Journal of Chromatography A, 1229 (2012) 63–71

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography A

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

Development of an ultrasound-assisted emulsification–microextraction method for the determination of the main compounds causing cork taint in wines

C. Pizarro*, Cristina Sáenz-González[1], Nuria Pérez-del-Notario[1], José María González-Sáiz[2]

Department of Chemistry, University of La Rioja, C/ Madre de Dios 51, 26006 Logroño, La Rioja, Spain

ARTICLE INFO

Article history:
Received 10 November 2011
Received in revised form 11 January 2012
Accepted 12 January 2012
Available online 20 January 2012

Keywords:
Ultrasound-assisted emulsification–microextraction
Haloanisoles
Halophenols
Cork taint
Wine
Experimental design

ABSTRACT

In this work, an ultrasound-assisted emulsification–microextraction method has been optimised for the determination in wine of haloanisoles (2,4,6-trichloranisole (TCA), 2,3,4,6-tetrachloroanisole (TeCA), 2,4,6-tribromoanisole (TBA) and pentachloranisole (PCA)) responsible for the so-called cork taint. Their halophenolic precursors (2,4,6-trichlorophenol (TCP), 2,3,4,6-tetrachlorophenol (TeCP), 2,4,6-tribromophenol (TBP) and pentachlorophenol (PCP)) have also been simultaneously determined. For this purpose, parameters affecting the USAEME–derivatisation procedure were exhaustively investigated. Firstly, extraction solvent, basic conditions and extraction time were selected to, subsequently, employ experimental design methodology for the simultaneous optimisation of the volumes of acetic anhydride and extraction solvent, temperature and ionic strength conditions. Once optimised, the evaluation of the analytical performance of the method confirmed its suitability for the determination of the studied compounds in wines. The proposed method showed satisfactory linearity (correlation coefficients over 0.981), repeatability (below 10.9%) and inter-day precision (below 11.0%). Detection limits obtained were similar or even lower than previously reported. In addition, the proposed method was successfully applied to the analysis of real samples. To our knowledge, this is the first time that USAME method has been optimised for the simultaneous determination of haloanisoles and halophenols in wine.

1. Introduction

During winemaking, chlorinated compounds present in the solutions used to bleach cork and wash barrels may react with lignin breakdown products forming chlorophenols such as 2,4,6-trichlorophenol (TCP), 2,3,4,6-tetrachlorophenol (TeCP) and pentachlorophenol (PCP) [1]. In addition, the treatment of waste waters with chlorine in presence of bromide and organic phenols may produce the formation of bromophenols such as 2,4,6-tribromophenol (TBP) [2]. TBP also could appear in cellars due to its use as flame retardant and fungicide [3,4]. Microorganisms present in wine can produce the biomethylation of these halophenols as a defensive reaction originating the corresponding haloanisoles [5–7]. The presence of haloanisoles in wine, even at low quantities, may cause the so-called cork taint, characterised by a mouldy-musty off-flavour [3,8]. Organoleptic properties are some of the main parameters in establishing the wine quality. Thus, the sensitive determination of haloanisoles and their halophenolic precursors in wine is of great interest to the wine industry in order to avoid economic losses derived from the commercialisation of contaminated wines.

Several methods such as liquid–liquid extraction [9–11], supercritical fluid extraction (SFE) [12], pressurised liquid extraction (PLE) [13], pervaporation [14], solid-phase extraction (SPE) [15–17], solid-phase microextraction (SPME) [18–21], stir bar sorptive extraction (SBSE) [22–24] and single drop microextraction (SDME) [25] have been employed for the extraction and concentration of haloanisoles and halophenols from wine previously to their determination. In addition, these approaches usually include a derivatisation step of the halophenols to transform them into less polar compounds, avoiding problems of broad and tailed peaks in their chromatographic determination.

Nevertheless, these techniques present certain non-negligible drawbacks such as the use of high volumes of solvent, the time required and the use of expensive devices with a limited lifetime which may entail carryover or cross-contamination problems. Consequently, in order to avoid these problems, dispersive liquid–liquid microextraction (DLLME) has been proposed as an efficient extraction–preconcentration method which has been successfully applied to the analysis of off-flavours responsible compounds in wine [26,27]. However, despite its advantages, such as simplicity, quickness and low solvent consumption, DLLME also presents some shortcomings. The use of disperser solvent can diminish the partition coefficient of analytes between the sample

* Corresponding author. Tel.: +34 941299626; fax: +34 941299621.
E-mail address: consuelo.pizarro@unirioja.es (C. Pizarro).
[1] Tel.: +34 941299636; fax: +34 941299621.
[2] Tel.: +34 941299634; fax: +34 941299621.

0021-9673/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2012.01.033

and the extraction solvent. In addition, manual shaking of the mixture, usually performed to facilitate the dispersion, may produce loss of sample or extraction solvent.

Taking this into account, in recent years, the ultrasound-assisted emulsification–microextraction method (USAEME) has been proposed as a modification of the DLLME method which uses ultrasonic radiation to achieve the dispersion of the extraction solvent in the sample thus avoiding the use of disperser solvent and manual shaking [28]. In the USAEME method, a mixture of aqueous sample and extraction solvent is submitted to ultrasonic radiation for a few minutes. The occurrence of cavitation phenomena caused by ultrasounds produces the emulsification of the extraction solvent in the sample thus increasing their contact surface and encouraging the transfer of the analytes from the sample to the extractant [29,30]. In addition, the homogenisation of the sample produced by ultrasounds and the variations produced by the cavitational collapse also affects the exchange of analytes between the phases [28,29,31,32]. This method has been successfully applied to the determination of, amongst others, bisphenol A in beverages [33] or fragrance allergens in water [34]. Furthermore, it has been described the simultaneous performance of derivatisation and USAEME of phenolic compounds in water [35]. In wine, the application of USAEME to the analysis of TCA, geosmin and 2-methylisoborneol, and ethyl and vinylphenols [36–38] has been reported.

Consequently, as a continuation to our previous endeavours in the exploration of novel sample preparation techniques, the aim of this study was the optimisation of the simultaneous USAEME–derivatisation method for the determination of cork taint responsible compounds in wine. Taking into account its efficiency, simplicity and low time consumption, aqueous acetylation with acetic anhydride in basic conditions was selected as derivatisation reaction [39]. The optimisation was performed by evaluating the influence of the main parameters which may affect the USAEME–derivatisation process. Firstly, the type of extraction solvent, the basic conditions and the extraction time were selected. Then, the effect of volumes of acetic anhydride and extraction solvent, temperature and ionic strength were simultaneously studied using experimental design methodology. Once optimised, the analytical performance of the method was studied and its linearity, limits of detection and quantification, precision and accuracy were established. Finally, the applicability of the method was evaluated analysing different real samples. To our best knowledge, the application of the USAEME method to the simultaneous determination of haloanisoles and halophenols in wines has not been previously reported.

