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Optimisation of a headspace solid-phase microextraction method for the direct determination of chloroanisoles related to cork taint in red wine

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Abstract

One of the most critical problems in the enological industry is associated with cork taint. The main compounds responsible for this off-flavour are some chloroanisoles: 2,4,6-trichloroanisole (TCA), 2,3,4,6-tetrachloroanisole (TECA) and pentachloroanisole (PCA). Headspace solid-phase microextraction (HS-SPME) coupled to gas chromatography-electron capture detection has been used for the direct determination of these chloroanisoles in wine samples. After the evaluation of some parameters (desorption conditions and salt addition) that affect extraction efficiency, a screening study of six types of fibres and five extraction temperatures was performed. Then, a Doehlert matrix including the exposure time, temperature and V_s (sample volume)/V_t (total volume) ratio as experimental factors was proposed. According to the results of this design and the kinetic profiles evaluated, an analytical procedure based on HS-SPME was optimised and validated. This method can be used for the simultaneous determination at the low ng/l level of all chloroanisoles involved in cork taint, and not only TCA, which is the only compound found in the literature when SPME is proposed as the analytical technique.
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Keywords: Wine; Solid-phase microextraction; Chloroanisoles; Cork taint; Optimisation

1. Introduction

The presence of organoleptic “diseases” in wine, perceived as “musty”, “mouldy” or “cork” taint, represents a significant source of financial loss for the wine industry. In the eighties, 2,4,6-trichloroanisole (TCA) was already suspected to be related to this alteration [1]. In 1996, the European project “QUERCUS”, whose object was to identify the sources of off-flavours associated with cork stoppers, concluded that the most prominent source compound was TCA due to its presence in most of the wines described as exhibiting a musty taint [2]. However, other different studies have shown that 2,3,4,6-tetrachloroanisole (TECA) and pentachloroanisole (PCA) may also contribute to this effect [3–6]. Therefore, it is of the utmost importance to fully study all of them. TCA has the lowest olfactory threshold (<10 ng/l) in wine samples [1] but, although TECA and PCA are less powerful compounds perceivable in wine [3,7], a synergism might occur and the effect produced by several components can be greater than the sum of their individual contributions.

The origin of these off-flavour compounds is related to the microbial degradation of the corresponding chlorophenols used as insecticides in different wood materials present in the wineries and the microflora and hypochlorite used to bleach wine corks [3–5].

Chloroanisoles have already been determined in several solid and liquid matrices. Cork stoppers [1,3,4,8–16], wine [1,3,4,7,9,10,15,17–20] and natural bark corks [19] have been processed because they are directly related to cork taint. Furthermore, some methods have been developed for the analysis of these three chloroanisoles found in freight containers, fibreboard cartons, packaging materials [21–23], food [23,24] and water [24,25].

Chloroanisole determination in these matrices usually includes a preconcentration step using liquid-liquid or solid-liquid extraction with organic solvents, generally hexane [4,11,12] and pentane [7,9,10,19], but also pentane/ethyl acetate [1] and pentane/dichlorometane [3]. This is followed by the analysis of the organic extract in a gas chromatograph and a subsequent detection with an electron-capture

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detector (GC–ECD) or mass spectrometer (GC–MS). Supercritical fluid extraction (SFE) [8] and Solid-phase extraction (SPE) [13] have also been used for the analysis of chloroanisoles in cork stoppers and other materials.

The latest studies have focused on the development of new extraction methods based on Solid-phase microextraction (SPME) because they are simpler than the traditional extraction techniques, eliminate the use of solvents, and reduce the sample preparation time. SPME methods have also been used for the analysis of various components, off-flavours and contaminants in wine samples (i.e. bouquet, sulphur aroma, diacetyl, methylisothiocyanate, pesticides) [26]. Previous research has determined cork taint only in wine based on HS-SPME by using a 100 μm polydimethylsiloxane (PDMS) fibre and 20–45 °C as temperature extraction [14,15,17,18]. That research has only focused on the presence of TCA. In some of these papers, other stationary phases and coating thickness have been tested to optimise the extraction conditions, but by using only a very narrow range of temperatures.

