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Determination of 24 Pesticide Residues in Fortified Wines by Solid-Phase Microextraction and Gas Chromatography−Tandem Mass Spectrometry

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ABSTRACT: The present work describes a solid-phase microextraction (SPME) gas chromatography−tandem mass spectrometry (MS/MS) method to quantify 24 pesticides in fortified white wine and fortified red wine. In this study "fortified wine" refers to a wine in which fermentation is arrested before completion by alcohol distillate addition, allowing sugar and alcoholic contents to be higher (around 80−100 g/L total sugars and 19−22% alcohol strength (v/v)). The analytical method showed good linearity, presenting correlation coefficients (R^2) ≥ 0.989 for all compounds. Limits of detection (LOD) and quantitation (LOQ) in the ranges of 0.05−72.35 and 0.16−219.23 μg/L, respectively, were obtained. LOQs are below the maximum residue levels (MRL) set by European Regulation for grapes. The proposed method was applied to 17 commercial fortified wines. The analyzed pesticides were not detected in the wines tested.

KEYWORDS: pesticides, fungicides, acaricides, SPME, GC-MS/MS, wine

INTRODUCTION

There is increasing interest regarding health and safety aspects associated with the use of pesticides and the presence of residues in processed foods and drinks.[1] The use of pesticides in viticulture is a major issue for grape protection, increasing grape productivity, and wine quality. The European Commission (EC) has set maximum residue levels (MRLs) for table and wine grapes through EC Regulation 396/2005,[2] but no harmonized MRLs have been set in the European Union for pesticides in wines.

The greatest impact on grape quality is caused by agents that attack berries directly. These include three of the major fungal grapevine pathogens, namely, *Botrytis cinerea* (gray rot), *Plasmopara viticola* (mildew), and *Uncinula necator* (oidium). Winegrowers may use different pesticides, mainly fungicides, to control pests.[3] Although the pesticide contents in wines are significantly lower than in grapes, due to the vinification process,[1,4−8] the consumer is generally worried about the possible presence of residues in the finished wine product. Several studies, namely, those developed by the research groups of Prof. Cabras[4,6,9] and Prof. Barba,[5] in Italy and Spain, respectively, have shown that some pesticides and/or their degradation products can be found in musts, and some of them can persist through the vinification process and be observed in the final wines.

With regard to some of the compounds included in the present study, Čuš et al.[10] have recently reported the presence of several pesticides in commercial wines from the Slovenia market, namely, cyprodinil (10−440 μg/L), fenhexamid (20−170 μg/L), metalaxyl (30−60 μg/L), procymidone (30−50 μg/L), azoxystrobin (40 μg/L), and iprodione (30 μg/L). According to these authors,[10] fenhexamid has been found in 44% of the 25 wines analyzed. Residues of cyprodinil (0.9−24.9 μg/L) have also been detected in commercial wines from Rías Baixas (Spain).[11] Azole fungicides were detected in >75% of the 103 wine samples, from 11 different countries, analyzed by Trösken et al.,[12] but all in concentrations below the known MRLs. Tebuconazole was the most commonly used fungicide, being detected in 53% of the samples and in samples of every country.[12] Conversely, other compounds such as deltamethrin,[13] chlorothalonil,[14] and folpet[6] are not detected in wines due to their degradation during the vinification process.

Solid-phase microextraction (SPME) eliminates problems associated with other extraction methods, such as liquid−liquid extraction (LLE) or solid-phase extraction (SPE). In SPME, solvents are completely eliminated, blanks are greatly reduced, and analytes' extraction can be performed in a few minutes.[15] This method does not require complete removal of the analyte from the liquid matrix[15,16] and can be applied to a wider range of applications than other techniques such as SPE, which requires an exhaustive extraction.

Several multiresidue methods for pesticide analysis in wines have been reported.[1,3−6,17−25] However, there is little information about multiresidue analysis of pesticides in fortified wines.[23] In this study "fortified wine" refers to a wine in which fermentation is arrested before completion by alcohol distillate addition, allowing sugar and alcoholic content to be higher, around 80−100 g/L total sugars and 19−22% alcohol strength (v/v). Complex matrices such as these types of wines are typically high-quality products, presenting important economic and cultural value. Efficient quality control is important and indispensable to secure the safety of these products in international food trade and the safety of the consumers.

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Table 1. Chemical Class, Mode of Action, Octanol/Water Partition Constant (K_{ow}), Molecular Weight, and Retention Time of the Analyzed Pesticides and MS/MS Conditions Used[a]

pesticide	chemical class	action[b]	log K_{ow}	MW	t_r (min)	isolation parent ion (m/z)	excitation voltage (V)	MS-MS fragments (m/z)
diazinon	organophosphorus	I, A	3.30	304.3	28.94	304	1.4	179, 162, 195
chlorothalonil	polychlorinated aromatic	F	2.89	265.9	29.36	266	1.1	266, 231
chlorpyriphos-methyl	organophosphorus	I, A	4.24	322.5	30.88	286	1.2	271, 241, 208, 172
vinclozoline	dicarboximide	F	3	286.1	30.89	212	1.2	172, 212, 145, 177
metalaxyl	phenylamide	F	1.75	279.3	31.50	160	1.3	145, 130, 160
fenitrothion	organophosphorus	I	3.5	277.2	32.06	260	1.2	125, 228, 150, 260
malathion	organophosphorus	I, A	2.75	330.3	32.52	173	1.4	99, 117, 127, 145
chlorpyriphos	organophosphorus	I	4.7	350.6	32.91	314	1.2	286, 258
fenthion	organophosphorus	I	4.84	278.3	32.92	278	1.2	245, 135, 151
cyprodinil	anilinopyrimidine	F	4.0	225.3	33.99	225	1.3	224, 208
procymidone	dicarboximide	F	3.14	284.1	34.98	283	1.1	255, 251, 96
folpet	phthalimide	F	3.11	296.6	34.87	130	1.3	102, 130
flusilazole	azole	F	3.74	315.4	37.18	233	1.1	233, 165, 152
kresoxim-methyl	strobilurin	F	3.4	313.4	37.25	206	1.1	116, 132
trifloxystrobin	strobilurin	F	4.5	408.4	39.74	131	1.1	130, 103, 90
fenhexamid	hydroxyanilide	F	3.5	302.2	39.75	266	1.1	170, 250, 266, 143
tebuconazole	azole	F	3.7	307.8	40.32	250	1.1	125, 179, 223, 250
iprodione	dicarboximide	F	3.0	330.2	41.30	314	1.1	245, 271
fenoxycarb	carbamate	I	4.07	301.3	41.66	255	1.1	186, 158, 129
λ-cyhalothrin	pyrethroid	I	7	449.9	43.82	181	1.1	181, 152
β-cyfluthrin	pyrethroid	I	5.9	434.3	46.97	206	1.1	206, 151, 179
cypermethrin	pyrethroid	I	6.6	416.3	47.28	181	1.1	181, 152
deltamethrin	pyrethroid	I	4.6	505.2	50.70	181	1.2	181, 152
azoxystrobine	strobilurin	F	2.5	403.4	51.37	344	1.1	329, 344

