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Miniaturized automated matrix solid-phase dispersion extraction of pesticides in fruit followed by gas chromatographic–mass spectrometric analysis

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Abstract

In this study a simple and fast miniaturized automated matrix solid-phase dispersion method for the sample preparation and quantitative extraction of pesticides was developed and evaluated. Only 25 mg of sample and 100 μl of organic solvent were used per analysis for this new miniaturized set-up. The extracts were subsequently analysed by GC–MS without any further purification. The method was optimized for oranges and tested for the determination of a variety of organophosphorus pesticides and a pyrethroid at concentration levels below the maximum residue levels set by the European Union and authorities in The Netherlands. The limits of detection were 4–90 μg/kg. The recoveries for pesticides in orange were 83–118% and the relative standard deviations for the total procedure were 10–13% ($n=4$) at the limit of quantification. The feasibility of the developed method for apple, pear and grapes was also studied. Equally good results were obtained, but for apple the washing step should be omitted.

Keywords: Matrix solid-phase dispersion; Extraction methods; Fruits; Automation; Food analysis; Pesticides; Organophosphorus compounds

1. Introduction

In agriculture, large quantities of chemicals are used to eliminate pests that damage fruits and vegetables. An unquestionable benefit for agricultural production is thereby achieved. However, after their application, pesticide residues remain in the crops, the soil and the groundwater and constitute a health risk because of their toxicity. To protect consumers' health, many countries have restricted the usage of these deleterious chemicals and have established legal directives to control their levels in food, through maximum residue levels (MRLs).

Most protocols dealing with the determination of pesticides in fruit involve several extraction, purifica-

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tion and concentration steps, which make them time consuming, expensive to perform when many samples must be analysed and not really suitable for routine analysis. Solid–liquid partitioning has often been used to extract organophosphorus pesticides (OPPs) [1,2] from fruit. Soxhlet extraction has also been recommended for carbamate pesticides and OPPs [3]. Other extraction techniques, such as supercritical fluid extraction (SFE) [4] and solid-phase extraction (SPE) with discs [5] or cartridges [6] have been developed for the same purpose. Many interferences are encountered when these techniques are used, as the solvents are non-selective and therefore tend to extract endogenous material from the sample, which interferes with the analysis. To obtain a satisfactory limit of detection a purification step is often necessary, e.g., an additional SPE step. The sample size for the analysis of pesticides with the stated extraction techniques is between 2 and 100 g and the solvent consumption varies from 5 to 200 ml.

Matrix solid-phase dispersion (MSPD) [7] can be regarded as a valuable alternative to the more classical sample preparation methods because it allows a significant reduction in both the sample size and solvent consumption needed for multiresidue analysis. In addition, depending on the nature of the sorbent selected, a simultaneous clean-up of the extract occurs, which in most cases allows the direct analysis of the collected extracts [8].

The MSPD-based methods for the determination of pesticides in fruit and vegetables published so far [9–11] are off-line methods. Even so, their advantages are striking: less time is required than with methods based on SPE; only relatively small amounts of sample (0.5–1.0 g), sorbent and organic solvent (10–15 ml) are used. MSPD has been used to extract several types of chemical residues (organochlorine pesticides, OPPs, carbamates, pyrethroids, sulfonamides, cephalosporins and benzimidazoles) from fruit, vegetables, liver, muscle tissue, kidney, milk and fat [7,9–13], but has also been used for many other applications [14]. Analysis of extracts is generally performed by gas chromatography (GC) with various detection methods [15] such as electron-capture (ECD) [9], nitrogen–phosphorus (NPD) and flame photometric (FPD) detection, and mass spectrometry (MS) [10]. Because of their thermal instability carbamates are analysed by liquid chromatography (LC)–UV [11], LC–fluorescence after post column derivatisation [16] or LC–MS [17].

The main objective of this study was to develop and evaluate a simple and fast miniaturized automated MSPD procedure for the sample preparation and quantitative extraction of pesticides in fruit. The method was optimized by testing desorption solvents and sorbents, with oranges as test matrix and a selected number of OPPs and a pyrethroid, ranging from the relatively polar parathion-methyl to apolar compounds such as bromophos-methyl at concentration levels below the MRLs allowed by the European Union (EU). The feasibility for the selected pesticides in pear, apple and grape analysis was also evaluated. All extracts were analysed by GC–MS.

