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Stir bar sorptive extraction polar coatings for the determination of chlorophenols and chloroanisoles in wines using gas chromatography and mass spectrometry

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ABSTRACT

The simultaneous determination of 14 chlorophenols (CPs) and chloroanisoles (CAs) in wine samples is carried out using stir bar sorptive extraction (SBSE) with thermal desorption and gas chromatography–mass spectrometry (TD–GC–MS), evaluating the preconcentration efficiency of two different polar extracting phases, ethylene glycol–silicone (EG–Silicone) copolymer and polyacrylate, which have recently become commercially marketed. The influence of several extraction variables on the preconcentration capacity of these two novel coatings was tested, as well as the variables affecting the thermal desorption step. The EG–Silicone extraction phase provided the best results, since it allowed the simultaneous preconcentration of both species the non-polar CAs, due to the silicone base, and the polar CPs, because of the ethylene glycol polymer. Consequently, under the finally selected conditions, CPs were determined without any derivatization step, reaching detection limits in the 0.3–1.4 ng L^{-1} range, depending on the compound. For CAs the detection limits ranged from 0.2 to 0.5 ng L^{-1}, with good precision and recovery. Five CAs and three CPs were found in several analyzed wines, some of which can be regarded as defective considering their contents in 2,4,6-TCA and 2,6-DCA.

1. Introduction

Stir bar sorptive extraction (SBSE) is a solvent-free sample preparation technique based on the extraction of target compounds from aqueous matrices onto a stationary phase-coated stir bar. For many years, polydimethylsiloxane (PDMS) was the only commercially available coating for stir bars, but its non-polar nature limited the applicability of SBSE to hydrophobic compounds. Since PDMS was unable to extract polar species, they usually showed poor recovery with SBSE, and transformation into less polar species by derivatization reactions, such as *in-situ* acetylation or in-tube silylation [1] was the only alternative.

The development of in-house coatings for SBSE using more polar extracting phases has extended the applicability of this technique to polar compounds. Several approaches have been successfully applied to species showing low affinity for PDMS coatings [2], such as sol–gel technology [3], monolithic materials [4], molecularly imprinted polymers [5] and polyurethane foams [6]. However, the lack of robustness of in-house coatings, which may lead to mechanical or thermal degradation, reducing their useful life and producing high bleeding rates, as well as the difficulties associated with the preparation of such coatings [7], involve significant limitations to their analytical application.

Recently, stir bars coated with polar friendly coatings, like ethylene glycol–polydimethylsiloxane copolymer (EG–Silicone) and polyacrylate (PA) [8] have reached the market, improving SBSE flexibility while maintaining robustness and ease of handling. These new commercial SBSE coatings were assayed to assess their suitability for the determination of the polar compounds, chlorophenols (CPs) and the related chloroanisoles (CAs), which are the main compounds responsible of the moldy aroma in wines.

Aroma is one of the most important characteristics of wine, since it is related with product quality and consumer acceptance. Thus, the appearance of corky, musty or earthy taints in wines, frequently related to the presence of some CPs and CAs [9], is a concern for the wine industry. The main compound responsible for this defect is 2,4,6-trichloroanisole (2,4,6-TCA), although other CAs, may also contribute to the off-flavors. These compounds are usually synthetized by fungal methylation of the corresponding CPs [10], which usually reaches wine samples by means of the natural cork used as bottle stoppers, or from contact with barrels. These species are generated during the treatment of the cork or wooden barrels with hypochlorite, although other sources, such as

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wood biocides, may also be responsible for spoilage [11]. Moreover, the control of the CP content in wines is of great importance because of their carcinogenic character and persistence.

Even though immunoassay tests have been used for the determination of CPs and CAs in wines [12,13], a more extensive use of gas chromatography (GC) is reported in the bibliography [11,14–29], coupled to a large variety of microextraction techniques, such as different liquid–liquid microextraction (LLME) modalities [26–28] and solid-phase microextraction (SPME) [16,18–24] with the aim of reaching the human olfactory and taste threshold ranges for haloanisoles. Although these ranges vary with the age of the wine and grape variety used in production, as well as with the sensitivity and training of judges, an interval of 0.03–50 ng L^{-1} has been proposed for 2,4,6-TCA (the TCA concentration considered to produce a defect in wine usually ranges from 10 to 40 ng L^{-1}) [14] and values of around 400 ng L^{-1} for 2,4-DCA, 40 ng L^{-1} for 2,6-DCA and 4 μg L^{-1} for PCA [10,17,26]. SBSE has previously been used for the determination of CP and CA-related taints in wine [11,15,23,25], as well as in cork [30–33] and other sample matrices, such as water [34] and soil [35].

