J. Sep. Sci. 2009, *32*

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Short Communication

Liquid-phase microextraction by solidification of floating organic microdrop and GC-MS detection of trihalomethanes in drinking water

A simple and sensitive methodology based on liquid-phase microextraction (LPME) followed by GC-MS, was developed for the determination of trihalomethanes (THMs) in drinking water. A microdrop of organic solvent was floated on the surface of the aqueous sample and it was agitated for a desired time. Then, the sample vial was cooled by inserting it into an ice bath for 4 min. The solidified solvent was transferred into a suitable vial and immediately melted. The extract was directly injected into the GC. Microextraction efficiency factors were investigated and optimized: 7 μL 1-undecanol microdrop exposed for 15 min floated on the surface of a 10.0 mL aqueous sample with the temperature of 60°C containing 3 M of NaCl and stirred at 750 rpm. Under the selected conditions, enrichment factors (EFs) up to 482-fold, LOD of 0.03–0.08 μg/L (S/N = 3) and dynamic linear ranges of 0.10–100 μg/L were obtained. A reasonable repeatability (RSD < 8.6%, $n = 8$) with satisfactory linearity ($r^2 \geq 0.9947$) of results illustrated a good performance of the present method. The protocol proved to be rapid, cost-effective, and is a green procedure for the screening purposes.

Keywords: Drinking water / Gas chromatography-mass spectrometry / Liquid-phase microextraction / Trihalomethanes

Received: June 29, 2008; revised: October 6, 2008; accepted: October 6, 2008

DOI 10.1002/jssc.200800369

1 Introduction

Nowadays, chlorination is the most widely used technique for the disinfection of drinking water. Chlorine is extensively applied because of its effectiveness against a broad range of pathogens, including bacteria, viruses, and protozoa. In addition, it is relatively inexpensive, allowing a residual disinfection power, which is important for the elimination of the possible contamination sources in the distribution system [1, 2]. However, scientists discovered that the chlorination of the organic matter in fresh water resulted in the formation of disinfection by-products (DBPs), including a variety of chlorinated compounds. Among these DBPs, trihalomethanes (THMs: chloroform, bromodichloromethane, dibromochloromethane, and bromoform) have been recognized as carcinogenic halogenated by-products and potentially hazardous to human health [3, 4]. In order to protect public health from the possible carcinogenic effects of these substances, the US Environmental Protection Agency (EPA) set the standard as 80 μg L^{-1} to regulate the THMs concentration in drinking water under the "safe drinking water act" [5, 6]. Therefore, the THMs determination in drinking water at trace level is of great importance.

The trace analysis of THMs and the other volatile compounds in water is usually performed by GC combined with a previous concentration step, including the traditionally used liquid–liquid extraction (LLE) [7] and the more newly developed headspace (HS) techniques [8]. The HS techniques can be divided into different subcategories, which are the static HS, the dynamic HS (purge and trap) [9, 10], and the HS-SPME [11]. The traditional LLE requires large amounts of high-purity organic solvents which are expensive, often hazardous, and harmful to the environment [7]. The static HS method is rapid and presents good repeatability. However, the disadvantage of the method is the handling problem of the samples and the standard solutions in an absolute contamination free atmospheric area. Also, a lower sensitivity is normally observed in the static HS technique compared with LLE [12, 13]. Furthermore, the purge and trap method is time consuming and the equipment is relatively expen-

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Abbreviation: IS, internal standard; **SFE,** supercritical fluid extraction; **TCM,** traditional Chinese medicine

sive [14]. HS-SPME, introduced by Pawliszyn and coworkers [15], is solvent free, easily automated and usually more feasibly combined with GC or HPLC [16]. Thus, it has been used for numerous applications in environmental [17], biological [18], and pharmaceutical analysis [19], such as the THMs monitoring [20].

Liquid-phase microextraction (LPME) refers to a class of sample preparation techniques based on using microliter level of extraction solvent. These methods usually integrate analyte isolation, purification, and enrichment into one single step. LPME is simple, fast, and is characterized by its affordability, and reliance on widely available apparatus [21]. Compared with SPME, LPME does not have the limitation of fiber connatural properties and reduced the possibility of carry-over [22]. Today, LPME has been applied to determine a broad variety of organic compounds from numerous types of samples [23, 24], including THMs [25, 26].