2. Experimental

2.1. Chemicals

Diazinon, parathion-methyl, fenitrothion, malathion, fenthion, chlorpyriphos-ethyl, bromophos-methyl, azinphos-methyl, permethrin and trifluralin, all >95%, were obtained from Riedel-de Haën (Seelze, Germany) and methidathion (>95%) from Dr. Ehrenstorfer (Augsburg, Germany). Individual solutions of 1 mg/ml of each pesticide were prepared in freshly distilled methyl acetate (Fluka, Steinheim, Switzerland). Working solutions containing 1, 5 or 10 μg/ml of each compound were prepared every 2 weeks by dilution in freshly distilled ethyl acetate (Riedel-de Haën, >99.5%). The internal standard (I.S.; trifluralin, 4 μg/ml) was also prepared in ethyl acetate. All solutions were stored at 4°C.

Quartz-distilled water was used in all experiments. The following sorbents were used: C_8-bonded silica (5 μm) from Merck (Darmstadt, Germany) and C_8-bonded silica (5 μm) from Chemie Uetikon Research Separation Labs. (Uetikon, Switzerland), C_{18}-bonded silica (5 μm) from Shandon (Runcorn, UK) and silica gel 60 (63–100 μm) from Merck. Silica was Soxhlet extracted (6 h) with ethyl acetate prior to use. The desorption solvents studied for MSPD were ethyl acetate and *n*-hexane, both from Riedel-de

Haën. Orange, apple, pear and grape samples were obtained from a local supermarket.

2.2. Instrumentation and MSPD procedure

A representative portion of the selected fruit sample, around 5 g, was cut into small pieces and homogenised in an omnimixer (Sorvall, Newtown, CT, USA) and kept in a freezer at −20°C until used. For practical reasons the whole fruit was analysed in the case of oranges and grapes, while initially only the peel was used for apple and pear analysis. Next, a 100-mg subsample was weighed for homogeneous sample preparation and further analysed using the proposed method. The MSPD sample preparation was done according to the off-line MSPD procedure proposed by Torres et al. [9], i.e., a fruit–sorbent (1:1, w/w) mixture was placed in a glass mortar and gently blended for a few minutes using a pestle to obtain a dry-powder-like homogeneous mixture.

Preliminary experiments were conducted to optimize the miniaturized MSPD method. In these experiments spiked orange samples (10 μg/g) were used and the main parameters affecting the extraction efficiency, were studied and optimized. C_8-bonded silica was selected as sorbent in these preliminary experiments and *n*-hexane and ethyl acetate were used as desorption solvents.

A 50-mg aliquot of the homogenised mixture was packed into a 10×4 mm I.D. stainless steel holder. The holder was closed with two 5-μm stainless steel screens held in PTFE rings, manufactured in the laboratory and connected to one of two Valco six-port valves used. After extraction, the cartridge was cleaned and re-used. All tubing was of stainless steel and 0.5–1 mm I.D. Clean-up of the sample in the holder was done by flushing with quartz-distilled water for 8 min at a flow-rate of 1 ml/min with a Gilson pump Model 302 (Gilson France, Villiers le Bel, France). After washing, the sample was dried under nitrogen (3 bar) for 30 min. Pesticides were desorbed with 100 μl of ethyl acetate pumped at a flow-rate of 100 μl/min with a Phoenix 20CU syringe pump (Carlo Erba, Milan, Italy). The extract was collected in a microvial. After adding the internal standard, 1 μl of the extract, which corresponds to 250 μg of the sample, was directly analysed by GC–MS. After optimization, C_{18}-bonded silica and silica gel were also studied.

The extraction efficiency of the MSPD method was evaluated by analysing orange samples spiked at the 0.5 μg/g level which is close to the EU MRLs for pesticides in fruit (Table 1) [18]. The fruit samples were spiked before sample preparation. Subsequently, the MSPD method was evaluated for the determination of pesticides in grape, apple and pear samples. Blank samples were analysed to check for contamination throughout the analytical procedure.

