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Cloud point extraction and gas chromatography with direct microvial insert thermal desorption for the determination of haloanisoles in alcoholic beverages

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ABSTRACT

A sensitive analytical procedure for the determination of four haloanisoles (2,4,6 trichloroanisole, 2,4,6-tribromoanisole, 2,3,4,6-tetrachloroanisole and pentachloroanisole) related with cork taint defects in wines, in different types of alcoholic beverages has been developed. The analytes were extracted from the matrix samples by cloud point extraction (CPE) using Triton X-114 heated to 75 °C, and the surfactant rich phase was separated by centrifugation. By means of direct microvial insert thermal desorption, 20 μL of the CPE obtained extract was submitted to gas chromatography-mass spectrometry (GC–MS) analysis. The parameters affecting the CPE and microvial insert thermal desorption were optimized. Quantification was carried by matrix-matched calibration using an internal standard. Detection limits ranged between 12.9 and 20.8 ng L^{-1}, depending on the compound, for beer and wine samples, whereas for whiskies values in the 46.3–48 ng L^{-1} range were obtained, since these samples were diluted for analysis. Recoveries for alcoholic beverages were in the 89–111% range, depending on the analyte and the sample.

1. Introduction

In recent years, cloud point extraction (CPE) has been widely used as a sample preparation technique in analytical chemistry [1]. CPE is based on the fact that a solution of non-ionic surfactants leads to the formation of micelles when it is heated above a certain temperature, known as cloud point temperature. In this way, a homogeneous solution provides a two phase disperse system, which can be separated [2]. Some compounds can be concentrated in the surfactant-rich fraction (coacervate), which is recovered by centrifugation. CPE is similar to other liquid–liquid microextraction (LLME) techniques, its main advantage being that it avoids the use of organic solvents, which mean it can be cataloged as an environmental friendly procedure [3].

CPE has been successfully applied for the determination of both inorganic [1,3–6] and organic species [2,7–10]. The enriched extracts obtained by CPE have usually been analyzed by atomic spectroscopic techniques for elementary determinations, and by liquid chromatography (LC) or electrophoresis (CE) [7] in the case of organic species. However, relatively few works have dealt with the coupling of this extraction technique with gas chromatography (GC) [11–20]. The low volatility of the coacervate makes its direct introduction into the GC system impossible using conventional injectors, since the injection of CPE extracts may not only pollute the inlet, but also cause capillary column blockage. However, two approaches have been proposed for this purpose, the most widely used being back-extraction (BE) [11–13,16,17,20], whereby the analytes preconcentrated in the surfactant rich phase are back extracted into an organic solvent immiscible with the CPE extract and compatible with GC. The efficiency and speed of this process is usually increased by the application of ultrasounds [12,16,20] or microwaves [13,16]. The other approach involves the derivatization of the surfactant contained in the coacervate [18,19] prior to its introduction into the GC. Silylation derivatization with N,O-bis (trimethylsilyl)fluoroacetamide (BSTFA) is usually applied, increasing the volatility of the surfactant, which is not retained in the injector or column. However, large quantities of surfactants are still introduced into the GC system, leading to large peaks that may overlap the analyte responses, or foul the ion source if MS is used as detector. Separation of the analytes from the surfactant has also been achieved using cation exchange columns [15].

In order to overcome this limitation, direct microvial insert thermal desorption [21] has been tested, thus avoiding additional steps after the CPE step. Such an approach is based on the use of a commercial thermal desorption unit (TDU) as interface to transfer the extracted analytes from the surfactant rich phase to the GC

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system. For this purpose, glass inserts containing up to 150 μL of liquid sample are placed in the thermal desorption tube. Microvial insert thermal desorption has previously been applied for the GC analysis of ionic liquid drops obtained through a microextraction step [22].

Cork taint is a defect related with musty, moldy or earthy aromas and off-flavors, which can be detected in wine, but also in other alcoholic beverages, such as beer or whisky [23]. 2,4,6-Trichloroanisole (TCA) has been suggested as being mainly responsible for this defect [24], although other haloanisoles (HAs), such as 2,4,6-tribromoanisole (TBA), 2,3,4,6-tetrachloroanisole (TeCA) and pentachloroanisole (PCA), may contribute to the off-flavors [25]. These species are generated by fungal methylation of their corresponding halophenols [26], which may be produced during the chlorine bleaching of the wood, or reach this material as a consequence of their use as biocides [27].

