Food Chemistry 111 (2008) 764–770

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Solid-phase microextraction and sample stacking micellar electrokinetic chromatography for the analysis of pesticide residues in red wines

Lidia M. Ravelo-Pérez [a], Javier Hernández-Borges [b], Teresa M. Borges-Miquel [a], Miguel Ángel Rodríguez-Delgado [a,*]

[a] Department of Analytical Chemistry, Nutrition and Food Science, University of La Laguna, 38071 La Laguna, Tenerife, Canary Islands, Spain
[b] Departamento de Protección Vegetal, Instituto Canario de Investigaciones Agrarias (ICIA), Apdo. 60, 38200 La Laguna, Tenerife, Islas Canarias, Spain

ARTICLE INFO

Article history:
Received 17 January 2008
Received in revised form 24 March 2008
Accepted 5 April 2008

Keywords:
Red wine
Solid-phase microextraction
Stacking
Pesticides
Micellar electrokinetic chromatography

ABSTRACT

Solid-phase microextraction (SPME) in combination with sample stacking micellar electrokinetic chromatography (MEKC) was studied for the simultaneous determination of 11 multi-class pesticides residues (pirimicarb, metalaxyl, pyrimethanil, procymidone, nuarimol, azoxystrobin, tebufenozide, fenarimol, benalaxyl, penconazole and tetradifon) in red wines samples. Pesticide residues present in the samples were preconcentrated by SPME using poly(dimethylsiloxane)/divinylbenzene (PDMS/DVB) fibers and the injection of large sample volumes into the capillary by reversed-electrode polarity stacking mode (REPSM). Validation of the method was carried out. The combination of both preconcentration procedures (SPME and REPSM) allowed the determination of 10 of these pesticides in red wines at concentrations between 0.049 and 1.69 mg/L (*i.e.*, levels well below the maximum residue limits (MRLs) allowed for these compounds in wine grapes, except for pirimicarb). Repeatability and accuracy of the SPME-MEKC-DAD method was verified by five consecutive extractions of spiked red wine samples at three levels of concentration. Apparent recovery values were in the range 90–107%. The potential of the method was demonstrated by analyzing multiple homemade red wine samples from the Canary Islands and two commercial samples. Only pyrimethanil, procymidone and azoxystrobin were found in the homemade samples; among them, the pesticide most frequently detected was procymidone.

1. Introduction

Wine can be considered one of the most consumed drinks in the world, subjected to strict regulations concerning its quality in regards to truth-to-label and absence of additives. The widespread use of pesticides in grape production has led to the presence of pesticide residues in wines offered commercially for public consumption (Driver, Ginevan, & Whitmyre, 1996; Fan & Jackson, 1989; Gaido et al., 1998). The appearance of pesticide residues in wine depends on several factors, as many as the number of stages comprising the production process (Otteneder & Majerus, 2005). It has even been reported that the aromatic quality of the wine can be modified by the presence of pesticide residues during the fermentation (Aubert et al., 1997; García et al., 2004; Oliva, García, Navarro, Pardo, & Barba, 2001) or that their presence or the presence of their degradation products may also influence negatively the stability of the finished wine, producing colloidal haze (Guguchkina & Ageeva, 1990). As a result, the increased use of agricultural defenses to control parasite attacks on grapes has made the determination and monitoring of the pesticides and their metabolites an important parameter in the quality control of wines.

From a legal point of view, maximum residue limits (MRLs) for grapes have been established by the national guidelines of residues. However no uniform limits have been set for wine (except in few countries around the world) and they are generally regulated through the various national standards for foodstuffs, as a MRL on the wine grapes (although today there is a worldwide trend to lower the MRL to be separated strictly for wine and the legislation is reducing the maximum permitted quantity of pesticides, with a clear intention to reach "zero tolerance" (Cooney, 1996; Pascual, Ros, Fernández, Bernal, & Lacasa, 2004)).

The analytical methods available for the determination of pesticides in wine are numerous and use both gas chromatography (GC) (Hyötyläinen, Jauho, & Riekkola, 1998; Hyötyläinen, Lüthje, Rautiainen-Rämä, & Riekkola, 2004; Jiménez, Bernal, del Nozal, Toribio, & Arias, 2001; Rial-Otero, Yagüe-Ruiz, Cancho-Grande, & Simal-Gándara, 2002; Schellin, Hauser, & Popp, 2004) and high-performance liquid chromatography (HPLC) (Goto et al., 2005; Miliadis, Tsiropoulos, & Aplada-Sarlis, 1999; Millán et al., 2003; Nozal, Bernal, Jiménez, Martín, & Bernal, 2005; Teixeira, Aguiar, Afonso, Alves, & Bastos, 2004). Capillary electrophoresis (CE) complements

* Corresponding author. Tel.: +34 922 31 80 46; fax: +34 922 31 80 03.
E-mail address: mrguez@ull.es (M. Á. Rodríguez-Delgado).

