Optimization of stir bar sorptive extraction applied to the determination of odorous compounds in drinking water

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Abstract The off-flavour compounds 2-methylisoborneol (MIB), geosmin, 2,4,6-trichloroanisole, 2,3,6-
trichloroanisole, 2,3,4-trichloroanisole and 2,4,6-tribromoanisole were analyzed in water samples by Stir Bar Sorptive Extraction (SBSE) followed by on-line thermal desorption (TD)-capillary GC/MS. Quantification was performed using MS in the single ion monitoring mode (SIM) with 2,4,6-trichloroanisol-D$_5$ as internal standard. Quantification limits are 0.1 ng/l to 0.2 ng/l for the haloanisoles, 0.5 ng/l for geosmin and 1 ng/l for MIB. The relative standard deviations at the quantification limit are ranging from 7 to 14.6%. SBSE-recovery was evaluated by spiking real water samples and varied from 87 to 117%. More than twenty samples per day can be analyzed by SBSE-TD-capillary GC-MS. The same technique in combination with olfactometry was used to elucidate unknown odorous compounds in water samples.

Keywords Capillary GC-MS; geosmin; haloanisoles; MIB; off-flavours; olfactometry; stir bar sorptive extraction

Introduction

The majority of complaints received by companies supplying drinking water are most often related to bad odour and taste. The presence in water of solutes creating an unpleasant but otherwise harmless odour or taste, is often linked by the consumer not only to an unacceptable quality but also to an unsafe product. In most of the cases, complaints concern chlorine and earthy/musty smelling compounds. A better understanding of the chemical sources of those off-flavours in drinking water supplies would help in the control of taste and odour problems.

It is now generally accepted that earthy/musty smelling of drinking water is associated with the presence of geosmin, 2-methylisoborneol (MIB) and/or haloanisoles (Jensen et al., 1994; Bruchet, 1999). MIB and geosmin have very strong odours that are detectable at extremely low threshold values. MIB has a woody or camphor odour with a threshold in water ranging from 5 to 10 ng/l while geosmin possesses a characteristic earthy odour detectable in water at a threshold ranging from 1 to 10 ng/l (Hrudey, 1992). Occurrence of these compounds in water has previously been associated with the presence of actinomycetes or their metabolic products (Hoehn, 1988; Suffet et al., 1999) in raw water, as well as with the presence of cyanobacteria and fungi (Dionigi et al., 1992). Haloanisoles have a musty odour with even lower threshold values. For instance, the threshold concentration of 2,4,6-trichloroanisole ranges from 0.05 to 4 ng/l. Haloanisoles are most probably formed by microbiological methylation of halophenols during water treatment or during transport through the distribution system (Nyström et al., 1992; Malleret and Bruchet, 2002). The halophenols can be formed by chlorine disinfection of drinking water and some of them have also been identified as natural halogenation products (Anselme et al., 1985).

The elucidation of these target compounds in water samples has been an analytical challenge for years, because accurate determination at the ppt (ng/l) level is not straightforward. From a chromatographic point of view, capillary gas chromatography (capillary GC) coupled with mass spectrometry (MS) is the only method combining the high separation
Several sample preparation techniques before capillary GC-MS analysis have been described. Most successfully applied until now is the so-called Close Loop Stripping Analysis (CLSA) (Grob and Zürcher, 1976; Krasner et al., 1983; APHA, 1995). With this method the organic substances are released from water in a hermetically closed circuit system by stripping or purging the water by air or an inert gas at 40°C. The liberated substances are transferred to a very small amount of charcoal localised in the closed circuit. Finally, the organic substances are eluted from the charcoal with a small quantity of solvent such as CS₂ and an aliquot is injected into the gas chromatographic system. Open stripping analysis (OSA), an alternative “purge and trap” technique to CLSA has been applied by Bruchet et al. to obtain higher recoveries (Bruchet and Hochereau, 1997). Notwithstanding their features, the “stripping” techniques CLSA and OSA are not efficient enough to enrich quantitatively low volatile and/or polar compounds and still lack sensitivity. Moreover, the application of CSLA and OSA is time consuming and labour intensive. Recently, Romero et al. validated the CLSA method according to EN-45001 and limit of quantification (LOQ) values of 15 ng/l (ppt) (S/N 5) for MIB and geosmin were reported (Romero et al., 2000). Bao et al. (1997) described a liquid–liquid extraction technique in combination with capillary GC-Ion Trap detection.