2. Material and methods

2.1. Chemicals and standard solutions

2,3,4,6-Tetrachloroanisole (TeCA) was supplied by Ultra Scientific (North Kingstown, RI, USA). 2,4,6-Trichloroanisole (TCA), 2,4,6-tribromoanisole (TBA), 2,4,6-tribromophenol (TBP) pentachlorophenol (PCP) and 4-iodoanisole (IA) (internal standard) were supplied by Aldrich Chemie (Steinheim, Germany). Pentachloroanisole (PCA), 2,4,6-trichlorophenol (TCP) and 2,3,4,6-tetrachlorophenol (TeCP) were supplied by Supelco (Belfonte, PA, USA). The purity of all standards was above 95%.

Chloroform and tetrachloroethylene were supplied by Scharlau (Barcelona, Spain). Carbon tetrachloride was purchased from Aldrich Chemie and chlorobenzene and carbon disulfide from Acros Organics (Geel, Belgium). Methanol was purchased from Merck (Darmstad, Germany) and acetic anhydride and sodium chloride from Aldrich Chemie. Ultrapure water was obtained from a Mili-Q system (Milipore, Bedford, MA, USA).

Individual stock standard solutions of each compound were prepared in methanol at a concentration level of 400 mg/L. Work solutions used for further studies were prepared by diluting different amounts of each stock standard solution. Standard and work solutions were stored in darkness at 4 °C.

2.2. Samples

Red and white wines were selected for the different studies. The absence of organoleptic defects in these samples was checked by sensory analysis. The synthetic wine solutions were prepared by dissolving 5 g/L of L-(+)-tartaric acid in a hydroalcoholic solution (13% (v/v) ethanol). The pH of these resulting solutions was adjusted to 3.5 with NaOH. Both real and synthetic samples were spiked with different amounts of work solutions containing the target analytes.

2.3. Sample preparation

To perform the simultaneous USAEME–derivatisation process, different amounts of extraction solvent, depending on the experiment, were dispersed with the aid of ultrasonic radiation in 5 mL of wine containing different amounts of sodium hydrogen phosphate, sodium chloride and acetic anhydride. The extraction–derivatisation process was carried out by immersing the mixture into an ultrasonic water bath ATU Utrasonidos (Valencia, Spain). The level of both liquids (water and sample) was the same. The ultrasound frequency and power were 40 kHz and 100 W respectively, and temperature and time conditions were controlled for each analysis. After the extraction, the organic phase was separated from the sample in the bottom of the conical test tube by centrifugation at 5000 rpm for 3 min in a Rotina 38 (Hettich, Tuttlingen, Germany). The extracts were collected with a microsyringe, and poured into a 0.15 mL glass insert that was placed into an autosampler vial.

2.4. Chromatographic conditions

Chromatographic analysis was performed with a Hewelett-Packard 5890 Series II gas chromatograph equipped with a splitless injector, electronic pressure control in the injector and an electron-capture detector. A capillary column HP-5MS (30 m x 0.25 mm I.D., 0.25 μm film thickness) from J&W Scientific (Folsom, CA, USA) was used. Helium at a flow of 1 mL min^{-1} was used as carrier gas. Oven temperature was programmed as follows: 50 °C for 1 min, heated at 15 °C min^{-1} to 115 °C, heated to 150 °C at 3 °C min^{-1} and kept for 10 min; and finally raised to 250 °C at 15 °C min^{-1} and maintained for 4 min. For each analysis, an injection of 0.5 μL of organic extract was performed in splitless mode for 1 min using an autosampler. Injector temperature was set to 250 °C. ECD temperature was kept at 300 °C. A chromatogram of a wine sample spiked with 150 ng/L of haloanisoles and halophenols is shown in Fig. 1.

2.5. Software

The construction and analyses of the experimental design and the response surfaces were carried out using the Nemrod-W statistical package [40].

3. Results and discussion

Optimisation of an extraction procedure involves the study of all the parameters which may have a significant effect on the efficiency of the process, i.e., the recovery of the target analytes. In this context, the optimisation of the USAEME procedure involves the

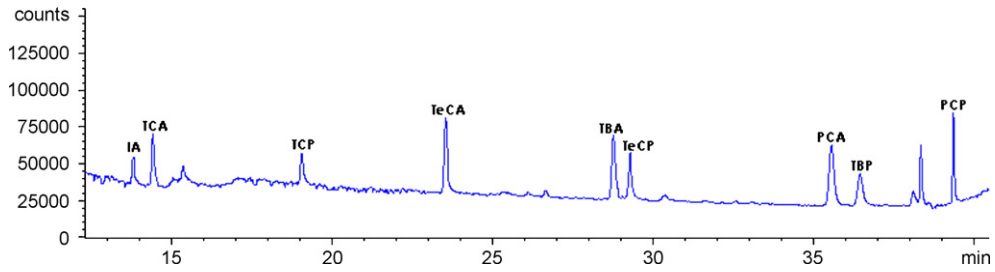

Fig. 1. GC-ECD chromatogram obtained from a spiked wine sample.

study of several factors such as type of extraction solvent, temperature and ionic strength conditions, volume of extraction solvent and extraction time. Moreover, in this study derivatisation conditions for the halophenols also have to be evaluated. For this purpose, preliminary experiments were performed in order to determine the optimal extraction solvent, basic conditions and extraction time. Then, experimental design methodology was applied to simultaneously evaluate the effect on the yield of the process of the volumes of extraction solvent and acetic anhydride, temperature and ionic strength. Then, the quality parameters of the optimised method were established and it was applied to the analysis of different real samples.

3.1. Preliminary experiments

3.1.1. Extraction solvent

One of the most important parameters that have to be studied in liquid–liquid extraction methods is the extraction solvent. In order to select the appropriate extraction solvent several considerations have to be taken into account. For liquid–liquid extractions, the extraction solvent has to have high affinity for the analytes and be immiscible with the aqueous medium that contains them. Furthermore, since the analytes will be determined by gas chromatography, it has to have good chromatographic behaviour. In addition, at the end of USAEME procedure the extraction solvent is separated by centrifugation at the bottom of a test tube. Thus, in order to facilitate the collection of the extracts, the extraction solvent has to have higher density than water. Taking these requirements into account, chloroform, carbon tetrachloride, tetrachloroethylene, chlorobenzene and carbon disulfide were evaluated as extraction solvents at different temperatures using spiked synthetic wine.

