Analysis of six fungicides and one acaricide in still and fortified wines using solid-phase microextraction-gas chromatography/tandem mass spectrometry

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ABSTRACT

A multiresidue gas chromatographic method for the determination of six fungicides (captan, chlorthalonil, folpet, iprodione, procymidone and vinclozolin) and one acaricide (dicofol) in still and fortified wines was developed. Solid-phase microextraction (SPME) was chosen for the extraction of the compounds from the studied matrices and tandem mass spectrometry (MS/MS) detection was used. The extraction consists in a solvent free and automated procedure and the detection is highly sensitive and selective. Good linearity was obtained with correlation coefficients of regression (R^2) > 0.99 for all the compounds. Satisfactory results of repeatability and intermediate precision were obtained for most of the analytes (RSD 6 20%). Recoveries from spiked wine ranged from 80.1% to 112.0%. Limits of quantification (LOQs) were considerably below the proposed maximum residue limits (MRLs) for these compounds in grapes and below the suggested limits for wine (MRLs/10), with the exception of captan.

Keywords: Fungicide, Acaricide, SPME, GC–MS/MS, Wine

1. Introduction

Monitoring of pesticide residues has received much attention in the last few years. As regards vineyard protection, the use of these compounds may result in the presence of residues in the wine, thus compromising the safety of this product (Correia, Delerue-Matos, & Alves, 2000; Oliva, Navarro, Barba, & Navarro, 1999; Patil et al., 2009). The presence of pesticides in grapes may result from direct applications and/or from indirect sources, such as contaminated agro-inputs. Besides the health hazards that may be caused by pesticide residues, the sensorial quality of wine may also be altered, affecting the marketability of the product (Patil et al., 2009).

The most common pests of vine are downy mildew (*Plasmopara viticola*), powdery mildew (*Uncicula necator*) and gray mold (*Botrytis cinerea*) (Cabras & Angioni, 2000; Garau et al., 2009; González-Rodríguez, Cancho-Grande, Torrado-Agrasar, Simal-Gándara, & Mazaira-Pérez, 2009). Fungicides are intensively used in the prevention/treatment of diseases of grapes for vinification. These compounds are typically applied close to harvest (Sandra et al., 2001). Among the fungicides, iprodione, procymidone and vinclozolin are commonly used in vineyard protection (Garcia, Melgar, & Fernández, 1999; Sandra et al., 2001). Captan and folpet (Cabras & Angioni, 2000; Cunha, Fernandes, Alves, & Oliveira, 2009) and chlorthalonil (Patil et al., 2009) are fungicides also used in the pest treatments of grapevines. Dicofol is widely used as an acaricide and it is also applied in vineyard protection (Soleas, Yan, Hom, & Goldberg, 2000). Although the correct use of pesticides does not cause a threat to human health and the environment, inappropriate treatments of crops may result in undesirable pesticide residues in grapes that can be transferred to the wine (González-Rodríguez et al., 2009).

During vinification, pesticides are subjected to a number of steps that reduce significantly the residue levels, so their contents in wines are significantly lower than in grapes (Cabras & Angioni, 2000; Cabras et al., 1997; Flamini & Panighel, 2006; Navarro, Barba, Oliva, Navarro, & Pardo, 1999; Otteneder & Majerus, 2005). Therefore, sensitive and selective analytical methods are required to detect pesticide residues in wine.

Until now, the European Commission (EC) has established pesticide maximum residue levels (MRLs) in grapes, but not in wine. Since 1st September 2008, a new legislative framework on pesticide residues is applicable (EC Regulation No. 396/2005) completing the harmonisation and simplification of the pesticide maximum residue levels throughout the European Union. However, pesticides MRLs for wine have been suggested in order to guarantee as much as possible the safety of the beverage. Otteneder and Majerus (2005) suggested that a limiting value could be estimated considering a reduction of 90% of the pesticide maximum residue levels in grapes, thus reaffirming the necessity of effective and sensitive methods to detect pesticide residues in wine.