In this paper GC analysis of the CPE extracts using microvial insert thermal desorption is proposed for the determination of four HAs in different alcoholic beverages. The surfactant rich phase drop is placed in a glass microvial by microsyringe. The microvial is introduced inside the TD tube, and the whole assembly is submitted to thermal desorption. A carrier gas propels the analytes to a programmed temperature vaporizator (PTV) injector, where they are focused before entering the chromatographic column. Next, the PTV is heated, and the retained compounds enter the GC system. In this way, the surfactant hardly reaches the GC system, and, even if some vapors are dragged by the gas flow, they are retained in the disposable PTV liner,

2. Experimental

2.1. Standards and reagents

2,4,6-Trichloroanisole (TCA, 99%) and 2,4,6-tribromoanisole (TBA, 99%) were supplied by Aldrich (Steinheim, Germany). 2,3,4,6-Tetrachloroanisole (TeCA, >95%) and pentachloroanisole (PCA, >95%) were provided by Ultra Scientific (Teddington, England) and Chem Service (West Chester, PA, USA), respectively. 5-Bromo-2-chloroanisole (97%), supplied by Aldrich, was used as internal standard (IS). Individual stock solutions of the compounds (1000 μg mL^{-1}) were prepared using pure grade acetone as the solvent, and stored in darkness at −10 °C. Working standard solutions were prepared daily by diluting with ultrapure water. The non-ionic surfactant Triton X-114, which was used as a 30% (w/v) aqueous solution, was provided by Fluka (Buchs SG, Switzerland). Sodium chloride, potassium dihydrogen phosphate and dipotassium hydrogen phosphate were obtained from Fluka. The water used was previously purified in a Milli-Q system (Millipore, Bedford, MA, USA). The carrier gas used for GC was helium (Air Liquide, Madrid, Spain).

2.2. Instrumentation

Samples were placed in disposable glass microvials (15 mm long, 1.9 mm i.d., 2.5 mm o.d., Gerstel, Mullheim an der Ruhr, Germany). 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) (Gerstel). The experimental conditions used for 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. In the selected conditions (Table 1), the analytes eluted with retention times between 5.0 and 7.4 min,

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

Thermal desorption unit	
Mode	Solvent venting
Temperature programme	75 °C, held 0.5 min 75–225 °C at 300 °C min^{-1}, held 5 min
Desorption flow	50 mL min^{-1}
Cooled Injector System	
Mode	Solvent Venting
Liner	Tenax, 2 mm i.d.
Temperature programme	15–250 °C (5 min) at 540 °C min^{-1}
GC–MS	
Capillary column	HP-5MS, 5% diphenyl-95% dimethylpolysiloxane (30 m x 0.25 mm, 0.25 μm)
Carrier gas	Helium (1 mL min^{-1})
Oven programme	80 °C, held 0.6 min 80–180 °C at 25 °C min^{-1}, held 0.6 min 180–210 °C at 25 °C min^{-1}, held 0.8 min 210–300 °C at 50 °C min^{-1}, held 1.4 min
Transfer line temperature	300 °C
Quadrupole temperature	150 °C
Ion source temperature	230 °C
Ionization	Electron-impact mode (70 eV)

Table 2
Retention times, monitored ions and enrichment factors.

Compound	RT, min	Monitored ions (*m/z*)	EF
2,4,6-Trichloroanisole (TCA)	5.0	167, 195 (98), 210 (63)	73.2
5-Bromo-2-chloroanisole- (IS)	5.3	222, 179 (89)	68.0
2,3,4,6-Tetrachloroanisole (TeCA)	6.2	203, 231 (84), 131 (80), 246 (64)	78.8
2,4,6-Tribromoanisole (TBA)	6.7	344, 346 (95), 329 (78), 301 (50)	76.0
Pentachloroanisole (PCA)	7.4	237, 265 (76), 280 (68), 167 (58)	73.2

Underlined numbers correspond to *m/z* of the target ion, and values in brackets represent the qualifier-to-target ion ratios in percentage.

corresponding to TCA and PCA, respectively (Table 2). The identification of the compounds was confirmed by comparison of retention times and MS-spectra with respect to pure standards. The analytes were quantified under the selected ion monitoring (SIM) mode using the target ions (Table 2). A domestic microwave oven was used in the sample treatment.

2.3. Samples and analytical procedure

Different wood-aged alcoholic beverages, including white and red wine, beer and whisky, were obtained from local supermarkets. Once opened, all samples were kept at 4 °C until analysis, in order to prevent losses of the more volatile analytes. Prior to analysis, a 1:4 sample: water dilution was applied for whisky samples, to decrease the alcohol content. For CPE, a 5 mL volume of beverage sample, in the presence of the IS at 0.25 ng mL^{-1}, was placed in a 10-mL screw cap glass tube with conical bottom, into which 0.2 g of NaCl had previously been weighed, and 0.5 mL of phosphate buffer solution (0.2 M, pH 7.2) was added. Next, 100 μL of 30% (w/v) Triton X-114 aqueous solution were injected into the sample solution using a microsyringe, and the mixture was gently shaken manually for several seconds. The resulting solution was heated at 75 °C in a microwave oven for 40 s, leading to the

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