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Short communication

Stir bar sorptive extraction for the analysis of wine cork taint

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Abstract

A magnetic stir bar with a polydimethylsiloxane coating was used to absorb 2,4,6-trichloroanisole, 2,3,4,5-tetrachloroanisole, pentachloroanisole and their respective phenols from synthetic and real wine samples. The stir bar sorptive extraction method was optimised to obtain the best extraction conditions in terms of temperature, time, pH and NaCl addition. The stir bar was desorbed in a thermal desorption system coupled to a gas chromatograph–mass spectrometer. The method proposed showed good linearity over the concentration range tested and correlation coefficients ranged from 0.96 to 0.99 for all the analytes. The reproducibility and repeatability of the method was estimated between 1.29 and 4.02%. With no a pre-concentration step and with a much reduced analysis time, all the analyzed compounds showed detection and quantification limits that were lower than those observed with other methods found in the bibliography. Except for pentachlorophenol due to its poor absorptivity in polydimethysiloxane, in red wines, LOD ranged between 7.56 and 61.56 pg/l, and LOQ ranged between 17.21 and 205.11 pg/l; while in white wines, the LOD ranged between 5.82 and 30.50 pg/l and LOQ ranged between 19.41 and 101.61 pg/l. These concentrations were always lower than their respective olfactory thresholds values.

Keywords: Stir bar sorptive extraction; Cork taint; Wine; Chloroanisoles; Chlorophenols

1. Introduction

Natural cork is the most widely used substance for closing wine bottles. Among its many valued properties are its lightness, impermeability to liquids and gases, resistance to wear, rot and temperature extremes and, perhaps above all, its renowned compressibility. However, there is growing concern within the wine industry about the possible flavour-damaging effect known as cork taint, which is usually perceived as a moldy, musty and/or earthy aroma that may mask the natural wine aroma and lessen its quality. About 1–5% of bottled wine is cork-tainted and cork stoppers are increasingly being replaced by synthetic substitutes. Several chemical compounds, including anisoles, guaicol and geosmine, and several aliphatic compounds, such as 1-octen-3-one or 1-octen-3-ol, have been related to this problem [1]. Anisoles, especially 2,4,6-trichloroanisole (TCA) and, to a lesser extent, 2,3,4,6-tetrachloroanisole (TeCA) and pentacloroanisole (PCA) are responsible for at least 80% cork taint cases reported [2,3]. The mechanisms whereby wine corks and other products used in wine production become infected with chloroanisoles and chlorophenol compounds to cause this musty taint are only partly understood. The use of polychlorophenolic biocides, especially pentachlorophenol, in cork-oak forests and the chlorine bleaching involved in the processing of barrels, among others, are thought to be the principal causes of this problem. In all cases, fungal methylation of chlorophenols to chloroanisoles is required [4,5]. These off-flavour compounds are present at extremely low concentrations and also have very low olfactory thresholds. For example, the TCA olfactory threshold ranges from 5 to 10 ng/l [1,6] or between 14 and 25 ng/l for TeCA and is around 4 μg/l for PCA [7]. Most analytical methods are not sensitive enough to detect such concentrations without an important pre-concentration step. A common analytical procedure to detect cork taint compounds, especially the presence of 2,4,6-trichloroanisole, includes a liquid–liquid extraction [3,8–10] or solid phase extraction with a C_{18} cartridge [11] followed by a reconcentration of the extract and direct injection into the standard GC-system. However, these techniques often produce more artefact compounds than the trace level analytes that are to be determined. An environment friendly approach was then considered to avoid sample

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manipulation and solvent consumption, by using thermally desorbed solid-phase microextraction (SPME) fiber [2,12]. Recently, to improve the absorption of analytes, a magnetic stir bar covered with a polydimethylsiloxane (PDMS) coating (Twister, Gerstel GmbH) has been introduced. When a liquid sample matrix is stirred, the analytes are partioned between the matrix and the PDMS phase on the stir bar according to their partitioning coefficients. The extraction theory of stir bar sorptive extraction (SBSE) is the same as for SPME but the higher phase ratio coating leads to an increase in sensitivity by a factor of more than 100 compared with SPME [13–15]. Furthermore, the analytes extracted by stirring the sample are recovered directly desorbed, and can be analyzed by GC/MS. Since its introduction, many trace analysis have been performed with this technique for in environmental [16–18], food [15,19], beverage [20,21] and many other samples.