Solid phase microextraction (SPME) is an alternative extraction method to traditional techniques, allowing complete elimination of

solvents, blanks reduction and a decrease in extraction time. This method does not require complete removal of the analyte from the liquid matrix (Kataoka, Lord, & Pawliszyn, 2000) and can be used for a wider range of applications than other techniques such as solid phase extraction (SPE), which requires an exhaustive extraction (Pawliszyn & Arthur, 1990). The SPME simplicity of use, relatively short sample processing time and fibre reusability have made this method an attractive choice for many analytical applications such as the analysis of environmental, food, aromatic, forensic and pharmaceutical samples (Ouyang & Pawliszyn, 2008). The need for higher selectivity and sensitivity, as well as the necessity for confirmation have been successfully achieved by coupling gas chromatography (GC) or liquid chromatography (LC) with mass spectrometry (MS) and tandem mass spectrometry (MS/MS) (Economou, Botitsi, Antoniou, & Tsipi, 2009; Flamini & Panighel, 2006; Hiemstra & Kok, 2007). The use of these chromatographic techniques coupled with MS or MS/MS, for the determination and/or confirmation of pesticides in still wine has been reported by some authors (Angioni et al., 2003; Cunha et al., 2009; González-Rodríguez et al., 2009; Likas, Tsiropoulos, & Miliadis, 2007; Patil et al., 2009; Rose, Lane, & Jordan, 2009; Sandra et al., 2001; Soleas et al., 2000; Vitali, Guidotti, Giovinazzo, & Cedrone, 1998; Zambonin, Quinto, De Vietro, & Palmisano, 2004).

Although many papers have been published regarding the determination of pesticide residues in wine, the application of these methods in the analysis of wines showing higher sugar and alcoholic levels is far more reduced. In this work, a SPME-GC–MS/MS method was validated for the determination of six fungicides (captan, chlorthalonil, folpet, iprodione, procymidone and vinclozolin) and one acaricide (dicofol) both in still and in fortified wines (white and red). 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)) while ‘‘still wine’’ refers to a wine obtained by complete fermentation, thus having lower sugar and alcoholic contents.

2. Materials and methods

2.1. *Solutions and reagents*

Captan (99.1%), chlorthalonil (99.2%), folpet (99.9%), procymidone (99.9%), vinclozolin (99.5%), iprodione (99.3%) and dicofol (97.6%) analytical standards were supplied by Riedel-de Häen (Seelze, Germany).

Individual pesticide stock solutions (1 g/L) where prepared in methanol (99.9%), supplied by Riedel-de Häen (Seelze, Germany), and stored under refrigeration (2–6 °C). A stock standard mixture solution containing all pesticides (7.97 mg/L captan, 7.98 mg/L chlorthalonil, 1.66 mg/L dicofol, 7.16 mg/L folpet, 28.97 mg/L procymidone, 3.82 mg/L vinclozolin and 104.7 mg/L iprodione) was also prepared in methanol, weekly, and stored under refrigeration (2–6 °C).

Matrix-matched standards (at six levels for captan, chlorthalonil, dicofol, procymidone and vinclozolin and at five levels for folpet and iprodione) were prepared in different types of wine, previously analyzed for the absence of compounds. Four experimental blends of wine (blends of still white wine, still red wine, fortified white wine and fortified red wine), characterized in Table 1, were obtained from commercial wines in order to achieve a representative composition and a sufficient volume of wine for all the study. The wine blend samples were spiked with different volumes of the standard mixture solution. The quantification was based on six-point external calibration graph (five-point in the case of folpet and iprodione) obtained by plotting the peak areas against the the-oretical standard concentration.

2.2. *SPME procedure*

SPME extraction was performed in a Combipal MH 01-00B autosampler (CTC Analytics AG, Zwingen, Switzerland). SPME fibres (Supelco, Bellefonte, USA) were conditioned according to the sup-plier's instructions. The extraction procedure was performed using 20 mL clear glass vials (La-Pha-Pack, Langerwehe, Germany). Wine blend samples of 19 mL were extracted by immersion of a 100 μm polydimethylsiloxane (PDMS) coated fibre. The extraction condi- tions were: extraction temperature 35 °C, agitator speed 250 rpm and extraction time 60 min. After extraction and desorption, fibre conditioning was performed for 5 min in the presence of nitrogen (99.995%).

