

In-Tube Extraction Dynamic Headspace (ITEX-DHS) sampling technique coupled to GC-MS for sensitive determination of odorants in water

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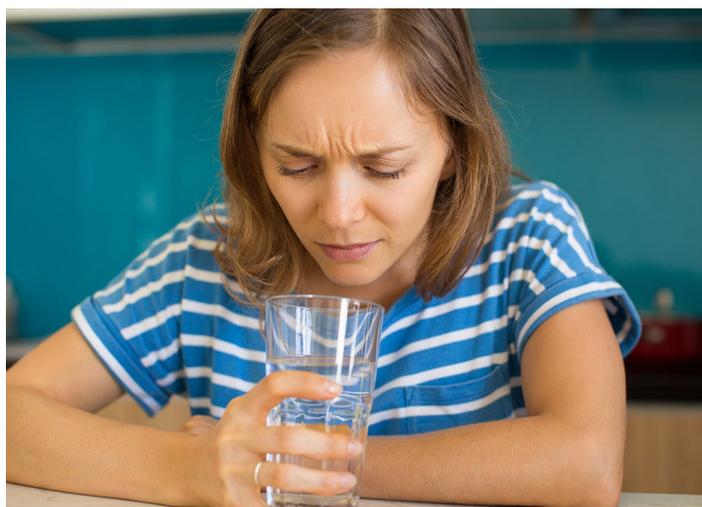
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Goal

This study aims to demonstrate the applicability of the In-Tube Extraction Dynamic Headspace (ITEX-DHS) technique coupled to gas chromatography-mass spectrometry (GC-MS) for the determination of trace-level odorants in water. Through dynamic headspace enrichment achieved by means of the ITEX technique, the target analytes isoborneol (IB), 2-methyl-isoborneol (2-MIB), 2,4,6-trichloroanisole (2,4,6-TCA), and geosmin were extracted and pre-concentrated to reach sub-ppt level limits of detection (LOD).

Introduction

The presence of malodorous compounds can impart an unpleasant smell to drinking water, negatively affecting consumer experience and generating complaints to water companies. Volatile organic compounds (VOCs) isoborneol, 2-methyl-isoborneol (2-MIB), 2,4,6-trichloroanisole (2,4,6-TCA), and geosmin (Figure 1) carry disagreeable earthy and musty olfactory notes. These malodors are perceived by the human nose at concentrations of a few



parts per trillion (ppt), whereas international standards ISO 17943¹ and GB 5749² request method detection limits of 10 ppt for 2-MIB and geosmin.

To reach such low detection levels, several approaches to extract and enrich the analytes prior GC-MS analysis have been published.³ Solventless extraction techniques such as Solid-Phase Microextraction (SPME) and Purge and Trap are currently successfully applied in routine water testing labs mainly due to the extraction efficiency, high level of automation, and extraction time compatible with routine sample throughput. In particular, the recent development of the SPME Arrow,⁴ capable of higher sorption volume and higher fiber robustness compared to classical SPME, offers a relatively inexpensive, more sensitive, and more reliable solution for odor compounds detection in water.

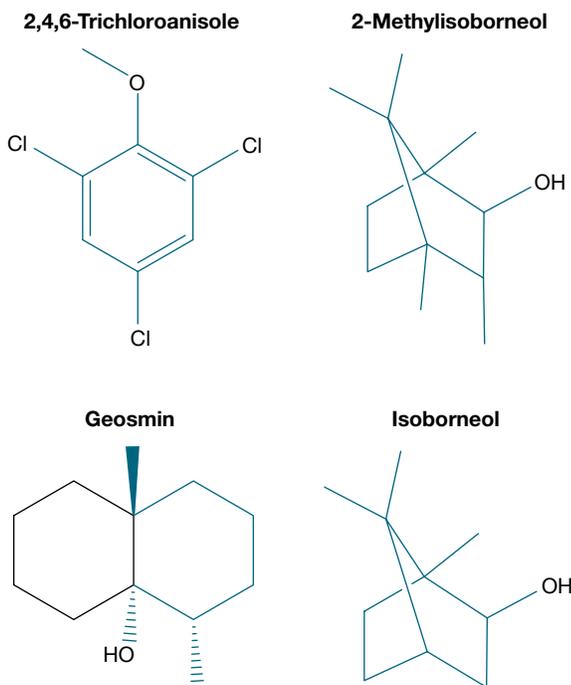


Figure 1. Compound structures of the target odorants

In this study, a different approach based on the In-Tube Extraction Dynamic Headspace (ITEX-DHS) sampling technique⁵ is used for trace determination of four target odor compounds in water samples. ITEX is a syringe-based headspace enrichment technique capable of offering advantages in terms of robustness and ease of use, combined with high sorbent capacity suitable for achieving sensitivity at ppt levels. Rapid and efficient enrichment and sample introduction, along with fast chromatography and sensitive mass spectrometric detection, made possible the determination of the target analytes at concentrations below their perception levels in water and with excellent linearity over a wide range of concentrations.