Due to the serious problems suffered by the wine industry as a result of cork taint, many experiments have been carried out in relation with 2,4,6-trichloroanisole but not with other related compounds, such as 2,3,4,5-tetrachloroanisole, pentachloroanisole and their respective phenols, which are also responsible for the mentioned off-flavour effect. Thus, the main aim of our research was to develop a fast and simple method able to quantify these compounds in a single chromatographic run based on thermal desorption-gas chromatography–mass spectrometry of the analytes absorbed by the stir bar sorptive extraction (SBSE). For this, SBSE technique was optimised by obtaining the best conditions to quantify, below or close to their olfactory threshold values, the six chlorinated compounds responsible for cork taint.

2. Experimental

2.1. Chemicals and reagents

Standards: 2,4,6-trichlorophenol, 2,3,4,6-tetrachlorophenol, 2,4,6-trichloroanisole and 2,3,4,6-tetrachloroanisole were obtained from Sigma–Aldrich (Madrid, Spain) and pentachlorophenol and pentachloroanisole from LGC Promochem (Molsheim, France). Exact masses of the chemical standards were dissolved in absolute ethanol.

Solvents: ethanol (analytical-reagent grade) was obtained from Merck (Damstard, Germany), while water was purified through a Milli-Q system (Millipore, Bedfords, MA, USA).

Synthetic wine samples were prepared by an ethanol solution at 12% (v/v) to which 5 g/l tartaric acid were added. Solution pH was adjusted to 3.6 with 1 M sodium hydroxide (Panreac, Barcelona, Spain).

2.2. Proposed extraction method

Compounds were extracted by introducing the polymethylsiloxane coated stir bar (0.5 mm film thickness, 10 mm length, Twister, Gerstel, Mülheim and der Ruhr, Germany) into 10 ml of sample (either commercial wine or synthetic wine solution), to which 2 μl of internal standard methyl octanoate solution at 6 μl/l in absolute ethanol was added. Samples were stirred at 700 rpm at room temperature for 60 min. The stir bar was then removed from the sample, rinsed with distilled water and dried with a cellulose tissue, and later transferred into a thermal desorption tube for GC/MS analysis.

2.3. GC/MS analysis

In the thermal desorption tube, the volatile compounds were desorbed from the stir bar at the following conditions: oven temperature at 330 °C; desorption time, 4 min; cold trap temperature, −30 °C; helium inlet flow 45 ml/min. The compounds were transferred into the Hewlett-Packard 6890 gas chromatograph coupled to an Hewlett-Packard LC 3D mass detector (Palo Alto, USA) with a fused silica capillary column (BP21 stationary phase 50 m length, 0.22 mm i.d., and 0.25 μm film thickness) (SGE, Ringwood, Australia). The chromatographic program was set at 50 °C (held for 5 min), rainsed to 180 °C at 2.5 °C/min (held for 2 min) and to 230 °C (5 °C/min) and held for 20 min. For mass spectrometry analysis, electron impact mode (EI) at 70 eV was used. The mass range varied from 35 to 500 u and the detector temperature was 150 °C. Identification was carried out using the NIST library and quantification was based on the calibration curves of respective standards in the synthetic wines.

2.4. Analytical method validation

For linearity study, calibration graphs were established with synthetic wine solution spiked with five different analytes concentration. Each level of concentration was analysed twice with two different stir bar, so there were a total of four replicates. The concentration ranges were from 1 to 50 ng/l for TCA, TeCA, PCA, TCP and TeCP and between 1 and 100 μg/l for PCP. The detection and quantification limits (LOD and LOQ, respectively) were calculated with the data generated in the linearity studies. LOD was defined as $(a + 3Sa/b)$ and LOQ as $(a + 10Sa/b)$, "a" being the origin ordinate, "Sa" the origin ordinate variance and "b" the slope. The limit of quantification was taken to be validated when within-batch relative standard deviation, using three replicate samples spiked with known LOQs, was under 20% according to Catice methodology [22].

The standard deviation for each compound (square root of the arithmetic mean of the variances) was calculated to obtain the repeatability (% R.S.D). The standard deviation of the three values for each compound multiplied by the square root of 3 was taken as the reproducibility value (if this value was higher than repeatability; if not, this last value was also taken as reproducibility) [23].

2.5. Application to wines

In order to see the matrix composition effect over the target compounds, calibration graphs were carried out also for red and white wines. For such purpose, five different analytes concentration were spiked to each wine. The concentration ranges were from 1 to 50 ng/l for TCA, TeCA, PCA, TCP and TeCP and between 1 and 100 μg/l for PCP. Method validation was studied as the synthetic wine solution. Each level of concentration was analysed twice with two different stir bar, so there were a total of four replicates.

