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A simple and sensitive vortex assisted liquid–liquid microextraction method for the simultaneous determination of haloanisoles and halophenols in wines

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ABSTRACT

A vortex assisted liquid–liquid microextraction (VALLME) method was developed and optimised for the determination of the main compounds that can cause cork taint in wines, 2,4,6-trichloroanisole (TCA), 2,3,4,6-tetrachloroanisole (TeCA), 2,4,6-tribromoanisole (TBA) and pentachloroanisole (PCA); and their corresponding halophenolic precursors. Target compounds were determined by gas chromatography combined with a micro-electron capture detector (GC–μECD) system. Halophenol extraction and derivatisation processes were performed at the same time. To optimise the VALLME method, the extraction solvent was selected. Then, the other parameters of influence, such as volume of extraction solvent and derivatisation agent, salt addition and vortex time were optimised using a central composite design combined with desirability functions. Once the optimal conditions had been determined, the method was validated, showing satisfactory linearity (with correlation coefficients over 0.983), repeatability (below 10.0%) and reproducibility (below 11.2%). Detection limits obtained were lower than the olfactory threshold of the studied compounds, being similar or even lower than previously reported with the advantage of reducing the extraction time. The analysis of real wine samples demonstrated the applicability of the method. To our knowledge, this is the first time that VALLME has been applied for the simultaneous determination of haloanisoles and halophenols in wine.

1. Introduction

The oenological industry stands out among food industries due to its economic impact. One of the main objectives of this industry is to find the best quality for its products. To achieve this objective, the processes that take place during winemaking have to be controlled, allowing better wines to be made that can be competitive in the current consumer market. Wine aroma is probably the most important characteristic of wine quality. Therefore, during winemaking it is extremely important to control its evolution to avoid the presence of certain compounds that can cause undesirable taste and odours, which can alter wine quality, causing serious economical losses [1,2].

Cork taint is one of the most common off-flavours that can be found in wines. Haloanisoles, 2,4,6-trichloroanisole (TCA), 2,3,4,6-tetrachloroanisole (TeCA), pentachloroanisole (PCA) and 2,4,6-tribromanisole (TBA) are the main compounds that can cause this musty off-flavour. It is believed that fungi, isolated from cellars, cork and barrels, may biosynthesise TCA together with other chlorophenols deriving from reactions between lignin breakdown products and chlorinated compounds, such as chlorinated solutions used to bleach cork and washing barrels, chlorinated biocides used in oak forests and wood preservatives [3,4]. On the other hand, 2,4,6-tribromophenol (TBP) may also appear due to its extensive use as a flame retardant and fungicide in cellars [5,6]. Halophenols may degrade into their corresponding haloanisoles via O-methylation by bacterial microorganisms [7,8].

Many techniques have been used for sample preparation prior to the determination of haloanisoles and halophenoles in wines. Traditionally, liquid–liquid extraction methods with organic solvents were employed [9,10], but these are hazardous and time consuming. For this reason, easier and more selective methods are used, such as solid phase extraction (SPE) [11–13], solid-phase microextraction (SPME) [14–17] and stir bar sorptive extraction (SBSE) [18,19]. Besides, dispersive liquid–liquid microextraction (DLLME) [20–22] and single drop microextraction (SDME) [23,24]

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have been used to determine the main compounds responsible for cork taint in wines. Nowadays, new extraction methods are used, such as ultrasound-assisted emulsification–microextraction (USAEME) [25–28], a simple, rapid, low cost and low solvent requeriment technique; and microextraction by packed sorbent (MEPS) [29], a miniaturisation of SPE, which reduces sample volume and extraction and washing solvent volumes used in it.

Nevertheless, some of these techniques have drawbacks that made them less appropriate for the determination of haloanisoles and halophenols in wines. Thereby, SPE uses relatively larger volumes of organic solvents, DLLME requires the use of a disperser solvent, SDME has relatively low precision, SPME involves the use of a relatively expensive and fragile device [30] and USAEME takes significantly longer than DLLME [31]. As an alternative, a new extraction method has been developed in recent years called vortex-assisted liquid–liquid microextraction (VALLME), which avoids some of these problems. VALLME is an efficient, fast, easy and economic method that allows repeatable measurements, requiring a small volume of extraction solvent.

VALLME was introduced by Yiantzi et al. in 2010 [32]. Thanks to the use of vortex mixing, the VALLME method enables the dispersion of the extraction solvent into the aqueous solution. The fine micro droplets that form during vortex mixing enable the extraction of analytes due to the formation of a larger specific surface area. This method has been previously used in different studies to analyse perfluorooctane sulphonate in water [33], aliphatic amines in complex sample solutions [34], mercury in sediments [35], pesticides in water [36] or phthalate esters in wine [37] and water [38].

