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Analysis of corky off-flavour compounds at ultra trace level with multidimensional gas chromatography-electron capture detection☆

Petra Slabizki, Hans-Georg Schmarr*

Dienstleistungszentrum Ländlicher Raum (DLR) Rheinpfalz, Competence Centre for Wine Research, Breitenweg 71, D-67435 Neustadt an der Weinstraße, Germany

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ABSTRACT

A robust method for routine quality control of corky off-flavour compounds in wine and cork soak matrices has been established. Based on an automated headspace solid phase microextraction (HS-SPME), the method needs only marginal sample preparation and achieves low (sub-ng L^{-1}) trace level detection limits (LODs) for the most relevant off-flavour compounds, such as 2,4,6-trichloroanisole (TCA), 2,3,4,6-tetrachloroanisole (TeCA) and 2,4,6-tribromoanisole (TBA). Particularly for wine matrix, reliable trace level quantification had only been achieved after applying heart-cutting multidimensional gas chromatography (MDGC). Using a halogen-sensitive electron capture detector (ECD) and quantification with a stable isotope dilution assay (SIDA), LODs of 0.1 ng L^{-1} for TCA, TeCA and TBA could be obtained. Since a SIDA based quantification method is used with a non-mass spectrometric detector, the necessary chromatographic resolution of internal standard and target analyte peaks resulted from the use of highly deuterated [$^2H_5$]-isotopologues.

1. Introduction

Musty cork taint is one of the most known off-flavours in wine, with 2,4,6-trichloroanisole (TCA) as the primary responsible compound [1,2]. However, other haloanisoles such as 2,3,4,6-tetrachloroanisole (TeCA) and 2,4,6-tribromoanisole (TBA) are also important for quality control in the cork and wine industry [3–5]. Known sources are from wood preservatives used in packaging or in the cellar surroundings [6,7]. Their sensory thresholds have been reported to be at the lower ng L^{-1} level in wine. Depending on the wine style, off-flavour can be detected at some 2–5 ng L^{-1} for TCA; *e.g.* in flavour-accentuated white wines with a particularly fruity character [3,7–9]. Therefore, aroma-relevant haloanisoles have to be monitored in a quality-control situation for the cork and wine industry at low levels, or in customer conflict situations, even at sub-ng L^{-1} levels. The cork industry usually monitors cork soaks (approximately 10% by volume ethanol solutions) for quality control, thus being relatively simple in matrix composition. In such situations, the applied analytical methods are often based on one-dimensional gas chromatographic analysis (^{1}D-GC) with mass spectrometric (MS) or electron capture detection (ECD) as standard procedures [10–18]. However, customer conflicts originate from rejected wines, due to "corkiness" detected during tasting. In such conflict situations, chemical analysis has to be performed on the much more complex and analytically demanding wine matrix. In our own control laboratory, an earlier-established method based on headspace solid phase microextraction (HS-SPME) and ^{1}D-GC-ECD analysis [10] failed to produce reliable data in certain trace-level (low ng L^{-1}) situations with wine matrices due to severe co-elution problems. We had been able to overcome such problems applying a method based on (off-line) solid phase extraction (SPE) and multidimensional GC (MDGC)–MS [19]. Detection limits of sub-ng L^{-1} levels could be achieved, but at the cost of relatively extensive sample preparation and clean-up procedures, also incorporating a large volume on-column injection. The procedure proved to be time consuming and somewhat demanding for the operator, thus not suitable for a routine application in our situation. For high-throughput analyses, a more practical and automated sample preparation had to be targeted. Based on previous methods using automated HS-SPME as extraction technique [10,20–23], we wanted to modify the original ^{1}D-GC-ECD system by increasing the chromatographic separation efficiency with a second separation dimension (^{2}D) and by heart-cutting the haloanisole fractions to this second dimension column. Reliable quantification for the HS-SPME-MDGC-ECD analytical method then should be assured by using highly deuterated isotopologues as internal standards, thus quantifying *via* a stable isotope dilution assay (SIDA) approach, which had been introduced by Rittenberg and Foster [24] and has found wide application, particularly in trace-level

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* Corresponding author. Tel.: +49 6321 671 289; fax: +49 6321 671 375.
E-mail address: hans-georg.schmarr@dlr.rlp.de (H.-G. Schmarr).

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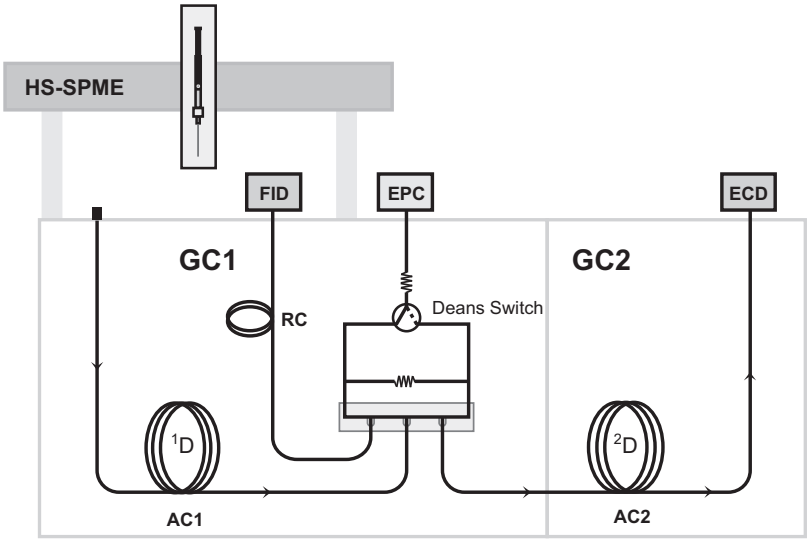

Fig. 1. Scheme of the automated HS-SPME-MDGC-ECD system. Heart-cuts are performed with a Deans Switch, transferring from ^{1}D analytical column (AC1; DB-XLB) to the ^{2}D analytical column (AC2; TG-1301MS); restrictor capillary (RC); electronic pressure controller (EPC).

flavour analyses since then [25]. In recent years, quantitative analyses of haloanisoles in wine, have often been based on SIDA methods published in literature, but, usually with MS or MS/MS detection [14,19,20,26–28].