2.3. *GC analysis*

Gas chromatographic analyses were performed in a FocusGC, equipped with a split/splitless injector (Thermo Fisher Scientific, Waltham, MA, USA). The analytical column used was a TR-5MS (30 m × 0.25 mm ID × 0.25 μm film thickness) coated with 5% phenyl methylpolysiloxane stationary phase (Thermo Fisher Scientific, Waltham, MA, USA). The carrier gas, high-purity helium (99.9999%), maintained at a constant pressure of 50 kPa, was also used as the collision gas at the ion trap chamber for MS/MS tests. The split/splitless injection port was maintained in splitless mode for 3 min and set at a fixed temperature of 250 °C. SPME desorption was carried out in the injector port for 6 min. The oven tempera- ture programme used for the analyses was the following: initial temperature 80 °C for 5 min, raised to 300 °C at a rate of 5 °C/ min and kept for 10 min.

Gas chromatographic conditions were based on the study devel- oped by Vitali et al. (1998) with few adaptations. The initial oven temperature was kept during 5 and not during 2 min, and the final oven temperature was 300 °C instead of 250 °C.

2.4. *Mass spectrometry detection*

Pesticide retention times were determined in full scan mode, after which the MS/MS conditions were optimized in order to allow the correct identification of each pesticide, as well as good signal to noise ratios for MS/MS detection. A PolarisQ ion trap mass spec- trometer (Thermo Fisher Scientific, Waltham, MA, USA) operated in the electron impact (EI) mode was used. The ion source and transfer line temperatures were set at 250 °C and 280 °C, respectively. The emission current of the ionization filament was set at 250 μA, generating electrons with 70 eV and the electrons multi- plier voltage was 1850 V. The 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. Some MS/MS conditions used are listed in Table 2. Quantification of each analyte was performed based on total ion count (TIC) after the fragmentation of the selected parent ion or on the base peak (Table 2). Instrument control and data acquisition were managed by a personal computer running the X-Calibur software (version 1.4).

The parent ions were chosen according to the MS spectra obtained in full scan mode (ions with the highest relative abundance), and considering the data on the pesticide's parent ions referred by other authors in the literature. Five excitation voltage values were studied (1, 1.1, 1.2, 1.3 and 1.4) for each pesticide and for the parent ion. The optimum values were selected.

Table 1

Characterization of the wine blends used in this study.

Wine blends	Alcoholometric title (%, v/v) (20 °C)	pH	Volatile acidity (g/L acetic acid)	Total acidity (g/L tartaric acid)	Reducing sugars (g/L)	Glycerol (g/L)
Still white	12.77	3.24	0.45	5.77	18.6	7.1
Still red	14.57	3.64	0.53	5.17	2.0	10.6
Fortified white	19.30	3.58	0.22	3.26	106.2	3.0
Fortified red	19.27	3.69	0.23	4.28	106.6	4.5

Table 2

MS/MS conditions used in the proposed SPME-GC–MS/MS method.

Pesticide	Isolation		Excitation		MS/MS quantification	MS/MS confirmation
	Parent ion	Time	Voltage	Excitation energy (q		
Captan	264	12	1.2	0.3	236[a]	236. 130. 204. 102
Chlorthaloni	266	12	1.1	0.3	TIC	266. 231. 170. 205. 133
Dicofol	139	12	1.1	0.3	TIC	111. 139. 75
Folpet	130	12	1.3	0.3	102[a]	102.115. 87. 75
Iprodione	314	12	1.1	0.3	TIC	245. 271. 188
Procymidon	283	12	1.1	0.3	TIC	255. 220. 173. 146
Vinclozolin	212	12	1.2	0.3	TIC	172, 177, 145, 212

[a] Base peak; TIC – total ion count.