Recently, Yamini and coworkers [27] have reported an effective LPME-based microextraction method, which was initially applied to the determination of polycyclic aromatic hydrocarbons (PAHs) in water. In this technique, a free microdrop of the organic solvent is floated on the surface of an aqueous sample while being agitated by a stirring bar in the bulk of the solution. Under the proper stirring conditions, the floated microdrop can remain in the top-center position of the aqueous sample. After the completion of the extraction, the sample vial was transferred into a cold water bath for a few minutes, in order to solidify the organic solvent. The solidified solvent was transferred into a small conical vial by the spatula and melted immediately at the room temperature. Finally, the analyte determinations in the extract can be performed by GC. This quantitative LPME method is green and a satisfactory analytical procedure, because excellent accuracy and precision are demonstrated.

The aim of the present study is to investigate the applicability of the technique for the determination of THMs in drinking water. The factors affecting the microextraction efficiency were studied in detail and the optimal conditions were established. The resulting method was validated for quantitative purposes and applied to real sample analysis in combination with GC-MS.

2 Experimental

2.1 Reagents

Chloroform ($CHCl_3$), bromodichloromethane ($CHBrCl_2$), dibromochloromethane ($CHBr_2Cl$), and bromoform ($CHBr_3$) were purchased from Riedel-de Haën (Germany). 1-Undecanol, 1-dodecanol, 2-dodecanol, and *n*-hexadecane were used as the extraction solvents, 1, 2-dibromopropane as the internal standard as well as sodium chloride which was purchased from Merck (Darmstadt, Germany). The stock standard solutions (2000.0 mg/L) of each compound were prepared in acetone and stored in a freezer at approximately −20°C. A fresh 2.00 mg/L standard solution, containing the four THMs in acetone, was prepared every week and stored at 4°C. The used reagent water was purified with a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.2 Instrumentation

The analysis was performed on a Hewlett-Packard (Agilent Technologies, Palo Alto, CA, USA) HP 6890 series GC, equipped with a split/splitless injector and an HP 5973 mass selective detector system. MS was operated at the electron impact (EI) mode (70 eV). Helium (99.999%) was employed as carrier gas at the flow rate of 0.8 mL/min. The analytes were separated on a 30×0.25 mm id $\times$ 0.25 μm film thickness DB-1 MS gas chromatographic column (J&W Scientific, Folsom, CA, USA) with the following oven temperature program: initial 35°C, from 35 (held 3 min) to 110°C at 10°C/min, increased at 25°C/min to 285°C and held for 5 min. The injection port was operated at 300°C and was used at the split mode with a split ratio of 1:10. The EI ion source, quadrupole mass analyzer and the interface temperature were maintained at 230, 150, and 280°C, respectively. MS was operated at the total ion current (TIC) mode, scanning from *m*/*z* 50 to 300 for identification purposes. To gain the highest possible sensitivity, the acquisition was performed at the SIM mode, based on the selection of some mass peaks of the highest intensity for each compound. Table 1 lists the retention times, the selected masses and the start scan times for each compound studied by GC-MS.

All injections were carried out using a 1.00 μL microsyringe (zero dead volume, cone tip needle, SGE, Australia). A magnetic heater-stirrer (IKA-Werke, Staufen, Germany) and an 8 mm $\times$ 1.5 mm PTFE coated stirring bar were used to stir the solutions. A simple water bath placed on the heater-stirrer was employed to control the temperature of the sample solutions.

2.3 Drinking water samples

Three drinking water samples: tap water 1 after ozonation process (Tehran Water and Sewerage Company, Tehran, Iran), tap waters 2 and 3 after chlorination process from two local areas (Tehran, Iran), were selected for validating the proposed method. Sampling was performed at the terminal point of the distribution system. Amber glass bottles (250 mL) with PTFE screw caps were used for the collection of the samples. The bottles were completely filled to avoid the volatile organic compounds evaporation. The samples were stored at 4°C and analyzed within 3 days after collection.