Even though the volatility and thermostability of CAs mean that they are suitable analytes for GC, a previous derivatization step is recommended in the case of CPs in order to improve sensitivity and to reduce peak tailing. These species, have also been determined by GC, without a derivatization step, which represents a saving of time and reagents, using SPME as preconcentration technique and the polar coating PA [19,36–39] and polyethylene glycol (PEG) fibers [40,41]. Similar extraction phases are available in SBSE but they have never been used for the determination of the compounds deemed responsible for cork taint. In this paper, 14 CPs and CAs were determined in wine samples using SBSE with thermal desorption and gas chromatography–mass spectrometry (TD–GC–MS), comparing the effectiveness of the two novel polar coatings, EG–Silicone and PA.

2. Experimental

2.1. Reagents

4-Chloroanisole (4-CA, 99%), 2,6-dichloroanisole (2,6-DCA, 97%), 2,4-dichloroanisole (2,4-DCA, 97%), 2,4,6-trichlorophenol (2,4,6-TCP, 98%), 2,4,6-trichloroanisole (2,4,6-TCA, 99%) and pentachlorophenol (PCP, 98%) were purchased from Aldrich (Steinheim, Germany). 4-Chlorophenol (4-CP, 99.5%), 2,4-dichlorophenol (2,4-DCP, 99.5%), 2,6-dichlorophenol (2,6-DCP, 99.5%), 2,4,5-trichloroanisole (2,4,5-TCA, 99.5%), 2,4,5-trichlorophenol (2,4,5-TCP), 2,3,4,6-tetrachlorophenol (2,3,4,6-TeCP, 98%) and 2,3,4,5-tetrachloroanisole (2,3,4,5-TeCA, 99%) were obtained from Dr. Ehrenstorfer (Ausburg, Germany) and pentachloroanisole (PCA, 99.3%) from Supelco (Bellefonte, PA, USA).

Individual stock solutions of the compounds (1000 μg mL^{-1}) were prepared using HPLC grade methanol and stored in darkness at −20 °C. Working standard solutions were freshly prepared in pure water and stored at 4 °C. Sodium hydroxide (99%) and sodium chloride (99.5%) were purchased from Sigma (St. Louis, MO, USA). L-(+)-Tartaric acid (99.5%) was provided by Merck (NJ, USA). Chromatographic quality methanol and ethanol were obtained from Sigma. Water was previously purified in a Milli-Q system (Millipore, Bedford, MA, USA) and the carrier gas used for GC was helium (Air Liquide, Madrid, Spain).

A synthetic wine containing 3.2 g L^{-1} of L-(+)-tartaric acid and 12% (v/v) of ethanol, with pH adjusted to 3.6 using a diluted NaOH solution, was used for the development and optimization of the method [15].

All the glass material was soaked with a detergent solution with added ethanol and dried in an oven.

2.2. Instrumentation

Commercial stir bars coated with polyacrylate (PA) and ethylene glycol–polydimethylsiloxane copolymer (EG–Silicone) layers (32 μL) were obtained from Gerstel (Mullheim an der Ruhr, Germany). Prior to use, the stir bars were conditioned in an empty thermal desorption tube at 200 °C for 0.5 h with helium at a flow desorption rate of 50 mL min^{-1}. The sample introduction system was composed of a Thermal Desorption Unit (TDU-2) equipped with an autosampler (MPS-2) and a Programmed Temperature Vaporization (PTV) Cooled Injector System (CIS-4) provided by Gerstel. The main experimental conditions used in the sample introduction system are summarized in Table 1. GC analyses were performed on an Agilent 6890N (Agilent, Waldbronn, Germany) gas chromatograph coupled to an Agilent 5973 quadrupole mass selective spectrometer equipped with an inert ion source. The total analysis time for one GC run was 27 min, the analytes being eluted with retention times between 10.1 and 25.4 min, as shown in Table 2. The ionization was carried out in the electron-impact (EI) mode (70 eV). The electron multiplier voltage was set automatically. The identification of the compounds was confirmed by injection of pure standards and comparison of the retention time and full MS-spectra. The analytes were quantified under the selected ion monitoring (SIM) mode using the most abundant ions (Table 2).