Experimental

Chromatographic separation and mass spectrometric detection were achieved with the Thermo Scientific™ ISQ™ 7000 GC-MS system. The Thermo Scientific™ TriPlus™ RSH robotic autosampler was equipped with the ITEX-DHS syringe tool for sample extraction and injection in an automated seamless workflow.

The ITEX technique uses a gas-tight syringe to collect the headspace and a micro trap positioned inside the syringe needle, filled with adsorbent material to efficiently trap and concentrate the volatile compounds (Figure 2). The collection of the headspace through repeated strokes of the syringe allows for a scalable sensitivity level according to the number of strokes, making this approach versatile towards low and high concentration samples. After the enrichment step, the ITEX tool moves to the GC injector and the micro trap is directly heated for desorption, releasing the compounds into the inlet very rapidly, and transferring the sample in a very narrow band so to preserve the column efficiency (Figure 3).

The simplicity of the hardware allows for easy access to the trap and the syringe for a straightforward trap replacement. This syringe-based approach does not add additional hardware to the system like transfer lines or switching valves, assuring higher robustness and ease of use. Moreover, it allows access to the injector and sharing it with other injection techniques.

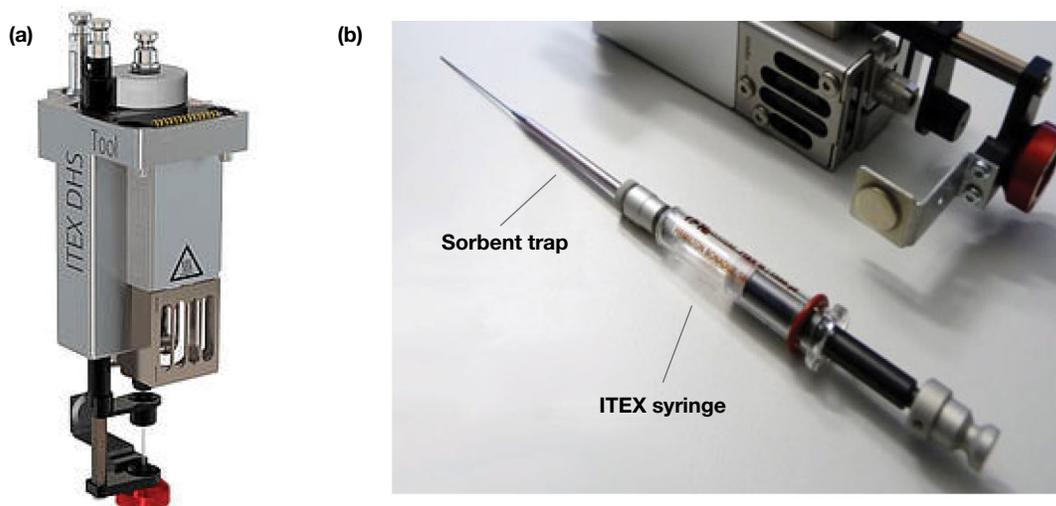


Figure 2. ITEX-DHS tool for the TriPlus RSH autosampler (a) and ITEX syringe (b)

