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Solid-phase microextraction – gas chromatography mass spectrometry: A fast and simple screening method for the assessment of organophosphorus pesticides residues in wine and fruit juices

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Abstract

A SPME-GCMS method for the determination of a mixture of organophosphorus pesticides (phorate, diazinon, methyl-parathion, fenitrothion, malathion, fenthion, ethyl-parathion and methidathion) in wine and different fruit juices was developed. The procedure is solvent-free, simple (direct SPME without further sample pre-treatment) and highly sensitive. Estimated LOD and LOQ ranged from 2 to 33 ng/ml and from 7 to 109 ng/ml, respectively, in wine, and from 2 to 90 ng/ml and from 7 to 297 ng/ml, respectively, in fruit juices. LOQ achieved by the present method are almost always below the maximum residue levels recommended by the European legislation.

Keywords: SPME; Organophosphorus pesticides; Wine; Fruit juices; GC-MS

1. Introduction

Organophosphorus (OPPs) are among the most widely employed pesticides world-wide. Their large use for crop protection has raised great concern since, due to their high half-life time, they can persist in the environment thus contaminating soils as well as surface and ground water. The incorrect use of OPPs may also result in the presence of residues of these substances in agricultural products and, hence, in derivate food commodities, i.e., wine, fruit juices and so on, thus compromising their safety.

The determination of pesticides is usually accomplished by chromatographic techniques and involves many preliminary steps like sampling, extraction, and clean-up for interference removal. Solid-phase microextraction (SPME) is a solvent-free extraction technique (Arthur & Pawliszyn, 1990) that represents a convenient alternative to conventional extraction methods. It allows simultaneous extraction and pre-concentration of analytes from sample matrix; furthermore, SPME eliminates some disadvantages of conventional extraction techniques such as plugging of cartridges in solid phase extraction and use of toxic solvents in liquid-liquid extraction. SPME has been widely applied to organophosphorus pesticides residue analysis. Most of these applications deals with OPPs determination in environmental samples such as ground water (Choudhury, Gerhardt, & Mawhinney, 1996; Eisert & Levsen, 1995a), waste water (Beltran, Lopez, Cepria, & Hernandez, 1998; Eisert & Levsen, 1995b; Valor, Moltò, Apraiz, & Font, 1997), river water (Eisert, Levsen, & Wunsch, 1994), lake water (Magdic, Boyd-Boland, Jinno, & Pawliszyn, 1996) and soil (Bouaid, Ramos, Gonzalez, Fernandez, & Camara, 2001; Ng, Mui, & Hans-Ake, 1999; Zambonin, Losito, Cilenti, & Palmisano, 2002). Most recent applications deal

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essentially with food analysis for the presence of OPPs residues. Correia, Delerue-Matos, and Alves (2000) described a multi-residue method based on SPME and GC with electron capture detector for insecticides (including some OPPs), acaricides and fungicides residues determination in wine; detection limits in the order of 5 ppb were found but four OPPs gave non linear responses. Headspace SPME combined with gas chromatography-mass spectrometry (GC-MS) has been reported for the rapid screening of seven OPPs in strawberries and cherries (Lambropolou & Albanis, 2003). SPME coupled to GC with flame ionisation detector has been used (Yang, Eisert, Lord, & Pawliszyn, 1999) for organonitrogen and organophosphorous pesticides added to home made orange and carrot juices. Simplicio and Vilas Boas (1999) have investigated the use of SPME coupled to GC with flame photometric detector (in the phosphorous mode) for the determination of seven OPPs in pears and apple or peach nectars. Low recoveries obtained for untreated samples could be improved (typically above 70%) by sample dilution making quantitation possible in the validated range of 25–250 ng/g.

The present work reports a SPME-GCMS method for the determination of height model organophosphorus pesticides (phorate, diazinon, methyl-parathion, fenitrothion, malathion, fenthion, ethyl-parathion and methidathion) in wine and different fruit juices (orange, grapefruit, lemon). It is demonstrated that recoveries are largely dependent on the sample matrix and for a given sample on the analyte. Quantitation limits for these pesticides (mainly insecticides) are below their maximum residues limits (MRLs) as regulated by the EC legislation. The use of MS detection in multiple ion monitoring mode (three fragments for each analyte) improves method selectivity.

