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Matrix solid-phase dispersion extraction procedure for multiresidue pesticide analysis in oranges

C.M. Torres*, Y. Picó, M.J. Redondo, J. Mañes

Laboratori de Toxicologia, Facultat de Farmàcia, Universitat de València, Av. Vicent Andrés Estellés s/n, 46100 Burjassot, València, Spain

Abstract

A multiresidue extraction method based on matrix solid-phase dispersion (MSPD) is optimized for the extraction and gas chromatographic screening of eighteen insecticides (aldrin, carbophenothion, captafol, chlorpyriphos, chlorfenvinphos, diazinon, dicofol, α-endosulfan, β-endosulfan, ethion, fenitrothion, folpet, methidathion, malathion, methyl-azinphos, methyl-parathion, phosmet, and tetradifon) from oranges. After optimization of different parameters, such as type of solid phase used and the amount of solid phase or eluent, recoveries ranged from 67 to 102% with relative standard deviations ranging from 2 to 10%. The limits of detection, calculated as 3 times the baseline noise ranged from 2 to 171 μg/kg. These limits of detection were about 10 times lower than the maximum residue levels established by the European Community. Compared with classical methods, the described procedure is simple, less labour intensive and does not require preparation and maintenance of equipment. Troublesome emulsions, such as those frequently observed in liquid–liquid partitioning did not occur.

1. Introduction

Present-day agricultural practice requires the use of many chemicals to increase crop yields [1]. When the pesticide application history is not known, or when a crop has been treated with several pesticides, the use of a multiresidue method of analysis is generally preferred because of reduced analysis time and cost [2,3].

Analytical methods used in pesticide residue monitoring programs should be capable of detecting the residues below the maximum residue limits (MRL) established by the European Community [4].

The extraction techniques used by governmental agencies [5–9] sometimes require large samples and large volumes of extracting solvents, back-washing and re-extraction and solvent evaporations. Such procedures are time-consuming and tedious, which limits their usefulness for residue screening, and have generally not kept pace with advances in analytical technology. The development of a rapid and efficient extraction techniques that minimize time and the use of expendable materials, especially solvents, could enhance residue monitoring protocols.

Matrix solid-phase dispersion (MSPD) isolation technology involves blending a small amount of matrix with C_{18} (octadecylsilyl derivatized silica) followed by washing with a small amount of solvent and elution to extract a wide

* Corresponding author.

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range of compounds [10]. This technology has been used to extract several compounds (e.g., organochlorine pesticides, sulfonamides, cephalosporins, and benzimidazoles) from liver, muscle tissue, kidney, milk and fat [11–13].

Stafford and Lin [14] explored the possibility of using MSPD for the determination of oxamyl and methomyl residues in apples, oranges and soybean leaves, insects and river water by high-performance liquid chromatography.

This paper describes the optimization of an MSPD method for the extraction and screening of 18 pesticides from oranges by quantitative gas chromatography with electron-capture detection (ECD). The pesticides selected are the most frequently used in orange crops. The method was applied to measure the levels of pesticides in orange samples taken from the market.

2. Experimental

2.1. Reagents

Organochlorine pesticide standards aldrin, captafol, dicofol, α- and β-endosulfan and folpet were purchased from Supelco (Bellefonte, PA, USA).

Organophosphorus pesticide standards carbophenothion, chlorfenvinphos, chlorpyriphos, diazinon, ethion, fenitrothion, malathion, methidathion, methyl-azinphos, methyl-parathion, phosmet and tetradifon were obtained from Promochem (Wesel, Germany).

Preparative C_{18}, C_8 and CN were supplied by Waters Associates (Milford, MA, USA), C_2 by Alltech (Deerfield, IL, USA), silica gel by Scharlau (Barcelona, Spain), florisil by J.T. Baker Chemicals (Deventer, Netherlands) and alumina by Merck (Darmstadt, Germany).

Ethyl acetate, hexane, acetone, acetonitrile, N,N'-dimethylformamide, petroleum ether and diethyl ether, were pesticide grade from Promochem.

