Method for trihalomethane analysis in drinking water by solid-phase microextraction with gas chromatography and mass spectrometry detection
S. Valencia, J. Marín and G. Restrepo

ABSTRACT
Headspace solid-phase microextraction coupled with gas chromatography and mass spectrometry detection (HS/SPME-GC/MSD) was optimized for trihalomethane (THM) determination in drinking water. A 75 μm fiber coated with carboxen/polydimethylsiloxane (75-CAR.PDMS) was used. Experimental parameters such as sample volume, time and temperature of extraction, time and temperature of desorption, magnetic stirring, and addition of sodium chloride (NaCl) were studied to determine their influence on THM extraction. Furthermore, analytical parameters such as linearity and detection limit were also evaluated. High sensitivity with detection limits in the μg/L range as well as a good linearity and reproducibility were achieved. Therefore, the HS/SPME-GC/MSD method with 75-CAR.PDMS is suitable for monitoring THMs in drinking water.

Key words | gas chromatography, mass spectrometry detector, solid-phase microextraction (SPME), trihalomethanes

INTRODUCTION
Natural organic matter (NOM) is a complex mixture of organic compounds, ranging from largely aliphatic compounds to highly colored aromatic molecules. It is formed by the decomposition of plants and animals in the environment (Matilainen et al. 2011; Uyguner-Demirel & Bekbolet 2011). Furthermore, NOM is the major source of organic precursors for disinfection byproducts (DBPs) in chlorinated or chloraminated drinking water (Hua & Reckhow 2007; Valencia et al. 2012). Approximately 600 DBPs have been identified, and some are highly toxic compounds such as those containing iodine and bromine (Krasner et al. 2006; Deborde & Gunten 2008). Among them, 11 are regulated by the US Environmental Protection Agency (EPA), including trihalomethanes (THMs) (DeMarini 2011). THMs are the major DBPs in drinking water (Matilainen & Sillanpää 2010; Valencia et al. 2011) and are well known to be mutagenic and carcinogenic (Eggins et al. 1997; Singer 2006; Richardson et al. 2007).

The general formula for THMs is CHX₃, where X may be any halogen or combination of halogens. They are a group of volatile organic compounds (VOCs). The THMs most commonly detected in chlorinated drinking water are chloroform (CHCl₃), bromodichloromethane (CHBrCl₂), dibromochloromethane (CHBr₂Cl), and bromoform (CHBr₃) (Pérez et al. 2008). Numerous methods for the extraction of THMs in drinking water have been reported in the literature; these include liquid–liquid extraction (LLE), solid-phase extraction, solid-phase microextraction (SPME), static headspace gas chromatography (GC), purge-and-trap GC (Smith 2005; San Juan et al. 2007; Pérez et al. 2008).

SPME is an expeditious, inexpensive, solvent-free, and simple sample preparation technique for chromatographic analysis (Pawliszyn 2012). Furthermore, it is a useful alternative to LLE and solid-phase extraction (Popiel & Sankowska 2011). The method also has remarkable advantages over conventional static headspace and purge-and-trap techniques in terms of reproducibility (Ai 1997). SPME has been widely used in environmental analyses, specifically for pre-concentration of analytes from water matrices. SPME technique have been developed not only to address the need for a reduction in solvent use and in the size of extraction
instrumentation but also to explore the ability of this approach to facilitate rapid and convenient sample preparation. Small extraction devices facilitate on-site applications, and allow for coupling to a variety of analytical micro-instrumentation. SPME is more convenient and cost effective compared with conventional exhaustive extraction approaches (Pawliszyn 2012).

In this technique, a coated fiber is placed in contact with the sample matrix for a predetermined period of time. It is left until the analyte concentration has reached the distribution equilibrium between the sample matrix and the fiber coating. Once this equilibrium is achieved, the extracted amount of analyte is independent of fiber exposure time (Lord & Pawliszyn 2000; Ouyang & Pawliszyn 2006; Spietzelm et al. 2011).

This work investigates the effect of experimental factors on headspace SPME (HS-SPME) for THM analysis in drinking water samples to optimize the procedure. These factors include extraction time, desorption time and temperature, sample volume, magnetic stirring, and salt addition (NaCl). Additionally, a 75 μm fiber coated with carboxen/polydimethylsiloxane (75-CAR.PDMS) was used for the analysis.