Preliminary MSPD experiments were carried out in duplicate and each extract was injected once, while the other MSPD experiments were done in fourfold and injected twice using trifluralin as internal standard. To avoid any possible matrix effects on

Table 1
MRLs (mg/kg) of the pesticides in relevant fruits [8,18]

Pesticide	MRL (mg/kg)[a]							
	Orange		Apple		Pear		Grape	
Diazinon	0.5	(E)	0.5	(E)	0.5	(E)	0.5	(E)
Parathion-methyl			0.2	(E)			0.2	(E)
Fenitrothion	2	(E)	0.2	(E)	0.2	(E)	0.2	(E)
Malathion	2	(E)						
Fenthion	0.05	(NL)						
Chlorpyriphos-ethyl	0.3	(E)	0.5	(E)	0.5	(E)	0.5	(E)
Bromophos-methyl			0.02	(NL)				
Methidathion	2	(E)			0.3	(E)	0.2	(E)
Azinphos-methyl	2	(E)	0.5	(NL)				
Permethrin					1	(NL)		

[a] E=EU MRL, NL=Dutch MRL.

analyte detectability [19], the calibration curves (five data points in the 0.025–0.20 μg/ml range) were constructed by standard addition to blank fruit extracts after checking that none of the selected pesticides was present.

2.3. GC–MS

Analysis of the MSPD extracts was performed by GC–MS (HP 6890 Series, Hewlett-Packard, Palo Alto, CA, USA; MSD, HP 5972). The injector was an Optic 2-200 (Ai Cambridge, Cambridge, UK). Extracts were injected in the cold splitless mode (splitless time 1.0 min, initial temperature 65°C, increased at 16°C/s to 240°C) on a HP-5MS column (30 m×0.25 mm I.D., d_f 0.25 μm). A three-step temperature programme was used from 60°C (3.5 min), then at 30°C/min to 200°C (2 min), next at 7°C/min to 210°C (2 min), and, finally, at 50°C/min to 280°C (4 min). The total run time was 19 min. Helium was used as the carrier gas using a pressure programme from 0.71 to 1.47 bar.

The mass spectrometer was operated in the electron ionization mode (EI, 70 eV); the temperature of the transfer line was 280°C. Analysis was carried out by selected ion monitoring (SIM). For each compound, two characteristic ions were monitored (Table 2), the first for quantification and the second as a qualifier. Since permethrin is a diastereomer, two peaks corresponding to its *cis* and *trans* configuration were detected. The former was selected for quantification. In some experiments and for confirmation purposes, the scan acquisition mode (m/z 50–450) was used.

3. Results and discussion

3.1. Optimization of the MSPD method

Preliminary experiments were carried out using C_8-bonded silica, to optimize the main parameters affecting the MSPD efficiency. For practical reasons the orange peel and pulp were homogenised together since the peel was too dry for separate homogenisation. Normally only the pulp is eaten; however the pesticide levels are expected to be higher in the peel than in the pulp.

Firstly, possible breakthrough of the most polar pesticides during the clean-up step was investigated. The washing solvent, water, was collected and extracted with ethyl acetate for 5 min. None of the compounds were detected in the washing solvent when 8 ml of quartz-distilled water at 1 ml/min were used for clean-up of the MSPD mixture. Therefore this clean-up procedure was selected for subsequent experiments.

Regarding the desorption speed, after several assays (with C_8-bonded silica as sorbent) a flow-rate of 100 μl/min was selected. This appeared to be low

Table 2
Molecular mass, selected ions and log P_{ow} of the analysed pesticides

Pesticide	Peak No.	M_r[a]	m/z[b]	Log P_{ow}[c]
Diazinon	1	304.4	179/199	3.11–3.81
Parathion-methyl	2	263.2	109/125	1.80–3.04
Fenitrothion	3	277.2	125/109	3.30–3.47
Malathion	4	330.4	125/173	2.84–2.94
Fenthion	5	278.3	278/125	4.09–4.17
Chlorpyriphos-ethyl	6	350.6	314/197	4.96–5.27
Bromophos-methyl	7	366.0	331/125	4.88–5.21
Methidathion	8	302.3	145/125	2.42
Azinphos-methyl	9	317.3	160/132	2.69
Permethrin (*cis*/*trans*)	10/11	391.3	183/163	5.84–6.60
Trifluralin	I.S.[d]	335.3	306/264	3.97

[a] Molecular mass.
[b] Two most abundant ions.
[c] Log partition coefficient *n*-octanol–water [20].
[d] Internal standard.

enough to allow both the extraction of the investigated pesticides without any unnecessary increase of the total solvent volume and the accurate collection of the desorption solvent volume. Although a solvent volume of around 75 μl was found to be sufficient for quantitative extraction of the selected compounds, 100 μl was finally used to be on the safe side and for improved precision. A mere 1-μl injection was sufficient to reach adequate detection limits for pesticides in fruit samples. In case of more dilute extracts or if lower detection limits are required, the present GC set-up would easily facilitate large-volume injection.