0308-8146/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2008.04.020

and for some applications even replaces classical chromatographic techniques (GC and HPLC) by combining automation with high separation efficiency for low sample amounts (miniaturization). The running costs in CE (low amount of running buffer, aqueous electrolyte and fused-silica capillary) are very low as compared with chromatographic packing materials with which high amounts of organic solvents have to be used (Brumley, 1995). Furthermore, a rapid conditioning of the separation system allows a high flexibility of the analysis methods within the same day. All these together make CE a rather "ecological" separation technique worth being developed for many specific routine applications. However, CE has almost not been explored in the routine analysis of these compounds in wine samples. In fact, to the best of our knowledge there only exist three works in the literature concerning the CE analysis of pesticides in such samples (Molina-Mayo, Hernández-Borges, Borges-Miquel, & Rodríguez-Delgado, 2007; Ravelo-Pérez, Hernández-Borges, Borges-Miquel, & Rodríguez-Delgado, 2007a, 2007b), two of them concerning the analysis of pesticides in white wine samples (Molina-Mayo et al., 2007; Ravelo-Pérez et al., 2007a) and one work relative to rose wines (Ravelo-Pérez et al., 2007b), but there does not exist any work in the literature concerning the CE analysis of pesticides in red wine samples which is a highly complex sample, due to the presence of several compounds like polyphenols, tannins, anthocyanins, etc. One of the main causes may have been the fact that one of the major limitations of CE compared to other techniques like GC or HPLC is its low sensitivity in terms of solute concentration. To overcome this problem, several off-line and on-line preconcentration strategies have been developed with success (Kim & Terabe, 2003; Quirino & Terabe, 2000).

In this work, the simultaneous determination of 11 pesticides (metalaxyl, pyrimethanil, procymidone, azoxystrobin, tebufenozide, fenarimol, benalaxyl and penconazole authorized in Europe and pirimicarb, nuarimol and tetradifon non authorized in Europe) – most of them fungicides, (see Table 1) – in red wines by micellar electrokinetic chromatography (MEKC) is proposed using solid-phase microextraction (SPME) and reversed-electrode polarity stacking mode (REPSM) as off-line and on-line preconcentration techniques, respectively. This methodology was applied to the analysis of 20 red wine samples from the Canary Islands (18 homemade samples and two commercial samples) in order to evaluate the presence of these compounds. The obtained results represent the first data of the literature concerning pesticides content in red wine of the Canary Islands.

2. Materials and methods

2.1. Chemicals and samples

All chemicals were of analytical reagent grade and used as received. Sodium dodecyl sulphate (SDS) and sodium tetraborate were from Sigma–Aldrich (Madrid, Spain). Sodium hydroxide and hydrochloric acid were from Merck (Darmstadt, Germany). Methanol and 1-propanol (HPLC-grade) were from Merck. Pirimicarb (2-dimethylamino-5,6-dimethylpyrimidin-4-yl dimethylcarbamate), metalaxyl (methyl *N*-(methoxyacetyl)-*N*-(2,6-xylyl)-DL-alaninate), pyrimethanil (*N*-(4,6-dimethylpyrimidin-2-yl) aniline), procymidone (*N*-(3,5-dichlorophenyl)-1,2-dimethylcyclopropane-1,2-dicarboximide), nuarimol ((±)-2-chloro-4′-fluoro-α-(pyrimidin-5-yl)benzhydryl alcohol), azoxystrobin (methyl (*E*)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate), tebufenozide (*N*-*tert*-butyl-*N*′-(4-ethylbenzoyl)-3,5-dimethylbenzohydrazide), fenarimol ((±)-2,4′-dichloro-α-(pyrimidin-5-yl)benzhydryl alcohol), benalaxyl (methyl *N*-phenylacetyl-*N*-2,6-xylyl-DL-alaninate), penconazole (1-(2,4-dichloro-β-propylphenethyl)-1H-1,2,4-triazole) and tetradifon (4-chlorophenyl 2,4,5-trichlorophenyl sulfone) obtained from Sigma–Aldrich were used without further purification (purity >99.0%). Standard solutions of each pesticide were prepared in methanol (approximately 1 mg/ml) and kept in the dark under refrigeration at 4 °C. Working mixtures of pertinent concentrations were prepared daily by appropriate combination and dilution. Water was purified by using a Milli-Q system A10 (Millipore, Bedford, MA, USA). All red wine samples were homemade and kindly supplied by local cultivators of the Canary Islands, except two of them (Samples 19 and 20) which were two commercial red wines from the provinces of La Rioja and Ciudad Real (Spain), respectively.

2.2. Capillary electrophoresis conditions

MEKC-DAD analyses were performed in a PACE/5510 CE apparatus (Beckman, Fullerton, CA, USA) equipped with a DAD detector working at 210 nm (except for pirimicarb which was 240 nm). System Gold Software was used for CE instrument control. Bare fused-silica capillaries with 50 μm i.d. were purchased from Composite Metal Services (Worcester, UK). The detection length was 50 cm and the total length was 57 cm. Injections were made at the anodic end using N_2 pressure. Electrophoretic separation was carried out at 25 °C and at +22 kV, using a BGE composed of 100 mM sodium tetraborate and 30 mM SDS at pH 8.5 plus 6% 1-propanol. Before first use, fused-silica capillaries were rinsed (20 psi) with 2 min 1 M hydrochloric acid, 2 min water, 5 min 0.1 M sodium hydroxide, 2 min water and 2 min running buffer. Capillary conditioning was done every morning rinsing at 20 psi with water for 1 min and with background electrolyte (BGE) for 1 min. To achieve a good reproducibility between runs, the following washing protocol was applied (all using 20 psi): 1 min with methanol, 1 min with water and 1 min with BGE. At the end of the day, methanol was passed through the capillary for 1 min and 2 min more with water.

