Development of a SPME-GC-ECD methodology for selected pesticides in must and wine samples

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Abstract
A method for the determination of some pesti- cide residues in must and wine samples was developed us- ing solid-phase microextraction (SPME) and gas chromatography – electron capture detection (GC/ECD). The procedure only needs dilution as sample pre-treatment and is therefore simple, fast and solvent-free. Eight fungicides (vinclozolin, procymidone, iprodione, penconazole, fenarimol, folpet, nuarimol and hexaconazole), one insecticide (chlorpyriphos) and two acaricides (bromopropylate and tetradifon) can be quantified. Good linearity was observed for all the compounds in the range 5–100 μg/L. The reproducibility of the measurements was found acceptable (with RSD's below 20%). Detection limits of 11 μg/L, on average, are sufficiently below the proposed maximum residue limits (MRL's) for these compounds in wine. The analytical method was applied to the determination of these compounds in Portuguese must and wine samples from the Demarcated Region of Alentejo, where any residues could be detected.

Introduction

One of the major fields in analytical chemistry is the development of faster and easier methodologies for characterization and quantification of trace compounds in complex matrices. A special attention is given to substances that can compromise food safety, such as pesticides.

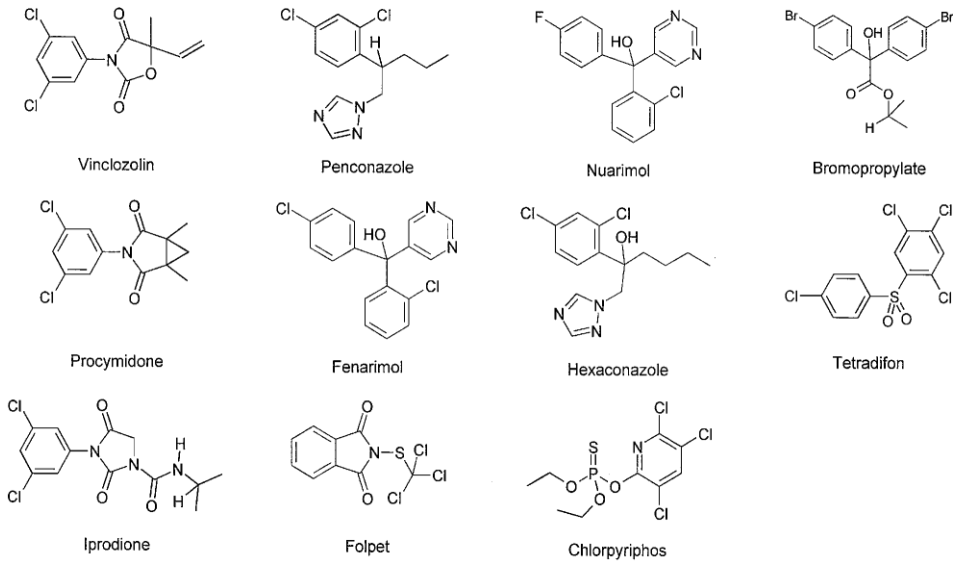
Pesticide contamination of wines has been assessed in numerous works [1–3], with different analytical methodologies being proposed, for many active substances. The contamination may arise because during the wine making process pesticides can eventually be transferred from treated grapes to the must, where they can remain or be transformed. It is currently accepted that the correct use of pesticides, particularly, the observation for dosages and pre-harvest interval, as well as the winemaking process definitely influence the decrease, and even the elimination of pesticide residues [4]. The generally low concentrations expected for pesticide residues in wines make necessary the use of sensitive analytical methods, where often the extraction/clean-up/concentration procedure is the limiting step. Most analytical methods in the literature involve extraction of pesticide residues by liquid-liquid [5, 6] or solid–phase extraction [5] and are usually tedious, time consuming and make use of environmental "unfriendly" organic solvents.

Solid-phase microextraction (SPME) constitutes a convenient alternative to other commonly used extraction methods because sampling can be done rapidly, directly, without any solvent and can be easily automated [7]. This technique has gained widespread acceptance, and has been successfully applied for the determination of a wide spectrum of analytes in a large variety of matrices [8].