2.2. Procedure

A representative portion of sample (200 g of whole orange) was prepared using a food processor and mixed thoroughly.

An aliquot of the sample (0.5 g) was placed into a glass mortar (50 ml capacity) and 0.5 g of C_{18} were added. The orange was then gently blended into the C_{18} material with a glass pestle, until a homogeneous mixture was obtained (ca. 1 min).

The homogenized sample was introduced onto a 100 × 9 mm I.D. glass chromatographic column containing 0.5 g of silica. A 10-ml volume of ethyl acetate was added to the column and the sample was allowed to elute dropwise by applying slight vacuum. The eluent was collected into a graduated conical tube (15 ml) and concentrated, under a stream of nitrogen, to 0.5 ml. A 1-μl portion of the extract was then directly analyzed by gas chromatography.

2.3. Recovery studies

To determine extraction efficiency, orange samples (0.5 g) were fortified with 500 μl of the pesticide stock solution in ethyl acetate.

Fortified samples were allowed to stand at room temperature for 12 h after pesticide addition. Samples were then blended with C_{18}.

Pesticide fortification levels resulted in a final concentrations in oranges of aldrin, 19 μg/kg; carbophenothion, 96 μg/kg; captafol, 625 μg/kg; chlorfenvinphos, 179 μg/kg; chlorpyriphos, 19 μg/kg; diazinon, 976 μg/kg; dicofol, 65 μg/kg; α-endosulfan 50 μg/kg; β-endosulfan, 36 μg/kg; ethion, 539 μg/kg; fenitrothion, 19 μg/kg; folpet, 51 μg/kg; methidathion, 179 μg/kg; methyl azinphos, 498 μg/kg; methyl-parathion, 115 μg/kg; phosmet, 230 μg/kg; and tetradifon 65 μg/kg.

The fortification levels used in this study were chosen because they fall within the ranges of European Union maximum residue levels for some of these pesticides in fruits and vegetables.

2.4. Gas chromatographic analysis

The gas chromatographic analyses were carried out on a Konik 2000-C gas chromatograph (Barcelona, Spain) equipped with an ECD. A

methyl silicone capillary column (DB-1, 30 m × 0.25 mm I.D., 0.25 μm) from J&W Scientific (Folsom, CA, USA).

The temperatures were as follows: injector temperature, 200°C; detector temperature, 300°C; initial oven temperature, 50°C, hold 0.8 min, linear temperature gradient 30°C/min to 100°C, hold 2 min, linear temperature gradient 10°C/min to 180°C, linear temperature gradient 4°C/min to 280°C and hold 1 min. Splitless time, 0.7 min.

The carrier gas was helium at a flow-rate of 1 ml/min.

3. Results and discussion

3.1. Study of the analytical variables

The following modifications were made to optimize the assay for orange tissue: (1) the amount of solid phase, (2) the type of solid phase, (3) the eluent and (4) the homogenization step. The modifications were tested in four different varieties of oranges (Lanelate, Valencia Late, Salustiana and Navelate).

Different amounts of C_{18} and silica have been used. The results reported in Table 1 shows that the best recoveries are obtained using 0.5 g of C_{18} and 0.5 g of silica. The results showed that the recoveries of all the pesticides were independent of the orange variety used.

The combined effect of C_{18} and silica makes this extraction column suitable for a large number of pesticides and a complex matrix, such as oranges. In this column, the orange being analyzed is combined with C_{18} to give a reversed-phase component which retains neutral compounds. Silica, the second component of the column removes polar compounds. Fig. 1 shows chromatograms obtained using only C_{18}, only silica and both components. As can be seen, the cleanest extracts are obtained using a non-polar (C_{18}) and a polar (silica) solid phase.