2. Experimental

2.1. Materials

An organophosphorus mix containing phorate, diazinon, methyl-parathion, fenitrothion, malathion, fenthion, ethyl-parathion and methidathion (Dr. Ehrenstorfer, Augsburg) dissolved in methylene chloride (1 μg/ml) was prepared and stored in the dark at 4 °C. More diluted solutions were prepared just before use. Methylene chloride was obtained from Labscan (Dublin, Ireland). Other chemicals were analytical grade reagents.

2.2. Instrumentation

GC-MS analysis was performed by an HP 5890 series II gas chromatograph equipped with a HP 5890 GC split/splitless injector and interfaced, by a GC transfer line, to a VG Trio-2000 quadrupole mass spectrometer (VG BIOTECH, Altrincham, UK).

The GC chromatographic column consisted of a Supelco fused silica SPB-5 capillary column (length 30 m, i.d. 0.20 mm with 0.25 μm film thickness) connected to the split/splitless injector. The injector liner i.d. was 0.75 mm. The carrier gas was helium.

2.3. Chromatographic and MS detection conditions

The oven temperature program was: from 50 °C (2 min) to 190 °C at 30 °C/min; from 190 °C (5 min) to 200 °C at 2 °C/min; from 200 °C (4 min) to 250 °C (5 min) at 15 °C/min. A column head pressure of 15 psi and an injector temperature of 250 °C were used. The injector was operated in splitless mode with 2 min (sampling time). The GC transfer line was maintained at 270 °C. The mass spectrometer was operated in the electron impact positive ion (EI^+) mode with a source temperature of 200 °C. The electron energy was 70 eV and the filament current 200 μA.

Chromatograms were acquired in "scan" mode scanning the quadrupole from m/z 50 to m/z 350 (scan time: 0.9 s, inter-scan time: 0.1 s) or in multiple ion monitoring mode using a dwell time of 0.2 s and a mass span of 0.4 a.m.u. In order to improve peak identification, three fragment ions were monitored for each analyte as specified in the following: m/z 97, 121, <u>260</u> (phorate); 137, 179, <u>304</u> (diazinon); 109, 125, <u>263</u> (methyl-parathion); 109, 125, <u>277</u> (fenitrothion); 93, 127, <u>173</u> (malathion); 109, 169, <u>278</u> (fenthion); 97, 139, <u>291</u> (ethyl-parathion); 85, 93, <u>145</u> (methidathion). Quantitation was performed extracting, for each analyte, a single ion trace (see underlined m/z above) from the total ion current chromatogram obtained in multiple ion monitoring.

2.4. Solid-phase microextraction

A silica fiber coated with a 85 μm thick polyacrylate (PA) film and a manual SPME device (Supelco) was employed as described elsewhere (Zambonin et al., 2002). Standard solutions were prepared by spiking 5 ml of triply distilled water with the pesticide mix in CH_2Cl_2 into 7 ml clear vials (Supelco). The vials were then sealed with hole caps and Teflon-faced silicone septa (Supelco). Extraction was carried out at room temperature under magnetic stirring (in order to improve mass transfer from the aqueous sample into the fibre coating) for 30 min; signals higher than 70% of the equilibrium values were obtained. Thermal desorption (5 min desorption time) was performed directly into the GC injection port maintained at 250 °C.

2.5. Real samples

Wine samples and orange, grapefruit and lemon juices were purchased from a local store. Aliquots of 5 ml of wine samples were transferred into a 7 ml clear vial and directly subjected to SPME. Fruit juice samples were first centrifuged for 60 s at 5000 rpm and, when required, diluted with water; then a 5 ml aliquot was transferred into a 7 ml clear vial and subjected to SPME.

