ISSN 1061-9348, Journal of Analytical Chemistry, 2017, Vol. 72, No. 8, pp. 890–896. © Pleiades Publishing, Ltd., 2017.

ARTICLES

Determination of Trace Amounts of Chlorobenzenes in Water Using Membrane-Supported Headspace Single-Drop Microextraction and Gas Chromatography–Mass Spectrometry[1]

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Received March 11, 2016; in final form, November 9, 2016

Abstract—A novel method based on the coupling of membrane-supported headspace single-drop microextraction with gas chromatography–mass spectrometry (GC–MS) is developed for the determination of chlorobenzenes in water samples. For the determination of five chlorobenzenes, a 15 μL toluene microdrop was placed inside the plastic membrane and exposed for 10 min for headspace extraction while stirring at 1000 rpm. The microdrop was then picked up by a microsyringe and directly injected into the injector block of the GC–MS instrument. Under the optimized operation conditions, the calculated calibration curves gave a high level of linearity for all targets with correlation coefficients range from 0.9945 to 0.9987. The limits of detection range from 0.01 to 0.05 μg/L and the RSDs for most of chlorobenzenes were below 7%. The method is simple, sensitive, and stable for single drop microextraction. Its applicability is demonstrated by the determination of chlorobenzenes in tap water samples.

Keywords: chlorobenzenes, membrane-supported, headspace single-drop microextraction, gas chromatography–mass spectrometry

DOI: 10.1134/S1061934817080135

Sample preparation is an important step in analytical process, especially when compounds must be determined at trace levels, since it requires both analyte preconcentration and sample matrix clean-up in order to separate potential interferences [1]. Among these techniques, liquid–liquid extraction, solid-phase extraction, solid-phase microextraction (SPME), liquid-phase microextraction (LPME) and single-drop microextraction (SDME) were developed [2]. Compared with SPME, SDME appears to have similar capabilities in terms of precision and efficiency, but it offers two distinct advantages. Firstly, the choice of solvents is wider than the limited number of phases currently available for SPME. Secondly, the cost of solvent is much lower than commercially available SPME fibers which makes SDME method more available [3]. Headspace SDME, in which the microdrop of high boiling extracting solvent is exposed to the headspace of a sample, is a development of the SDME. Compared with direct SDME, headspace SDME can shorten the time of extraction significantly because of the faster diffusion rate of the analytes in gaseous phase than in aqueous phase [4]. It can be used in any matrix since there is no direct contact between the sample and organic solvent. However, this method still has some limitations [3]. The biggest problem is the instability of the single-drop. Since the single-drop is suspended on the tip of the syringe, any slight movement or non-standard operation may result in the falling of the droplet, which affects the reproducibility of this method. Moreover, the sensitivity and the efficiency of SDME method need further improvement by prolonged extraction time and fast stirring rate. However, increasing these two main factors may result in drop instability.

Chlorobenzenes (CBs) are a class of environment pollutants used as industrial solvents, pesticides, dielectric fluids, deodorant and chemical intermediates. Their presence in the environment is a result of uncontrolled release of solid/liquid effluents as well as industrial atmospheric discharges. Because CBs are hazardous and persistent under the anaerobic conditions, they were ranked as priority pollutants by the US Environmental Protection Agency (EPA) [5]. Maximum allowed levels of CBs are 1,2-dichlorobenzene 0.6 mg/L, 1,4-dichlorobenzene 0.075 mg/L, 1,2,4-trichlorobenzene 0.07 mg/L) [6]. In light of this, developing a simple, inexpensive and efficient method to detect the trace level of CBs in water samples is very important. Headspace SDME is especially suitable to be used to determine CBs, as they can easily diffuse

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into headspace from a liquid matrix due to their high volatility. Several papers using headspace SDME technique to determine the CBs in water have been published in recent years [1, 7–10].

