.* **1991**, *54*, 41–46.
- Itak, J. A.; Selisker, M. Y.; Herzog, D. P.; Fleeker, J. R.; Bogus, E. R.; Mumma, R. O. Determination of captan in water, peaches, and apple juice by a magnetic particle-based immunoassay. *J. AOAC Int.* **1994**, *77*, 86–91.
- Sannino, A.; Bandini, M.; Bolzoni, L. Determination of pyrethroid pesticide residues in processed fruits and vegetables by gas chromatography with electron capture and mass spectrometric detection. *J. AOAC Int.* **2003**, *86*, 101–108.
- Khrolenko, M.; Dzygiel, P.; Wiecezorek, P. Combination of supported liquid membrane and solid-phase extraction for sample pre-treatment of triazine herbicides in juice prior to capillary electrophoresis determination. *J. Chromatogr. A* **2002**, *975*, 219–227.
- Hankin, L.; Pylipiw, H. M., Jr. Pesticides in orange juice sold in Connecticut. *J. Food Prot.* **1991**, *54*, 310–311.
- Perret, D.; Gentili, A.; Marchese, S.; Sergi, M.; D'Ascenzo, G. Validation of a method for the determination of multiclass pesticide residues in fruit juices by liquid chromatography/tandem mass spectrometry after extraction by matrix solid-phase dispersion. *J. AOAC Int.* **2002**, *85*, 724–730.
- Shaw, I. Pesticides in food. In *Pesticide Chemistry and Bio-science*; Brooks, G. T., Roberts, T. R., Eds.; Royal Society of Chemistry: Cambridge, U.K., 1999; pp 421–428.
- Cai, C. P.; Liang, M.; Wen, R. R. Rapid multiresidue screening method for organophosphate pesticides in vegetables. *Chromatographia* **1995**, *40*, 417–420.
- De Paoli, M.; Barbina, M. T.; Mondini, R.; Pezzoni, A.; Valentino, A. Determination of organophosphorus pesticides in fruits by on-line size-exclusion chromatography-liquid chromatography-gas chromatography-flame photometric detection. *J. Chromatogr. A* **1992**, *626*, 145–150.
- Schenck, F. J.; Howard-King, V. Rapid solid-phase extraction cleanup for pesticide residues in fresh fruits and vegetables. *Bull. Environ. Contam. Toxicol.* **1999**, *63*, 277–281.
- Pihlström T.; Österdahl B. Analysis of pesticide residues in fruit and vegetables after cleanup with solid-phase extraction using ENV+ (polystyrene-divinylbenzene) cartridges. *J. Agric. Food Chem.* **1999**, *47*, 2549–2552.
- Simplício, A. L.; Vilas Boas, L. Validation of a solid-phase microextraction method for the determination of organophosphorus pesticides in fruits and fruit juice. *J. Chromatogr. A* **1999**, *833*, 35–42.
- Tena, M. T.; Rios, A.; Valcárcel, M.; Sánchez-Alarcón, M. Supercritical fluid extraction of organophosphorus pesticides from orange samples: Effect of solid additives on recovery. *Chromatographia* **1997**, *46*, 524–528.
- Barker, S. A. Matrix solid-phase dispersion. *LC-GC Int.* **1998**, *11*, 719–724.
- Kristenson, E. M.; Haverkate, E. G. J.; Slooten, C. J.; Ramos, L.; Vreuls, R. J. J.; Brinkman, U. A. Th. Miniaturized automated matrix solid-phase dispersion extraction of pesticides in fruit followed by gas chromatographic-mass spectrometric analysis. *J. Chromatogr. A* **2001**, *917*, 277–286.
- Torres, C. M.; Picó, Y.; Marín, R.; Mañes, J. Evaluation of organophosphorus pesticide residues in citrus fruits from the Valencian community (Spain). *J. AOAC Int.* **1997**, *80*, 1122–1128.
- Albero, B.; Sánchez-Brunete, C.; Tadeo, J. L. Multiresidue determination of pesticides in honey by matrix solid-phase dispersion and gas chromatography with electron-capture detection. *J. AOAC Int.* **2001**, *84*, 1165–1171.
- Schenck, F. J.; Wagner, R. Screening procedure for organochlorine and organophosphorus pesticide residues in milk using matrix solid-phase dispersion (MSPD) extraction and gas chromatographic determination. *Food Addit. Contam.* **1995**, *12*, 535–541.

- (20) Wylie, P. L.; Uchiyama, K. Improved gas chromatographic analysis of organophosphorus pesticides with pulsed splitless injection. *J. AOAC Int.* **1996**, *79*, 571–577.
- (21) Laganà, A.; D'Ascenzo, G.; Fago, G.; Marino, A. Determination of organophosphorus pesticides and metabolites in crops by solid-phase extraction followed by liquid chromatography/diode array detection. *Chromatographia* **1997**, *46*, 256–264.
- (22) Jiménez, J. J.; Bernal, J. L.; del Nozal, M. J.; Toribio, L.; Arias, E. Analysis of pesticide residues in wine by solid-phase

extraction and gas chromatography with electron capture and nitrogen–phosphorus detection. *J. Chromatogr. A* **2001**, *919*, 147–156.

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