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Solid-phase microextraction–gas chromatographic–mass spectrometric method for the determination of the fungicides cyprodinil and fludioxonil in white wines

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Abstract

A solid-phase microextraction (SPME) followed by a gas chromatographic–mass spectrometric (GC–MS) determination has been developed and validated for the determination of cyprodinil and fludioxonil in white wine samples. Extraction parameters such as the selection of SPME coating, the effect of the temperature, the effect of the headspace volume and the salt addition were studied and optimized, together with GC–MS analytical conditions. The divinylbenzene–Carboxen–polydimethylsiloxane (DVB–CAR–PDMS) fiber was the most appropriate for the determination of the two pesticides in wine. The quality parameters of the proposed method demonstrated a good precision (RSD about 5%), with detection limits of 0.1 and 0.2 μg/l for cyprodinil and fludioxonil, respectively. Fifteen commercial white wine samples produced in Rías Baixas area in Galicia (N.W. Spain) were analyzed with the SPME–GC–MS procedure. Some of the commercial wines (75%) presented the two pesticides in concentrations ranging from 0.9 to 28.6 μg/l. In conclusion, SPME–GC–MS has a great potential for fungicide determination in wines. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Wine; Solid-phase microextraction; Cyprodinil; Fludioxonil; Pesticides

1. Introduction

Two new fungicides, cyprodinil and fludioxonil, have recently been used to control gray mold (*Botrytis cinerea*) in grapes for wine production, due to their high level of activity against this fungus [1]. Cyprodinil is an anilinopyrimidine (Fig. 1a); its mechanism of action is based on the inhibition of methionine biosynthesis [2]. Fludioxonil (Fig. 1b), a non-systemic fungicide, is a phenylpyrrole which affects the transport processes in the plasmatic membrane [3].

Although the correct use of pesticides does not cause problems of public concern in health and environmental areas, if inappropriate abusive treatments are applied without respecting safety recommendations, undesirable residues can remain on grapes after harvest. These residues may be taken into the wine producing important negative consequences, such as a decrease in the quality of wine because of the production of off-flavors [4,5], the production of stuck and sluggish fermentation, and problems in malolactic fermentation [6]. Nevertheless others authors pointed that yeasts can decrease

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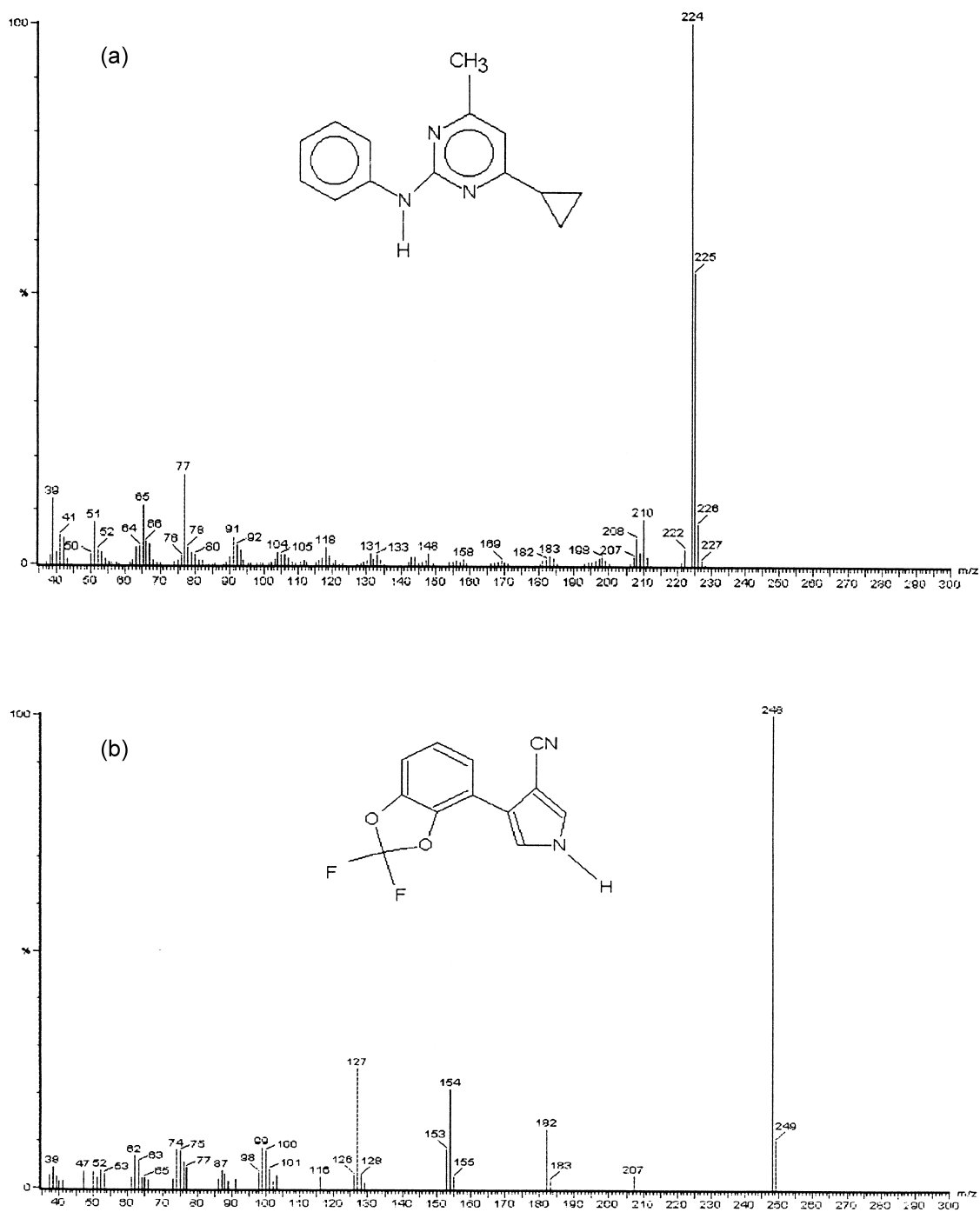*E-mail address:* jsimal@uvigo.es (J. Simal Gándara).

