Journal of Chromatography A, 1218 (2011) 1576–1584

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Development of a dispersive liquid–liquid microextraction method for the simultaneous determination of the main compounds causing cork taint and Brett character in wines using gas chromatography–tandem mass spectrometry

C. Pizarro*, C. Sáenz-González, N. Pérez-del-Notario, J.M. González-Sáiz

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

ARTICLE INFO

Article history:
Received 29 November 2010
Received in revised form 14 January 2011
Accepted 17 January 2011
Available online 22 January 2011

Keywords:
Dispersive liquid–liquid microextraction
Gas chromatography–tandem mass spectrometry
Cork taint
Brett character
Wine
Experimental design

ABSTRACT

A novel dispersive liquid–liquid microextraction (DLLME) method, coupled to gas chromatography–tandem mass spectrometry (GC–MS/MS), was developed for simultaneously determining the main compounds responsible for cork taint (2,4,6-trichloranisole (TCA), 2,3,4,6-tetrachloroanisole (TeCA), 2,4,6-tribromoanisole (TBA) and pentachloranisole (PCA)) and Brett character (4-ethylguaiacol (EG), 4-ethylphenol (EP), 4-vinylguaiacol (VG) and 4-vinylphenol (VP)) in wines. Optimisation of DLLME procedure was performed by evaluating the type of disperser and extraction solvents and the temperature and salt addition effects. The volumes of disperser and extraction solvents were also optimised by means of a central composite design combined with desirability functions. Under optimum conditions, 5 mL of wine were extracted with an extraction mixture consisting of 1.43 mL of acetone, and 173 μL of chloroform at room temperature. The analytical characteristics of the method were evaluated. Satisfactory linearity (with correlation coefficients over 0.992), repeatability (below 11.6%) and between-days precision (below 11.0%) were obtained for all target analytes. Detection limits attained were at similar levels or even lower than the olfactory threshold of the studied compounds. Finally, the developed method was successfully applied to the analysis of wine samples. To our knowledge, this is the first time that DLLME has been applied to simultaneously determine the compounds responsible for cork taint and Brett character in wine.

1. Introduction

Wine is an alcoholic beverage made from fermented grape juice. During winemaking several physical, chemical and microbiological processes take place affecting the final quality of the resulting wine, which is mostly determined by its organoleptic characteristics. Quality of wine may be negatively affected by the apparition during its production of some compounds producing undesirable taste and odour. Cork taint and Brett character are two of the most important off-flavours that might appear during winemaking [1,2]. Hence, their simultaneous determination is of great interest in the enological industry to guarantee wine quality and avoid economic losses.

Cork taint, characterised by mouldy-musty off-flavours, is associated with the presence of haloanisoles (2,4,6-trichloroanisole (TCA), 2,3,4,6-tetrachloroanisole (TeCA), pentachloroanisole (PCA) and 2,4,6-tribromoanisole (TBA)) [3–6]. Haloanisoles are the product of the O-methylation of their corresponding halophenols, performed by some microorganisms as a defensive mechanism [5–9]. Volatile phenols, 4-ethylphenol (EP), 4-ethylguaiacol (EG), 4-vinylphenol (VP) and 4-vinylguaiacol (VG), are usually present in wine contributing positively to its aroma. However, at high concentrations these compounds may reduce desirable fruity and flowery notes of wine [10] and lend to the wine the so-called Brett character, depicted as horse-sweat or medicinal flavour [2,11–13]. Volatile phenols appear in wine because of the generation of vinylphenols through the decarboxylationreaction of p-coumaric acids. Subsequently, vinylphenols are reduced by the Brettanomyces/Dekkera yeast into ethylphenols [12,14].

Several approaches have been proposed for the quantitative determination of haloanisoles and volatile phenols in wine. These determinations are commonly performed by gas chromatography coupled to an adequate detection system. Due to the complexity of the matrix and the low concentrations of these compounds in wine, an extraction and/or preconcentration step is usually accomplished prior to the chromatographic analysis. Regarding the

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

0021-9673/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2011.01.055

extraction step, several sample treatments have been reported such as liquid–liquid extraction (LLE) [15–18], solid-phase extraction (SPE) [19–22], solid-phase microextraction (SPME) [23–28], stir bar sorptive extraction (SBSE) [29–32] and single drop microextraction (SDME) [33].

New investigations go for more efficient, more economical and faster sample preparation methods. In this context, dispersive liquid–liquid microextraction (DLLME) has been proposed as an economical, simple and fast microextraction procedure based on a ternary solvent mixture [34]. In DLLME an organic solvent (extraction solvent) is dispersed into an aqueous sample with the help of an auxiliary solvent, namely disperser solvent. The dispersion allows the formation of a large contact surface between the sample and the extractant thus encouraging the extraction of the analytes in the organic phase. This method has been successfully applied to the determination of several organic and inorganic compounds in different matrices [35–37]. In wines, DLLME has been previously used to analyse ethylphenols [38], fungicides [39] and, more recently, haloanisoles and halophenols [40,41].

The aim of this study was the optimisation of a dispersive liquid–liquid microextraction method, coupled with gas chromatograpy–tandem mass spectrometry (GC–MS/MS), for the simultaneous determination of the cork taint and Brett character responsible compounds in wine. The influence of the type of solvent, temperature and salt addition on the DLLME extraction efficiency for haloanisoles and volatile phenols was evaluated. In addition, experimental design methodology was used in the optimisation of the volumes of extraction and disperser solvents. The analytical characteristics of the optimised DLLME method were evaluated and, subsequently, the optimised and validated method was applied to the analysis of the compounds responsible for the studied off-flavours in real wines. To our best knowledge, no approaches applying DLLME procedure to the simultaneous determination of cork taint and Brett character responsible compounds in wine have previously been published.