Taking into account the advantages of the VALLME method, this study aimed to develop a vortex-assisted liquid liquid microextraction–derivatisation method to determine haloanisoles and halophenols in wines. Target compounds have high volatility and halogenated groups. Therefore, the analytical technique used for their determination was gas chromatography coupled to a micro-electron capture detector (GC–μECD). However, due to the high polarity of halophenols, which may cause problems of broad and tailed peaks in their chromatographic determination, a derivatisation step must be included to transform the halophenols into less polar compounds. For this purpose, aqueous acetylation with acetic anhydride in basic conditions was selected for the derivatisation reaction [39], due to its simplicity, low time consumption and efficiency.

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 disulphide from Acros Organics (Geel, Belgium). Ethanol, acetone, acetonitrile and tartaric acid were purchased from Merck (Darmstad,Germany). Acetic anhydride and sodium chloride were purchased from Aldrich Chemie and sodium phosphate di-basic was purchased from Panreac (Barcelona, Spain).

Ultrapure water was obtained from a Mili-Q system (Milipore, Bedford, MA, USA).

2.2. Samples and standard solutions

Individual stock standard solutions of each compound were prepared in methanol at a concentration level of 400 mg L^{-1}. Working 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.

Red and synthetic wines were selected for the different studies. The synthetic wine solutions were prepared by dissolving 5 g L^{-1} of L-(+)-tartaric acid in a hydroalcoholic solution (13% (v/v) ethanol). The pH of the resulting solutions was adjusted to 3.5 with NaOH to be similar to that of the analysed wine. Both real and synthetic samples were spiked with different amounts of work solutions containing the target analytes.

2.3. Sample preparation

For each analysis, the extraction–derivatisation processes were performed simultaneously. For each VALLME analysis, an aliquot of 5 ml of red wine spiked with haloanisoles and halophenols was placed in a 10 ml glass test tube with conical bottom. According to the specific experiment proposed in the experimental plan, different amounts of sodium chloride were dissolved in the sample. 0.28 g of sodium phosphate di-basic were added to the wine sample to create alkaline conditions required for acetylation reaction (pH=7.6). Then, different amounts of extraction solvent and acetic anhydride were injected into the sample depending on the experiment. The mixture was dispersed at different times in a Heidolph Reax Top Vortex (Schwabach, Germany) at 2500 rpm. The dispersion formed during vortex extraction was disrupted by centrifugation at 5000 rpm for 3 min in a Rotina 38 (Hetthich, Tutlingen, Germany). Then, the organic phase was removed from the sample, the extracts were collected from the bottom of the glass tube with a microsyringe and added to a 0.15 mL glass insert that was placed into an autosampler vial. Finally, the extracts were analysed by GC–μECD. All the experiments were performed in triplicate.

2.4. Chromatographic conditions

Chromatographic analysis was performed with a Varian 3800 gas chromatograph (Walnut Creek, CA, USA) equipped with a programmable temperature vaporising injector (Varian 1079) and connected to an micro-electron capture detector (μECD). Compounds were separated using a VF-5ms capillary column (30 m × 0.25 mm i.d. and 0.25 μm film thickness) from Varian. A split liner packed with CarboFrit™ (Restek, Bellefonte, PA, USA) was placed into the injection port. The injected volume was 5 μl. The injector temperature was programmed as follows: 100 °C for 0.5 min, heated at 100 °C min^{-1} to 300 °C and kept for 10 min. The split valve was opened until 0.5 min (split flow 50 ml min^{-1}), then closed for 3 min and finally opened again (split flow 100 ml min^{-1}). Helium at 1 ml min^{-1} was used as carrier gas. Oven temperature was programmed as follows: 100 °C for 3.5 min, heated at 15 °C min^{-1} to 115 °C, heated to 150 °C at 1 °C min^{-1}; and finally raised to 250 °C at 25 °C min^{-1} and maintained for 2 min. μECD temperature was kept at 300 °C.

2.5. Multivariate optimisation

The VALLME procedure was optimised by evaluating the influence of different parameters (volume of extraction solvent, ionic strength, volume of derivatisation agent and vortex time) on the recoveries obtained for the target compounds using an experimental design methodology combined with desirability functions. 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 [40].

3. Results and discussion

The purpose of optimising the VALLME method is to determine the best experimental conditions to enable adequate recoveries of the target analytes. For this purpose, it is necessary to control different factors which may have a significant effect on the efficiency of the process, such as type of extraction solvent and its volume, ionic strength conditions, volume of acetic anhydride and vortex time. Firstly, as a key step in the optimisation, the most appropriate extraction solvent was chosen. Once the extraction solvent was selected, its volume, ionic strength, volume of anhydride acetic and vortex time were examined simultaneously using an experimental design methodology to evaluate their effect on the yield of the response.

