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TECHNICAL NOTE

Liquid chromatography time-of-flight mass spectrometry following sorptive microextraction for the determination of fungicide residues in wine

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Abstract This work evaluates the suitability of sorptive microextraction, using disposable silicone sorbents, and liquid chromatography time-of-flight mass spectrometry (LC-TOF-MS) for the determination of 15 fungicides in wine. Under optimized conditions, wine samples (10 mL) were diluted with the same volume of ultrapure water and poured in a glass vessel containing a magnetic stirrer and 4 g of sodium chloride. Extractions were performed at room temperature for 4 h, using an inexpensive silicone disk (12 μL volume) exposed directly to the sample. Thereafter, analytes were recovered with 0.2 mL of acetonitrile. The electrospray ionization (ESI) source was operated in the fast polarity switching mode obtaining, in the same injection, selective LC-MS records (extracted with a mass window of 10 ppm) of compounds rendering $[M+H]^+$ and $[M-H]^-$ ions. The method provided limits of quantification (LOQs) between 0.1 and 2.2 ng mL^{-1}, linear response ranges up to 500 ng mL^{-1}, relative recoveries from 75% to 117% and an inter-day variability below 15% for all analytes in red and white wine samples. The feasibility of in situ sample enrichment followed by delayed desorption and analysis is also assessed.

Keywords Sorptive microextraction · Silicone sorbents · Fungicides · Wine · Liquid chromatography · Time-of-flight mass spectrometry

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Introduction

Fungicides constitute one of the major classes of pesticides sprayed on vineyards [1]. With the exception of a few compounds, the maximum residue levels of these agrochemicals in wine have not been regulated yet. Nevertheless, knowing their background concentrations is of interest (1) to assess human exposure through wine ingestion and (2) to evaluate their dissipation rates during wine elaboration [2, 3]. Mass spectrometry techniques, following a previous chromatographic separation step, show excellent features for the determination of fungicide residues in wine samples [4–7]. However, despite the dramatic advances in the determination step, sample preparation still has an upmost importance in wine analysis for determination of fungicides at trace levels.

Besides liquid-liquid extraction (LLE) [8–10] and solid-phase extraction (SPE) [5, 11, 12], several microextraction techniques have been proposed for the extraction of fungicides from wine samples. Solid-phase microextraction (SPME), in combination with gas chromatography (GC) [13], liquid chromatography (LC) [14] and even capillary electrophoresis (CE) [15], has been widely applied to the determination of different families of fungicides in wine. Stir bar sorptive extraction (SBSE), followed by thermal

[16] and organic solvent desorption [17], has also been proposed for fungicides extraction from wine and other liquid food stuffs. However, the routine application of SPME and SBSE in fungicide monitoring studies, dealing with the analysis of a large number of wine samples, still requires to solve some practical drawbacks, such as the limited stability of some SPME coatings, cross-contamination risks and the important cost of SPME fibers and polydimethylsiloxane (PDMS) coated bars.

For the past years, the applicability of technical grade silicone sorbents for the microextraction of organic species from liquid matrices has been thoroughly evaluated [18, 19]. The sorbent employed in these studies is constituted by a siloxane skeleton with a variable percentage of different organic substituents (basically methyl, phenyl, and vinyl moieties), and it mimics the extraction behavior of PDMS-covered fibers and stir bars [20]. This means high extraction yields for medium and low polar species and a reversible absorption mechanism. In addition, bulk silicone sorbents have a negligible cost allowing (1) to concentrate simultaneously as many samples as required and (2) to discard the material after each use, preventing cross-contamination problems between samples. Silicone materials, in several different formats (rods, tube and disks), have been successfully tested for the concentration of organic pollutants in water matrices [21, 22] and also phenolic species, related with the organoleptic quality of wine [23, 24]; however, to the best of our knowledge, their efficiency for the extraction of fungicides from wine has not been reported.

Herein, we investigate the usefulness of technical grade silicone, in a disk format, for the sensitive and selective determination of a broad group of fungicides, belonging to different chemical classes, in commercial wine samples. After sorptive extraction, fungicides were recovered with a small volume of suitable solvent and further determined by liquid chromatography–mass spectrometry (LC-MS), using electrospray ionization (ESI) and accurate mass determination of ionized species.