2.4 Analytical procedure

Aqueous THMs solution (10.0 mL) (containing 50.00 μg/L of each THM) were transferred into an 11.0 mL vial. 1-Undecanol (10.0 μL) was floated on the surface of the aqueous sample using a 10.0 μL model 701 N microsyringe (Reno, NV, USA). The vial was sealed and then the magnetic stirrer was turned on. Under the proper stirring conditions, the floated microdrop could remain in the top-center position of the aqueous sample. In an immiscible liquid–liquid system with the proper interface tension, the microdrop would not break up even in the absence of any support from the microsyringe needle, polymer rod or other supporting material like hollow fibers. On the other hand, the microdrop movement was affected by the flow field, which favored the promotion of the mass transfer inside the microdrop. After the desired extraction time, the sample vial was transferred into an ice beaker and the organic solvent was solidified after 4 min. Then, the solidified solvent was transferred into a conical vial and it melted immediately. Finally, 1.00 μL of the extract was injected into the GC-MS system. In order to improve the precision and accuracy of the method in all of experiments, 1, 2-dibromopropane with the concentration of 1.00 mg/L was used as the internal standard and was added into the extracting solvent.

3 Results and discussion

A univariate approach was employed to optimize the influential factors in this method. Quantifications were based on the relative peak area of the analyte to the internal standard from the average of three replicate measurements.

3.1 Selection of the extracting solvent

The selection of an appropriate extraction solvent is of great importance for the optimization of the LPME process. To choose a suitable organic solvent, the following points should be considered. Firstly, the chosen solvent should be immiscible with water and illustrate a high boiling point with a low vapor pressure in order to reduce the evaporation risk [28]. Secondly, it should exhibit a good chromatographic behavior [29] and thirdly, improve appropriate extraction efficiency to get high extraction recoveries and enrichment factors (EFs) [26]. Finally, it must demonstrate a melting point near the room temperature (in the range of 10–30°C) [27]. According to these considerations, three solvents, including 1-undecanol, 2-dodecanol, and *n*-hexadecane were considered. The experimental results (Fig. 1) revealed that 1-undecanol presented the highest extraction efficiency and thus was selected as the extracting solvent.

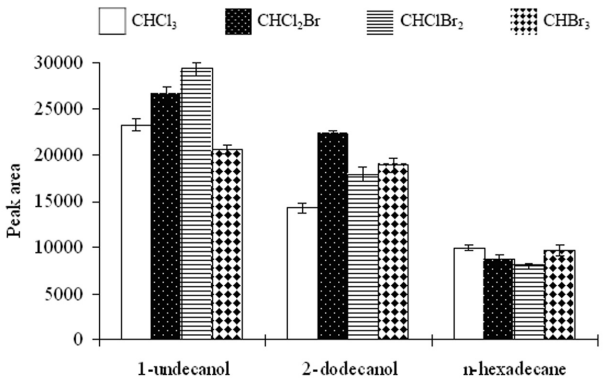

Figure 1. Effect of the organic solvent type on the extraction efficiency. Conditions: sample volume: 10.0 mL; extraction temperature: 60°C; extraction solvent volume: 10.0 μL; stirring rate: 600 rpm; extraction time: 25 min, and without salt addition.

3.2 Sample solution temperature

Solution temperature affects extraction kinetics. At higher temperatures, diffusion coefficients of analytes increase, therefore, this process facilitated the mass transfer of the analyte from the sample to the organic solvent and the time required to reach equilibrium decreased [30, 31]. The effect of sample solution temperature on the extraction efficiency was studied in the range of 20–60°C by floating a 1-undecanol microdrop for 25 min in the surface of the water samples. Figure 2 clearly exhibits that by increasing the temperature, the extraction efficiency increased for all of the analytes. However, at higher temperatures (>60°C), the over-pressurization of the sample vial made the extraction system unstable. Thus, the sample solution temperature was held at 60°C for the subsequent experiments.

