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Trace level analysis of corky off-flavor compounds: Development of a new analytical method based on solid phase extraction and analysis by multidimensional gas chromatography with mass spectrometric detection☆

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ABSTRACT

This work describes the development of a trace level (<1 ng L^{-1}) analysis of haloanisoles in complex wine matrix. The suggested method involves sample preparation based on solid phase extraction, a clean-up to remove acidic compounds, concentration of the haloanisole fraction and large volume on-column injection into a multidimensional GC–MS system. Mass spectrometric detection in the selected ion mode allowed reliable quantification of 2,4,6-trichloroanisole (TCA) or 2,4,6-tribromoanisole (TBA), via their highly deuterated ([$^2H_5$]) isotopologues as internal standards (stable isotope dilution analysis; SIDA), which had prior been synthesized in house. The development of this new method had become necessary, as a one-dimensional HS-SPME–GC-ECD method, routinely applied for analysis of TCA in cork soaks, had to be extended for TeCA and TBA determination, but failed due to co-elutions within wine matrices. The newly developed SPE//MDGC–MS method provided detection limits well below olfactory thresholds of the analytes with 0.05 ng L^{-1} (LOD), 0.19 ng L^{-1} (LOQ) for TCA, 0.06 ng L^{-1} (LOD), 0.21 ng L^{-1} (LOQ) for TeCA, and 0.09 ng L^{-1} (LOD), 0.34 ng L^{-1} (LOQ) for TBA.

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1. Introduction

The corky taint of wines is one of the most known off-flavors to the wine industry, and has recently been reviewed by several authors [1–3]. Although the compounds primarily important for this off-flavor, such as 2,4,6-trichloroanisole (TCA) or 2,4,6-tribromoanisole (TBA), are known for many years [4], their reliable trace level analysis still is a challenging task for the analytical chemist. This can be seen in method optimization steps found in numerous publications which are still produced on that topic [5–14], citing only a few. Sensory thresholds of haloanisoles have been reported to be extremely low [15,16] and cork taint in wine can easily be detected by consumers with TCA concentrations as low as a few ng L^{-1}, depending on wine style and alcohol content [17–19]. Therefore, analytical procedures for quality control have to work on concentration levels even down to the sub-ng L^{-1} level.

Reliable quantification can best be achieved using isotopic labeled internal standards [20,21], in a stable isotope dilution assay [22]. In our situation we started using highly deuterated [$^2H_5$]-isotopologues of TCA and TBA for quantification, and a common sample preparation approach using headspace solid phase microextraction (HS-SPME) [21,23–31]. The [$^2H_5$]-isotopologues provide good chromatographic separation from the non-deuterated target compounds, allowing the use of the selective and sensitive electron capture detector (ECD) for quantitative analysis [32]. However, the complex wine matrix caused co-elution problems in the classical (one-dimensional, 1D) HS-SPME–GC-ECD analysis (Fig. 1) which had to be overcome by an alternative approach.

Besides the fact that most recent applications found in literature use headspace SPME techniques for the analysis of haloanisoles in cork or wine matrix, alternative methods have been described. Static headspace with cryo-trapping [33] or dynamic headspace [34] have also been described in the literature. Supercritical fluid extraction (SFE) [35] or pressurized liquid extraction [36] have been described for direct cork analysis. An alternative method to SPME is stir bar sorptive extraction (SBSE), having the advantage of higher capacities of the sorbent material, suitable for trace level analysis and allowing multiple extractions in parallels [8,11,37–40]. Furthermore, Maggi et al. [41] compared immersion (IS) SPME,

