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Multi-residue methodology for pesticide screening in wines

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Abstract

A multi-residue solid-phase microextraction/gas chromatography/electron-capture detection method for pesticide screening (nine organochlorine, eleven organophosphorous and a tiadiazine insecticides, a benzoylurea acaricide and a halogenated sulfamide fungicide) in wine was developed. Fiber coating, extraction time and temperature, exposure of the fiber to the headspace or to the liquid phase and the ethanol effect on pesticides extraction were the parameters studied. The best results were obtained for a 100 μm poly(dimethylsiloxane) fiber, with 30 min immersion, in a 3 ml sample, at 45°C. Pesticides extraction yield from aqueous spiked solutions was studied, for different ethanol concentrations (9%, 12.5% and 20%, v/v), which are representative of the main types of Portuguese wines. Calibration curves for extracted standards gave linear responses for all the pesticides, except dichlorvos, mevinphos, disulfoton and methidathion. Average detection limits were lower than 5 ppb and no significant interference from the matrix was found in the conditions studied, except for buprofezin.

Keywords: Wine; Food analysis; Pesticides; Organochlorine compounds; Organophosphorous compounds

1. Introduction

Pesticide determination in food, and particularly in wine, has received much attention in the last few years, because the incorrect use of pesticides for crop protection may result in the presence of residues of these substances in wine, thus compromising the safety of this product [1–3]. On the other hand, this fact is sometimes associated with stuck and sluggish fermentations and several studies have shown that some compounds exhibit a negative effect on the growth and metabolism of yeasts [3,4].

It is currently accepted that the correct use of pesticides, particularly, the respect for dosages and pre-harvest interval, and the winemaking process definitely influence the decrease, and even the elimination of pesticide residues. In this case, pesticide levels in wine are expected to be much lower than the ones that can produce the problems pointed above [5].

In most of the countries there are no established maximum residue limits (MRLs) for pesticides in

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wines and OIV (Office International de la Vigne et du Vin) [6] has recently proposed MRLs in wine for some pesticides used on vine treatment.

Even the generally low concentrations expected for pesticides residues in wines justify the use of sensitive analytical methods, where often the extraction/concentration procedure is the limiting step.

Solid-phase microextraction (SPME) constitutes a convenient alternative to other commonly used extraction methods (purge and trap, liquid and solid-phase extractions, etc.) because sampling can be done rapidly, directly, without any solvent and can be easily automated [7]. SPME has gained widespread acceptance in many areas, in recent years, and has been applied for the determination of a wide spectrum of analytes for a large variety of matrices [8]. SPME application to extract pesticides from wine samples is still under development, because it is a recently introduced technique and these complex matrices may cause interference in the extraction procedure [9].

The potential of SPME for pesticide determination in wine has been investigated, particularly, the interference of the matrix and its alcoholic content [10,11], but a lot of information is still lacking for most of the compounds included in this work. Arthur et al. [12] and Eisert and Levsen [13], have studied the effect of methanol concentration on compounds extraction by SPME, and Batlle et al. [14] optimised SPME of pesticides in ethanol–water mixtures that are used as food simulants. They all conclude that the presence of organic solvents in the liquid matrices may act as a co-solvent for partitioning of pesticides in the phases involved.

In this study, a SPME–GC–electron capture detection (ECD) method was developed for 22 insecticides and acaricides (dichlorvos, mevinphos, diazinon, dimethoate, lindane, flufenoxuron, heptachlor, disulfoton, aldrin, parathion-methyl, malathion, endosulfan, DDE, dieldrin, methidathion, buprofezin, endrin, DDD, ethion, DDT, azinphos-ethyl, phosalone) and a fungicide (dichlofluanid). This method was based on two previous works [10,11].

Special attention was paid to the pesticide extraction yield from aqueous spiked solutions, of different ethanol concentrations (9%, 12.5% and 20%, v/v), which are representative of the main types of Portuguese wines.