In the present study, a new microextraction technique termed membrane-supported headspace single-drop microextraction (MS-HS-SDME) was developed. In this method, a hemispherical membrane is used to hold the drop of the organic solvent, while the membrane is supported by a custom-made wire frame and exposed to the headspace of the sample. After the solution is stirred for a prescribed period of time, the membrane is withdrawn from the sample vial, the microdrop is extracted by a microsyringe and injected into the GC for quantification. The supporting part is made of a disposable plastic tube by removing its bottom, and the hemispherical tip is then used to support the extraction solvent. The objective of the present work is to study the applicability of the proposed MS-HS-SDME approach followed by GC–MS system for the determination of CBs in water samples.

EXPERIMENTAL

Reagents and materials. The five CBs considered in this work were: 1,2-dichlorobenzene (1,2-DCB), 1,3-dichlorobenzene (1,3-DCB), 1,4-dichlorobenzene (1,4-DCB), 1,2,4-trichlorobenzene (1,2,4-TCB), 1,2,4,5-tetrachlorobenzene (1,2,4,5-TeCB). All these CBs were obtained from Aldrich (Milwaukee, WI, USA). Toluene and methanol were obtained from TEDIA (Ohio, USA). All the compounds used in this study were of HPLC grade. Nanopure water was prepared on a water purification system (Millipore, Milford, MA, USA).

Standard stock solutions of 1000 mg/L target compounds with respect to five CBs were prepared in methanol and refrigerated at 4°C. Working solutions were freshly prepared by diluting the standard stock solution to a mixture of 50 μg/L with nanopure water. Tap water samples (directly potable) were freshly collected in the laboratory, after allowing the water to flow for at least 10 min, and directly analyzed by MS-HS-SDME coupled with GC–MS.

The material of SDME membrane used in the experiment was obtained from a disposable plastic tube (Fisherbrand, 5 mL, 12 × 75 mm, Mexico). The bottom part (2 cm) of the plastic tube was cut to a hemispherical shape and fixed on a small wire holder.

GC–MS analysis. All analyses were performed on a Shimadzu (Tokyo, Japan) QP 2010 gas chromatography–mass spectrometry system equipped with a 30 m × 0.25 mm 0.25 μm DB-5 MS capillary column (J&W Scientific, Folsom, CA). The injection temperature was maintained at 200°C and operated in the splitless mode. Helium (>99.999% pure) was used as the carrier gas at a flow-rate of 1.8 mL/min. The column oven was initially set at 50°C for 1 min, programmed to 106°C at a 8 grad/min rate, and finally increased to 240°C at 15 grad/min rate, where it was held for 2 min. The interface temperature was set at 240°C. A 5 min solvent cut time was allowed for all analyses. The ionization mode was electronic impact. A selected ion monitoring (SIM) mode was constructed for GC–MS acquisition and quantification. Prior to quantification in the SIM mode, the full scan mode (m/z 40–350) was used for identification of all target compounds based on their mass spectra and retention times. Overall, quantification was based on the following target ions (m/z): 1,3-DCB – 146; 1,4-DCB – 146; 1,2-DCB – 146; 1,2,4-TCB – 180; 1,2,4,5-TeCB – 216. The total time of a single GC run was 16.93 min.

Analytical procedure. In this MS-HS-SDME, in order to eliminate volatilization losses of CBs, all aqueous samples were freshly prepared before each headspace SDME extraction. The working solutions were prepared by diluting the standard stock solution to 50 μg/L with nanopure water in a 250 mL volumetric flask, and then 15 mL of the sample solution was transferred to a 20 mL volume crimp top glass vial with a septum using glass pipette. In the investigation of the influence of agitation, a 2 cm stirring bar was used. The membrane was prepared previously by removing the bottom 2 cm of a disposable plastic tube and fixing it to a wire holder by rubberized fabric. It should be precleaned in methanol and dried in air before use. The wire holder was affixed to the vial by piercing through the septum, and 15 μL toluene was added into the membrane as the extraction solvent. After the whole apparatus was clamped, the magnetic stirrer was turned on with stirring speed of 1000 rpm at room temperature (24°C, air-conditioned). After extracting for 10 min, the membrane holder was removed from the vial, 2 μL of the extraction solvent was withdrawn into a 10 μL microsyringe and injected into GC–MS system for analysis. Figure 1 shows the apparatus used for this method.