3. Results and discussion

The effect of the most important parameters (e.g., extraction time, temperature, pH, ionic strength) influencing the SPME extraction efficiency and the desorption conditions, has been already discussed elsewhere (Zambonin et al., 2002) and can be summarised as follows. Ionic strength: significant; pH: not significant (range explored from 4 to 11); extraction temperature: not significant (range explored from 20 to 80 °C); desorption temperature and time: significant (no carry over for 5 min desorption time at $T \geqslant 250$ °C); extraction time: significant (equilibrium reached at $t \geqslant 60$ min). As for the choice of the fibre, it was proved that with the exception of phorate the extraction efficiency of PA coating was better than that of PDMS coatings. PA fibers were then chosen for further investigations.

The fibre-solution distribution coefficients, K_{fs}, of each analyte have been calculated as the ratio between the concentration of the analyte in the fibre coating and in the solution following an approach already described by Fromberg et al. (1996). As it can be seen from Table 1, high $\log(K_{fs})$ values were obtained indicating the high affinity of the target analytes for the PA fibre. The K_{fs} values give also a measure of the preconcentration efficiency achievable by the SPME techniques and the consequent strong improvement of sensitivity.

Fig. 1 reports an SPME-GCMS chromatogram, acquired in multiple ion monitoring mode, relevant to a white wine sample spiked at 50 ng/ml level. Unspiked samples revealed the absence of OPPs residues naturally contaminating the analyzed samples as well as the absence of matrix components coeluting with the target analytes. This was a clear indication of the high selectivity of the employed technique. Average ($n = 3$) percent recoveries, calculated by spiking wine samples with an OPPs mix, as described in Section 2, are reported in Table 2. The high recoveries observed for the investigated analytes indicate a substantial absence of matrix effects.

Table 1
Fibre-solution distribution coefficients, K_{fs}, of the investigated pesticides (85 μm thick polyacrylate coated fibre)

Analyte	$\log(K_{fs})$
Diazinon	3.97
Ethyl-parathion	4.73
Fenitrothion	4.43
Fenthion	4.46
Malathion	3.58
Methidathion	4.09
Methyl-parathion	4.05
Phorate	4.06

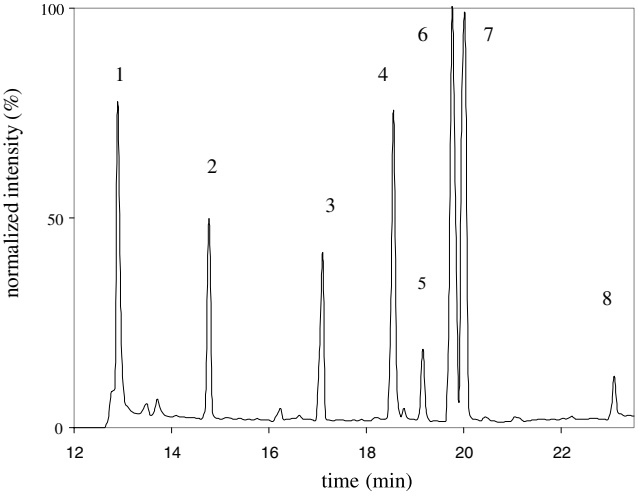

Fig. 1. SPME-GCMS chromatogram relevant to a spiked (50 ng/ml) wine sample. Acquisition in SIM mode as specified under Section 2. Elution order: (1) phorate, (2) diazinon, (3) methyl-parathion, (4) fenitrothion, (5) malathion, (6) fenthion, (7) ethyl-parathion and (8) methidathion.

Table 2
Average percent recovery ± standard deviation of the target organophosphorus pesticides from white wine samples spiked at 50 ng/ml level and analyzed in triplicate

Analyte	Percentage recoveries ± SD
Diazinon	67 ± 5
Ethyl-parathion	87 ± 4
Fenitrothion	87 ± 3
Fenthion	97 ± 6
Malathion	82 ± 5
Methidathion	87 ± 4
Methyl-parathion	90 ± 5
Phorate	100 ± 4

Calibration curves were constructed spiking wine samples with an OPPs mix in order to cover the range from 10 to 500 ng/ml; three replicates for each concentration were performed. Calibration curve resulted linear with correlation coefficients better than 0.996 and intercept not significantly different from zero at 95% confidence level.