The polarity of the sorbent and the desorption solvent are known to be key factors in MSPD since they determine both the efficiency of the extraction and the cleanness of the final extract. Therefore, the influence of both parameters on the proposed MSPD method was evaluated. Two desorption solvents, *n*-hexane and ethyl acetate, were tested using 5 μm C_8-bonded silica from Chemie Uetikon. Fig. 1 shows the *m*/*z* 125 fragmentograms of the pesticides obtained with ethyl acetate and *n*-hexane. The results are seen to be rather similar. However, remarkably extractions with hexane gave a two times higher background. In addition, fenitrothion was not very well separated from an interference from the orange matrix present when hexane was used for desorption. In general, this finding agrees with those previously published by other authors who concluded that ethyl acetate gives cleaner extracts than apolar solvents such as *n*-hexane [9]. On the basis of these results, ethyl acetate was selected for all further work.

3.1.1. MSPD sorbent selection

Based on the preliminary experiments with C_8-bonded silica, quantitative analysis was performed using C_8-bonded silica, C_{18}-bonded silica and silica gel. When comparing the recovery data, rather different results were found for the two alkyl-bonded sorbents as compared with silica. As is evident from Fig. 2, without exception the recoveries found using silica were lower than those obtained with the hydrophobic sorbents. The five most polar OPPs (for

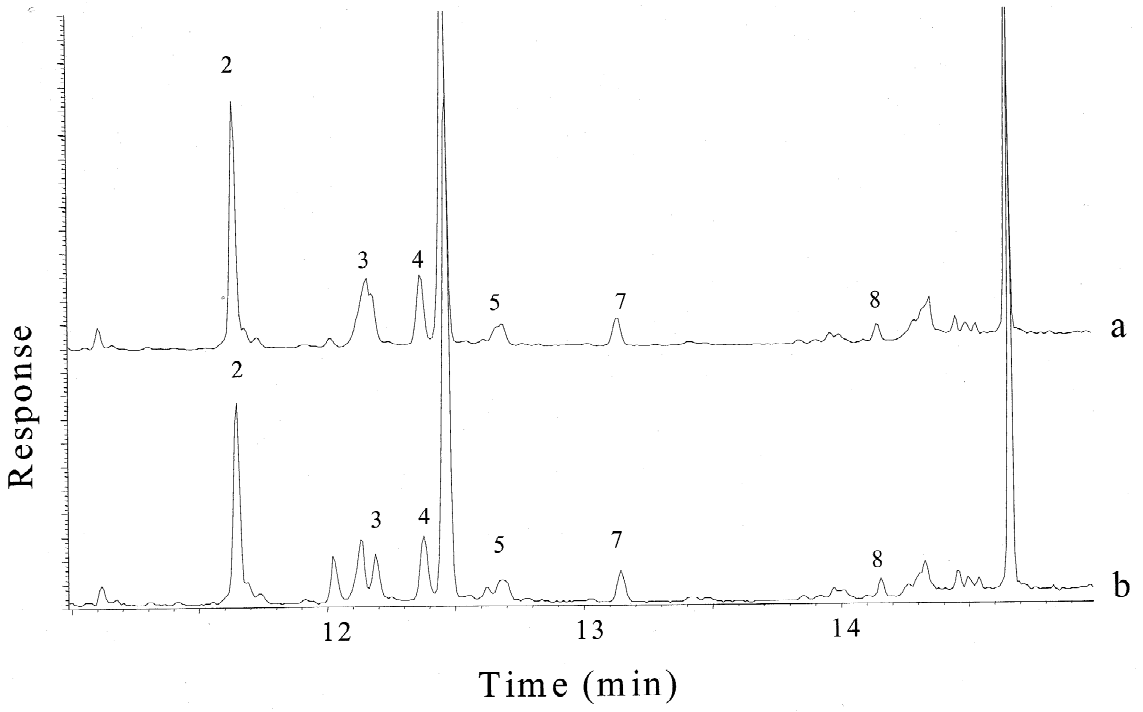

Fig. 1. Fragmentograms (*m*/*z* 125) of orange extract prepared with C_8-bonded silica as sorbent and with (a) *n*-hexane and (b) ethyl acetate as desorption solvent. See Table 2 for peak numbering.