2. Experimental

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), 4-ethylphenol (EP), 4-ethylguaiacol (EG), 4-vinylphenol (VP), p-cresol (internal standard for the volatile phenols) and 4-iodoanisole (internal standard for the haloanisoles) were supplied by Aldrich Chemie (Steinheim, Germany). Pentachloroanisole (PCA) was supplied by Supelco (Belfonte, PA, USA) and 4-vinylguaiacol (VG) was purchased from Alpha Aesar (Ward Hill, MA, 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, ethanol, acetone, acetonitrile and tartaric acid were purchased from Merck (Darmstad, Germany) 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 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. DLLME procedure

For DLLME analysis, an aliquot of 5 mL of spiked wine was placed in a 10 mL glass test tube with a conical bottom. For each analysis, an appropriate extraction mixture, containing different volumes of disperser solvent and extraction solvent (depending on the experiment), was prepared immediately before injection and rapidly injected into the aqueous sample by using a micropipette, thus forming the cloudy solution. The mixture was gently shaken to assure the complete dispersion of the extraction solvent in the sample. Then, the mixture was centrifuged for 2 min at 5000 rpm in a Rotina 38 (Hettich, Tuttlingen, Germany). The centrifugation allowed the separation of organic phase in the bottom of the conical test tube, which was then totally removed with a microsyringe. The extracts were added with a fixed concentration of internal standards and poured into a 0.15 mL glass insert which was placed into an autosampler vial to be analysed by gas chromatography coupled to mass spectrometry. All experiments were performed in triplicate.

2.4. Chromatographic conditions

Chromatographic analyses were performed with a Varian 3800 gas chromatograph (Walnut Creek, CA, USA) equipped with a CombipalAutosampler (CTC Analytics, Zwingen, Switzerland) and connected to an ion-trap mass spectrometer (Varian Saturn 2200). Compounds were separated using a CP-WAX 52-CB column (30 m × 0.25 mm I.D., 0.25 μm film thickness) from Varian.Helium, at a flow of 1 mL/min, was used as carrier gas. Oven temperature was programmed as follows: 35 °C for 1 min, heated at 20 °C/min to 170 °C and kept for 1 min and finally raised to 210 °C at 3 °C/min and held for 12 min. Injection was performed in splitless mode for 1 min and then split was set at 40 mL/min. An inlet of 3.4 mm I.D. was used and injector temperature was fixed at 250 °C. The manifold, GC/MS interface and ion trap temperatures were set at 60 °C, 280 °C and 200 °C, respectively. Mass spectra were obtained using electron impact ionisation (70 eV). Precursor ions were isolated using a 3 amu isolation window and subjected to collision-induced dissociation (CID). For operating in MS/MS mode, the emission current was fixed at 80 μA and scan time at 0.50 s/scan. The rest of MS/MS parameters are summarised in Table 1 Table 1 Table 1. A chromatogram of a wine sample spiked with 250 ng/L of haloanisoles and 100 μg/L of volatile phenols is shown in Fig. 1.

2.5. Software

The construction and analyses of the experimental design, the response surface and the desirability functions for reaching the optimum conditions were carried out using the Nemrod-W statistical package [42].

3. Results and discussion

3.1. Optimisation of DLLME conditions

The goal of the DLLME procedure is the extraction in the organic phase of the greatest amount of analyte from the aqueous sample. In order to maximise the proportion of haloanisoles and volatile phenols extracted from wine, several factors affecting the DLLME

Table 1
Retention time and MS/MS detection parameters using the proposed method.

Compound	Retention time (min)	Precursor ion (m/z)	Quantification ions (m/z)	CID parameters	
				Storage level	Amplitude
2,4,6-Trichloroanisole	9.668	195	167	100	90
2,3,4,6-Tetrachloroanisole	12.933	246	203 + 231	100	85
2,4,6-Tribromoanisole	16.176	346	303 + 331	110	89
Pentachloroanisole	17.158	280	237	100	84
4-Ethylguaiacol	11.976	137	94	75	77
4-Ethylphenol	14.135	107	91	60	68
4-Vinylguaiacol	14.51	150	77	80	72
4-Vinylphenol	18.213	120	107	65	64
4-Iodoanisole	11.192	234	191	90	87
p-Cresol	12.678	107	77	60	68

procedure were evaluated in different steps for a fixed wine volume of 5 mL. Firstly, an exhaustive study was carried out by examining all combinations of disperser and extraction solvents considered. Then temperature and salting-out conditions were selected to, finally, simultaneously examine the effect of the volumes of disperser and extraction solvent by applying an experimental design methodology.

3.1.1. Solvent selection

A critical step in DLLME method development is the selection of the most suitable extraction and disperser solvents. Methanol, acetone and acetonitrile were evaluated as disperser solvents since they have good solubility in water and organic solvents. Otherwise, extraction solvent must have low water solubility. Moreover, due to the reduced volume used, and to facilitate its collection at the conical bottom of a test tube, the extraction solvent must have higher density than water. Taking this into account, chloroform, carbon tetrachloride, tetrachlorethylene, chlorobenzene and carbon disulfide were examined as extraction solvents.

In order to determine the combination of solvents, namely extraction mixture, which provides better recovery results, DLLME efficiency was examined for the target analytes using all combinations of disperser and extraction solvents studied. For this purpose, extraction mixtures consisting of 1 mL of disperser solvent and 100 μL of extraction solvent were used to extract 5 mL of synthetic wine spiked with the target analytes. All the experiments were performed in triplicate.