Fig. 1. Chemical structures and EI mass spectra for cyprodinil (a) and fludioxonil (b).

the amount of pesticides by degradation and adsorption processes [7–12]. In any case, the presence of fungicides in wines is a matter of public health concern.

The 93/58/ECC European Directive has established maximum residue limits (MRLs) for cyprodinil (2 mg/kg) and fludioxonil (1 mg/kg) in viniferous grapes [13]. No MRLs have been established in wines but the Office International de la Vigne et du Vin (OIV, France) has recently proposed MRLs in wine for some pesticides used on vine treatment [14] taking into account that levels are known to decrease considerably during vinification [15,16].

Analytical methods for determining pesticide residues in wine include commonly gas chromatography (GC) [17–21] or high-performance liquid chromatography (HPLC) [22–29]. An efficient clean-up of wine samples is always necessary before the chromatographic analysis using liquid–liquid extraction (LLE) with non-polar solvents [17,18,21,22,30] and solid-phase extraction with C_8 [27], C_{18} [19,23] or XAD-2 [20] cartridges. These sample pre-treatments are tedious and increase the risk of sample loss and contamination. Much attention has been paid to the development of tandem liquid chromatography (LC–LC) [31], LC–GC [32,33] and other automated sample pre-treatment methods. Experimental works found in literature related to cyprodinil and fludioxonil proposed LLE with acetone–light petroleum or dichloromethane followed by GC–nitrogen–phosphorus detection (NPD) [34] or HPLC–UV [35], respectively.

Solid-phase microextraction (SPME), the extraction technique developed by Pawliszyn and co-workers [36–39], has become popular for the analysis of organic compounds because it combines sampling and pre-concentration in a single step. It requires no solvents or complicated apparatus and provides good results over a wide range of analyte concentrations. SPME coupled with GC and electron-capture detection (ECD), NPD or mass spectrometry (MS) has been applied to the analysis of herbicides [40], pesticides [41–44] and polychlorinated biphenyls [45]. SPME was also applied for the determination of organic compounds in wine samples such as thiols, sulfides and disulfides [46].

The applicability of SPME followed by GC–MS was assessed for the determination of cyprodinil and fludioxonil residues in wines. Six commercial SPME fibers were considered; the parameters affecting the adsorption of these two compounds onto the fiber (temperature, headspace volume, salt addition and sampling time) and the desorption process (time and temperature of GC injector) were evaluated and optimized by analysis of spiked synthetic wines (a matrix as similar as possible to the real white wine samples analyzed). Once the SPME procedure was optimized, quality parameters of the method such as precision, linearity, and detection limits were evaluated with the spiked wines. Finally, 15 commercial white wines produced in Rías Baixas area (Galicia, N.W. Spain) were analyzed in order to assess the performance of the method with real samples and to screen the presence of these fungicides.

2. Experimental

2.1. Chemicals, solvents and disposables

Cyprodinil and fludioxonil pestanal grade from Riedel-de Haën (Seelze, Germany) were used without further purification (degrees of purity were >99.9% for both pesticides). Lindane from Aldrich (Steinheim, Germany), with a purity of 97%, was used as internal standard (I.S.).

Other reagents used were ethyl acetate suprasolv grade from Merck (Darmstadt, Germany); methanol for organic trace analysis, sulfur dioxide solution (94.5%), succinic acid (99.5%) and magnesium sulfate heptahydrate (99%) from Fluka (Steinheim, Germany); DL-(±)-malic acid (99%) and L-(±)-tartaric acid (99%) from Riedel-de Haën; ethanol, acetone, acetic acid, D-(+)-glucose (99%), D-(−)-fructose (99%), citric acid (99%) and sodium hydroxide (98%) from Panreac (Barcelona, Spain). Ultrapure water was from a Milli-Q water purification system (Millipore, USA).