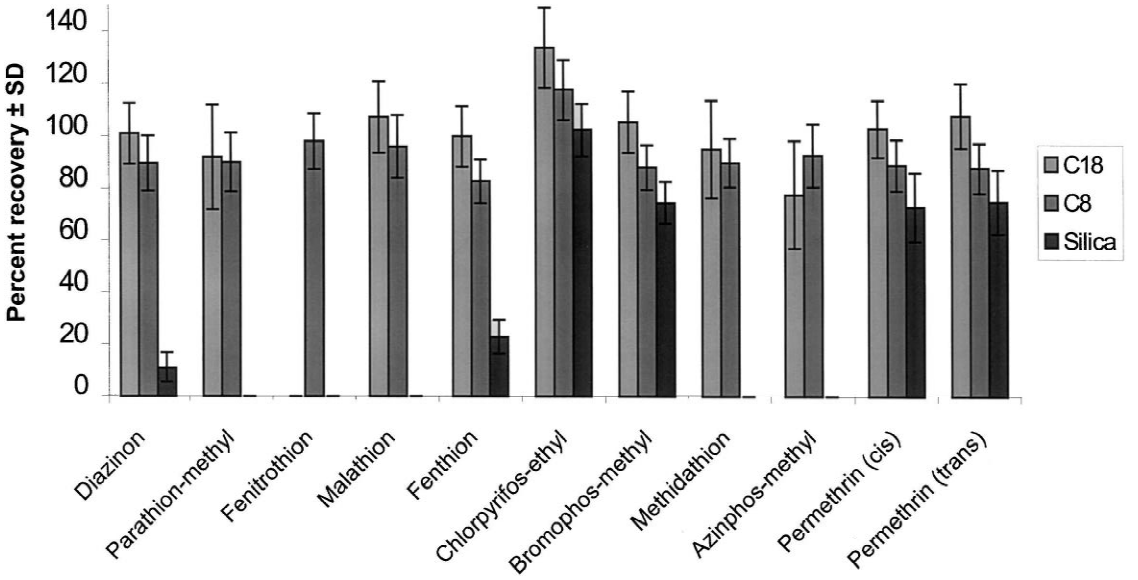

Fig. 2. Percent recoveries±SD (n=4) of the analytes, obtained for oranges, spiked at 0.5 mg/kg, using different sorbents.

log P_{ow} values, see Table 2) were even lost completely, and the two analytes of intermediate polarity, diazinon and fenthion, showed poor recoveries of 10–20%. The insufficient retention power of silica was clearly demonstrated in an experiment where two identical portions of spiked orange/silica, added to two separate holders, were either directly desorbed with ethyl acetate or, according to the standard procedure used in this study, washed with water and dried with nitrogen before desorption with ethyl acetate. The polar compounds from parathion-methyl through to fenitrothion could all be detected by GC–MS when directly desorbed with ethyl acetate, but not when the washing step was included. Unfortunately, when washing was omitted, the simultaneous extraction of the endogenous matrix material by the organic solvent led to a much higher background.

With the two alkyl-bonded sorbents, the analyte recoveries were closely similar, viz. 82–117% and 78–134% for C_8- and C_{18}-bonded silica, respectively (for all but one OPP). However, the RSDs (n=4) for C_8-bonded silica (10–13%) were lower than for C_{18}-bonded silica (11–27%). Despite the small sample size, both recovery and RSD data were comparable to those previously reported in the literature for methods involving a much larger amount of sample and solvent [9,11,15]. A drawback of using C_{18}-bonded silica was that fenitrothion was co-eluted with interferences from the orange matrix and therefore could not easily be detected or quantified. Further, C_8-bonded silica provided cleaner extracts. Finally, when calibration curves were constructed by means of the standard-addition method (spiking of the final extract), essentially the same results were obtained for both alkyl-bonded silicas. The correlation coefficients for all pesticides were closely similar, i.e., between 0.989 for fenitrothion and 0.998 for malathion for C_8-bonded silica, and between 0.982 and 0.997 for C_{18}-bonded silica (five data points in duplicate; test range, 0.025–0.20 μg/ml). The only difference was that inter-day reproducibility appeared to be slightly superior for C_8-bonded silica.