The final aim of this work was to optimise a HS-SPME method for the direct analysis of cork taint in red wine samples. The main parameters that influence the process were studied in order to reach the highest extraction yields for the three chloroanisoles involved. This research included the evaluation of new specific stationary phases for the trace analysis of semi-volatile compounds such as chloroanisoles and the comparison of these to those used in previous works. Moreover, the range of extraction temperatures considered was wider. On the other hand, no SPME method has yet been proposed for the simultaneous analysis of all chloroanisoles involved in cork taint. Another goal of this work was to develop a method to analyse all three compounds simultaneously as this is the only way to establish possible synergies that have not yet been evaluated and to provide a global solution for the industrial sector.

2. Experimental

2.1. Chemicals

2,4,6-Trichloroanisole (TCA) was supplied by Aldrich Chemie (Steinheim, Germany), 2,3,4,6-tetrachloroanisole (TeCA) by Ultra Scientific (North Kingstown, RI, USA) and pentachloroanisole (PCA) by Supelco (Bellefonte, PA, USA). The suppliers stated purity of all standards was above 95%. Sodium chloride was purchased from Aldrich Chemie (Steinheim, Germany) and methanol, ethanol and L(+)-tartaric acid from Merck (Darmstadt, Germany).

2.2. Standard solutions and samples

Individual stock standard solutions of each chloroanisole were prepared in methanol. They were stored in darkness at 4 °C. Work solutions used in further studies were prepared by diluting different amounts of each stock standard solution.

Red wine without cork taint was spiked with different amounts of these solutions to prepare the samples for the different studies. The synthetic wine used for matrix effect study was obtained by dissolving 5 g/l of L(+)-tartaric acid in an hydroalcoholic solution (13%, v/v, ethanol) with the same pH as the wine sample.

2.3. SPME procedure

The fibres evaluated were purchased from Supelco (Bellefonte, PA, USA) and were coated with different stationary phases: polydimethylsiloxane (PDMS, 100 μm), polydimethylsiloxane/divinylbenzene (PDMS/DVB, 65 μm), polyacrylate (PA, 85 μm), carboxen™/polydimethylsiloxane (CAR/PDMS, 75 μm), carbowax®/divinylbenzene (CW/DVB, 65 μm) and divinylbenzene/carboxen™/polydimethylsiloxane (DVB/CAR/PDMS, 50/30 μm). They were conditioned under the specifications of the producer before use.

The main parameters that affect the SPME process (i.e. desorption time, ionic strength, type of fibre, time and extraction temperature, sample volume and stirring) were studied. All the vials used had the same shape and size (20 ml headspace vials).

The preliminary experiments were aimed at selecting the desorption conditions and studying the influence of salt addition. These preliminary analyses were carried out by exposing a PDMS coating in the headspace of the vial at 25 °C. The type of fibre and temperature were chosen based on the results of previous works [14,15,17,18]. Headspace extraction was preferred to direct extraction to prevent the direct contact of the fibre with the complex matrix and related matrix effects. Moreover, headspace gets better sensitivity for the volatile, relatively non-polar chloroanisoles, and thus obtains a far better signal to noise than does immersion SPME. The samples were prepared by adding 5 ml of wine spiked with the chloroanisoles considered in this study into a 20 ml headspace vial sealed with a PTFE septum. The final concentrations in the samples were between 3.8 and 4.5 μg/l. Depending on the experiment, 0–2 g of NaCl was added in order to alter the ionic strength of the matrix. Before the extraction, the vials were equilibrated for 5 min and the sampling time was 30 min.

For the fibre and temperature screening study extraction was carried out for 30 min. Sealed vials were immersed in a water bath. Every day, before using the fibres, a blank desorption was run to ensure that the fibres and needles were free of contaminants.

Considering the previous results, an optimisation step was performed using an experimental design. Depending on the experiment, different conditions of extraction temperature (50–80 °C), exposure time (30–90 min) and V_s (sample volume)/V_t (total volume) (4/20–12/20) ratio were

evaluated. Time extraction profiles were obtained by preparing a set of vials containing samples and then extracting them for progressively longer periods of time, and the influence of agitation was evaluated using an 8 × 3 mm stir bar at 1000 rpm.

2.4. Equipment and chromatographic conditions

The SPME procedure was carried out using an IKAMAG RET control visc (Staufen, Germany) with heating and stirring features. After the extraction, the SPME device was removed from the vial and inserted immediately into the injection port for thermal desorption for 5 min.