In this study six SPME fibers were considered: 7 μm polydimethylsiloxane (7-PDMS), 85 μm polyacrylate (PA), 65 μm polydimethylsiloxane–divinylbenzene (PDMS–DVB), 65 μm Carbowax–divinylbenzene (CW–DVB), 65 μm Carboxen–polydimethylsiloxane (CAR–PDMS) and 50/30 μm Stable Flex divinylbenzene–Carboxen–polydimethylsilox-

ane (DVB–CAR–PDMS). The commercially available SPME device and fibers were purchased from Supelco (Bellefonte, PA, USA). Fibers were initially conditioned according to the manufacturer's instructions in order to remove contaminants and to stabilize the polymeric phase. Conditioning was carried out in an extra injection port (split open) with helium carrier gas prior to each extraction.

For the SPME, wine samples were placed in 40-ml EPA vials (Wheaton, USA) equipped with stir bars and sealed with PTFE-faced silicone septum, and stirred with a magnetic stirrer (Raypa, Spain).

2.2. Standard solutions and synthetic wine preparation

A stock standard solution (ca. 1000 mg/l) of each fungicide was prepared in methanol by weighing approximately 0.025 g of the analyte into a 25-ml volumetric flask and diluting to volume. An intermediary standard solution was prepared by dilution in ethyl acetate of the stock standard solutions to give a concentration of 100 mg/l of both fungicides. Stock and intermediary standard solutions of the internal standard were prepared in the same way. All standard solutions were stored in the dark at 4°C.

Working standard solutions for further studies were prepared by spiking different volumes of the intermediary standard solution in a synthetic wine solution in order to obtain a matrix as similar as possible to real white wine samples, and these samples were always spiked with internal standard. The synthetic wine solution was prepared as follows: ethanol (120 ml), L-(+)-tartaric acid (2.5 g), DL-(±)-malic acid (4.0 g), citric acid (0.5 g), succinic acid (1.0 g), acetic acid (0.3 g), D-(+)-glucose (1.0 g), D-(−)-fructose (1.0 g) and sulfur dioxide (0.07 g) were made-up in ultrapure water (1 l) and, finally, the pH value was adjusted to 3.2 with 5 M NaOH solution. This synthetic wine presented the mean concentrations of the major characteristic compounds of the Rías Baixas white wines according to the manufacturers.

2.3. SPME procedure

Commercial or synthetic wine samples (30 ml) were placed into 40-ml EPA glass vials equipped with PTFE-coated magnetic bars, and capped with a PTFE-faced silicone septum. To each sample lindane was added as an internal standard (3 μl of the intermediary solution of 100 mg/l). The holder needle was inserted through the septum and the fiber was directly immersed in the sample solution for 30 min under magnetic stirring at room temperature (22°C). Magnetic stirring facilitates mass transport of the analyte between wine and fiber, reducing equilibration times. After extraction, the fiber was withdrawn into the holder needle, removed from the vial and immediately introduced into the GC injector port for 5 min at 240°C for thermal desorption.

2.4. Gas chromatography–mass spectrometry system and conditions

A Fisons (Rodano, Italy) GC 8000 series gas chromatograph equipped with a mass-selective detector MD 800 was used for GC analysis. Chromatographic separations were performed using a Supelco MDN-5S (30 m×0.25 mm I.D.) fused-silica capillary column with 5% diphenyl–95% dimethylsiloxane liquid phase (0.25 μm film thickness). The oven temperature was programmed as follows: 50°C for 1 min, ramped at 10°C/min to 275°C and held for 10 min. A split/splitless injector was used in the splitless mode (5 min). Carrier gas was helium with a column head pressure of 100 KPa. Injector temperature was 240°C, and transfer line temperature was 275°C.

MS detection was performed in single ion monitoring (SIM) mode, the ion energy used for the electron impact (EI) was 70 eV; selected ions (m/z) used for the quantitation of each fungicide and the internal standard were: 225±1 for cyprodinil; 248±1.5 for fludioxonil; and 183±2 for lindane. Confirmation of fungicides in commercial wines was performed by direct comparison between the full mass spectral scans of the reference sample and the standard recorded within the same analytical conditions.

3. Results and discussion

3.1. Method optimization

3.1.1. SPME
In order to develop the SPME described method

for cyprodinil and fludioxonil extraction in wine samples, several parameters such as selection of SPME coating, effect of temperature and salt addition, sample volume, extraction time and desorption conditions were studied. Direct SPME was selected rather than headspace SPME due to the high polarity of analytes studied and their affinity to the wine matrix.

3.1.1.1. Selection of SPME fiber

The choice of an appropriate fiber is essential for the establishment of an SPME method and it is dependent of the chemical nature of the target analytes (polarity and volatility) [47]. PDMS coating presents a non-polar phase which efficiently extracts non-polar analytes. PA phase is suitable for more polar compounds. In mixed phases (CAR–PDMS, CW–DVB, PDMS–DVB and DVB–CAR–PDMS), porous carbon (CAR) or microspheres of the DVB polymer are immobilized onto the fiber by using either PDMS or CW coating.