Soon after its introduction, head space Solid Phase Microextraction (SPME) was applied to the analysis of geosmin and MIB in water samples. Brand used a PDMS (polydimethylsiloxane) fiber and reported detection limits for geosmin and MIB of 100–200 ng/l (Brand, 1995). The method was further refined and Lloyd et al. (1998) reported limits of detection in algal cultures of 10 ng/l. McCallum et al. (1998) evaluated different SPME fibers for the enrichment of geosmin and MIB. A divinylbenzene fiber was giving the highest yield (ca. 85%) for both solutes compared to 50% for PDMS and 80% for Carboxen. The authors also stated that for PDMS, headspace sampling was much more efficient than immersion sampling. The method showed good linearity over the concentration range 5–40 ng/l with LODs of 1 ng/l. The disadvantage is that CI-MS had to be applied for MIB and EI-MS for geosmin.

Stir bar sorptive extraction (SBSE), a recently introduced solventless extraction technique (Baltussen et al., 1999) can be an alternative choice to conventional stripping methods and SPME. The principle of SBSE is similar to SPME on polydimethylsiloxane (PDMS) fibres but the higher sorbent quantity applied results in higher enrichment factors. The principle of SBSE is as follows: a magnetic stirring rod is incorporated into a glass jacket coated with a 0.5 mm layer of polydimethylsiloxane (PDMS). Extraction is performed by placing a suitable sample amount in a vial, adding a PDMS stir bar and stirring for 30 to 120 min. After extraction, the stir bar is introduced in a glass desorption tube, placed in a thermal desorption unit, and thermally desorbed on-line with the capillary GC-MS system. SBSE has been applied by Nakamura et al. (2001) for geosmin and MIB and by Ochiai et al. (2001) for geosmin, MIB and 2,4,6 TCA. Sub-ppt (ng/l) sensitivities were reported with good reproducibilities.

The aim of the present study was to evaluate the possibilities of SBSE to quantify, below or close to their odour threshold values (Table 1), six odorous organic compounds in water considered responsible for the earthy/musty smell. Moreover, SBSE was also combined with olfactometric detection to confirm the smell of the target solutes and/or to elucidate other off-flavour smells.
**Experimental**

**Chemicals and reagents**

Methanol (pesticide grade), acetic acid anhydride and potassium carbonate were obtained from Merck (Darmstadt, Germany). Spring water was used to prepare blanks and standards. 2-methylisoborneol (MIB), 2,4,6-trichloroanisole (246-TCA), 2,3,6-trichloroanisole (236-TCA), 2,3,4-trichloroanisole (234-TCA), 2,4,6-tribromoanisole (246-TBA), geosmin and 2,4,6-trichloroanisole-D5 (246-TCAD5) were obtained from LGC Promochem (Molsheim, France). Stock solutions for MIB, geosmin and haloanisols were prepared in spring water at 1 µg/l and for 246-TCAD5 at 20 µg/l. The stock solutions stored at 4°C are stable for at least one month.

**Extraction procedures**

The compounds were extracted from 100 ml of water sample with PDMS coated stir bars of 20 mm × 0.5 mm (48 µl) of PDMS. The stir bars, called Twisters™, are commercially available from Gerstel GmbH, Mülheim a/d Ruhr, Germany. The water was placed in a 125 ml vial and 5 ml methanol together with 40 µl of the internal standard (246-TCAD5) solution were added. Methanol is added to minimize wall adsorption effects for the target solutes. The samples were stirred for 2 hours at room temperature and at 1,000 rpm. The Twisters were then dried on tissue paper. In some applications i.e. for quantitation of the target solutes, two water samples originating from the same water were SBSE enriched and the two Twisters were placed in a single glass thermal desorption tube.

For *in-situ* acylation of phenolic compounds, 1 g of potassium carbonate and 0.5 ml of acetic acid anhydride were added to the 100 ml water sample and SBSE sampling, as described above, was then performed immediately.