[a] Excitation Energie (q value) = 0.3. [b] A, acaricide; F, fungicide; I, insecticide.

The aim of this study was to set up a multiresidue methodology for the determination of 24 pesticides (azoxystrobin, β-cyfluthrin, chlorothalonil, chlorpyriphos, chlorpyriphos-methyl, cypermethrin, cyprodinil, deltamethrin, diazinon, fenhexamid, fenitrothion, fenthion, fenoxycarb, flusilazole, folpet, iprodione, kresoxim-methyl, λ-cyhalothrin, malathion, metalaxyl, procymidone, tebuconazole, trifloxystrobin, and vinclozolin) in fortified wines, by SPME and gas chromatography−tandem mass spectrometry (GC-MS/MS). The method is simple and fully automated and, to the best of our knowledge, was validated for the first time for the set of pesticides selected in fortified wines (white and red).

MATERIALS AND METHODS

Solutions and Reagents. All pesticide standards were of high purity (>95%) supplied by Sigma-Aldrich (Seelze, Germany). Individual pesticide stock solutions (1 g/L) were prepared in methanol (99.9%), supplied by Sigma-Aldrich, and stored under refrigeration (2−6 °C). A stock standard mixture solution containing all pesticides was also prepared in methanol, weekly, and stored under refrigeration (2−6 °C).

Experimental blends of fortified wine (blends of fortified white wine and fortified red wine) were obtained from a mixture of commercial wines previously analyzed for the absence of pesticides. The wine blend samples were spiked with different volumes of the stock standard mixture solution.

SPME Procedure. SPME was performed using a Combipal MH 01-00B autosampler (CTC Analytics AG, Zwingen, Switzerland). SPME fibers (Supelco, Bellefonte, PA) were conditioned according to the supplier's instructions. Samples of 19 mL were placed in 20 mL dark glass vials (La-Pha-Pack, Langerwehe, Germany) and extracted by immersion of a 100 μm polydimethylsiloxane (PDMS) coated fiber. The extraction was performed at 35 °C, with an agitator speed of 250 rpm, for 60 min. Desorption was carried out in the injector port for 6 min, at 250 °C. After extraction and desorption, the fiber was conditioned for 5 min, in the presence of nitrogen (99.995%).

GC-MS/MS Analysis. A FocusGC coupled to a PolarisQ ion trap mass spectrometer (Thermo Fisher Scientific, Waltham, MA) was used, interfaced to a computer running Xcalibur 1.4 software. Analytes were separated in a TR-5MS column (30 m × 0.25 mm i.d. × 0.25 μm film thickness) coated with 5% phenylmethylpolysiloxane stationary phase (Thermo Fisher Scientific). The split/splitless injector was maintained in splitless mode for 3 min. The GC oven temperature program was as follows: 80 °C hold for 5 min, increased to 300 °C at a rate of 5 °C/min and hold for 10 min, with a total acquisition time of 59 min. Helium (99.9999%) was used as carrier gas, at a constant pressure of 50 kPa, and as collision gas in the ion trap chamber. The mass spectrometer operated in electron impact (EI) mode. The ion source and transfer line temperatures were set at 250 and 280 °C, respectively. Analyses were carried out with a filament-multiplier delay of 5 min. The mass spectrometer was calibrated frequently to perfluorotributylamine (PFTBA) through an automatic tune process. Retention times of each pesticide were determined in full scan mode (m/z 50−650). For the determination of the target pesticides, MS/MS mode was selected. Compound properties and MS/MS conditions used are presented in Table 1.

The quantification was based on at least a five-point external calibration graph obtained by plotting the individual peak areas against the concentration of the calibration standards.

Table 2. Analytical Data for the Analyzed Pesticides in Fortified White Wine (FWW) and Fortified Red Wine (FRW) Using the Proposed Method, LODs and LOQs for Other Multiresidue Methods Reported in the Literature, and MRLs for Grapes According to EC Regulation 396/2005