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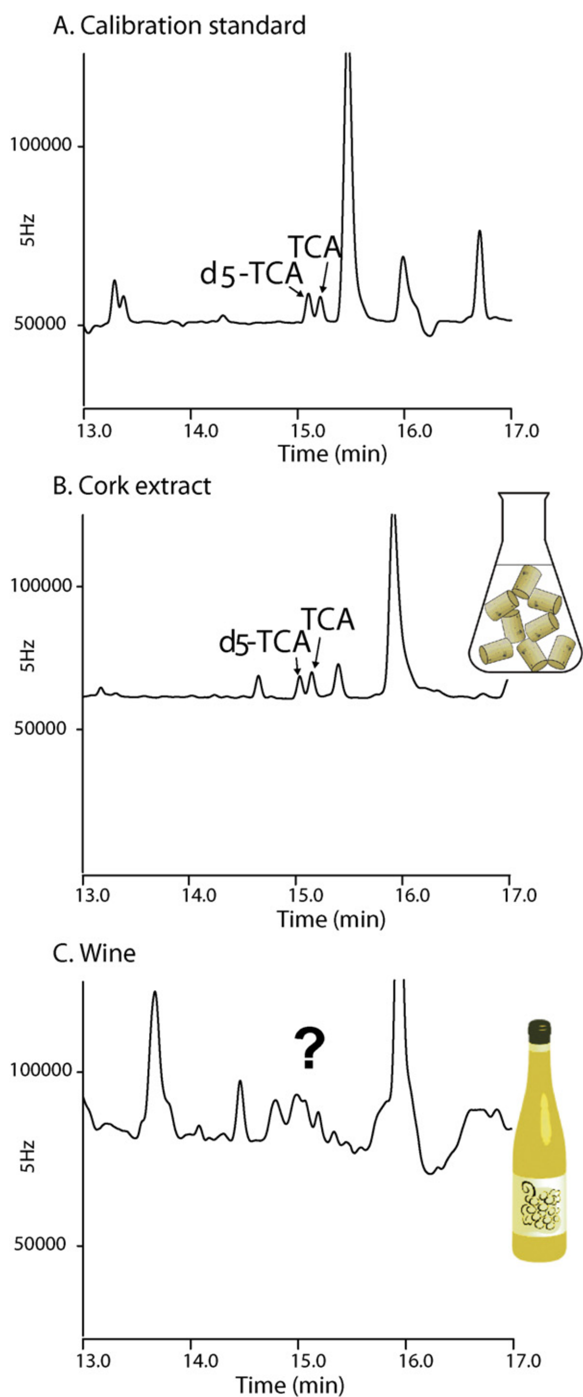


Fig. 1. HS-SPME–GC-ECD chromatogram of a calibration standard (A), a cork soak sample (B) and a wine sample (C).

HS-SPME and SBSE (usually applied in immersion mode) for the analysis of haloanisoles in different liquid matrices, and found best results for trace level analysis with SBSE. In their study, detection was performed with ion-trap tandem mass spectrometry (MS/MS).

Amongst the liquid extraction methods, solid phase extraction (SPE) is a good alternative, with applications for the analysis of haloanisoles in wine [13,42]. SPE materials can be made very selective, e.g. with the incorporation of immunosorbents as described recently [14]. SPE sorbents are available in various polarities suiting dedicated purposes, and liquid extracts obtained can be further processed and concentrated for trace level analysis.

Still, wine matrix problems encountered with target component analysis, are common and may be further resolved by more specific detection [43] or increased separation efficiency, e.g. by multidimensional gas chromatographic (MDGC) separation [44]. The latter has been successfully applied for trace level determination of methoxypyrazines [45] or 2-aminoacetophenone [46] in our laboratory before. Therefore, we wanted to develop a reliable method for trace level analysis of some important haloanisoles based on SPE and MDGC and compare its suitability particularly for wine and cork soak analysis with a 1D HS-SPME–GC-ECD method.

2. Experimental

2.1. Chemicals and reagents

Acetonitrile and 2,3,4,6-tetrachloroanisole (TeCA) were from LGC Promochem (Wesel, Germany), calcium hypochlorite (technical), 2,4,6-trichloroanisole (TCA), [$^2H_6$]-phenol were from Sigma–Aldrich (Steinheim, Germany), [$^2H_3$]-iodomethane, dichloromethane, N,N-dimethylformamide (anhydrous), tetrabutyl ammoniumbromide were from Fluka (Buchs, Switzerland), diethylether, ethanol (absolute) (30%) hydrogen peroxide, sodium hydroxide, sodium chloride, sodium carbonate (anhydrous), sodium hydrogencarbonate, vanadium(V) oxide, silica gel and LiChrolut® EN solid phase extraction cartridges (200 mg, 3 mL) were from VWR (Darmstadt, Germany), magnesium sulfate (anhydrous) and phosphoric acid were from Riedel de Häen (Seelze, Germany). Aluminum oxide (basic) was from J.T. Baker (Griesheim, Germany). A vacuum manifold from Agilent (Waldbronn, Germany) was used for SPE preparation. Commercial chemicals were usually of analytical grade. Method calibration data were calculated with DINTEST, vers. 2005 DE software (Georg Schmitt, Inst. f. Rechtsmedizin, Universiätsklinikum Heidelberg, Germany; www.analytiksoft.de) according to DIN 32645.

2.2. Cork soaks and wine samples

Cork samples (100 individual corks) were submersed in an ethanol/water solution (10% vol.). The flasks were covered with aluminum foil and left at room temperature over night. Samples were from routine quality control, or when sensory analysis of cork samples suggested chemical analysis.

Wine samples were taken from tanks, respectively barrels or from bottled wines, when a cork taint was suspected during quality control or sensory evaluation.