On the basis of these overall results, C_8-bonded silica was considered the optimum choice for MSPD experiments. Fig. 3 shows the merged fragmentograms of the eight OPPs and permethrin added at the 0.2 μg/ml level to an orange extract prepared according to the method discussed above, which corresponds to 0.8 mg/kg in orange. This result demonstrates that the miniaturized MSPD+GC–MS procedure allows the unambiguous determination of the test compounds at levels below the MRLs even if

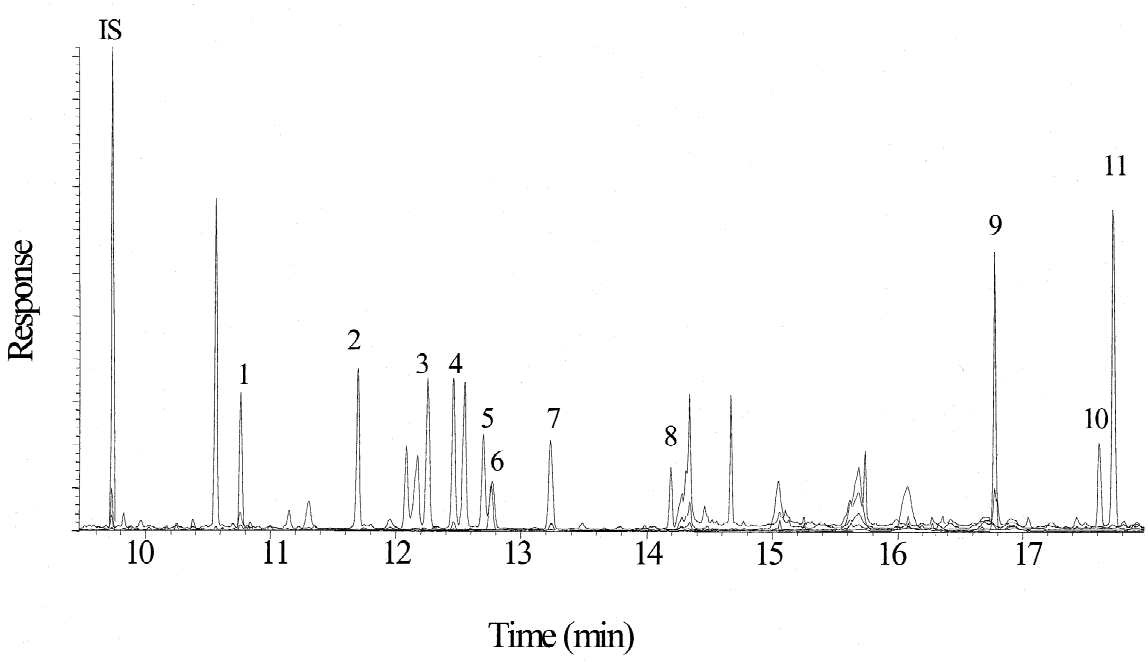

Fig. 3. Merged GC–MS fragmentograms (*m/z* 125, 199, 160, 183 and 314) of orange extract spiked at the 0.2 μg/ml level: the concentration of the I.S. was 0.2 μg/ml. See Table 2 for peak identification. The blank extract did not contain any of the pesticides.

as small a sub-sample as 25 mg is used for the analysis. To confirm the validity of the above approach, experiments were also carried out with a larger, i.e., a 30×3 mm I.D. holder, and correspondingly larger sample sizes of 70 mg. This change did not improve the practicality of the procedure and the average recovery of 82±7% (obtained with 100–200 μl of ethyl acetate) was the same as of the miniaturized procedure, 86±5%. Secondly, a comparison was made with solid–liquid partitioning. To that end, 100 mg of orange was vortex-mixed with 500 μl of ethyl acetate. After injection of 1 μl of the extract, the results were much poorer than with MSPD. The average recovery of the OPPs and the pyrethroid dropped to 28% with an RSD of 15% ($n=4$).

3.2. Analytical data and applications

The MSPD method was optimized for orange analysis, but it was also used for the determination of the same pesticides in grapes, apple and pear.

Recovery data for the various fruits are summarized in Table 3. The results for orange are highly satisfactory, being in the range 83–98% for all but one OPP, chlorpyriphos-ethyl (118%). With one exception (fenthion; see below), the recoveries for pear (72–80%) and grapes (47–62%) fall within short ranges and can be called satisfactory even in the latter case because of the excellent RSD values. The overall results are in the range of those previously published by Torres and co-workers [9,15] who reported recoveries of these pesticides of 79–101% (orange) and 58–93% (pear) when using off-line MSPD involving much larger samples (500 mg) and more desorption solvent (10 ml) than in the present study. For all fruits studied, the recoveries of fenthion were lower than for the other pesticides. No reference has been found in the literature about the exceptional behaviour of this pesticide in MSPD and no further explanation can as yet be offered.