3. Results and discussion

Because of the low concentration of the chlorinated compounds in the wine samples, an enrichment step is always necessary prior to the analysis. This step normally requires considerable time and in very labour intensive [3,9,11]. However, according to the numerous bibliographic references, stir bar requires lower analysis time and, more importantly, sample manipulation is considerably reduced in comparison with other extractives techniques. Special attention was taken with TCA due to its low volatile conditions [1,6] and also with PCP as may not be readily partitioned into the non-polar PDMS phase [15]. The portioning theory for SPME, extended to SBSE, establishes that the parameters, temperature, sample pH and salt content during the extraction, should be considered to enhance the sensitivity of the PDMS phase with polar analytes, such as phenols or anisoles [12,15,24]. The sorption kinetics for the target analytes were evaluated by analyzing 10 ml of synthetic wine solution spiked with 1 μg/l of each compound at different conditions. At times ranging from 30 to 120 min, the maximum sorption time was achieved at 60 min, no significant differences being found at higher extraction times.

Evans et al. [12] increased the detection limits of TCA by increasing the temperature from room temperature to 45 °C, but not improvement was observed by adding salt (1 g of sodium chloride), as was clearly observed for trichlorophenol in water samples by Buchholz and Pawliszyn [25]. When both approaches were assayed with the stir bar, neither higher temperatures (60 °C) nor the use of sodium chloride significantly favoured the sensitivity of the target compounds (measured as peak area), not even in the case of TCA. It seems that, ionized organic species such as phenols are not readily partitioned into the non-polar PDMS phase, an effect that may be mitigated by adjusting the sample pH before the extraction step [15]. However, lowering the sample pH from 3.6 (normal wine pH) to pH 2 did not significantly enhance the extraction of chlorinate phenols into the stir bar phase.

The optimum stir bar sorptive extraction conditions were therefore fixed as: 10 ml of the sample spiked with the target analytes and stirred at 700 rpm with a stir bar at room temperature for 60 min. Sample pH was adjust to 3.6. After absorption in the stir bar, the analytes were thermally desorbed and determined by GC/MS. To avoid wine matrix interferences between the aromatic and the chlorinated compounds, the MS analysis was carried out in single ion

Table 1
Analytical characteristics of the method using synthetic, red and white wines

Parameters	TCA	TeCA	PCA	TCP	TeCP	PCP
Compound ions (*m*/z)	195, 210	203,231,246	237,265,280	132, 160,196	166, 232	165,230, 266
Selected ions (*m*/z)	195	246	280	196	232	266
Concentration range (ng/l)	1–50	1–50	1–50	1–50	1–50	100–1000
Synthetic wines						
Linearity curve (r^2)	0.9926	0.9949	0.9871	0.9632	0.9899	0.9897
Detection limit (LOD) (pg/l)	0.34	1.70	1.54	5.84	3.97	$2.31E^{+6}$
Quantification limit (LOQ) (pg/l)	1.08	5.66	5.13	19.31	13.16	$6.01E^{+6}$
Reproducibility (%)	2.11	1.53	3.13	2.22	1.58	4.02
Repeatability (% R.S.D.)	2.11	1.53	1.29	2.23	1.58	3.05
White wines						
Linearity curve (r^2)	0.9844	0.9861	0.9876	0.9751	0.9942	0.9853
Detection limit (LOD) (pg/l)	30.50	12.96	5.82	9.90	15.64	$3.31E^{+6}$
Quantification limit (LOQ) (pg/l)	101.61	43.19	19.41	32.89	52.13	$7.01E^{+6}$
Reproducibility (%)	3.01	1.62	3.32	2.73	1.63	3.52
Repeatability (% R.S.D.)	2.47	1.59	1.42	2.53	1.63	3.12
Red wines						
Linearity curve (r^2)	0.9954	0.9841	0.9828	0.9652	0.9826	0.9721
Detection limit (LOD) (pg/l)	61.56	7.57	11.20	24.38	11.77	$4.15E^{+6}$
Quantification limit (LOQ) (pg/l)	205.11	25.26	17.21	114.05	39.23	$10.11E^{+6}$
Reproducibility (%)	3.12	1.83	2.82	2.56	1.57	3.35
Repeatability (% R.S.D.)	3.12	1.83	2.47	2.51	1.53	2.96

Note: TCP (2,4,6-trichlorophenol); (TeCP) 2,3,4,6-tetrachlorophenol; (PCP) pentachlorophenol; TCA (2,4,6-trichloroanisole); (TeCA) 2,3,4,6-tetrachloroanisole; (PCA) pentachloroanisole.

monitoring (SIM) mode using their characteristics *m*/*z* values (Table 1).The internal standard was also quantified in the single ion monitoring (SIM) mode at its *m*/*z* 74.