2.3. Synthesis of deuterated reference substances

Whereas deuterated [$^2H_5$]-TCA is available from various suppliers in mg amounts, no source for the deuterated [$^2H_5$]-TBA was listed in the chemical abstracts database (CAS). This prompted us to synthesize the standards in-house, as outlined hereafter.

2.3.1. Synthesis of [$^2H_2$]-2,4,6-trichlorophenol ([$^2H_2$]-TCP)

Basically following the procedure described earlier [47], 3.24 g calcium hypochlorite were added to a solution of 300 mg [$^2H_6$]-phenol in 60 mL water and 30 mL 0.1 M NaOH. The solution was then stirred for 18 h at room temperature. Then, 20 mL of an aqueous solution of sodium thiosulfate (250 g L^{-1}) were added and pH was brought to 3.5 with 0.45 M phosphoric acid. This mixture was extracted 3 times with 30 mL diethyl ether and once with 30 mL dichloromethane. The combined organic extracts were washed with half saturated sodium hydrogen carbonate solution and brine, and finally dried with anhydrous magnesium sulfate. After filtration, the solvent was evaporated using a rotavapor and the product was dried in an exsiccator, yielding 304 mg (51%) [$^2H_2$]-TCP (CAS

no. 93951-80-5). Purity as determined by GC–MS was >98%; linear retention index (LRI, based on *n*-alkanes and determined as described earlier [48]: 1354 (ZB-5); *m/z* **198** (100), **200** (99), **99** (61), **134** (45), **136** (37), **202** (34), **64** (22), **101** (19), **162** (17), **62** (16); *M* = 199.46.

2.3.2. Synthesis of [$^2H_5$]-2,4,6-trichloroanisole ([$^2H_5$]-TCA, d_5-TCA)

Under argon atmosphere, 120 μL of [$^2H_3$]-iodomethane were added via microsyringe to a solution of 0.3 g anhydrous sodium carbonate, 240 mg [$^2H_2$]-TCP and 3 mL anhydrous dimethylformamide. After stirring over night at room temperature this solution was extracted twice with 10 mL diethyl ether. The organic extracts were washed with half saturated sodium hydrogen carbonate solution and water and finally dried with anhydrous magnesium sulfate. After filtration, the solvent was evaporated using a rotavapor and the product was dried in an exsiccator, yielding 190 mg (73%) [$^2H_5$]-TCA (CAS no. 352439-08-8). Purity was determined by GC–MS, using commercial TCA as standard and a calculation based on equal response. Purity as determined by GC–MS was >98%; LRI 1310 (ZB-5), m/z **197** (100), **199** (99), **215** (76), **217** (75), **169** (51), **171** (50), **99** (36), **201** (35), **219** (27), **64** (22); *M* = 216.51.

2.3.3. Synthesis of [$^2H_2$]-2,4,6-tribromophenol ([$^2H_2$]-TBP)

In our hands, the analogous synthesis of TBP from [$^2H_5$]-phenol with calcium hypobromite as described above gave low yields and di- and tri-brominated compounds as by-products. Therefore, we looked into an alternative way for this synthesis. We followed the route, Bora et al. had described in detail earlier, applying a new regioselective bromination of organic substrates with tetrabutylammonium bromide promoted by V_2O_5–H_2O_2 under mild conditions, which occurs with high selectivity and yield [49].

In short: to a stirred solution of 670 mg V_2O_5 in a 50 mL acetonitrile/water mixture (1:1, v:v) 17.5 mL 30% hydrogenperoxide were added in portions over some 10 min. Temperature was maintained at 0–5 °C using an ice–water bath. Then, tetrabutylammonium bromide (4.7 g) and [$^2H_6$]-phenol (350 mg) were added to the solution, which was further stirred for about 48 h at room temperature. The solvent was evaporated using a rotavapor and the residue was extracted three times (about 30 mL each) with ethyl acetate. The organic phase was washed with 5% sodium thiosulfate solution (2 × 10 mL) and water (2 × 10 mL), and dried with $MgSO_4$. After filtration, the solvent was evaporated using a rotavapor and the product was dried in an exsiccator, yielding crude [$^2H_2$]-TBP (CAS no. 1219795-42-2), which was further purified by silica gel chromatography (petrol ether:ethyl acetate; 24:1; v:v). Yield: 495 mg (43%); purity as determined by GC–MS was >98%; LRI 1637 (ZB-5), *m/z* **64** (100), **332** (98), **334** (97), **62** (55), **65** (39), **143** (37), **145** (37), **336** (35), **330** (33), **38** (22); *M* = 332.8.

