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Short communication

Trace analysis of ten chlorinated benzenes in water by headspace solid-phase microextraction

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Abstract

Headspace solid-phase microextraction (SPME), as a simple, solvent-free method, has been applied to the analysis of 10 chlorinated benzenes (CBs) present at trace levels in water samples. An SPME fibre coated with 100-μm thick poly(dimethylsiloxane) was used for extraction. The analytical data exhibited a relative standard deviation (RSD) range of 1.19% (for pentachlorobenzene) to 8.19% (for hexachlorobenzene) for the 10 CBs; the RSD of most compounds was under 6%. The sensitivity of the method was enhanced with agitation and with addition of salt to the sample solutions. With mass spectrometric detection, the limit of detection was below 0.006 μg/l for all 10 CBs after a 30-min sampling time. The linearity range was 0.02–20 μg/l for the compounds studied. Water samples collected from a reservoir, and from the tap in a laboratory were analysed using the optimised conditions.

Keywords: Headspace analysis; Water analysis; Extraction methods; Chlorobenzenes

1. Introduction

As an alternative to conventional sample preparation methods such as liquid–liquid extraction (LLE) [1], solid-phase microextraction (SPME), which has achieved tremendous success since its advent, is simple, solvent-free, rapid, portable and has been automated [2]. Published papers on its applications have been increasing dramatically in the last several years.

Since it was first introduced in 1989, SPME has seen a rapid development with respect to the following features:

1. it has found numerous applications in the environmental, food, pharmaceutical, clinical and biological areas [3–13].
2. Various techniques have been coupled with SPME to achieve different analytical goals. These techniques include GC, GC–MS, HPLC, Raman spectrometry [14] and capillary zone electrophoresis [15,16], etc.
3. Novel SPME fibre coatings, such as bonded sol–gel layer of poly(dimethylsiloxane) (sol–gel PDMS) [17], silica-bonded phases [18] and activated carbon fibre (ACF) [19] have been introduced as extraction materials. Furthermore, the extracting phase coated inside a capillary tubing for in-tube SPME has been developed [20].

Headspace SPME, in which the fibre is exposed to the sample headspace to extract target analytes which are partitioned between the gaseous and aqueous phase, is another development of the technique.

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Compared with direct SPME, headspace SPME can shorten the time of extraction significantly because of the faster diffusion rate of the analytes in gaseous phase than in aqueous phase [21]. It can be used in any matrix [22] since there is no direct contact between the fibre coating and the sample matrix. Furthermore, it can totally eliminate the possibility of introducing trace-level water caused by the wick effect in direct SPME. Headspace SPME is especially suitable to be used in the analysis of volatile and semivolatile organic compounds because these compounds can easily diffuse into the sample headspace from a liquid matrix.

Chlorinated benzenes (CBs), which can enter the aquatic environment as solvents, by-product materials of phenol and pesticide manufacturing, chemical contamination, etc., are ubiquitous due to their widespread use during the last several decades [23]. They are hazardous to health and have been ranked as priority pollutants by the US Environmental Protection Agency (EPA) [24]. CBs existing in aquatic environments can be dechlorinated by photodegradation and biodegradation to form less chlorinated isomers. For example, in an anaerobic enrichment culture, hexachlorobenzene (HCB) can be reductively dechlorinated to pentachlorobenzene (pCB), tetrachlorobenzene (TeCB) isomers and trichlorobenzene (TCB) isomers. TeCB and TCB can be dechlorinated further to form dichlorobenzenes (DCBs) [23]. There have been some publications reporting the use of headspace SPME to the analysis of CBs in spiked soil samples, but the analytes investigated were limited to two or three types of CBs [25,26]. Since a wide variety of CBs exist in the environment, a broad-spectrum analysis covering a wider range of these compounds seems necessary to obtain a clearer picture of their ubiquity.

The main objective of our work is to optimise headspace SPME for the analysis of 10 CBs, including isomers, in aqueous samples and evaluate it as an alternative technique to conventional analytical methods.

2. Experimental

2.1. Standards and reagents

The 10 CBs considered in this work are: 1,3-, 1,4-, 1,2-dichlorobenzene (DCB), 1,3,5-, 1,2,4-, 1,2,3-trichlorobenzene (TCB), 1,2,4,5-, 1,2,3,4-tetrachlorobenzene (TeCB), pentachlorobenzene (pCB) and hexachlorobenzene (HCB). 1,4-Dibromobenzene was used as the internal standard (I.S.) for the GC–MS analysis. All chemicals were obtained from Aldrich (Milwaukee, WI, USA). A 10 mg/l standard solution containing the 10 CBs was prepared in methanol. Sodium chloride, used to adjust the ionic strength of the aqueous samples, and was heated to 300°C to eliminate possible organic contaminations before use.