The data of Table 3 also show that the analyte recoveries were unexpectedly low in the case of apple, viz. about 20–25% with, moreover, RSDs of 15–30% indicating poor precision. Obviously, in this case, re-optimization was required. Further work showed that omitting the washing procedure dramatically improved the results. As can be seen from the

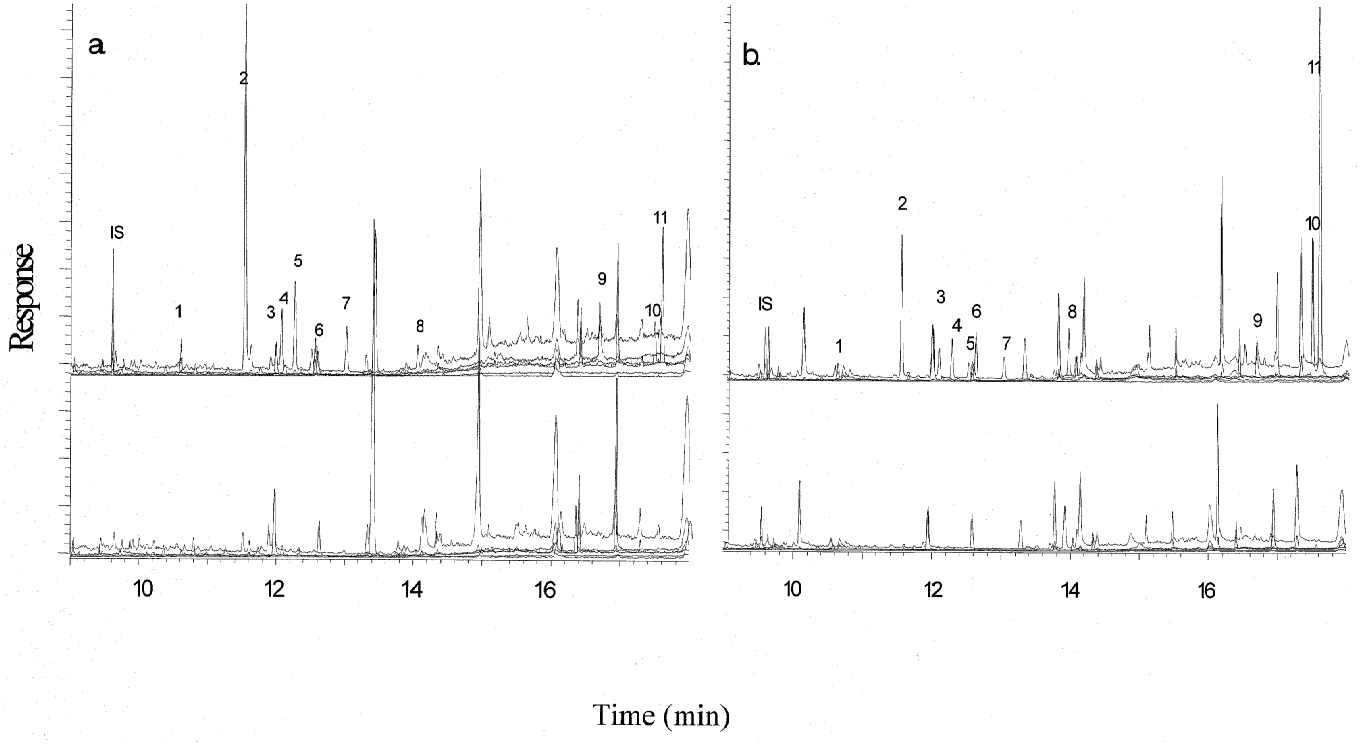

Fig. 4. GC–MS fragmentograms (m/z 125) of (a) grapes and (b) pear peel extracts without (lower fragmentograms) and with (upper fragmentograms) 0.5 mg/kg spiking. See Table 2 for peak numbering.