2.3.4. Synthesis of [$^2H_5$]-2,4,6-tribromoanisole ([$^2H_5$]-TBA, d_5-TBA)

The derivatization of [$^2H_2$]-TBP (400 mg) proceeded according to the procedure described for the synthesis of [$^2H_5$]-TCA and yielded 368 mg (91%) [$^2H_5$]-TBA (CAS no. 1219795-33-1); purity as determined by GC–MS was >98%; LRI 1618 (ZB-5), m/z **64** (100), **351** (99), **349** (97), **331** (65), **333** (65), **62** (54), **76** (42), **143** (39), **145** (37), **353** (36); *M* = 349.86.

The overall synthesis pathway for [$^2H_2$]-TBP and [$^2H_5$]-TBA is summarized in Scheme 1.

2.4. HS-SPME–GC-ECD

Based on a procedure described by Fischer and Fischer [26] with some modifications, gas chromatographic analysis was done with a model HP 6890 Series GC (Agilent, Waldbronn, Germany), equipped with an electron capture detector (ECD; kept at 230 °C, using nitrogen as make-up gas). Separation was with a 30 m × 0.25 mm i.d. fused silica capillary, coated with a film thickness of 0.25 μm of DB-XLB (J&W; Agilent). Carrier gas used was nitrogen at a constant flow of 0.9 mL/min. Oven temperature was programmed from 50 °C (2 min hold) with 20 °min^{-1} to 150 °C, then with 8 °C/min to 190 °C and finally with 30 °min^{-1} to 250 °C (4.75 min hold). Splitless injection (1 min splitless) was done at 250 °C. For solid phase microextraction (SPME), a 100 μm PDMS fibre was used (Supelco, Steinheim, Germany), which was desorbed for 2 min in the GC injector at a temperature of 250 °C. Automation was with a CombiPal CTC-autosampler (Chromtech, Idstein, Germany) using the following conditions: 1 min pre-incubation at 35 °C, then 20 min extraction at 250 rpm using a single magnet mixer (Chromtech); agitation 50 s on; 2 s off. Instrument control and data handling was done with Cycle Composer Version 1.5.2 (CTC, Zwingen, Switzerland) and ChemStation Rev. A. 10. 02 (Agilent) software, respectively. HS-SPME analysis was done on 5 mL sample volumes in a 10 mL headspace vial with a silicone/PTFE septum, to which 1 g sodium chloride (previously heated at 180 °C overnight) and a magnetic stir bar were added. Internal standards ([$^2H_5$]-TCA and [$^2H_5$]-TBA) were added as ethanolic solution in concentrations of 2 ng L^{-1}, each.

2.5. SPE//MDGC–MS

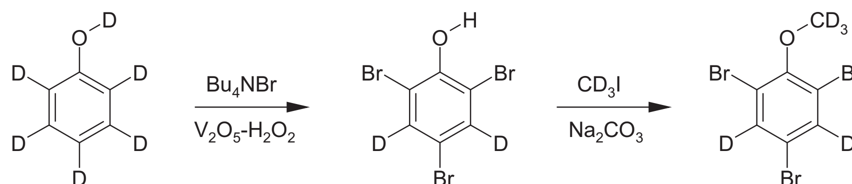
With the off-line sample preparation by SPE, the method abbreviation is used as follows: SPE//MDGC–MS (the forward slashes representing off-line coupling of techniques according to the suggestion given by Peter Schoenmakers on behalf of the HTC-11 symposium, Bruge, Belgium, 26–29 January 2010).

2.5.1. SPE

Inspired by a SPE procedure described earlier [13], we developed an SPE approach using 3 mL cartridges with 200 mg of LiChrolut® EN material, as this sorbent is often used in our lab in other fields of wine aroma analysis. Samples (100 mL volumes) were loaded onto the cartridges, which had been previously conditioned with dichloromethane, methanol and 12.5% aqueous ethanolic solution (6 mL each). The samples were spiked prior to analysis with internal standards [$^2H_5$]-TCA and [$^2H_5$]-TBA, about 2 ng L^{-1} each. Cartridge loading utilized a large volume adapter and a vacuum manifold. After drying the cartridges with an argon flow in a specialized home-made drying station, desorption of the haloanisole containing fraction was done with 1.5 mL of *n*-pentane/dichloromethane mixture (4/1; v/v). This eluate was applied onto a second SPE cartridge filled with 0.5 g of basic aluminum oxide. Finally, the haloanisole fraction was recovered with an additional application of 0.3 mL of the eluent described above. It was recovered in a GC vial which was previously charged with 0.15 mL of *n*-hexane, serving as co-solvent [50,51] for trapping of volatiles in the final concentration step, in which the volatile solvent was simply allowed to freely evaporate in the fume hood. Fig. 2 gives an overview of the sample preparation steps. For MDGC–MS analysis, 20 μL of this concentrated extract was analyzed as described below.