As far as fruit juices are concerned, Fig. 2 shows an example of single-ion GC traces, extracted from the total ion current chromatogram acquired in multiple ion monitoring for a lemon juice sample spiked at 50 ng/ml level. Table 3 reports the average ($n = 3$) percent

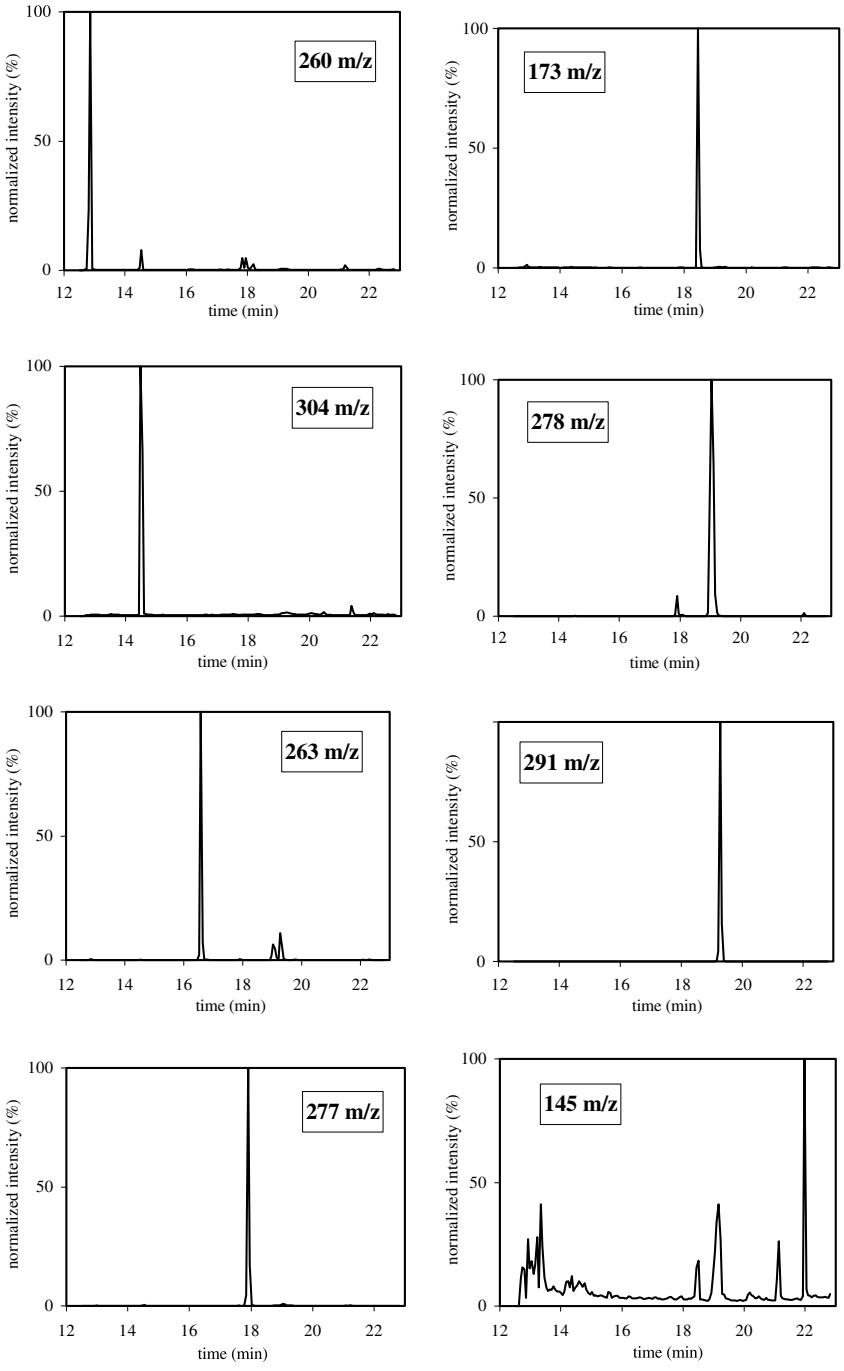

Fig. 2. Single-ion chromatographic traces relevant to a spiked lemon juice sample. m/z 260 (phorate), m/z 304 (diazinon), m/z 263 (methyl-parathion), m/z 277 (fenitrothion), m/z 173 (malathion), m/z 278 (fenthion), m/z 291 (ethyl-parathion) and m/z 145 (methidathion).

recoveries for orange, grapefruit and lemon juice samples spiked at 50 ng/ml level. As can be seen recoveries are generally lower than those obtained for wine samples. The extraction efficiency was found highly dependent on both the nature of the matrix and the particular analyte considered. For instance, passing from grapefruit to lemon juice the extraction yield of ethyl-parathion increased from 38% to 79% while that of fenthion decreased from 62% to 5%.

Simplicio and Vilas Boas (1999) have clearly shown that suspended matter as well as dissolved matter (in particular pectins) are the main factors responsible for a reduced extraction efficiency by forming micelles, adsorbing the analytes and/or slowing down their diffusion towards the fiber. In this respect, it should be pointed out that the use of headspace SPME which for solid sample (e.g., fruits) is the only possibility, is not completely free from inconvenience. As shown by Lambro-

Table 3
Average percent recovery ± standard deviation of the target organophosphorus from spiked orange, grapefruit and lemon juice samples

Analyte	Percentage recoveries ± SD		
	Orange	Grapefruit	Lemon
Diazinon	74 ± 5	30 ± 9	38 ± 8
Ethyl-parathion	60 ± 5	38 ± 9	79 ± 4
Fenitrothion	58 ± 6	44 ± 9	66 ± 5
Fenthion	39 ± 8	62 ± 6	5 ± 10
Malathion	21 ± 10	98 ± 4	63 ± 5
Methidathion	32 ± 8	28 ± 12	31 ± 9