Chromatographic analyses were performed with a Hewlett-Packard 5890 Series II gas chromatograph equipped with a split/splitless injector, electronic pressure control in the injector and an electron capture detector (ECD). A capillary column HP-5MS (30m × 0.25mm i.d., 0.25 μm film thickness) from J&W Scientific (Folsom, CA, USA) was used. Helium at a flow of 1 ml/min was used as carrier gas. Oven temperature was programmed as follows: 50 °C for 2 min, heated at 15 °C/min to 115 °C, heated to 150 °C at 3 °C/min and kept for 8 min; finally raised to 250 °C at 15 °C/min and held for 1 min. Injection was performed in the splitless mode for 2 min and then the split flow was set to 30 ml/min. An inlet of 0.75 mm i.d. was used and the injector temperature was different depending on the type of coating: 280 °C for PA and CAR/PDMS, 270 °C for PDMS, PDMS/DVB and DVB/CAR/PDMS and 230 °C for CW/DVB. ECD temperature was held at 260 °C.

3. Results and discussion

3.1. Preliminary experiments

Before optimising the desorption conditions, the needle exposure depth was adjusted to place the fibre in the centre of the hot injector zone, which is typically located at the centre of the insert. The maximum allowable coating temperature was chosen as desorption temperature and then desorption time was set to 5 min (2 min of splitless). In order ensure a proper selection of parameters, a wine sample with a high concentration of chloroanisoles was prepared and processed. In a subsequent exposure to the sample, the fibre was placed inside the injector. After the predetermined desorption time, the fibre was removed and sealed in the needle with a piece of septum. When the chromatographic run was completed, the fibre was immediately injected again to confirm the absence of peaks.

The influence of altering the ionic strength of the matrix was also studied by addition of different amounts of NaCl. Analyte solubility usually decreases as ionic strength increases. A decrease in analyte solubility improves sensitivity by promoting analyte partitioning into the stationary phase, but the "salting-out" effect is compound dependant.

Table 1
Influence of the addition of NaCl (expressed as area counts) on the HS-SPME of 2,4,6-trichloroanisole (TCA), 2,3,4,6-tetrachloroanisole (TECA) and pentachloroanisole (PCA) in spiked samples of wine using a 100 μm PDMS fibre at 25 °C ($n = 3$)

(I) g NaCl (ml)	(J) g NaCl (ml)	Differences between means ($I - J$) ($\times 10^{-4}$)		
		TCA	TECA	PCA
0	0.2	4.968[a]	21.80[a]	37.73[a]
	0.4	3.731[a]	25.23[a]	49.07[a]
0.2	0	−4.968[a]	−21.80[a]	−37.73[a]
	0.4	−1.238	3.435	11.34[a]
0.4	0	−3.731[a]	−25.23[a]	−49.07[a]
	0.2	1.238	−3.435	−11.34[a]

[a] Significance: 5% level.

NaCl was added under (0.2 g/ml) and over (0.4 g/ml) the supersaturation level for wine samples. Each experiment was performed in triplicate. Levene's Test was performed to verify the homogeneity of variances before evaluating the Analysis of Variances (ANOVA) between groups. The results obtained by means of multiple comparisons based on least significant difference (LSD) — 5% level are summarised in Table 1. As it can be seen, salt addition did not improve the extraction efficiency of chloroanisoles in wine samples. Therefore, no salt addition was performed in further studies.

In this research six fibres (PDMS 100 μm, PDMS/DVB 65 μm, PA 85 μm, CAR/PDMS 75 μm, CW/DVB 65 μm and DVB/CAR/PDMS 50/30 μm) and five temperatures (25, 40, 60, 80 and 100 °C) were evaluated and compared. The results of the fibre screening summarised in Fig. 1 show that, in general, the highest responses for the considered chloroanisoles were associated with the DVB/CAR/PDMS 50/30 μm fibre but only when high extraction temperatures were used. The behaviour of PA, PDMS/DVB and PDMS for the analysis of spiked wine samples was similar and the lowest extraction efficiency was obtained by CAR/PDMS. The CW/DVB coating could not be assessed because, after five extractions in the headspace of the vial, the stationary phase came off from the flexible fused silica core. The profiles obtained when the extraction was performed at different temperatures can be seen in Fig. 2 and show that the less volatile the chloroanisole, the higher the number of chlorines, the bigger the temperature associated with the highest response: 40 °C for TCA, 60 °C for TECA and 80 °C for PCA. These temperatures were optimum to obtain the most efficient extraction for each analyte with any fibre except for DVB/CAR/PDMS. The ideal temperature for the SPME process developed with this coating was always one step higher. The interaction between the type of fibre and extraction temperature could be established by analysing Figs. 1 and 2. Temperature was a much more relevant parameter when DVB/CAR/PDMS was used. The same change in extraction temperature caused a larger difference in the response when the extraction was performed with that coating.