Six SPME fiber coatings were evaluated to select the most appropriate for the method. A fortified synthetic wine sample (40 ml spiked at level of 5 μg/l with each fungicide) was analyzed twice with each fiber. Previously to extraction process, magnesium sulfate (3 g) was added. The extraction time was 30 min at room temperature for all fibers. The desorption time was 3 min (splitless mode) at 240°C for all fibers. The fiber was immersed for 10 min in a vial containing deionized water in order to remove small amounts of adhering salt from the coating prior other extraction process.

Areas obtained for each fungicide with the different fibers are shown in Table 1. The PDMS fiber (a non-polar phase) is recommended in the literature

Table 1
Extraction efficiencies of commercial SPME fiber coatings for sampling fungicides

Fiber coating	Peak area counts[a] ($\cdot 10^3$)	
	Cyprodinil	Fludioxonil
7 μm PDMS	26	4
85 μm PA	10	47
65 μm CAR–PDMS	403	84
65 μm CW–DVB	214	1061
65 μm PDMS–DVB	622	1594
50/30 μm DVB–CAR–PDMS	906	1915

[a] (n=2) mean of determinations.

because it is a rugged liquid coating able to withstand high injector temperatures, up to 300°C [47]; however, in this case resulted to be the worst performance coating due to its low capacity to extract analytes. Poor results were also obtained with PA fiber. The mixed SPME phase coatings have complementary properties compared with the last two fibers and showed better results for both fungicides. DVB–CAR–PDMS was found to be the most effective due to the presence of the two adsorbents DVB and CAR and it was selected for the method optimization.

3.1.1.2. Effect of temperature

The extraction temperature has two opposing effects on the SPME technique. An increase of temperature reduces the coating/sample distribution constant of the analyte when the adsorption is an exothermic process, or it can enhance the sorption kinetics of analytes [47].

To evaluate the effect of temperature, a fortified synthetic wine sample (40 ml spiked at a level of 5 μg/l with each fungicide) was analyzed twice with the DVB–CAR–PDMS fiber under the extraction and desorption conditions described above. It was observed that the extraction efficiency was lower at 50°C, specially for fludioxonil. Then, further experiments were performed at room temperature (22°C).

3.1.1.3. Effect of headspace volume

To optimize the extraction procedure of the two fungicide compounds, the effect of the relationship between the synthetic wine volume and the gaseous phase or headspace volume was studied. This experiment was performed using EPA 40-ml vials and increasing the synthetic wine volume of a fortified synthetic wine sample (spiked at 200 ng for each fungicide) from 30 to 40 ml. Samples were analyzed twice with the DVB–CAR–PDMS fiber. The extraction time was 30 min at room temperature; the desorption time was 3 min (splitless mode) at 240°C.

SPME theory dictates that high extraction can be achieved when no volume of the headspace exist in order to avoid the loss of analytes [47]. Nevertheless, a significant increase in the extraction yield (%) was observed with the presence of a headspace volume. Some authors have reported the negative influence of ethanol, one of the major constituents of wine, on the pesticide extraction efficiency [14,48]. The observed

behavior could be explained by the displacement of ethanol to the gaseous phase during the extraction process due to its high volatility; in this way, ethanol competition to be sorbed into the coating could decrease respect to cyprodinil and fludioxonil. Further experiments were performed using 30 ml of sample.

3.1.1.4. Effect of salt addition

The addition of salt into the samples can modify the extraction efficiency, because the partition coefficients are partially determined by matrix–analyte–fiber interactions [47]. The effect of increasing

the ionic strength of the sample was determined with samples containing no salt, 7.5 and 15% (w/v) of magnesium sulfate.

Results showed that the amount of compounds extracted decreased when the salt concentration increased. The best results were obtained when no $MgSO_4$ was added; these data are in good agreement with those of Mestres et al. [49] who reported the possibility of formation of a thin layer of salt around the fiber, which decreases the extraction efficiency.

3.1.1.5. Sorption and desorption time profiles

The extraction of cyprodinil and fludioxonil with the DVB–CAR–PDMS fiber is an adsorption process due to the presence of DVB and CAR sorbents in the fiber. The sorption time profile for the selected fiber was obtained by plotting the detector response versus the extraction time for each fungicide in order to obtain the partition equilibrium curve (Fig. 2a). Synthetic wine samples spiked at 5 μg/l were analyzed at experimental conditions described in the SPME procedure.

Sorption time profiles indicated that a sampling time higher than 120 min is necessary to reach the equilibrium for both compounds. The sorption time can be shortened by working in non-equilibrium conditions. Ai [50,51] proposed a dynamic model of SPME adsorption, indicating that the amount of analyte adsorbed from the sample onto the fiber is proportional to the initial concentration in the sample matrix, if the agitation and the sampling time are held constants amongst samples. According to Ai, SPME quantitation is feasible at non-equilibrium conditions. Thus, considering a compromise between the extraction time and the chromatographic analysis time, an extraction time of 30 min was selected for further experiments.