Experimental

Standards, solvents, and sorbents

HPLC-grade acetonitrile and methanol were acquired from Merck (Darmstadt, Germany). Ultrapure water was obtained from a Milli-Q system (Millipore, Billerica, MA, USA). Ammonium acetate (98%) was from Riedel de Haën (Seelze, Germany). Standards of target fungicides were purchased from Riedel de Haën and Sigma-Aldrich (Milwaukee, WI, USA). Their abbreviated names and octanol–water partition coefficients (K_{ow}) are shown in Table 1. Tebuconazole labeled with deuterium (TEB D6, 100 μg mL^{-1} in acetone) was provided by Dr. Ehrenstorfer (Augsburg, Germany). This standard was diluted with acetonitrile and added to wine samples, as internal surrogate (IS), before the extraction step. Stock solutions of the analytes and further dilutions were also prepared in acetonitrile. Calibration standards, considered during optimization of LC-MS determination conditions, were dissolved in acetonitrile/water (1:1).

The disposable silicone sorbent was purchased from Goodfellow (Bad Nauheim, Germany) in sheets with a thickness of 0.6 mm. Disks (5 mm diameter×0.6 mm thickness, 12 μL volume) were cut and conditioned as described elsewhere [23].

Samples and sample preparation

Wine samples considered in this work were obtained from local supermarkets. Extraction conditions were optimized with aliquots of a pool of red wines (*Tempranillo*, *Mencía* and *Cabernet Sauvignon*) spiked with target analytes at 100 ng mL^{-1}. Method validation was carried out with red and white wine samples spiked at different concentrations from 0.5 to 500 ng mL^{-1}.

Extractions were carried out in 22 mL vessels furnished with PTFE-lined septa and crimp caps. A magnetic stirrer (10 mm× 3 mm) followed by a given amount of sodium chloride and an aliquot of diluted wine were poured in each vessel.

The highest extraction efficiencies were attained for 4 g of sodium chloride, 10 mL of wine and the same volume of ultrapure water. The silicone sorbent was exposed directly to the stirred (900 rpm) sample for 4 h at room temperature (20±2 °C). Thereafter, disks were removed with tweezers, rinsed with ultrapure water, dried with a lint-free tissue and transferred to small volume (1.5 mL) vessels. Analytes were recovered with 200 μL of acetonitrile, soaking the closed vessel for 30 min. The extract was then diluted with water (1:1) and injected (15 μL) in the LC-MS system.

Unless otherwise stated, the IS was added to wine samples at a constant concentration of 20 ng mL^{-1}.

LC-MS parameters

Analytes were determined using a liquid chromatography–electrospray ionization–quadrupole time-of-flight system, acquired from Agilent (Wilmington, DE, USA). The LC instrument was an Agilent 1200 Series, consisting of a vacuum degasser unit, an autosampler, two isocratic high pressure mixing pumps and a chromatographic oven. The QTOF mass spectrometer was an Agilent 6520 model, furnished with a Dual-Spray ESI source.

Table 1 Retention times (RT), quantification ions, and instrumental performance of the LC-TOF-MS system for fungicide standards