3.1. Solvent selection

A critical step in the development of a VALLME method is the selection of an appropriate solvent to extract the analytes from the sample. In a VALLME process a fine liquid–liquid dispersion system is formed. Thus, a good VALLME solvent must facilitate the formation of the micro droplets using vortex mixing and, after centrifugation, restore them as a single micro drop. Taking these requirements into account, carbon tetrachloride, chlorobenzene, chloroform, carbon disulphide and tetrachloroethylene were used to evaluate their extraction efficiency in wine.

To select the most appropriate solvent, 5 ml of wine spiked with 250 ng L^{-1} of haloanisoles and halophenols were used. Then, sodium chloride at 3% (w/v), 0.28 g of sodium phosphate di-basic, 150 μL of each solvent and 100 μL of anhydride acetic were added to the sample. The vortex time applied was 6 min.

Carbon tetrachloride, chloroform and chlorobenzene were discarded due to the precipitate formed when they were used, which hindered the collection of the extracts. However, carbon disulphide and tetrachloroethylene allowed the extracts to be easily collected from the bottom of the conical test tube. Fig. 1 shows the results obtained for the target compounds when they were extracted with carbon disulphide and tetrachloroethylene. Both solvents provided values for area counts that were statistically similar for target compounds. However, the repeatability of the method was negatively affected by the use of carbon disulphide, making the extractions unreliable. Probably, the formation of carbon disulphide micro droplets in the aqueous phase is more difficult than the formation of tetrachloroethylene droplets, decreasing the reproducibility of the process. This could be due to the combination of its physical properties such as surface tension, density and viscosity, which are directly related to micro droplets formation in a dispersion process. For this reason, to improve the precision of the method tetrachloroethylene was selected as extraction solvent.

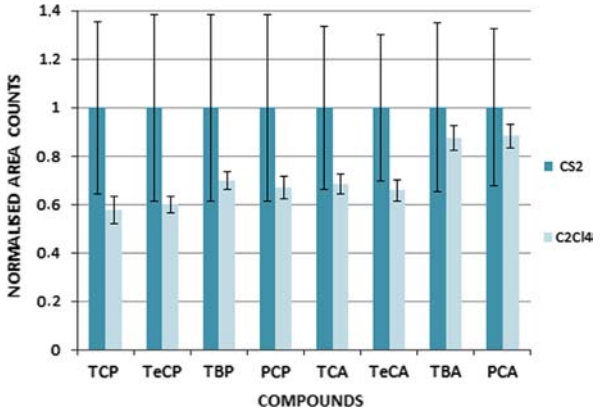

Fig. 1. Influence of solvent extraction on the efficiency of VALLME for haloanisoles and halophenols using the VALLME method ($n=3$). Responses were normalised to the maximum signal achieved for each response.

3.2. Multivariate optimisation

Once the extraction solvent had been selected, the rest of the VALLME influential parameters were established and evaluated. The ratio between solvent extraction volume and sample volume, acetic anhydride volume, ionic strength and vortex time could produce different effects in the VALLME process. Thus, solvent extraction volume must be high enough to achieve a good recovery of the analytes and thus allow the collection of the extract. However, if it is too high, it can lead to excessive dilution of the analytes. Moreover, the salting out effect can prompt a decrease in the solubility of the analytes in the sample, encouraging their extraction in the organic solvent [41]. On the other hand, the addition of salt can also produce an increase in the viscosity of the aqueous phase, which decelerates the mass transfer kinetics [42]. Acetic anhydride can also have a different effect in both groups of compounds [25]. Finally, low amounts of vortex time cannot produce the correct dispersion of the analytes, resulting in lower rates of recovery [32,42]. Bearing in mind all of the foregoing, a chemometrical approach based on experimental design and response surface methodology was applied to study factor effects and their interactions on the yield of the process. For this purpose, a Central Composite Design (CCD) type 2^4 plus star, involving 24 runs, 5 central points and 5 test points was selected to evaluate VALLME–derivatisation process efficiency. All experiments were performed in triplicate and randomly to minimise the effects of uncontrolled factors that may introduce bias into the measurements.

To define the experimental domain for each factor, preliminary studies and operative limits were considered. Since sample volume was fixed at 5 mL, the ratio between extraction solvent and the sample ($V_{\text{extractant}}$ (μL)/V_{sample} (ml)) was evaluated from 20 to 50, namely, from 100 to 250 μL of extraction solvent. These limits were selected to enable the proper collection of the organic extract from the bottom of the conical tube. Another factor that was taken into account was the derivatisation agent. Thus, in order to achieve the complete derivatisation of the halophenols, 50 μL was set as the minimum volume of acetic anhydride necessary to complete the process. The upper value was fixed at 100 μL. In terms of the amount of NaCl, this was evaluated from 0 to 10% (w/v). Finally, vortex time was studied from 1 to 10 min to guarantee the complete formation of the dispersion. The experimental matrix, experimental conditions and recoveries obtained are presented in Table 1.