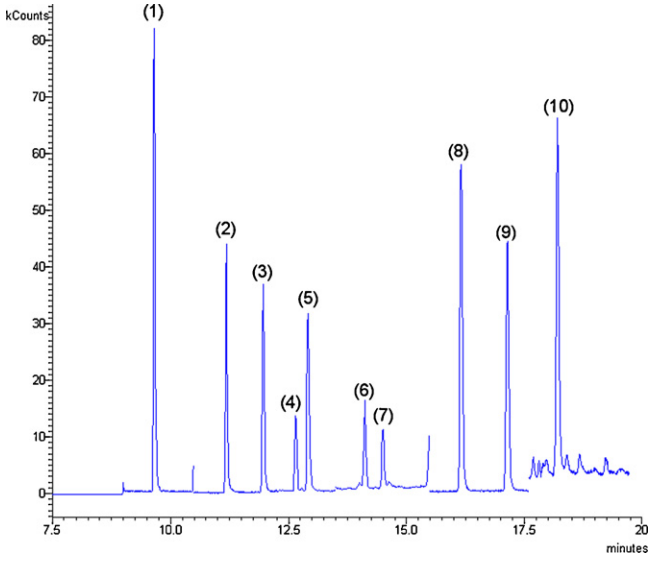

Fig. 1. GC–MS/MS chromatogram of a spiked wine sample. (1) 2,4,6-Trichloroanisole, (2) 4-iodoanisole, (3) 4-ethylguaiacol, (4) p-cresol, (5) 2,3,4,6-tetrachloroanisole, (6) 4-ethylphenol, (7) 4-vinylguaiacol, (8) 2,4,6-tribromoanisole, (9) pentachloroanisole and (10) 4-vinylphenol.

Whichever the disperser solvent, no statistical differences were found for recoveries of haloanisoles regardless of the extraction solvent employed. Nevertheless, significantly lower recoveries were found for volatile phenols when tetrachloroethylene or carbon disulfide was used as extractant. Moreover, in case of vinylphenols, carbon tetrachloride also provided poor yields. As a result, these extraction solvents were discarded. This behaviour can be observed in Fig. 2a, which shows the results obtained using all combinations employing acetone as disperser solvent. On the other hand, as it can be appreciated in Fig. 2b and c, when using chloroform or chlorobenzene as extractants, DLLME clearly yielded better recoveries for haloanisoles when acetone or methanol were employed as disperser solvents. In addition, recoveries of 4-ethylphenol and 4-vinylphenol were significantly lower by using methanol as disperser solvent. Thus, acetone was selected as disperser solvent. Since the combination acetone–chloroform lead to higher enrichment factors than the combination acetone–chlorobenzene (data not shown), it was selected as the extraction mixture.

3.1.2. Temperature effect

Temperature affects the solubility between phases in such a way that the increase of the temperature produces a decrease of the extract volume. This could result in higher recoveries of the analytes at low temperatures, and conversely at high temperatures. However, when reducing the temperature, the rise in viscosity of the extraction solvent decelerates the mass transfer kinetics, and the opposite effect occurs when temperature increases [43]. As a result, temperature is a parameter which could have different effects on the yield of the extraction procedures depending on the system and the type of analytes studied.

The influence of the temperature in the recoveries of haloanisoles and volatile phenols from wine by using DLLME was studied by performing extractions at three levels: in an ice bath (0 °C), at room temperature and at 40 °C. Spiked synthetic wine was used in these extractions and each experiment was performed in triplicate.

Fig. 3 shows the recoveries obtained for all the compounds at the three temperature levels studied. It can be observed that recoveries of volatile phenols decreased, along with the extract volume, by raising the temperature. On the other hand, recoveries of haloanisoles also decreased when the temperature went up to 40 °C. Nevertheless, at low temperature, the recoveries of the haloanisoles decreased in spite of the higher extract volume. Taking these results into account, room temperature was selected as the working temperature.