Methyl-parathion	86 ± 4	50 ± 7	77 ± 4
Phorate	88 ± 4	53 ± 7	48 ± 7

Values are calculated on three replicate determinations performed at a concentration level of 50 ng/ml.

polou and Albanis (2003) for strawberries and cherries, recoveries are strongly dependent on the amount of water and organic solvent added to solid samples and of their ratio; recoveries could be increased by increasing the amount of methanol but less clean chromatograms were obtained.

In the presence of strong matrix effects in liquid samples (e.g., fruit juices as in the present case) direct SPME could still be used provided a sample dilution is performed. Assuming the pesticide distributed in the sample between a "free" form and a "bound" form with matrix components, a sample dilution should increase the recoveries as a consequence of the displacement of the equilibrium towards the free form of the pesticide due to a reduced matrix effect. This hypothesis is supported by data reported in Fig. 3 (lemon juice), where the percentage recoveries are plotted versus the dilution factor (recoveries were calculated by comparing the peak areas of the chromatograms relevant to the spiked lemon juice and an aqueous solution at the same OPPs concentration level, that was progressively subjected to the same dilution). As apparent, a dilution of 1:25 brought the recovery of fenthion from 5% to 65% and recoveries of the remaining pesticides to values comprised between 80% and 100%; a similar trend was also observed for other juices. This dilution factor was chosen as best compromise between increased extraction efficiency and loss of sensitivity, and was then adopted for further experiments on fruit juice samples.

Calibration curves resulted linear for all the investigated range (10–500 ng/ml) with correlation coefficients better than 0.992 and intercept not significantly different from zero at 95% confidence level.