			FWW		FRW				
pesticide	linearity (μg/L)	R^{2a}	LOD (μg/L)	LOQ (μg/L)	LOD (μg/L)	LOQ (μg/L)	LOD_{wines} lit.	LOQ_{wines} lit.	MRL (μg/kg)
diazinon	1.59−13.24	0.997	0.87	2.65	0.87	2.63	6.84 μg/L;[b] 2 μg/L;[c] 10 μg/L;[e] 1.3 μg/L;[f] <1.0 mg/L[g]	44 μg/L;[d] 10 μg/L[c]	10
chlorothalonil	14.77−123.11	0.996	7.99	24.20	8.12	24.62	1.0 mg/L[g]		3000
chlorpyriphos-methyl	0.64−5.35	0.996	0.21	0.64	0.35	1.06	2 μg/L;[c] 1.3 μg/L;[f] <1.0 mg/L[g]	10 μg/L[c]	200
vinclozoline	18.07−150.56	0.993	5.77	17.50	5.96	18.07	5 μg/L;[c] 50 μg/L;[e] 3.3 μg/L;[i] 6.3 μg/L;[f] 1.0 mg/L[g]	17.8 μg/L;[h] 10 μg/L[c]	5000
metalaxyl	42.30−527.49	0.994	23.27	70.50	34.81	105.50	25 μg/L;[i] 2.5 μg/L;[f] 1.0 mg/L[g]		1000
fenitrothion	0.95−7.93	0.995	0.31	0.95	0.52	1.58	1.0 mg/L[g]	26 μg/L[d]	10
malathion	8.14−98.38	0.997	2.69	8.14	4.33	13.12	5.63 μg/L;[b] 50 μg/L;[e] 6.3 μg/L;[f] <1.5 mg/L[g]	96 μg/L[d]	5000
chlorpyriphos	1.27−10.56	0.995	0.42	1.27	0.42	1.27	50 μg/L;[e] 4.7 μg/L;[i] 5.0 μg/L;[f] 1.0 mg/L[g]	15.8 μg/L[h]	500
fenthion	0.16−1.35	0.995	0.05	0.16	0.05	0.16	5.5 μg/L;[f] <1.5 mg/L[g]		10
cyprodinil	7.11−59.27	0.997	2.35	7.11	3.91	11.84	6.3 μg/L;[f] <1.5 mg/L[g]		5000
procymidone	22.40−186.63	0.993	7.39	22.40	7.39	22.41	2 μg/L;[c] 50 μg/L;[e] 6.5 μg/L;[f]1.0 mg/L[g]	12.06 μg/L;[h] 10 μg/L[c]	
folpet	119.58−747.38	0.996	39.46	119.58	65.56	198.66	90 μg/L;[e] 6.3 μg/L;[f] 15 mg/L[g]	9.96 μg/L[h]	5000
flusilazole	11.29−84.71	0.997	2.29	6.94	3.73	11.29			200
kresoxim-methyl	10.19−84.91	0.998	3.36	10.18	3.36	10.19	0.2 mg/L;[j] 0.2 mg/L[k]	0.7 mg/L;[j] 0.6 mg/L[k]	1000
trifloxystrobin	11.40−94.18	0.997	3.73	11.30	6.27	19.00	0.2 mg/L;[j] 0.2 mg/L[k]	40 μg/L;[l] 0.6 mg/L;[j] 0.6 mg/L[k]	5000
fenhexamid	172.41−862.03	0.992	56.82	172.18	56.89	172.41			5000
tebuconazole	92.47−462.35	0.995	18.27	55.35	30.52	92.47	1.5 mg/L[g]		2000
iprodione	147.00−1234.21	0.997	48.51	147.00	48.87	148.11	2 μg/L;[c] 20 μg/L;[e] 6.0 μg/L;[f] 5.0 mg/L[g]	9.52 μg/L;[h] 10 μg/L[c]	
fenoxycarb	41.75−260.96	0.989	13.78	41.75	13.40	40.60			1000
λ-cyhalothrin	5.79−48.70	0.996	1.91	5.79	3.21	9.74			200
β-cyfluthrin	13.42−110.93	0.995	4.43	13.42	7.32	22.19			300
cypermethrin	6.30−52.11	0.993	2.08	6.30	3.44	10.42	100 μg/L;[e] 2.0 mg/L[g]		500
deltamethrin	6.76−54.31	0.993	3.72	11.27	3.58	10.86	6.5 μg/L;[f] 8.0 mg/L[g]		200
azoxystrobin	131.40−1098.62	0.996	43.51	131.83	72.35	219.23	0.2 mg/L;[j] 0.1 mg/L[k]	0.8 mg/L;[j] 0.5 mg/L[k]	2000

[a] Typically, the R^2 values are different for FWW and FRW. The R^2 values given in the table are the minimum values. [b] SPME-GC-ECD.[18] [c] SPE-GC-MS.[17] [d] SPME-GC-MS.[21] [e] SPE-GC-MS.[1] [f] QuEChERS and LP-GC/MS.[23] [g] SPE-GC-MS.[20] [h] SPME-GC-ECD.[19] [i] GC-MS (for metalaxyl) and GC-ECD (for chlorpyrifos, penconazole, fenarimol, and vinclozoline).[5] [j] LLE-HPLC-DAD.[27] [k] LLE-GC-MS.[27] [l] LC-MS.[9]