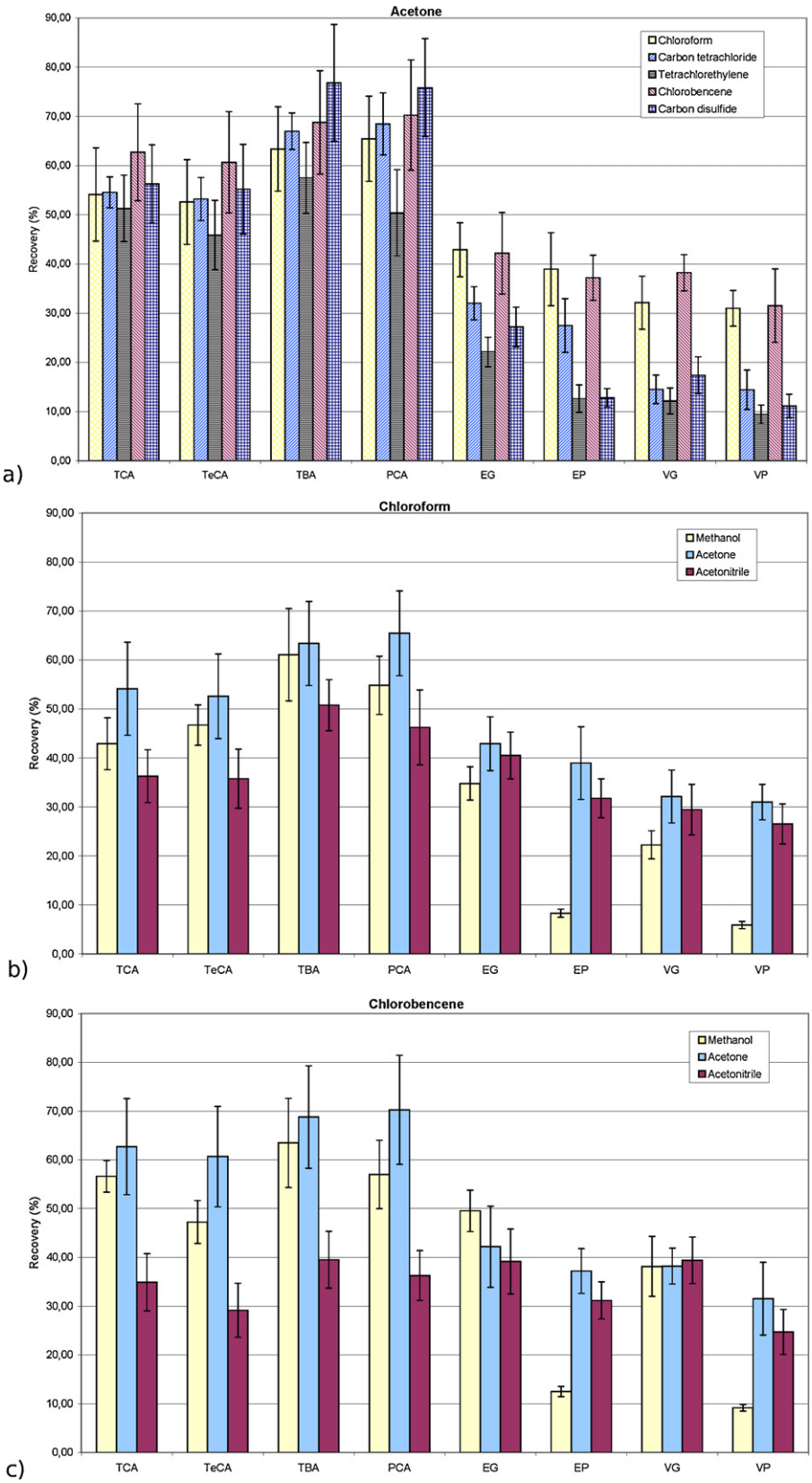

Fig. 2. (a) Recoveries of the target analytes obtained by using acetone as disperser solvent depending on the extraction solvent employed. (b) Recoveries of the target analytes by using chloroform as extraction solvent depending on the disperser solvent employed. (c) Recoveries of the target analytes by using chlorobenzene as extraction solvent depending on the disperser solvent employed.

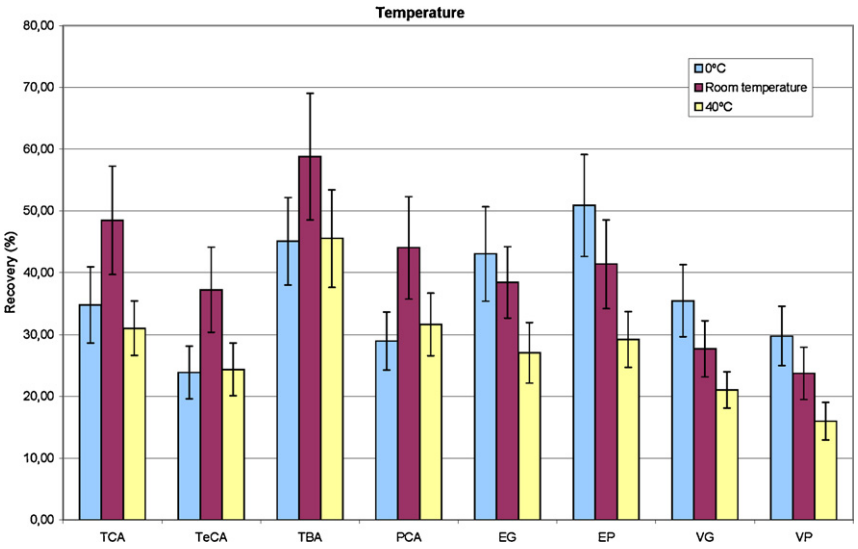

Fig. 3. Effect of temperature on the recoveries obtained for haloanisoles and volatile phenols by DLLME procedure ($n = 3$).

3.1.3. Salting-out effect

Another parameter that modifies the solubility and viscosity of the phases is the addition of salt [43,44]. Analyte solubility in the aqueous phase usually decreases as ionic strength increases. Moreover, solubility of the extraction solvent in the sample also decreases. Accordingly, extraction efficiency should enhance. However, it must be taken into account that the addition of salt produces an increase in the sample viscosity, which negatively affects the mass transfer phenomena. Additions of different amounts of sodium chloride were tested in order to investigate the effect of the ionic strength on the DLLME efficiency for the extraction of haloanisoles and volatile phenols from wine. For this purpose, extractions of spiked synthetic wine were carried out, in triplicate, at three different sodium chloride concentrations (no addition, 5% and 10%). Despite the increment in the extract volume by increasing the ionic strength (data not shown), Fig. 4 shows that there were no significant differences on the recoveries obtained for 4-ethylphenol, 4-ethylguaicol and 4-vinylphenol when the ionic strength was modified. Nevertheless, when larger amounts of sodium chloride were added, recoveries significantly decreased for haloanisoles and 4-vinylguaiacol, probably due to the slowdown in mass transference rate. According to these results, no salt addition was carried out in further experiments.

3.1.4. Experimental design: optimisation of the volumes of disperser and extraction solvents

Volumes of disperser and extraction solvents are key parameters to the proper formation of the dispersion and the maximisation of the recoveries of the target analytes. Therefore, once the type of solvents, the salt addition and the temperature conditions have been fixed, optimum disperser and extraction solvents volumes must be selected. Taking this into account, the influence of the volumes of disperser and extraction solvents on the recoveries of the target analytes from wine by DLLME procedure was evaluated by applying an experimental design methodology by means of a central composite design type 2^2 plus star, involving 8 runs, 3 central points and 3 test-points.