3. Results and discussion

3.1. SPME conditions

In a multiresidue method, the operational conditions used hardly match the optimum conditions for each analyte. Thus, compromise conditions have to be selected. Several types of fibre coatings are commercially available for SPME. The affinity of each type of fibre depends on the compounds characteristics (principle of ‘‘like dissolves like’’). Non-polar PDMS fibre is preferred for the extraction of non-polar analytes. However, it can also be applied successfully to more polar compounds. This fibre coating is very rugged and is able to withstand high injector temperatures, up to about 300 °C (Kataoka et al., 2000). Besides these reasons, the PDMS fibre has been chosen because of its very low carryover between samples when compared with other type of coatings (Reyzer & Brodbelt, 2001).

The use of PDMS fibre coating for the extraction of pesticides in wine has been reported by some authors. Vitali et al. (1998) reported a SPME-GC–MS method for the determination of fourteen pesticide residues in wine, such as vinclozoline, procymidone, captan and folpet, using a 100 µm PDMS coated fibre. Correia, Delerue-Matos, and Alves (2001) developed a SPME-GC-ECD methodology for eight pesticides, including vinclozolin, procymidone, iprodione and folpet, in must and wine samples. The PDMS fibre coating was selected after preliminarily comparing the 100 µm PDMS and 85 µm polyacrilate (PA) fibres. Despite of the slightly higher performance of the PA coating over the PDMS for extracting the analytes from the wine matrix, the relative standard deviations were higher for the PA fibre (Correia et al., 2001). Sandra et al. (2001) reported a successful extraction procedure for the analysis of dicarboximide fungicides (iprodione, procymidone and vinclozoline) in wine using a PDMS coated stir bar sorptive extraction (SBSE) in combination with thermal desorption-capillary GC–MS analysis (TD-cGC–MS). Based on the referred studies, the PDMS fibre was selected for the present study.

Although SPME has a maximum sensitivity when equilibrium conditions are attained, full equilibration is not necessary for accurate and precise analysis (Kataoka et al., 2000). In this study an extraction time of 60 min was chosen enabling reproducible results and adequate sensitivity. An agitator speed of 250 rpm was suggested by the Combipal MH 01-00B autosampler supplier, allowing good reproducibility and at the same time to enhance the life time of the SPME fibre.

3.2. *Confirmation of the residues*

Pesticides were identified by retention time windows of the tar- get compound and comparison of the product ion mass spectra (MS/MS spectra) with the product ion mass spectra of standards, in wine matrix.

3.3. *Calibration and linearity*

Calibration curves were obtained for all pesticides by spiking the wine blend samples at six concentration levels for captan, chlorthalonil, dicofol, procymidone and vinclozolin and at five con- centration levels for folpet and iprodione. This procedure was re- peated in five different days and for the four different wine matrices. At the end, five calibration curves were obtained for each pesticide and for each matrix. The calibration ranges were chosen in order to include values lower than one tenth of the MRLs estab- lished for grapes (MRL/10), with the exception of captan which presented simultaneously a low response signal and a low MRL that did not permit to achieve that concentration level. Pesticides retention times and calibration ranges used, as well as the calcu-lated LOQs for each pesticide and the respective MRLs for grapes (EC Regulation No. 396/2005) are shown in Table 3.

The values of the slope and the correlation coefficient of regres- sion (R^2) of representative calibration curves obtained for each pes- ticide in the matrices studied are presented in Table 4. Good linearity was achieved for the majority of the pesticides in all matrices ($R^2 > 0.99$).

3.4. *Repeatability and intermediate precision*

The repeatability was assessed using wine blend samples spiked with pesticides at two different concentration levels. The tests were performed at least in four independent preparations and for all wine matrices. The results were expressed as relative standard deviation (RSD, %) (data not shown). According to EC SANCO (2009), good results were obtained for the majority of the analytes

Table 3

Retention times, calibration ranges, limits of quantification for each pesticide and respective MRLs for grapes according to EC Regulation No. 396/2005.