2.5.2. MDGC–MS

The system for heart-cut two-dimensional GC (MDGC) was based on the "*Moving Capillary Stream Switching*" (MCSS) and has been described in detail in an earlier application [46]. Two GC instruments (model 8560, Mega II series) from C.E. Instruments (today ThermoFisher Scientific, Dreieich, Germany) were connected via a heated transfer line. The 1D GC was equipped with the MCSS device allowing heart-cutting of GC fractions and an AS 800 autosampler (Fisons, now ThermoFischer) equipped with a large volume injection



Scheme 1. Synthesis of deuterated 2,4,6-tribromophenol and 2,4,6-tribromoanisole.

syringe (Hamilton, Bonaduz, Switzerland), injecting into a cold on-column injector (Fisons). Modifications of the system described earlier are outlined hereafter.

1st *Dimension system* (Scheme and annotations as in Fig. 3): carrier gas (helium) was supplied by a pressure regulator P1 (on-column injector), pressure applied to the switching device (glass dome; P2) was supplied via an additional line from the regulator of the unused split/splitless injector. Inlet pressures were 130 kPa (P1) and 40 kPa (P2), respectively. The actual inlet pressure for the second dimension column was given by the read-out on the pressure gauge P2* and was at 25 kPa. A flame ionization detector (FID) was used as monitor detector and set to 250 °C. Control of the MCSS system as well as data processing was achieved by the Chromcard data acquisition software, version 2.2 (ThermoFisher). Temperature was programmed from 80 °C (8 min isothermal), at 20 ° min^{-1} to 125 °C, at 2 ° min^{-1} to 150 °C, then at 8 °min^{-1} to 250 °C (15 min isothermal). The 1st dimension column configuration consisted of a 3.5 m × 0.53 mm i.d. phenylmethylsilylated pre-column, which was connected via a press-fit to a 30 m × 0.25 mm i.d. analytical column, coated with 0.5 μm of a polyethylene glycol phase (ZB-Wax, Phenomenex, Aschaffenburg, Germany). A deactivated fused silica capillary (1.5 m × 0.25 mm i.d., Phenomenex) was guided through a heated transfer line (200 °C), connecting the two GC ovens. Cut intervals were set to 26.4–28.2 min (d_5-TCA/TCA), 32.4–33.5 min (TeCA) and 35.5–37.6 min (d_5-TBA/TBA).

2nd *Dimension system:* the 2D GC was coupled to a quadrupole mass spectrometer, model MD 800 (Fisons). The transfer capillary from the first oven was connected via a press-fit to the 2D analytical column (15 m × 0.25 mm i.d. fused silica capillary with 0.5 μm of a 35% phenylmethypolysiloxane (ZB-35, Phenomenex). The oven temperature program and MS acquisition were initiated via external event activation at the time of the first heart-cut. The oven temperature was raised from 55 °C (22 min hold) at 20 ° min^{-1} to 125 °C, at 2 ° min^{-1} to 150 °C, then at 4 ° min^{-1} to 250 °C (10 min isothermal). For MS detection, the positive electron ionization mode at 70 eV was used. Haloanisoles were monitored with selected ion mode. Mass channels were m/z = 195 (197), 167 (169) and **210** (**215**) for TCA (d_5-TCA); m/z = 203, 231, **246** (TeCA) and 301 (303), 329 (331), **344** (**349**) for TBA (d_5-TBA); with 133 ms dwell times (quantifier ions given in bold). Temperatures for ion source and MS transfer line were at 230 °C. MS data acquisition was via Xcalibur software, version 1.2 (ThermoFisher).

2.6. Method validation

Calibration was done with n-hexane solutions containing known amounts of the standards in varying concentrations. With a sample volume of 100 mL used for analysis, a concentration range of 0.1–8.25 ng L^{-1} (TCA, TBA), and 0.1–7.4 ng L^{-1} (TeCA) was