The effect of each studied factor and their interactions were fitted 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: $V_{\text{extractant}}/V_{\text{sample}}$; X_2: $V_{\text{acetic anhydride}}$; X_3: % NaCl; X_4: vortex time) and the response Y was the recovery values for each compound. The obtained model coefficients were estimated by least squares linear regression and validated by analysis of variance (ANOVA) and by using test points using NEMROD-W software [40]. For all compounds, the proposed mathematical models were significant and correctly

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

No. exp	V_{extr}/V_{sample} (μL)	$V_{ac.anh.}$ (μL)	NaCl conc. (% w/V)	Time (min)	Recovery (%)							
					TCA	TeCA	TBA	PCA	TCP	TeCP	TBP	PCP
1	0.030	66.0	3.0	3.8	58.83	43.92	71.63	47.47	72.14	61.93	72.72	65.21
2	0.040	66.0	3.0	3.8	29.21	16.89	51.20	20.59	37.26	35.62	50.55	33.10
3	0.030	85.0	3.0	3.8	62.87	47.46	63.34	48.67	74.23	58.79	73.53	73.21
4	0.040	85.0	3.0	3.8	55.31	44.56	61.87	40.85	43.04	42.00	53.03	38.13
5	0.030	66.0	7.0	3.8	61.16	34.14	62.67	41.74	33.74	39.50	51.76	33.94
6	0.040	66.0	7.0	3.8	52.40	28.09	53.05	29.33	19.38	31.20	37.54	17.89
7	0.030	85.0	7.0	3.8	60.37	49.94	66.82	49.18	40.05	49.18	59.06	56.24
8	0.040	85.0	7.0	3.8	37.75	19.29	51.92	23.57	30.67	31.07	42.38	32.62
9	0.030	66.0	3.0	7.2	86.35	83.17	89.50	79.53	69.77	77.44	86.05	64.79
10	0.040	66.0	3.0	7.2	70.57	42.20	65.82	49.51	42.30	45.19	60.12	52.93
11	0.030	85.0	3.0	7.2	95.29	75.73	89.87	88.31	67.51	75.85	90.87	86.94
12	0.040	85.0	3.0	7.2	70.90	29.51	62.23	45.37	41.63	51.44	67.04	45.26
13	0.030	66.0	7.0	7.2	61.23	69.26	65.99	58.23	51.51	48.39	56.63	34.01
14	0.040	66.0	7.0	7.2	33.27	33.60	41.83	30.43	36.56	30.01	29.92	20.68
15	0.030	85.0	7.0	7.2	62.64	50.79	71.69	57.20	62.36	53.50	72.71	71.47
16	0.040	85.0	7.0	7.2	35.11	14.03	49.29	46.15	33.59	33.37	48.17	22.50
17	0.020	75.0	5.0	5.5	81.01	84.45	90.69	89.23	94.13	91.77	95.57	89.91
18	0.050	75.0	5.0	5.5	25.11	9.69	47.08	15.81	12.64	21.31	29.82	6.21
19	0.035	50.0	5.0	5.5	65.09	43.27	59.89	33.66	40.52	35.77	46.06	25.84
20	0.035	0.02	5.0	5.5	74.04	48.39	68.42	49.74	37.40	48.48	62.24	54.45
21	0.035	75.0	0.0	5.5	66.67	49.27	79.72	57.88	90.01	69.71	82.84	66.93
22	0.035	75.0	10.0	5.5	39.85	32.85	57.16	34.64	35.05	35.43	39.18	27.05
23	0.035	75.0	5.0	1	38.85	24.47	48.09	25.68	30.29	31.58	44.97	43.20
24	0.035	75.0	5.0	10	54.29	52.73	60.03	54.89	55.65	51.67	63.40	45.76
Central	0.035	75.0	5.0	5.5	61.35	34.80	70.55	38.34	58.35	47.26	63.04	43.84
Central	0.035	75.0	5.0	5.5	54.49	21.63	59.41	34.30	48.11	50.79	65.61	46.43
Central	0.035	75.0	5.0	5.5	54.28	25.19	59.47	30.27	53.37	40.70	57.02	45.97
Central	0.035	75.0	5.0	5.5	56.50	39.27	64.50	45.21	42.20	45.15	56.82	47.02
Central	0.035	75.0	5.0	5.5	70.11	51.21	69.75	59.29	47.22	59.79	65.67	51.45
Test 1	0.030	71.0	4.5	5.1	51.86	31.66	66.24	47.52	56.52	68.70	75.93	66.27
Test 2	0.040	71.0	4.5	5.1	55.95	22.87	55.27	19.51	44.22	40.94	53.50	47.73
Test 3	0.035	84.0	4.5	5.1	66.78	52.90	72.01	56.66	47.00	63.34	69.23	61.6
Test 4	0.035	75.0	7.0	5.1	60.71	43.23	63.01	50.18	36.47	34.67	46.98	29.95
Test 5	0.035	75.0	5.0	7.2	61.04	46.30	75.17	57.40	62.91	50.93	66.72	66.13

Table 2
Estimates of the model coeficients.