2. Experimental

2.1. Materials

Pesticide analytical standards used were obtained from PolyScience (Niles, IL, USA): lindane (LIN), heptachlor (HEP), aldrin (ALD), dieldrin (DIE), endrin (END), endossulfan (ENS), DDE, DDD, DDT, malathion (MAL), diazinon (DIA), dichlorvos (DIC), mevinphos E (MEV), dimethoate (DIM), parathion-methyl (PAR), azinphos-methyl (AZI), phosalone (ZOL), disulfoton (DIS), ethion (ETI), and from Dr. Ehrenstorfer (Augsburg, Germany): methidathion (MET), buprofezin (BUP), flufenoxuron (FLU) and dichlofluanid (DIF). Pesticides were used without further purification (degrees of purity were>95%, for all pesticides, except for DDD (70%) and mevinphos E (90%)).

For the preparation of standard stock solutions, ethanol LiChrosolv (Merck, Darmstadt, Germany) was used. Working solutions of pesticides were prepared daily with deionised and bidistillate water.

Seven Portuguese red wine and five white wine samples were analysed.

2.2. Apparatus and chromatography

Gas chromatographic analyses were performed with an HP5890 gas chromatograph equipped with a ^{63}Ni electron-capture detector and a split/splitless injector. The column used was a HP-PAS 1701 capillary column [low/mid polarity, 14% (cyanopropylphenyl)methylpolysiloxane] (25 m×0.32 mm I.D., 25μm film). The split/splitless injector and detector temperatures were set at 250°C and 300°C, respectively. Both carrier and make-up gases were argon–methane (95:5), at 1.35 ml/min and 50.8 ml/min, respectively. The initial oven temperature was kept at 80°C for 2 min, which was increased to 212°C at 40°C/min, held for 7 min, and then raised to 252°C at 6°C/min and kept for 1 min. The temperature was finally increased to 280°C, at 5°C/min, held for 2 min. The total run time was 27.56 min. Injection volume was 1 μl, when direct injections were made. Chromatographic data were recorded in a Chromatography Data Station for Windows (CSW 1.7) software (DataApex, Prague, Czech Republic). Pesticide quantification was per-

formed by external standard method. Initially, the detector's linearity for direct injection of ethanolic standard solutions was checked and detector calibration curves were obtained.

2.3. SPME procedure

A SPME fiber holder for manual use, and 7 μm and 100 μm poly(dimethylsiloxane) (PDMS) fibers were obtained from Supelco. Fibers' conditioning was performed according to supplier's information. During extraction, a Corning stirrer/hot plate (Supelco) was used to heat and agitate samples, using a magnetic stirrer (4 ml vials were filled with 3 ml samples). After absorption, the fiber was inserted into the GC injector for 3 min, in splitless mode, at 250°C.

Individual solutions of about 1 g/l of each pesticide were prepared in ethanol. A stock standard solution containing all the pesticides (~10 mg/l in organophosphorous insecticides, BUP, DIF and FLU and ~1 mg/l in organochlorine insecticides) was also prepared in ethanol. SPME calibration curves were obtained by extracting the compounds from different aqueous solutions [0, 9, 12.5 and 20% (v/v) ethanol aqueous solutions], with concentrations of approximately 1, 5, 10, 25 and 50 μg/l in each analyte, in the case of organophosphorous compounds BUP, DIF and FLU, and about ten times lower for organochlorines insecticides. Peak areas of the extracted standards were plotted against the initial standard concentration to obtain calibration curves. The SPME extraction yield was determined as the ratio between the extracted amount (calculated from calibration curves of standards directly injected) and the initial concentration of the standards.

3. Results and discussion

3.1. Gas chromatographic determination

The identification of the compounds was done by comparison of the retention times with those obtained for standard solutions directly injected into the gas chromatograph (Fig. 1). All pesticides studied were injected separately and, under the conditions used, there were no co-eluting compounds. If a pesticide is identified in the sample, standard addition must be performed to avoid matrix interference. Nevertheless, it should be stressed that positive cases in wine samples have to be confirmed by SPME–GC–MS or, for some of the pesticides, by HPLC–diode array detection (DAD).