2.3. Samples, analytical procedure and recovery studies

A total of 30 wines (samples 1–8 were white, 9–26 red and 27–30 rosé wines) were obtained from local wine merchants. Taking into account that cork taint is very unusual in large-scale industrial produced wines [26], craft wines, from small local productions and aged in barrels, were chosen for sample selection. Samples were kept at 4 °C until analysis, in order to prevent losses of the most volatile analytes.

Table 1
Experimental conditions of the TD–GC–MS procedure.

Thermal Desorption Unit	
Mode	Splitless
Temperature program	50 (0.5 min)–220 °C (12.7 min) at 300 °C min^{-1}
Gas flow and pressure	95 mL min^{-1} and 7.5 psi
Cooled Injector System	
Mode	Solvent venting
Liner	Poly(2,6-diphenyl-p-phenylene oxide), 2 mm i.d.
Temperature program	15–150 °C at 840 °C min^{-1}150–330 °C (5 min) at 630 °C min^{-1}
GC–MS	
Capillary column	HP-5MS, 5% diphenyl–95% dimethylpolysiloxane30 m × 0.25 mm, 0.25 μm film thickness
Carrier gas	Helium (1 mL min^{-1})
Oven program	50 (2.5 min)–120 °C (6 min) at 10 °C min^{-1}120–170 °C (10 min) at 33 °C min^{-1}
Transfer line temperature	280 °C
Quadrupole temperature	150 °C
Ion source temperature	230 °C
Ionization	Electron-impact mode (70 eV)

Table 2
Method characteristics.

Compound	Retention time (min)	Monitorized ions (*m*/*z*)	RSD[a] (%)	DL[b] (ng L^{-1})
4-CA	10.1	142, 127	3.4	0.2
2,6-DCP	11.5	162, 126	5.7	0.5
2,6-DCA	11.8	176, 161	4.8	0.4
2,4-DCP	12.1	162, 126	6.0	0.5
4-CP	12.4	128, 100	6.4	1.4
2,4-DCA	14.7	176, 161	3.5	0.4
2,4,6-TCA	16.0	195, 210	4.3	0.4
2,4,5-TCA	16.2	195, 210	5.1	0.3
2,4,6-TCP	16.7	196, 132	5.2	0.4
2,4,5-TCP	16.9	196, 132	6.4	0.3
2,3,4,6-TeCP	20.1	232, 133	5.3	0.3
2,3,4,5-TeCA	22.3	246, 203	3.7	0.5
PCA	24.1	280, 267	3.9	0.5
PCP	25.4	266, 167	5.5	0.5

[a] $n=10$.
[b] Corresponding to S/N=3.

An SBSE stir bar was placed in a 15 mL glass vial containing an aliquot of 10 mL of the sample and 1.0 g of NaCl, and stirred at 600 rpm for 2 h until equilibrium was reached. Next, the stir bar was removed from the vial, rinsed with Milli-Q water in order to eliminate salt residues and dried with a lint-free tissue before being introduced into a glass desorption tube. The analytes were thermally desorbed from the stir bar by placing the desorption tube in the TDU.

Since no reference materials were available, spiked samples were prepared at two different concentration levels for validation purposes. Three wine samples (white, rosé and red) were spiked at 0.1 and 0.2 μg L^{-1}. Three replicates were analyzed in each case.

3. Results and discussion

In order to evaluate the performance of the PA and EG–Silicone SBSE coatings for the preconcentration of CAs and CPs, and the influence of some experimental variables, both during the extraction step and during the desorption process, a synthetic wine spiked at 1 μg L^{-1} was used.

3.1. SBSE extraction parameters

The following variables were studied individually: ionic strength, pH of the extraction medium and extraction time. The addition of chemical modifiers such as methanol, a commonly procedure in SBSE, was discarded since the ethanol content of the samples was high enough to avoid any adsorption of non-polar compounds on the inner walls of the sample vials.

The influence of the ionic strength of the extraction medium was evaluated at different sodium chloride concentrations (0, 2, 5, 10 and 15% w/v). The addition of NaCl usually increases the extraction efficiency of PDMS coatings, since a decrease in the water solubility of polar organic compounds increases their partitioning coefficients between the coating and aqueous extraction medium. In the case of the polar coatings evaluated, the relationship between salt addition and extraction efficiency may not be so clear. However, regardless of the extracting phase, high salt concentrations may decrease the extraction efficiency because the increased viscosity of the solution hinders diffusion. Maximum extraction efficiency for the EG–Silicone coating was attained with 10% (w/v) NaCl, except in the case of PCP, for which the highest sensitivity was attained with 15% (w/v). Because the enhancement of sensitivity (from 10 to 15% w/v) for PCP was not important, a 10% (w/v) NaCl concentration was chosen. For the PA coating, most of the analytes reached their maximum extraction efficiency with a 10% (w/v) NaCl concentration, the salt having little effect in the case of the less polar analytes TeCA, TeCP, PCA and PCP.