MATERIALS AND METHODS

Reagents

The THM standard solution: THMs calibration mix: chloroform, bromodichloromethane, dibromochloromethane, and bromoform (2,000 μg/mL each component in methanol, analytical standard). An internal standard of 2,000 μg/mL fluoride benzene (FB) in methanol and a surrogate standard of 2,000 μg/mL 1-bromo-4-fluorobenzene (BFB) were also prepared. All reagents were purchased from Supelco (Sigma-Aldrich). Methanol (gradient grade) and sodium chloride (99.5%) were purchased from Merck. The water that was used was filtered by a Milli-Q system.

Gas chromatography system and instrumentation

Chromatography analysis was performed using an Agilent Technologies 7890 A GC system gas chromatograph coupled to an Agilent Technologies 5975C VI mass detector (GC/MSD). The gas chromatography/mass spectrometer interface temperature was held at 180 °C while the source temperature was kept at 230 °C. For each GC run, the temperature was first set at 60 °C for 2 min, ramped at 12 °C/min to 180 °C, and held at this temperature for 2 min. Next, the temperature was ramped at 20 °C/min to 240 °C. A post-run column flush at 240 °C was then performed. Sample injection was operated in pulsed splitless mode at 15 mL/min for 5 min. The helium carrier gas flow rate was maintained at 1.3 mL/min. The injection liner (i.d. 0.75 mm, Supelco) was used in pulsed splitless mode at 26 psi for 5 min. An Rtx VMS analytical column (60 m, i.d. 0.25 mm, and 1.4 μm film thickness) was used for these runs (Román & Nogueroles 2007). The MS detector was operated in full scan mode. Quantification of THMs was performed in selective ion monitoring (SIM) mode, which gave more reliable results than the most selective conventional chromatographic detectors.

SPME extraction was performed manually using the 75 μm carboxen/polydimethylsiloxane-coated fiber (75-CAR.PDMS), which has been shown to possess the best extraction efficiency (Campillo et al. 2005; Ganeshjeevan et al. 2007). This fiber was conditioned at 200 °C for 1 h in the injection port of GC prior to use, following the manufacturer’s recommendations. Additionally, an SPME manual holder, inlet guide, and sampling stand, as well as 15 mL vials, were used.

Standard solution for calibration

Intermediate calibration standard solutions of THMs (200 mg/L) were prepared by diluting 1.0 mL of the THMs standard solution (2,000 μg/mL) to 10 mL with methanol. In addition, internal and surrogate standards (200 mg/L) were prepared using the same procedure. These solutions were stored at −15 °C with minimal headspace in bottles with Teflon lined septa and screw caps. A series of working standard solutions (10–150 μg/L) were prepared daily by diluting the intermediate THM standards in 50 mL volumetric flasks using Milli-Q water. Furthermore, they were spiked with the internal and surrogate standards at a concentration of 20 μg/L. The internal standard was used for each analysis to confirm the stability of the dissolved compounds. Additionally, the surrogate standard was analyzed...
daily as a way to check method performance (Kudlejova et al. 2012).

**Solid-phase microextraction procedure**

For GC/MS analysis, volumes of working standard solutions (depending on the experiment) were transferred into 15 mL vials. In all experiments, the fiber was lowered into the vial with the fiber suspended in the HS above the liquid layer to sample the volatile THMs. Additionally, the aqueous solution was replaced after each measurement. To optimize analytical conditions, different conditions such as extraction time, sample volume, desorption time, desorption temperature, magnetic stirring, and NaCl addition were varied (Kudlejova et al. 2012). All experiments were performed in triplicate.

**RESULTS AND DISCUSSION**

**Effect of extraction time**

The selection of the optimum extraction time is one of the critical steps in SPME method development. The equilibrium time is the duration after which the amount of extracted analyte remains constant. It has been shown that the lowest detection limits and the highest reproducibility are obtained at the equilibrium time (Soh & Abdullah 2005). To determine the optimal extraction time, the period the fiber was allowed to absorb volatile THMs was varied between 1 and 20 min at 25 °C.

Figure 1 shows the effect of extraction time on the peak areas of HS/SPME-GC/MSD for each THM. The maximum extraction was achieved at 10 min for all THMs. Beyond this time, the response area decreased for all TMHs analyzed. This decrease might be due to the so-called displacement effect. This effect occurs for fibers extracting the analytes by adsorption rather than by absorption. Moreover, this decrease is also due to the limited amount of sites on the surface of the coating for adsorption. If this area is substantially occupied, then the displacement of the compounds with low distribution ratios by those with high distribution ratios occurs (Górecki et al. 1998; Lord & Pawlisyn 2000). These results were similar to those received from VOCs analyzed by SPME-GC/FID (flame ionization detector; Alonso et al. 2003). Therefore, there is no actual equilibrium time for this system, and careful timing of extractions must be employed to obtain high precision. Thus, experiments were performed using an extraction time of 10 min in all subsequent trials.