2.3. Reversed-electrode polarity stacking mode (REPSM) conditions

In this on-line preconcentration procedure, the capillary is first filled with the BGE (100 mM sodium tetraborate and 30 mM SDS at pH 8.5 with 6% 1-propanol). Then a large plug of sample is hydrodynamically injected for 11 s at 20 psi (1 psi = 6894.76 Pa). The standard stock solutions and the extracted red wine samples were diluted and reconstituted, respectively with a 1:3 dissolution made of water:100 mM sodium tetraborate at pH 8.5 v:v). A high voltage (−22 kV) is then applied and the electric current monitored to control sample matrix removal from the capillary. When the current becomes 95–99% of the value obtained with the BGE, the voltage is turned off and the polarity reversed to run the separation (Ravelo-Pérez, Hernández-Borges, Cifuentes, & Rodríguez-Delgado, 2007).

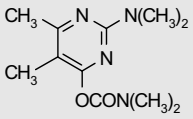
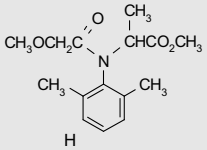
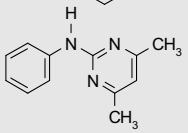
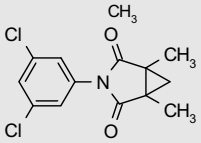
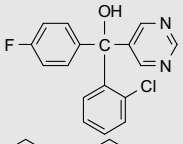
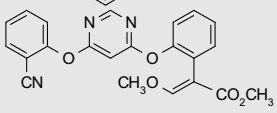
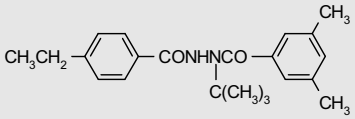
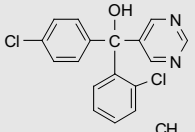
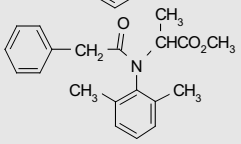
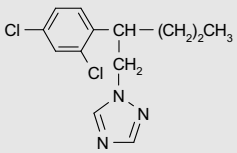
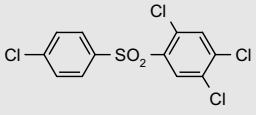
2.4. Software

The StatGraphics Plus Software Version 5.1 (Statistical Graphics, Rockville, USA) was used for data processing.

2.5. SPME procedure

The fiber coatings used in this work were made of poly(dimethylsiloxane)/divinylbenzene (PDMS/DVB, 60 μm). The SPME device for manual extraction, consisting in a holder assembly and several replaceable fibers, was purchased from Supelco (Madrid, Spain). Before extraction each fiber was conditioned in methanol with stirring for 30 min at 500 rpm and, between extractions, they were cleaned with methanol for 20 min. SPME extraction of the pesticides was carried out with the following procedure: 10.0 ml of a wine sample previously filtered with 0.22 μm filters

Table 1
Chemical structure, family and MRLs for wine grapes of the selected pesticides

Pesticide	Family	Structure	Codex alimentarius' MRLs (mg/kg)[a]	Spain's MRLs (mg/kg)[b]	EU's MRLs (mg/kg)[c]
Pirimicarb (1)	Carbamate		–	0.5	–
Metalaxyl (2)	Acylalanine		1.0	1.0	1.0
Pyrimethanil (3)	Anilinopyrimidine		–	5.0	–
Procymidone (4)	Dicarboximide		5.0	5.0	–
Nuarimol (5)	Pyrimidine		–	0.2	–
Azoxystrobin (6)	Methoxyacrylate		–	2.0	2.0
Tebufenozide (7)	Diacylhydrazine		2.0	0.5	–
Fenarimol (8)	Pyrimidine		0.3	0.3	–
Benalaxyl (9)	Acylalanine		0.2	0.2	–
Penconazole (10)	Triazole		0.2	0.2	–
Tetradifon (11)	Organosulphurus		–	2.0	–

[a] Taken from Pesticide Residues in Food, Codex Alimentarius. Available at <http://www.codexalimentarius.net/mrls/pestdes/jsp/pest_q-e.jsp>.
[b] Taken from the Spanish Ministry of Agriculture, Fishering and Food. Official maximum residue limits for pesticides 2007. Available at: <http://www.mapa.es/es/agricultura/agricultura.htm>.
[c] Taken from EU MRLs sorted by pesticide. Available at <http://www.ec.europa.eu/food/plant/protection/pesticides/index_en.htm>.

(Millex-GV13 Millipore, Bedford, MA, USA) was placed into a 16.0 ml screw-cap vial containing a magnetic stirring bar and mixed with 3.0 g of sodium chloride (30% w/v). The pH was adjusted to 9.5 with 1 M sodium hydroxide solution. The PDMS/DVB SPME fiber was immersed directly into the sample solution and the extraction was carried out at ambient temperature for 143 min with continuous stirring at 900 rpm. Then, desorption of the pesticides from the fiber was carried out with 1.0 ml of methanol by stirring for 13 min at 1000 rpm. The extract obtained from the SPME procedure was evaporated to dryness on a rotary evaporator (Rotavapor R-200, Vacuum Controller V-800 and Vacuum Pump V-500 from Büchi Labortechnik, Flawil, Switzerland) at 40 °C and 250 mbar and reconstituted with 1.0 ml of the mixture 1:3 water:100 mM sodium tetraborate at pH 8.5 (v/v). Injection

was carried out following the REPSM procedure. Repeatability and accuracy provided by the SPME procedure were determined by spiking free wine samples with the working mixtures at appropriate concentrations (0.20–8.0 mg/L).