3.3 Effect of the ionic strength

Ionic strength modification is a very useful tool for the enhancement of the coefficient of the partitioning between extracting phase and sample matrix [32]. The NaCl concentration effect (0–4 M) was investigated and the extraction efficiency was monitored. The results are presented in Fig. 3, and revealed that the extraction efficiency gradually increased with the increase in the NaCl concentration. The maximum signal was achieved at the NaCl concentration of 3 M. Increasing extraction extent can be explained by the engagement of water molecules in the hydration spheres around the ionic salt and, hence, decreasing the available amount of water to dissolve analytes. In fact, the salt presence increases the ionic strength of the solution and reduces the solubilities of the analytes due to the salting-out phenomena [33, 34].

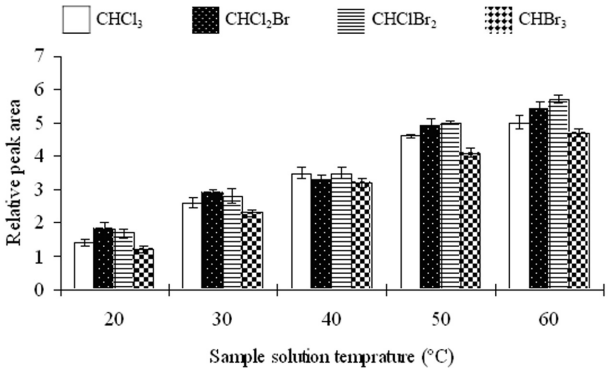

Figure 2. Influence of the aqueous sample temperature on the relative peak area. Extraction conditions as with Fig. 1 and extraction solvent: 1-undecanol.

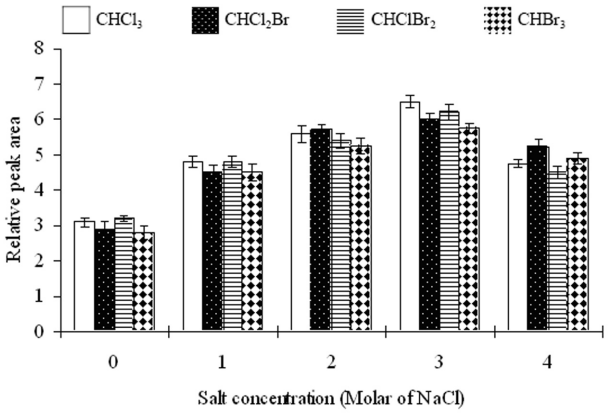

Figure 3. Effect of the salt addition on the relative peak area. Extraction conditions as with Fig. 2 and aqueous sample temperature 60°C.

Thus, further extractions were carried out in the NaCl presence of 3 M.

3.4 Organic solvent volume

The effect of the organic solvent volume on the responses was tested in the range of 6.0–12.0 μL. Experimental results show that the THMs extraction efficiencies increase by increasing the solvent volume to 7 μL and diminished afterward. Furthermore, at larger volumes (*i.e.*, >7 μL), due to dilution effect, concentration of the analytes in the organic phase decreased [30]. So, to enhance the sensitivity of the LPME procedure, volume of 7 μL was selected as the optimum.

3.5 Stirring rate

Agitation of the sample solution has been used universally to improve microextraction efficiency [35]. The stirring can regenerate a new sample solution surface, so, accelerating the mass transfer from the donor phase to the acceptor phase [22, 24]. In this work, samples were agitated at different stirring rates (0, 150, 300, 450, 600, and 750). According to obtained results, the relative peak area of all analytes increases with the stirring rate increase up to 750 rpm. Higher stirring rates (>750) were not used because in this case the microdrop would become spattered and damaged. Hence, for the following studies, the stirring rate of 750 rpm was chosen.