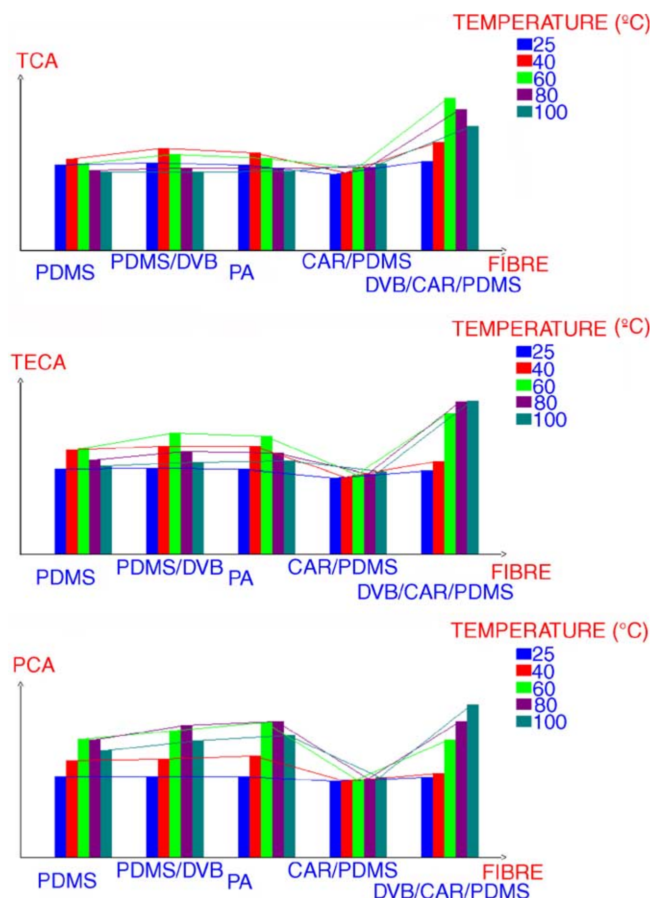

Fig. 1. Influence of type of fibre on the efficiency of extraction developed at different temperatures.

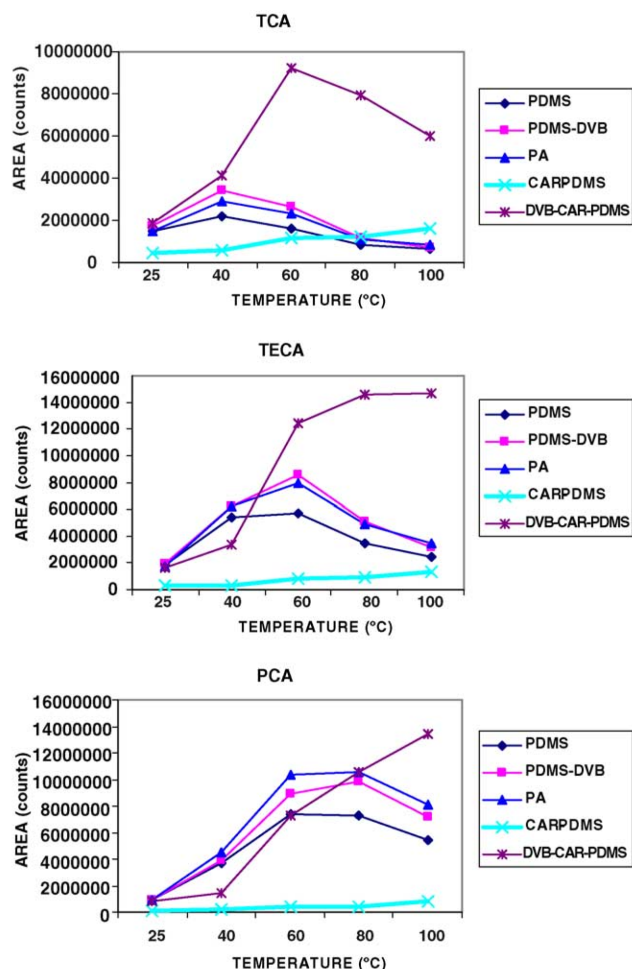

Fig. 2. Influence of temperature on the efficiency of extraction developed with different coatings.

