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Direct sample introduction-gas chromatography-mass spectrometry for the determination of haloanisole compounds in cork stoppers

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ABSTRACT

A solventless analytical method is proposed for analyzing the compounds responsible for cork taint in cork stoppers. Direct sample introduction (DSI) is evaluated as a sample introduction system for the gas chromatography-mass spectrometry (GC–MS) determination of four haloanisoles (HAs) in cork samples. Several parameters affecting the DSI step, including desorption temperature and time, gas flow rate and other focusing parameters, were optimized using univariate and multivariate approaches. The proposed method shows high sensitivity and minimises sample handling, with detection limits of 1.6–2.6 ng g^{-1}, depending on the compound. The suitability of the optimized procedure as a screening method was evaluated by obtaining decision limits (CCα) and detection capabilities (CCβ) for each analyte, which were found to be in 6.9–11.8 and 8.7–14.8 ng g^{-1}, respectively, depending on the compound. Twenty-four cork samples were analysed, and 2,4,6-trichloroanisole was found in four of them at levels between 12.6 and 53 ng g^{-1}.

1. Introduction

Cork taint is a wine defect related with a musty, mouldy or earthy aroma and off-flavours, leading to a poor quality wine and a decrease in consumer acceptance. The number of wine bottles affected by this taint has been estimated in the 1–5% range [1], which represents large economic losses to a winery, whose reputation may also suffer. The compound suggested as being mainly responsible for this defect is 2,4,6-trichloroanisole (TCA) [1], although other haloanisoles (HAs), such as 2,4,6-tribromoanisole (TBA), 2,3,4,6-tetrachloroanisole (TeCA) or pentachloroanisole (PCA) may also contribute to the off-flavour [2]. Other compounds have been suggested as being co-responsible for cork taint, including 1-octen-3-ol, 1-octen-3-one, 2-methylisoborneol, geosmin and guaicol [3]. HAs are generated as a result of fungal activity in cork, through methylation of their corresponding halophenols [4], which may be produced during the chlorine bleaching of the bark cork or may already be in this material as a consequence of their addition as biocides to wood [5] or as cleaning agents during manufacture of cork stoppers and oak barrels [6,7].

A wide range of analytical methods have been proposed for the determination of cork taint-related compounds in corks. Most employ their separation by gas chromatography (GC) and detection by mass spectrometry (MS) [8–18] or electron capture detector (ECD) [19–27], although biosensors [28] have also been proposed.

Different sample preparation techniques have been applied for the extraction and preconcentration of HAs present in corks. Extraction from the solid matrix is usually carried out by means of solid-liquid extraction (SLE), or different modifications of this procedure such as pressurized fluid extraction (PFE) [12] or microwave assisted extraction (MAE) [18,22,29], followed by preconcentration by liquid–liquid extraction (LLE) [20], or solid phase extraction (SPE) [17,19]. SLE is usually time-consuming and requires large amounts of sample and organic solvents, which brings with it a risk of environmental contamination, resulting in additional costs for the treatment of residues. In recent years, this technique has been replaced by others which are solvent-free, offer automation and are cleaner, more selective, rapid and efficient. For example, headspace solid-phase microextraction (HS-SPME) [8,11,12,23–27,30] and headspace sorptive extraction (HSSE) [15] have been used for the direct HA extraction and preconcentration from corks. Other miniaturized techniques have been used to preconcentrate the obtained extracts, such as SPME [16,19] and stir bar sorptive extraction (SBSE) [9,18] under direct immersion modes, as well as dispersive liquid–liquid microextraction (DLLME) [22].

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In order to avoid the use of organic solvents for extracting the analytes, Amirav and Dagan developed direct sample introduction (DSI) [31], a rapid, sensitive, simple and inexpensive procedure in the context of large volume injection (LVI). In DSI, the solid sample, with a diameter up to 5 mm and a length of 30 mm, is placed in a glass desorption tube and introduced into the programmed temperature vaporizator (PTV) or into a thermodesorption unit (TDU) attached to the PTV inlet. The solid matrix components remaining in the desorption tube are removed and discarded after the assay, while volatile compounds are vaporized and transferred to the GC column for separation [32]. DSI has previously been used for the determination of pesticides in eggs, milk and vegetables [33,34] after conventional SLE and to determine odour-related compounds other than HAs in juices and wines, after extraction with SPE [35,36]. Despite the interesting advantages of DSI, to the best of our knowledge, this methodology has not previously been applied to the determination of HAs in corks that, as already pointed, is especially relevant in the winery industry.