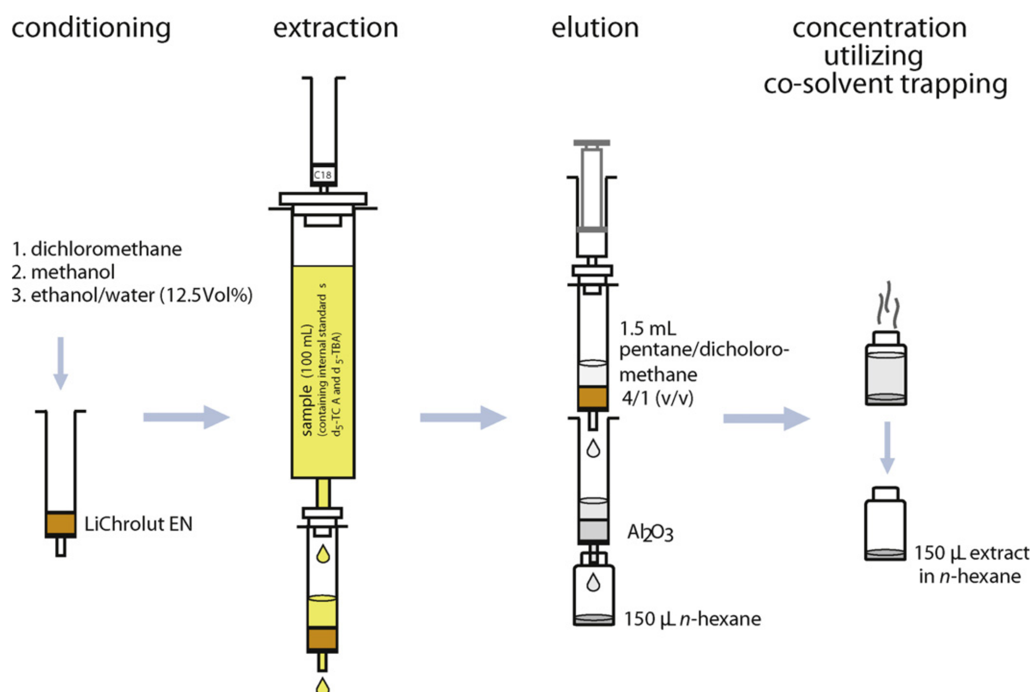


Fig. 2. Sample preparation scheme, including conditioning of LiChrolut EN cartridge, extraction of the sample, elution with a mixture of n-pentane and dichloromethane followed by basic aluminum oxide (Al_2O_3) filltration and concentration utilizing co-solvent trapping with n-hexane.

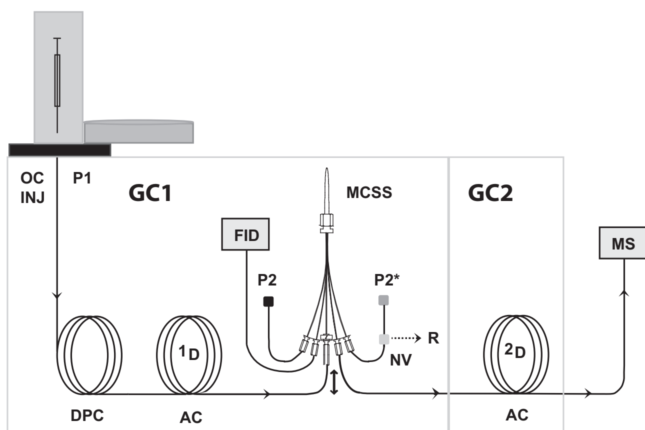


Fig. 3. MDGC system equipped with an autosampler for large volume on-column injection (OC INJ) and a quadrupole mass spectrometer. The 1D capillary system consists of a deactivated pre-column (DPC) and an analytical column (AC), detector for the 1D is a flame ionization detector (FID). Heart-cuts are performed with a moving capillary stream switching device (MCSS), transferring the desired fractions to the 2D analytical column (AC). P1 and P2 (pressure regulators), NV (needle valve), R (restrictor), P2* (manometer); for detailed description see text and elsewhere [46].

calibrated. TCA and TBA were quantified via their isotopologous standards, TeCA was quantified via d_5-TCA.

3. Results and discussion

3.1. HS-SPME–GC-ECD

Applying the earlier established HS-SPME–GC-ECD method [26] to wine matrix failed due to co-elutions with unknown matrix compounds as can be seen in Fig. 1. This problem could not be solved with changing chromatographic parameters or an alternative separation column and was even more severe for including other analytes, such as TeCA or TBA (data not shown).

3.2. SPE using LiChrolut® EN//MDGC–MS

In order to achieve the sensitivity at (sub-)ng L^{-1} level, a large volume injection and SPE eluate concentration had to be applied. This was easily achieved by adding a higher boiling alkane (*n*-hexane) serving as co-solvent for trapping of volatiles [50,51]. However, during earlier steps of method development, the FID chromatograms of the 1D-GC pre-separation were overloaded with matrix compounds and resulted in non-detectable peaks for the haloanisoles in the 2D-chromatogram (data not shown here). Disturbing matrix compounds could finally be eliminated by a kind of a “polarity-filtration”, using basic aluminum oxide as sorbent material. This step compares to the alkaline washing described by Insa et al. [13], as the abundant acidic compounds in the wine aroma matrix can be eliminated. We preferred the additional chromatographic separation with the aluminum oxide. Although it introduces another step and handling with more material, it increases overall separation power. The cartridges are easily packed by hand and can then be re-used. In earlier studies of method development (data not shown) we also compared simple neutralization (pH 8) of the wine with the additional aluminum oxide filtration. Since the basic aluminum oxide filtration gave cleaner extracts, we preferred this version. In summary, the sample preparation based on solid phase extraction and purification, combined with the higher separation efficiency of heart-cut MDGC solved the wine matrix problems. This can be seen with the chromatograms shown in Fig. 4. It is also noteworthy, that with MDGC alone, such clean chromatographic zones in the target compound regions could not be achieved. In particular, the additional filtration through basic Al_2O_3 contributed significantly to the removal of troublesome wine matrix substances.