Analyte	Abbreviation	Log K_{ow}	Retention time (min)	Quantification ion (m/z)	Linear range (ng mL^{-1})	R^2	LOQ (ng mL^{-1})	Precision (RSDs, %)		
								Intra-day[a]		Inter-day[b]
								5 ng mL^{-1}	50 ng mL^{-1}	10 ng mL^{-1}
Metalaxyl	MET	1.76	10.31	280.1543[c]	LOQ-500	0.9992	0.7	1.4	0.6	2.0
Triadimenol	TRI	2.97	13.16	296.1160[c]	LOQ-500	0.9998	0.6	4.1	1.8	9.4
Fludioxonil	FLD	3.67	14.54	247.0325[d]	LOQ-1000	0.9957	0.9	2.1	1.4	7.7
Iprovalicarb	IPR	3.56	14.97	321.2173[c]	LOQ-500	0.9952	0.3	1.6	0.9	2.5
Myclobutanil	MYC	3.07	15.08	289.1215[c]	LOQ-1000	0.9968	0.9	5.8	2.9	3.8
Azoxystrobin	AZO	5.12	15.53	404.1241[c]	LOQ-500	0.9983	0.2	2.5	0.6	2.6
Tebuconazole	TEB	3.58	16.52	308.1524[c]	LOQ-1000	0.9995	0.5	1.9	1.7	4.9
Flusilazole	FLU	3.70	17.22	316.1076[c]	LOQ-500	0.9989	0.9	3.0	1.0	4.9
Penconazole	PEN	4.64	17.42	284.0716[c]	LOQ-1000	0.9966	1.7	2.7	1.8	3.9
Procymidone	PRC	2.93	18.61	284.0240[c]	LOQ-1000	0.9996	2.6	4.9	3.4	13.8
Diniconazole	DIN	4.34	18.78	326.0822[c]	LOQ-1000	0.9999	1.1	2.3	1.5	5.7
Propiconazole	PRO	3.65	19.04	342.0771[c]	LOQ-1000	0.9994	1.8	1.5	2.2	4.4
Cyprodinil	CYP	4.00	19.72	226.1339[c]	LOQ-500	0.9982	0.5	1.5	0.9	13.5
Benalaxyl	BEN	3.26	21.91	326.1751[c]	LOQ-500	0.9974	0.3	1.1	0.7	10.2
Difenoconazole	DIF	4.92	22.59	406.0720[c]	LOQ-1000	0.9991	1.0	3.2	1.4	6.4

[a] n=5 injections in the same day

[b] n=12 injections in three consecutive days

[c] $[M+H]^+$ ion

[d] $[M-H]^-$ ion

LC separations were carried out in a Zorbax Eclipse XDB-C18 column (100 mm×2.1 mm, 3.5 μm), acquired from Agilent, and connected to a C18 (4 mm×2 mm) guard cartridge supplied by Phenomenex (Torrance, CA, USA). Ultrapure water (A) and acetonitrile (B), both containing ammonium acetate 1 mM, were used as mobile phases. LC conditions were adapted from a previous work using LC-MS/MS determination after SPE of wine samples [25].

Nitrogen (99.999%), used as nebulization (40 psi) and drying gas (300 °C, 9 L min^{-1}) in the dual ESI source, was provided by a high purity generator (ErreDue srl, Livorno, Italy). The TOF instrument was operated in the 2-GHz Extended Dynamic Range resolution mode, using the fast polarity switching option to record the ESI (+) and ESI (−) chromatographic traces in the same injection. Capillary and fragmentor voltages were set at 4,000 V and 160 V in both polarities modes. Full scan (from 55 to 950 m/z units) MS spectra were recorded using an acquisition rate of 1.2 spectra s^{-1}.

Selected ion chromatograms were extracted using a mass window of 10 ppm centered in the exact (theoretical) m/z ratios of $[M+H]^+$ and $[M-H]^-$ ions of each analyte. The ratio between peak areas obtained for target fungicides and the IS (m/z 314.1895) was used as variable response to assess the performance of the developed method (linear response range, precision and accuracy) and also to quantify selected analytes levels in non-spiked wine samples, using matrix-matched standards of red and white wine.

Results and discussion

Performance of the LC-TOF system instrumental parameters

Table 1 summarizes retention times, exact masses for quantification ions and the most relevant features of the LC-TOF-MS system, without considering the sample preparation process and without IS correction, for fungicide standards. Peak area versus concentration plots followed a linear dependence, with determination coefficients (R^2) from 0.9952 to 0.999. LOQs, calculated as the concentration of each compound producing a chromatographic peak with a signal to noise ratio of 10 (S/N=10), varied between 0.2 ng mL^{-1} for AZO and 2.6 ng mL^{-1} for PRC, Table 1. Globally, these LOQs are similar to those reported for fungicide compounds using LC-QqQ systems, operated in the MS/MS mode [5, 11] and LC-TOF, maintaining the ESI source in the positive mode during the whole chromatographic run [7]. The variability in the system response was