Fig. 2 shows the results of the extraction solvent screening. As can be observed, carbon tetrachloride provided the lowest recoveries for haloanisoles whereas for halophenols the worst results were obtained with chloroform. The recoveries obtained when using chlorobenzene and tetrachloroethylene were significantly higher than when using carbon disulfide. Although tetrachloroethylene yielded slightly lower recoveries than chlorobenzene, it was selected as extraction solvent due to the precipitate formed when using chlorobenzene, which hindered the collection of the extract at the bottom of the test tube.

This screening study allowed not only the selection of the extraction solvent but also the definition of the experimental domain for temperature for the subsequent optimisation step. As it can be observed, at low temperatures the recoveries obtained for all the target compounds significantly decreased for all the studied solvents. The decrease of the temperature produces an increase on the viscosity of the solvents. This produces a negative effect on the cavitation phenomena and the mass transference which may be responsible for the decrease of the recoveries at low temperatures [31,41]. Thus, the lower temperature limit of the experimental domain was set at 20 °C.

3.1.2. Basic conditions and extraction time

Once the extraction solvent had been selected, other preliminary experiments were performed to investigate the best basic conditions and determine the extraction time.

Derivatisation reaction used in the proposed USAEME–derivatisation method is the aqueous acetylation with acetic anhydride under basic conditions. Usually, aqueous acetylation is accomplished in presence of carbonate salts to adjust the pH. To avoid problems in the separation of the organic extracts caused by the carbon dioxide bubbles formed during reaction, the use of non-carbonate salts, such as sodium hydrogen phosphate, has previously been proposed [36]. The derivatisation process was studied adding different amounts of this salt providing pH from 7.6 to 8.1. As it can be appreciated in Fig. 3, no significant differences in the recoveries were observed for any of the studied analytes. Thus, 7.6 was selected as the working pH.

The emulsification process was also studied at different extraction times in order to determine the minimum time necessary to achieve the emulsification without lengthening the time of experimentation. USAEME was evaluated at 5, 10 and 15 min of extraction. The results obtained are shown in Fig. 4. Neither the recoveries of the haloanisoles nor those of the halophenols showed significant differences when time of extraction was increased. Therefore, it can be considered that 5 min was enough time to perform the extraction.

3.2. Multivariate optimisation

Volume of extraction solvent and acetic anhydride, temperature and ionic strength conditions are key parameters in the emulsification–derivatisation process. Too high ratios between the solvent extraction volume and the sample volume could negatively affect the emulsification. Temperature and ionic strength have opposite effects in the viscosity of the phases. Growing ionic strength and decreasing temperatures produce an increase in the viscosity, which interferes with the cavitation, ultrasounds propagation and mass transfer phenomena. On the other hand, the solubility of the phases increases when the temperature is raised. Thus, part of the extraction solvent is diluted in the aqueous phase and there is a decrease on the recoveries. Furthermore, the salting out effect produces a decrease in the solubility of the analytes in the sample, encouraging their extraction in the organic solvent [41,42]. Regarding all of this, the variation of these factors could produce different effects in USAEME and their interactions may not be negligible.

Taking this into account, optimum conditions for these parameters were simultaneously found using a chemometrical approach based on experimental design and response surface methodology. For this purpose, a Doehlert design involving 20 experiments, 4 central points and 5 test points was employed. Experimental domain for each factor was established taking into account preliminary experiments and instrumental and operative limits. The volume ratios $V_{\text{extractant}}$ (μL)/V_{sample} (mL) and $V_{\text{ac.anh.}}$ (μL)/V_{sample}

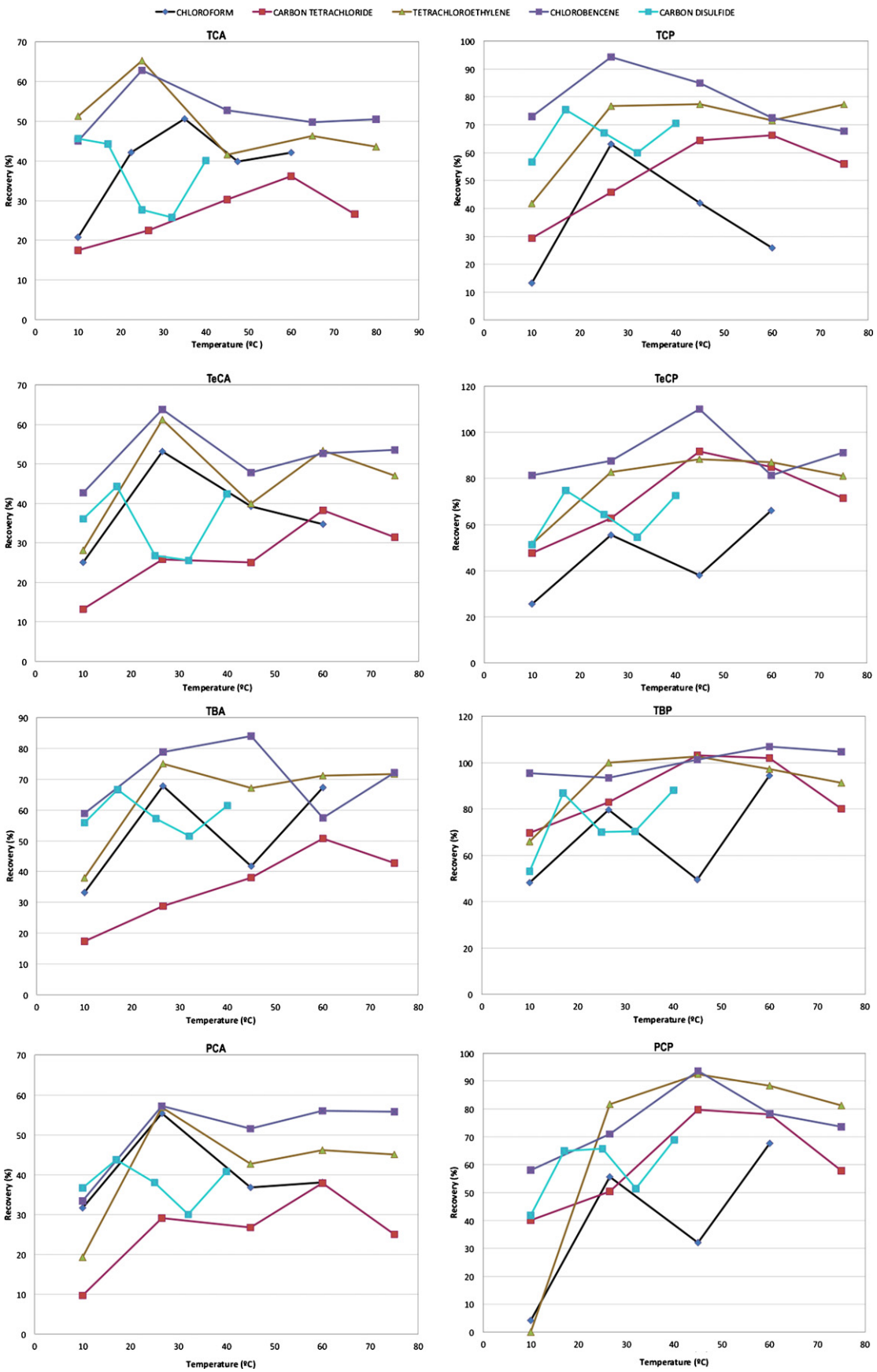

Fig. 2. Influence of solvent extraction type and temperature on the recoveries obtained for haloanisoles and halophenols by the USAEME–derivatisation method ($n = 3$).