The recoveries observed using only silica for clean-up are worse than those obtained using

Table 1
Effect on the pesticide recoveries of different polar and non-polar solid-phase amounts

Pesticides	g C_{18} + g silicagel						
	0.25 + 0.25	0.25 + 0.5	0.5 + 0.25	0.5 + 0.5	0.5 + 1	1 + 0.5	1 + 1
Aldrin	35 ± 12	48 ± 7	91 ± 6	101 ± 5	75 ± 4	50 ± 5	42 ± 8
Carbophenothion	27 ± 13	39 ± 9	76 ± 5	86 ± 6	85 ± 3	71 ± 6	60 ± 9
Captafol	69 ± 15	24 ± 12	80 ± 7	87 ± 4	73 ± 7	57 ± 9	52 ± 9
Chlorfenvinphos	41 ± 10	67 ± 12	80 ± 11	94 ± 9	97 ± 7	72 ± 10	58 ± 13
Chlorpyriphos	23 ± 11	36 ± 8	99 ± 6	108 ± 5	82 ± 5	74 ± 8	45 ± 8
Diazinon	34 ± 12	37 ± 10	90 ± 8	94 ± 6	72 ± 3	44 ± 5	40 ± 7
Dicofol	34 ± 12	22 ± 9	106 ± 9	105 ± 7	76 ± 4	31 ± 9	59 ± 9
α-Endosulfan	34 ± 13	36 ± 9	103 ± 7	96 ± 5	90 ± 8	50 ± 4	46 ± 6
β-Endosulfan	28 ± 10	24 ± 7	93 ± 5	95 ± 4	101 ± 10	73 ± 6	54 ± 7
Ethion	32 ± 15	38 ± 7	80 ± 7	93 ± 7	90 ± 4	78 ± 8	62 ± 8
Fenitrothion	42 ± 12	47 ± 8	90 ± 6	98 ± 2	93 ± 6	97 ± 5	51 ± 9
Folpet	27 ± 13	21 ± 7	96 ± 8	91 ± 5	87 ± 8	68 ± 6	49 ± 2
Phosmet	40 ± 13	29 ± 6	70 ± 5	66 ± 2	75 ± 5	63 ± 5	61 ± 6
Malathion	40 ± 16	70 ± 12	80 ± 7	87 ± 10	89 ± 2	90 ± 8	52 ± 7
Methidathion	33 ± 12	27 ± 10	81 ± 4	85 ± 2	90 ± 4	79 ± 3	65 ± 6
Methyl-azinphos	–	–	60 ± 10	57 ± 5	86 ± 9	91 ± 6	95 ± 9
Methyl-parathion	43 ± 15	71 ± 6	92 ± 8	97 ± 5	92 ± 8	80 ± 7	48 ± 8
Tetradifon	20 ± 12	28 ± 8	98 ± 6	98 ± 5	94 ± 5	73 ± 9	46 ± 9

Recoveries are expressed as ($\bar{x}$ ± R.S.D.)% for $n = 5$.