3. Results and discussion

3.1. SPME-REPSM-MEKC-DAD method

The SPME-REPSM-MEKC-DAD method used in this work was previously developed by our group for the analysis of the same group of pesticides in white wine samples (Ravelo-Pérez et al., 2007a). The MEKC electrolyte consisted of 100 mM sodium tetraborate and 30 mM SDS at pH 8.5 with 6% v/v 1-propanol. SPME parameters (pH, extraction time, desorption time and percentage of sodium chloride) were optimized by means of an experimental design providing a selective and clean extraction of the target analytes (see Section 2 for details). Matrix matched calibration was necessary since significant differences between calibration curves obtained with spiked aqueous hydroalcoholic solutions (12% v/v ethanol) and with spiked white wine samples were observed.

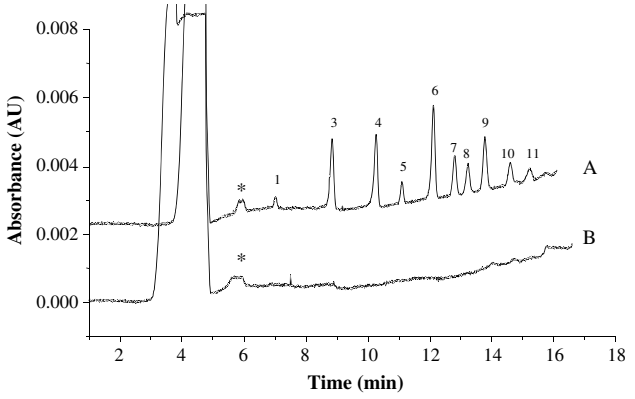


Fig. 1. REPSM-MEKC-DAD electropherograms of (A) spiked and (B) non spiked red wine sample after SPME (pH 9.5, 30% NaCl (w/v), 143 min extraction at 900 rpm, 13 min desorption at 1000 rpm). Injection: 11 s at 20 psi. Sample dissolved in 1:3 (v/v) water:100 mM sodium tetraborate at pH 8.5. Separation electrolyte: 100 mM sodium tetraborate, 30 mM SDS, pH 8.5 and 6% 1-propanol. Total length of the capillary 57 cm (50 cm effective length, 50 μm id). Voltage, +22 kV. Temperature, 25 °C. UV detection at 210 nm. Peak identification and concentration of the fortified sample: (*) Unknown peak, (1) pirimicarb (8.00 mg/L), (3) pyrimethanil (1.45 mg/L), (4) procymidone (0.75 mg/L), (5) nuarimol (0.80 mg/L), (6) azoxystrobin (1.45 mg/L), (7) tebufenozide (0.25 mg/L), (8) fenarimol (0.60 mg/L), (9) benalaxyl (0.60 mg/L), (10) penconazole (0.60 mg/L) and (11) tetradifon (0.70 mg/L). Pirimicarb was detected at 240 nm (electropherogram not shown).

However, up to now, the method has not been validated for the analysis of red wine samples (which is a more complex sample) and, to our knowledge, no previous works dealing with the CE analysis of pesticides in red wines have been developed.

When the previously developed SPME method was applied to red wines, we found that it could also be applied to these more complex samples. Fig. 1 shows the electropherogram of the SPME extract of a spiked and non spiked red wine sample. As it can be seen, good separation was achieved in less than 16 min. As for white wine samples, all the pesticides could be perfectly extracted (except metalaxyl) without any interferences from the sample matrix. Metalaxyl was not also extracted from white wine samples, although it was extracted from hydroalcoholic solutions, which clearly shows that the sample matrix highly inhibits/influences the extraction of this pesticide (Ravelo-Pérez et al., 2007a).

3.2. Method validation

Repeatability of the REPSM-MEKC-DAD method was evaluated in terms of intraday and interday precision. Intraday precision was assessed by the injection on the same day of an intermediate concentration level (concentrations of the pesticides between 0.460 and 1.25 mg/L) injected in triplicate. Interday precision was assessed for 33 consecutive days (triplicate injections in each case). The results, expressed as relative standard deviation (RSD) of the peak areas and migration times, are given in Table 2. As it can be observed, acceptable precision was obtained in all cases: intraday RSD values were below 3.4% for peak areas and below 0.6% for migration times, while interday RSD values were below 6.7% for peak areas and below 2.4% for migration times.

Matrix matched calibration curves based of the peak areas at the working ranges indicated in Table 2 were obtained by spiking red wine samples free of pesticides ($n = 5$). Each concentration level was injected in triplicate. Statistic parameters calculated from the least-square regression are presented in Table 2. In all cases, determination coefficients (R^2) higher than 0.990 were obtained. In order to clearly evaluate the matrix effect, matrix matched calibration graphs were statistically compared with calibration curves obtained following the same procedures but with an hydroalcoholic solution (SPME extraction of spiked Milli-Q water containing 12% ethanol v/v). A statistical program that calculates *F*- and *p*-values for the comparison of the slopes and the intercepts was used. For all the pesticides, statistical differences were observed (*p*-values for the comparison of the slopes or intercepts were $\leqslant 0.1$) and as a result, quantification should be developed using the calibration curves obtained with the red wine samples. Similar matrix effects have also been reported previously (Ravelo-Pérez et al., 2007a, 2007b).