The ECD response is linear over the range studied (~1–10 mg/l for organophosphorous, BUP, DIF and

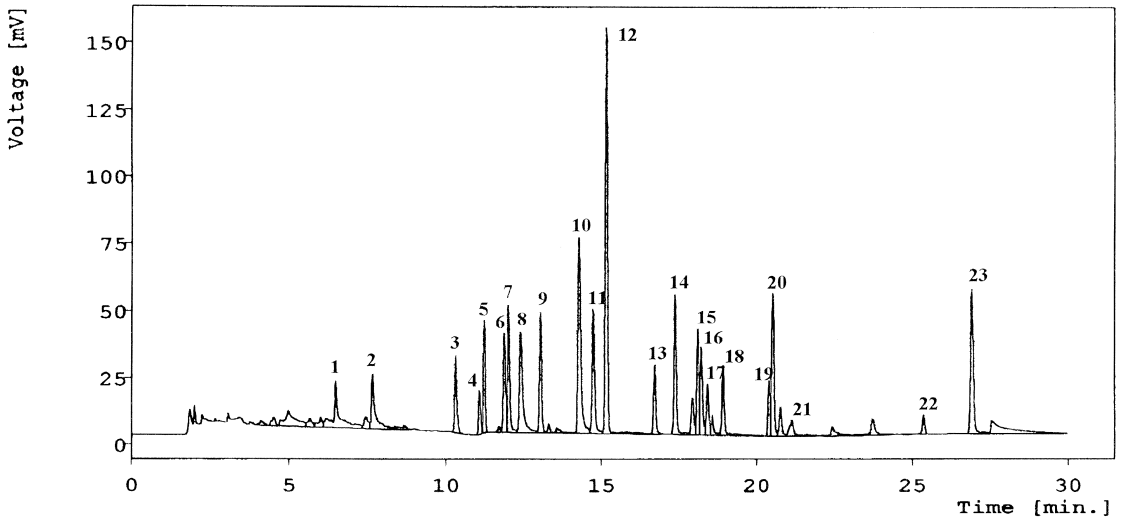

Fig. 1. GC–ECD chromatogram of a standard solution ~1 mg/l in organophosphorous insecticides, BUP, DIF and FLU and ~0.1 mg/l in organochlorine insecticides (1 μl, direct injection). For peak identification, see Table 1.

FLU and ~0.1–1 mg/l for organochlorine insecticides). Table 1 shows compounds retention times as well as other validation parameters of the SPME–GC–ECD method. It should be noticed that the response factor of ECD is considerably higher for organochlorines than for organophosphorous.

The total run time is 27.56 min. The intermediate precision for direct injection of standards was 10%, on average, expressed by the relative standard deviation obtained for 8 independent analyses (same method and operator, same equipment, in different days) of a standard solution ~1 mg/l in organophosphorous, BUP, DIF and FLU and ~0.1 mg/l in organochlorine insecticides (16.1% maximum for phosalone and 2.8% minimum for DDE). The repeatability (same method and operator, same equipment, within the same day) was 8.4%, on average, calculated for three replicates, with a maximum of 16% azinphos-ethyl and a minimum of 2.6% for DDE).

3.2. Method development

Optimal conditions for SPME were studied using either ethanol–water or wine spiked samples ~20 μg/l in organophosphorous insecticides, BUP, DIF and FLU, and ~2 μg/l in organochlorine insecticides. Parameters studied were fiber coating, immersion versus headspace sampling, temperature effect and extraction time, and ethanol effect.

3.2.1. Fiber selection

A preliminary and qualitative assay was performed in order to select one of the two available fibers: 7 μm and 100 μm PDMS. As expected, the 100 μm PDMS fiber exhibits better results for all the compounds (Table 2). Dichlorvos, mevinphos and disulfoton could not be detected in the extracted standards. Methidathion could not be quantified because separation from dieldrin was not complete. Also dieldrin has a response factor, both for ex-