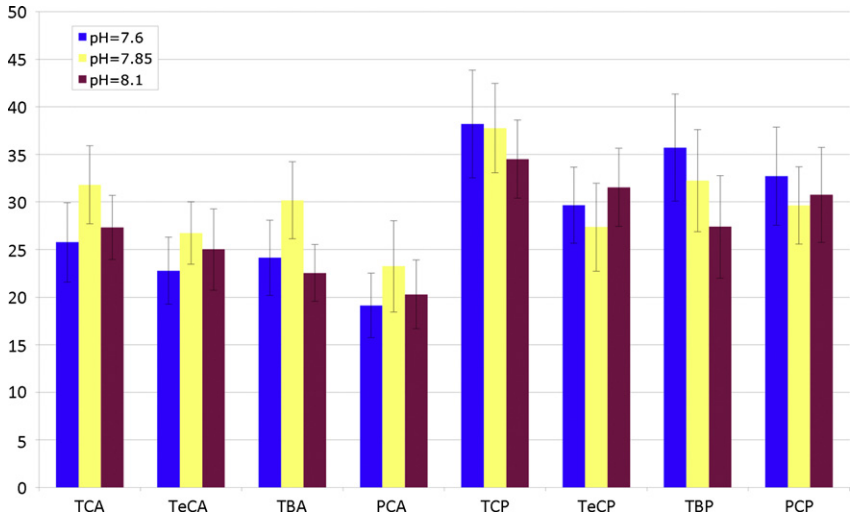

Fig. 3. Influence of pH on the recoveries obtained for haloanisoles and halophenols by the USAEME–derivatisation method ($n = 3$).

(mL) were evaluated from 10 to 40 and from 10 to 20 respectively. Since the sample volume was fixed at 5 mL, the volume of extractant was studied from 50 to 200 μL and the acetic anhydride from 50 to 100 μL. Temperature limits defined in the solvent screening ranged from 20 to 80 °C and salt concentration was studied between 0 and 10%. The experimental matrix, experimental conditions and recoveries obtained are presented in Table 1. All experiments were performed randomly and in triplicate to minimise the effects of uncontrolled factors that may introduce bias into the measurements.

The experimental results were fitted, by least-squares linear regression, to a polynomial quadratic equation with the form:

$$Y = b_0 + \sum_{i=1}^{n} b_i x_i + \sum_{i=1}^{n}\sum_{j=1}^{n} b_{ij} x_i x_j \quad (1)$$

where X_i were the studied factors (X_1: $V_{ac.anh.}$; X_2: $V_{extractant}$; X_3: temperature; X_4: NaCl concentration) and the response Y was the recovery values obtained for each compound. Then, models were analysed and validated by analysis of variance (ANOVA) and the test points. Once the models validity had been assured for each compound, model coefficients and response surfaces fitting the data were used to evaluate the effect of each factor on the recovery and the interaction effects between factors.

Analysing the estimates of the model coefficients, indicated in Table 2, it is possible to identify the significant factors for each response. All the studied responses show several significant interaction factors. Consequently, the effects of the factors cannot be studied separately. From the analysis of the response surfaces it can be concluded that recoveries of haloanisoles and halophenols are differently influenced by the factors variation. The main difference between both groups of compounds is the effect of the acetic anhydride. The addition of acetic anhydride has a negative effect on the recoveries of haloanisoles. This is probably due to the increase in the acidity of the organic phase caused by the hydrolysis of acetic anhydride. Nevertheless, the role of acetic anhydride on the derivatisation of the halophenols produced an increase in their response. Regarding the volume of extraction solvent, it has a positive effect on the recoveries of all the studied compounds. Responses of haloanisoles and halophenols also increase with raising temperatures. This increase is more pronounced at low temperatures but at high temperatures this effect diminished especially for haloanisoles. Finally, the addition of salt also produced a slight increase in the recoveries of all the studied analytes.

Due to the different influence of the factors on the two groups of compounds studied, it is difficult to find common optimum conditions. Therefore, it is necessary to find a compromise optimum at

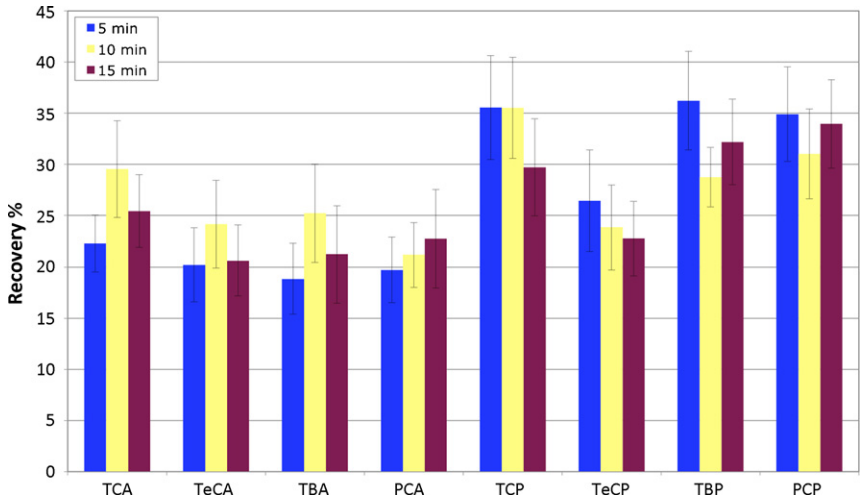

Fig. 4. Influence of extraction time on the recoveries obtained for haloanisoles and halophenols by the USAEME–derivatisation method ($n = 3$).

Table 1
Experimental design matrix and average recoveries for studied compounds ($n = 3$).