Coefficients	TCA	TeCA	TBA	PCA	TCP	TeCP	TBP	PCP
b_0	**59.995**	**36.379**	**64.751**	**43.614**	**48.580**	**48.747**	**61.844**	**48.431**
b_1	**−10.184**	**−14.316**	**−8.598**	**−13.132**	**−13.272**	**−11.930**	**−11.608**	**−15.088**
b_2	1.776	0.362	**1.371**	**2.550**	0.715	**2.265**	**3.507**	**6.354**
b_3	**−6.472**	**−4.488**	**−5.042**	**−5.217**	**−9.549**	**−7.797**	**−9.159**	**−9.770**
b_4	**4.603**	**5.982**	**2.954**	**7.284**	**4.211**	**3.728**	**4.012**	1.615
b_{11}	−0.915	**1.626**	0.505	1.385	0.497	**1.018**	0.117	−0.050
b_{22}	**1.468**	**1.565**	−0.134	−0.076	**−1.586**	**−1.020**	**−1.109**	−1.203
b_{33}	−0.869	0.883	0.479	0.600	**1.818**	0.432	−0.149	−0.269
b_{44}	**−1.859**	0.508	**−1.571**	−0.273	−0.959	**−1.133**	**−1.112**	−0.549
b_{12}	−0.154	0.132	0.697	−0.252	−0.309	0.793	0.243	**−4.088**
b_{13}	−0.704	−0.222	0.119	1.050	**3.187**	1.841	0.660	0.469
b_{23}	**−3.270**	−2.114	1.001	−1.639	1.300	0.965	**1.952**	**3.746**
b_{14}	−1.779	**−6.520**	**−3.228**	**−3.326**	−0.501	−1.948	**−1.701**	−1.262
b_{24}	−0.171	**−5.475**	0.235	−1.244	−1.268	0.361	**1.906**	0.925
b_{34}	**−8.564**	**−3.317**	**−4.083**	**−4.543**	**4.107**	**−2.634**	**−2.304**	−2.620

Bold numbers indicate significant effects (5%).

explained the behaviour of the compounds in the experimental domain. Therefore, the models were accepted and the results analysed in detail. The models were explained by their coefficients, enabling the identification of the significant factors and their interactions for each response in Table 2. The presence of significant interaction factors showed that these cannot be studied separately. When analysing the response surfaces, a similar behaviour was observed for all the studied responses. To illustrate the obtained results, the response surfaces for the most relevant significant interactions for TCP, TBP, PCP and TeCA are presented in Fig. 2. High volumes of extraction solvent have a negative effect on the recoveries of both groups of compounds (Fig. 2a and d). This may probably be due to the fact that when the volume of extraction solvent increases, it is more difficult to achieve a correct dispersion to extract the analytes applying the same vortex agitation speed. The derivatisation agent has a similar effect for both groups of compounds. Thus, using low volumes of acetic anhydride combined with a high percentage of salt or combined with low vortex time decreases process yield (Fig. 2b and c). In the case of haloanisoles, excessively high volumes of derivatisation agent had negative effects on the yields obtained, probably due to an increase in the acidity of the organic phase, caused by the