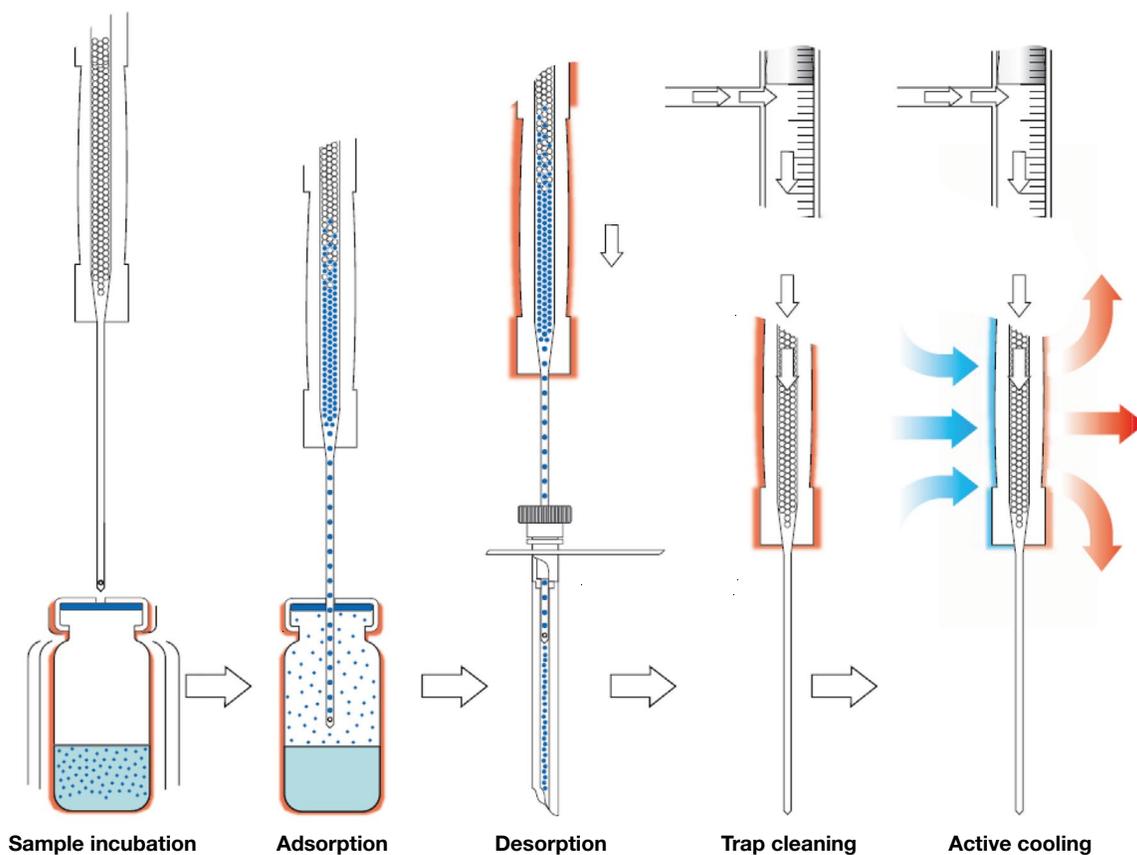


Figure 3. Schematic of the ITEX-DHS sample extraction and desorption process

Materials

Powder standards of 2,4,6-trichloroanisole (P/N 235393) and isoborneol (P/N I13901) with a purity of $\geq 95\%$ and a certified reference material solution containing geosmin and 2-methylisoborneol (P/N CRM47525) at 100 $\mu\text{g}/\text{mL}$ in methanol were purchased from Sigma-Aldrich. Additional volatile organic compounds were considered in the study for trapping comparison: standard p-xylene (P/N 317195), 1-chloroheptane (P/N 109746), (R)-(+)-limonene (P/N 183164), nitrobenzene (P/N N10950), ethyl caprylate (P/N 112321), methyl jasmonate (P/N 392707), and tridecane (P/N T57401) with a purity $\geq 95\%$, all purchased from Sigma-Aldrich. Methanol was purchased from Fisher Scientific. Ultrapure water was obtained from an ELGA water purification system. Tap water samples have been collected from three different locations and analyzed against the standard solutions.

Standards preparation

Stock standard solutions were prepared at 1000 mg/L for 2,4,6-trichloroanisole and isoborneol in methanol. An intermediate stock solution containing all four compounds was prepared at 1 mg/L in methanol, which was further diluted to the range of 1–100 ng/L with water for the calibration curve setup. Stock solutions of additional VOC standard were prepared at 1000 mg/L in methanol and then diluted to 50 ng/L with water.

A volume of 1 mL of standard in water was put into a 20 mL vial for sampling (Thermo Scientific™ Vials, P/N 20-CV, and Caps, P/N 20-MCB-ST3).

Instrument and method setup

ITEX-DHS conditions

Dynamic headspace extraction and enrichment were performed using the TriPlus RSH autosampler equipped with a vial incubation oven and agitator, and the ITEX-DHS sampling tool. Optimized autosampler parameters are shown in Table 1.

GC-MS conditions

Gas chromatography (GC) analysis was performed using a Thermo Scientific™ TRACE™ 1300 GC with a split/splitless injector equipped with a split/splitless single taper liner (1 mm ID, P/N 453A1335) and operating in splitless mode. Chromatographic separation was obtained using a Thermo Scientific™ TraceGOLD™ TG-5MS column 30 m x 0.25 mm, 0.25 µm (P/N 26098-1420). MS analysis was performed on a Thermo Scientific™ ISQ™ 7000 Single Quadrupole equipped with an Extractabrite electron impact (EI) ionization source. GC-MS parameters are listed in Table 2.