Temperature of GC injector and desorption time were tested in order to guarantee the complete desorption of fungicides and to avoid carryover. For the DVB–CAR–PDMS fiber, temperatures ranging between 230 and 260°C were tested. High desorption temperatures can enhance the process but they can also degrade analytes. Desorption at 230°C was not capable of desorbing completely the analytes; they were completely removed from the coating at 240–260°C and no significant differences were observed within this range of temperatures. 240°C was select-
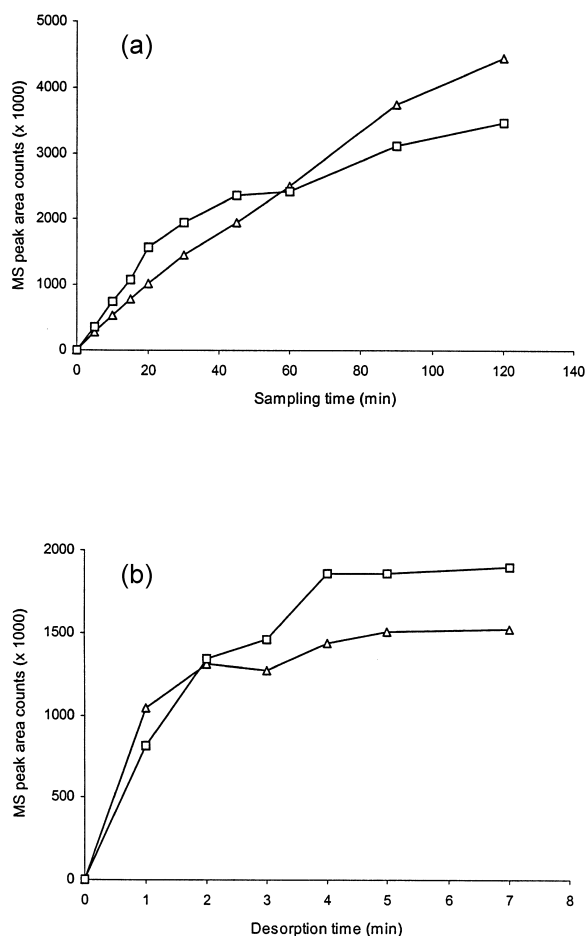

Fig. 2. Sorption time profiles (a) and desorption time profiles (b) for cyprodinil (△) and fludioxonil (□) by direct-SPME using the DVB–CAR–PDMS fiber. Synthetic wine samples (30 ml) containing both fungicides (5 μg/l of each compound).

ed due to high temperatures can shorten the coating lifetime and can result in the bleeding of the polymer, causing problems in separation and quantitation [47].

Desorption profiles of cyprodinil and fludioxonil were obtained by plotting the detector response versus different desorption times (1, 2, 3, 4, 5 and 7 min). Desorption profiles showed that a 4-min period was sufficient to desorb both fungicides in the GC injector port (Fig. 2b); therefore a 5-min period was chosen to guarantee a reproducible desorption. When chromatographic analysis was completed, the fiber was immediately thermally desorbed again at the same conditions to determine carryover; no peaks corresponding to cyprodinil and fludioxonil were registered.

3.1.2. GC–MS

Direct comparison between the full mass spectral scans of a reference standard and the sample recorded within the same analytical conditions represents the highest level of positive identification obtainable by MS. Fig. 1a and b present the mass of both fungicides. However, the full mass spectral scans are not usually used for quantitation in residue analysis due to the low specificity and sensitivity obtained. To increase selectivity and sensitivity, SIM offers a convenient method for ignoring potential interferences by concentrating efforts on specific ions that belong to the compound under investigation. This fact has been manifested by comparison of the low response observed in full scan mode (tested at 0.5 mg/l for both fungicides) vs. SIM mode (tested at 5 μg/l for both fungicides).

The confirmation of the identity of fungicides in commercial wines was carried out using the 125–250 m/z full scan mode by comparison of full mass

spectral scans with a reference standard analyzed under the same instrumental conditions.

3.2. Method performance

With the selected conditions for the SPME procedure, quality parameters of the SPME–GC–MS method such as linearity, limits of detection and quantitation, and precision were calculated.

The linearity of the method was evaluated by plotting calibration lines of the analyte area relative to that of the internal standard (lindane) vs. the analyte concentration. Linear ranges and determination coefficients (r^2) obtained for each fungicide are given in Table 2. The 10-point calibration line was found to have good linearity with a r^2 higher than 0.99. The loss of linearity observed at higher concentrations can be justified due to overloading of the SPME fiber capacity.

Limits of detection and quantitation were evaluated on the basis of the signal obtained with the analysis of unfortified synthetic wine samples ($n=7$), following the recommendations of the American Chemical Society [52]. As tested experimentally detection and quantitation limits were 0.1 and 0.2 μg/l, respectively, for cyprodinil; 0.9 and 2.0 μg/l, respectively, for fludioxonil (Table 2).