3.2. Doehlert experimental design: evaluation of the influence of the exposure time, extraction temperature and V_s/V_t

Taking the results of the preliminary experiments as a starting point, the optimum coating for an efficient extraction of the chloroanisoles, i.e., DVB/CAR/PDMS 50/30 μm, and a narrower range of temperatures (50–80 °C) that provided the best extraction yield were selected. Considering that the optimum temperature for each analyte is different, it was necessary to achieve a compromise. Therefore, this parameter was included in the optimisation process. The other factors evaluated were the extraction time and V_s/V_t ratio. In order to optimise the three responses, a Doehlert's experimental matrix [27,28] with 21 experiments was applied. Five of them were performed at the centre to estimate experimental repeatability (these experiments are useful to detect the curvature of the domain). Four test points fixed at the vertices of a regular tetrahedron within a sphere with a radius of 0.5 were also included. All the experiments were randomly performed. Doehlert's experimental matrix, the corresponding experimental conditions and experimental responses studied are shown in Table 2.

This study was aimed at optimising chloroanisole extraction in wine samples; therefore, area counts for each chloroanisole were selected as responses of interest. The quadratic model parameters related to the different experimental responses were estimated by multi-linear regression; the model was analysed and validated by ANOVA and the test points using NemrodW software [29]. After calculating the model coefficients, it was necessary to find out whether they were meaningful or whether the deviations of the data points from a constant value were simply due to chance, a random variation in the response, measurement errors or variations or drifts in uncontrolled factors. For TCA and PCA, the proposed model provided an appropriate description of the studied responses and thus the mathematical models were accepted. For the TECA-associated response, a transformation by Box and Cox method [30] was proposed because a not significant regression in the analysis of variance was found out. After a theoretical choice, the transformations were tested to check whether this improved the results. The logarithm transformation of the response provided the best results in terms of significance in the proposed model. The model coefficients of each response are shown in Table 3. The effects

Table 2
Doehlert's experimental matrix, the corresponding experimental conditions for exposure time, extraction temperature and V_s (sample volume)/V_t (total volume) and response values for the three variables optimised

No. experiment	Coded experiments matrix			Experimental plan			Results (area counts)		
	t_{exp} (x_1)	T (x_2)	V_s/V_t (x_3)	t_{exp} (min)	T (°C)	V_s/V_t	TCA	TECA	PCA
1	1.0000	0.0000	0.0000	90	65	8/20	1712470	2474950	2096110
2	−1.0000	0.0000	0.0000	30	65	8/20	1010100	1465080	1142620
3	0.5000	0.8824	0.0000	75	80	8/20	1211490	1812730	1594000
4	−0.5000	−0.8824	0.0000	45	50	8/20	679699	750442	471698
5	0.5000	−0.8824	0.0000	75	50	8/20	801642	805996	531339
6	−0.5000	0.8824	0.0000	45	80	8/20	1270690	2212910	1947840
7	0.5000	0.2941	0.8000	75	70	12/20	1586070	2414040	2150080
8	−0.5000	−0.2941	−0.8000	45	60	4/20	1087560	1409870	1007660
9	0.5000	−0.2941	−0.8000	75	60	4/20	1524060	2065910	1419680
10	0.0000	0.5882	−0.8000	60	75	4/20	1236930	2115730	1891460
11	−0.5000	0.2941	0.8000	45	70	12/20	1237810	1625990	1356010
12	0.0000	−0.5882	0.8000	60	55	12/20	1395830	1958420	1309900
13	0.0000	0.0000	0.0000	60	65	8/20	1393950	2279770	1849570
14	0.0000	0.0000	0.0000	60	65	8/20	1417960	1969850	1646600
15	0.0000	0.0000	0.0000	60	65	8/20	1301110	1733130	1414990
16	0.0000	0.0000	0.0000	60	65	8/20	1272370	1583360	1309980
17	0.0000	0.0000	0.0000	60	65	8/20	1450930	1863500	1371640
18	−0.4000	−0.2353	−0.2000	48	61	7/20	1313260	1904870	1398670
19	0.4000	−0.2353	−0.2000	72	61	7/20	1489750	2226180	1762590
20	0.0000	0.4706	−0.2000	60	73	7/20	1205980	2341810	2012590
21	0.0000	0.0000	0.6000	60	65	11/20	1542400	2381280	1955210

of the evaluated factors for the three responses were similar. The V_s/V_t ratio had no influence on the yield extraction for any compound. However, exposure time and extraction temperature had a positive effect on them. A long exposure time and high extraction temperature resulted in a maximisation of these responses.