Table 2
Repeatability and calibration data from standards prepared in red wine after SPME-MEKC-DAD

Peak	Pesticide	Intraday precision ($n = 3$)		Interday precision ($n = 33$)		Range of concentration tested (mg/L)	Calibration curve matrix ($n = 5$)	R^2
		t_R	Area	t_R	Area			
1	Pirimicarb	0.3	0.8	1.1	3.3	4.75–8.94	$y = (0.004 \pm 0.000)x + (0.020 \pm 0.002)$	0.999
2	Metalaxyl	0.2	0.8	1.3	4.7	–	–	–
3	Pyrimethanil	0.4	2.8	1.6	4.9	0.419–2.50	$y = (0.087 \pm 0.008)x + (0.097 \pm 0.012)$	0.996
4	Procymidone	0.5	2.2	2.4	4.3	0.270–2.50	$y = (0.165 \pm 0.008)x + (0.129 \pm 0.010)$	0.999
5	Nuarimol	0.4	1.1	1.6	4.8	0.523–1.41	$y = (0.040 \pm 0.005)x + (0.032 \pm 0.005)$	0.995
6	Azoxystrobin	0.3	1.1	1.5	2.8	0.295–1.65	$y = (0.080 \pm 0.013)x + (0.108 \pm 0.015)$	0.992
7	Tebufenozide	0.5	1.0	1.9	5.6	0.165–1.00	$y = (0.307 \pm 0.037)x + (0.040 \pm 0.025)$	0.996
8	Fenarimol	0.4	0.6	1.9	6.2	0.367–1.00	$y = (0.035 \pm 0.006)x + (0.080 \pm 0.004)$	0.992
9	Benalaxyl	0.4	1.5	1.8	6.7	0.210–1.00	$y = (0.111 \pm 0.018)x + (0.122 \pm 0.012)$	0.992
10	Penconazole	0.5	3.4	2.2	4.9	0.331–1.00	$y = (0.082 \pm 0.008)x + (0.068 \pm 0.006)$	0.997
11	Tetradifon	0.6	1.2	2.2	2.4	0.700–2.13	$y = (0.007 \pm 0.001)x + (0.073 \pm 0.002)$	0.990

R^2: determination coefficient.

Validation for spiked wine samples was carried out by using a one-sample test (Student's *t*-test) (Miller & Miller, 2002). Samples without pesticides were fortified at three levels of concentrations and analyzed by the proposed method five times ($n = 5$) at each level. Some of these levels have been selected in order to demonstrate the applicability of the method to concentrations equivalent or very near to those of the MRLs established for wine grapes (although, as previously indicated, no uniform MRLs have been fixed in wines). Table 3 shows the results obtained. In all cases *t* values were lower than the tabulated one (2.78 for $n = 5$) and thus the null hypothesis might be accepted (no significant differences were observed between the real and the experimental value). In all cases apparent recovery values ranged between 90% and 107% for all the pesticides. Recoveries did not show dependence with concentration for the different spiked levels assayed. The term "apparent recovery" refers to pesticides concentrations determined rather than the actual percent of analytes extracted by the SPME analysis, since in any SPME procedure extraction can be developed under equilibrium or non equilibrium conditions, which does not necessarily mean 100% extraction. Apparent recoveries are frequently provided in SPME analysis (Cai, Gong, Chen, & Wu, 2006; Navalón, Prieto, Araujo, & Vilchez, 2002). The LODs of the method which were calculated as the lowest extractable concentrations that yielded a signal to noise ratio of 3, ranged between 0.049 mg/L (tebufenozide) and 1.69 mg/L (pirimicarb). These values are lower than the MRLs established for these compounds in wine grapes except for pirimicarb. Extraction at the LOD level was corroborated by the duplicate analysis of fortified samples (data not shown). We could effectively confirm that all the pesticides could be extracted at the LOD level. The above mentioned results clearly show that the proposed method can be feasibly applied to the analysis of these pesticides in red wine samples at the required MRLs levels.

3.3. Analysis of red wine samples

To demonstrate that the SPME-REPSM-MEKC method can be applied for routine analysis of red wines samples and in order to evaluate the pesticide content of wine from the Canary Islands 20 samples were analyzed. Among them, 18 samples were homemade wines kindly supplied by local cultivators (16 from the island of Tenerife, one from Lanzarote and one from La Palma islands) while two of them were commercial (Rioja and Ciudad Real). Table 4 shows the results of the analysis. Only pyrimethanil, procymidone and azoxystrobin (fungicides) were found in the samples which are frequently used in the islands. Identification of the pesticides was carried out by fortifying the samples with the mixtures of pesticides and also by comparison of the DAD spectra of both samples and standards. Pyrimethanil was found in six of the homemade samples, in five cases below the LOQ of the method (which was calculated as the lowest extractable concentration that yielded a signal to noise ratio of 10). Azoxystrobin was only found in one of the samples, at a concentration below the LOQ of the method while procymidone, however, was found in 12 of the homemade samples, in 10 cases below the LOQ. The highest concentration of fungicides was found in sample number 6 with 1.18 mg/L of pyrimethanil and 0.72 mg/L of procymidone. In the commercial wines none of the selected pesticides were found. Fig. 2 shows the electropherogram of one of the analyzed samples (sample *n*13) which contained procymidone. Similar electropherograms (clean and without interferences from the sample matrix) were obtained for the rest of the samples. In general, the levels of these

Table 3
Results of assays to check the accuracy of the proposed method for the selected pesticides in red wine samples