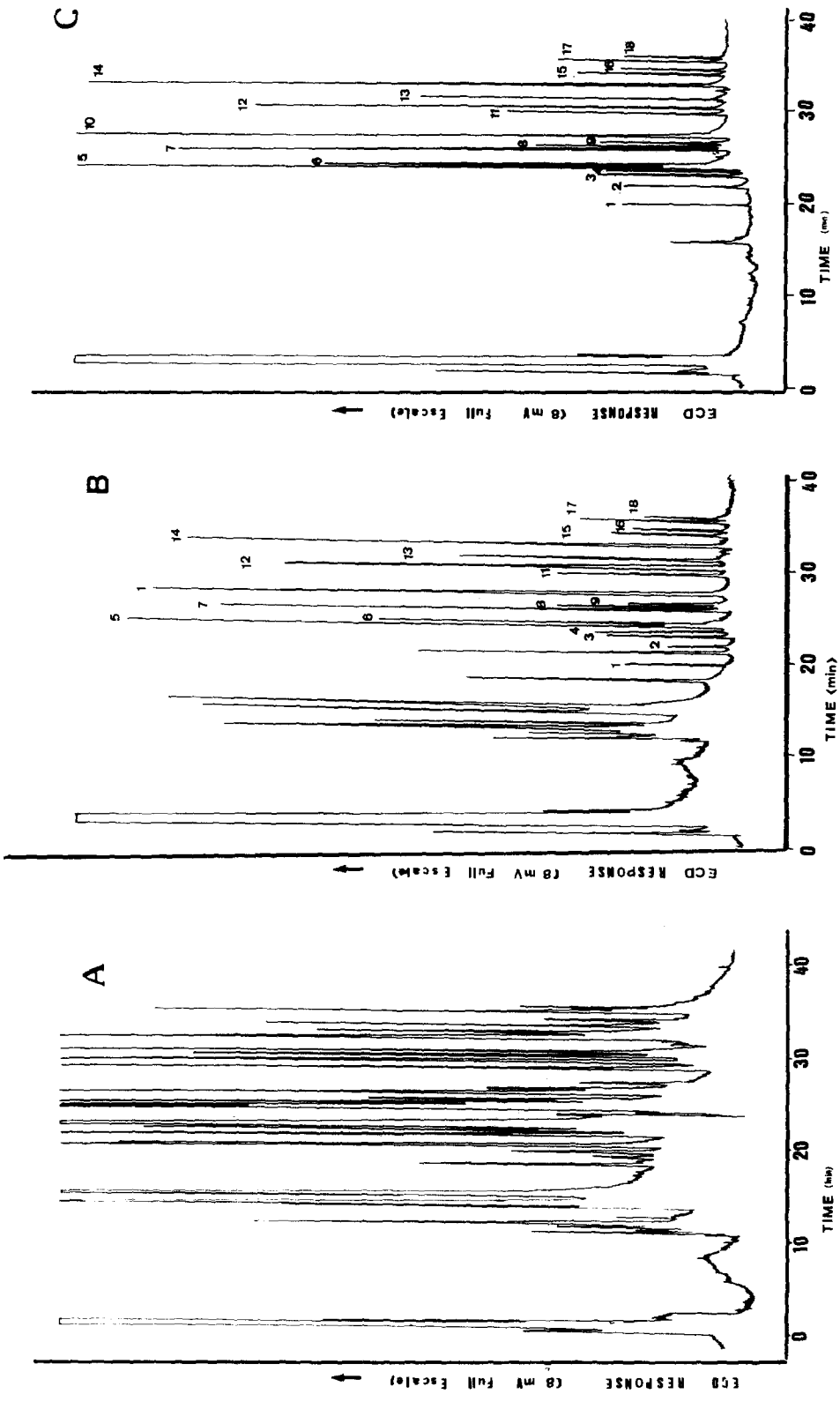

Fig. 1. Chromatograms obtained from 0.5-g aliquots of orange samples spiked with 0.5 ml of the stock solution. Orange was extracted by (A) homogenization with C_{18} alone, (B) put on a silica chromatographic column alone, and (C) the proposed method. Peaks: 1 = diazinon, 2 = methyl-parathion, 3 = fenitrothion, 4 = malathion, 5 = aldrin, 6 = chlorpyriphos, 7 = chlorfenvinphos, 8 = folpet, 9 = metidathion, 10 = α-endosulfan, 11 = β-endosulfan, 12 = ethion, 13 = carbophenothion, 14 = captafol, 15 = phosmet, 16 = dicofol, 17 = tetradifon, 18 = methyl-azinphos.

both phases (see Fig. 1B). This fact also confirms the previous remark about the effect of blending with C_{18}. Fig. 1A shows that the extraction performed only with C_{18} results in a large number of interfering peaks which avoid identification of the pesticide peaks.

The influence of the homogenization step was also checked. The results obtained by the proposed procedure were compared with those obtained with either homogenization with silica gel, as was proposed by several researchers [15–17], and clean-up by C_{18} or homogenization with a silica gel–C_{18} mixture. Not only the best recoveries, but also the cleanest extracts were obtained with the method proposed.

A probable explanation of this could be that the C_{18} acts as a better orange dispersant than the silica due to its hydrophobic characteristics. It is also known that C_{18} has a high affinity for non-polar compounds, whereas silica has been found to be more efficient in retaining polar compounds [1]. A column prepared from a C_{18}–matrix blend in combination with a co-column of silica and eluted with ethyl acetate produces and extract that shows minimal interferences, as can be seen in chromatograms (Fig. 1C).