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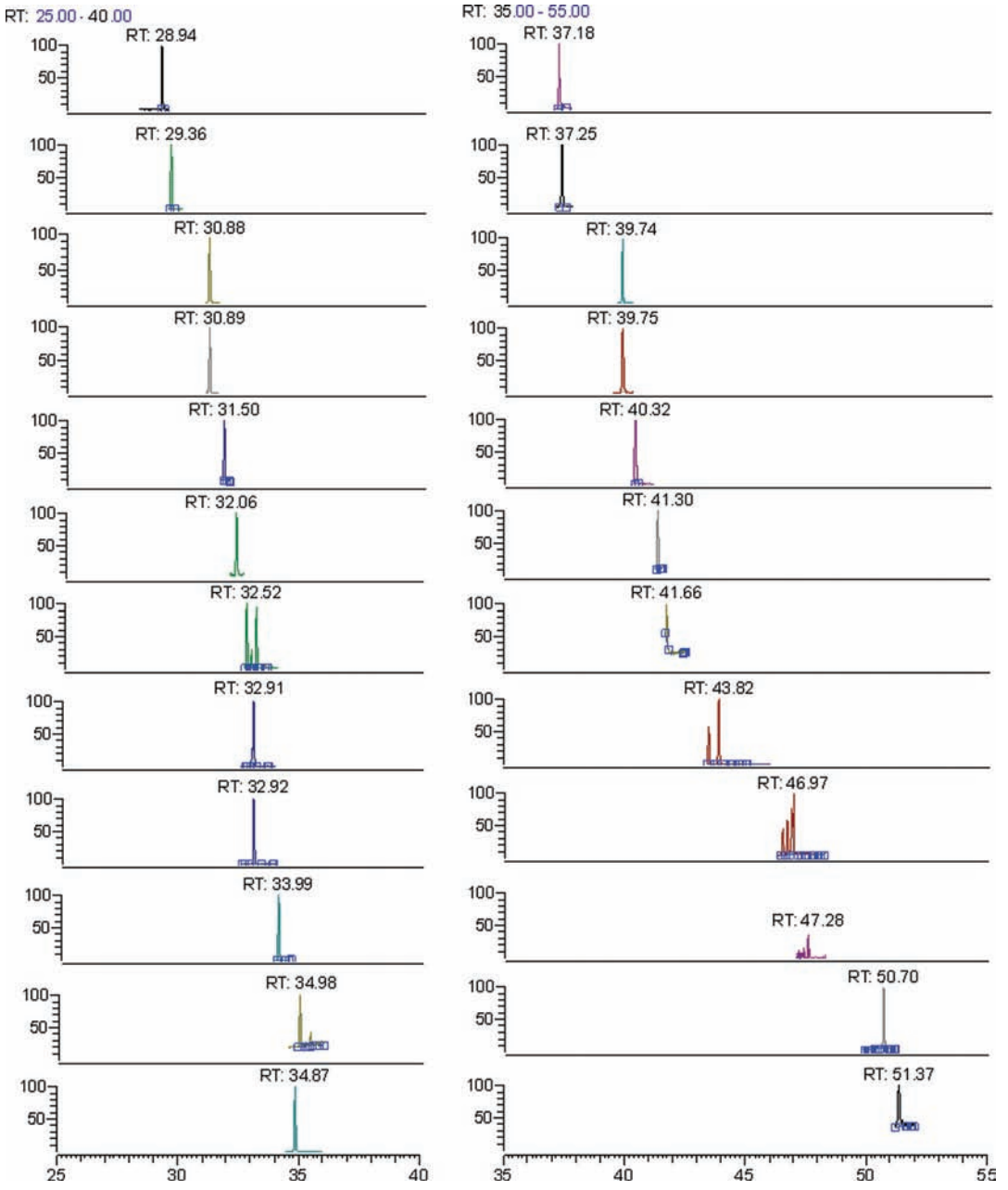

Figure 1. Representative GC-MS/MS chromatograms of the studied compounds (for peak assignment, refer to Table 1).

RESULTS AND DISCUSSION

Calibration. Matrix effects are known to be problematic in pesticide residue analysis.[26] In GC, response enhancement effect can occur when coextractives fill active sites in the chromatographic system. On the other hand, matrix diminishment effects may be due to buildup of nonvolatile materials in the GC inlet.[26] The most common way to avoid matrix effects is to use matrix-matched calibration standards, and this was selected for this study. Blends of commercial fortified red and white wines, spiked with pesticides, were used for the preparation of the calibration curves. Good linearity was achieved for all of the compounds for the concentration ranges selected, in fortified white wine (FWW) and in fortified red wine (FRW), with correlation coefficients $R^2 \geq 0.989$ for all of the compounds (Table 2). The lowest value of R^2 was obtained for fenoxycarb in FRW (0.989) and the highest value for λ-cyhalothrin in FWW (0.999). Representative GC-MS/MS chromatograms are presented in Figure 1.

The ranges of concentrations that were selected were not the same for all of the compounds. Physical−chemical characteristics affect the analytical signal and for some compounds higher concentrations had to be used to detect the compound. Additionally, some MRLs are also "relatively high" for some compounds, and, for this reason, higher concentrations were tested (Table 2). However, in the analytical conditions used, the method shows good linearity for all of the compounds in the entire calibration range, and apparently no carry-over was observed for the higher concentrations tested.

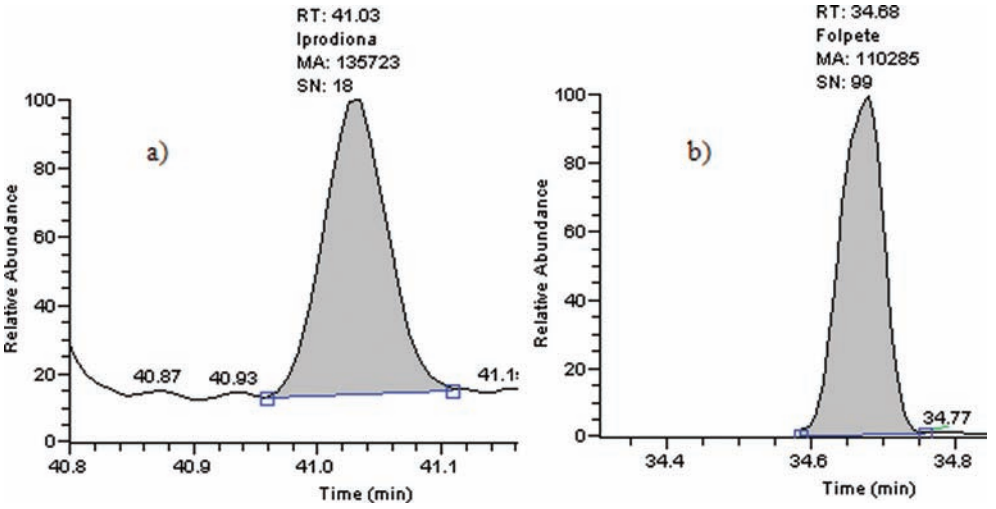

Figure 2. GC-MS/MS chromatograms obtained for (a) iprodione, spiked at 148.11 μg/L, and (b) folpet, spiked at 198.66 μg/L, in a fortified red wine sample.