**Instrumental conditions**

The gas chromatograph used is an Agilent 6890-5973 MSD (Agilent Technologies, Palo Alto, CA, USA) -olfactometric detection combination (Gerstel GmbH, Mülheim a/d Ruhr, Germany). The system is equipped with a thermal desorption unit type TDSA (Gerstel) and a PTV injector (CIS-4 from Gerstel). The operating conditions were as follows. Stir bars were thermally desorbed in the splitless mode by programming the TDS from 30°C (0.8 min) at 60°C/min to 280°C (5 min). The desorbed solutes were cryofocused in the PTV at −100°C. After desorption, the PTV injector was programmed to 300°C at 10°C/s and held for 2 min. Injection was done in the solvent vent mode. The carrier gas was helium at 1.5 ml/min constant flow. The compounds were separated on a 30 m × 0.25 mm i.d. × 0.25 µm HP5-MS capillary column (Agilent Technologies). The oven was programmed from 50°C (2 min) to 200°C at 10°C/min then to 300°C at 25°C/min (2 min). MS detection was achieved in the single ion monitoring (SIM) mode for quantitative analysis and in scan mode for qualitative analysis. The olfactometer transfer line was heated at 250°C. The column outlet was split with one third of the effluent to the mass spectrometer and two thirds to the olfactometer.

**Table 1** Target odorous compounds

<table>
<thead>
<tr>
<th>Name</th>
<th>Abbreviation</th>
<th>Odour</th>
<th>Threshold</th>
</tr>
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<tbody>
<tr>
<td>2-methylisoborneol</td>
<td>MIB</td>
<td>Earthy</td>
<td>5–10 ng/l</td>
</tr>
<tr>
<td>2,4,6-trichloroanisole</td>
<td>246TCA</td>
<td>Musty</td>
<td>0.1–2 ng/l</td>
</tr>
<tr>
<td>2,3,6-trichloroanisole</td>
<td>236TCA</td>
<td>Musty</td>
<td>0.1–2 ng/l</td>
</tr>
<tr>
<td>geosmin</td>
<td>geosmin</td>
<td>Camphor</td>
<td>1–10 ng/l</td>
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</tbody>
</table>
Tuning the Mass Spectrometric Detector (MSD)

For qualitative analyses, the MSD was tuned using the autotune macro. Using this macro, the relative abundances for ions 219 and 502 relative to the abundance of ion 69 using PFTBA as calibrant, are typically around 60% and 3%, respectively.

For quantitative analysis of MIB, geosmin and haloanisols, a target tune was used. Ions 69, 219 and 414 were used as target ions. The repeller voltage was set to give an optimum response for ion 219. The target tune resulted in relative abundances of respectively 110% and 10% for ions 219 and 414, relative to ion 69. Figure 1 shows the mass spectra of the target compounds for quantitation. For monitoring ions in the SIM mode, 95, 108 and 110 were chosen for MIB, 112 and 125 for geosmin, 210 and 212 for the three chloroanisoles and, 346 and 344 for 2,4,6-tribromoanisole. The internal standard, 2,4,6-trichloroanisole-D₅, was monitored at 217.

Method validation

The method was validated according to the AFNOR regulation XP T 90-210.

The validation consists in defining:

- the linearity range:
- the limit of quantification (LOQ):
- the repeatability:
- the accuracy:
- the reproducibility:

Results and discussion

Successful analysis of odorous organic compounds in aqueous environmental samples requires high enrichment factors for a broad range of solutes. SPME and SBSE, both using

![Mass spectra of target compounds](https://iwaponline.com/wst/article-pdf/49/9/161/421153/161.pdf)
PDMS coatings are solventless sorptive extraction techniques that offer several features such as predictable recoveries, absence of displacement effects and fast and mild desorption. With PDMS coatings, SPME and SBSE are by nature equilibrium techniques based on the partitioning of the solutes between the PDMS phase and the aqueous (or gas) matrix. In fact, the principle of both techniques is the same as liquid–liquid extraction (LLE) but with a very low quantity of solvent i.e. 0.5 µl of PDMS for SPME with a 100 µm fibre and 48 µl of PDMS for SBSE with a 20 mm × 0.5 mm coating (Baltussen et al., 2002).