Pesticide	Retention time (min)	Range (Ig/L)	LOQ (Ig/L)	MRL
Captan	35.70	10.48–204.39 [ad]; 20.92–	52.10	20
Chlorthalonil	29.73	6.30–204.70	20.95	3000
Dicofol	33.86	1.31–42.68	4.37	2000
Folpet	35.71	37.59–753.72 [abc]; 18.82–	93.60	5000
Iprodione	41.91	137.53–2683.65	274.70	10.000
Procymidone	35.24	22.85–742.85	76.04	5000
Vinclozolin	31.19	3.01–97.97	10.03	5000

[a] Still white wine.

[b] Still red wine.

[c] Fortified white

Table 4

Slopes and correlation coefficients of regression (R^2) of representative calibration curves obtained, for each pesticide, in still and fortified wines.

Pesticide	Still white wine		Still red wine		Fortified white wine		Fortified red wine	
	Slope	R^2	Slope	R^2	Slope	R^2	Slope	R^2
Captan	175.7	0.9989	136.5	0.9978	253.4	0.9969	114.9	0.9977
Chlorthalonil	28515.4	0.9999	12054.0	0.9933	15336.4	0.9998	15505.9	0.9978
Dicofol	163128.0	0.9924	142562.0	0.9997	90520.0	0.9957	82582.4	0.9979
Folpet	362.3	0.9987	311.6	0.9941	506.9	0.9939	260.9	0.9939
Iprodione	152.5	0.9994	122.0	0.9937	43.6	0.9958	42.6	0.9989
Procymidone	6791.6	0.9994	4824.7	0.9991	2749.1	0.9964	2993.4	0.9934
Vinclozoline	77567.4	0.9996	57594.3	0.9999	33211.4	0.9981	35767.2	0.9996

Table 5

Intermediate precision (*n* P 4), expressed as RSD (%), and recovery, Rec. (%), for the target pesticides in white and red wines.

Pesticide	Conc. (Ig/L)	Still white wine			Still red wine			Fortified white wine			Fortified red wine		
		RSD% (area)	RSD% (est	Re	RSD% (area)	RSD% (est	Re	RSD% (area)	RSD% (est	Re	RSD% (area)	RSD% (est	Re
Captan	419.55				31.9	1.5	101.	41.8	2.6	99.			
	204.40	30.0	6.6	95.	33.6	6.3	95.	30.3	12.1	104.	61.8	22.0	92.
	103.52	23.5	7.3	89.	36.2	8.3	90.	40.1	16.4	91.	38.7	9.0	96.
	52.10	15.0	9.9	94.	61.1	13.0	97.	38.7	7.5	94.	26.3	13.5	95.
Chlorthalon	204.70	8.6	0.8	100.	21.2	0.8	101.	37.7	1.5	100.	54.4	1.1	99.
	103.68	8.4	2.7	97.	21.2	2.9	94.	33.6	5.2	98.	53.1	4.1	102.
	52.18	8.9	4.4	94.	23.8	3.3	95.	26.3	6.4	101.	53.5	2.1	105.
	20.95	11.2	7.8	97.	28.9	5.6	97.	30.0	7.9	102.	55.8	5.2	93.
Dicofol	42.68	17.6	2.3	100.	12.5	1.5	99.	61.4	2.3	98.	36.9	1.7	98.
	21.62	11.7	6.6	100.	12.7	5.3	100.	46.0	7.4	106.	36.5	3.3	103.
	10.88	8.3	4.9	102.	14.5	4.5	104.	39.4	8.0	107.	38.5	2.4	103.
	4.37	16.7	18.9	98.	18.4	2.0	90.	50.5	14.0	92.	39.7	6.3	91.
Folpet	753.72	44.7	2.0	101.	33.3	2.2	100.	40.1	3.3	98.			
	367.20	29.7	8.8	96.	32.1	9.2	97.	25.1	14.4	111.	39.9	2.4	100.
	185.98	27.7	4.6	93.	35.8	5.7	91.	48.7	22.8	88.	32.1	11.8	95.
	93.60	22.3	6.8	99.	43.8	9.8	97.	41.7	10.9	92.	33.1	7.5	103.
Iprodione	2683.65	10.2	0.8	101.	16.7	0.7	101.	88.1	1.6	100.	18.4	1.4	98.
	1359.25	7.0	2.8	94.	12.1	3.4	94.	76.4	7.2	96.	12.1	4.6	103.
	684.07	4.1	5.3	92.	13.9	2.0	96.	63.8	6.1	98.	17.9	10.6	112.
	274.70	9.0	6.0	104.	14.3	6.5	103.	56.0	21.7	103.	22.2	18.0	80.
Procymido	742.85	7.1	0.6	99.	7.9	1.0	98.	53.0	0.7	98.	17.1	1.0	98.
	376.25	9.1	2.8	100.	5.9	4.0	103.	50.7	2.5	104.	15.5	3.5	106.
	189.35	9.0	3.4	101.	9.1	4.0	107.	45.4	3.7	106.	14.7	2.1	105.
	76.04	8.1	4.4	99.	11.9	4.8	91.	49.0	9.6	95.	15.1	4.8	95.
Vinclozolin	97.97	7.9	0.6	100.	6.2	0.4	99.	54.1	1.0	99.	23.2	0.8	98.
	49.62	7.8	2.2	99.	7.4	1.4	101.	47.2	3.2	101.	21.5	2.5	103.
	24.97	8.9	3.7	98.	6.8	2.9	103.	42.3	4.7	104.	21.8	3.5	105.