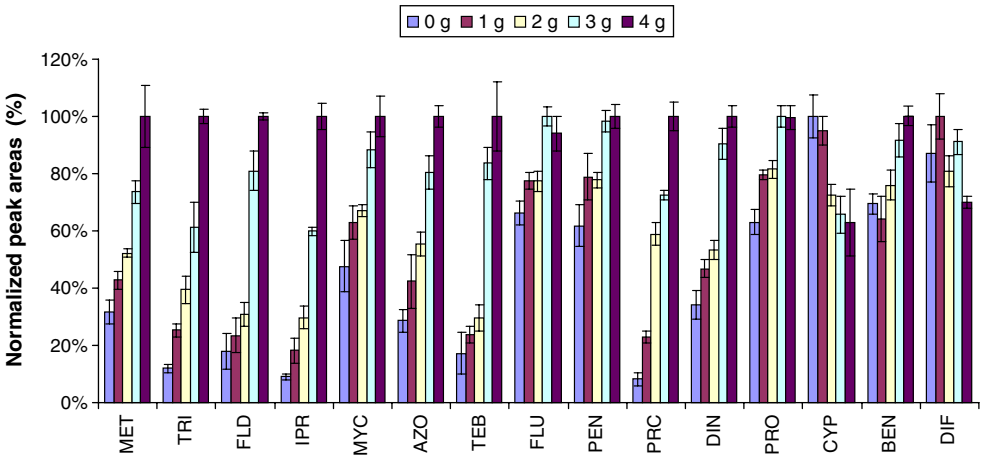

Fig. 1 Normalized responses for each compound corresponding to different amounts of NaCl, $n=3$ replicates

investigated with standards at two concentrations (5 and 50 ng mL^{-1}) for intra-day precision, and at 10 ng mL^{-1} for inter-day precision studies, Table 1.

Sample preparation conditions

Desorption solvent and volume

Methanol and acetonitrile were considered as desorption solvents on the basis of their compatibility with LC reversed-phase separations. Analytes were first incorporated in the silicone disks (direct sampling at room temperature, 3 h) and further recovered by soaking them with consecutive 0.2 mL aliquots of the considered solvent for 30 min. Above 85% of the normalized response for all compounds corresponded to the first 0.2 mL fraction, data not given. Taking into account the reduced cost of silicone disks (less than 0.1 Euro per unit), they were discarded after each extraction–desorption cycle and the volume of desorption solvent was limited to 0.2 mL. These compromise conditions prevent unnecessary dilution of the extracts and minimize organic solvent consumption. Although acetonitrile and methanol provided similar desorption efficiencies, the former was chosen as desorption solvent since it is also used in the LC separation step.

Extraction conditions

Extraction mode, temperature, and stirring Series of experiments ($n=3$ replicates) were performed pouring 2.5 mL aliquots of a spiked pool of red wines and 7.5 mL of ultrapure water plus 1 g of salt in the extraction vessel. HS experiments were carried out at room temperature, 60 °C and 80 °C. Direct extractions were performed at room temperature and 60 °C. In all cases, the sampling time was 3 h. Whatever the sample temperature, responses (peak areas without IS correction) obtained in the HS mode remained below 10% of those attained for direct sampling. As regards direct exposure, IPR and CYP were the only species showing higher responses at 60 °C than at room temperature, data not given. Thus, direct sampling at room temperature was adopted as working mode for the rest of the study. Moreover, in order to reduce the HS in the extraction vessel, sample (wine) and water volumes were upscale to 5 and 15 mL, respectively. Under these conditions, higher responses were obtained for stirred versus non-stirred samples; however, significant differences were not observed considering three different stirring rates: 400, 900, and 1,400 rpm. An intermediate value of 900 rpm was adopted for further experiments.

Sample dilution and ionic strength The ethanol content of wine increases the analytes solubility in the sample, reducing their affinity for silicone type sorbents [26]. Therefore, high dilution factors are expected to increase the efficiency of the extraction. On the other hand, the concentration of fungicides in the extraction vessel decreases with the dilution factor. Consequently, despite a higher extraction yield, the concentration of analytes in the final acetonitrile extract, obtained from the silicone disk, might be reduced. Experimental data, obtained for spiked aliquots of red wine, showed that slightly higher responses were obtained for a 1:1 dilution factor (10 mL of spiked wine plus 10 mL of ultrapure water) versus a 1:4 one (5 mL