A first optimisation approach was adopted by graphical analysis. As only two significant parameters appeared, the choice of plotting graphs was simplified; the factor x_3 (V_s/V_t) could be fixed at any level and each response studied was fixed on the x_1x_2 plane. Fig. 3 shows the evolution of chloroanisole-related responses throughout the experimental domain when the V_s/V_t ratio was fixed at the lowest level, 4/20. All the responses could be simultaneously optimised by setting the temperature at 70 °C. However, the exposure time necessary to achieve the best efficiency of extraction was different depending on the compounds, and the maximum response was reached at 90 min for TCA, 50 min for TECA and 30 min for PCA. It is necessary to set, as extraction time, a point where the sensitivity and precision were maximised over an acceptable experimental time. Sensitivity was established by TCA as this chloroanisole has the lowest threshold concentrations and is often present in wine at concentrations much lower than the rest. In order to define this compromise, some experiments were carried out to describe the extraction time profile when temperature and the rest of experimental conditions were ideal.

Table 3
Response variables and estimates of the model coefficients for the optimisation of the HS-SPME method

Coefficient	y_1 TCA (area counts)	y_2 log TECA (area counts)	y_3 PCA (area counts)
b_0	**1386.13**	**3.30**	**1630.78**
b_1	**275.15**	**0.09**	**360.45**
b_2	**176.89**	**0.18**	**696.90**
b_3	85.35	0.02	112.59
b_{11}	−12.82	−0.02	−0.69
b_{22}	**−511.59**	**−0.26**	**−622.32**
b_{33}	88.68	0.04	43.45
b_{12}	−97.34	−0.07	−241.02
b_{13}	−11.39	0.03	317.27
b_{23}	−87.93	−0.14	−393.02

Significant effects (5%) are printed in bold type. b_0: constant term; b_1, b_2, b_3: linear terms; b_{11}, b_{22}, b_{33}: quadratic terms; b_{12}, b_{13}, b_{23}: interaction terms.

3.3. Determination of the extraction time

The aim was to evaluate extraction time by determining the time required for an analyte to reach equilibrium between the sample matrix and the stationary phase. The results were represented in graphs that included a comparison of the peak area against extraction time. In this research, the extraction time profile for DVB/CAR/PDMS at 70 °C (Fig. 4) was also studied. As it can be seen, the HS-SPME process was very slow when the extraction was performed with this fibre. This can be due to the porosity of the coating that enables it to retain a bigger amount of analyte. None of the chloroanisoles reached equilibrium in the studied time periods except TCA, which reached equilibrium after one-hour and a half. The selection of an extraction time at equilibrium for all chloroanisoles was virtually impossible because