	10.03	11.8	7.1	97.	9.9	2.1	96.	49.7	7.9	98.	21.9	5.4	95.

est. conc. – estimated concentration.

(RSD ≤ 20%). Captan and folpet were the most problematic compounds, regarding the repeatability results, presenting RSD ≥ 20% for some concentration levels (RSD in the range 13.4–33.4% and 14.1–37.9%, for captan and folpet, respectively). This was probably due to a partial degradation in the injector or/and during the chromatographic analysis. Values of RSD ≤ 5% were obtained for some pesticides, such as vinclozolin and procymidone, even at low concentration levels. The lowest RSD was obtained for chlorthalonil, in still red wine fortified at 204.70 Ig/L (1.2%), while the highest value was obtained for folpet, in still red wine, fortified at 367.20 lg/L (37.9%).

The intermediate precision was assessed using at least four independent preparations for each wine matrix. Each preparation was performed in a different day. Intermediate precision results, expressed as RSD (%), are shown in Table 5. These results are presented according to two different calculation options: (i) RSDs

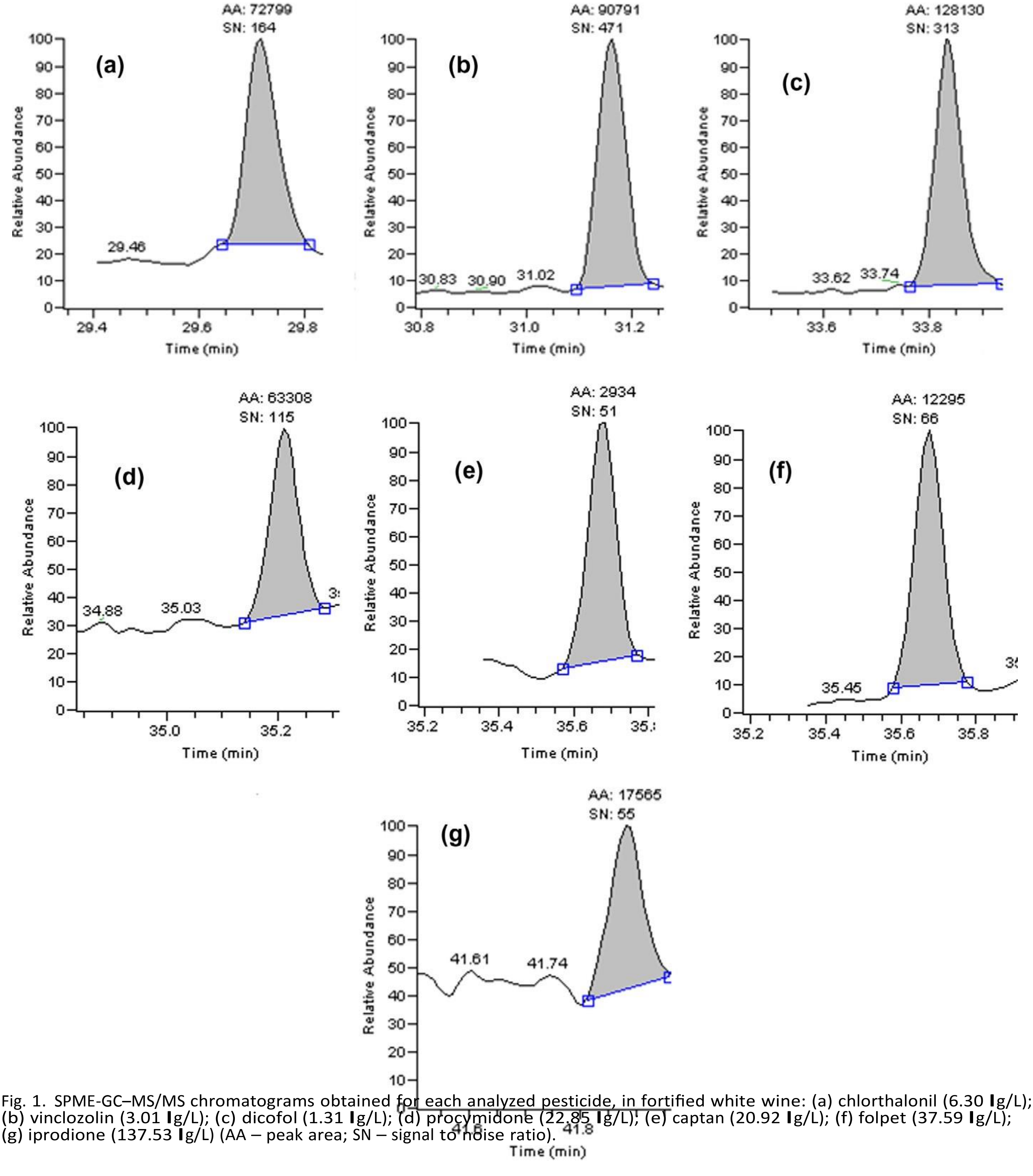

Fig. 1. SPME-GC–MS/MS chromatograms obtained for each analyzed pesticide, in fortified white wine: (a) chlorthalonil (6.30 Ig/L); (b) vinclozolin (3.01 Ig/L); (c) dicofol (1.31 Ig/L); (d) procymidone (22.85 Ig/L); (e) captan (20.92 Ig/L); (f) folpet (37.59 Ig/L); (g) iprodione (137.53 Ig/L) (AA – peak area; SN – signal to noise ratio).