Different calibration curves were made in a concentration range below or close to the olfactory threshold values of the analytes commonly found in wine samples [3,11]. The method showed good linearity over the concentration ranges tested and the correlation coefficients were higher than 0.98 for all the analytes, except trichlorophenol (0.96). It is important to point out that the excellent signal-to-noise ratio of the individual ions observed. Blank runs of the stir bar were made before and after each analysis and no memory effect occurred for the target solutions at concentrations lower than μg/l levels. For the reproducibility of a method (% R.S.D.) to be considered acceptable, its value should be less than 20%. In all cases, the reproducibility of the compounds ranged from 1.29% (PCA) to 3.05% (PCP). The same limit (20%) was taken to represent good repeatability, in this case ranging from 1.53% (TeCA) to 4.02% (PCP). In conclusion, the method proposed shows a very good repeatability and reproducibility. Note, too, that the total analysis time per sample was approximately 2 h and while stir bar enrichment is time consuming but it is not labour intensive.

The quantification and detection limits given in the synthetic extract were always lower than their known olfactory thresholds. For 2,4,6-trichloroanisole (TCA), different analytical detection limits have been reported in wine, depending on the extraction method used. For example, when liquid–liquid extraction combined with GC/MS [9], the detection limit has been estimated as 0.5–2 ng/l, while it is around 0.1 ng/l by solid–liquid extraction [11] and from 2.9 to 5 ng/l by SPME [12]. Hoffmann et al. [26] reported that with SBSE is was possible to detect concentration as low as 9.5 ng/l in Riesling or 0.3 ng/l in Welschriesling wines. In this work, the detection limit of TCA was calculated as 0.34 pg/l, whereas its quantification limit was 1.08 pg/l, both lower than the olfactory threshold (4–10 ng/l).

For the other chloroanisoles, TeCA and PCA, detection limits (LOD) of 2.4 and 2.7 ng/l, respectively, have been reported by liquid–liquid extraction combined with GC/MS [9], no other extraction techniques having been found in the literature. In the case of stir bar sorptive extraction, the LOD and LOQ for tetrachloroanisole (TeCA) were established as 1.70 and 5.66 pg/l, respectively. For PCA, LOD and the LOQ concentrations were 1.54 and 5.13 pg/l, respectively. The concentrations of both compounds, then, lower than their