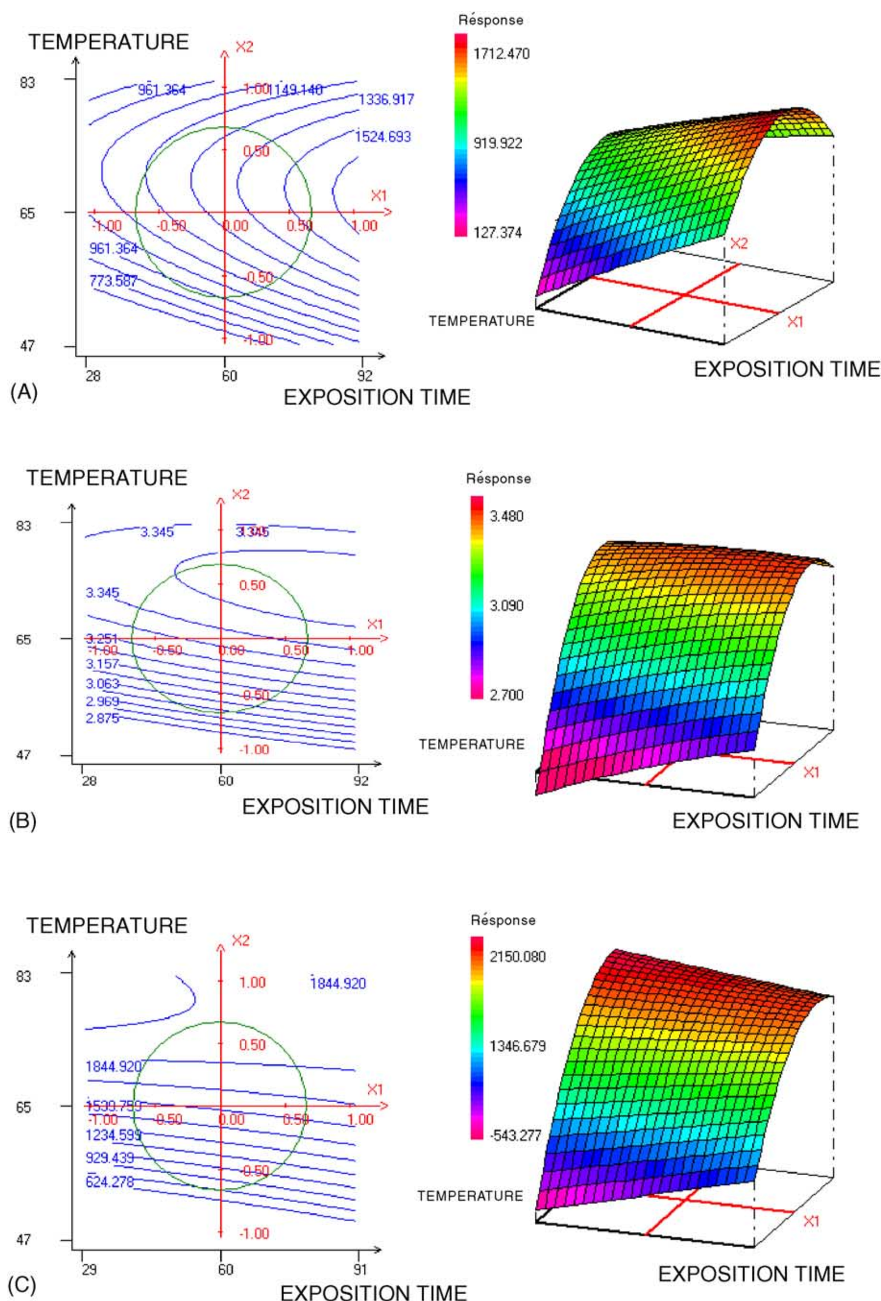

Fig. 3. Response surfaces extraction temperature vs. exposition time for the doehlert design: (A) TCA response; (B) log TECA response; (C) PCA response.

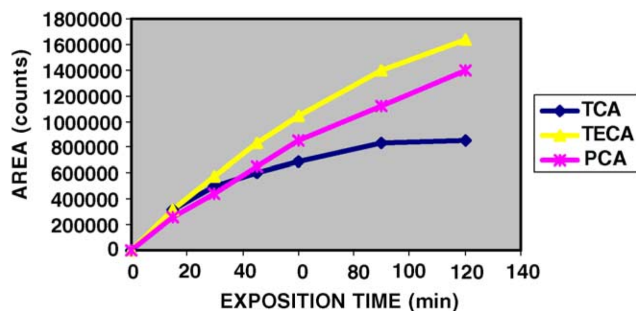

Fig. 4. Extraction time profile obtained by HS-SPME at 70 °C using a 50/30 μm DVB/CAR/PDMS fibre.

there were many differences among them and 90 min prolonged excessively the analysis time. While the extraction conditions were kept constant, working in a non-equilibrium situation did not cause any problem for SPME quantification. Therefore, 60 min was selected as exposure time.

3.4. Effect of agitation sample

According to the slow kinetics profiles described for these chloroanisoles when the extraction was performed using this coating, sample stirring was proposed as an alternative to improve the sensitivity of the proposed method. Agitation

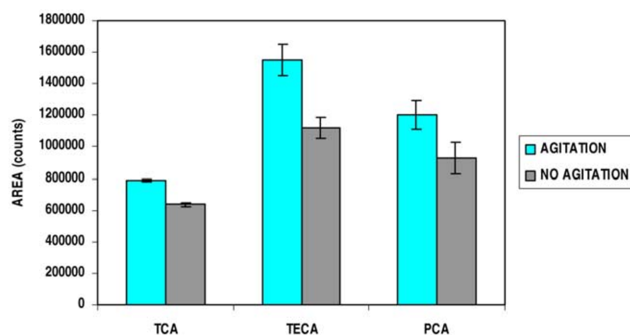

Fig. 5. Effect of stirring on the HS-SPME of 2,4,6-trichloroanisole (TCA), 2,3,4,6-tetrachloroanisole (TECA) and pentachloroanisole (PCA) using a 50/30 μm DVB/CAR/PDMS fibre ($n = 3$).

accelerates the transfer of analytes from the sample matrix to the coating fibre and magnetic stirring is widely used for agitation. Each experiment was performed in triplicate, setting the experimental conditions according to previous results. The results obtained (Fig. 5) were analysed by one factor-ANOVA once the homogeneity of variances between groups had been confirmed by Levene's test. In line with this study, agitation had a significant positive effect on the responses. Therefore, wine samples were stirred in subsequent experiments.