Since CPs are weakly acidic, the influence of pH on the extraction efficiency was considered. Wine samples generally show pH values close to 3, and so CPs will probably remain in their neutral form and so be effectively extracted. However, because such a low pH may reduce the extraction efficiency of the tested coatings, the influence of the extraction medium pH was evaluated at two levels by incorporating 1 mL of two different buffer solutions: citric acid/sodium citrate (0.2 M, pH 3.5) or acetic acid/sodium acetate (0.2 M, pH 4.8). Since no significant differences were observed for either coating in the pH range studied, the use of a buffer solution was discarded.

The most important parameter affecting SBSE is extraction time. Therefore, the optimum extraction time was investigated from 0.5 to 6 h. The extraction time profiles (equilibration curves) are shown in Fig. 1. Note that in order to assess the relevance of this variable for the different analytes in the optimization process, regardless of their different sensitivities; the analytical signals were normalized with regard to average areas for each compound. Equilibrium was reached for all the compounds at about 2 h using the two extraction phases tested, so this time was chosen to ensure high extraction efficiencies.

3.2. Thermal desorption conditions

Since the number of variables involved in the thermal desorption step is large, their effect and significance were tested using a Plackett–Burman multivariate design (PBD). The use of this screening test allows the most important variables to be identified, and the most suitable values to be selected for the rest of the variables assayed. Once the most important parameters had been

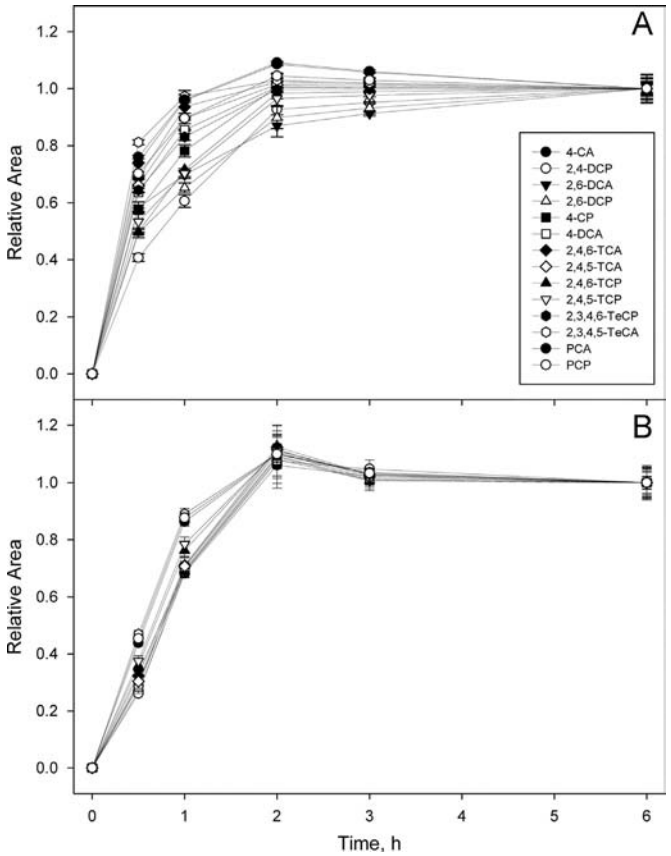

Fig. 1. Influence of the SBSE extraction time on the analytical responses for (A) EG–Silicone and (B) PA coatings.

identified, they were submitted to a Central Composite Design (CCD), which provided the optimum values.

The PBD (12 experiments, in duplicate) was similar for both coatings, and included the following variables: TDU desorption time (5 and 10 min), TDU desorption temperature (200 and 220 °C), inert gas flow rate (50 and 100 mL min^{-1}), CIS heating temperature (280 and 330 °C) and inert gas vent pressure (6 and 8 psi). The results of using EG–Silicone or PA are summarized by means of the Pareto charts in Fig. 2A and B, respectively.