No. exp	$V_{ac.anh.}$ (μL)	$V_{extractant}$ (μL)	Temperature (°C)	NaCl conc. (%, w/v)	Recovery (%)							
					TCA	TeCA	TBA	PCA	TCP	TeCP	TBP	PCP
1	100.0	125.0	50.0	5.0	35.84	55.67	49.55	68.98	60.25	54.76	59.61	62.66
2	50.0	125.0	50.0	5.0	60.26	87.22	79.73	90.66	59.24	40.01	51.92	41.42
3	87.5	200.0	50.0	5.0	59.11	78.36	38.51	87.26	93.21	83.85	83.43	83.75
4	62.5	50.0	50.0	5.0	34.74	61.28	36.88	60.50	47.86	44.21	56.31	42.65
5	87.5	50.0	50.0	5.0	28.14	41.73	39.21	41.68	33.81	36.35	45.58	40.02
6	62.5	200.0	50.0	5.0	79.33	76.93	73.58	77.87	78.27	61.39	64.74	62.30
7	87.5	150.0	80.0	5.0	40.90	58.74	51.35	53.79	74.56	82.92	81.68	71.50
8	62.5	100.0	20.0	5.0	30.60	43.64	29.49	37.96	25.50	28.66	46.01	23.62
9	87.5	100.0	20.0	5.0	18.18	23.85	20.60	20.03	33.57	25.43	31.92	30.17
10	75.0	175.0	20.0	5.0	31.91	35.66	19.70	48.81	34.03	26.84	38.49	30.84
11	62.5	150.0	80.0	5.0	50.54	64.71	60.93	56.05	82.40	63.36	58.44	64.16
12	75.0	75.0	80.0	5.0	22.30	39.41	35.33	47.29	44.02	45.01	50.93	46.82
13	87.5	150.0	57.5	10.0	26.87	45.27	29.91	56.78	75.77	61.20	62.72	68.72
14	62.5	100.0	42.5	0.0	41.70	46.16	34.82	43.25	41.73	33.22	42.06	30.43
15	87.5	100.0	42.5	0.0	28.01	31.22	23.91	27.51	40.10	33.32	37.76	35.06
16	75.0	175.0	42.5	0.0	57.92	55.11	45.41	62.21	65.63	56.34	55.16	49.48
17	75.0	125.0	72.5	0.0	46.21	56.59	46.95	49.79	41.91	55.06	64.65	46.22
18	62.5	150.0	57.5	10.0	36.94	52.71	46.01	52.46	72.12	52.25	54.30	55.90
19	75.0	75.0	57.5	10.0	26.03	41.06	37.35	50.47	51.33	48.36	57.05	49.29
20	75.0	125.0	27.5	10.0	22.46	30.76	23.25	35.99	26.68	31.50	53.80	32.78
Central	75.0	125.0	50.0	5.0	30.66	49.77	44.70	63.97	68.19	58.80	63.58	62.09
Central	75.0	125.0	50.0	5.0	32.03	51.61	36.98	59.97	51.66	49.29	59.00	48.72
Central	75.0	125.0	50.0	5.0	34.24	47.63	46.66	59.48	63.12	55.11	61.42	59.42
Central	75.0	125.0	50.0	5.0	36.20	51.17	38.15	56.88	56.50	48.93	61.45	51.98
Test 1	65.0	105.2	44.1	4.2	36.43	50.85	40.30	60.04	49.45	43.82	55.78	42.46
Test 2	84.9	105.2	44.1	4.2	27.21	41.85	34.92	54.91	51.25	45.31	53.45	50.11
Test 3	75.0	164.5	44.1	4.2	45.50	55.37	44.95	68.41	66.37	56.95	63.57	58.35
Test 4	75.0	125.0	67.8	4.2	35.51	53.69	47.21	58.18	64.89	62.02	64.32	60.90
Test 5	75.0	125.0	50.0	8.2	28.34	44.99	37.42	57.53	59.08	51.83	61.44	55.60

which all the responses have an acceptable value. For this purpose, desirability functions methodology was applied. In this methodology, each individual response is transformed in a dimensionless function, called partial desirability function, d_i, which varies from zero (undesirable response) to one (optimal response). Depending on the objective of the study, the optimal values of response and the most appropriate form of the desirability function have to be selected for each response. Once the partial desirability functions have been defined, it is possible to calculate the global desirability function D which represents the quality of the compromise solution and it is calculated as the weighted geometric average of n individual desirability functions (Eq. (2)):

$$D = \left[\prod_{i=1}^{n} d_i^{p_i}\right]^{1/n} \quad (2)$$

where p_i is the weighting of the ith, normalised so that $\sum_{i=1}^{n} p_i = 1$. Weighting of partial desirability functions allows optimisation to take into account the relative importance of each response. The examination of the form of the global desirability function enabled us to find the region where the function was close to 1 and determine the compromise optimum conditions.

In this research, calculation of global desirability function was performed defining linear partial desirability functions for each response. When constructing these functions, the minimum acceptable response was fixed at 50% whilst 100% was set as the optimum recovery. Amongst the studied compounds, TCA and TBA have the lowest olfactory threshold. Therefore, the weight of their partial desirability functions was set at 10 whereas 1 was the weight of the rest of the responses. Fig. 5 shows the plots of the global desirability function calculated. In this figure it is possible to locate the maximum desirability at high volumes of tetrachloroethylene, medium-high temperatures and medium low

Table 2
Estimates of the model coeficients.

Coefficients	TCA	TeCA	TBA	PCA	TCP	TeCP	TBP	PCP
b_0	**33.234**	**49.493**	**41.330**	**60.835**	**59.750**	**53.121**	**61.353**	**55.401**
b_1	**−12.154**	**−12.652**	**−13.650**	**−8.035**	0.703	**6.940**	**3.644**	**9.437**
b_2	**19.970**	**14.713**	**10.229**	**17.508**	**25.506**	**18.034**	**12.115**	**17.329**
b_3	**6.699**	**13.331**	**16.086**	**10.779**	**21.728**	**22.616**	**14.959**	**20.161**
b_4	**−9.711**	**−3.003**	**−2.281**	2.017	**5.671**	**2.376**	**4.486**	**7.162**
b_{11}	**14.840**	**21.831**	**23.125**	**19.178**	−0.022	**−5.744**	**−5.553**	−3.422
b_{22}	**17.850**	**12.723**	−0.077	1.724	**4.689**	**6.322**	**3.463**	3.463
b_{33}	**−9.461**	**−16.377**	**−13.391**	**−30.518**	**−17.182**	**−11.683**	**−14.724**	**−16.273**
b_{44}	−0.586	**−11.080**	**−10.557**	**−19.655**	**−10.122**	**−8.545**	**−9.294**	**−11.854**
b_{12}	**−7.862**	**11.852**	**−21.780**	**15.921**	**16.566**	**17.509**	**17.004**	**13.741**
b_{13}	4.486	4.001	**7.072**	3.577	**−15.777**	**7.775**	**16.864**	−4.540
b_{23}	**12.332**	**11.155**	**10.013**	**−14.648**	**22.866**	**24.324**	**17.037**	**14.474**
b_{14}	4.009	−0.897	2.634	5.543	1.181	−2.791	−2.496	1.159
b_{24}	**−14.596**	**−12.452**	**−22.615**	**−19.011**	−5.698	**−12.593**	**−11.192**	**−13.976**
b_{34}	**−10.301**	**−15.084**	**−11.211**	**−11.809**	**26.176**	0.152	**−18.861**	**14.845**

Bold numbers indicate significant effects (5%).