Table 3
Percent recovery (RSD; $n=4$) of the selected pesticides in orange, pear, grape and apple[a]

Pesticide	Orange	Pear	Grape	Apple	
				Clean-up	No clean-up
Diazinon	90 (12)	73 (8)	51 (4)	22 (15)	80 (4)
Parathion-methyl	90 (12)	72 (4)	47 (5)	22 (21)	94 (8)
Fenitrothion	98 (11)	78 (8)	52 (7)	23 (18)	88 (6)
Malathion	96 (13)	79 (4)	51 (6)	23 (21)	82 (2)
Fenthion	83 (10)	43 (2)	31 (32)	9 (16)	76 (3)
Chlorpyrifos-ethyl	118 (10)	76 (4)	51 (6)	22 (18)	78 (3)
Bromophos-methyl	88 (10)	75 (3)	62 (5)	21 (21)	90 (3)
Methidathion	90 (10)	79 (11)	47 (3)	22 (24)	97 (4)
Azinphos-methyl	93 (13)	79 (2)	48 (3)	27 (22)	74 (7)
Permethrin (*cis*)	89 (11)	80 (3)	54 (2)	20 (28)	87 (8)
Permethrin (*trans*)	88 (11)	80 (1)	53 (5)	21 (25)	89 (8)

[a] Spiking level, 0.5 mg/kg.

data added in Table 3, the recoveries increased to 85±5%. Omitting the clean-up step, somewhat surprisingly, caused little increase of the background in GC–MS analysis and no additional interfering peaks showed up.

Table 4 summarizes the limits of detection (LODs) for the pesticides of interest in extracts of all fruits. It is clear that, with one or two exceptions, LODs are in the 10–50 μg/kg range, and are essentially the same for all sample types studied. The small differences in LODs among them can be attributed to differences in interfering compounds or intensity of the background signal. In all cases, the LODs were much lower than the MRLs established by the EU and Dutch legislation for these fruits (cf. Table 2) which indicates the suitability of the proposed method for the determination of pesticides in fruit.

Table 4
LODs of all pesticides in fruits

Pesticide	LOD (μg/kg)			
	Orange	Pear	Grape	Apple
Diazinon	40	50	30	25
Parathion-methyl	10	10	4	6
Fenitrothion	50	40	50	8
Malathion	40	40	10	4
Fenthion	10	20	6	60
Chlorpyrifos-ethyl	10	7	7	25
Bromophos-methyl	10	80	4	90
Methidathion	10	10	20	8
Azinphos-methyl	30	30	40	30
Permethrin (*cis*)	30	50	30	155
Permethrin (*trans*)	10	20	10	20

3.2.1. Application to real-life samples

As a final application, the optimized procedure was used to analyse a number of oranges, apples, pears and grapes. In each instance fruit extracts spiked at the 0.5 mg/kg level were also analysed. Two typical examples, for grapes and pear, are shown in Fig. 4. In orange, chlorpyriphos-ethyl was found at a level of 0.27±0.04 mg/kg, which is close to the MRL of 0.3 mg/kg. None of the pesticides in the test set were detected in the other fruit extracts investigated.

It is important to add that, as regards the maintenance of the set-up, no clogging of either frit or tubing was observed during 6 months of constant use. There were no major experimental problems and it is justified to call the overall procedure user-friendly.

4. Conclusions

A miniaturized automated MSPD method with subsequent GC–MS analysis has been developed and its feasibility for the trace-level determination of pesticides in fruit demonstrated. The procedure is simple and rapid with a total analysis time of around 1 h and requires only small amounts of sample (100 mg for sample preparation and only 25 mg for

sample analysis) and solvent (100 μl). C_8-bonded silica was preferred as sorbent because of the lower background in GC–MS, higher recoveries and better repeatability than found with C_{18}-bonded silica and silica gel. The method, which was optimized for oranges, showed fully satisfactory recoveries for oranges, pears and grapes. The much lower recoveries obtained with apple (20–27%) could be considerably improved to satisfactory values of over 80% by omitting the clean-up step of washing with distilled water. Since this procedural change helps to increase sample throughput, further study in this area is indicated. The LODs for the pesticides in fruit were 4–90 μg/kg, which is below the MRLs (average 0.5 mg/kg). Analytical performance (linearity, repeatability, etc.) were fully satisfactory, and maintenance problems were essentially absent. In other words, miniaturized MSPD can be considered as a valuable alternative to more classical large-scale methods typically used for routine analysis which usually involve longer analysis times and larger amounts of sample and organic solvents.

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