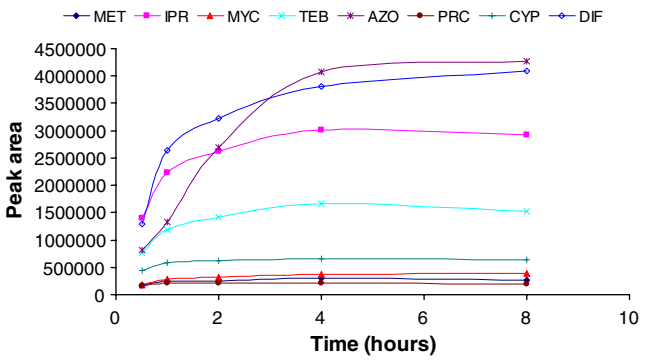

Fig. 2 Kinetics of the sorptive extraction for selected compounds. Average responses for duplicate extractions

Table 2 Linearity, limits of quantification (LOQs), intra- and inter-day precision of the proposed methodology for white and red wines

Analyte	Linear range (ng mL^{-1})	Determination coefficient, R^2		Slope white wine/ slope red wine	LOQs (ng mL^{-1})		Intra-day precision (RSDs, %)[a]				Inter-day precision (RSDs, %)[b]	
		White wine	Red wine		White wine	Red wine	10 ng mL^{-1}		50 ng mL^{-1}		25 ng mL^{-1}	
							White wine	Red wine	White wine	Red wine	White wine	Red wine
MET	2.5–500	0.9987	0.9985	1.15	2.2	2.2	3.8	7.2	7.2	4.2	4.5	5.8
TRI	2.5–250	0.9991	0.9998	1.09	1.0	1.2	5.9	5.2	11.1	4.1	13.1	12.7
FLD	2.5–500	0.9966	0.9932	1.01	0.8	1.2	8.4	14.8	15.3	8.9	12.7	13.8
IPR	2.5–500	0.9971	0.9986	1.07	0.7	0.7	4.9	7.5	10.7	1.3	6.1	10.9
MYC	2.5–500	0.9910	0.9927	1.07	0.8	1.0	3.2	7.3	7.3	4.7	7.1	9.5
AZO	0.5–500	0.9912	0.9978	1.02	0.2	0.2	2.1	8.1	10.4	4.4	7.2	9.1
TEB	0.5–500	0.9964	0.9977	1.01	0.3	0.3	1.5	6.6	10.2	2.8	9.4	12.8
FLU	0.5–250	0.9993	0.9999	1.05	0.2	0.2	5.3	8.1	7.2	5.3	9.2	12.9
PEN	0.5–250	0.9999	0.9999	1.06	0.5	0.4	6.6	9.5	9.3	5.2	8.2	14.9
PRC	2.5–500	0.9966	0.9932	1.19	1.9	2.2	1.9	6.1	6.4	5.0	6.1	6.0
DIN	0.5–500	0.9920	0.9977	1.03	0.3	0.3	6.7	10.6	10.8	5.0	8.8	13.1
PRO	0.5–500	0.9960	0.9960	1.04	0.4	0.5	4.2	5.1	10.3	4.2	8.9	14.8
CYP	0.5–500	0.9996	0.9949	1.01	0.4	0.6	5.9	9.9	13.4	6.1	3.1	6.8
BEN	0.5–250	0.9966	0.9978	1.06	0.2	0.2	5.8	6.1	3.6	3.0	5.4	10.2
DIF	0.5–500	0.9990	0.9955	1.25	0.1	0.1	5.1	7.2	9.3	5.9	13.0	11.1

[a] n=3 samples processed in the same day

[b] n=9 samples processed in three consecutive days

Table 3 Accuracy (given as relative recoveries,%) of the proposed methodology for red and white wine samples spiked at two different concentration levels (10 and 50 ng mL^{-1}), n=3 replicates

Analyte	Recovery (%)±SD			
	White wine		Red wine	
	10 ng mL^{-1}	50 ng mL^{-1}	10 ng mL^{-1}	50 ng mL^{-1}
MET	109.5±3.1	112.4±4.0	74.8±0.3	115.3±2.9
TRI	102.8±6.3	105.9±3.6	77.1±2.1	105.4±5.1
FLD	111.4±0.6	102.4±1.1	78.8±4.9	117.4±9.3
IPR	92.5±4.5	104.9±1.4	106.7±0.2	97.5±3.4
MYC	107.9±1.1	107.8±1.1	90.9±0.7	95.0±7.2
AZO	105.3±1.7	103.8±1.5	87.6±2 .5	99.4±6.1
TEB	94.2±1.2	101.8±4.8	94.2±0.3	90.7±4.9
FLU	100.5±0.7	104.6±8.2	102.3±2.2	80.8±3.4
PEN	98.7±2.3	102.5±8.0	103.8±0.9	86.6±2.2
PRC	96.1±2.5	100.2±6.7	99.0±0.5	89.3±5.1
DIN	105.7±3.1	112.0±4.8	111.8±2.2	102.4±7.5
PRO	97.6±5.5	100.7±12.4	106.6±1.8	101.1±7.1
CYP	89.6±4.8	92.3±0.7	115.6±2.9	74.6±4.4
BEN	97.3±2.5	102.8±9.4	101.5±1.2	98.4±5.1
DIF	95.8±3.4	96.6±9.3	102.8±5.9	113.2±4.4