Peak	Pesticide	MRL in Spain (mg/kg)[a]	Spiked level (mg/L)	Found[b]	Recovery (%)	*t*	LODs[c] (mg/L)
1	Pirimicarb	0.5	8.00	8.08 ± 0.24	101	2.33	1.69
			6.80	6.72 ± 0.10	99	0.70	
			5.60	5.55 ± 0.14	99	0.54	
3	Pyrimethanil	5	1.25	1.21 ± 0.18	97	2.45	0.126
			0.83	0.81 ± 0.08	105	1.40	
			0.40	0.42 ± 0.06	98	1.79	
4	Procymidone	5	1.25	1.23 ± 0.10	98	1.87	0.081
			0.78	0.77 ± 0.04	93	2.54	
			0.30	0.28 ± 0.07	99	1.48	
5	Nuarimol	0.2	1.40	1.38 ± 0.14	99	1.36	0.157
			0.95	0.96 ± 0.05	96	1.53	
			0.50	0.48 ± 0.09	101	1.41	
6	Azoxystrobin	2	1.45	1.42 ± 0.12	98	2.39	0.089
			0.88	0.87 ± 0.08	107	1.66	
			0.30	0.32 ± 0.07	100	0.27	
7	Tebufenozide	0.5	1.00	0.97 ± 0.09	97	2.37	0.049
			0.60	0.60 ± 0.04	100	0.31	
			0.20	0.20 ± 0.05	100	0.19	
8	Fenarimol	0.3	1.00	1.00 ± 0.11	100	0.04	0.110
			0.70	0.70 ± 0.06	105	1.54	
			0.40	0.42 ± 0.09	100	0.01	
9	Benalaxyl	0.2	1.00	1.01 ± 0.08	101	0.66	0.063
			0.60	0.59 ± 0.09	94	0.98	
			0.20	0.19 ± 0.04	99	0.96	
10	Penconazole	0.2	1.00	1.01 ± 0.07	101	1.16	0.099
			0.65	0.66 ± 0.06	90	1.49	
			0.30	0.27 ± 0.06	101	0.54	
11	Tetradifon	2	2.12	2.14 ± 0.15	101	1.15	0.298
			1.56	1.51 ± 0.13	95	0.46	
			1.00	0.95 ± 0.10	97	0.36	

t: Experimental *t* value.
[a] Taken from the Spanish Ministry of Agriculture, Fishering and Food. Official maximum residue limits for pesticides 2007. Available at: <http://www.mapa.es/es/agricultura/agricultura.htm>.
[b] Average value ± standard deviation of 5 determinations (95% confidence level).
[c] LODs of the SPME-MEKC-DAD procedure.

Table 4
Results of the application of the proposed methodology to the analysis of different red wine samples

Sample	Zone	Year	pH	Pesticide detected	Concentration (mg/L)
n1	Lanzarote (Masdache)	2007	3.91	–	–
n2	Tenerife (Ycoden-Daute-Isora)	2006	2.87	Pyrimethanil	<LOQ
				Procymidone	0.36 ± 0.09
n3	Tenerife (Valle de la Orotava)	2006	3.17	Pyrimethanil	<LOQ
				Procymidone	<LOQ
n4	Tenerife (Valle de Güímar)	2006	3.21	Pyrimethanil	<LOQ
				Procymidone	<LOQ
n5	Tenerife (Tacoronte-Acentejo)	2006	2.99	Procymidone	<LOQ
n6	Tenerife (Valle de la Orotava)	2006	2.92	Pyrimethanil	1.18 ± 0.18
				Procymidone	0.72 ± 0.10
n7	Tenerife (Tacoronte-Acentejo)	2006	3.02	–	–
n8	Tenerife (Tacoronte-Acentejo)	2006	2.88	Pyrimethanil	<LOQ
				Procymidone	<LOQ
n9	Tenerife (Tacoronte-Acentejo)	2006	3.08	Procymidone	<LOQ
n10	Tenerife (Valle de la Orotava)	2006	3.02	Pyrimethanil	<LOQ
				Procymidone	<LOQ
n11	Tenerife (Tacoronte-Acentejo)	2004	2.99	–	–
n12	Tenerife (Ycoden-Daute-Isora)	2006	3.15	Procymidone	<LOQ
n13	Tenerife (Ycoden-Daute-Isora)	2006	3.08	Procymidone	<LOQ
n14	La Palma (Fuencaliente)	2006	3.10	Azoxystrobin	<LOQ
n15	Tenerife (Tacoronte-Acentejo)	2005	2.87	–	–
n16	Tenerife (Tacoronte-Acentejo)	2006	3.09	Pyrimethanil	<LOQ
				Procymidone	<LOQ
n17	Tenerife (Tacoronte-Acentejo)	2006	3.12	Procymidone	<LOQ
n18	Tenerife (Abona)	2006	3.48	–	–
n19 (commercial)	Rioja (Haro)	2003	2.95	–	–
n20 (commercial)	Ciudad Real (Valdepeñas)	2006	3.02	–	–