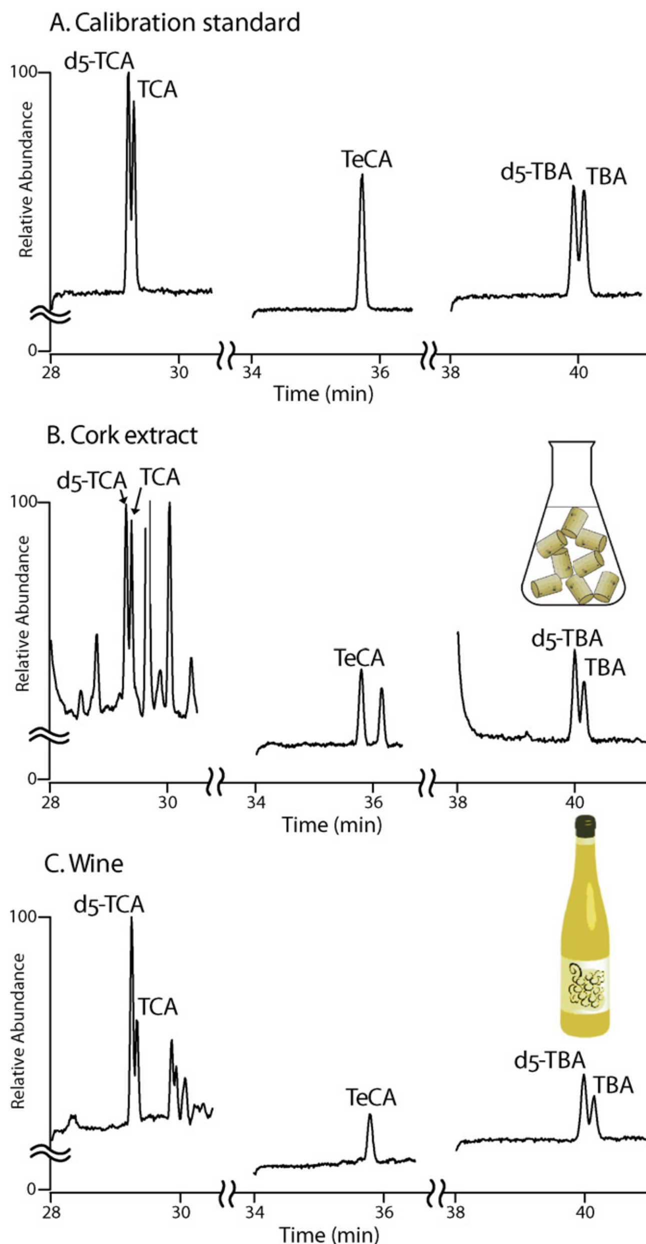


Fig. 4. SPE//MDGC–MS chromatogram (SIM mode) of a calibration standard (A), a cork extract sample (B) and a wine sample (C).

3.3. Method validation

Calibration data for SPE//MDGC is summarized in Table 1. Calibration graphs express good linearity in the targeted concentration

Table 1
Calibration curve. LODs and LOQs for analysis of TCA. TeCA and TBA with SPE//MDGC–MS (8 calibration points; $n = 4$ each; 0.10–8.25 ng L^{-1} (TCA and TBA) and 0.1–7.35 ng L^{-1} (TeCA)).

Haloanisole	Calibration curve	R^2	LOD [ng L^{-1}]	LOQ [ng L^{-1}]
TCA	$y = 0.347x + 0.035$	0.9999	0.05	0.19
TeCA	$y = 0.458x + 0.039$	0.9998	0.06	0.21
TBA	$y = 0.409x + 0.077$	0.9997	0.09	0.34

Table 2
Validation data of SPE//MDGC–MS method for quantification of TCA, TeCA and TBA in a Riesling wine matrix. Validation occurred with spiked wines over a low ng L^{-1} range ($n = 3$).