Data obtained using matrix-matched calibration

of the same spiked wine plus 15 mL of water). Therefore, 10 mL of wine and the same volume of water were used in further extractions.

The influence of the ionic strength on the efficiency of the sorptive extraction was evaluated at five levels (n=3 replicates), considering a sampling time of 2 h. The global trend of data depicted in Fig. 1 is an increase in the efficiency of the extraction with the amount of NaCl in the extraction vessel. The above pattern can be explained on the basis of the salting out effect. On the other hand, salt addition increases the viscosity of the solution, reducing the migration rates (extraction kinetics) of less polar compounds between the sample and the sorbent. Likely, kinetics factors are responsible for the anomalous behavior of CYP and DIF, Fig. 1. In further experiments, 4 g of sodium chloride were added to samples in the extraction vessel.

Extraction kinetics Sorptive extraction kinetics was investigated between 0.5 and 8 h. Figure 2 shows the time course of the process for selected fungicides. Equilibrium times ranged from 2 to 4 h, depending on the considered compound. In general, analytes with high K_{ow} values, such as DIF and AZO (log K_{ow} 4.92 and 5.12, respectively), required longer times than the most polar ones, e.g., MET and PRC (log K_{ow} 1.76 and 2.93, respectively), to achieve equilibrium conditions, Fig. 2. An extraction time of 4 h was adopted as working value of this factor. Rial Otero and co-workers [13] have reported equilibrium times above 2 h

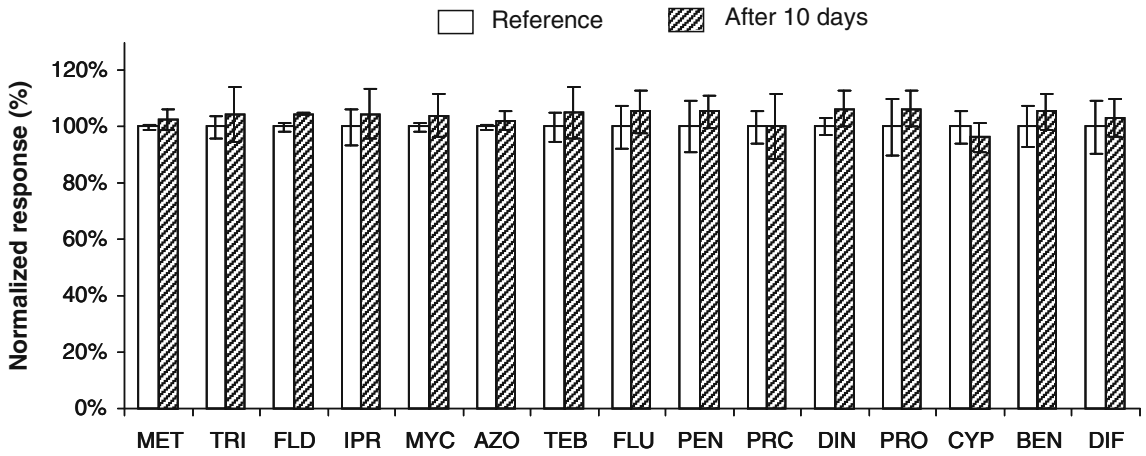

Fig. 3 Comparison of responses for silicone disks desorbed immediately after the extraction step versus those stored at 4 °C for 10 days, n=4 replicates

for the SPME of CYP and FLD from 30 mL wine samples. In another application of the same technique, an extraction period of 140 min was proposed for 10 mL wine samples [15]. Thus, the sampling time used in this study remains in the same range of values as those employed in SPME protocols; moreover, the use of inexpensive, disposable silicone disks allows extracting simultaneously several samples, leading to a higher sample throughput than SPME.