All compounds used in this study were of analytical-reagent grade. The water used was purified using a Milli-Q water purification system (Millipore, Bedford, MA, USA).

Natural water from a reservoir was collected for this work. The water samples were filtered (Whatman, UK, filter paper) and 0.45-μm membrane (Millipore) to eliminate particulate matter before analysis.

Tap water samples (directly potable) were collected from a laboratory. It was freshly collected, after allowing the water to flow for at least 10 min, and analyzed by headspace SPME–GC–MS directly.

The SPME fibre used in the experiments was a poly(dimethylsiloxane)-coated fused-silica fibre with a 100-μm film thickness (Supelco, Bellefonte, PA, USA).

2.2. Analytical conditions

Analysis was performed with a Shimadzu (Tokyo, Japan) QP-5000 GC–MS system equipped with a 30-m×0.32-mm capillary column coated with a 0.25-μm DB-1 stationary phase (J&W, Folsom, CA, USA). The carrier gas was helium. The GC conditions were as follows: injector temperature 200°C; initial oven temperature 30°C for 2 min, programmed to 220°C at rate of 8°C/min, then maintained at 220°C for 1 min. Splitless injection mode was used. The total time of a single GC run was 26.75 min.

2.3. Headspace solid-phase microextraction procedure

For headspace SPME, 5-ml ultrapure water was transferred into the sample vial. In the case of the optimisation experiments of the effect of salt, 5 ml

NaCl solutions of 10%, 20% and saturated concentrations (w/w), instead of ultrapure water, were used. In the investigation of the influence of agitation, a 2-cm stirring bar was used. The vial was sealed with a PTFE-silicon septum and a hole-top aluminium cap. The standard solution and I.S. were spiked into the water directly. Adoption of such a method to prepare an aqueous sample prevented loss of volatile and semi-volatile organic analytes. Our experiments also showed that the peak area of each compound after spiking for 10 h decreased much more than that of the freshly prepared samples. In order to avoid the loss of the compounds, the aqueous sample was freshly prepared for use. Generally, the aqueous samples were prepared no more than 40 min before headspace extraction. This period of time also allowed the compounds to attain equilibrium between the gaseous and the aqueous phases.

The procedure of SPME headspace extraction is similar to what has been described in detail previously [21]. Care was taken to ensure that the fibre did not contact the aqueous sample. The fibre was transferred into the GC injector for desorption as soon as the sampling was completed. The desorption time of the fibre was set at 10 min based on our experimental results.

The fibre was conditioned in the GC injector at 200°C for at least 20 min before use every day. A fibre blank was performed to ensure that there were no contaminants on the fibre.

3. Results and discussion

3.1. Optimisation of headspace microextraction

Initially, the MS full-scan mode was used to characterise the 10 CBs in an aqueous sample containing 50 μg/l of each compound in the mixture. The mass range scanned for this analysis was *m/z* 40–350. Basic information such as the retention times of each of these compounds, the ion sets and the ions selected for identification and quantitation were obtained. The SIM (selected ion monitoring) mode was then used for quantitation. The analytes were identified by three selected ions for each compound and quantified by the most abundant ion. Fig. 1 is a typical chromatogram of a spiked water extract, obtained in total ion mode.

The development of a SPME method generally consists of a number of stages. In this study, we paid particular attention to such factors as the extraction time, influence of temperature, ionic strength of the sample solution and whether stirring made a significant difference to the extraction.