To examine the feasibility of the MSPD method, the phase polarity is tested using C_{18}, C_8, C_2 and CN with silica as non-polar phases, and silica, florisil and alumina with C_{18} as polar-phases. The use of C_8, C_2 and CN failed to extract many of the pesticides considered. In particular, a poor recovery of the pesticides was observed when C_2 extraction was performed.

Using florisil, alumina or silica also gave clean extracts with a minimum of ECD-sensitive compounds which did not interfere with the pesticides. However, the higher adsorption strength of florisil and alumina [1] gave a decrease in pesticide recovery compared to silica.

Acetone, N,N-dimethylformamide, methanol, *n*-hexane, petroleum ether, diethyl ether and ethyl acetate were tested as elution solvents. Elution of the MSPD column, prepared as described, with acetone, N,N-dimethylformamide and methanol instead of ethyl acetate produced

Table 2
Comparison of the pesticide recoveries obtained by the proposed MSPD method and three classical methods of analysis

Pesticides	Method proposed by Mills [5]	Method proposed by Andersson [8]	Method based on ethyl acetate [9]	MSPD method proposed
Aldrin	63 ± 13	88 ± 12	85 ± 9	101 ± 5
Carbophenothion	98 ± 6	99 ± 5	93 ± 5	76 ± 6
Captafol	–	69 ± 4	83 ± 12	77 ± 4
Chlorfenvinphos	47 ± 6	102 ± 22	81 ± 12	84 ± 9
Chlorpyriphos	72 ± 16	81 ± 7	85 ± 6	108 ± 5
Diazinon	61 ± 8	62 ± 19	72 ± 8	94 ± 6
Dicofol	19 ± 7	23 ± 4	26 ± 5	105 ± 7
α-Endosulfan	54 ± 11	96 ± 6	90 ± 2	96 ± 5
β-Endosulfan	47 ± 10	86 ± 6	96 ± 2	95 ± 4
Ethion	91 ± 12	67 ± 7	93 ± 8	73 ± 7
Fenitrothion	88 ± 13	99 ± 4	84 ± 8	98 ± 2
Folpet	–	20 ± 3	23 ± 4	91 ± 5
Phosmet	85 ± 8	75 ± 5	81 ± 7	66 ± 2
Malathion	64 ± 13	73 ± 5	88 ± 7	87 ± 10
Methidation	90 ± 11	99 ± 11	99 ± 9	85 ± 2
Methyl-azinphos	8 ± 14	146 ± 6	69 ± 10	67 ± 5
Methyl-parathion	33 ± 13	67 ± 12	80 ± 17	97 ± 5
Tetradifon	62 ± 8	107 ± 3	86 ± 8	48 ± 5

Recoveries are expressed as ($\bar{x}$ ± R.S.D.)% for $n = 5$.

large quantities of interferences, as evidenced by the colour of the residue observed after solvent evaporation and by the number and intensity of peaks recorded by GC–ECD analysis of the extracts. Although ethyl acetate, *n*-hexane, petroleum ether and diethyl ether all extract pesticides from the dispersed orange without extracting large quantities of observable interferences, elution with ethyl acetate gave the highest recoveries.

3.2. Analytical parameters of MSPD

Table 1 shows the recovery and precision of the proposed method. The recovery ranged from 67% for methyl-azinphos to 107% for chlorpyriphos and the relative standard deviations (R.S.D.s) ranged from 2% for methidathion to 10% for fenitrothion.