Small volumes of extraction solvent provide small volumes of sedimented phase, which could result in little recoveries of the analytes and problems in handling the extract. Nevertheless, if the extraction solvent volume is too high, a deficient formation of the dispersion and/or a dilution of the analytes in the extract

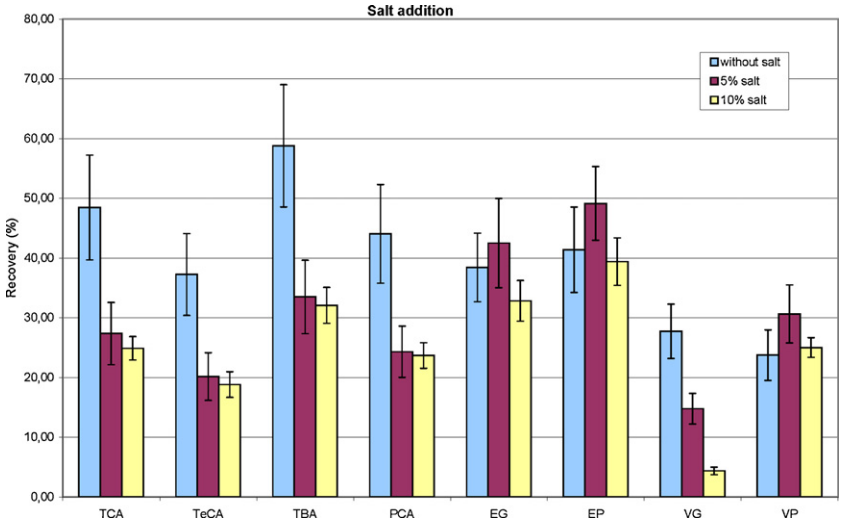

Fig. 4. Effect of salt addition on the recoveries obtained for haloanisoles and volatile phenols by DLLME procedure ($n = 3$).

Table 2
Experimental design matrix and average recoveries for studied compounds ($n = 3$).

No. exp.	Disperser solvent volume (mL)	Extraction solvent volume (μL)	Extraction efficiency							
			TCA	TeCA	TBA	PCA	EG	EP	VG	VP
1	0.72	89	63.56	54.44	57.75	59.01	48.15	41.16	39.25	53.72
2	1.78	89	47.26	50.58	54.07	65.86	13.05	11.72	7.98	19.62
3	0.72	181	69.27	64.54	64.59	68.45	86.28	77.24	84.76	85.42
4	1.78	181	96.60	92.96	96.09	103.13	77.15	65.54	70.17	82.02
5	0.50	135	59.29	52.57	51.56	53.46	63.29	54.96	62.47	79.57
6	2.00	135	70.32	69.32	72.34	82.02	36.47	30.50	36.42	59.50
7	1.25	70	50.08	46.92	53.96	61.74	24.47	20.08	11.99	18.26
8	1.25	200	85.47	84.02	85.22	92.12	98.05	89.19	89.76	82.42
Central	1.25	135	90.71	86.62	91.85	100.11	71.48	60.41	79.59	80.61
Central	1.25	135	95.70	87.39	92.08	98.82	73.50	64.52	78.40	84.21
Central	1.25	135	98.34	90.10	93.80	100.43	73.82	64.33	74.79	79.63
Test 1	0.93	119	84.70	73.74	77.80	82.76	66.13	59.50	68.50	76.19
Test 2	1.57	119	82.54	76.07	81.18	97.54	54.25	44.93	53.07	62.78
Test 3	1.25	167	97.50	94.01	96.51	100.71	87.08	77.90	89.94	91.18

may occur. On the other hand, the volume of disperser solvent must be high enough to form the dispersion properly but, as it increases, the solubility of the extraction solvent in the aqueous phase increases, producing lower volumes of extract and, thus, a lower extraction efficiency. Taking this into account, experimental domain for extraction solvent volume ranged from 70 to 200 μL while the volume of disperser solvent was studied from 0.5 to 2 mL. The experimental matrix, experimental conditions and recoveries obtained are presented in Table 2 Table 2 Table 2. All experiments were performed randomly to minimise the effects of uncontrolled factors that may introduce bias into the measurements.

The results obtained were used to estimate, by means of least squares linear regression, the coefficients of second order polynomial models following the Eq. (1):

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

where X_i were the studied factors (X_1: disperser solvent volume; X_2: extraction solvent volume) and the response Y was the recovery obtained for each compound. These models were analysed and validated by analysis of variance (ANOVA) and the test points using Nemrod-W software [42]. For all compounds, the proposed mathematical models were significant and correctly explained the behaviour of the compounds in the experimental domain. Therefore, the models were accepted.

Once the models were accepted, model coefficients and response surface fitting the data for each response were used to evaluate the effects of each factor and the interaction effects between factors. Model coefficients for each response are shown in Table 3 Table 3 Table 3. All the coefficients were significant for all compounds, including the interaction coefficient, thus, the effects of the factors cannot be studied separately. By examining the response surfaces, it can be appreciated that the rise in volumes of disperser and extraction solvents had a positive effect on the recoveries of all target analytes but, when both factors continued rising, the recoveries diminished. This could be due to an increase in the solubility of the analytes in the sample [34]. However, two different behaviours could be observed for the two groups of compounds studied. Regarding the disperser solvent, optimum volume was found at lower values for volatile phenols than for haloanisoles. The optimum volume of extraction solvent was also different for the two kinds of analytes studied, being lower for haloanisoles than for volatile phenols. As a result, it was difficult to find a common optimum for all of the analytes. In these cases, desirability functions are a very useful tool to find experimental conditions of compromise, so that each one of the responses is within an acceptable range [45,46].

When multiple responses have to be optimised simultaneously, desirability functions methodology allows finding the common optimum by the maximisation of a function, the global desirability D, which represents the quality of the compromise solution. For this purpose, 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 our 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 as the weighted geometric average of n individual desirability functions (Eq. (2)) [47]:

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

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.