3.5. Summary of the set up conditions

To sum up, the HS-SPME-based method proposed can be described by the following parameters: 4 ml of wine into a 20 ml headspace vial sealed with a PTFE septum were prepared and immersed in a water bath at 70 °C. Extraction was performed with a 50/30 μm DVB/CAR/PDMS for 60 min while the sample was being stirred. After that, the fibre was immediately inserted into the injector for thermal desorption at 270 °C for 5 min. For the first 2 min, the splitless mode was used.

3.6. Method performance

Red wine samples spiked with the three chloroanisoles were prepared to validate the method. The linearity of the proposed model was established with samples fortified at five concentrations (5–100 ng/l). The presence of TCA and TECA in red wine at a higher level would suggest that wine is completely contaminated. In this case, the human nose would be an instrument good enough to detect it. These samples were analysed in triplicate. The correlation coefficients (r^2) obtained were 0.996, 0.994 and 0.994 for TCA, TECA and PCA, respectively, so a directly proportional relationship between the extract amount of chloroanisoles and initial sample concentration was demonstrated. Repeatability studies were performed at three levels. The results are shown in Table 4 as relative standard deviations (R.S.D.) and ranged from 2.62 to 9.47. The quantification and detection limits shown in Table 4 were calculated for a signal-to-noise ratio of 10 and 3, respectively. Detection limits for TCA and TECA are of the same order of magnitude as TCA and TECA olfactory thresholds in wine for an expert taster. These limits vary from 1.4 ng/l in a Pinot Noir wine [31] up to 4.6 ng/l for TCA, depending on the wine style and panel [32] and 25 g/l in white wine for TECA [33]. PCA has less odorous power in wine than the others chloroanisoles.

Table 4
Quality parameters of the proposed analytical procedure

Compound	Precision ($n = 5$) R.S.D (%)			Quantification limits (S/N = 10, ng/l)	Detection limits (S/N = 3, ng/l)
	5 ng/l	25 ng/l	100 ng/l		
2,4,6-TCA	5.16	3.10	3.49	8.4	2.5
2,3,4,6-TECA	4.33	2.78	2.62	6.2	1.9
PCA	9.47	3.06	3.02	4.3	1.3

The decision of which quantification approach to choose depend on the sample matrix complexity. In order to study the relevance of the matrix effect, the slopes of the standard addition curves for spiked red wine and synthetic wine were compared using a Student's *t*-test (data not shown). This comparison demonstrated significant differences between them for all chloroanisoles so the standard addition technique is the proposed method for the analytes quantification.

4. Conclusions

In this paper, the influence of different parameters on the HS-SPME process has been assessed in order to optimise a procedure for the analysis of cork taint in red wine. The preliminary experiments focused on the evaluation of desorption conditions and salt addition. A screening study of the different types of coatings and a wide range of temperatures was also performed. The last study allowed us to select the most suitable fibre for chloroanisole extraction, i.e., DVB/CAR/PDMS 50/30 μm, and limit the range of adequate temperatures. Taking into account these preliminary results, a Doehlert experimental design was proposed to optimise the extraction temperature, exposure time and ratio V_s/V_t ratio. Only the first two factors had a significant influence on the three chloroanisole-related responses. Thus, the V_s/V_t ratio was fixed at the lowest level, i.e., 4/20. If the extraction temperature was set at 70 °C, it would be possible to optimise simultaneously the yield of all of them. As the optimum exposure time for each compound did not coincide, a complementary study of kinetics at this temperature was performed for an adequate selection of a compromise. Therefore, 60 min was chosen as exposure time. Finally, the positive effect of stirring wine samples when the experiments were carried out under optimised conditions was proven. To our knowledge, this proposed method based on HS-SPME is the first application of this technique for the direct extraction of all chloroanisoles involved in cork taint, and not only TCA.

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