Table 1
Validation parameters of the SPME–GC–ECD methodology

Number in Fig. 1	Compound	t_r (min)	Linear range (μg/l)	Limit of detection (μg/l)	Repeatability RSD (%) (n=5)	Intermediate precision RSD (%) (n=5)	Recovery[a] (%)
1	DIC	6.47	–	–	–	–	–
2	MEV	7.65	–	–	–	–	–
3	DIA	10.32	1.0–49.8	6.84	4.5	6.0	86
4	DIM	11.09	1.1–53.9	5.26	6.1	10.6	94
5	LIN	11.24	0.1–5.05	0.30	6.0	10.3	113
6	FLU	11.88	1.0–50.4	13.0	4.6	7.9	54
7	HEP	12.01	0.1–6.60	1.42	11.0	15.1	85
8	DIS	12.40	–	–	–	–	–
9	ALD	13.05	0.1–5.15	1.30	18.3	19.7	102
10	PAR	14.30	1.0–50.6	6.45	2.3	9.6	119
11	MAL	14.75	1.0–51.0	5.63	0.2	6.0	95
12	DIF	15.17	1.0–49.7	2.83	2.6	8.1	76
13	ENS	16.73	0.1–5.2	0.60	9.1	14.6	62
14	DDE	17.39	0.12–6.00	1.28	5.1	10.3	79
15	DIE	18.12	0.11–5.25	0.46	8.3	17.7	60
16	MET	18.22	–	–	–	–	–
17	BUP	18.43	2.0–99.2	9.66	20.1	18.4	14
18	END	18.93	0.13–6.70	0.60	12.3	16.0	71
19	DDD	20.43	0.11–5.25	0.76	8.0	11.8	63
20	ETI	20.54	0.50–24.85	2.40	16.3	16.6	60
21	DDT	20.79	0.11–5.40	0.61	12.4	9.9	78
22	AZI	25.37	1.0–51.0	9.65	10.3	8.4	69
23	ZOL	26.93	1.0–51.6	6.12	3.3	7.6	145

[a] Spiked ethanol–water solution (12.5%, v/v, ethanol) ~10 μg/l in organophosphorous insecticides, BUP, DIF and FLU, and ~1 μg/l in organochlorine insecticides.

Table 2
Variation of peak areas with fiber coating (30 min immersion in a 3 ml spiked wine sample ~20 μg/l in organophosphorous insecticides, BUP, DIF and FLU, and ~2 μg/l in organochlorine insecticides, at 20°C)

Pesticide	Peak area (mV s)	
	7 μm PDMS	100 μm PDMS
Dichlorvos	317	343
Mevinphos E	390	522
Diazinon	–	398
Dimethoate	148	1908
Lindane	581	8489
Flufenoxuron	995	3439
Heptachlor	9447	11 915
Disulfoton	31	645
Aldrin	8579	10 622
Parathion-methyl	108	2131
Malathion	59	698
Dichlofluanid	77	2814
Endossulfan	8131	11 437
DDE	8905	10 303
Dieldrin	9346	11 898
Methidathion	–	–
Buprofezin	4032	7016
Endrin	9644	12 155
DDD	11 801	14 914
Ethion	2595	9294
DDT	7338	8390
Azinphos-methyl	1369	7761
Phosalone	160	5287

traction and chromatographic processes, much higher than methidathion.

3.2.2. Immersion versus headspace

The SPME procedure has been applied both to liquid (immersion) and to the vapour in equilibrium with it (headspace sampling). The last one is preferable when samples exhibit undissolved particles or low volatile compounds, which may interfere, either in the chromatographic analysis or by saturating the fiber. As wine matrix is very complex, sampling the headspace could be an interesting alternative that was investigated. The results obtained using the 100 μm PDMS fiber (30 min extraction of a 3 ml spiked wine sample ~ 20 μg/l in organophosphorous insecticides, BUP, DIF and FLU, and 2 μg/l in organochlorine insecticides, at 45°C) reveal that, with the exception of DIC, MEV, DIS and MET, for which the response was null in both cases, the headspace sampling provides a significantly lower extraction than the immersion.

Similar results are obtained when ethanol–water solutions 12.5% (v/v) spiked at the same level are used, even for different temperatures (20°C and 62°C).

3.2.3. Temperature effects

Considering that immersion was preferred over the headspace sampling, different extraction temperatures were tested in order to compare the effect of this parameter on extraction yields.

The temperature effect, which influences the partitioning constants between the two phases (liquid phase and the fiber), is not the same for all the pesticides (Table 3). Considering that the extraction was improved for 11 of the 19 pesticides studied, the temperature of 45°C was chosen for the subsequent analyses.