In this study, linear partial desirability functions were selected for each response. In these functions, the optimum recovery value was 100% and recoveries under 50% were considered unacceptable. The weight for all the compounds was fixed at 1 except for TCA

Table 3
Estimates of the model coefficients.

Coefficients	TCA	TeCA	TBA	PCA	EG	EP	VG	VP
b_0	**94.957**	**87.618**	**92.078**	**100.005**	**73.196**	**63.715**	**77.483**	**81.797**
b_1	**3.228**	**5.930**	**7.051**	**10.603**	**−10.041**	**−9.547**	**−10.413**	**−8.242**
b_2	**13.083**	**13.461**	**11.890**	**10.967**	**25.585**	**23.346**	**27.212**	**23.152**
b_{11}	**−14.329**	**−12.867**	**−14.585**	**−15.582**	**−11.421**	**−10.353**	**−13.924**	**−6.066**
b_{22}	**−12.877**	**−10.371**	**−10.592**	**−11.155**	**−5.870**	**−4.477**	**−13.206**	**−15.613**
b_{12}	**10.979**	**8.143**	**8.869**	**6.695**	**6.332**	**4.494**	**4.225**	**7.683**

Bold numbers indicate significant effects (5%).

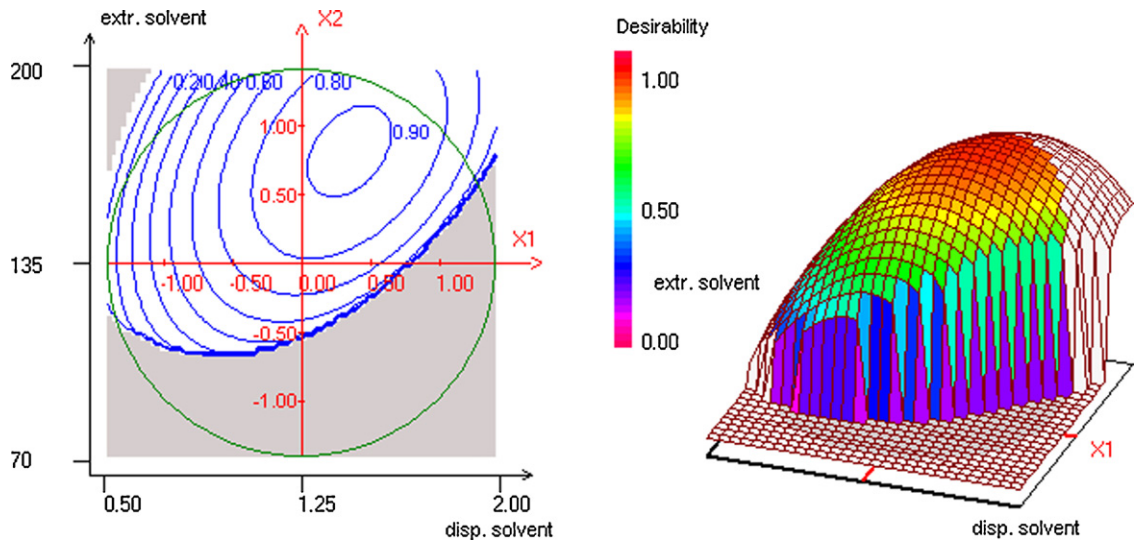

Fig. 5. Response surfaces of global desirability as a function of the volume of extraction and disperser solvents.

and TBA. These two compounds presented lower olfactory threshold than the rest of studied compounds so it was decided to give more weight to their recoveries. Therefore, the weight of the partial desirability functions for TCA and TBA was fixed at 10. Fig. 5 shows the plots of the global desirability obtained. Compromise optimum conditions were those in which global desirability was close to 1 and were set at 1.43 mL of acetone (disperser solvent) and 173 μL of chloroform (extraction solvent).

3.2. Method performance

For the first time, cork taint and Brett character responsible compounds have been simultaneously determined in wine by using a DLLME procedure. Red wine samples spiked with the target compounds were used in order to establish the quality parameters of the proposed method. Linearity was evaluated at five levels. Linear range for the haloanisoles ranged from 10 to 500 ng/L whereas volatile phenols were studied from 1 to 1800 μg/L. Mandel's fitting test was applied to evaluate the linearity of the data obtained using internal standard. In Table 4 Table 4 Table 4 it can be observed that significance higher than 0.05 (confidence level 95%) verified that the linear model is the best fit for all calibration data. Thus, linear regression was performed achieving correlation coefficients ranging from 0.992 to 0.996 (Table 4). Quantification and detection limits were calculated at the lowest concentration level for a signal/noise ratio (S/N) of 10 and 3, respectively. Detection limits close to or even lower than the perception threshold were obtained of all the compounds, including TCA and TBA, whose concentrations causing defect in wine are considered to be around 10–40 ng/L for TCA [27,48] and 7.9 ng/L for TBA [6]. These detection limits were similar or even lower than those obtained with other analytical methods [19,21,27,33]. Moreover, the detection limits achieved for the ethylphenols with the DLLME method proposed were lower than those obtained when DLLME was applied to the analysis of only these two compounds in wine [38].

Five extractions at three concentration levels were performed on the same day under optimum conditions in order to evaluate the repeatability of the proposed procedure. Between-days precision was assessed by performing extractions at the same levels on five different days. Table 5 Table 5 Table 5 shows the RSDs obtained for both of them. Acceptable precision, ranging from 4.3 to 11.6%, was obtained for all the compounds. Recoveries for red and white wines spiked with the target analytes, were analysed in triplicate. As it can be observed in Table 5, recoveries higher than 80% were obtained for all compounds. According to these results DLLME can be proposed as a simple, fast and economical method which may be used for the simultaneous determination of haloanisoles and volatile phenols in wine at suitable concentration levels and with adequate recoveries and precision.