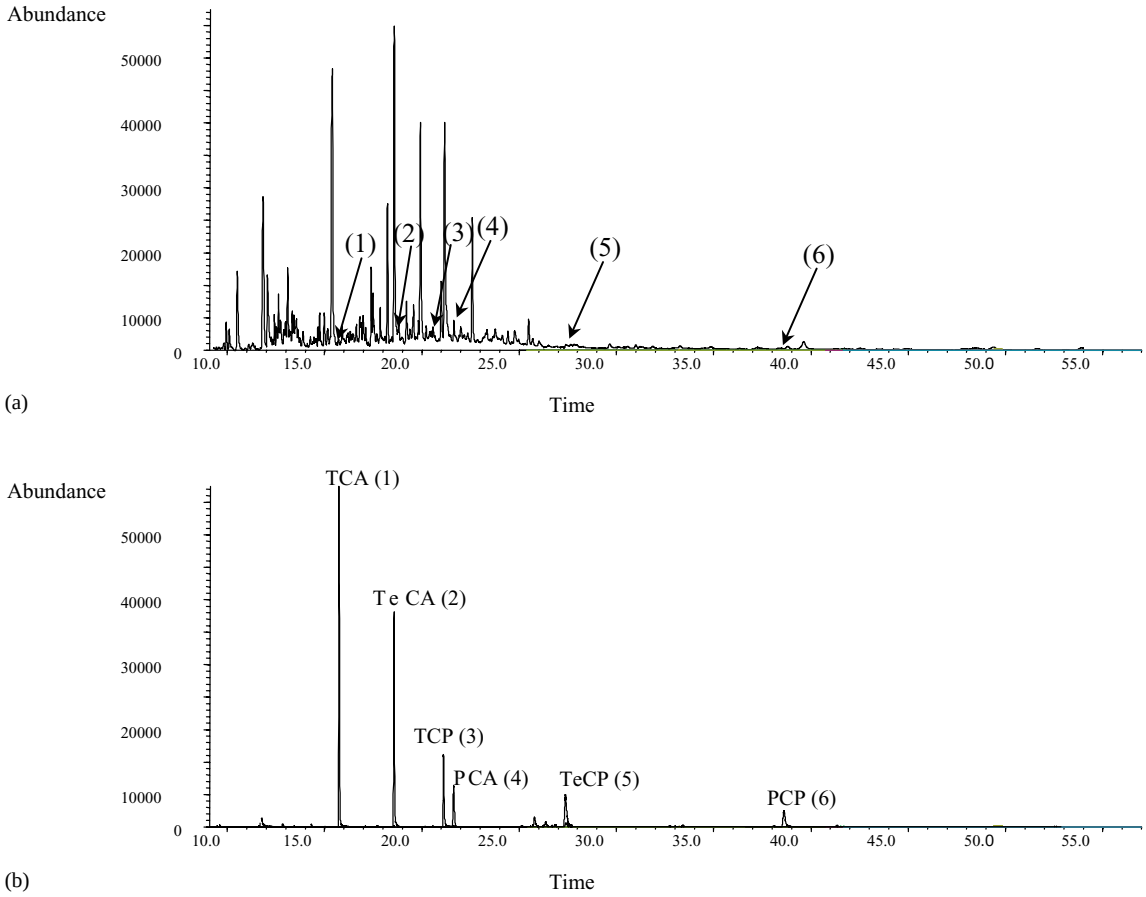

Fig. 1. (a) Red wine chromatogram analyzed by stir bar sorptive extraction (SBSE) with gas chromatography–mass spectrometry technique; (b) overlaid selected ion chromatograms of the six target compounds at 2 ppb in red wine; (1) TCA (2,4,6-trichloroanisole); (2) (TeCA) 2,3,4,6-tetrachloroanisole; (4) (PCA) pentachloroanisole; (3) TCP (2,4,6-trichlorophenol); (5) (TeCP) 2,3,4,6-tetrachlorophenol; (6) (PCP) pentachlorophenol.

respective wine olfactory thresholds of 14–25 and 4000 ng/l [7]. It should be pointed out that these values are one order of magnitude lower than those found in a standard operation with SPME, and almost two orders of magnitude lower than those found with liquid–liquid extraction.

We have only found one reference about the detection limit of trichlorophenol (TCP), 0.7 ng/l, as determined by solid–liquid extraction [11]. With the stir bar sorptive extraction methodology, a LOD of 5.84 pg/l and LOQ of 19.3 pg/l were calculated. No bibliographic references to the detection limits of TeCP and PCP were found, although they have been determined in wine samples. Their wine olfactory thresholds were not found, either. The SBSE method, provided a TeCP detection limit of 3.97 ng/l and a quantification limit of 13.16 ng/l. Among all the analytes, PCP had the highest LOQ and LOD values (μg/l levels), whereas the other chlorinated compounds had ng/l levels. Such differences may be due to the different polarities between PCP and the polymethylsiloxane phase. It is also known that pentachlorophenol, the most commonly chlorophenol biocide used in oak forests, also contains smaller amounts of TeCP and TCP [27]. Pentachlorophenol may be degraded to less substituted chlorophenolic (tetra- and tri-) compounds, which may in terms methlylated by fungal activity to their respective anisoles. In the literature, no direct relationship between this compound and cork taint has been reported, although, it can cause an unpleasant flavour easily detected in a sensorial evaluation [3].

In complex samples, like wines, the matrix composition affects the determination of the target compounds. As it can be observed in Fig. 1a, there is a great number of aromatic compounds but they do not interfere with the six target compounds (Fig. 1b) when they are detect at their selected *m*/*z* (Table 1). Among all the compounds within the wine matrix, it is believe that parameters such as ethanol and polyphenols can have some influence on the quantification results [28]. The red and white wines used in this study had the same ethanol content (approximately 12%), but the amount of polyphenols ranged considerably. The polyphenolic content of such wines was measured by the total polyphenol index (TPI) (expressed as gallic acid) [29], resulting in 4.3 g/l for red wine and 2.1 g/l for white wine. The comparison of red and white wines calibration curves for each compound showed significant differences between the slopes and therefore in the LOD and LOQ values (Table 1). This confirms that the matrix effect is of great importance when chlorinate compounds are analyzed, and also that red wine interferences are higher than the white ones. Although, the reproducibility and repeatability parameter values were very good as they were below 4%. It should be point out that the new LOD and LOQ values in real wine samples are still below their respective olfactory threshold.

The results presented in this report indicate that the stir bar sorptive extraction technique is an excellent technique as all compounds responsible for cork taint are clearly detected. With no a pre-concentration step and with a much reduced analysis time, all the analyzed compounds showed detection and quantification limits that were lower than those observed with other methods found in the bibliography and, more importantly, with concentrations lower than their olfactory thresholds values.

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