The potential of solid-phase micro-extraction for pesticide determination in wine has been investigated, particularly, the interference of the matrix and its alcoholic content [3, 9, 10]. The presence of organic solvents in the liquid matrices (as ethanol in wines) may act as a co-solvent for partitioning of pesticides in the phases involved. This could be critical when comparing results obtained from samples with different ethanol contents, as in the case of this study. In order to minimize ethanol and other interference arising from matrix compounds [11], dilution of samples was performed prior to extraction.

A SPME-GC-ECD methodology was developed for eight fungicides (vinclozolin, procymidone, iprodione, penconazole, fenarimol, folpet, nuarimol and hexaconazole), one insecticide (chlorpyriphos) and two acaricides (bromopropylate and tetradifon) and was applied to Portuguese must and wine samples. The chemical structures of these

Fig. 1 Chemical structure of the studied compounds



pesticides are presented in Fig. 1. Total analysis time is around 60 min (30 min for extraction and 30 min for chromatographic separation). The detection limits of the method (11 μg/L, on average) are bellow the maximum residue limits proposed for these compounds in wine samples.

Experimental

Reagents and pesticides stock solutions

Pesticide analytical standards used were obtained from Dr. Ehrenstorfer (Augsburg, Germany): vinclozolin, chlorpyriphos, penconazole, folpet, procymidone, hexaconazole, nuarimol, bromopropylate, iprodione, tetradifon, and fenarimol. Pesticides were used without further purification (degrees of purity were > 95%, for all pesticides).

Individual standard stock solutions of about 1 g/L of each pesticide were prepared by exact weighing and dissolution in ethanol (LiChrosolv, Merck, Darmstadt, Germany). A stock standard solution containing all the pesticides ~20 mg/L was also prepared in ethanol. Working solutions of pesticides were prepared daily by appropriate dilutions with deionized and bidistillate water.

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 DB 1701 (J&W Scientific, Folsom, USA) capillary column (30 m × 0.32 mm i.d. ×25 μm 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 1.36 mL/min and 50.7 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 for7 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 10 °C/min, held for 8 min. The total run time was 30.56 min.

The detector's linearity for direct injection (1 μL) of ethanolic standard solutions was checked and detector calibration curves were obtained. Chromatographic data were recorded in a Chromatography Data Station for Windows (CSW 1.7) software (DataApex, Ltd, Prague, The Czech Republic). Pesticide quantification was performed by external standard method.

SPME procedure

A SPME fiber holder for manual use and 100 μm PDMS (polydimethylsiloxane) 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 agitate samples, using a magnetic stirrer (4 mL vials were filled with 3 mL samples). After absorption (20 °C), the fiber was inserted into the GC injector for 3 min, in splitless mode, at 250 °C.

SPME calibration curves were obtained by extracting the compounds from different spiked solutions (ethanol-water 12.5%(v/v), red and white wines and must samples), after dilution with water (1:50). Peak areas of the extracted standards were plotted against the initial solution concentration (approximately 5, 10, 20, 50 and 100 μg/L in each analyte, before dilution). SPME extraction yield was determined as the ratio between the extracted amount (calculated from calibration curves of standards directly injected) and the initial amount present in the standards.

Results and discussion

Method development

Optimal conditions for SPME were studied using either ethanol-water or wine spiked samples ~20 μg/L. Parameters studied were immersion vs. headspace sampling, salt addition and extraction time. Immersion was preferred against headspace sampling (10 mL diluted sample ~20 μg/L in a 15 mL vial; 6 g/L NaCl; 45 °C) because the last provided a significantly lower extraction (almost null for most of the analytes) than immersion.