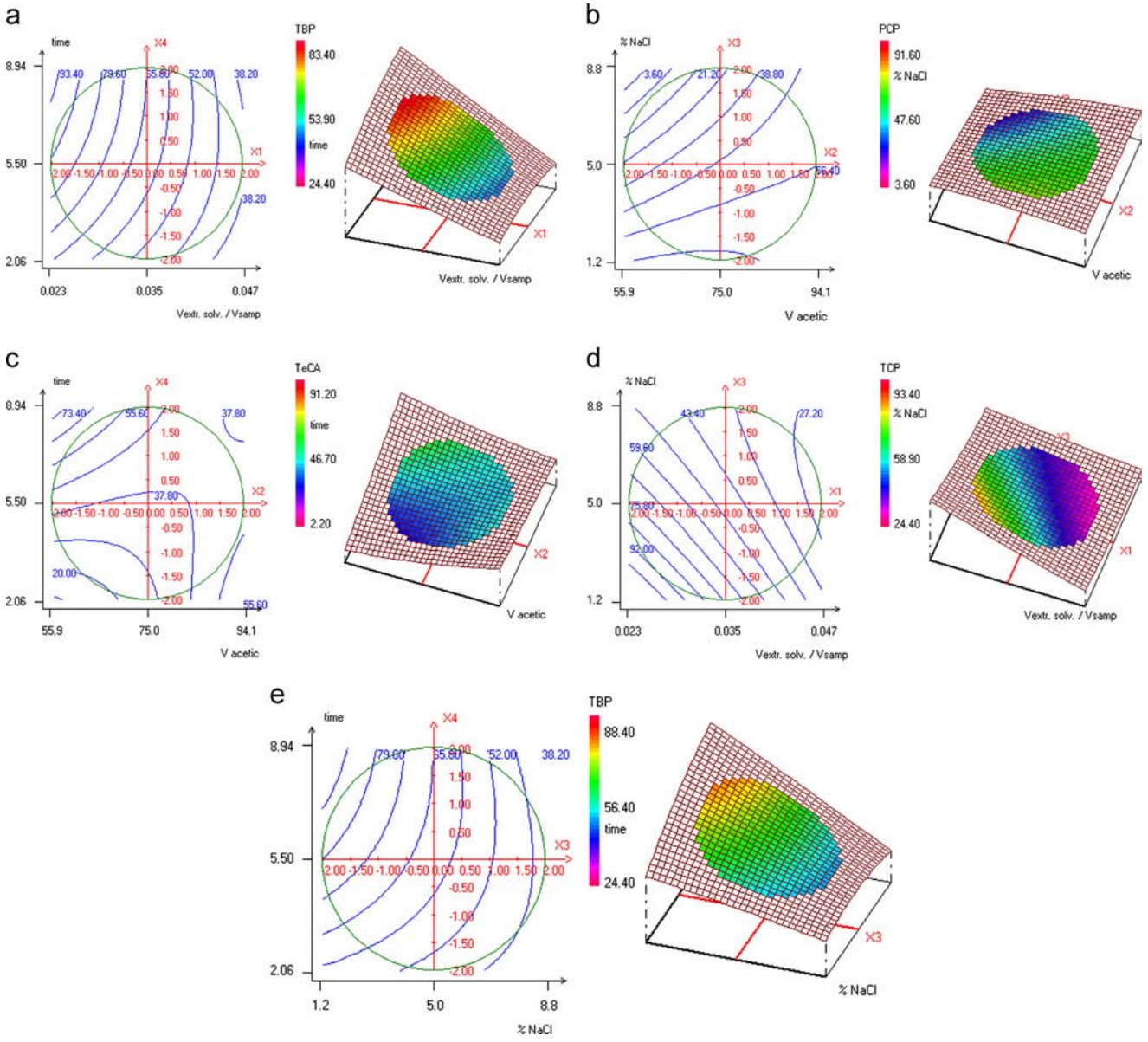

Fig. 2. Response surfaces obtained for significant interactions for TBP, PCP and TeCA (a) time vs V_{extr}/V_{sample} ($V_{ac.anh}$: 75 μL, 5% NaCl) for TBP; (b) %NaCl vs $V_{ac.anh}$ (V_{extr}/V_{sample}: 0.035, 5.5 min) for PCP; (c) time vs $V_{ac.anh}$ (V_{extr}/V_{sample}: 0.035, 5% NaCl) for TeCA; (d) %NaCl vs V_{extr}/V_{sample} ($V_{ac.anh}$: 75 μL, 5.5 min) for TCP; (e) time vs %NaCl (V_{extr}/V_{sample}: 0.035, $V_{ac.anh}$: 75 μL) for TBP.

hydrolysis of acetic anhydride [21] (Fig. 2c). On the other hand, the addition of large amounts of NaCl (combined with low volumes of acetic anhydride or tetrachloroethylene, and regardless the time employed) decreased the extraction efficiency of the process (Fig. 2b, d and e). This may be due to an increase in the viscosity of the aqueous solution that may hinder the formation of a correct dispersion into the sample. Finally, when using vortex times slightly higher than the mean value, the extraction process provided high recoveries for all compounds (Fig. 2a, c and e). Although the studied compounds presented similar behaviour, optimum conditions were different for both groups of compounds and desirability functions were necessary in order to find optimum compromise experimental conditions.