2. Experimental

2.1. Chemicals and reagents

2,3,4,6-Tetrachloroanisole (CAS no. 938-22-7) was from LGC Promochem (Wesel, Germany), 2,4,6-trichloroanisole (CAS no. 87-40-1) and 2,4,6-tribromoanisole (CAS no. 607-99-8) were from Sigma–Aldrich (Steinheim, Germany), ethanol (absolute) was from VWR (Darmstadt, Germany), and sodium chloride and ethanol (denaturated with 1% methylethylketone) were from Roth (Karlsruhe, Germany). The deuterated reference substances [$^{2}H_5$]-2,4,6-trichloroanisole (TCA-d_5; CAS no. 352439-08-8) and [$^{2}H_5$]-2,4,6-tribromoanisole (TBA-d_5; CAS no. 1219795-33-1) were synthesized in-house as described earlier [19]. Commercial chemicals were usually of analytical grade.

2.2. HS-SPME conditions

HS-SPME extraction was done on 5 mL sample volumes, using 10 mL headspace vials with silicone/polytetrafluoroethylene (PTFE) septa and metallic screw caps. Sample preparation involved addition of 1 g of sodium chloride (previously conditioned at 180 °C), a glass-coated magnetic stir bar, and the internal standards TCA-d_5 and TBA-d_5 in a concentration of 2 ng L^{-1} each in an ethanolic solution (10 μL of 1 pg μL^{-1}).

Table 1
Method validation data for HS-SPME-MDGC-ECD analysis. Calibration graphs based on 8 calibration points (n = 3), calculated with equal weighting according to DINTEST, with ranges from 0.1 to <10 ng L^{-1} (details in Section 2.4).

Analyte	Calibration graph	R^2	LOD	LOQ
TCA	$y = 0.407x - 0.002$	0.9994	0.1 ng L^{-1}	0.4 ng L^{-1}
TeCA	$y = 0.709x + 0.047$	0.9998	0.1 ng L^{-1}	0.4 ng L^{-1}
TBA	$y = 0.572x - 0.014$	0.9994	0.1 ng L^{-1}	0.5 ng L^{-1}

SPME utilized a 1 cm fibre coated with 100 μm of polydimethylsiloxane (PDMS; Supelco, Steinheim, Germany). Automation was done with a CombiPal autosampler (CTC, Zwingen, Switzerland), comprising a single magnet mixer (Chromtech, Idstein, Germany) for agitation and incubation. Since no fibre conditioning station was available, the SPME fibre was conditioned for 10 min in the GC injector at 250 °C, prior to starting an analytical sequence. Extraction conditions involved a 1 min pre-incubation at 35 °C, and extraction for 20 min at 35 °C and 250 rpm agitation speed. Desorption of the fibre was done in the GC injector at 250 °C utilizing a 2 min splitless time and a liner dedicated for SPME application (Supelco). Instrument control was with the Cycle Composer Software version 1.5.2 (CTC).

2.3. Gas chromatographic conditions

Heart-cutting MDGC was based on the Deans switch principle [29], using the capillary flow technology from Agilent (Waldbronn, Germany). The MDGC system consisted of two HP 6890 Series GCs (Agilent) equipped with a flame ionization detector (FID) for ^{1}D and an ECD for ^{2}D detection. The two GC instruments were connected *via* a heated transferline (kept at 230 °C). The ^{1}D separation column was a fused silica capillary (30 m × 0.25 mm i.d.) with a film thickness of 0.25 μm of DB-XLB (J&W; Agilent), the ^{2}D separation column was a fused silica capillary (15 m × 0.25 mm i.d.) coated with 0.25 μm TG-1301MS (ThermoFisher Scientific, Dreieich, Germany). The restrictor column between the Deans switch and FID consisted of a 2 m phenylmethyl deactivated fused silica capillary (0.15 mm i.d.). The ^{1}D and ^{2}D analytical columns were connected *via* a phenylmethyl deactivated fused silica capillary (1.2 m × 0.25 mm i.d.). Deactivated capillaries were from Agilent. The carrier gas used was hydrogen in constant pressure mode at 129 kPa (^{1}D inlet pressure). Mid-point pressure for the Deans switch was applied at 80 kPa *via* an auxiliary electronic pressure regulator (EPC). ^{1}D oven temperature was programmed from 50 °C (2 min isothermal) with 20 °C min^{-1} to 120 °C (0.5 min hold), and then with 5 °C min^{-1} to 250 °C (5 min hold). ^{2}D oven temperature was programmed from 50 °C (20 min isothermal) with 25 °C min^{-1} to 85 °C (0.5 min hold), then with 2 °C min^{-1} to 140 °C and finally with 40 °C min^{-1} to 250 °C (5 min hold). FID and ECD were each kept at 250 °C, using nitrogen as make-up gas in both cases. A scheme