RESULTS AND DISCUSSION

The headspace microextraction theory indicates that analytes in headspace are transferred into the organic solvent which exposed in the membrane, a dynamic equilibrium is finally established between the concentration of the analytes in headspace and that of analytes in the organic solvent drop. The amount of the analyte, n, extracted by the microdrop at equilibrium is described by the following equation [11]:

$$n = \frac{k_{odw} V_d c_0 V_s}{k_{odw} V_d + k_{hs} V_h + V_s},$$

where k_{odw} and k_{hs} are the organic drop-water (sample) and the headspace-water distribution constants, respectively; c_0 is the initial concentration of the analyte in the matrix; and V_d, V_s and V_h are the volumes of the drop, the sample, and the headspace respectively. From this equation we can easily find the relationships

between the amount of extractant and other parameters such as k_{odw}, V_d, V_s and V_h. These parameters are very important for optimizing the extraction conditions.

In this study, we explored the applicability of MS-HS-SDME to the determination of CBs in aqueous matrices. The material of membrane and the effect of a number of variables including the type of solvent, the volume of solvent, the stirring rate and extraction time were examined.

Selection of membrane material. It is very important to select suitable membrane material for this newly developed method. In this experiment, 7 kinds of plastic membranes were investigated with various properties including 5 types of soft plastic film and 2 types of hard plastic film. In fact, most of the soft plastic membranes presented difficulties in placement onto the wire support. Finally, the bottom end of the disposable plastic tube was selected as a supporting membrane. Compared with other materials it has the following advantages: (1) does not react with the extraction solvent, (2) has enough strength to support the organic drop, (3) has enough hardness in case of being pierced by microsyringe tip when withdrawing the extractant, (4) is easy to make and get, (5) is inexpensive.

Selection of extraction solvent. Organic solvent is a very important factor in LPME. In general, the solvent should meet four requirements. Firstly, it should have a high boiling point and low vapor pressure in order to reduce the risk of evaporation. Secondly, the solvent should have good chromatographic behavior. Thirdly, its partitioning coefficient should be high. Finally, the solvent should be of high-purity [5]. Accordingly, high-purity toluene, cyclohexane and *n*-octanol were considered as extraction solvents. Among them, it is reported that octanol did not appear to be suitable chromatographically, because the solvent peak interfered with those of the target compounds [5]. Of the other solvents, toluene had lower vapor pressure (3786 Pa) than cyclohexane (12918 Pa) being more resistant to evaporation. Compared with cyclohexane, toluene is more compatible with the principle of "like dissolves like." Overall, toluene was selected as the extraction solvent.

Sample and organic drop volume. In general, increasing the amount of aqueous sample volume resulted in an increase of analytical signal for the reason of the larger amount of target pollutants transfer into the headspace. In addition to this, appropriately decreasing headspace volume also improves the sensitivity of extraction [12]. In general, for HS-SDME, the optimized condition is 4 mL vial with sample size of 3 mL and a headspace of 1 mL [13]. According to the above analysis, finally, 20 mL vials with sample volume of 15 mL and a headspace of 5 mL were used.