**Effect of sample and headspace volumes**

Sample volume is directly related to sensitivity. This may be because increasing sample volume increases the amount of analyte extracted. This effect only occurs up to a particular volume, after which sensitivity no longer increases with sample volume. Additionally, the smaller headspace-volume/sample-volume ratio leads to faster analyte transport from the sample to the fiber (Román & Nogueroles 2007; Kudlejova et al. 2012). Because of this variability, the sample volume used for the assay had to be optimized.

Figure 2 shows the dependence of THM peak areas from HS/SPME-GC/MSD analysis on the sample volume. Different sample volumes between 1 to 6 mL were tested using 15 mL vials (corresponding to headspace volumes from 14 to 9 mL). THM samples (100 μg/L) were extracted for 10 min at 25 °C while stirring at 250 rpm and desorbed for 10 min at 230 °C. All THMs presented the same response to sample volume variation, with the highest response area at a sample volume of 5 mL. Subsequent experiments were then performed using 5 mL of sample (corresponding to a headspace volume 10 mL).
Effect of desorption temperature

The increase of desorption temperature enhances the diffusion coefficient of the analytes in the fiber while decreasing the distribution constant between the fiber and the carrier gas (Román & Nogueroles 2007). Figure 3 shows the effect of desorption temperature on the THM peak areas from HS/SPME-GC/MSD analysis. Desorption temperature was increased from 180 to 230 °C. Higher temperatures were not assayed to avoid rapid fiber degradation. The response peak areas decreased when desorption temperature was increased. However, the peak widths decreased at higher temperature, implying better selectivity. These results were similar to those received from VOCs analyzed by SPME-GC/MS such as CHCl₂, C₂H₄Cl₂, C₆H₆, C₂HCl₃, CHCl₂Br, CHClBr₂, C₂Cl₄, and CHBr₃ (Lara-Gonzalo et al. 2008). Therefore, 230 °C was selected as the desorption temperature for further experiments.

Effect of desorption time

Figure 4 shows the effect of desorption time on THM peak area. The desorption time was increased from 1 to 10 min at 230 °C. It was found that after 3 min, each THM was completely purged. To further demonstrate this effect, the fiber was analyzed a second time after desorption and prior to re-exposure to a sample, and no peaks were found in the resulting chromatogram. Therefore, 3 min at 230 °C was sufficient to remove all THMs from the fiber. Further experiments were performed using these conditions.

Effect of magnetic stirring on extraction

Agitation is required to increase the transport of analytes from the bulk of the solution to the vicinity of the fiber (Alonso et al. 2003). Figure 5 shows the effect of magnetic stirring on THM peak areas. Samples were stirred with a spin bar on a magnetic stirrer. The magnetic stirring was increased from 0 to 1200 rpm. The highest peak area was reached at 800 rpm for all THMs. However, the peak area decreased for Br₃CH above 800 rpm and for Cl₃CH, BrCl₂CH, and Br₂ClCH above 1000 rpm. This might be due to liquid droplets depositing on the surface of the fiber. Therefore, further experiments using magnetic stirring were performed at 800 rpm.
Effect of salt addition (NaCl)

The addition of salt into the aqueous sample prior to the extraction process can increase or decrease the amount of analyte extracted. This depends on the concentrations of the compound and salt. In general, salt addition increases peak area due to the increase of ionic strength of the solution, which reduces the solubility of analytes. Moreover, this process changes vapor and partial pressures, thermal conductivity, density, and surface tension of the solution-analyte system causing a variation in the vapor/liquid equilibrium (Banat et al. 1999; Pawliszyn 2005). In HS-SPME, sodium chloride or sodium sulfate can be used.

Figure 6 shows the effect of NaCl on THM peak areas without magnetic stirring. NaCl weight was increased from 0.0 to 1.8 g (saturation limit of 0.36 g/mL). The highest peak area was attained using 1.8 g NaCl in 5 mL samples.

