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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)