The repeatability and reproducibility of the SPME–GC–MS method were assessed by analyzing seven spiked synthetic wine samples on the same day ($n=7$) and a total of three samples per day along 2 different weeks ($n=9$), respectively. All samples were spiked at a concentration of 5 μg/l of each fungicide (fungicides and internal standard). Results are reported in Table 2. The relative standard deviation (RSD) for repeatability was about 5% for both compounds; and for reproducibility, ranged from 6 to

Table 2
Repeatability, reproducibility, linear dynamic ranges, determination coefficients (r^2), limits of detection (LODs) and limits of quantitation (LOQs) of the optimized method using the DVB–CAR–PDMS fiber

Fungicide	Repeatability[a] (relative recovery (%)±RSD)	Reproducibility[b] (relative recovery, (%)±RSD)	Linearity range[c] (μg/l)	Determination coefficient (r^2)	LOD[a] (μg/l)	LOQ[a] (μg/l)
Cyprodinil	100±5.0	100±5.9	0.5–37	0.999	0.1	0.2
Fludioxonil	100±5.2	100±9.9	2.5–35	0.995	0.9	2.0

[a] ($n=7$) mean of determinations.
[b] ($n=9$) mean of determinations.
[c] ($n=10$).

10%. These values allow to confirm the good precision of the method. Relative recoveries are of about 100% (Table 2) because real samples and standard-spiked real samples are processed in the same way.

3.3. Matrix effects assessment

The SPME–GC–MS method was applied to the determination of cyprodinil and fludioxonil by spiking samples of synthetic and commercial Rías Baixas wines free of the selected fungicide traces as found by previous analysis. Duplicate samples of synthetic and commercial wines spiked at 5 μg/l for each fungicide were analyzed. Results for wines are given in Table 3. Quantitation was performed using the calibration line for each compound with lindane as internal standard.

Significant differences were found between results obtained for the different matrices. These differences can be attributed to matrix effects and were also detected by other authors [14,53]. As an important fact, it was observed that matrix effects for both fungicides varied as a function of the commercial wine origin. As a conclusion, the standard addition method was used in the quantitation process in order to avoid matrix effects. Therefore, it is not necessary the estimation of an accuracy or recovery figure; this would be a relative recovery of about 100% (Table 2) because real samples and standard-spiked real samples are processed in the same way.

Standard addition method was applied as follows. Wine samples were directly analyzed twice and subsequently two standard additions of cyprodinil and fludioxonil were performed into the wine at levels of 8 and 10 μg/l for further analysis. The four-point calibration equation was calculated in order to estimate the fungicide concentrations in the

wine as well as the corresponding error according to Miller and Miller [54]. Unspiked wines with high fungicide levels were previously diluted with HPLC water with an ethanolic content of 12% (v/v) as recommended in the literature [14,46].

3.4. Analysis of commercial wine samples

Galician white wines with VQPRD (Vino di Qualitá Prodotto in Regione Determinata) or denominación de origen certifications Rías Baixas have been studied. Five different growing districts, named and numbered in Fig. 3 as O Rosal (1), Val do Salnés (2), Condado do Tea (3), Ribeira do Ulla (4) and Soutomaior (5), compose the total producing Rías Baixas area.

The most important and prestigious variety of grape in the area is Albariño, although there are other high quality authorized grapes such as Treixadura, Loureira Blanca, Caiño Blanco, Torrontés and Godello. The majority of white wines produced in all the districts are albariño wines (100% single variety), but wines from each district can be produced by blending albariño with the authorised grapes cited above. As a result, in O Rosal, the minimum is 70% albariño blended with loureira with the remaining percentage from the authorized varietals produced in this district. In Val do Salnés and Ribeira do Ulla, the minimum is 70% albariño and the rest from the authorized varietals. In Condado do Tea, the minimum is 70% albariño blended with treixadura with the remaining percentage from the authorized varietals produced in this district. In Soutomaior, for the time being, white wines are always 100% albariño. These Rías Baixas white wines are characterized by a very rich mosaic of qualities including a straw-colored or yellowish hue with golden and greenish iridescence, strong fruity and floral bouquet, average

Table 3
Measured concentrations and standard deviations of cyprodinil and fludioxonil in spiked wines and commercial Rias Baixas white wines at 5 μg/l of each fungicide determined by direct-SPME–GC–MS in order to assess matrix effects