Previous studies[18] point out that some wine constituents such as ethanol, sugar, tartarates, and phenols may interfere in SPME and that ethanol may have one of the most significant effects. In the present work, and with regard to some of the analytes, namely, the organophosphorus compounds, a study was conducted comparing the calibration curves obtained for FWW, FRW, and ethanol−water solution (5%, v/v). For chlorpyriphos, chlorpyriphos-methyl, malathion, and fenthion, there was not a significant difference in the slopes of the three calibration curves (data not show), and therefore the matrix effect can be neglected. For diazinon a significant negative effect was observed in the wine matrices when compared to the hydroalcoholic solution. Conversely, a moderate positive matrix effect was observed for fenitrothion. These results are in line with many SPME studies that show that this technique is sensitive to matrix effects and that the use of matrix-matched calibration standards is advisible.

Limits of Detection and Quantification. The estimated limits of detection (LODs) and limits of quantification (LOQs) are presented in Table 2. LODs and LOQs were calculated from the calibration curves according to the method of Miller and Miller.[28] LOQs were validated by taking into account the results obtained for the intermediate precision (IP) and recovery (R) studies and the French Standard NF T 90-210.[29]

Fenthion presented the lowest LOD and LOQ values, 0.05 and 0.16 μg/L respectively, in FWW, whereas the highest LOD and LOQ values, 72.35 and 219.23 μg/L, respectively, were obtained for azoxystrobin, in FRW. Besides azoxystrobin, other analyzed compounds presented low detector responses, such as folpet, iprodione, and fenhexamid. Higher LODs and LOQs were obtained for some pesticides in FRW, possibly due to the higher contents of polyphenolic compounds, making FRW a more complex matrix.

The LODs and LOQs presented on this study were compared with those reported in the literature for other multiresidue methods (Table 2); the values provided by this method are generally lower than the ones from the literature. Moreover, the LOD and LOQ values reported in the literature were obtained for still wines, which are less complex matrices, with lower amounts of phenolic compounds, sugars, and alcohol. Lower LOQs were reported for some compounds when SPE[17] and QuEChERS[23] extraction methods were used. However, SPME has many advantages over the other extraction methods, allowing a very simple, solvent-free extraction and an automated procedure. For routine analysis, the only step necessary is to measure the sample for the extraction vial and to place it in the autosampler.

Correia et al.[19] proposed a more sensitive methodology for some of the studied compounds by SPME-GC-ECD. However, and even though electron capture detection is highly sensitive for some compounds, another methodology would be required for confirmation. Nevertheless, all of the LOQ values validated in this study are far below of the MRLs set for grapes by the European Community,[2] and almost all of the values are below of the MRLs that have been suggested for wine by Otteneder and Majerus, which correspond to $^{1}/_{10}$ of the MRLs set for grapes.[7] The exceptions are the pesticides diazinon, metalaxyl, fenitrothion, and azoxystrobine. Even so, LOQs obtained for these compounds are close to MRL values suggested for wine.

By analysis of the chromatograms (Figure 2) obtained for relatively low concentrations (148 and 199 μg/L, in each pesticide) of two of the most problematic compounds, iprodione and folpet, it is possible to verify that the equipment sensitivity allows achieving lower LOQ values than those estimated, if a concentration level presenting a signal-to-noise ratio of 10 is considered.

Precision and Accuracy. Precision in conditions of repeatability (r) was determined for the wine blend samples spiked with pesticides at three different concentration levels, in FWW and FRW matrices. Data from three analyses, performed on the same day, were used in the calculations. Different concentration levels were chosen for the different pesticides based on the MRLs set for grapes and also on the analytical signal of the compound. The spiking levels used for the precision and accuracy studies ranged between 0.16 and 1.01 μg/L for fenthion and between 147 and 926 μg/L for iprodione. The r data, expressed as relative standard deviation (RSD, %), are summarized in Table 3. Good results were obtained for almost all of the tests (RSD $\leq$ 20%), according to EC SANCO/2009/10684.[30] Values of RSD $\leq$ 5% were obtained for some pesticides, even at low concentration levels. The lowest value of r was obtained for iprodione in FRW, spiked

Table 3. Results of Repeatability (r), Intermediate Precision (IP), and Recovery (R) Studies for the Analyzed Pesticides in Fortified White Wines (FWW) and Fortified Red Wines (FRW) (n = 3)