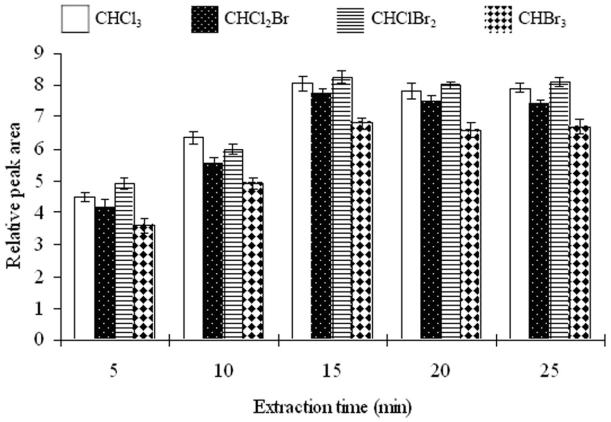

Figure 4. Influence of the extraction time on the extraction efficiency. Extraction conditions as with Fig. 3 and: 3 M of NaCl, extraction solvent (1-undecanol) volume of 7.0 μL with stirring rate of 750 rpm.

3.6 Extraction time

Like SPME, LPME is a process dependent on equilibrium rather than exhaustive extraction [30]. To increase repeatability of the extraction, it is necessary to choose an extraction time during which the equilibrium between the aqueous and the organic phase is reached. The time for reaching equilibrium determines the maximum amount of the analytes that can be extracted by the microdrop [36]. This effect was studied in the range of 5–25 min at the optimized experimental conditions. Figure 4 shows that the relative peak area increases with sampling time up to 15 min and remained nearly constant afterwards. Therefore, the extraction time period of 15 min was selected to obtain a reasonable sensitivity.

3.7 Evaluation of the method performance

Under the selected optimum experimental conditions, the suggested methodology was applied to a series of standard solutions containing various analytes concentrations, in order to develop the respective calibration curves. For each level, three replicate extractions were conducted. The LODs based on the S/N of three, the regression coefficient (r^2), the linear ranges (LRs), the RSDs, and the EFs are calculated and summarized in Table 1.

Table 1. Retention times, selected ions, scan start time, and some quantitative data obtained after the LPME and GC-MS determination of the THMs

Compound	Retention time (min)	Selected ions (*m*/*z*)	Scan start time (min)	LOD[b] (μg/L)	Regression coefficient (r^2)	LR[c] (μg/L)	EF[d]	RSD%[e] (*n* = 8)
$CHCl_3$	2.63	83, 85	2.00	0.06	0.9947	0.25 – 100	397	6.8
$CHCl_2Br$	3.78	83, 85	3.00	0.04	0.9959	0.10 – 100	448	5.6
$CHClBr_2$	5.42	127, 129	4.50	0.03	0.9953	0.10 – 100	482	5.9
$CHBr_3$	7.21	171, 173	7.00 [a]	0.08	0.9960	0.25 – 100	366	8.7

[a] The MS detector was OFF after the time point of 8.00 min.
[b] LOD for S/N = 3.
[c] Linear range.
[d] EF.
[e] RSD at the concentration of 5.00 μg/L of each THM.

Table 2. The results obtained from the analysis of the real water samples.

Sample		$CHCl_3$	$CHCl_2Br$	$CHClBr_2$	$CHBr_3$
Tap water 1 (0.50 μg/L added)	Concentration (μg/L)	ND[a]	ND	ND	ND
	Found (μg/L)	0.56	0.55	0.47	0.53
	Relative recovery (%)	113	110	95	106
	RSD% (*n* = 8)	8.2	7.7	6.9	9.2
Tap water 2 (5.00 μg/L added)	Concentration (μg/L)	19.29	14.66	11.20	0.92
	Found (μg/L)	23.86	19.96	15.51	6.39
	Relative recovery (%)	91	106	86	109
	RSD% (*n* = 8)	7.4	6.9	7.3	8.2
Tap water 3 (50.00 μg/L added)	Concentration (μg/L)	31.21	10.01	16.22	3.76
	Found (μg/L)	88.04	58.17	62.91	47.43
	Relative recovery (%)	114	96	93	87
	RSD% (*n* = 8)	9.1	6.4	5.7	8.8

[a] Not detected.

For the EF calculation of each analyte, three replicate extractions were performed at the optimal conditions from the aqueous solution, containing 5.0 μg/L of the analytes. EF was calculated as the ratio of the final analyte concentration in the microdrop and its concentration in the original solution. The THMs standard solutions were prepared in 1-undecanol as a solvent and calibration curves were drawn in the concentration range of 0.5 – 5.0 mg/L with three replicate direct injections. The actual concentration of each extracted analyte in 1-undecanol was calculated from the calibration curves and the EF values were determined.