Combined effect of magnetic stirring and salt addition

Figure 7 shows the combined effect of magnetic stirring and salt addition on THM peak areas. The interaction of magnetic stirring and salt addition produced a synergistic effect. Therefore, subsequent experiments were conducted with 800 rpm and 1.8 g NaCl.

Together, the optimal conditions for THM analysis with HS/SPME-GC/MSD using 75 μm fibers coated with carboxen/polydimethylsiloxane (75-CAR.PDMS) were concluded to be as follows: an extraction time of 10 min, an extraction temperature of 25 °C, a sample volume of 5 mL, a desorption time of 3 min, a desorption temperature of 230 °C, magnetic stirring at 800 rpm, and an addition of 1.8 g NaCl (solution saturation).

Method validation

Calibration studies were conducted with the given optimized conditions to validate the HS/SPME-GC/MSD method and to demonstrate its suitability for THM analysis. The following parameters were calculated: the linearity with the correlation coefficient ($R^2$), the precision as the relative standard deviations (RSD) of three replicates for each concentration level, and the accuracy as the percent relative recovery. In addition, the limits of detection (LOD) and quantification (LOQ) were also determined.

Calibration curves of each THM in aqueous solution were prepared using the same technique as the internal standard. FB and BFB were used as internal and surrogate
standards, respectively. The extraction time profile for the internal standard was also performed to determine the equilibrium time for extraction, which was less than 10 min (data not shown). A series of THM solutions with concentrations ranging from 10 to 150 μg/L were prepared. They were spiked with the internal and surrogate standards at 80 μg/L. The linearity of the HS/SPME-GC/MSD method was evaluated by plotting the ratio of the analyte peak area to the internal standard peak area versus the THM concentration in solution (Figure 8). The correlation coefficients ($R^2$) are shown in Table 1. These values suggest a strong linear response of the analytes.

To check if there was a statistically significant correlation between the peak areas and THM concentrations, a hypothesis test was performed. The null hypothesis ($H_0$) $\beta = 0$ and the alternative hypothesis ($H_A$) $\beta \neq 0$ were used. Table 1 shows that for the calibration curve $t_{obs}$ was greater than $t(\alpha/2, n-2)$. Therefore, the null hypothesis is rejected, and the alternative hypothesis is accepted. This implies that there is a significant correlation between concentration and the areas.

The LOD was calculated based on the THM concentration that produced a signal-to-noise ratio (S/N) of 3 using Milli-Q water spiked with low levels of THMs. Similarly, LOQ was calculated based on a signal-to-noise ratio (S/N) of 10 (Mitra & Brukh 2003; Huber 2007). The LOD and LOQ values are shown in Table 1. All THMs exhibited low LOD and LOQ values. Therefore, the proposed HS/SPME-GC/MSD method is highly sensitive.

The precision of the method was evaluated by calculating the relative standard deviation (%RSD) of five replicates using THM standards at 100 μg/L. %RSD for precision ranged between 1.3 and 3.1% (Table 2). For SPME-GC applications, typical standard deviation values should be below 5% (Food and Drug Administration 2001). Additionally, these values are within the %RDS limit for a 100 μg/L standard given by the Association of Official Analytical Chemistry as below 15% (AOAC 2003).

The accuracy was evaluated by the percent relative recovery (%REC) (Table 1). %REC for accuracy ranged between 94.4 and 107.8%. These values are within the given limits of the AOAC (80–110%). Additionally, it was found that $t_{obs}$ was less than $t(\alpha/2, n-2)$ with a 99% confidence level. This result implies that the HS/SPME-GC/MSD

### Table 1 | Linearity range (RL), slope (A), intercept (B), correlation coefficient ($R^2$), limits of detection (LOD), and limits of quantification (LOQ) of the optimized HS/SPME-GC/MSD method with 75-CAR.PDMS fibers