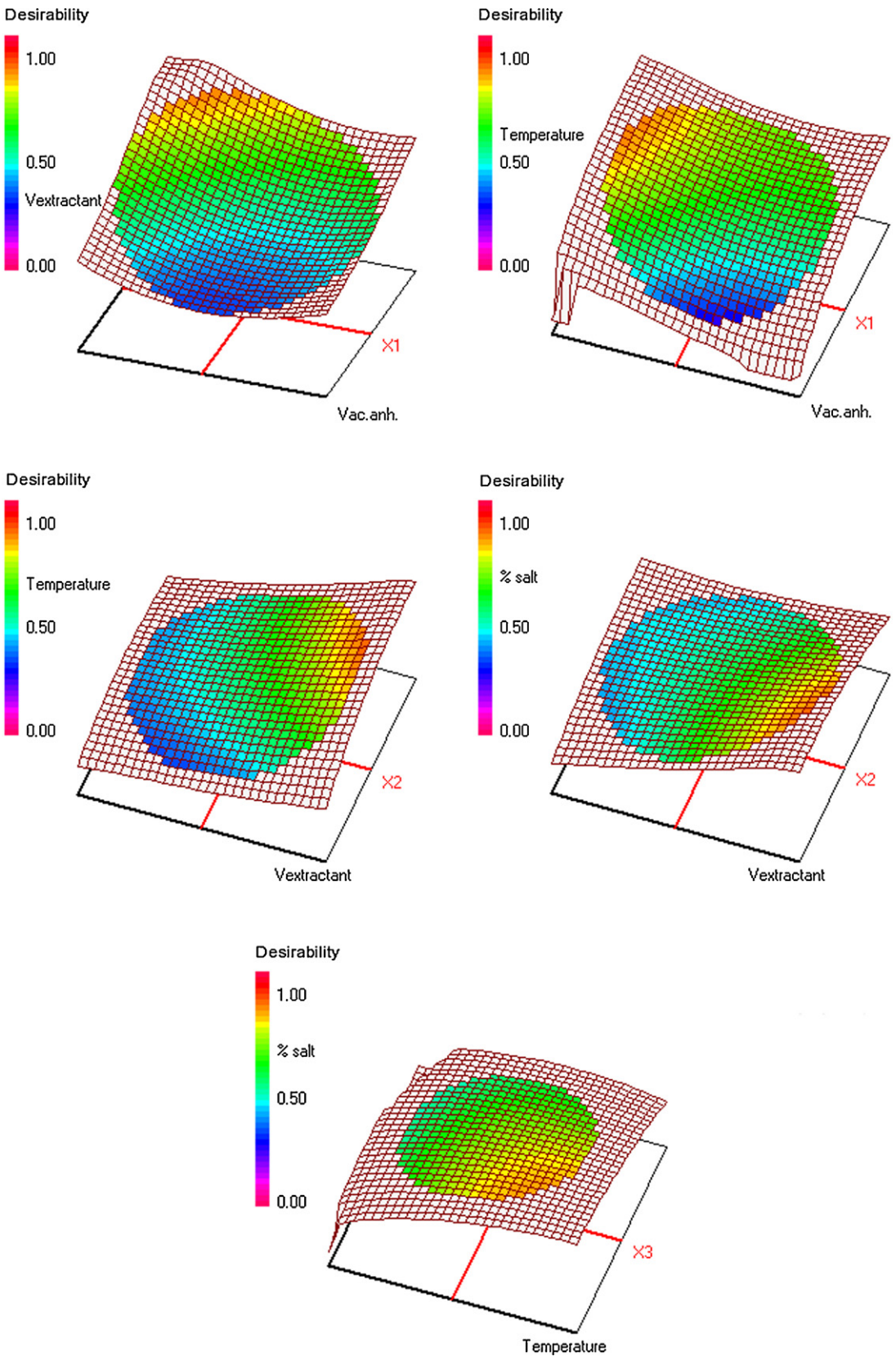

Fig. 5. Response surfaces of global desirability obtained for all significant interactions under optimised conditions.

Table 3
Significance values for Mandel's fitting test, correlation coefficients of linear regressions, detection and quantification limits of the proposed method.

Compound	Mandel's fitting test p	Correlation coefficient R^2	LOD S/N = 3 (ng/L)	LOQ S/N = 10 (ng/L)
TCA	0.132	0.981	1.9	6.3
TeCA	0.091	0.990	2.1	7.0
TBA	0.201	0.985	2.3	7.7
PCA	0.099	0.984	2.4	8.0
TCP	0.365	0.991	4.0	13.3
TeCP	0.177	0.994	3.7	12.3
TBP	0.287	0.993	4.9	16.3
PCP	0.120	0.992	4.8	16.0

Table 4
Comparison of USAEME with other extraction methods for determination of haloanisoles and halophenols in wine.

Method	V_{sample} (mL)	$V_{solvent}$	LOD (ng/L)								Ref.
			TCA	TeCA	TBA	PCA	TCP	TeCP	TBP	PCP	
LLE	200	15 mL pentane	0.5	0.5	n.a.	0.5	10	10	n.a.	10	[9]
SPE	1000	3 mL methanol + 2 mL hexane	2.4	0.3	n.a.	0.4	0.5	0.2	n.a.	0.3	[16]
SDME	20	2 μL	8.1	n.a.	6.1	n.a.	n.a.	n.a.	n.a.	n.a.	[25]
DLLME	5	150 μL carbon tetrachloride + 1.3 mL acetone	2.3	2.2	2.7	2.6	3.9	4.2	5.3	5.2	[26]
DLLME	5	173 μL carbon tetrachloride + 1.43 mL acetone	5	8	7	12	n.a.	n.a.	n.a.	n.a.	[27]
USAEME	5	180 μL carbon disulfide	1.9	2.1	2.3	2.4	4.0	3.7	4.9	4.8	Present study

n.a.: not available.

volume of acetic anhydride and salt concentration. Taking this into account it was found that optimum USAEME–derivatisation of haloanisoles and halophenols in wine was performed adding 180 μL of tetrachloroethylene and 65 μL of acetic anhydride to 5 mL of wine with 3% of salt concentration at 60 °C.