As multiple responses had to be optimised simultaneously, it was necessary to achieve experimental conditions that allowed each response to be obtained within an acceptable range. For this purpose, desirability functions methodology was applied. This consisted in transforming each response into a dimensionless partial desirability function, d_i, which varied from zero (undesirable response) to one (optimal response). Firstly, the most appropriate form of the desirability function and its behaviour within the fixed limits of the domain for each response was set. Once all the partial desirability functions had been defined, the next step was to evaluate the global desirability function D, defined as the weighted geometric average of n individual desirability functions [43]

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

where p_i is the weighting of the ith, normalised so that $\sum_{i=1}^{n} p_i = 1$. The highest value that global desirability can have is 1 and the desirability function for all parts of the domain where an individual response is outside the acceptable range is therefore zero. This allows optimisation to take into account the relative importance of each response, while selecting the most appropriate form of the partial desirability functions. A linear partial desirability function was selected for each response. In these functions, the optimum recovery value was set to 100% and recoveries under 50% were

considered unacceptable. Since, among the compounds studied, TCA and TBA had the lowest detection thresholds, they were given greater weight in the calculation of the overall desirability function. Thus, the weight of the partial desirability functions for TCA and TBA was fixed at 10. Establishing compromise optimum conditions in which global desirability was close to 1, the factors were set to 135 μL of tetrachloroetylene, 78 μL of acetic anhydride, 3% (w/v) of NaCl in the wine and 7 min of vortex time.

3.3. Method performance

This was the first time that VALLME was applied to determine cork taint responsible compounds in wine. For this reason, the suitability of the proposed method was evaluated in terms of linearity, detection and quantification limits, precision and recoveries. Linearity was evaluated at five concentrations levels of red spiked wine from 10 to 500 ng L^{-1}. The linearity of the data obtained using the internal standard was tested using Mandel's fitting test, obtaining significances higher than 0.05 (confidence level 95%). Once linearity was established, linear regression was established achieving correlation coefficients ranging from 0.985 to 0.994, as shown in Table 3. Quantification (LOQ) and detection (LOD) limits were calculated for ratio S/N of 10 and 3, respectively, at the lowest concentration level of the studied linear range (Table 3). These limits show that the method can be used to determine haloanisoles, along with their halophenols precursors, in wines since it is capable of revealing the presence of those compounds in wines below their perception thresholds (3 ng L^{-1} for TCA and TBA, 15 ng L^{-1} for TeCA and 10,000 ng L^{-1} for PCA) [44]. Furthermore, the method presents similar or even lower detection limits than those obtained in previous studies, as shown in Table 4.

The precision of the method was studied in terms of repeatability and inter-day precision. In both studies, red wine samples spiked at three concentration levels were employed. For the repeatability study, five extractions were performed on the same day, ranging from 3.5 to 10.0% (RSD). An inter-day precision study was performed with extractions on five different days, and ranged from 6.1 to 11.2% (Table 5). These results were considered acceptable, particularly taking into account that the procedure included a derivatisation step. The recoveries obtained for all compounds are also shown in Table 5; their values ranged from 67.7 to 99.1%.

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^{-1})	LOQ $S/N=10$ (ng L^{-1})
TCA	0.075	0.990	2.0	6.7
TeCA	0.186	0.983	2.7	9.0
TBA	0.192	0.993	1.9	6.3
PCA	0.163	0.994	2.9	9.7
TCP	0.082	0.992	2.3	7.7
TeCP	0.125	0.985	3.0	10.0
TBP	0.126	0.990	3.9	13.1
PCP	0.089	0.985	4.4	14.7

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

Method	V_{sample} (mL)	Time	$V_{solvent}$	LOD (ng L^{-1})								Ref.
				TCA	TeCA	TBA	PCA	TCP	TeCP	TBP	PCP	
LLE	200	n.a.	15 mL pentane	0.5	0.5	n.a.	0.5	10	10	n.a.	10	[9]
SPE	1000	n.a.	3 methanol+2ml hexane	2.4	0.3	n.a.	0.4	0.5	0.2	n.a.	0.3	[13]
SPME	5	85 min	–	0.4	0.3	0.5	0.4	3.3	2.5	3.8	3.7	[17]
SBSE	10	120 min	–	0.4	n.a.	n.a.	0.5	0.4	0.3	n.a.	0.5	[19]
DLLME	5	Few seconds	150 μL carbon tetrachloride+1.3 mL acetone	2.3	2.2	2.7	2.6	3.9	4.2	5.3	5.2	[21]
SDME	20	25 min	2 μL	8.1	n.a.	6.1	n.a.	n.a.	n.a.	n.a.	n.a.	[24]
USAEME	5	5 min	180 μL tetrachloroethylene	1.9	2.1	2.3	2.4	4.0	3.7	4.9	4.8	[25]
MEPS	5	15 min	50 μL ethanol (+1 ml ethanol washing step)	3.2	2.1	5.8	8.3	n.a.	n.a.	n.a.	n.a.	[29]
VALLME	5	7 min	135 μL tetrachloroethylene	2.1	2.7	2.1	2.9	2.3	3.0	3.9	4.4	Present study