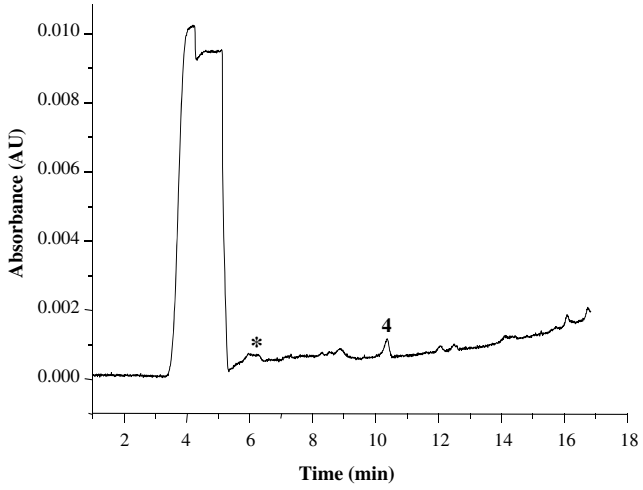


Fig. 2. REPSM-MEKC-DAD electropherogram of a homemade red wine sample (sample n13) after SPME under optimum extraction conditions (pH 9.5, 30% NaCl (w/v), 143 min extraction at 900 rpm, 13 min desorption at 1000 rpm). Injection: 11 s at 20 psi. Sample dissolved in 1:3 (v/v) water:100 mM sodium tetraborate at pH 8.5. Separation electrolyte: 100 mM sodium tetraborate, 30 mM SDS, pH 8.5 and 6% 1-propanol. Total length of the capillary 57 cm (50 cm effective length, 50 μm i.d.). Voltage, +22 kV. Temperature, 25 °C. UV detection at 210 nm. Peak identification: (*) Unknown peak, (4) procymidone.

residues cannot be considered a serious public health problem since they are below the MRLs established from wine grapes. These results represent the first data of the literature concerning pesticides content in red wine of the Canary Islands.

When these results are compared with a previous preliminary study carried out by our group for homemade white and rose wine samples from Tenerife (Ravelo-Pérez et al., 2007a, 2007b) – only 10 samples were analyzed in these cases – we can clearly observe that the same pesticides (procymidone and pyrimethanil) occurred in the three types of wine and that the pesticide content of red wines is lower than the ones from rose or white wines. This fact can be associated to the red wine making process, which is more complex and more time consuming (in general, white and rose wines can reach the market in the same year of production while red wines can be in the market a year after). Besides, this relatively high persistence of the pesticides in wines can be associated to the relatively high pH value of the wine: in general pesticides are more stable at low pH values.

4. Conclusions

In this work a new method is proposed for the analysis of a group of 11 multi-class pesticides residues in red wine samples using SPME and REPSM-MEKC. After suitable validation of the method in terms of linearity, precision, accuracy and selectivity, 20 red wine samples (18 of them homemade and two commercial) were analyzed. The results revealed that the concentrations of studied pesticide residues in the samples were in the permissible limits. This work represents the first data of the literature concerning the pesticide content of red wines from the Canary Islands. The proposed method helps to cover some of the most important research and development needs in this area of pesticides in foods to assess the state of food pollution.

Acknowledgements

L.M.R.P. wishes to thank the Spanish Ministry of Education and Science for the FPU grant. J.H.B. wishes to thank the Spanish Ministry of Education and Science for the Juan de la Cierva contract in the Instituto Canario de Investigaciones Agrarias (ICIA). Authors would also like to thank Dr. Francisco J. García-Montelongo for his indications and suggestions. This work has been supported by the Spanish Ministry of Education and Science (AGL2005-02924/ALI project).

References

Aubert, C., Baumes, R. L., Günata, Y. Z., Lepoutre, J. P., Cooper, J. F., & Bayonove, C. L. (1997). Effects of fluzilazole, a sterol biosynthesis inhibitor fungicide, on the