	FWW				FRW			
pesticide	spiking (μg/L)	r (RSD, %)	IP (RSD, %)	R (%)	spiking (μg/L)	r (RSD, %)	IP (RSD, %)	R (%)
diazinon	1.59	15.7	6.8	72.7	1.58	17.9	27.8	102.7
	2.65	12.5	8.6	80.0	2.63	14.6	17.4	94.7
	9.92	2.7	11.0	84.1	9.87	12.3	16.7	83.5
chlorothalonil	14.52	20.7	7.0	92.2	14.77	4.2	19.5	88.2
	24.20	8.3	4.8	99.6	24.62	4.4	16.4	97.9
	90.75	6.9	10.2	100.4	92.33	11.6	16.3	93.9
chlorpyriphos-methyl	0.46	16.6	13.0	92.0	0.64	5.2	13.2	95.1
	1.07	8.9	5.6	98.6	1.06	1.4	9.0	101.8
	4.01	6.2	5.1	98.6	3.98	6.5	13.5	94.1
vinclozoline	17.50	14.5	3.5	104.1	18.07	7.4	7.4	101.7
	29.16	4.1	6.5	102.8	30.11	1.8	7.1	101.2
	109.36	4.6	3.7	99.4	112.92	2.4	10.1	105.5
metalaxyl	42.30	10.7	15.1	121.3	70.33	5.0	24.3	89.8
	70.50	5.7	6.9	104.0	105.50	9.9	15.8	104.3
	264.39	3.9	9.3	94.5	263.74	5.3	12.1	115.3
fenitrothion	0.95	17.7	10.0	104.7	1.58	4.4	12.4	101.5
	1.59	6.4	14.3	100.8	2.38	2.8	19.6	98.2
	5.95	5.2	11.0	97.8	5.94	2.1	10.2	111.7
malathion	8.14	13.3	9.7	112.6	13.12	12.7	14.2	114.1
	13.57	6.5	13.9	108.1	19.68	6.7	14.1	113.6
	27.14	2.7	13.6	99.6	49.19	6.1	7.0	102.6
chlorpyriphos	1.27	7.8	12.5	95.7	1.27	6.4	11.8	99.0
	2.11	5.2	3.6	101.2	2.11	2.1	7.0	98.3
	7.92	6.0	3.5	99.2	7.92	8.6	9.1	103.3
fenthion	0.16	13.4	11.1	105.5	0.16	15.7	6.0	97.9
	0.27	10.8	7.4	97.5	0.27	6.2	7.1	98.9
	1.01	6.3	6.4	98.9	1.01	6.1	10.2	103.1
cyprodinil	7.11	9.4	16.3	98.9	7.11	6.5	19.0	94.5
	11.85	6.5	5.7	98.5	11.84	4.8	15.9	100.2
	44.45	2.8	5.3	98.5	44.41	14.5	14.5	95.3
procymidone	22.40	13.3	18.2	87.1	22.41	10.2	15.3	92.7
	37.33	8.0	9.4	101.2	37.35	7.0	11.5	98.8
	139.97	2.5	4.9	97.0	140.05	1.1	5.5	102.5
folpet	119.58	18.7	6.1	91.9	119.19	27.3	12.7	77.8
	199.30	15.3	7.7	80.4	198.66	19.4	15.1	76.2
	747.38	19.0	7.3	69.9	744.96	14.3	13.4	83.7
flusilazole	6.94	7.8	12.8	96.2	11.29	4.1	18.5	102.0
	11.57	7.6	8.6	94.6	16.94	2.5	20.2	98.1
	43.40	2.5	2.6	98.4	42.35	6.1	13.3	103.6

Table 3. Continued

	FWW				FRW			
pesticide	spiking (μg/L)	r (RSD, %)	IP (RSD, %)	R (%)	spiking (μg/L)	r (RSD, %)	IP (RSD, %)	R (%)
kresoxim-methyl	10.18	12.2	7.3	92.1	10.19	1.4	8.3	99.1
	16.97	7.0	4.9	97.1	16.98	2.5	8.7	92.7
	63.64	4.2	8.1	100.5	63.68	6.4	13.0	106.6
trifloxystrobin	11.30	7.0	16.4	96.0	11.40	6.0	14.8	108.7
	18.84	6.8	8.0	97.7	19.00	0.8	15.9	100.7
	70.63	4.6	3.8	96.02	71.24	5.6	13.8	111.9
fenhexamid	103.31	10.9	20.3	99.0	172.41	3.4	17.2	104.2
	172.18	9.1	17.3	114.0	258.61	3.3	20.7	95.2
	645.67	8.2	7.5	101.7	646.53	1.1	12.4	114.7
tebuconazole	92.25	24.5	19.6	91.3	92.47	5.1	20.6	98.1
	138.38	11.9	15.8	91.9	138.71	1.1	23.8	127.5
	345.94	5.9	9.9	100.4	346.77	2.9	18.3	117.7
iprodione	147.00	4.5	9.9	105.9	148.11	4.8	17.7	103.0
	245.00	2.8	6.2	106.0	246.84	0.8	13.2	94.2
	918.74	1.0	5.5	101.1	925.66	5.3	13.2	100.5
fenoxycarb	41.75	8.7	11.3	87.5	40.60	6.3	10.6	93.0
	69.59	4.0	3.2	96.7	67.67	11.0	8.9	87.6
	260.96	9.9	11.9	85.5	253.75	16.0	13.6	82.4
λ-cyhalothrin	5.79	15.4	17.3	102.8	5.84	8.2	19.2	98.7
	9.64	11.7	7.1	100.2	9.74	3.3	10.4	108.4
	36.15	11.0	4.8	94.6	36.53	10.8	5.3	96.6
β-cyfluthrin	13.42	12.9	13.9	108.0	13.31	7.3	13.2	98.8
	22.37	10.1	6.3	102.2	22.19	5.7	15.1	97.2
	83.88	8.1	7.9	93.3	83.20	8.4	11.8	103.1
cypermethrin	6.307	15.4	12.5	99.4	6.25	9.7	10.0	105.5
	10.50	10.3	5.0	97.3	10.42	9.2	16.2	97.2
	39.39	6.1	6.9	94.7	39.08	10.8	12.1	100.9
deltamethrin	6.76	4.8	19.0	111.5	6.52	3.2	17.6	107.6
	11.27	2.6	1.1	96.8	10.86	5.3	14.3	97.7
	42.25	1.8	8.9	102.6	40.73	9.7	10.5	98.5
azoxystrobine	131.83	14.1	18.0	103.7	131.40	5.0	5.1	115.4
	219.72	10.6	9.2	96.9	219.23	3.9	5.0	91.0
	823.96	14.1	7.3	89.5	822.11	5.7	7.2	99.8

at 246.84 μg/L (0.8%), whereas the highest value was obtained for folpet, also in FRW, spiked at 119.19 μg/L (27.3%).

Intermediate precision (IP), using data from three analyses performed on different days, was assessed in wine samples spiked with pesticides at three different concentration levels. IP results, expressed as RSD (%), are also presented in Table 3. As in the case of repeatability, good results were obtained for almost all of the tests (RSD $\leq$ 20%). The lowest value of IP was obtained for deltamethrin in FWW, spiked at 11.27 μg/L (1.1%), and the highest value for IP was obtained for diazinon, in FRW, spiked at 1.59 μg/L (27.8%).