3.3. Application of the method to real samples

The applicability of the proposed method was evaluated by analysing the content of cork taint and Brett character responsible compounds in different samples of red and white wines. Two red and two white wines from different origins were extracted under the optimised DLLME conditions. Each determination was made in triplicate. The results of the analyses are shown in Table 6 Table 6 Table 6. Regarding the red wines, both of them contained TeCA, EP and EG. In case of EG, its concentration was slightly over its odour threshold in red wine A. Red wine B also presented contamination of TCA, PCA, VP and VG. Only TCA and TeCA were higher than its

Table 4
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 (μg/L)	LOQ S/N = 10 (μg/L)
TCA	0.079	0.995	0.005	0.017
TeCA	0.121	0.994	0.008	0.027
TBA	0.523	0.992	0.007	0.025
PCA	0.176	0.992	0.012	0.041
EG	0.683	0.996	0.068	0.225
EP	0.248	0.994	0.051	0.169
VG	0.069	0.995	0.066	0.221
VP	0.081	0.992	0.075	0.250

Table 5
Repeatability, reproducibility 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	7.1	6.3	5.2	5.6	6.4	4.3	99.5 ± 7.2	96.6 ± 2.9	93.3 ± 9.1	99.0 ± 5.8
TeCA	6.0	10.2	8.0	11.0	10.0	8.3	94.5 ± 8.1	95.1 ± 7.8	94.2 ± 8.8	90.3 ± 4.8
PCA	8.1	10.6	7.0	10.9	10.9	6.5	97.9 ± 2.2	92.3 ± 4.8	97.4 ± 8.0	92.8 ± 7.5
TBA	5.0	13.6	8.7	10.5	9.2	6.8	102.2 ± 2.7	91.7 ± 3.0	98.7 ± 8.7	99.5 ± 7.8
TCP	8.9	11.6	7.1	8.0	10.8	6.2	87.0 ± 8.6	92.5 ± 6.7	95.4 ± 6.6	88.4 ± 6.3
TeCP	10.3	9.5	4.9	9.6	10.0	6.6	81.4 ± 8.1	82.0 ± 7.6	85.1 ± 9.1	88.1 ± 8.2
PCP	8.3	8.8	6.7	10.2	9.5	4.7	86.8 ± 3.5	90.2 ± 6.3	89.6 ± 8.0	87.9 ± 3.9
TBP	7.9	10.5	7.5	9.0	9.4	6.1	88.9 ± 4.6	88.8 ± 7.1	86.3 ± 5.1	89.0 ± 9.0

[a] Haloanisoles: 10 ng/L; volatile phenols: 10 μg/l.
[b] Haloanisoles: 250 ng/L; volatile phenols: 100 μg/l.
[c] Haloanisoles: 500 ng/L; volatile phenols: 1000 μg/l.

Table 6
Results of an analysis of wine samples by the DLLME proposed method ($n = 3$).

Compound	Concentration ± SD			
	Red wine A	Red wine B	White wine A	White wine B
TCA[a]	–	24 ± 3	30 ± 5	–
TeCA[a]	84 ± 6	98 ± 9	72 ± 3	–
TBA[a]	–	–	28 ± 2	–
PCA[a]	–	153 ± 7	–	–
EG[b]	39 ± 4	25 ± 2	–	–
EP[b]	83 ± 8	113 ± 6	–	–
VG[b]	–	153 ± 8	72 ± 4	29 ± 5
VP[b]	–	179 ± 7	117 ± 7	63 ± 3

[a] ng/L.
[b] μg/L.

olfactory threshold. White wine B only displayed low concentration levels of VG and VP. However, white wine A presented not only these compounds, but also TCA, TBA and TeCA at concentrations over their olfactory thresholds.

4. Conclusions

In this study, a novel approach based on the DLLME method has been presented for the simultaneous determination of cork taint and Brett character responsible compounds in wines. Optimum conditions for the DLLME extraction of 5 mL of wine were found at 1.43 mL of acetone and 173 μL of chloroform, at room temperature and without salt addition. Linearity, precision and detection limits of the developed DLLME method confirmed its suitability for the determination of the studied wine defects and it was applied for analysing different samples of red and white wines. To our knowledge, the proposed method is the first application of a DLLME procedure to the simultaneous determination of cork taint and Brett character responsible compounds in wine. The satisfactory results obtained prove that this method could be a suitable alternative to previously reported methods.

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. The authors also thank the ConsejoRegulador de la Denominación de Origen Calificada Rioja for supplying wine for the study.