calculated using the obtained areas in the different days of analysis and (ii) RSDs calculated using ''*estimated concentration values*''. As mentioned before, five independent calibration curves were obtained for each pesticide in each wine matrix. As expected, different calibration curves were obtained in different days as a consequence of the variation in the response of the chromatographic system. Thus, considerable variations were notorious when comparing the values of the areas obtained for a pesticide, in a matrix, at the same concentration level, in different days. This situation was overcome working with ''*estimated concentration values*'', obtained substituting the values of the areas in the calibration curve of the respective day of analysis.

The RSDs obtained using ''*estimated concentration values*'' (Table 5) were lower than those based in the peak areas since the first method takes into account the variations in the equipment response, as referred above. The RSDs based on area values are much larger and show clearly the inter-day variation. Thus, considering the RSDs based on ''*estimated concentration values*'', good results were obtained for the intermediate precision for most of the pesticides (RSD ≤ 20%). Generally, the RSD values decreased as the concentration increased, like it would be expected. An exception was observed for captan and folpet possibly due to their instability during the extraction and/or chromatographic steps, as above mentioned. As regards the intermediate precision, the lowest RSD value was obtained for vinclozolin in still red wine fortified at 97.97 µg/L (0.4%) and the highest value was obtained for folpet, in fortified white wine at 185.98 µg/L (22.8%).