Accuracy data were provided by recovery experiments, R, expressed in percent (Table 3). Good recoveries were achieved for the majority of the studied pesticides, according to EC SANCO values reported (recovery values between 70 and 120%).[30] R values ranged between 69.9% for folpet in FWW,

Table 4. Results Obtained for the Analytes Included in Four Successive Interlaboratory Essays (ILE)

	ILE 1, red wine			ILE 2, red wine			ILE 3, red wine			ILE 4, white wine		
pesticide	concn found[a] (μg/L)	concn[b] (μg/L)	Z score	concn found (μg/L)	concn (μg/L)	Z score	concn found (μg/L)	concn (μg/L)	Z score	concn found (μg/L)	concn (μg/L)	Z score
azoxystrobin	202	120	1.46	111	67	1.57	95	44	3.00			
chlorpyriphos	63	57	0.50	141	141	0.39	107	102	0.30	14	19	−0.80
cyprodinil	72	92	−0.53	32	25	0.56	288	180		36	60	−0.94
fenitrothion	119	102	0.40	170	170	0.40	26	19	1.33	65	81	−0.27
fenhexamid	121	67	1.86	89	90		203	188	0.31	<103	42	
fluzilazole	160	134	0.28	45	47	−0.06	80	58	1.47	520	758	−1.31
iprodione	112	24	2.00	191	131	0.60	129	96	1.24	<147	44	
metalaxyl	102	87	0.62	57	26	3.75	60	36	3.00	54	75	−0.64
procymidone	76	64	0.80	168	149	0.72	102	82	0.89	102	120	−0.35
tebuconazole	141	107	0.55	130	131	0.26	<45	36				
vinclozoline	45	43	0.13	40	41	0.08	153	118	0.96	87	99	−0.14

[a] concentration estimated by the proposed method. [b] Accepted analyte concentration.

Table 5. Characterization of the Analyzed Wines

	minimum	maximum
alcoholometric title (%, v/v) (20 °C)	19.00	22.00
pH	3.20	3.70
volatile acidity (g/L acetic acid)	0.20	0.90
total acidity (g/L tartaric acid)	3.5	5.5
reducing sugars (g/L)	90.0	110.0
glycerol (g/L)	4.0	8.0

spiked at 747.38 μg/L, and 127.5% for tebuconazole in FRW, spiked at 138.38 μg/L.

Application to Analysis of Real Wine Samples. The proposed method has been used in our laboratory as part of international interlaboratory proficiency studies promoted by Bureau Inter-Professional d'Etudes Analytiques (BIPEA), Gennevilliers, France. The testing scheme followed the recommendations found in the ISO 43-1 guide "Proficiency testing of laboratories by intercomparison". These interlaboratory studies did not apply to all of the compounds tested, but just to some of them as listed in Table 4. The results show that the method can be applied to the qualitative and quantitative determination of these pesticide residues in real wine samples, with very acceptable *Z* scores (most of them <2.0), in both red and white wines.

The presented methodology was applied to 17 commercial fortified wines, and residues of the analyzed pesticides were not detected. Some characteristics of the wine samples are presented in Table 5. No additional information regarding whether the studied compounds were used by the producers was obtained. However, many of these compounds are allowed to be used in grape production, and as discussed in the Introduction, some of them have been reported in previous studies in other wine samples. The results from the interlaboratory studies indicate the method is reliable for the determination of the compounds; their absence in the samples is reassuring for consumers, and the security of the wines, with regard to these parameters, is guaranteed.

Nowadays viticulture and wine production assume an important contribution to society, in both economic and social aspects, around the world. Pesticide residues in wine have been under great scrutiny in the past few decades. The development of analytical methods for pesticide residue analysis is therefore an ongoing task of utmost importance, especially for high-quality products.

Taking into account factors such as the method's performance, and the use of SMPE, an environmentally friendly and automated technique, the proposed multiresidue methodology is considered to be appropriate for the determination of the 24 studied pesticides in fortified red and white wines, with minimal sample preparation. Although matrix effects are likely to occur in SPME, the use of matrix-matched calibration standards allows minimizing these effects. Additionally, MS/MS detection permits a high degree of certainty in analyte identification. The method yields recoveries between 69.9 and 127.5%, and the limits of quantitation (0.16−219.23 μg/L) were, in all cases, significantly lower than the MRLs established for grapes by European regulation and in almost all cases lower than the suggested limits for wine (MRLs/10). The proposed method was applied to 17 commercial fortified wines, and the analyzed pesticides were not detected.

REFERENCES

(1) Wong, J. W.; Halverson, C. A. Multiresidue analysis of pesticides in wines using C-18 solid-phase extraction and gas chromatography−mass spectrometry. *Am. J. Enol. Vitic.* **1999**, *50*, 435–442.

(2) European Parliament and Council. Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in products of plant and animal origin and amending Council Directive 91/414/ECC. *Off. J. Eur. Communities* **2005**, *L 70*,1−16.

(3) Millán, S.; Sampedro, M. C.; Unceta, N.; Goicolea, M. A.; Rodríguez, E.; Barrio, R. J. Coupling solid-phase microextraction and high-performance liquid chromatography for direct and sensitive determination of halogenated fungicides in wine. *J. Chromatogr., A* **2003**, *995*, 135–142.

(4) Cabras, P.; Angioni, A.; Garau, V. L.; Minelli, E. V.; Melis, M.; Pirisi, F. M. Pesticides in the distilled spirits of wine and its byproducts. *J. Agric. Food Chem.* **1997**, *45*, 2248–2251.

(5) Navarro, S.; Barba, A.; Oliva, J.; Navarro, G.; Pardo, F. Evolution of residual levels of six pesticides during elaboration of red wines. Effect of wine-making procedures in their dissappearance. *J. Agric. Food Chem.* **1999**, *47*, 264–270.

(6) Cabras, P.; Angioni, A. Pesticide residues in grapes, wine and their processing products. *J. Agric. Food Chem.* **2000**, *48*, 967–973.

(7) Otteneder, H.; Majerus, P. Pesticide residues in wine, transfer from grapes. *Bull. O.I.V.* **2005**, *78*, 173–181.