In the present work, we develop an analytical method using DSI coupled to GC–MS for the determination of HAs responsible for cork taint (TCA, TBA, TeCA and PCA) in different types of cork stoppers.

2. Materials and methods

2.1. 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 (Middlesex, UK) and Chem Service (West Chester, PA, USA), respectively. 5-Bromo-2-chloroanisole (97%) was used as internal standard (IS), being supplied by Aldrich. Individual stock solutions of the compounds (1000 mg mL^{-1}) were prepared using pure grade acetone as solvent, and stored in darkness at −10 °C. Working standard solutions were freshly prepared in the same solvent and stored at 4 °C. The carrier gas used for GC was helium (Air Liquide, Madrid, Spain).

2.2. Instrumentation

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) from Gerstel (Mullheim an der Ruhr, Germany). 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. The instrumental conditions, which are summarized in Table 1, provided retention times (Table 2) of between 5.0 and 7.4 min, corresponding to TCA and PCA, respectively. Compounds were identified by comparing their retention time and relevant MS-spectra with those obtained for the injection of pure standards (Table 2). The analytes were quantified under the selected ion monitoring (SIM) mode using the target ion.

2.3. Samples, analytical procedure and recovery studies

Twenty-one used corks were obtained from wine bottles from different origins, while three different batches of unused cork were obtained from a local cellar. All stoppers were also classified according to their manufacturing process in natural (7 stoppers) or agglomerated (17 stoppers), made of cork granulated material mixed with chemical agglutinating compounds. A cork borer was used for sampling purposes, and cylindrical portions (5 × 10 mm) were obtained from each stopper. Prior to analysis each cork portion was weighed on an analytical balance, and was seen to weigh, on average, 20 mg.

Spiked samples were prepared by applying different volumes of the standard solution mixture, to the bored cork, using a microsyringe at different points of the sample, including its surface and the inner part. The IS was also added at a concentration level of 100 ng g^{-1}. Samples were set aside for 60 min at room temperature in 1.5 mL microcentrifuge tubes to allow the solvent to evaporate while the analytes were retained in the cork before being submitted to the DSI procedure.

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

Thermal Desorption Unit	
Mode	Splitless
Temperature programme	40–141 °C at 200 °C min^{-1}, held 2.1 min
Desorption flow	35 mL min^{-1}
Cooled Injector System	
Mode	Solvent Venting
Liner	Fiberglass, 2 mm i.d.
Temperature programme	15–260 °C (5 min) at 540 °C min^{-1}
GC–MS	
Capillary column	HP-5MS, 5% diphenyl-95% dimethylpolysiloxane (30 m × 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 time and monitored ions.

Compound	RT(min)	Monitored ions (*m*/*z*)
2,4,6-Trichloroanisole (TCA)	5.0	169 (61), 195, 197 (98), 210 (63), 212 (60)
5-Bromo-2-chloroanisole (IS)	5.3	179 (59), 222
2,3,4,6-Tetrachloroanisole (TeCA)	6.2	201 (69), 229 (80), 231, 233 (48), 244 (58), 246 (71)
2,4,6-Tribromoanisole (TBA)	6.8	301 (45), 303 (42), 329 (77), 331 (72), 344, 346 (91)
Pentachloroanisole (PCA)	7.4	235 (61), 237 (66), 263 (52), 265, 278 (37), 280 (58)

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

3. Results and discussion

3.1. Direct sample introduction

Direct sample introduction is a complex step that may be influenced by a large number of experimental variables. The effect of five of these variables (desorption time and temperature, gas flow-rate, PTV liner filling, and heating temperature) in the response of the HAs was evaluated at different levels. For this purpose, cork cylinders of about 20 mg containing the analytes at 100 ng g^{-1} were placed in the desorption tube and introduced into the gas chromatograph using the TDU-CIS system.

Five different desorption temperatures (from 100 to 180 °C) and times (from 0.5 to 4 min) were tested. In general, higher temperatures and longer times facilitated the vaporization of analytes from