By the nature of the SPME methodology, based on partition equilibrium between phases, recovery of the analytes from the samples is normally far from 100%. Dilution of samples may improve the extraction yield results by lowering the concentration of interfering compounds (adsorption of analytes, forming micelles and/or making it difficult for analytes to reach the fiber) [11] and/or by lowering the concentration of the co-solvent. Standard solutions with different ethanol contents were diluted at different ratios (5, 10, 25 and 50 X) and the extraction yield was found to improve with dilution of the matrix. Dilution

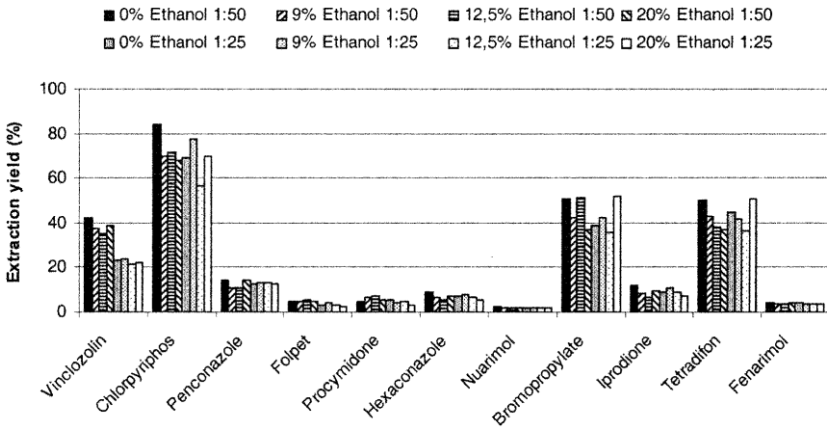


Fig. 2 Effect of dilution on the extraction yield of SPME for aqueous solutions with different ethanol concentrations (% (v/v)) (spiking level ~20 μg/L). For each compound, extraction yield was calculated by dividing the amount extracted (ng), obtained from the detector's calibration curve (for direct injection), by the initial amount (ng) present in the sample

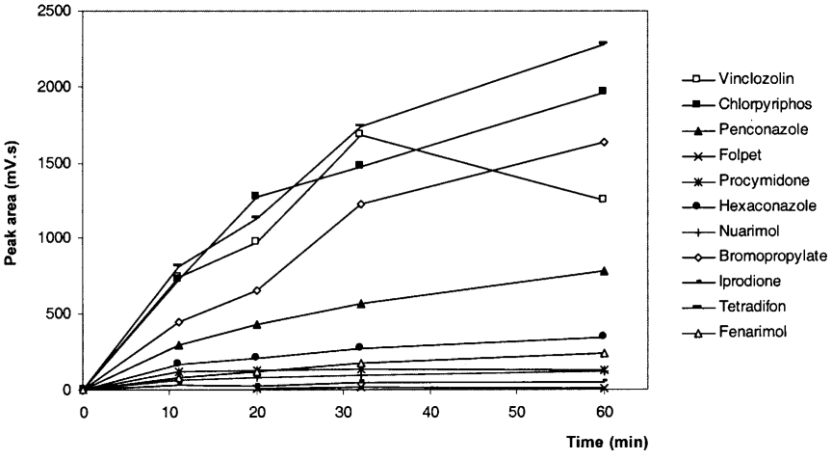


Fig. 3 Variation of peak areas with extraction time (100 μm PDMS fiber, immersion in a 3 mL spiked wine sample ~20 μg/L, diluted 1:50, at 20 °C)