The equilibration time for the analytes between the

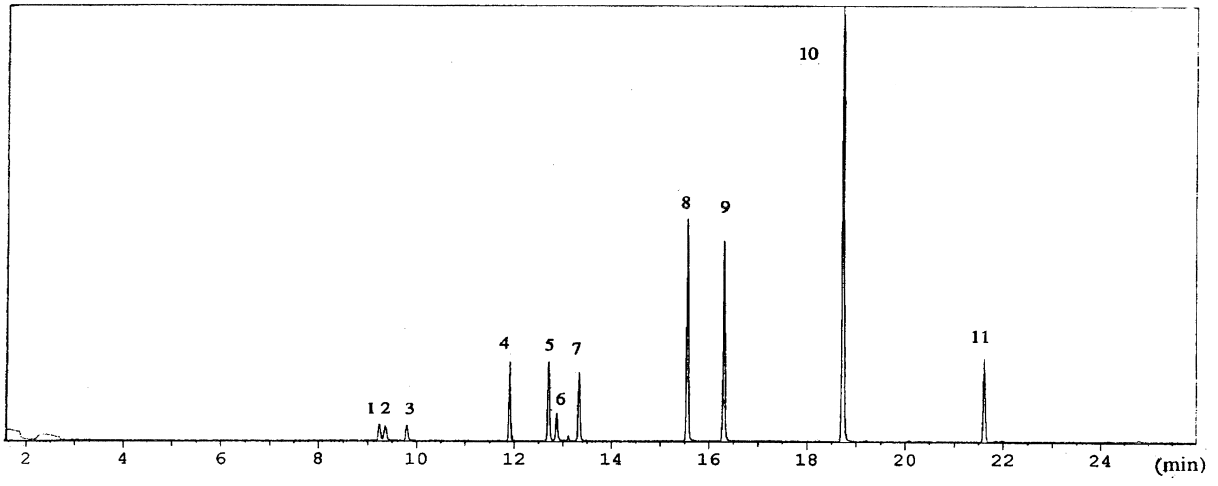

Fig. 1. Total ion chromatogram obtained from headspace SPME of 10 chlorobenzenes (10 μg/l for each analyte) and internal standard (I.S.), from a spiked water sample. Peaks: (1) 1,3-dichlorobenzene; (2) 1,4-dichlorobenzene; (3) 1,2-dichlorobenzene; (4) 1,3,5-trichlorobenzene; (5) 1,2,4-trichlorobenzene; (6) 1,4-dibromobenzene (I.S.); (7) 1,2,3-trichlorobenzene; (8) 1,2,4,5-tetrachlorobenzene; (9) 1,2,3,4-tetrachlorobenzene; (10) pentachlorobenzene; (11) hexachlorobenzene. GC–MS conditions are given in the text.

stationary phase and the sample headspace was determined. The range of extraction time was from 5 to 40 min at room temperature (26°C). Our results show that rapid absorption occurred for the first 10 min for the DCBs, TCB and I.S., after which equilibrium was attained (or the rate of absorption slowed down). However, TeCB, pCB and HCB needed a longer time (nearly 30 min) to attain equilibrium, because they are less volatile than the DCBs and TCBs [27]. The consequence of the time dependence of the absorption process is that the extraction time must be long enough for equilibrium to occur or for the rate of absorption to have slowed. Thus, reasonably precise timing was essential. In our experiments, 30 min extraction time was selected.

To obviate the possibility that some compounds were not desorbed completely from the fibre, a sufficiently long desorption time was considered, by checking sample carryover; a 10-min desorption time was determined to be satisfactory.

In SPME, the salting-out effect [28] can be employed to modify the matrix by adding salt, e.g., NaCl, to increase the ionic strength of the water so as to decrease the solubility of analytes and release more analytes into the headspace, thereby contributing to enhanced absorption on the fibre.

Aqueous samples (containing 10 μg/l of each analyte) with different NaCl concentrations (w/w) of 10%, 20%, and saturated solution were studied at room temperature to determine the level of dissolved salt at which extraction was optimum.

It is apparent that the addition of NaCl improves the extraction and optimum adsorption on the fibre was observed at 20% NaCl concentration for most analytes except for HCB. Salting-out effect on analytes has relationship with their solubilities in the aqueous phase [29]. The greater the solubility of analytes in water, the greater the influence on absorption will be by adding salt, i.e., addition of salt improves the extraction of those compounds which are comparatively more soluble in water. This explains why adding NaCl did not increase the extraction of HCB as significantly as other analytes because HCB has the lowest water solubility of all the compounds considered (Table 1) [27].

The influence of temperature was studied at 26°C (ca. room temperature, controlled by ambient air conditioning), 40 and 50°C. Compared with the results at 26 and 40°C, greatly increased extraction efficiency for pCB and HCB only (nearly two times for HCB, versus extraction at 26°C) were observed. They have lower vapour pressures than the other analytes. The influence of extraction temperature on 1,2,4,5-, 1,2,3,4-TeCB was not obvious. The other compounds showed decreased extraction. Changing the temperature from 40 to 50°C did not lead to any further improvement to the extraction of HCB and the extraction of the other compounds dropped further because of the decrease in the partition coefficient between the coating and the headspace at the elevated temperature. Thus, the addition of NaCl proved to be a better option to improve overall analyte extraction from the sample matrix. For subsequent extraction, the temperature was kept at ambient (26°C in this particular case).