Data acquisition and processing

Data acquisition and processing were performed using Thermo Scientific™ Chromeleon™ CDS 7.2 software, offering the full instrument control and interactive, customizable reporting capability.

Results and discussion

Method development

To optimize the extraction and recovery of the target compounds, key parameters were assessed including trap material, number of extraction strokes, and the extraction and desorption parameters such as temperatures and flows. The optimization of the extraction strokes was driven by the international standards ISO 17943 and GB 5749 as a balance between sensitivity and analysis time. For the analysis of a standard mixture in water of the four target compounds at 100 ng/L, the trap material was saturated after about 30 strokes, and further increasing the number of strokes did not improve the detection of the compounds (Figure 4). To ensure satisfactory recovery in as few extractions as possible, potentially limiting the process to ten strokes, further optimization of the parameters was required. A low trap temperature during extraction and a low extraction flow rate were applied, allowing a sufficient interaction of the compounds with the trap material and their adsorption within the trap.

Table 1. ITEX-DHS sampling parameters

| Parameter | Setting |
|-------------------------------|--|
| Vial size | 20 mL |
| Sample volume in vial | 1 mL |
| ITEX-DHS trap | Tenax® GR: Carbosieve SIII (bottom:top 1:1) (P/N 1R77010-1148) |
| Incubation time | 10 min |
| Incubation temperature | 80 °C |
| Agitator speed | 250 rpm |
| Trap pre-cleaning temperature | 250 °C |
| Trap pre-cleaning time | 20 s |
| Extraction strokes | 10 |
| Trap extraction temperature | 50 °C |
| Syringe temperature | 50 °C |
| Extraction volume | 1000 µL |
| Extraction aspirate flow rate | 50 µL/s |
| Extraction dispense flow rate | 500 µL/s |
| Sample prefill ratio | 40% |
| Water removal | Enabled |
| Desorb temperature | 150 °C |
| Injector aspirate flow rate | 100 µL/s |
| Post injection delay | 20s |
| Desorb flow rate | 500 µL/s |
| Sample volume | 1000 µL |
| Injector penetration speed | 50 mm/s |
| Trap post cleaning time | 60 s |

Table 2. GC-MS parameter settings

| Parameter | Setting |
|---------------------------------|---|
| Inlet | Split/splitless |
| Inlet temperature | 250 °C |
| Inlet mode | Splitless |
| Splitless time | 0.5 min |
| Split flow | 10 mL/min |
| Carrier gas | Helium (99.999% purity) |
| Carrier gas flow rate | 1.0 mL/min |
| Oven temperature program | 60 °C (0 min), 25 °C/min to 120 °C (1 min), 10 °C/min to 165 °C (1 min) |
| GC run time | 9 min |
| MS transfer line temperature | 300 °C |
| Ionization mode | EI, 70 eV |
| MS acquisition mode, <i>m/z</i> | SIM, @ 2 min 95, 107, 110, 154, 168 SIM, @ 6.50 min 112, 149, 167, 182, 195, 210 |
| Ion source temperature | 300 °C |