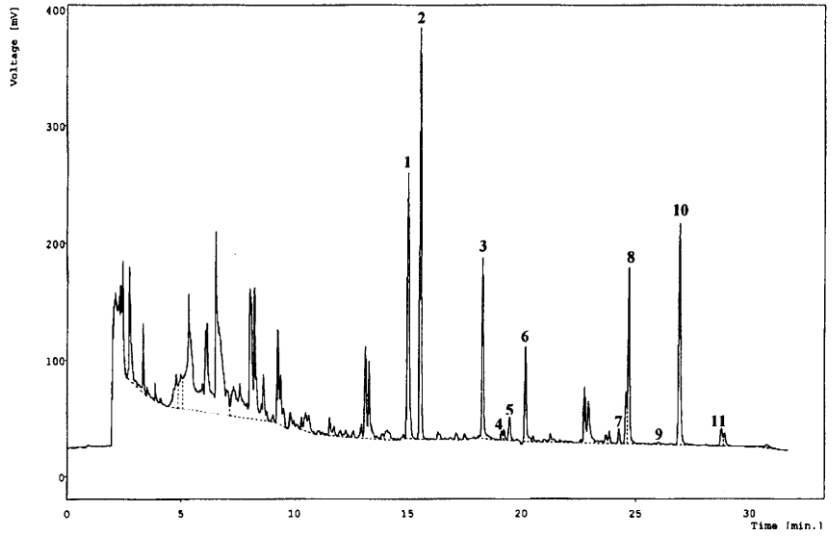


Fig. 4 GC-ECD chromatogram after SPME extraction of a spiked red wine sample ~20 μg/L (dilution 1:50; 100 μm PDMS fiber; 30 min immersion; 20°C)

had the advantage of making possible to obtain similar responses from samples with different ethanol content, as is shown in Fig. 2.

Different extraction times were studied, at the conditions described in Fig. 3. For tetradifon, chlorpyriphos, bromopropylate and penconazole equilibrium is reached for extraction times higher than 60 min; for the other pesticides equilibrium is reached after about 30 min. Considering this and a compromise between the duration of the analysis and the time of extraction, the extraction time of 30 min was chosen for subsequent analyses.

The ionic strength was modified by the addition of NaCl 6 g/L. Salt addition had a positive effect on the extraction of pesticides from ethanol:water standard solu-

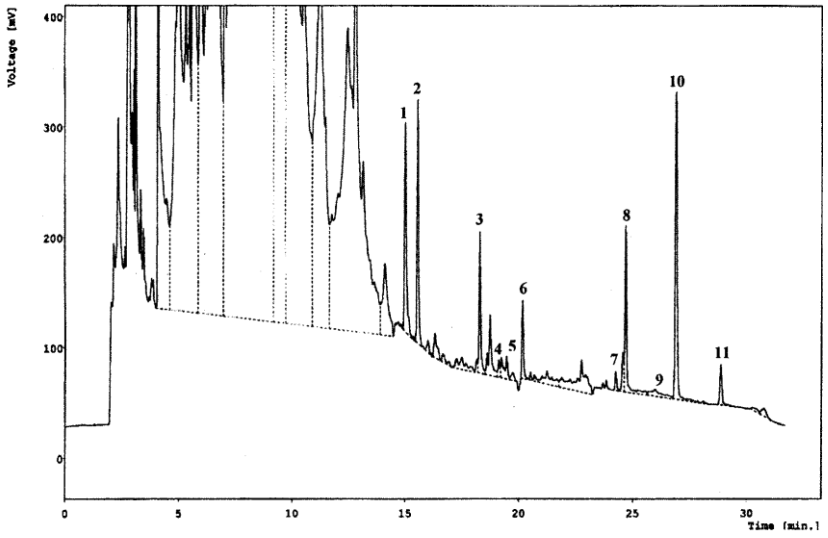


Fig. 5 GC-ECD chromatogram after SPME extraction of a spiked red must sample ~20 μg/L, first day of fermentation (dilution 1:50; 100 μm PDMS fiber; 30 min immersion; 20 °C)