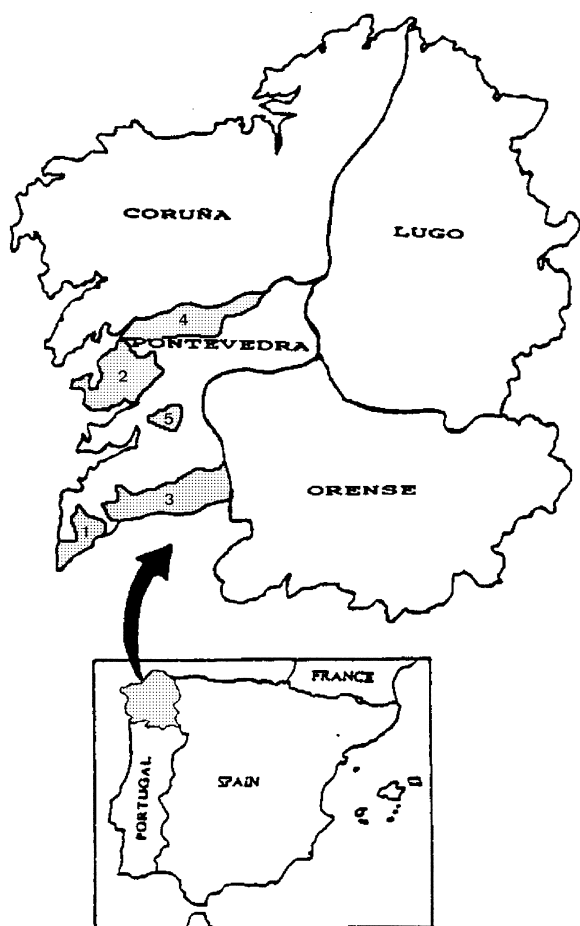|	Wine sample concentration (μg/l)±SD			
	Synthetic wine	O Rosal	Ribeira do Ulla	Val do Salnés
Cyprodinil	5.2±0.2	9.8±0.1	7.3±0.1	8.1±0.2
Fludioxonil	5.0±0.1	5.2±0.1	3.2±0.02	6.9±0.04

(n=2) mean of determinations.

Fig. 3. Location of five growing districts which compose the VQPRD of Rias Baixas: (1) O Rosal, (2) Val do Salnés, (3) Condado do Tea, (4) Ribeira do Ulla and (5) Soutomaior.

alcohol content of 12 degrees proof, well-balanced acidity and a youthful quality which, in this wine, is a definite asset.

In this work, 15 commercial Rías Baixas wines were analyzed with the proposed SPME–GC–MS method described above (Fig. 4). Three white wines (A–C) were produced in O Rosal, six white wines (D–I) in Val do Salnés, three white wines (J–L) in Condado do Tea, two white wines (M–N) in Ribeira do Ulla and, finally, one white wine (O) in Soutomaior. The viniferous grapes were treated with the commercial formulation Switch (37.5% cyprodinil and 25% fludioxonil).

The results obtained have contributed to the

monitoring of these fungicides in Spanish wines due to the lack of investigation of this subject. As shown in Table 4, the most of wine samples analyzed presented the two monitored fungicides, independently of the growing district considered. The levels found for cyprodinil in wines ranged between 0.9 and 24.9 µg/l; and for fludioxonil, ranged between 2.4 and 28.6 µg/l. These concentrations are lower respect to the maxima concentrations established in wines in Swiss legislation: 0.5 mg/l for cyprodinil and fludioxonil, separately [55]. Although cyprodinil percentage in the formulation is higher than fludioxonil percentage, concentrations determined in wines were in the same order of magnitude or even a little higher for the fludioxonil fungicide. This fact was also observed by Cabras et al. [1] which evaluated these fungicide residues on grapes and their decay rates after application to vines, as well as residues from vine to wine. Cyprodinil decay rate in grapes was quicker showing a half-life of 12 days meanwhile fludioxonil has a half-life of 24 days [1].

Cyprodynil and fludioxonil residues in wines determined by other authors are in the same order. Scarponi and Martinetti determined by LLE–HPLC–UV these residues in white and rose wines from Italy at levels of 30 µg/l for cyprodinil and 34 µg/l for fludioxonil [35]. Cabras et al. found cyprodinil residues which varied between 0.70 and 0.20 mg/kg in wines elaborated with grapes collected on the same day of the treatment and 28 days after, respectively; variation of fludioxonil residues in wines in the same conditions was 0.6 and <0.23 mg/kg, respectively [34].