Even though the desorption temperature was identified by the PBD as being the most relevant desorption parameter for EG–Silicone (the higher the temperature, the higher the response as shown in Fig. 2A), the thermolability of the EG–Silicone coating prevented temperatures higher than 220 °C from being applied, so this variable was not subjected to further study and the maximum assayed value was selected. The other relevant parameters for the thermal desorption process when using EG–Silicone coating, according to Fig. 2A, were TDU desorption time and gas flow rate, which were carefully studied in the 6.6–13.5 min and 55–110 mL min^{-1} ranges, respectively, using a CCD ($\alpha = 1.5$, $n = 10$). The obtained response surface (Fig. 3A) showed its adequacy to experimental results ($r^2 > 0.95$) and the relevance of the assayed variables ($p < 0.01$). Therefore, a desorption time of 12.7 min and an inert gas flow rate of 95 mL min^{-1} were adopted. Other less relevant parameter values were set. A pressure of 7.5 psi, corresponding to column pressure, was chosen as gas vent pressure to avoid longer pressure equilibration times, while a temperature of 330 °C was selected for CIS heating. The application of a fast heating program to achieve this temperature in the PTV injector provided sharper chromatographic peaks, and thus better peak resolution. Taking account that a high ramp temperature as 840 °C min^{-1} can be only applied until 150 °C is attained, a heating program with two ramps at almost the highest heating temperature rates (Table 1) was selected for further experiences.

When a PA coating was evaluated, the most relevant thermal desorption parameters were desorption time and inert gas flow rate, as shown by the Pareto charts (Fig. 2B). Consequently, these parameters were optimized by means of a CCD ($\alpha = 1.5$, $n = 10$), between 3.75 and 8 min for desorption time and between 55 and 110 mL min^{-1} for the gas flow. The response surface obtained (Fig. 3B) fitted the experimental results ($r^2 > 0.95$) and pointed the relevance of the assayed variables ($p < 0.01$). Consequently, the desorption of PA coated stir bars was carried out by heating the TDU at 200 °C for 5 min, while an inert gas flow of 105 mL min^{-1} impelled the analytes to the PTV. The CIS heating temperature was set to 330 °C and a 7.5 psi gas pressure was used for the vent gas.

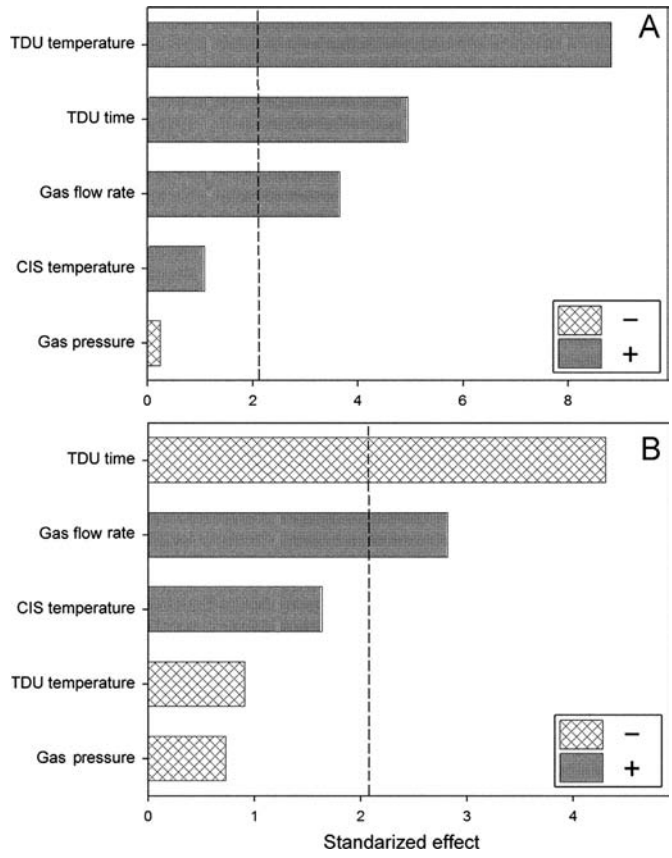

Fig. 2. The Pareto charts obtained for the analysis of effects through the Plackett–Burman designs for thermal desorption step using (A) EG–Silicone and (B) PA extraction phases.