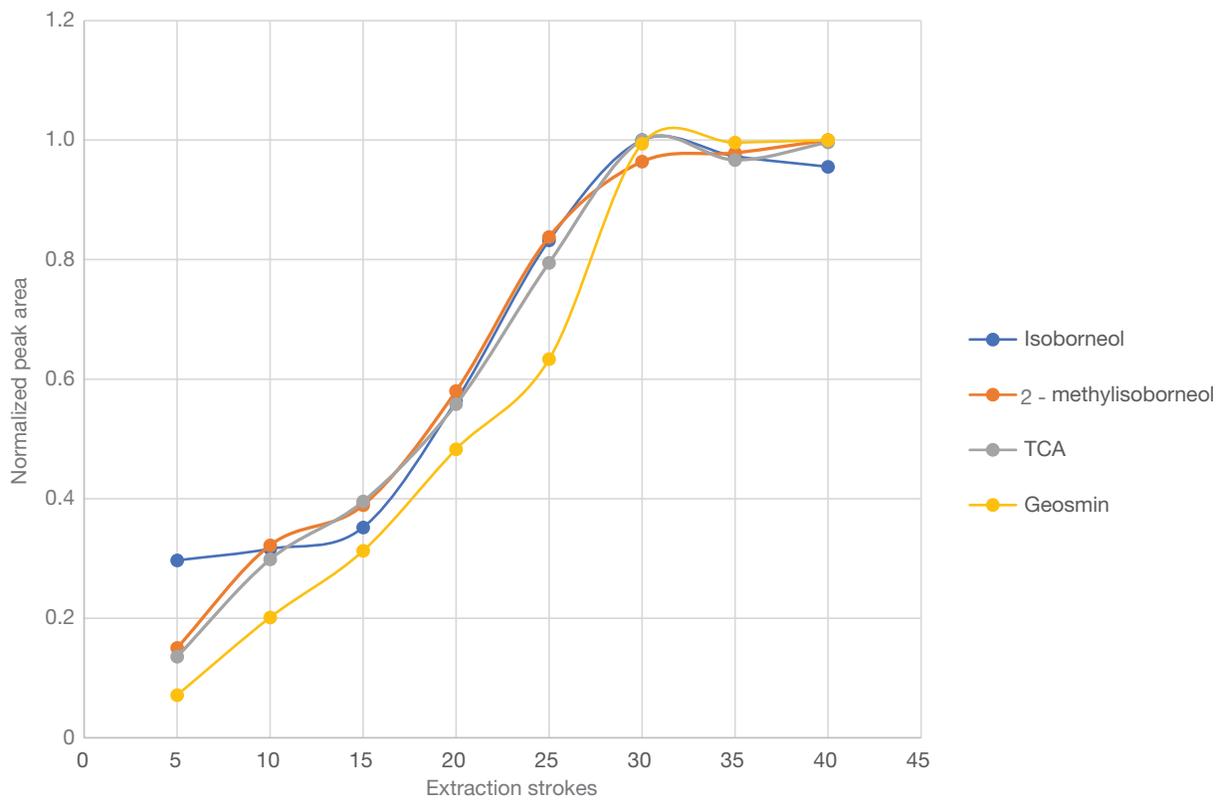


Figure 4. Effects of extraction strokes on peak areas for the target odorants

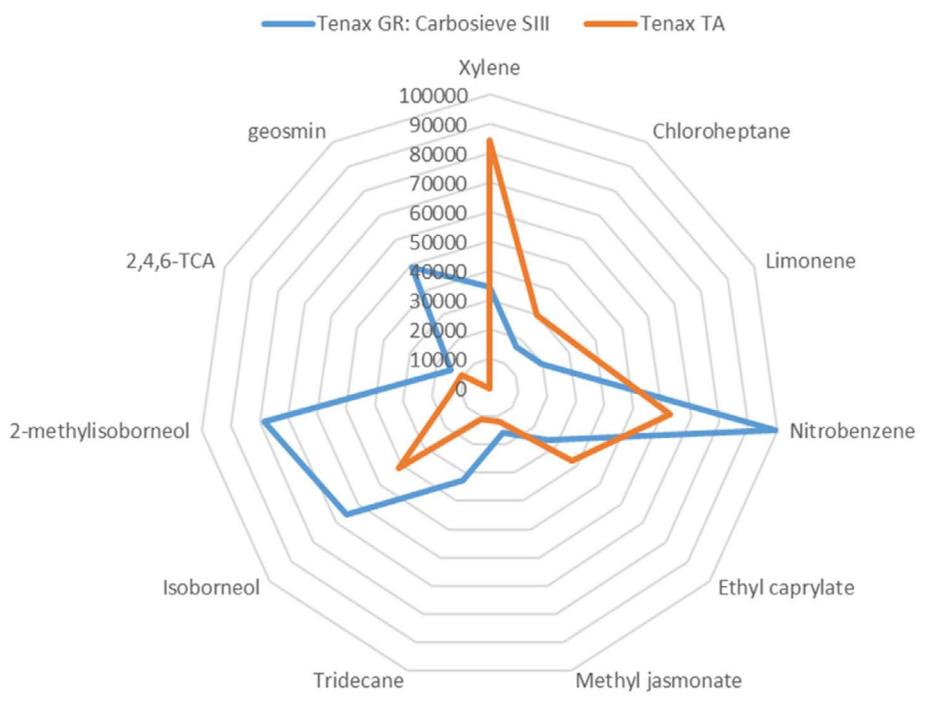


Figure 5. Comparison of Tenax TA and combined Tenax GR:Carbosieve SIII traps

To remove possible amounts of water that may accumulate on the trap during the extraction, the water removal step was enabled. In contrast, a high desorption temperature and speed were used to ensure rapid desorption and prevention of peak broadening. Unlike the SPME technique where the desorption step is achieved into the injector, the ITEX trap desorption temperature and flow can be optimized independently from the inlet settings, to achieve a highly efficient desorption process. The use of a high desorption flow rate combined with a SSL liner with a small inner diameter, allowed the injection in splitless mode for maximum sensitivity without compromises on peak shape, as shown in the chromatograms reported in Figures 7 and 8. The selection of trap material was based on the paper by Jens Laaks *et al.*,⁶ which indicated that a combination of Tenax GR and Carbosieve SIII gave the best selectivity for the compounds of interest. The Tenax GR:Carbosieve SIII trap was evaluated against the standard Tenax TA trap for the four compounds plus a selection of other volatile organic compounds (Figure 5). The mixed-sorbent trap was confirmed to give a higher response for the four compounds of interest, although it is not optimal for xylene, chloroheptane, and limonene.

A general approach for the method optimization is possible maintaining a low extraction temperature and flow rate combined with a high desorption temperature and speed. Incubation temperature and time are sample dependent while the equilibrium conditions are continuously shifted in favor of the gas phase at every stroke. The only trade-off is between the number of syringe strokes for the required sensitivity and the overall cycle time. In this case the extraction time plus the GC separation required a cycle time per sample of 12 min.

Method performance

The method was tested for linearity, repeatability, limit of detection and quantification, and carryover. Excellent linearity with $r^2 \geq 0.999$ and AvCF %RSD between 2.9% and 11.1%, was obtained for all four odorant compounds across the range of 1–100 ng/L for geosmin and 2-methylisoborneol and 5–100 ng/L for 2,4,6-trichloroanisole and isoborneol, applying 10 extraction strokes. The linear plots are shown in Figure 6.