Stirring the water sample undergoing headspace SPME can generally increase the extraction efficiency [30]. Experiments were performed at room temperature (26°C) and without NaCl. The aqueous samples were stirred at 750 and 1500 rpm. Progressively improved extraction was observed for all compounds at greater stirring rates, especially for the lower CBs. The absorption of the compounds with comparatively lower vapour pressure, i.e., those that were less volatile, such as HCB and pCB, were notably enhanced by stirring because the transfer of the compounds from water to headspace could conceivably be speeded up through agitation. A

Table 1
Quantitative analysis of chlorinated benzenes in aqueous samples using headspace SPME

Compound	RSD (%)	Limit of detection (μg/l)	Limit of detection of EPA method 8121 [1] (μg/l)
1,3-Dichlorobenzene	4.65	0.006	0.250
1,4-Dichlorobenzene	5.97	0.006	0.890
1,2-Dichlorobenzene	5.17	0.006	0.270
1,3,5-Trichlorobenzene	5.73	0.004	0.012
1,2,4-Trichlorobenzene	3.97	0.004	0.130
1,2,3-Trichlorobenzene	6.78	0.004	0.039
1,2,4,5-Tetrachlorobenzene	2.45	0.003	0.010
1,2,3,4-Tetrachlorobenzene	1.86	0.003	0.010
Pentachlorobenzene	1.19	0.004	0.038
Hexachlorobenzene	8.19	0.006	0.006

stirring rate of 1500 rpm was selected as the optimum.

In summary, the extraction efficiency of the present method is enhanced with agitation (stirring rate of 1500 rpm) and with the addition of salt (20%) to the aqueous sample.

3.2. Quantitative analysis

Linear calibration curves were obtained under the optimised conditions. Linearity was observed over the range 0.02–20 μg/l. Coefficients of correlation (r^2) were better than 0.991 except for HCB (0.988).

The limits of detection (LODs), defined at a signal-to-noise ratio (S/N) of 3, ranged from 0.003 to 0.006 μg/l (Table 1). When determining the LOD, fibre blanks and solution blanks were carried out every time to ensure that there was no sample carryover on the fibre and the background of the NaCl aqueous matrix that was spiked was satisfied. Compared with EPA method 8121 [1], except for HCB, the LOD achieved by headspace SPME is superior.

3.3. Water analysis

Natural water from a reservoir and tap water from a chemistry laboratory were analyzed using the method developed, by GC–MS-SIM. Trace levels of 1,2-DCB was detected in the reservoir water. The concentration was nearly at the limit of detection, 0.006 μg/l. It has previously been determined that tap water in Singapore was generally free of chlorinated benzene contamination [31]. The present results for this type of sample are consistent with this observation, although the present method was more sensitive than the previous method used (solid-phase extraction with GC–MS) [31].

Since the genuine water samples are free from contamination, reservoir and tap water, fortified with CBs, was analyzed to check the matrix effect on determination. Because SPME is a non-exhaustive extraction procedure, the relative recovery, which is determined as the peak area ratio of real sample and ultra pure water sample spiked with analytes at the same level (instead of absolute recovery as used in exhaustive extraction procedures), was employed. Results of relative recovery and RSD (%) of environmental water samples fortified at the 1.0 μg/l level are shown in Table 2. The data for reservoir and tap water samples with spiked analytes show that for all CBs, the relative recovery is higher than 90%, illustrating that the matrix in real samples, in our present context, has little effect on the analysis under the headspace-based SPME method. For the analyte detected, 1,2-DCB in reservoir water, the same conclusion could be deduced from the data obtained for its isomers.

Table 2
Summary of results from analysis of chlorinated benzenes in spiked reservoir and tap water samples[a]

Analyte	Reservoir water		Tap water	
	Relative recovery[b] (%)	RSD (%)	Relative recovery[b] (%)	RSD (%)
1,3-Dichlorobenzene	94.4	5.8	91.8	9.4
1,4-Dichlorobenzene	100.4	8.3	101.7	9.2
1,2-Dichlorobenzene	(103.3[c])	–	93.3	4.1
1,3,5-Trichlorobenzene	99.8	6.2	101.7	5.2
1,2,4-Trichlorobenzene	100.4	4.7	100.2	3.7
1,2,3-Trichlorobenzene	100.2	5.4	99.7	2.1
1,2,4,5-Tetrachlorobenzene	97.9	7.2	95.3	3.6
1,2,3,4-Tetrachlorobenzene	98.7	6.1	98.5	6.1
Pentachlorobenzene	99.6	3.8	104.5	7.2
Hexachlorobenzene	92.0	3.8	107.7	7.2

[a] Water samples containing 1.0 μg/l of each analyte.
[b] Mean values from three determinations.
[c] Natural samples contain 1,2-dichlorobenzene.

4. Conclusion

This paper describes a headspace SPME method that is applied to the analysis of 10 chlorinated benzenes in environmental water samples. The results of this study illustrate that the method provides high relative recoveries for all analytes with acceptable RSD (%) in the analysis of reservoir and tap water samples. This method is fast, simple and highly sensitive.

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