References

[1] A.P. Pollnitz, K.H. Pardon, D. Liacopoulos, G.K. Skouroumounis, M.A. Sefton, Aust. J. Grape Wine Res. 2 (1996) 184.
[2] P.X. Etievant, in: H. Maarse (Ed.), Volatile Compounds in Foods and Beverages, first ed., Marcel Dekker, New York, 1991, p. p483.
[3] H.R. Buser, C. Zanier, H. Tanner, J. Agric. Food Chem. 30 (1982) 359.
[4] P. Chatonnet, G. Guimberteau, D. Dubourdieu, J.N. Boidron, J. Int. Sci. VigneVin. 28 (1994) 131.
[5] T.H. Lee, R.F. Simpson, in: G.H. Flete (Ed.), Wine Microbiology and Biotechnology, vol. 353, Harwood Academic Press, Chur, 1993 (Chapter 12).
[6] P. Chatonnet, S. Bonnet, S. Boutou, M.D. Labadie, J. Agric. Food. Chem. 52 (2004) 1255.
[7] M. Czaplicka, Sci. Total. Environ. 322 (2004) 21.
[8] F.B. Whitfield, J.L. Hill, K.J. Shaw, J. Agric. Food Chem. 45 (1997) 889.
[9] A.S. Allard, M. Remberger, A.H. Neilson, Appl. Environ. Microbiol. 53 (1987) 839.
[10] V. Gerbaux, B. Vincent, Revue des Œnologues 99 (2001) 15.
[11] P. Chatonnet, D. Dubourdieu, J.N. Boidron, M. Pons, J. Sci. Food Agric. 60 (1992) 165.
[12] J.P. Towey, A.L. Waterhouse, Am. J. Enol. Vitic. 47 (1996) 163.
[13] J.L. Licker, T.E. Acree, T. Henick-Kling, in: S.E. Ebeler, A.L. Waterhouse (Eds.), Chemistry of Wine Flavor (ACS Symposium Series, No. 714), American Chemical Society, Washington, DC, 1998, p. 96.
[14] T. Heresztyn, Arch. Microbiol. 146 (1986) 96.
[15] 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.
[16] V. Ferreira, R. López, J.F. Cacho, J. Sci. Food. Agric. 80 (2000) 1659.
[17] R. Juanola, D. Subirà, V. Salvadó, J.A. GarcíaRegueiro, E. Anticó, J. Chromatogr. A 953 (2002) 207.
[18] T. Garde-Cerdán, S. Rodríguez, C.A. Azpilicueta, Food Res. Int. 35 (2002) 603.
[19] A. Martinez-Uruñuela, I. Rodríguez, R. Cela, J.M. Gonzalez-Saiz, C. Pizarro, Anal. Chim. Acta 549 (2005) 117.
[20] S. Insa, E. Anticó, V. Ferreira, J. Chromatogr. A 1089 (2005) 235.
[21] C. Domínguez, D.A. Guillén, C.G. Barroso, Anal. Chim. Acta 458 (2002) 95.
[22] D. Hernanz, V. Gallo, A.F. Recamales, A.J. Melendez-Martínez, F.J. Heredia, Talanta 76 (2008) 929.
[23] N. Martorell, M.P. Martí, M. Mestres, O. Busto, J. Guasch, J. Chromatogr. A 975 (2002) 349.
[24] A. Martínez-Uruñuela, J.M. González-Sáiz, C. Pizarro, J. Chromatogr. A 1056 (2004) 49.
[25] C. Pizarro, N. Pérez-del-Notario, J.M. González-Sáiz, J. Chromatogr. A 1143 (2007) 26.
[26] C. Pizarro, N. Pérez-del-Notario, J.M. González-Sáiz, J. Chromatogr. A 1143 (2007) 176.

[27] C. Pizarro, N. Pérez-del-Notario, J.M. González-Sáiz, J. Chromatogr. A 1166 (2007) 1.
[28] S. Boutou, P. Chatonnet, J. Chromatogr. A 1141 (2007) 1.
[29] J. Díez, C. Domínguez, D.A. Guillén, R. Veas, C.G. Barroso, J. Chromatogr. A 1025 (2004) 263.
[30] A. Zalacain, G.L. Alonso, C. Lorenzo, M. Iñiguez, M.R. Salinas, J. Chromatogr. A 1033 (2004) 173.
[31] L. Maggi, A. Zalacain, V. Mazzoleni, G.L. Alonso, M.R. Salinas, Talanta 75 (2008) 753.
[32] C. Franc, F. David, G. de Revel, J. Chromatogr. A 1216 (2009) 3318.
[33] E. Martendal, D. Budziak, E. Carasek, J. Chromatogr. A 1148 (2007) 131.
[34] M. Rezaee, Y. Assadi, M.R.M. Hosseini, E. Aghaee, F. Ahmadi, S. Berijani, J. Chromatogr. A 1116 (2006) 1.
[35] C. Bosch-Ojeda, F. Sánchez-Rojas, Chromatographia 69 (2009) 1149.
[36] A. Sarafraz-Yazdi, A. Amiri, TrAC: Trends Anal. Chem. 29 (2010) 1.
[37] S. Dadfarnia, A.M. Haji Shabani, Anal. Chim. Acta 658 (2010) 107.
[38] L. Fariña, E. Boido, F. Carrau, E. Dellacassa, J. Chromatogr. A 1157 (2007) 46.
[39] R. Montes, I. Rodriguez, M. Ramil, E. Rubí, R. Cela, J. Chromatogr. A 1216 (2009) 5459.
[40] C. Pizarro, C. Sáenz-González, N. Pérez-del-Notario, J.M. González-Sáiz, J. Chromatogr. A 1217 (2010) 7630.
[41] N. Campillo, P. Viñas, J.I. Cacho, R. Peñalver, M. Hernández-Córdoba, J. Chromatogr. A 1217 (2010) 7323.
[42] D. Mathieu, J. Nony, R. Phan-Tan-Luu, NEMROD-W 2001, LPRAI, Marseille, 2000.
[43] H. Kuhn, H.-D. Försterling, D.H. Waldeck, Principles of Physical Chemistry, Wiley, New York, 2009.
[44] R. Montes, I. Rodríguez, E. Rubí, R. Cela, J. Chromatogr. A 1216 (2009) 205.
[45] M.J. Sáiz-Abajo, J.M. González-Sáiz, C. Pizarro, Anal. Chim. Acta 528 (2005) 63.
[46] C. Pizarro, J.M. González-Sáiz, N. Pérez-del-Notario, J. Chromatogr. A 1132 (2006) 8.
[47] G.A. Lewis, D. Mathieu, R. Phan-Tan-Luu, Pharmaceutical Experimental Design, Marcel Dekker, New York, 1999.
[48] R. Alzaga, L. Ortiz, F. Sánchez-Baeza, M.P. Marco, J.M. Bayona, J. Agric. Food Chem. 51 (2003) 3509.