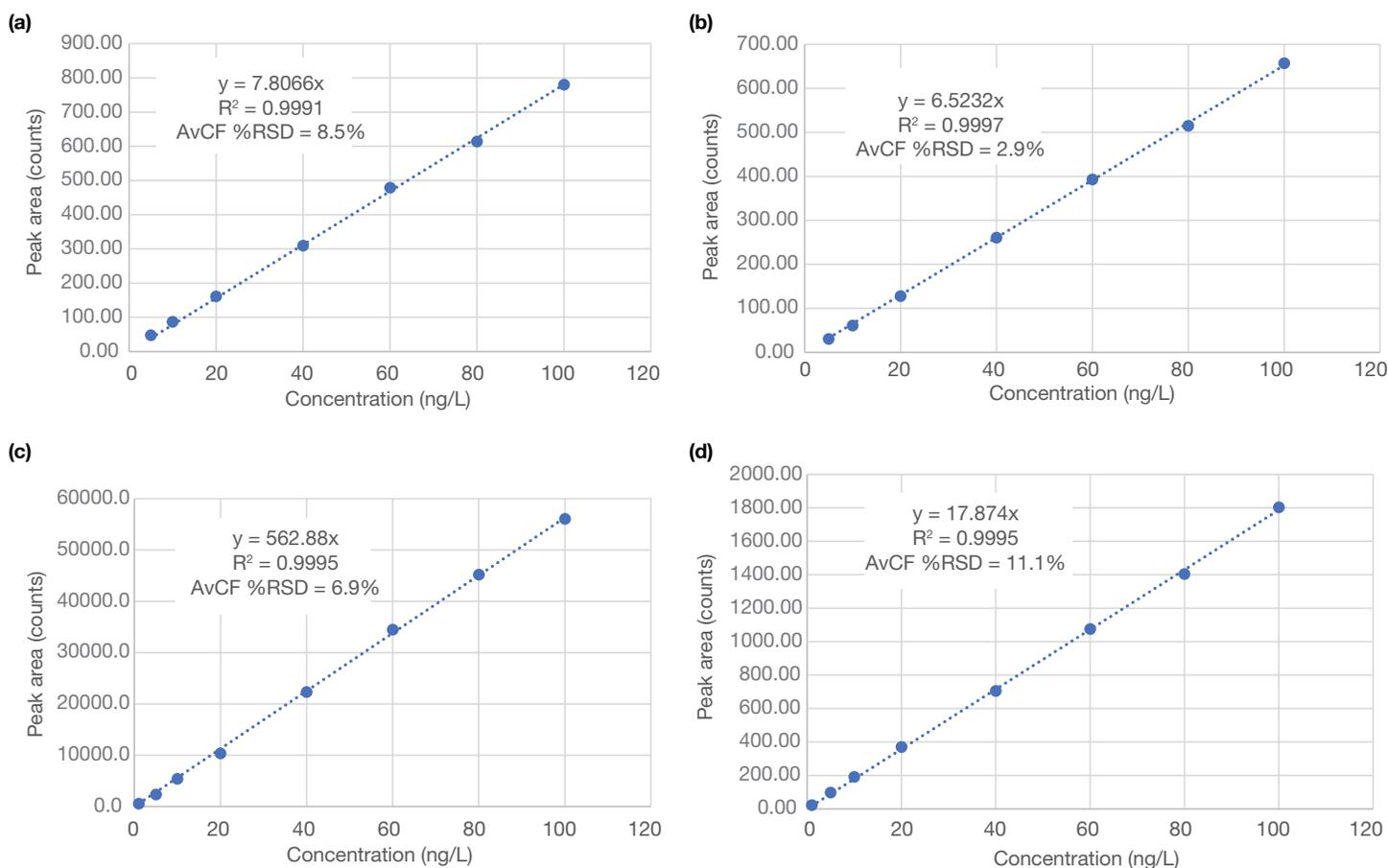


Figure 6. Calibration curves assessed over a concentration range of 5–100 ng/L (a–b) and 1–100 ng/L (c–d) for a) isoborneol, b) 2,4,6-trichloroanisole, c) 2-methylisoborneol, and d) geosmin in water by ITEX-DHS-GC-MS

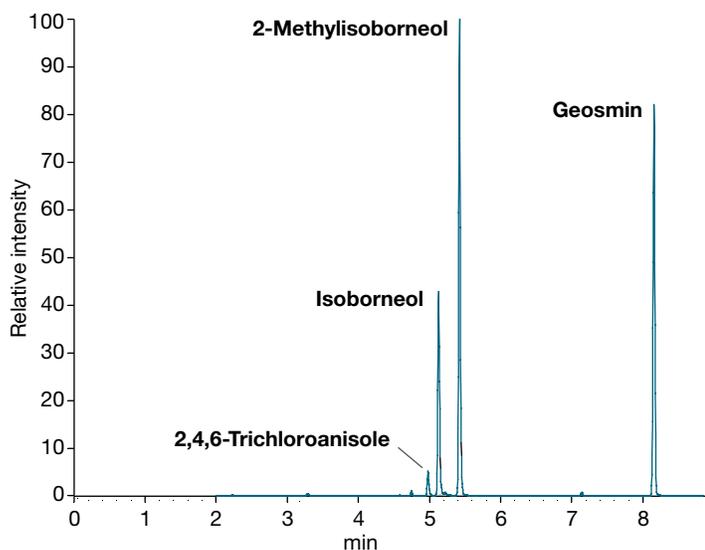


Figure 7. SIM chromatogram of 5 ng/L standard solution in water

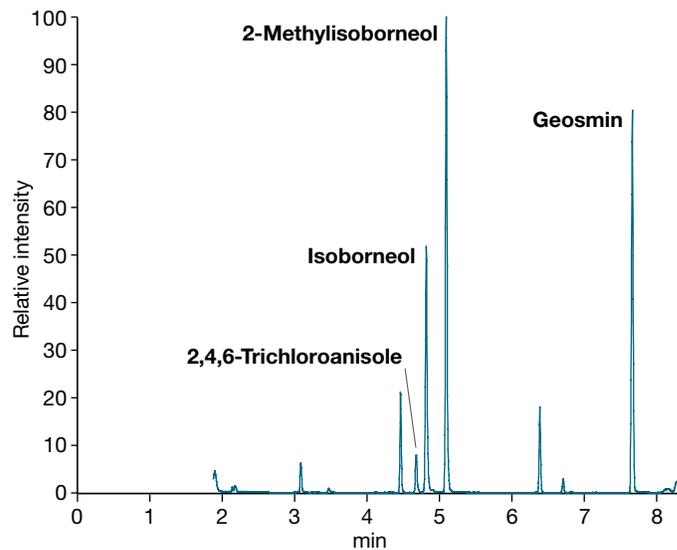


Figure 8. SIM chromatogram of water sample 3

Repeatability testing was performed with three replicates at three points within the calibration range: the lowest, highest, and a mid-range standard. Method detection limits (MDL) were calculated by spiking standards at 10 ng/L, using the following equation (Table 3):

$$MDL = t \times S$$

Where t is the single tailed t -value and S is the standard deviation of the measured concentrations over seven repetitions.

Carryover was assessed by injecting $n = 3$ standard solutions at a concentration of 100 ng/L followed by water blanks. Carryover was not observed in the blank injections.