The partitioning coefficient between PDMS and water \((K_{PDMS/W})\) can be approximated by the octanol–water partitioning coefficient \((K_{O/W})\) \([19]\). The equilibrium is then depicted by Eq. (1) and the recovery by Eq. (2) where \(m_{PDMS}\) is the quantity sorbed on PDMS, \(m_W\) the quantity non extracted, \(\beta\) the ratio volume of water/volume of PDMS and \(m_0\) the initial quantity.

\[
K_{O/W} = K_{PDMS/W} = \frac{C_{PDMS}}{C_W} = \frac{m_{PDMS}}{m_W} \times \frac{V_W}{V_{PDMS}} = \frac{m_{PDMS}}{m_W} \times \beta
\]

(1)

\[
R = \frac{m_{PDMS}}{m_0} = \left(\frac{K_{O/W}}{\beta}\right) \left(1 + \frac{K_{O/W}}{\beta}\right)
\]

(2)

The ratio of the distribution coefficient and the phase ratio are controlling the extraction recovery. In SPME, the maximum volume of PDMS coated on the fibre (100 µm) is 0.5 µl. For a sample volume of 10 ml, the phase ratio equals \(2 \times 10^4\). This implies that quantitative extraction is only obtained for compounds with a \(\log K_{O/W}\) larger than 5. Only a limited number of components exhibit such high \(K_{O/W}\) values. In SBSE, on the other hand, the situation is more favourable. A stir bar coated with 48 µl of PDMS gives a \(\beta\) factor of ca. 200 which implies that solutes with \(\log K_{O/W}\) in excess of 4 are quantitatively extracted into the PDMS coated stir bar. This ensures high sensitivity. Figure 2 compares theoretical curves for SPME with a 0.5 µl fibre for 10 and 100 ml water samples versus SBSE with a 48 µl coating for 10, 100 and 200 ml water samples.

The \(\log K_{O/W}\) of the target solutes were calculated with the SRC-KOWWIN software package (Syracuse Research, Syracuse, NY, USA) according to a fragment constant estimation methodology \([23]\) and are 2.85 for MIB, 3.57 for geosmin, 4.01 for the trichloroanisoles and 4.75 for tribromoanisole. For a compound with \(\log K_{O/W}\), 3 and present at the 1 ng/l level in water, the absolute theoretical recoveries at equilibrium are: for SPME 0.5 pg for a 10 ml sample and <0.1 pg for a 100 ml sample and for SBSE 8.3 pg for a 10 ml sample, 32 pg for a 100 ml sample and 38 pg for a 200 ml sample. A 100 ml sample size was therefore selected for further experiments. For some experiments two twisters

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**Figure 2** Theoretical SPME recovery curves and experimental results with SBSE
placed in two 100 ml aliquots of the same water sample were used to increase detectability (64 pg versus 38 pg for 200 ml sample directly). Experimental enrichment curves as a function of the water volume were made and, as an illustration, Figure 3 shows the quantity sorbed on the 48 µl stir bar for spring water volumes between 10 to 200 ml spiked with 1 ng of MIB and extraction for 120 min (see further). The experimental results are in accordance with theory. The extracted amount increases up to 100 ml of the sample volume and then levels off.

Until now, the discussion has been limited to equilibrium conditions. In SBSE the thickness of the coating (0.5 mm) strongly influences the speed of extraction and thus the required equilibration time. According to equation 3 (Pawliszyn, 1997).

\[ t_{95\%} = \frac{d_{PDMS}^2}{2D_{PDMS}} \]  

in which \( t_{95\%} \) is the time required to reach 95% extraction, \( d_{PDMS} \) is the thickness of the PDMS layer used and \( D_{PDMS} \) is the diffusion coefficient of the analyte under investigation in PDMS, the time for equilibrium is ca. 25 times longer for SBSE than for SPME. In practice, however, full equilibration is not essential for accurate and sensitive determination as long as the equilibration-time curves are rising rapidly.

The kinetics of sorption for the target solutes were evaluated by analyzing 100 ml spring water samples spiked with 2 ng/l of each compound in a time frame ranging from 15 min to 300 min. Figure 4 shows the relationship between the extraction time and the response obtained for the target compounds.

For all solutes, the sorption kinetics are fast up to 120 min and are then slowing down without reaching a plateau. For routine analysis 120 min was empirically chosen. Figure 5 shows a SIM chromatogram at the 2 ng/l level for a 100 ml water sample, enriched with one Twister of 20 mm \( \times \) 0.5 mm PDMS at room temperature and 120 min stirring at 1,000 rpm.

To reach even higher detectability, two Twisters originating from two SBSE extractions of twice 100 ml aliquots of the same water sample were placed in the thermal desorption tube for quantitative analysis. The results of the validation study for these experiments are summarised in Table 2 and calibration curves are shown in Figure 6. The AFNOR regulation XP T 90-210 validation criteria were met for all target compounds.