4. Conclusions

The developed SPME–GC–MS method is suitable for monitoring cyprodinil and fludioxonil fungicides in white wine samples. The DVB–CAR–PDMS fiber was found to be the most appropriate to extract both analytes quantitatively. Non-equilibrium conditions were adopted in order to reduce the total extraction time. A headspace volume was left in order to minimize interference effects, probably due to ethanol. The method has good linearity, good precision and accuracy, and is highly sensitive. Matrix effects
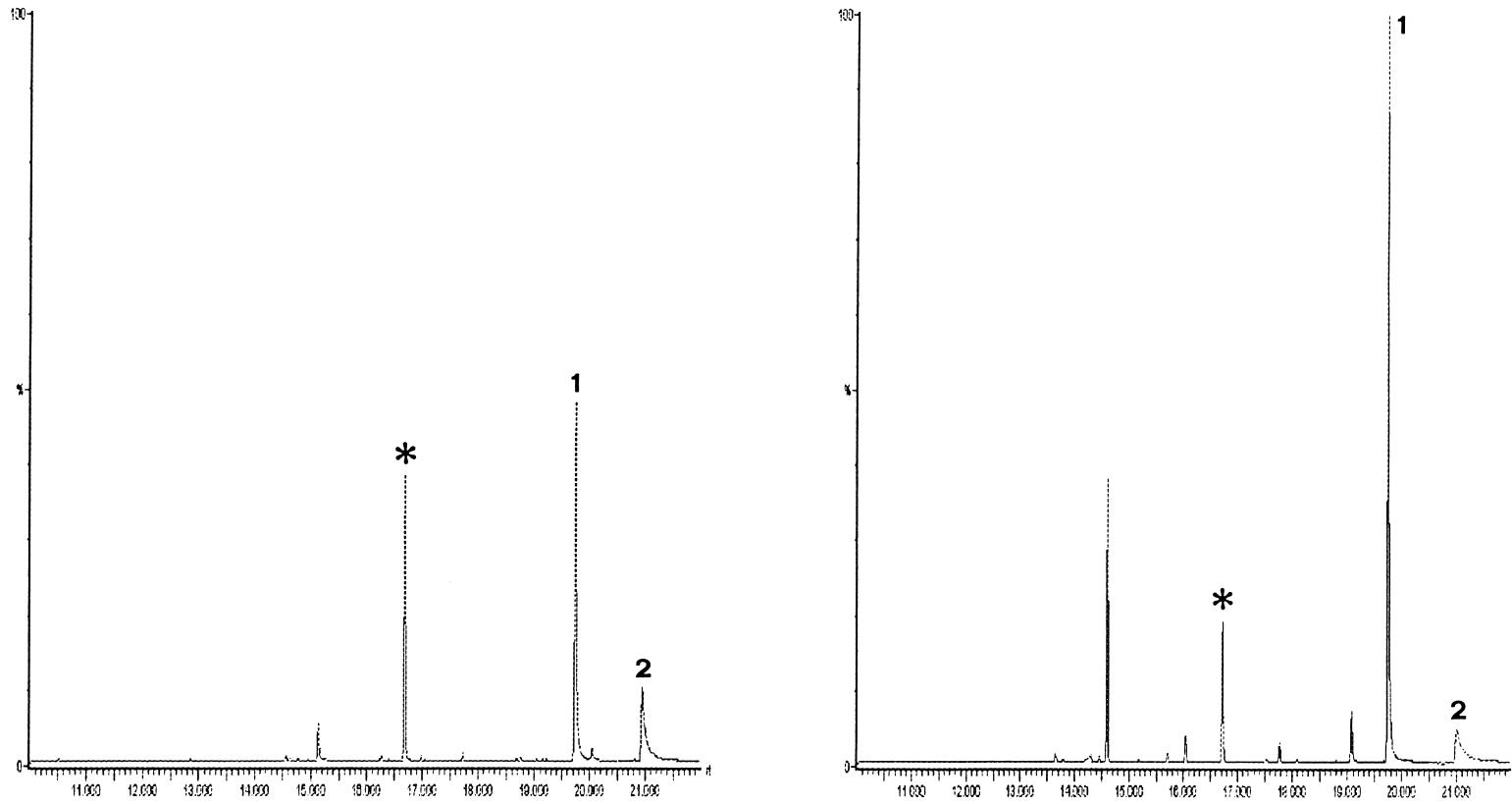
Fig. 4. SPME–GC–MS chromatograms registered in SIM mode for cyprodinil (*m/z* 225), fludioxonil (*m/z* 248) and lindane as internal standard (*m/z* 183) of synthetic wine spiked at 5 μg/l of each fungicide (left) and of a commercial Rias Baixas white wine (right) at the optimized and validated conditions. Peaks: *=internal standard, lindane; 1=cyprodinil and 2=fludioxonil. (Chromatographic conditions as described in Section 2.4).

Table 4
Analysis of 15 Rias Baixas wines from cyprodinil and fludioxonil by SPME–GC–MS

Rias Baixas area	White wine	Concentration (μg/l)$\pm$SD	
		Cyprodinil	Fludioxonil
O Rosal	A	24.7$\pm$0.7	10.3$\pm$1.2
	B	n.d.	n.d.
	C	5.6$\pm$0.1	13.0$\pm$0.9
Val do Salnés	D	n.d.	n.d.
	E	3.9$\pm$0.4	2.5$\pm$0.1
	F	1.8$\pm$0.1	2.4$\pm$0.5
	G	7.8$\pm$0.7	16.2$\pm$1.1
	H	3.0$\pm$0.2	7.9$\pm$0.5
	I	7.7$\pm$0.4	15.4$\pm$0.5
Condado do Tea	J	10.3$\pm$1.0	4.0$\pm$0.5
	K	0.9$\pm$0.2	n.q.
	L	1.5$\pm$0.2	2.7$\pm$0.6
Ribeira do Ulla	M	24.9$\pm$0.4	28.6$\pm$0.6
	N	n.d.	n.d.
Soutomaior	O	2.2$\pm$0.4	2.6$\pm$0.8

n.d.: Not detected, n.q.: not quantified.

can be avoided with the use of the addition standard method for quantitation.

This method can be considered as a good alternative to other analytical methods for the determination of the fungicides cyprodinil and fludioxonil in white wines. Its advantages are: solvents are not needed, intensive manual labor of LLE or solid-phase extraction techniques are avoided and, finally, it is more sensitive.

Acknowledgements

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