Under the chromatographic conditions selected and extracting 0.5 g of orange the limits of

Table 3
Content of pesticides in orange samples

Variety	Total number of samples	Number of samples without residues	Number of sample with residues	Residue	Amount (mg/kg)
Navelate	8	4	Sample 3	Methyl-parathion	0.035
				Malathion	0.078
			Sample 4	Methyl-parathion	0.036
				Malathion	0.074
				Chlopyriphos	0.013
			Sample 5	Chlopyriphos	0.011
			Sample 6	Methyl-parathion	0.054
				Chlorpyriphos	0.001
Lanelate	10	6	Sample 1	Methyl-parathion	0.032
				Folpet	0.026
			Sample 7	Chlorpyriphos	0.004
			Sample 8	Methyl-parathion	0.078
			Sample 10	Methyl-parathion	0.035
				Malathion	1.138
				Chlorpyriphos	0.028
				Folpet	0.250
				Methidathion	1.017
Salustiana	8	6	Sample 1	Chlorpyriphos	0.005
				Chlorfenvinphos	0.318
				Folpet	0.098
			Sample 6	Methyl-parathion	0.069
Valencia late	8	3	Sample 2	Malathion	0.627
				Chlorpyriphos	0.103
			Sample 3	Methyl-parathion	0.046
				Malathion	0.216
				Chlorpyriphos	0.032
			Sample 4	Methyl-parathion	0.038
				Chlorpyriphos	0.069
			Sample 7	Fenitrothion	0.006
				Malathion	0.777
				Chlorpyriphos	0.133
			Sample 8	Fenitrothion	0.024
				Chlorpyriphos	0.252

detection (signal-to-noise ratio = 3) for 18 pesticides considered were: aldrin, 2 μg/kg; carbophenothion, 16 μg/kg; captafol, 171 μg/kg; chlorfenvinphos, 28 μg/kg; chlorpyriphos, 26 μg/kg; diazinon, 15 μg/kg; dicofol, 10 μg/kg; α-endosulfan, 15 μg/kg; β-endosulfan, 15 μg/kg; ethion, 95 μg/kg; fenitrothion, 3 μg/kg; folpet, 9 μg/kg; malathion, 27 μg/kg; methyl-azinphos, 8 μg/kg; methyl-parathion, 16 μg/kg; phosmet, 48 μg/kg; and tetradifon, 17 μg/kg. Most of these values are 10–25 times lower than the MRL legislated by the EC [4].

3.3. Method comparison

The extraction efficiency of MSPD was compared with those obtained by using three classical liquid–liquid extraction methods [5,8,9]. These methods have been recommended for the analysis of organophosphorus compounds in sev-