3.2.4. Extraction time

Different extraction times were studied, at the conditions described in Fig. 2. Ethion was the

Table 3
Variation of peak areas with temperature (100 μm PDMS fiber, 30 min immersion in a 3 ml spiked wine sample ~50 μg/l in organophosphorous insecticides, BUP, DIF and FLU, and ~5 μg/l in organochlorine insecticides, at 20°C and at 45°C)

Pesticide	20°C	45°C
Diazinon	951	637
Dimethoate	1102	979
Lindane	1529	1031
Flufenoxuron	2222	2699
Heptachlor	4921	6663
Aldrin	3318	5270
Parathion-methyl	956	532
Malathion	425	245
Dichlofluanid	4476	2297
Endossulfan	2951	3426
DDE	2782	4781
Dieldrin	4183	5015
Buprofezin	610	872
Endrin	3564	4314
DDD	1337	2052
Ethion	5504	6114
DDT	955	883
Azinphos-methyl	281	333
Phosalone	1007	835

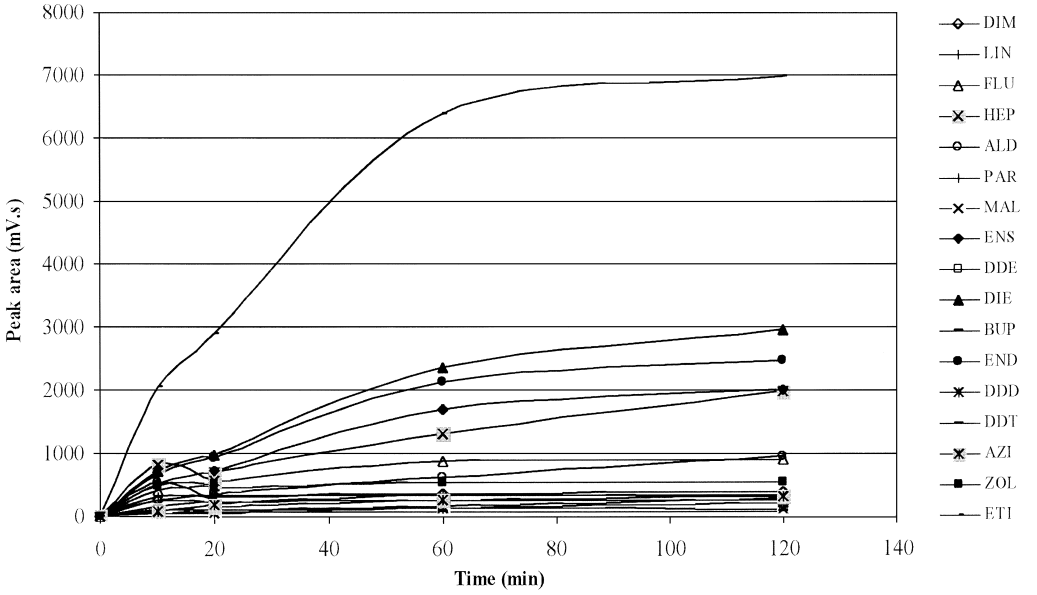

Fig. 2. Variation of peak areas with extraction time (100 μm PDMS fiber, 30 min immersion in a 3 ml spiked wine sample ~20 μg/l in organophosphorous insecticides, BUP, DIF and FLU, and ~2 μg/l in organochlorine insecticides, at 45°C).

pesticide for which the effect of the extraction time is markedly significant, when time exceeds 30 min. A group of five other pesticides, all of them organochlorine insecticides (DIE, END, HEP, ENS and ALD) also need higher extraction times to reach equilibrium. Considering a compromise between the duration of the analysis and the time of the extraction and considering also that those pesticides are not the most currently applied to vines, the extraction time of 30 min was chosen for subsequent analyses.

3.2.5. Effect of ethanol content

Previous results in literature describe the influence of ethanol on the efficiency of the SPME method [10,11]. For some of the pesticides studied, as phosalone, it is referred by Montury et al. [10] that their behaviour toward this extraction is not the same in the 10% ethanol water solution, in a red wine or in a white wine. It is considered that other constituents, as sugar, tartarates, phenols, etc., interfere with the extraction.