3.3. Method performance

Once optimised, the method performance of the proposed USAEME method was evaluated and its quality parameters were determined. Red wine spiked samples were used to determine the linear range from 10 to 500 ng/L. Mandel's fitting test was applied to confirm the linearity of the experimental data, obtaining significances higher than 0.05 (confidence level 95%) for each compound. Therefore, linear regressions were calculated for all calibration data achieving correlation coefficients from 0.981 to 0.994. Significances for Mandel's fitting test and correlation coefficients are shown in Table 3. The lowest concentration level of the linear range was employed to calculate the quantification and detection limits for each compound using a signal/noise ratio (S/N) of 10 and 3, respectively. Detection limits obtained ranged from 1.9 to 4.9 ng/L for all the studied compounds (Table 3), being lower than their perception thresholds. This results especially noticeable for TCA and TBA, which have very low perception thresholds in wine. In addition, as can be seen in Table 4, these detection limits were similar or even lower than those reported for other analytical techniques including DLLME [9,25,26].

Precision and recovery results are indicated in Table 5. Precision of the method was evaluated using red and white wine samples spiked at three concentration levels. Five extractions on the same day were performed to calculate repeatability ranging from 6.1 to 10.9% (RSD). Inter-day precision ranging from 6.0 to 11.0% was calculated performing extractions on five different days. Recoveries higher than 75.9% were obtained for all the compounds.

According to these results, recoveries obtained for cork taint responsible compounds are slightly higher when using DLLME than when using the USAEME method [26,27]. However, the USAEME method presents better precision assuring the dispersion formation with the application of ultrasonic energy and avoiding the manual shaking step. In addition, the use of only extraction solvent, without the aid of the disperser solvent, allows the USAEME method to be more sensitive, yielding lower detection limits (except for TCP). Taking these into account, the proposed USAEME method for the determination of haloanisoles and halophenols in wine constitutes a suitable alternative that allows saving solvent and time to the previously reported DLLME methods.

Table 5
Repeatability, inter-day precision and recovery studies of the proposed method.

Compound	Repeatability RSD%			Reproducibility RSD%			Recoveries ± RSD (%)			
	Low level[a]	Medium level[b]	High level[c]	Low level[a]	Medium level[b]	High level[c]	Red wine		White wine	
							Low level[a]	High level[c]	Low level[a]	High level[c]
TCA	10.5	9.4	6.1	7.9	9.0	7.5	90.3 ± 3.2	90.6 ± 3.9	93.3 ± 9.1	99.0 ± 5.8
TeCA	9.5	10.0	6.6	10.3	9.6	6.9	91.5 ± 8.1	92.1 ± 4.8	94.2 ± 8.8	90.3 ± 4.8
TBA	6.3	6.4	9.3	7.1	5.6	8.2	93.9 ± 6.2	94.3 ± 5.9	98.7 ± 8.7	99.5 ± 7.8
PCA	10.6	10.8	6.2	8.9	8.0	7.1	92.2 ± 7.2	94.7 ± 6.0	97.4 ± 8.0	92.8 ± 7.5
TCP	10.6	10.9	6.5	8.1	8.9	7.0	78.0 ± 6.6	75.9 ± 4.2	82.6 ± 6.6	88.4 ± 6.3
TeCP	8.8	9.5	7.7	8.3	10.2	6.7	82.1 ± 5.1	88.1 ± 7.1	88.9 ± 8.1	88.1 ± 10.2
TBP	10.2	10.0	8.3	6.0	11.0	8.0	76.8 ± 4.9	79.3 ± 6.1	81.9 ± 8.0	80.3 ± 3.9
PCP	9.6	9.2	6.8	6.2	10.5	8.7	81.9 ± 4.6	86.3 ± 5.1	87.6 ± 6.1	84.5 ± 8.7

[a] 25 ng/L.
[b] 150 ng/L.
[c] 400 ng/L.

Table 6
Results of the analysis of wine samples by the USAEME-derivatisation proposed method ($n = 3$).

Compound	Concentration ± SD (ng/L)			
	Red wine A	Red wine B	White wine A	White wine B
TCA	12 ± 4	56 ± 6	–	–
TeCA	–	87 ± 8	–	51 ± 4
TBA	–	28 ± 3	–	–
PCA	–	–	–	–
TCP	–	–	182 ± 7	–
TeCP	98 ± 5	–	177 ± 9	–
TBP	–	119 ± 6	105 ± 6	–
PCP	113 ± 6	–	91 ± 6	68 ± 8

3.4. Application of the method to real samples

Once optimised, the applicability of the proposed USAEME–derivatisation method was evaluated analysing the content of haloanisoles and halophenols in different samples of wine. For this purpose, two red and two white wines were analysed in triplicate. The concentrations of cork taint responsible compounds found in these wines are indicated in Table 6. By sensory analysis, it was found that, amongst the analysed samples, only white wine A was not affected with cork taint off-flavour. Indeed, the analysis of the wines revealed that none of the haloanisoles were present in this wine. As can be appreciated, TCA was only found in red wines and its concentration was above its odour threshold. Red wine B also contained TeCA and TBA at concentrations higher than its organoleptic threshold. White wine B presented TeCA slightly above its odour threshold too. Regarding the halophenols, white wine A contained all of them whereas white wine B only contained PCP, which was also present in red wine A together with TeCP. In red wine B, it only was found TBP.

4. Conclusions

In this work, the different parameters that may affect the USAEME–derivatisation method have been evaluated and extraction conditions have been optimised for the sensitive determination of cork taint responsible compounds in wine. Once optimised, the satisfactory results obtained in terms of linearity, precision and detection and quantification limits confirmed the suitability of the proposed method for the analysis of haloanisoles and halophenols in wine. In addition, its applicability was also proved by analysing different real samples of red and white wines. The quality of these results shows that USAEME improves the performance of DLLME avoiding the use of disperser solvent and applying ultrasonic energy to achieve the dispersion. Therefore, the proposed method constitutes a simple, fast and inexpensive extraction and preconcentration method that reduces the organic solvent consumption and extraction time. To our knowledge, this is the first application of a USAEME procedure to the simultaneous determination of haloanisoles and halophenols in wine.

Acknowledgements

The authors thank the Autonomous Government of La Rioja – *Consejería de Educación, Cultura y Deporte* – and the Spanish Government – *Ministerio de Ciencia e Innovación* – for their financial support (research grant FPI-2007 and project CTQ2008-03493/BQU) and Professor R. Phan-Tan-Luu of the University of Marseille (France) for providing the software NEMROD-W.