Estimated limits of detection (LOD) and quantitation (LOQ) calculated (Long & Winefordner, 1983) as 3× and 10× the standard deviation of the intercept obtained by unweighted linear regression analysis are reported in Table 4 for each analyzed matrix and compared with the MRLs fixed by the European legislation (Council Directives 90/642/EEC, 1990). It should be stressed that these MRLs values are referred to fruits and not to derivate products (wine or juices); then they must be corrected for the dilution factor achieved during the preparation process (in this respect consider that analyzed juices contained ca. 50% of water). It is worth noting that LOQ levels are well below the MRLs even taking into account the dilution factor.

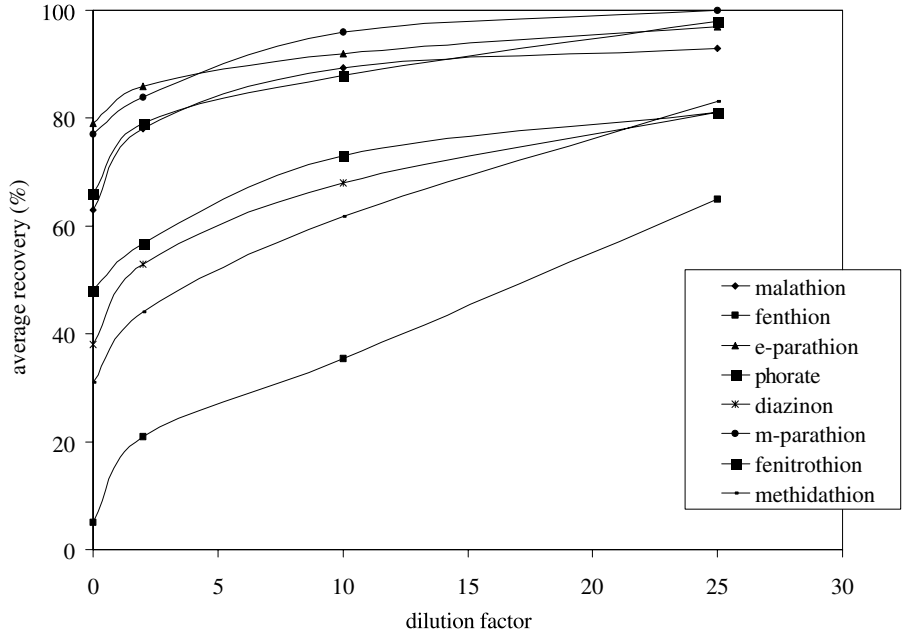

Fig. 3. Percentage recoveries plotted versus the dilution factor; recoveries were calculated by comparing the peak areas of the chromatograms relevant to a spiked lemon juice sample and a standard solution at the same OPPs concentration level, that were progressively subjected to the same dilution.

Table 4
Estimated limits of detection (LOD) and quantitation (LOQ) calculated as 3× and 10× the standard deviation of the intercept obtained by unweighted linear regression analysis for each analyzed matrix and compared with the maximum residue limits (MRLs) fixed by the European legislation

Analyte	LOD (ng/ml)				LOQ (ng/ml)				MRL (μg/g)	
	Wine	Orange	Grape-fruit	Lemon	Wine	Orange	Grape-fruit	Lemon	Citrus	Wine grapes
Diazinon	13	14	2	22	44	47	7	75	0.02	0.02
Ethyl-parathion	3	18	9	14	10	61	30	46	0.05	0.05
Fenitrothion	8	20	15	17	26	68	51	58	0.5	0.5
Fenthion	2	16	11	8	7	54	37	27	0.3	0.5
Malathion	28	42	62	65	96	145	215	225	2	0.5
Methidathion	33	50	80	90	109	165	264	297	2	0.5
Methyl-parathion	16	26	41	4	54	89	141	13	0.2	0.2
Phorate	7	10	12	16	23	33	40	54	0.05	0.05

4. Conclusions

The present SPME-GCMS procedure was successfully employed for the determination of organophosphorus pesticides in wine and fruit juices. It possesses the advantages of SPME (fast, simple, highly sensitive and solvent free) and could be potentially extended to other matrices. The obtained LOQ are almost always below the maximum residue limits (MRLs) fixed by the European legislation (Council Directives 90/642/EEC, 1990).

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