3.5. *Recovery*

Recovery results based on ''*estimated concentration values*'' are also presented in Table 5. Good recoveries were achieved for all the studied pesticides, according to EC SANCO (2009) (recovery values between 70% and 120%). Recovery values ranged between 80.1%, for iprodione in fortified red wine at 274.70 µg/L, and 112.0%, for the same compound at 684.07 µg/L. As an example, the graphical representation of the ''*estimated concentration values*'' versus theoretical concentration for the pesticide vinclozolin, in still red wine, based on the results obtained for five different calibration curves in five different days, was described by the following equation: *estimated concentration* = *theoretical concentration* $+ 3 \times 10^{-14}$, with $R^2 = 0.9989$. The intercept is close to zero and the slope value is 1, demonstrating that the estimated concentrations obtained are closed to the theoretical concentration. Similar results were obtained for the other compounds.

3.6. *Limits of quantification*

Limits of quantification (LOQ) were estimated considering the repeatability and intermediate precision studies. For each pesticide, the lowest concentration level tested with RSD values ≤20% was considered (EC SANCO, 2009). LOQ values, expressed as µg/L, are presented in Table 3, as well as the MRLs established for grapes according to EC Regulation No. 396/2005. The two values can be easily compared considering that the density of the wine samples used in this study is very close to 1 (the density of the wine samples ranged between 0.9921–0.9974 for still wines and 1.0208–1.0233 for fortified wines). Otteneder and Majerus (2005) have proposed as MRLs for wine one tenth of the MRLs established for grapes, based on an average pesticide concentration reduction of 90% of the initial levels in grapes, due to the vinification process.

Good LOQs were obtained for all pesticides with the exception of captan. This LOQ (52.10 µg/L) was above the MRL established by the EC Regulation for grapes (20 µg/kg) and consequently above the proposed limit of MRL/10. LOQ values ranged between 4.37 µg/L for dicofol and 274.70 µg/L for iprodione. Analyzing chromatographic peaks obtained for low concentration levels (Fig. 1), it can be seen that the equipment sensitivity allows achieving lower LOQ values than the estimated, if LOQs based in a signal to noise ratio of 10 are considered.

Interlaboratory comparisons, organized by the *Bureau Interprofessionnel d'Études Analytiques* (BIPEA, Gennevilliers, France) showed Z-Score values <2, for three pesticides using the proposedmethod (iprodion, procymidone and vinclozoline), in an organicred wine sample. The other analytes were not included in the inter-laboratory study.

3.7. *Limitations of the proposed method*

Regarding the results presented in Table 4, a significant matrix effect was observed and, for this reason, the analysis using matrix-matched standards is necessary.

Other limitations are related to compounds behaviour during analysis. Among the studied pesticides, some present low detector responses, such as captan, folpet and iprodione. Degradation of thepesticides captan and folpet may occur during GC analysis (Büttler and Hörmann, 1981) and decomposition of iprodione can occur in the GC column above 200 °C, during thermal desorption at 300 °C and in the hot transfer line (Flamini & Panighel, 2006). Nevertheless, folpet and iprodione do not represent a concern to this method because LOQs are 53 and 36 times lower, respectively, than theMRLs for these compounds.

Generally, the estimated LOQs allow ensuring that the MRLs established for grapes and also the suggested MRLs for wine (MRLs for established for grapes/10) are respected, except for captan. Still, captan and folpet do not seem to be problematic compounds inwine quality control since, according to literature, these compounds are totally or almost totally degraded during winemaking(Angioni et al., 2003; Flamini & Panighel, 2006).

4. Conclusions

The proposed method allows the simple, rapid and automated determination of six fungicides and one acaricide in red and white wines, still or fortified. The method showed good recoveries (80.1– 112.0%) and precision, with an overall average repeatability of 11%. With the exception of captan, the limits of quantification were significantly lower than the MRLs established for grapes and than the suggested limits for wine (MRLs/10) making this a very convenient method for the analysis of the selected pesticides in wine samples.

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