Analytical performance

The absolute efficiency of the sorptive microextraction method was defined as the difference between measured responses for spiked (50 ng mL^{-1}) and non-spiked aliquots of a red wine sample divided by those obtained for an extract of the latter matrix (non-spiked wine) fortified after extraction and multiplied by 100. The efficiency of the sample preparation process ranged from 1% for MET up to 30% for BEN, data not shown. Data related with linearity, precision and LOQs of the procedure for red and white wine samples are compiled in Table 2. Depicted values correspond to white (mixture of *Viura* and *Airen* varieties) and red (*Tempranillo*) wines, which did not contain detectable levels of target analytes. For the linearity study, aliquots of these samples were spiked at eight different levels between 0.5 and 500 ng mL^{-1}, covering the range of concentrations reported for target analytes in wine samples [11, 13, 27]. Internal standard calibration curves (analyte/IS responses versus concentration) fitted a linear model between the LOQs and 250 ng mL^{-1} for TRI, FLU, PEN, and BEN, and up to 500 ng mL^{-1} for the rest of fungicides, with determination coefficients (R^2) higher than 0.991, Table 2. The ratio between calibration curves slopes for white and red wines stayed between 1.01 and 1.25, indicating higher extraction efficiency for the first matrix, which could not be completely compensated using TEB D6 as internal surrogate, Table 2.

Precision was investigated with samples processed in the same day and spiked at two different levels (10 and 50 ng mL^{-1}), and in different days with aliquots fortified at 25 ng mL^{-1}. The relative standard deviations (RSDs) of the corrected responses (analyte/IS peak areas) ranged from 1.3% to 15.3%, for intra-day precision and between 3.1% and 14.9% for inter-day precision, Table 2.

The analysis of procedural blanks, corresponding to synthetic wine samples [23], demonstrated the absence of contamination problems during sample preparation; thus, the LOQs of the method were estimated from the lowest level of calibration curves obtained for wine samples. Achieved values varied between 0.1 ng mL^{-1} for DIF to 2.2 ng mL^{-1} for MET and PRC, Table 2. These LOQs are far below EU regulations for grapes used for vinification

Table 4 Levels of fungicides in non-spiked wine samples. Average concentrations (ng mL^{-1}) with their standard deviations, $n=3$ replicates

	Sample code												
	W1 Albariño	W2 Albariño	W3 Albariño	W3[a] Albariño	W4 Albariño	W5 Ribeiro	W6 Valdeorras	W7 Valdeorras	R1 Ribeira Sacra	R2 Ribeira Sacra	R3 Ribeiro	R4 Valdeorras	R4[a] Valdeorras	R5 Ribeiro
MET	40.4±0.6	38.4±4.3	47.3±1.9	52.1±2.3	31.1±0.8	22.3±0.5	3.11±0.07	10.0±0.3	2.5±0.3	2.9±0.4	3.1±0.3	7.4±1.0	6.7±0.9	3.8±0.9
TRI	n.d.	n.d.	29.2±1.1	27.3±5.0	n.d.	n.d.	3.6±0.6	n.d.	n.d.	n.d.	n.d.	<LOQ	n.d.	n.d.
FLD	11.6±0.4	35.7±3.3	21.5±1.3	19.6±1.1	1.3±0.2	0.84±0.01	<LOQ	n.d.	5.6±0.6	n.d.	1.73±0.05	n.d.	n.d.	n.d.
IPR	39.4±0.3	90.6±5.0	27.6±1.2	22.9±3.0	3.2±0.2	3.24±0.2	3.1±0.2	<LOQ	0.71±0.07	53.2±8.6	3.1±0.2	2.8±0.5	3.2±0.6	3.4±0.1
MYC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ	<LOQ	5.6±0.8	3.9±0.2	n.d.	n.d.	6.1±0.5
AZO	1.48±0.02	2.5±0.3	0.67±0.03	0.94±0.07	27.7±0.6	0.32±0.01	n.d.	<LOQ	n.d.	n.d.	0.21±0.01	1.2±0.2	1.0±0.1	n.d.
TEB	2.5±0.1	0.52±0.02	2.36±0.03	2.74±0.08	<LOQ	n.d.	n.d.	n.d.	n.d.	14.2±2.7	n.d.	n.d.	n.d.	n.d.
PRC	n.d.	2.58±0.02	4.7±0.2	5.0±0.2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
CYP	17.9±0.8	42.5±2.5	12.9±0.6	13.2±0.3	<LOQ	<LOQ	<LOQ	n.d.	5.62±0.02	<LOQ	0.83±0.01	n.d.	n.d.	n.d.
BEN	0.42±0.01	<LOQ	0.69±0.06	0.5±0.1	n.d.	n.d.	<LOQ	0.87±0.08	n.d.	n.d.	0.32±0.01	<LOQ	n.d.	<LOQ