<table>
<thead>
<tr>
<th>Compound</th>
<th>RL (μg/L)</th>
<th>A</th>
<th>B</th>
<th>$R^2$</th>
<th>$t_{obs}$</th>
<th>$t_{0.025,3}$</th>
<th>LOD (μg/L)</th>
<th>LOQ (μg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl₃CH</td>
<td>10–80</td>
<td>0.0072</td>
<td>-0.009</td>
<td>0.9941</td>
<td>22.43</td>
<td>3.182</td>
<td>0.020</td>
<td>0.058</td>
</tr>
<tr>
<td>BrCl₂CH</td>
<td>10–80</td>
<td>0.0065</td>
<td>-0.0176</td>
<td>0.9932</td>
<td>20.92</td>
<td>0.021</td>
<td>0.021</td>
<td>0.061</td>
</tr>
<tr>
<td>Br₂ClCH</td>
<td>10–80</td>
<td>0.0058</td>
<td>-0.0185</td>
<td>0.9945</td>
<td>23.39</td>
<td>0.019</td>
<td>0.019</td>
<td>0.065</td>
</tr>
<tr>
<td>Br₂CH</td>
<td>10–80</td>
<td>0.0045</td>
<td>-0.0119</td>
<td>0.9953</td>
<td>25.21</td>
<td>0.030</td>
<td>0.030</td>
<td>0.085</td>
</tr>
</tbody>
</table>

### Table 2 | Evaluation of the precision, accuracy, and %REC of the optimized HS/SPME-GC/MSD method with 75-CAR.PDMS fibers

<table>
<thead>
<tr>
<th>Compound</th>
<th>Parameter</th>
<th>Precision $(n = 5)$</th>
<th>Accuracy $(n = 5)$</th>
<th>%REC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%RSD</td>
<td>Confidence limit</td>
<td>$t_{obs}$</td>
<td>$t_{0.025,4}$</td>
</tr>
<tr>
<td>Cl₃CH</td>
<td>1.7</td>
<td>6809667.647</td>
<td>2.769</td>
<td>2.776</td>
</tr>
<tr>
<td>BrCl₂CH</td>
<td>3.1</td>
<td>12973836.77</td>
<td>1.157</td>
<td>97.6</td>
</tr>
<tr>
<td>Br₂ClCH</td>
<td>1.3</td>
<td>5505581.402</td>
<td>2.489</td>
<td>95.7</td>
</tr>
<tr>
<td>Br₂CH</td>
<td>1.9</td>
<td>5976976.496</td>
<td>2.178</td>
<td>107.8</td>
</tr>
</tbody>
</table>

Figure 8 | Calibration curves of each THM. Extraction time of 10 min at 25°C, desorption time of 3 min at 230°C, 15 mL vial, sample volume of 5 mL, magnetic stirring at 800 rpm, and salt addition of 1.8 g NaCl.

The LOD was calculated based on the THM concentration that produced a signal-to-noise ratio (S/N) of 3 using Milli-Q water spiked with low levels of THMs. Similarly, LOQ was calculated based on a signal-to-noise ratio (S/N) of 10 (Mitra & Brukh 2003; Huber 2007). The LOD and LOQ values are shown in Table 1. All THMs exhibited low LOD and LOQ values. Therefore, the proposed HS/SPME-GC/MSD method is highly sensitive.

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The reproducibility of the HS/SPME-GC/MSD method with 75-CAR.PDMS fibers was evaluated by peak area using 100 μg/L THM standards in five replicate samples, Figure 9. The reproducibility is expressed as the relative standard deviation (%RSD) (Mitra & Brkhu 2003). The %RSD was in the range of 3.7–6.9%. Thus, the method is precise enough to analyze THMs.

This result is similar to those of Cho et al. (2003) who have reported %RSD values of 0.8–6.2%. In contrast, Campillo et al. (2005) found a lower LOD (0.0005–0.0014 μg/L) and better reproducibility (<2.6%) with 85 μm CAR/PDMS fibers than 75-CAR.PDMS. Also contrary to our results, San Juan et al. (2007) found that using 75-CAR.PDMS fibers presented rather irreproducible results, giving the shortest linear range than other fibers and presenting %RSD values of 6–15% along with the worst LOD and LOQ. They selected the PDMS-DVB fiber as the most appropriate for determining THMs due to its lower LOD and LOQ, better reproducibility, and a broader linear range with a high correlation coefficient.

**CONCLUSIONS**

Headspace solid-phase microextraction coupled with gas chromatography and mass spectrometry detection (HS/SPME-GC/MSD) using a 75-CAR.PDMS has been successfully calibrated for the determination of THMs in drinking water. 75-CAR.PDMS presents high sensitivity and a reasonable analysis time. The optimal conditions for the HS/SPME-GC/MSD method were concluded to be as follows: an extraction time of 10 min, an extraction temperature of 25 °C, a sample volume of 5 mL, a desorption time of 3 min, a desorption temperature of 230 °C, magnetic stirring at 800 rpm, and an addition of 1.8 g NaCl (solution saturation).

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**REFERENCES**


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