In this study, at the extraction conditions defined in Table 4, the ethanol effect was compared for ethanol–water solutions at 0%, 9%, 12.5% and 20% (v/v) ethanol. The amount of each pesticide extracted is generally lower than 30%, even for water

Table 4
Variation of the extraction yield (%) with the ethanol content (v/v) of standard aqueous solutions (100 μm PDMS fiber, 30 min immersion in a 3 ml spiked wine sample ~10 μg/l in organophosphorous insecticides, BUP, DIF and FLU, and ~1 μg/l in organochlorine insecticides, at 45°C)

Pesticide	Extraction yield (%)			
	Ethanol content (%, v/v)			
	0	9	12.5	20
Diazinon	12	6.9	5.1	2.9
Dimethoate	16	9.9	8.8	4.7
Lindane	6.9	3.9	3.1	1.7
Flufenoxuron	7.9	13	18	9.4
Heptachlor	13	16	25	17
Aldrin	7.6	12	17	15
Parathion-methyl	2.5	1.2	1.1	0.5
Malathion	2.5	1.2	1.1	0.5
Dichlofluanid	8.2	3.4	2.7	0.8
Endossulfan	23	19	23	12
DDE	5.9	10	13	10
Dieldrin	22	22	25	14
Buprofezin	11	11	12	8.3
Endrin	22	16	22	10
DDD	7.4	13	19	11
Ethion	12	16	19	9.8
DDT	12	12	12	5.3
Azinphos-methyl	8.1	15	13	5.0
Phosalone	7.5	1.8	3.4	0.1

solutions. A consistent decrease in the extraction yield (%) was observed with increasing ethanol content for seven of the pesticides, namely, DIA, DIM, LIN, PAR, MAL, DIF and DDT. On the other hand, an increase in the extraction yield (%) with increasing ethanol content, particularly in the range 0–12.5% (v/v), was also observed for other seven pesticides (FLU, HEP, ALD, DDE, BUP, DDD and ETI) (Table 4). This conclusion reveals the necessity of taking into account the percentage of ethanol in each wine analysed, in order to obtain the calibration curve with standards at the same ethanol content or correct the response for each compound studied.

3.2.6. Matrix effects assessment

Considering other matrix effects than the ethanol content, a red and a white wine were previously analysed by this method and no traces of the selected pesticides were detectable. New samples of the same wines were spiked, extracted in duplicate and linearity of response to increasing added amounts verified (Fig. 3). Good linearity was obtained ($r^2 \geq 0.99$ for most of the compounds). Recovery (%) was calculated, for the samples ~10 μg/l in organophosphorous insecticides, BUP, DIF and FLU, and ~1 μg/l in organochlorine insecticides, dividing the amount extracted (g), obtained from the calibration curve of the extracted spiked wine, by the amount extracted (g) obtained from the calibration curve of the ethanol–aqueous solution 12.5% (v/v), similar to the ethanol content of the wine samples used (Table 5).

Table 5
Comparison between recovery obtained in red wine and white wine samples spiked with pesticide standards ~10 μg/l in organophosphorous insecticides, BUP, DIF and FLU, and ~1 μg/l in organochlorine insecticides (100 μm PDMS fiber, 30 min immersion in a 3 ml sample, at 45°C)

Pesticide	Recovery (%)	
	Red wine	White wine
Diazinon	93	79
Dimethoate	107	81
Lindane	121	104
Flufenoxuron	72	36
Heptachlor	101	70
Aldrin	118	85
Parathion-methyl	121	117
Malathion	108	83
Dichlofluanid	76	75
Endossulfan	74	49
DDE	80	79
Dieldrin	73	48
Buprofezin	17	12
Endrin	87	55
DDD	79	47
Ethion	77	42
DDT	96	59
Azinphos-methyl	67	72
Phosalone	143	148