free and bound aroma fraction of Muscat of Alexandria wines. *Journal International des Sciences de la Vigne et du Vin, 31*, 57–64.
Brumley, W. C. (1995). Environmental applications of capillary electrophoresis for organic pollutant determination. *LC–GC, 13*, 556–568.
Cai, L., Gong, S., Chen, M., & Wu, C. (2006). Vinyl crown ether as a novel radical crosslinked sol–gel SPME fiber for determination of organophosphorus pesticides in food samples. *Analytica Chimica Acta, 559*, 89–96.
Cooney, C. M. (1996). New pesticide law drops "zero-tolerance" standard, focuses on exposures to children. *Environmental Science & Technology, 30*, A380.
Driver, J. H., Ginevan, M. E., & Whitmyre, G. K. (1996). Estimation of dietary exposure to chemicals: A case study illustrating methods of distributional analyses for food consumption data. *Risk Analysis, 16*, 763–771.
Fan, A. M., & Jackson, R. J. (1989). Pesticides and food safety. *Regulatory Toxicology and Pharmacology, 9*, 158–174.
Gaido, K., Dohme, L., Wang, F., Chen, I., Blankvoort, B., Ramamoorthy, K., et al. (1998). Comparative estrogenic activity of wine extracts and organochlorine pesticide residues in food. *Environmental Health Perspectives, 106*(Suppl. 6), 1347–1351.
García, M. A., Oliva, J., Barba, A., Cámara, M. A., Pardo, F., & Díaz-Plaza, E. M. (2004). Effect of fungicide residues on the aromatic composition of white wine inoculated with three *Saccharomyces cerevisiae* strains. *Journal of Agricultural and Food Chemistry, 52*, 1241–1247.
Goto, T., Ito, Y., Oka, H., Saito, I., Matsumoto, H., Sugiyama, H., et al. (2005). The high throughput analysis of *N*-methyl carbamate pesticides in wine and juice by electrospray ionization liquid chromatography tandem mass spectrometry with direct sample injection into a short column. *Analytica Chimica Acta, 531*, 79–86.
Guguchkina, T. I., & Ageeva, N. M. (1990). Influence of pesticide residues on wine stability. *Sadovodstvo i Vinogradarstvo i Vinodelie Moldovy, 12*, 34–35.
Hyötyläinen, T., Jauho, K., & Riekkola, M. L. (1998). Analysis of pesticides in red wines by on-line coupled reversed-phase liquid chromatography gas chromatography with vaporiser/precolumn solvent split/gas discharge interface. *Journal of Chromatography A, 813*, 113–119.
Hyötyläinen, T., Lüthje, K., Rautiainen-Rämä, M., & Riekkola, M. L. (2004). Determination of pesticides in red wines with on-line coupled microporous membrane liquid–liquid extraction-gas chromatography. *Journal of Chromatography A, 1056*, 267–271.
Jiménez, J. J., Bernal, J. L., del Nozal, M. J., Toribio, L., & Arias, E. (2001). Analysis of pesticide residues in wine by solid-phase extraction and gas chromatography with electron capture and nitrogen–phosphorus detection. *Journal of Chromatography A, 919*, 147–156.
Kim, J. B., & Terabe, S. (2003). On-line sample preconcentration techniques in micellar electrokinetic chromatography. *Journal of Pharmaceutical and Biomedical Analysis, 30*, 1625–1643.
Miliadis, G. E., Tsiropoulos, N. G., & Aplada-Sarlis, P. G. (1999). High-performance liquid chromatographic determination of benzoylurea insecticides residues in grapes and wine using liquid and solid-phase extraction. *Journal of Chromatography A, 835*, 113–120.
Millán, S., Sampedro, M. C., Unceta, N., Goicolea, M. A., Rodríguez, E., & Barrio, R. J. (2003). Coupling solid-phase microextraction and high-performance liquid chromatography for direct and sensitive determination of halogenated fungicides in wine. *Journal of Chromatography A, 995*, 135–142.
Miller, J. C., & Miller, J. N. (2002). *Statistics and chemometrics for chemical analysis. 4th ed.* Madrid: Prentice Hall.
Molina-Mayo, C., Hernández-Borges, J., Borges-Miquel, T. M., & Rodríguez-Delgado, M. A. (2007). Determination of pesticides in wine using micellar electrokinetic chromatography with UV detection and sample stacking. *Journal of Chromatography A, 1150*, 348–355.
Navalón, A., Prieto, A., Araujo, L., & Vilchez, J. L. (2002). Determination of pyrimethanil and kresoxim-methyl in green groceries by headspace solid-phase microextraction and gas chromatography–mass spectrometry. *Journal of Chromatography A, 975*, 355–360.
Nozal, M. J., Bernal, J. L., Jiménez, J. J., Martín, M. T., & Bernal, J. (2005). Determination of azolic fungicides in wine by solid-phase extraction and high-performance liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry. *Journal of Chromatography A, 1076*, 90–96.
Oliva, J., García, M. A., Navarro, S., Pardo, F., & Barba, A. (2001). Incidencia de los residuos de ciprodinil, fludioxonil y pirimetanil en vinos obtenidos por fermentación con distintas especies de Saccharomyces. *Enólogos, Investigación y Ciencia, 9*, 18–25.
Otteneder, H., & Majerus, P. (2005). Pesticide residues in wine, transfer from grapes. *Bulletin de l'O.I.V., 78*, 173–181.
Pascual, J. A., Ros, M., Fernández, P., Bernal, A., & Lacasa, A. (2004). Future of compost as an alternative to chemical compounds in ecological agriculture. *Waste Management and the Environment II* (Vol. 78, pp. 251–262). United Kingdom: Wit Press.
Quirino, J. P., & Terabe, S. (2000). Sample stacking of cationic and anionic analytes in capillary electrophoresis. *Journal of Chromatography A, 902*, 119–135.
Ravelo-Pérez, L. M., Hernández-Borges, J., Borges-Miquel, T. M., & Rodríguez-Delgado, M. A. (2007a). Multiple pesticide analysis in wine by MEKC combined with solid-phase microextraction and sample stacking. *Electrophoresis, 28*, 4072–4081.
Ravelo-Pérez, L. M., Hernández-Borges, J., Borges-Miquel, T. M., & Rodríguez-Delgado, M. A. (2007b). Pesticide analysis in rose wines by micellar electrokinetic chromatography. *Journal of Separation Science, 30*, 3240–3246.
Ravelo-Pérez, L. M., Hernández-Borges, J., Cifuentes, A., & Rodríguez-Delgado, M. A. (2007). MEKC combined with SPE and sample stacking for multiple analysis of pesticides in water samples at the ng/L level. *Electrophoresis, 28*, 1805–1814.
Rial-Otero, R., Yagüe-Ruiz, C., Cancho-Grande, B., & Simal-Gándara, J. (2002). Solid-phase microextraction-gas chromatographic–mass spectrometric method for the determination of the fungicides cyprodinil and fludioxonil in white wines. *Journal of Chromatography A, 942*, 41–52.
Schellin, M., Hauser, B., & Popp, P. (2004). Determination of organophosphorus pesticides using membrane-assisted solvent extraction combined with large volume injection-gas chromatography–mass spectrometric detection. *Journal of Chromatography A, 1040*, 251–258.
Teixeira, M. J., Aguiar, A., Afonso, C. M. M., Alves, A., & Bastos, M. M. S. M. (2004). Comparison of pesticides levels in grape skin and in the whole grape by a new liquid chromatographic multiresidue methodology. *Analytica Chimica Acta, 513*, 333–340.