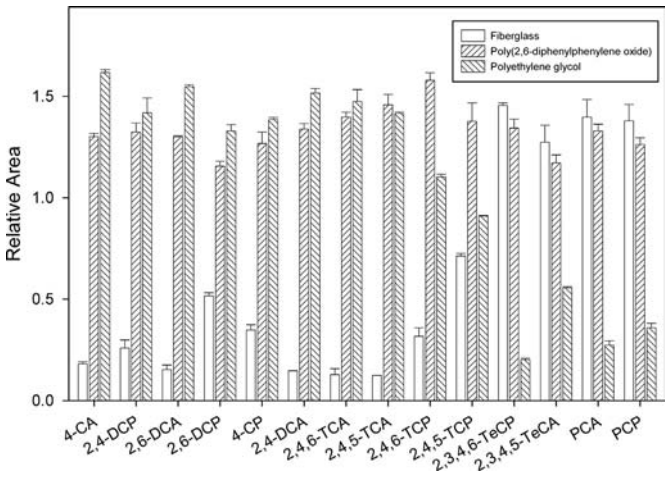

Fig. 4. Influence of CIS liner filling material on the analyte sensitivity.

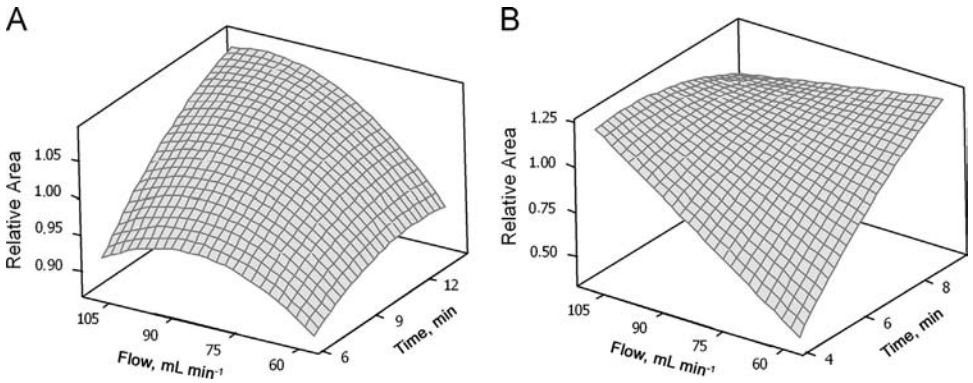

Fig. 3. Response surfaces showing the effects on relative responses obtained with (A) EG–Silicone and (B) PA coatings.

The PTV focusing temperature was set to 15 °C by means of a Peltier Unit. Different filling materials for the PTV liner were checked in order to facilitate the retention of the analytes: fiberglass, poly(2,6-diphenylphenylene oxide) and polyethylene glycol. As can be observed in Fig. 4, fiberglass retained most of the heavier compounds and so provided low recoveries for the most volatile compounds, whereas polyethylene glycol showed the opposite behavior. The liner filled with poly(2,6-diphenylphenylene oxide) showed a balanced retention power for the studied compounds, providing good recoveries both for the more volatiles and the heavier analytes, therefore it was selected.

3.3. Coating evaluation

After optimizing the SBSE extraction and desorption conditions, the extraction capabilities of EG–Silicone and PA coatings were evaluated by comparing the slopes of calibration graphs using a synthetic wine. As shown in Table 3, the use of EG–Silicone was nearly 5 times more sensitive than the PA coating (5.3 times for CAs and 4.5 times for CPs). The higher preconcentration power of the EG–Silicone phase may result from its copolymeric composition, the high extraction efficiency for CPs being due to the hydrogen bond interactions with EG. Moreover, this coating phase also ensures the efficient extraction of CAs due to its silicone base. Considering the results obtained, EG–Silicone coated stir bars were selected for the determination of CPs and CAs in wine samples.

3.4. Analytical characteristics of the method

The standard additions method was applied to three different wine samples (white, rosé and red wine) and also to a synthetic wine, by spiking these samples at six concentration levels, which were submitted in duplicate to the optimized procedure. The representation of peak area *versus* the analyte concentration was linear in the range 25–1750 ng L^{-1}, with correlation coefficients higher than 0.99 in all cases. When the slopes obtained were compared using an analysis of variance (one-way ANOVA), no statistically significant differences were observed ($p > 0.05$), calibration by using the synthetic wine solution was used for quantification purposes.

Repeatability tests were performed by submitting to the proposed procedure ten aliquots of a spiked red wine sample at a 500 ng L^{-1} concentration level, providing RSD values of 4.7 ± 0.8%. The detection (DLs) and quantification (QLs) limits of the method were calculated as three and ten times the signal-to-noise ratio, respectively (Table 2). QLs in the range 0.7–4.7 ng L^{-1}, depending on the compound, were obtained. The DL values obtained are lower than the sensory thresholds reported in the literature for the analytes studied. A slight increase of sensitivity was obtained for TeCA with EG–Silicone stir bars related to the use of non-polar PDMS phase, whereas an important increase of sensitivity (about 6500 times) was attained for PCP [28].