The total analysis time per sample is ca. 2.30 h. Due to the fact that several Twister enrichments can be performed simultaneously the throughput is ca. 20 samples per day. Note that Twister enrichment is time consuming but not labour intensive. The analysis of off-flavours in water samples is, however, not required every day and it was interesting to evaluate whether sampled stir bars could be stored for batch analysis at a later time. This
Figure 4 Relationships between extraction times and responses of target compounds

Figure 5 SIM chromatogram at 2ng/l level for 100 ml water sample

Figure 6 Calibration curves for SBSE extractions of target compounds
was evaluated by extracting seven samples originating from the same spring water spiked with 2 ng/l of each compound by SBSE. One twister was analyzed immediately and the others, stored at 4°C in closed vials, were analyzed on the following days. Figure 7 shows the influence of storage on the response for all compounds. The results show that no loss of compounds occurs during one week of storage. This is important because enrichment can be done immediately after collection of the water sample so that wall adsorption effects are minimized. This also opens perspectives for in field sampling.

Different water samples were analyzed following complaints for taste and odour problems. Three specific cases are presented to illustrate the possibilities of the described methodologies.

**Case 1**
A sample collected at a consumer’s home gave a very pronounced musty odour combined with hospital-like and solvent odours. The sample was in the first instance analyzed by SBSE-TD-capillary GC/MS in the SIM mode to quantify MIB, geosmin and haloanisols. The quantitative results and chromatogram are shown in Table 3 and in Figure 8.

The concentration levels of 246-TCA, geosmin and 246-TBA explain the musty odour. In order to elucidate other smelling compounds, the experiment was repeated with olfactometric evaluation and, the musty odours at the retention times of 246-TCA, geosmin and 246-TBA were confirmed. The hospital-like smell and the solvent smell were elucidated around 8.4 and 13.9, respectively (Figure 9). Based on the mass spectral data and isotope ratios, the hospital-like smell corresponds to dibromoiodobutane (8.4 min) and the solvent smell to tetrachlorobenzene (13.9 min).

**Conclusion**
SBSE enrichment combined with thermal desorption-capillary GC/MS/olfactometry is a powerful technique to detect off-flavours in water samples. The well-known off-flavours 2-methylisoborneol (MIB), geosmin, 2,4,6-trichloroanisole, 2,3,6-trichloroanisole,
2,3,4-trichloroanisole and 2,4,6-tribromoanisole can be quantified in the SIM mode with 2,4,6-trichloroanisol-D$_5$ as internal standard at their organoleptic threshold values. For those solutes the described method has been validated according to the AFNOR standard XP T 90-210. Although SBSE enrichment takes 120 min, several samples can be prepared simultaneously in a simple and non labour intensive manner resulting in a throughput of ca. 20 samples per day. Moreover, stir bars can be stored after sampling allowing batch analysis or in-field sampling. The use of olfactometric detection offers opportunities to elucidate other off-flavour solutes that can be identified by operating the MS in the scan mode.

**Figure 8** Scan chromatogram for unknown off-flavours in Case 1

**Table 3** Concentration of target compounds in sample Case 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>[C] (ng/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-methylisoborneol</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>2,4,6-trichloroanisole</td>
<td>8.9</td>
</tr>
<tr>
<td>2,3,6-trichloroanisole</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>geosmin</td>
<td>5.2</td>
</tr>
<tr>
<td>2,3,4-trichloroanisole</td>
<td>&lt; 0.2</td>
</tr>
<tr>
<td>2,4,6-tribromoanisole</td>
<td>0.4</td>
</tr>
</tbody>
</table>

**Figure 9** SIM chromatogram of chlorinated and filtered water for Case 2

2,3,4-trichloroanisole and 2,4,6-tribromoanisole can be quantified in the SIM mode with 2,4,6-trichloroanisol-D$_5$ as internal standard at their organoleptic threshold values. For those solutes the described method has been validated according to the AFNOR standard XP T 90-210. Although SBSE enrichment takes 120 min, several samples can be prepared simultaneously in a simple and non labour intensive manner resulting in a throughput of ca. 20 samples per day. Moreover, stir bars can be stored after sampling allowing batch analysis or in-field sampling. The use of olfactometric detection offers opportunities to elucidate other off-flavour solutes that can be identified by operating the MS in the scan mode.
References