(8) Flamini, R.; Panighel, A. Mass spectrometry in grape and wine chemistry. Part II: The consumer protection. *Mass Spectrom. Rev.* **2006**, *25*, 741–774.

(9) Garau, V. L.; Abreu, S. M.; Caboni, P.; Angioni, A.; Alves, A.; Cabras, P. Residue-free wines: fate of some quinone outside inhibitor (QoI) fungicides in the winemaking process. *J. Agric. Food Chem.* **2009**, *57*, 2329–2333.

(10) Čuš, F.; Česnik, H. B.; Bolta, Š.V.; Gregorčič, A. Pesticide residues and microbiological quality of bottled wines. *Food Control* **2010**, *21*, 150–154.

(11) Rial Otero, R.; Yague Ruiz, C.; Cancho Grande, B.; Simal Gándara, J. Solid-phase microextraction−gas chromatographic−mass spectrometric method for the determination of the fungicides cyprodinil and fludioxonil in white wines. *J. Chromatogr., A* **2002**, *942*, 41–52.

(12) Trösken, E. R.; Bittner, N.; Völkel, W. Quantitation of 13 azole fungicides in wine samples by liquid chromatography−tandem mass spectrometry. *J. Chromatogr., A* **2005**, *1083*, 113–119.

(13) Jiménez, J. J.; Bernal, J. L.; del Nozal, M. J.; Bernal, J.; Toribio, L. Persistence and degradation of metalaxyl, lindane, fenvalerate and deltamethrin during the wine making process. *Food Chem.* **2007**, *104*, 216–223.

(14) Čuš, F.; Česnik, H. B.; Bolta, Š.V.; Gregorčič, A. Pesticide residues in grapes and during vinification process. *Food Control* **2010**, *21*, 1512–1518.

(15) Pawliszyn, J.; Arthur, C. Solid phase microextraction with thermal desorption using fused silica optical fibers. *Anal. Chem.* **1990**, *62*, 2154–2148.

(16) Kataoka, H.; Lord, H.; Pawliszyn, J. Applications of solid-phase microextraction in food analysis. *J. Chromatogr., A* **2000**, *880*, 35–62.

(17) Soleas, G. J.; Yan, J.; Hom, K.; Goldberg, D. M. Multiresidue analysis of seventeen pesticides in wine by gas chromatography with mass-selective detection. *J. Chromatogr., A* **2000**, *882*, 205–212.

(18) Correia, M.; Delerue-Matos, C.; Alves, A. Multi-residue methodology for pesticide screening in wines. *J. Chromatogr., A* **2000**, *889*, 59–67.

(19) Correia, M.; Delerue-Matos, C.; Alves, A. Development of a SPME-GC-ECD methodology for selected pesticides in must and wine samples. *Fresenius' J. Anal. Chem.* **2001**, *369*, 647–651.

(20) Wong, J. W.; Webster, M. G.; Halverson, C. A.; Hengel, M. J.; Ngim, K. K.; Ebeler, S. E. Multiresidue pesticide analysis in wines by solid-phase extraction and capillary gas chromatography−mass spectrometry detection with selective ion monitoring. *J. Agric. Food Chem.* **2003**, *51*, 1148–1161.

(21) Zambonin, C. G.; Quinto, M.; De Vietro, N.; Palmisano, F. Solid-phase microextraction−gas chromatography mass spectrometry: a fast and simple screening method for the assessment of organophosphorous pesticides residues in wine and fruit juices. *Food Chem.* **2004**, *86*, 269–274.

(22) Likas, D. T.; Tsiropoulos, N. G.; Miliadis, G. E. Rapid gas chromatographic method for the determination of famoxadone, trifloxystrobin and fenhexamid residues in tomato, grape and wine samples. *J. Chromatogr., A* **2007**, *1150*, 208–214.

(23) Cunha, S. C.; Fernandes, J. O.; Alves, A.; Oliveira, M. B. P. P. Fast low-pressure gas chromatography−mass spectrometry method for the determination of multiple pesticides in grapes, musts and wines. *J. Chromatogr., A* **2009**, *1216*, 119–126.

(24) Economou, A.; Botitsi, H.; Antoniou, S.; Tsipi, D. Determination of multi-class pesticides in wines by solid-phase extraction and liquid chromatography−tandem mass spectrometry. *J. Chromatogr., A* **2009**, *1216*, 5856–5867.

(25) Patil, S. H.; Banerjee, K.; Dasgupta, S.; Oulkar, D. P.; Patil, S. B.; Jadhav, M. R.; Savant, R. H.; Adsule, P. G.; Deshmukh, M. B. Multiresidue analysis of 83 pesticides and 12 dioxin-like polychlorinated biphenyls in wine by gas chromatography−time-of-flight mass spectrometry. *J. Chromatogr., A* **2009**, *1216*, 2307–2319.

(26) Lehotay, S. J.; Son, K. A.; Kwon, H.; Koesukwiwat, U.; Fu, W.; Mastovska, K.; Hoh, E.; Leepipatpiboon, N. Comparison of QuEChERS sample preparation methods for the analysis of pesticide residues in fruits and vegetables. *J. Chromatogr., A* **2010**, *1217*, 2548–2560.

(27) Abreu, S. M. F. M. Estudo dos fungicidas QoI na Vinha e nos Vinhos Portugueses; PhD thesis, University of Porto, 2007.

(28) Miller, J. C.; Miller, J. N. *Statistics for Analytical Chemistry*, 2nd ed.; Wiley: New York, 1989.

(29) NF T 90-210: Qualité de l'eau − Protocole d'évaluation initiale des performances d'une méthode dans un laboratoire, 2009.

(30) European Commission SANCO/2009/10684 − Method validation and quality control procedures for pesticide residues analyses in food and feed, http://www.crl-pesticides.eu/library/docs/fv/guidefinalversion.pdf (accessed Feb 2010).