Table 3
Analytical characteristics obtained using EG–Silicone and PA as SBSE extracting phase.

Compound	EG–Silicone		PA	
	Slope[a] (L mg^{-1})	Regression coefficient	Slope[a] (L mg^{-1})	Regression coefficient
4-CA	901 ± 35	0.991	153 ± 6	0.991
2,4-DCP	639 ± 27	0.993	141 ± 7	0.986
2,6-DCA	982 ± 43	0.988	152 ± 6	0.991
2,6-DCP	319 ± 13	0.993	75 ± 3	0.994
4-CP	353 ± 13	0.992	64 ± 2	0.994
2,4-DCA	827 ± 27	0.993	146 ± 4	0.995
2,4,6-TCA	1159 ± 36	0.994	193 ± 5	0.995
2,4,5-TCA	1465 ± 39	0.996	286 ± 7	0.996
2,4,6-TCP	913 ± 22	0.993	175 ± 4	0.996
2,4,5-TCP	1153 ± 40	0.993	258 ± 10	0.990
2,3,4,6-TeCP	1398 ± 38	0.993	357 ± 10	0.995
2,3,4,5-TeCA	1331 ± 28	0.997	385 ± 1	0.996
PCA	1141 ± 40	0.993	261 ± 9	0.992
PCP	1510 ± 48	0.994	315 ± 10	0.995

[a] Mean value ± standard deviation ($n=6$).

3.5. Analysis of wines and validation of the method

Thirty samples, including red, rosé and white wines, were analyzed using the optimized procedure and, some of the studied compounds were found in seven samples (Table 4). 4-CA and 2,4,6-TCA were the most abundant analytes, being present in six of the samples at concentrations in the range 30–106 and 32–82 ng L^{-1}, respectively. The content of 2,4,6-TCA in two of these samples was higher than human threshold reported, 50 ng L^{-1}. 2,4-DCA was also found in four samples, at concentrations between 35 and 95 ng L^{-1}, which are lower than its sensory threshold (400 ng L^{-1}), whereas 2,6-DCA was found in three samples at concentrations higher than the corresponding threshold level (40 ng L^{-1}). Other species such as 2,4-DCP, 2,4,6-TCP, 2,3,4,6-TeCP and 2,3,4,5-TeCA were detected in some samples. The presence of these compounds in wine samples at similar concentrations has been reported previously [19,22].

A typical chromatogram obtained by SBSE–TD–GC–MS under SIM mode for a red wine fortified at 500 ng L^{-1} in the selected conditions is shown in Fig. 5. The chromatogram showed the absence of

Table 4
Results obtained in the analysis of the samples (ng L^{-1}).

Compound	Wine 2	Wine 3	Wine 9	Wine 12	Wine 14	Wine 21	Wine 24
4-CA	97 ± 7	38 ± 2	106 ± 3	30 ± 2	53 ± 3	ND	50 ± 5
2,6-DCP	146 ± 8	ND	ND	52 ± 4	ND	ND	ND
2,6-DCA	ND	162 ± 9	ND	ND	ND	64 ± 7	116 ± 8
2,4-DCA	75 ± 6	35 ± 2	ND	95 ± 13	48 ± 2	ND	ND
2,4,6-TCA	44 ± 5	40 ± 4	57 ± 5	82 ± 9	32 ± 2	ND	38 ± 3
2,4,6-TCP	ND	ND	ND	47 ± 2	ND	ND	ND
2,3,4,6-TeCP	26 ± 2	ND	ND	20 ± 1	ND	ND	ND
2,3,4,5-TeCA	ND	ND	ND	51 ± 5	ND	ND	ND

Values are mean ± standard deviation ($n=3$).
ND means not detected.