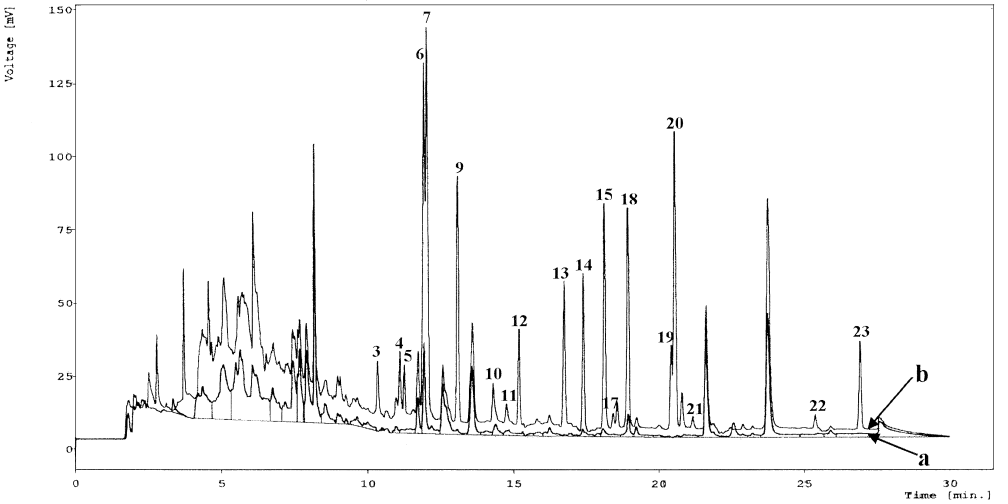

Fig. 3. GC–ECD chromatograms after SPME extraction of (a) a red wine sample and (b) the corresponding spiked wine ~5 μg/l in organophosphorous insecticides, BUP, DIF and FLU and ~0.5 μg/l in organochlorine insecticides. For peak identification, see Table 1.

The worse results were obtained for buprofezin (14%) and for phosalone (145%) indicating that some matrix effect may occur for these compounds.

Linearity of response to increasing added amounts of each pesticide to red and white wine proves that there is no saturation of the fiber for the concentration range studied. Fibers could be used for over than 100 injections, when analysing aqueous solutions and over than 50 times when analysing wine samples. This represents a real advantage over solid-phase extractions, considering that the later is much more time and reagent consuming (expensive residue grade solvents).

3.3. Validation of the analytical method

According to the method development described above, the following analytical conditions were adopted: use of a 100 μm PDMS fiber, 30 min of immersion, at 45°C, in a 3 ml sample.

Under these conditions, the intermediate precision (RSD) was 8.5%, on average calculated for five replicate extractions of a standard solution (12.5% ethanol) ~10 μg/l in organophosphorous insecticides, BUP, DIF and FLU and ~ 1 μg/l in organochlorine insecticides. The reproducibility was 11.5%, on average, expressed by the RSD obtained for five independent analyses of the same standard solution (with a maximum of 19.7% for aldrin and a minimum of 6.0% for diazinon and malathion).

3.4. Application to wine samples

Twelve wine samples were analysed (seven Portuguese red wine and five white wine samples) but any trace of the pesticides studied was detected. This fact points out one of the advantages of this screening methodology, that is the use of a simpler, less expensive and quicker technique than GC–MS. Even considering that, at the level of the detection limits, *noise* may interfere with results, it always compensates than the use of GC–MS applied to all the samples to be analysed.

4. Conclusions

The improved analytical methodology by SPME–GC–ECD enables the detection of 19 from the 23 pesticides studied in wine samples. Dichlorvos, mevinphos, disulfoton and methidation could not be efficiently extracted with the procedure adopted – 100 μm PDMS fiber, immersion during 30 min at 45°C. Total analysis time is 60 min (30 min for extraction plus 28 min for chromatography).

It is concluded that ethanol content of samples affects the extraction yield and calibration curves with extracted standards are recommended.

Although the extraction yield did not exceed 30%, the detection limits obtained (around 5 μg/l, on average) are considerably below the recommended maximum levels for these pesticides in wine samples. This methodology has the advantage of being easily implemented in a wine quality control laboratory, as a screening method. However it should be noticed that positive cases should be confirmed by a complementary detection method, or the analysis performed by GC–MS.

Future work to be done includes the study of other matrix effects than the ethanol content.

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