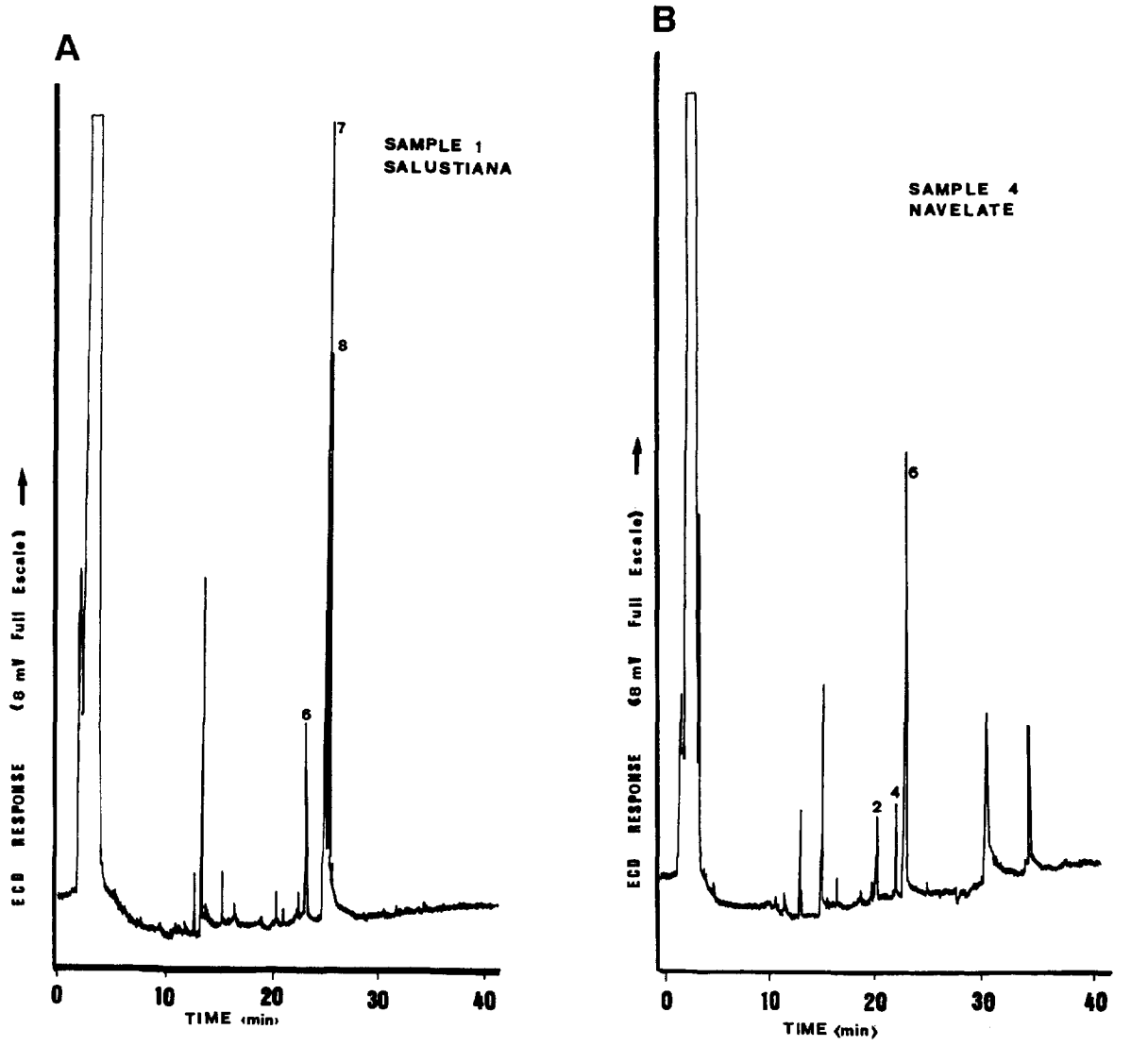

Fig. 2. Chromatogram obtained from (A) sample 1, variety Salustiana and (B) sample 4, variety Navelate. Peak numbers as in Fig. 1.

eral studies [18–20]. For these experiments, samples of 100 g were supplemented with the pesticide stocks solutions at the same concentrations as those used in the MSPD assays. After application of the three procedures, the solvent reduction step was performed in the same way as that adopted for MSPD, however until a final volume of 5 ml. Because of the high amount of sample processed and the co-extraction of interfering substances, split injection is used with a flow-ratio of 1/32.

The recoveries are listed in Table 2. Although some of the classical liquid–liquid extraction methods demonstrated their efficiency for pesticide residue analysis, their results are always comparable with those obtained with the proposed MSPD procedure.

3.4. Application to real market samples

To verify the MSPD procedure, 34 orange samples taken from the Valencia central market were analyzed. The samples were taken during the spring of 1994 and corresponded to the Valencia Late, Navelate, Lanelate and Salustiana varieties, which are the latest varieties that appeared on the market.

Table 3 shows the contents of positive samples and Fig. 2A shows a chromatogram obtained from sample 1, variety Salustiana, while Fig. 2B shows a chromatogram obtained from sample 4, variety Navelate. The concentrations found in oranges were always lower than the limits established by the EU [4] which demonstrated the good quality of the Spanish oranges for human consumption.

To confirm the identities of the pesticides found, in case the concentrations established by the EU are exceeded, a confirmatory test should be performed by checking the retention time on a second chromatographic column of different polarity or by using other specific detectors, such as mass spectrometry.

4. Conclusion

The MSPD method was successfully applied to the extraction of 18 selected pesticides from oranges. The procedure is simple and rapid and requires only small sample sizes and small solvent volumes. As demonstrated by the results obtained for real samples of oranges obtained from the market, the method can be used as a routine technique in the laboratory.

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