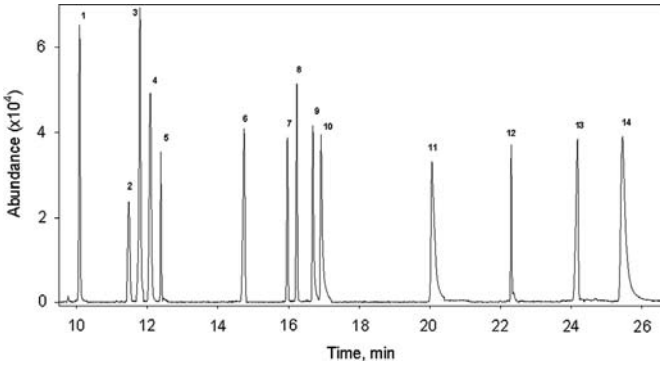

Fig. 5. SBSE–TD–GC–MS chromatogram obtained for a spiked synthetic wine fortified at 0.5 μg L^{-1} under SIM mode. Peaks correspond to: (1) 4-CA, (2) 2,6-DCP, (3) 2,6-DCA, (4) 2,4-DCP, (5) 4-CP, (6) 2,4-DCA, (7) 2,4,6-TCA, (8) 2,4,5-TCA, (9) 2,4,6-TCP, (10) 2,4,5-TCP, (11) 2,3,4,6-TeCP, (12) 2,3,4,5-TeCA, (13) PCA, (14) PCP.

Table 5
Results obtained from the recovery assays using the proposed method.

Compound	Spiked level[a] ($\mu g\ L^{-1}$)	Concentration found[b] ($\mu g\ L^{-1}$)		
		White wine	Rosé wine	Red wine
4-CA	0.1	0.095 ± 0.003	0.103 ± 0.004	0.092 ± 0.006
	0.2	0.194 ± 0.007	0.202 ± 0.007	0.190 ± 0.014
2,6-DCP	0.1	0.097 ± 0.005	0.095 ± 0.005	0.103 ± 0.010
	0.2	0.197 ± 0.011	0.198 ± 0.010	0.203 ± 0.021
2,6-DCA	0.1	0.096 ± 0.004	0.097 ± 0.004	0.094 ± 0.008
	0.2	0.193 ± 0.009	0.196 ± 0.009	0.190 ± 0.015
2,4-DCP	0.1	0.102 ± 0.006	0.091 ± 0.005	0.105 ± 0.013
	0.2	0.202 ± 0.011	0.196 ± 0.011	0.203 ± 0.021
4-CP	0.1	0.104 ± 0.005	0.094 ± 0.005	0.106 ± 0.012
	0.2	0.205 ± 0.011	0.199 ± 0.011	0.206 ± 0.023
2,4-DCA	0.1	0.091 ± 0.004	0.092 ± 0.004	0.088 ± 0.009
	0.2	0.188 ± 0.009	0.190 ± 0.008	0.180 ± 0.016
2,4,6-TCA	0.1	0.089 ± 0.004	0.102 ± 0.004	0.090 ± 0.008
	0.2	0.184 ± 0.009	0.203 ± 0.009	0.185 ± 0.015
2,4,5-TCA	0.1	0.088 ± 0.004	0.095 ± 0.004	0.092 ± 0.008
	0.2	0.183 ± 0.008	0.195 ± 0.008	0.186 ± 0.017
2,4,6-TCP	0.1	0.105 ± 0.005	0.087 ± 0.004	0.107 ± 0.011
	0.2	0.206 ± 0.009	0.195 ± 0.009	0.200 ± 0.018
2,4,5-TCP	0.1	0.108 ± 0.004	0.105 ± 0.005	0.111 ± 0.010
	0.2	0.211 ± 0.009	0.206 ± 0.009	0.218 ± 0.020
2,3,4,6-TeCP	0.1	0.099 ± 0.005	0.095 ± 0.004	0.102 ± 0.010
	0.2	0.201 ± 0.009	0.192 ± 0.009	0.196 ± 0.018
2,3,4,5-TeCA	0.1	0.084 ± 0.003	0.091 ± 0.003	0.093 ± 0.006
	0.2	0.178 ± 0.006	0.186 ± 0.006	0.188 ± 0.012
PCA	0.1	0.086 ± 0.002	0.094 ± 0.002	0.096 ± 0.004
	0.2	0.181 ± 0.004	0.193 ± 0.004	0.200 ± 0.008
PCP	0.1	0.092 ± 0.004	0.113 ± 0.005	0.110 ± 0.009
	0.2	0.209 ± 0.009	0.220 ± 0.010	0.210 ± 0.020

[a] Values in brackets correspond to spiking levels for red wine samples.
[b] Mean value ± standard deviation ($n=3$).

interfering peaks at the analyte retention times. The analytes were identified by comparing their retention times, and by identifying mass spectra of the peaks in samples and standard solutions.

To check the accuracy of the proposed method, and since no reference materials were commercially available for the validation of the method, recovery assays were performed using three different wine samples (red, rosé and white) by fortifying at two concentration levels (100 and 200 $ng\ L^{-1}$). The recoveries obtained ranged from 84 to 116% ($n=126$) at the lower level and from 89 to 113% ($n=126$) for the higher level (Table 5).

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