As it is illustrated in Table 2, LODs for the THMs were found to be 0.03 up to 0.08 μg/L. The linearity values varied from 0.10 to 100 μg/L with regression coefficient of 0.9947 – 0.9960. The precision of the method was investigated with a 5.00 μg/L THMs mixed standard solution. Regarding the RSDs for eight replicates, they varied from 5.6 to 8.7%, while the EF values ranged from 366 to 482.

3.8 Analysis of drinking water samples

The performance of this procedure was tested by analyzing the THMs in three drinking water samples. The results listed in Table 2 indicate that tap water 1 was free of the target analytes, while total THM concentrations were found to be 46.07 and 61.20 μg/L for tap water 2 and 3, respectively. All of the real samples were spiked with THMs standards at different concentration levels to assess the matrix effects. LPME is a nonexhaustive extraction procedure and the relative recovery (determined as the ratio of the concentrations found in real sample and reagent water sample, spiked with the same amount of analytes), instead of the absolute recovery (used in exhaustive extraction procedures), was employed. The relative recoveries of the analytes are given in Table 2 and varied from 86 to 114%, which indicated that the real matrices in our present context had little effect on the LPME. The chromatogram obtained by GC-MS of tap water 1 spiked at the concentration level of 0.50 μg/L of

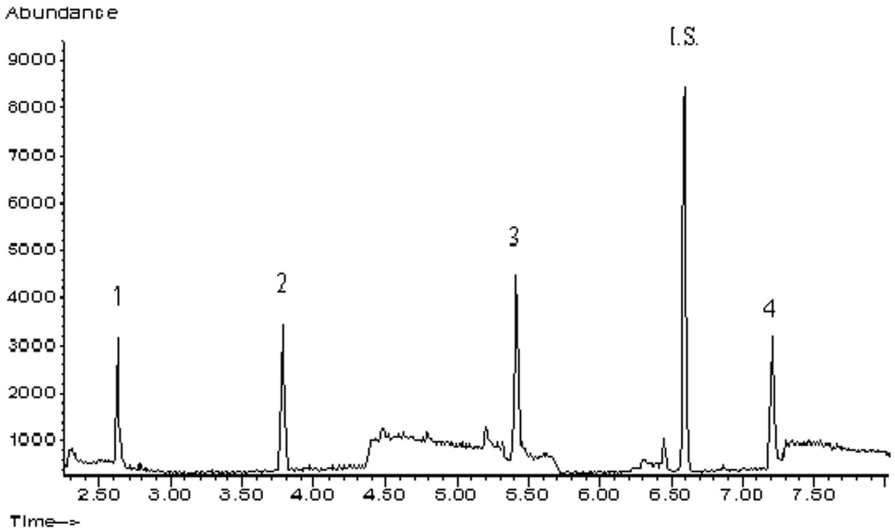

Figure 5. Chromatogram obtained by GC-MS after performing LPME of tap water one, spiked with THMs (0.50 μg/L). Peak numbers correspond to (1) $CHCl_3$, (2) $CHCl_2Br$, (3) $CHClBr_2$, I.S.: Internal standard-1, 2-dibromopropane, and (4) $CHBr_3$.

each analyte after the developed method at optimum conditions is shown in Fig. 5.

4 Conclusion

This paper outlined the successful development and application of a method based on the LPME technique, combined with the capillary GC-MS, for the qualitative and quantitative analysis of THMs in drinking water samples. The designed method was concluded to be precise, reproducible, and linear over a broad range with sufficient selectivity and high sensitivity. Compared with the other conventional sample preparation methods, the analytical technique offered numerous advantages, such as simplicity, low cost, ease of operation, no possibility of sample carry-over, and high EFs. In addition, the technique required only a small volume of organic solvent, being therefore an environmentally friendly approach. The performance of this procedure in THMs extraction from different real water samples with various matrixes was excellent. Subsequently, it can be extended to other applications.

This work has been supported by grants from the University of Tehran Research Council, which is hereby gratefully acknowledged.

The authors declared no conflict of interest.

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