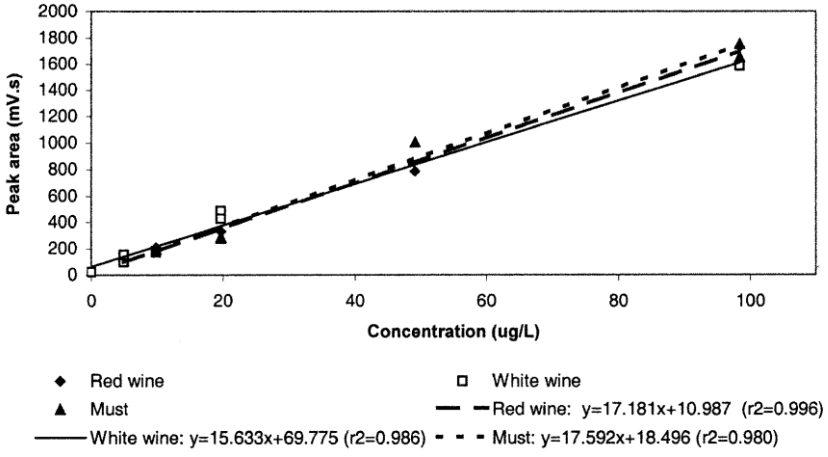


Fig. 6 Relation between the SPME/GC-ECD response and hexaconazole spike level for wines and a red must (dilution 1:50; 100 μm PDMS fiber; 30 min immersion; 20 °C)

Table 1 Validation parameters of the SPME-GC-ECD methodology. Good linearity was observed for all compounds in the range 5–100 μg/L

Number in Figs.	Analyte	t_R (min)	Limit of quantification (μg/L)	Intermediate precision RSD (%) (n = 5)	Reproducibility RSD (%) (n = 5)	Extraction yield (%)
1	Vinclozolin	14.95	17.8	15.6	19.67	24.9
2	Chorpyriphos	15.51	15.8	9.92	12.64	44.6
3	Penconazole	18.25	10.1	8.50	10.1	27.8
4	Folpet	19.09	9.96	1.86	4.20	4.21
5	Procymidone	19.43	12.06	9.88	13.41	5.08
6	Hexaconazole	20.13	10.16	8.81	10.7	17.0
7	Nuarimol	24.23	12.02	1.49	4.86	3.19
8	Bromopropylate	24.65	9.35	6.17	11.4	20.3
9	Iprodione	25.65	9.52	15.74	13.58	1.37
10	Tetradifon	26.85	10.14	8.60	9.30	33.4
11	Fenarimol	28.82	9.17	1.58		4.02

tions and from musts, with an increase in the detector's of about 20% on average, but had a negative effect in the case of wines and it was decided not to perform salt addition. Preliminary studies were conducted in order to compare the 100 μm PDMS and 85 μm PA fibers. The results obtained indicate that the polyacrilate fiber has a slightly higher performance than the 100 μm PDMS fiber for extracting the analytes from the wine matrix, but the relative standard deviations were higher in the first case, and the 100 μm PDMS fiber was chosen for subsequent analyses.

Figures 4 and 5 show the GC-ECD chromatograms obtained for the extraction of spiked wine and must samples.

Calibration curves were obtained by spiking different must and wine samples with 5, 10, 20, 50 and 100 μg/L of each pesticide and applying the analytical procedure described. Response is linear for all compounds in the range 5–100 μg/L. A similar response is obtained for diluted samples of the different matrixes (Fig. 6). Table 1 summarizes the validation parameters obtained for this methodology.

Real sample analysis

Ten white and fifteen red wines from the Demarcated Alentejo Region (South Portugal) were analyzed by the proposed method. The samples were filtered prior to extraction. Centrifuged samples from the fermentation of three different red grape varieties were also analyzed. To assess the validity of the method for the quantification of the pesticide residues during the fermentation (where not only the ethanol content but also other changes occur in the composition of the must) samples from successive fermentation days were spiked with the same standard solution to obtain a final concentration of 20 μg/L. The differences found in the detector's response are within the precision of the method. The pesticides included in this study were not detected in the real samples analyzed.

Conclusions

The proposed method is appropriate for the determination of the selected pesticide residues in must and wine samples, with a minimum pre-treatment of samples (filtration/centrifugation and water dilution). Dilution of samples improves the extraction yield results possibly by lowering the concentration of the co-solvent, making possible to obtain similar responses from samples with different ethanol content. Although extraction yields are not very high they are acceptable considering the precision of the method. Detection limits (11 μg/L, on average) are bellow the proposed maximum residue limits, for most of the compounds and others and can be improved, for example, by using a higher volume of diluted sample, if it is applicable.

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