Quantification of odor compounds in real samples

Tap drinking water samples collected from three different locations were analyzed for the presence of the target odorant compounds. Direct analysis of the water was performed with no pretreatment by pipetting 1 mL of water sample into a 20 mL headspace vial. Each sample was prepared in triplicate. The method allowed the detection of the presence of all four compounds in all the water samples (Figure 8) with very good repeatability ($RSD\% < 2.5$, $n=3$). The quantitation of target odor compounds was achieved against the calibration curves (Table 4). Target ion ratios are reported in Table 5, showing a deviation from the calibration standard within $\pm 20\%$, in compliance with EPA 6040 guidelines.⁷

Table 3. Method performance results

| Compound | Repeatability (RSD%) | | | Method detection limit (ng/L) |
|------------------------|----------------------|---------------|-----------------|-------------------------------|
| | Low (1 or 5 ng/L) | Mid (50 ng/L) | High (100 ng/L) | |
| Isoborneol | 4.0 | 2.3 | 2.8 | 1.0 |
| 2-Methylisoborneol | 3.7 | 3.0 | 3.2 | 0.008 |
| 2,4,6-Trichloroanisole | 3.7 | 3.8 | 3.4 | 1.1 |
| Geosmin | 1.2 | 2.0 | 1.6 | 0.10 |

Table 4. ITEX-DHS-GC-MS analysis of water samples

| | Isoborneol | | 2-Methylisoborneol | | 2,4,6-Trichloroanisole | | Geosmin | |
|----------|--------------|------|--------------------|------|------------------------|------|--------------|------|
| | Conc. (ng/L) | %RSD | Conc. (ng/L) | %RSD | Conc. (ng/L) | %RSD | Conc. (ng/L) | %RSD |
| Sample 1 | 16.5 | 0.3 | 2.17 | 2.1 | 23.6 | 1.4 | 2.09 | 1.0 |
| Sample 2 | 15.5 | 1.8 | 2.19 | 1.9 | 12.2 | 0.8 | 4.80 | 0.7 |
| Sample 3 | 3.78 | 1.4 | 3.12 | 1.5 | 8.27 | 2.1 | 6.15 | 2.4 |

Table 5. Intensity ratio of the qualifier and quantifier ions of the target analytes in the calibration standard and the samples. The intensity ratio in the samples are within $\pm 20\%$ of those in the standard, as required by the EPA 6040 guidelines.

| | | | Standard | Sample 1 | | Sample 2 | | Sample 3 | |
|------------------------|----------------------|---------------------|--------------------------|--------------------------|-------------|--------------------------|-------------|--------------------------|-------------|
| | Quantifier ion (m/z) | Qualifier ion (m/z) | Relative abundance ratio | Relative abundance ratio | Delta (%) | Relative abundance ratio | Delta (%) | Relative abundance ratio | Delta (%) |
| Isoborneol | 95 | 110 154 | 5.26 50.0 | 5.55 50.0 | 5.5 0.0 | 5.88 50.0 | 11.8 0.0 | 5.88 50.0 | 11.8 0.0 |
| 2-Methylisoborneol | 95 | 107 168 | 4.00 25.0 | 4.00 25.0 | 0.0 0.0 | 4.35 25.0 | 8.7 0.0 | 4.17 25.0 | 4.2 0.0 |
| 2,4,6-Trichloroanisole | 195 | 210 167 | 1.51 1.61 | 1.47 1.59 | 2.6 1.2 | 1.56 1.64 | 3.3 1.8 | 1.51 1.66 | 0.0 3.1 |
| Geosmin | 112 | 149 182 | 9.09 9.09 | 8.33 10.0 | 8.4 10.0 | 8.33 10.0 | 8.4 10.0 | 8.33 9.09 | 8.4 0.0 |

Conclusions

A sensitive method was developed for the analysis of four odorants commonly found in water: isoborneol, 2-methylisoborneol, 2,4,6-trichloroanisole, and geosmin. Key parameters for the optimization of ITEX-DHS were identified, namely extraction strokes, extraction trap temperature, volume and speed, and desorption trap temperature and speed. The sensitivity obtained exceeds the requirement of 10 ng/L of the international standards ISO 17943 and GB 5749, maintaining a limited cycle time of 12 min. The following conclusions can therefore be stated:

- The ITEX method can be considered a very suitable alternative to SPME and P&T for the direct analysis of water samples, providing high robustness, long trap lifetime, and high sensitivity.
- The TriPlus RSH autosampler with the ITEX-DHS tool allows for a fully automated sampling procedure with no sample pre-treatment required, reducing the overall analysis time and minimizing manual operations.

- Chromeleon CDS ensures a full control of all the ITEX parameters as well as of the GC-MS system and streamlines the entire workflow thanks to the simplified reprocessing and reporting features.

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