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

Compound	Repeatability RSD%			Inter-day precision RSD%			Recoveries ± RSD (%)			
							Red wine		White wine	
	Low level[a]	Medium level[b]	High level[c]	Low level[a]	Medium level[b]	High level[c]	Low level[a]	High level[c]	Low level[a]	High level[c]
TCA	6.0	6.3	9.1	6.1	9.1	9.3	79.6 ± 6.1	88.6 ± 9.4	84.3 ± 5.8	86.8 ± 7.5
TeCA	7.8	10.0	6.5	9.9	8.9	8.2	94.9 ± 7.9	94.3 ± 8.1	97.6 ± 8.0	99.1 ± 5.2
TBA	6.3	9.2	6.4	8.6	9.4	7.9	67.7 ± 8.5	86.2 ± 8.0	75.4 ± 5.5	83.3 ± 6.0
PCA	4.9	8.6	7.2	8.9	7.9	9.7	85.8 ± 9.8	87.1 ± 7.5	90.4 ± 8.3	94.1 ± 3.9
TCP	7.7	7.8	6.9	8.8	10.5	8.8	71.5 ± 9.2	82.5 ± 7.2	80.9 ± 7.7	79.4 ± 6.2
TeCP	8.3	8.0	8.1	9.9	11.2	6.0	88.1 ± 7.0	87.2 ± 8.4	83.4 ± 9.0	90.5 ± 6.7
TBP	3.5	8.4	6.5	6.2	9.0	8.0	89.0 ± 6.0	84.1 ± 7.3	83.6 ± 6.9	89.6 ± 8.3
PCP	4.4	7.5	6.7	6.8	8.0	10.7	82.1 ± 6.6	97.1 ± 8.7	75.4 ± 10.6	94.2 ± 7.2

[a] 25 ng L^{-1}.
[b] 150 ng L^{-1}.
[c] 400 ng L^{-1}.

Table 6
Results of the analysis of wine samples using the VALLME–derivatisation method proposed ($n=3$).

Compound	Concentration ± SD (ng L^{-1})			
	Red wine A	Red wine B	White wine A	White wine B
TCA	15 ± 4	–	13 ± 3	–
TeCA	–	–	–	88 ± 10
TBA	12 ± 2	20 ± 6	–	–
PCA	–	32 ± 7	–	–
TCP	95 ± 8	–	–	–
TeCP	–	–	153 ± 6	130 ± 8
TBP	–	98 ± 5	90 ± 7	–
PCP	–	125 ± 9	–	–

These results show that the method has good linearity, precision and recoveries. The low detection and quantification limits obtained for all compounds show that the method is suitable and sensitive for the analysis of haloanisoles, along with their halophenols precursors, below their perception thresholds in wine. In addition, it is important to highlight that most of the methods previously reported required longer extraction times than those based on VALLME (Table 4). Moreover, the use of a mixing vortex is more cost-effective than an ultrasonic bath [45]. Thus, the use of a vortex, along with the reduction of extraction time and the low solvent consumption obtained, could yield relevant economic benefits for oenological laboratories.

3.4. Application of the method to real samples

Once the proposed VALLME-derivatisation method had been optimised and validated, its applicability was evaluated by analysing two different red and white wine samples, in which cork taint defect was detected by sensory analysis. These analyses were performed in triplicate (Table 6). Red wine samples had haloanisoles above their perception thresholds, excluding PCA in red wine B, which was below its perception threshold. The tasters defined red wine A as one of the most defective, and also presented TBA and TCA over their perception thresholds. Additionally, red wine B was defined as a highly defective wine due to the presence of a high concentration of TBA. The tasters also detected that both white wines were cork tainted, perhaps due to the presence of TCA in white wine A and TeCA in white wine B at concentrations above their perception thresholds. As regards the halophenols, TCP was present in red wine A, TeCP was present in white wines A and B, red wine B and white wine A contained TBP and PCP was only found in red wine B.

4. Conclusions

This study presents a novel approach based on the VALLME method coupled to GC–μECD for the simultaneous determination of the main compounds causing cork taint in wines. The VALLME procedure was optimised by evaluating the influence of different parameters on the recoveries obtained for the target compounds using an experimental design methodology. Once the method was optimised, it was validated by studying its linearity, precision and recoveries, yielding satisfactory results. Furthermore, the detection and quantification limits achieved showed that VALLME is a suitable method that allows studied compounds to be determined below their olfactory thresholds. The applicability of the method was also verified by analysing four real wine samples. In conclusion, VALLME is presented as a low cost, fast and efficient alternative to previously reported methods for the early determination of haloanisoles and halophenols in wines. Furthermore, to the best of our knowledge, this is the first time that the VALLME method has been used for the simultaneous determination of haloanisoles and halophenols in wine.

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