[a] Values obtained for SPE of 10 mL wine samples [25]

n.d. not detected, *<LOQ* below the limit of quantification of the method, Codes *W* and *R* correspond to white and red wines, respectively

(from 0.05 to 2 μg g^{-1}). Furthermore, they are in the same range of values as those achieved combining SPME [13] or LLE [10] with GC-MS, as well as SPE followed by LC-MS/MS using triple quadrupole [5, 11] and quadrupole time-of-flight hybrid mass spectrometers [25].

Table 3 shows the relative recoveries obtained for aliquots of white (*Godello*) and red (*Mencía*) wines, quantified against the calibration curves obtained for *Viura* and *Airen* white and *Tempranillo* red wines, respectively. All samples were fortified with TEB D6 as IS at 20 ng mL^{-1}. In the case of white wine, the accuracy of the procedure was excellent with relative recoveries comprised between 90% and 112%, and standard deviations below 12%. For red wine, the relative recoveries varied from 75% to 117%, which can be still considered acceptable for screening purposes. Overall, the above data suggest the possibility of using matrix-matched standards of red and white wine (submitted to the whole sample preparation process as in the case of any micro-extraction technique), instead of the more time-consuming standard addition methodology, as quantification approach.

Delayed desorption

In situ sample preparation followed by delayed desorption of the sorbent in the laboratory is particularly attractive when low-cost traps (extraction devices) are used in combination with simplified sample preparation schemes (without requiring an exhaustive control of experimental variables, e.g., temperature, stirring speed, pH, etc.), which can be carried out by personnel without specific training. Figure 3 compares the relative responses measured for silicone disks desorbed after finishing the sampling step, performed with wine samples spiked at 50 ng mL^{-1}, and those stored at 4 °C for 10 days. Depicted data demonstrate the stability of target fungicides in silicone disks.

Application to wine samples

The developed procedure was applied to a total of 12 young wines elaborated in 2010 and corresponding to different geographic denominations from the Northwest of Spain. Five of the analytes considered in this research (FLU, PEN, DIN, PRO, and DIF) remained undetected in all samples. On the other hand, MET, FLD, IPR, and CYP were found in a significant percentage of samples at concentrations above 10 ng mL^{-1} (Table 4). Two of the analyzed samples were also processed using SPE, following a previously published method [25]. Found values were in reasonable agreement with those measured using sorptive extraction (Table 4), which confirms the accuracy of the matrix-matched calibration strategy used in this study. A chromatogram for a real sample is provided as Electronic Supplementary Material (Fig. S1).

Conclusions

A low-cost (less than 0.1 Euro per sorbent unit), simple and robust sample preparation method has been proposed as a convenient alternative for determining trace levels of 15 fungicides in wines samples by LC-TOF-MS. Under optimized working conditions, the developed methodology provides LOQs low enough for real samples analysis, with suitable precision and linear response ranges. Additionally, it shows a lower organic solvent consumption than most SPE methodologies (0.2 versus 2–3 mL), it is free of cross-contamination problems and sample preparation can be performed in situ and then disks stored at 4 °C for more than 1 week. The extraction time (4 h) is similar to that employed in SPME methods; nevertheless, the possibility of processing simultaneously several samples improves the usefulness of the method in screening studies, involving the analysis of many samples. Data obtained in this research indicate the possibility of using matrix-matched standards, instead of standard addition, as quantification technique. Analysis of real samples confirmed the frequent occurrence of significant residues of MET, IPR, CYP, and FLD in wines produced in the Northwest of Spain.

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