References

[1] P. Chatonnet, G. Guimberteau, D. Dubourdieu, J. Int. Sci. Vigne Vin. 28 (1994) 131.
[2] M. Czaplicka, Sci. Total Environ. 322 (2004) 21.
[3] P. Chatonnet, S. Bonnet, S. Boutou, M.D. Labadie, J. Agric. Food Chem. 52 (2004) 1255.
[4] F.B. Whitfield, J.L. Hill, K.J. Shaw, J. Agric. Food Chem. 45 (1997) 889.
[5] T.H. Lee, R.F. Simpson, in: G.H. Fleet (Ed.), Wine Microbiology and Biotechnology, vol. 353, Harwood Academic Press, Chur, 1993, 353 pp.
[6] A.S. Allard, M. Remberger, A.H. Neilson, Appl. Environ. Microbiol. 53 (1987) 839.
[7] J.L. Hill, A.D. Hocking, F.B. Whitfield, Food Chem. 54 (1995) 161.
[8] A.P. Pollnitz, K.H. Pardon, D. Liacopoulos, G.K. Skouroumounis, M.A. Sefton, J. Aust, Grape Wine Res. 2 (1996) 184.
[9] A. Peña-Neira, B. Fernández de Simón, M.C. García Vallejo, T. Hernández, E. Cadahía, J.A. Suarez, Eur. Food Res. Technol. 211 (2000) 257.
[10] R. Juanola, D. Subirà, V. Salvadó, J.A. García Regueiro, E. Anticó, J. Chromatogr. A 953 (2002) 207.
[11] N. Campillo, N. Aguinaga, P. Viñas, I. López-García, M. Hernández-Córdoba, J. Chromatogr. A 1061 (2004) 85.
[12] M.K. Taylor, T.M. Young, C.E. Butzke, S.E. Ebeler, J. Agric. Food Chem. 48 (2000) 2208.
[13] J.L. Gómez-Ariza, T. García-Barrera, F. Lorenzo, A.G. González, Anal. Chim. Acta 540 (2003) 17.
[14] J.L. Gómez Ariza, T. García-Barrera, F. Lorenzo, J. Chromatogr. A 1049 (2004) 147.
[15] G.J. Soleas, J. Yan, T. Seaver, D.M. Goldberg, J. Agric. Food Chem. 50 (2002) 1032.
[16] A. Martinez-Uruñuela, I. Rodríguez, R. Cela, J.M. Gonzalez-Saiz, C. Pizarro, Anal. Chim. Acta 549 (2005) 117.
[17] S. Insa, E. Anticó, V. Ferreira, J. Chromatogr. A 1089 (2005) 235.
[18] A. Martínez-Uruñuela, J.M. González-Sáiz, C. Pizarro, J. Chromatogr. A 1048 (2004) 141.
[19] A. Martínez-Uruñuela, J.M. González-Sáiz, C. Pizarro, J. Chromatogr. A 1056 (2004) 49.
[20] C. Pizarro, N. Pérez-del-Notario, J.M. González-Sáiz, J. Chromatogr. A 1143 (2007) 26.
[21] C. Pizarro, N. Pérez-del-Notario, J.M. González-Sáiz, J. Chromatogr. A 1166 (2007) 1.
[22] Y. Hayasaka, K. MacNamara, G.A. Baldock, R.L. Taylor, A.P. Pollnitz, Anal. Bioanal. Chem. 375 (2003) 948.
[23] A. Zalacain, G.L. Alonso, C. Lorenzo, M. Iñiguez, M.R. Salinas, J. Chromatogr. A 1033 (2004) 173.
[24] L. Maggi, A. Zalacain, V. Mazzoleni, G.L. Alonso, M.R. Salinas, Talanta 75 (2008) 753.
[25] E. Martendal, D. Budziak, E. Carasek, J. Chromatogr. A 1148 (2007) 131.
[26] C. Pizarro, C. Sáenz-González, N. Perez-del-Notario, J.M. González-Sáiz, J. Chromatogr. A 1217 (2010) 7630.
[27] C. Pizarro, C. Sáenz-González, N. Perez-del-Notario, J.M. González-Sáiz, J. Chromatogr. A 1218 (2011) 1576.
[28] J. Regueiro, M. Llompart, C. Garcia-Jares, J.C. Garcia-Monteagudo, R. Cela, J. Chromatogr. A 1190 (2008) 27.
[29] B. Abismail, J.P. Canselier, A.M. Wilhelm, H. Delmas, C. Gourdon, Ultrason. Sonochem. 6 (1999) 75.
[30] M.D. Luque de Castro, F. Priego-Capote, Analytical Applications of Ultrasound, Elsevier, Amsterdam, 2006.
[31] S.N. Antonov, V.V. Shemet, YuS. Kupets, Acoust. Phys. 52 (2006) 633.
[32] B. Agranat, M. Dubrovin, N. Javski, Fundamentos de la física y técnica de los ultrasonidos, Mir, Moscow, 1990.
[33] A.R. Fontana, M. Muñoz de Toro, J.C. Altamirano, J. Agric. Food Chem. 59 (2011) 3559.
[34] E. Becerril-Bravo, J. Pablo Lamas, L. Sánchez-Prado, M. Lores, C. Garcia-Jares, B. Jiménez, M. Llompart, Chemosphere 81 (2010) 1378.
[35] J. Regueiro, M. Llompart, E. Psillakis, J.C. Garcia-Monteagudo, C. Garcia-Jares, Talanta 79 (2009) 1387.
[36] C. Cortada, L. Vidal, A. Canals, J. Chromatogr. A 1218 (2011) 17.
[37] C. Pizarro, C. Sáenz-González, N. Pérez-del-Notario, J.M. González-Sáiz, J. Chromatogr. A 1218 (2011) 8975.
[38] A.R. Fontana, S.H. Patil, K. Banerjee, J.C. Altamirano, J. Agric. Food Chem. 58 (2010) 4576.
[39] I. Rodriguez, M.I. Turnes, M.C. Mejuto, R. Cela, J. Chromatogr. A 721 (1996) 297.
[40] D. Mathieu, J. Nony, R. Phan-Tan-Luu, NEMROD-W 2001, LPRAI, Marseille, 2000.
[41] H. Kuhn, H.-D. Försterling, D.H. Waldeck, Principles of Physical Chemistry, Wiley, New York, 2009.
[42] T.J. Mason, J.P. Lorimer, Applied Sonochemistry: Uses of Power Ultrasound in Chemistry and Processing, Wiley, Weinheim, 2002.