TCA		TeCA		TBA	
[a]	[b]	[a]	[b]	[a]	[b]
0.6	0.8 ± 0.0	0.5	0.4 ± 0.1	0.6	1.0 ± 0.1
1.1	1.4 ± 0.1	1.0	0.8 ± 0.2	1.1	1.5 ± 0.1
3.3	3.7 ± 0.1	2.9	2.1 ± 0.3	3.3	3.9 ± 0.1
5.5	5.6 ± 0.4	4.9	3.7 ± 0.1	5.5	6.5 ± 0.2

[a] Spiked.
[b] Determined (numbers in ng L^{-1}).

range. Repeatability was determined ($n = 3$) on a wine spiked with low levels of target compounds as 3.7 ± 0.1 ng L^{-1} (TCA), 2.1 ± 0.3 ng L^{-1} (TeCA) and 3.9 ± 0.1 ng L^{-1} (TBA). Recoveries were determined in a white wine free of TCA, TeCA and TBA ($n = 3$). The spiked amount of the target analytes were in the range of their odor thresholds and was at 2 ng L^{-1}. Recoveries obtained were 64 ± 12% (TCA), 47 ± 18% (TeCA) and 63 ± 5% (TBA). In literature, such values are often generated at much higher levels, yielding better numbers, but we preferred to evaluate our method at the relevant concentration level around odor thresholds. Results for method validation with spike experiments in a Riesling wine over the targeted concentration range are listed in Table 2, indicating good suitability of the described method.

3.4. Application of SPE//MDGC–MS method to wine samples

Haloanisole concentrations summarized in Table 3 represent white wine samples which had been chosen due to sensory problems. Samples 1–6 were from a cellar, which had a contamination history with haloanisoles. These samples were analyzed before and after cellar cleansing, venting and exchange of rubber gaskets or polymer stoppers which had been in contact with the wine. It has been shown earlier, that haloanisoles do not exclusively originate from the cork material but may originate from formerly applied wood preservatives (e.g. pentachlorophenol treated panels) or flame retardants (e.g. used for storage/transportation pallets) [52–54]. Under contaminated cellar conditions, the problem of migration of haloanisoles into sorbtive, respectively sorptive materials, such as gaskets or stoppers is critical, as further migration into the wine is possible.

Wine sample 7 represents a case, where the sensory evaluation revealed a musty rather than a classical cork taint. The concentration for the most abundant TeCA is lower than its olfactory threshold of 14–25 ng L^{-1} (determined in a red wine) [40]. However, it has been shown, that wine style (e.g. red or white) possesses a distinctive influence on sensory threshold levels as well

Table 3
Haloanisole concentrations found in white wine samples, which were suspicious for cork taint problems.

Sample	TCA		TeCA [ng L^{-1}]		TBA	
1	0.7[a]	0.4[b]	<LOQ[a]	1	0.7[a]	0.4[b]
2	0.8[a]	0.5[b]	<LOQ[a]	2	0.8[a]	0.5[b]
3	0.6[a]	0.8[b]	<LOD[a]	3	0.6[a]	0.8[b]
4	0.9[a]	0.7[b]	<LOQ[a]	4	0.9[a]	0.7[b]
5	0.9[a]	0.8[b]	<LOQ[a]	5	0.9[a]	0.8[b]
6	0.6[a]	0.6[b]	<LOD[a]	6	0.6[a]	0.6[b]
7[c]	0.6		2.8	7[c]	0.6	
8[d]	5.1		<LOD	8[d]	5.1	

[a] Prior cellar treatment.
[b] After cellar treatment (see text).
[c] Wine had a musty taint.
[d] Wine was rejected by wine control (LWK-RLP [55]).

as consumer rejection thresholds [17–19]. Therefore, the observed sensory defect may still be attributed to the TeCA concentration found, at least together with the additional presence of TCA and TBA (although also below sensory threshold). Often, such compounds contribute synergistically to a sensory defect. An obvious case was found with wine sample 8, which had a distinct cork taint and a TCA concentration above its olfactory threshold value for a white (Riesling) wine [17].

4. Conclusion

A routinely applied method for cork soak analysis, based on 1D HS-SPME–GC-ECD method, failed due to matrix (co-elution) problems of the complex wine matrix. These problems could be solved through the increased separation power found in a combined sample clean-up and MDGC approach. In our case, disturbing matrix compounds were eliminated via an additional clean-up of the primary haloanisole SPE extract, using basic aluminum oxide as an additional step. The desired sensitivity for the haloanisole detection limit of sub-ng L^{-1} was obtained with a large volume injection technique after concentration of the final extract. The increased separation efficiency achieved by heart-cut MDGC and mass spectrometric detection in the selected ion mode allowed reliable quantification of the analytes on the targeted trace level range. The method described shows good repeatability and appropriate recoveries. It allows the analysis of these important off-flavor compounds in the relevant concentration range even below their olfactory threshold levels, which is a pre-requisite for quality control investigations.

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