The organic drop volume is also a very important factor in this microextracion method. The theoretical relationship between the amount of analyte extracted and the organic drop volume is described by Eq. (1). The amount of analyte extracted by the microdrop is related to the volume of the drop, and the sensitivity improves as the volume of the drop increases. For the previous method of HS-SDME, the maximum volume of organic solvent is 3 μL because of the instability of the single drop [7]. In fact, the small volume of organic solvent limited the efficiency of this method [13]. Compared with HS-SDME, one feature of the developed method is increasing the volume of the solvent without concerning about the stability of the drop. Therefore, toluene volume from 10, 15, 20, 25 to 30 μL were exposed separately for 10 min at 24°C to the headspace of 15 mL aqueous solution spiked at 50 μg/L with all target analytes and stirred at 1000 rpm. Figure 2 shows that increasing the organic drop volume from 10 to 15 μL resulted in a dramatically increasing peak area for all the analytes except 1,2,4,5-TeCB that did not show much difference. However, a further increase of the toluene drop from 20 to 30 μL made the peak area decreased sharply for all the compounds. The unfavorable effect of larger organic drop volumes is attributed to insufficient equilibration time [11, 12]. In general, diffusion coefficients in the gas phase are much larger than the corresponding diffusion coefficients in condensed phases, and mass transfer in the headspace is a fast process [14]. Furthermore, during headspace SDME, headspace convection is induced due to stirring of the aqueous phase. Nonetheless, the microdrop is expected to be stagnant and consequently mass transfer into the drop is by diffusion alone, representing thus a slow step in the overall extraction procedure and explaining the extended equilibration times needed for larger organic solvent drops [8]. Based on these considerations and the experiment results, a volume of 15 μL toluene was used for all subsequent experiments.

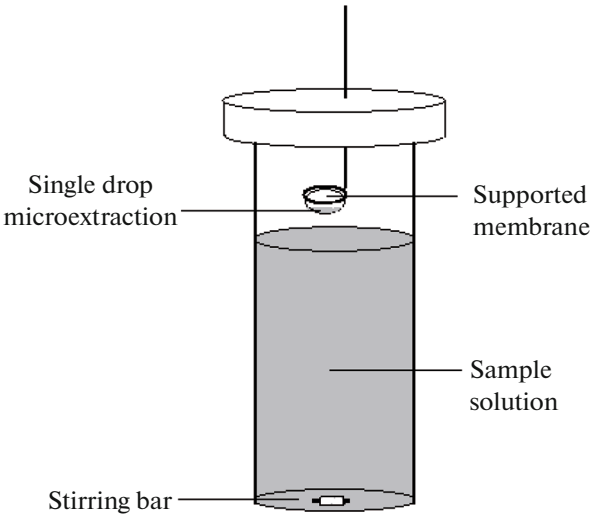

Fig. 1. Schematic diagram of the membrane-supported headspace single-drop microextraction apparatus.

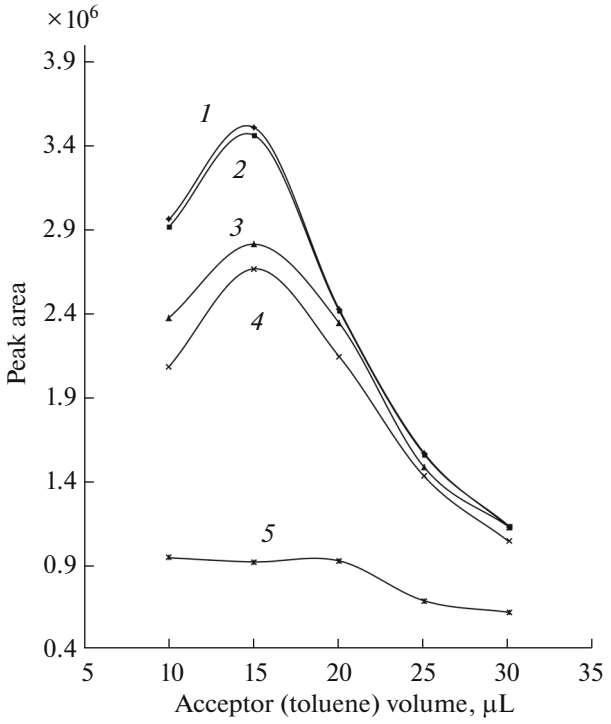

Fig. 2. Influence of acceptor volume on the relative peak area of chlorobenzenes; 50 μg/L concentration level; 15 mL aqueous sample in 20 mL glass vial; 1000 rpm stirring rate; 24°C; extraction time 10 min; injection volume 1.4 μL; *1*, 1,3-DCB; *2*, 1,4-DCB; *3*, 1,2-DCB; *4*, 1,2,4-TCB; *5*, 1,2,4,5-TeCB.

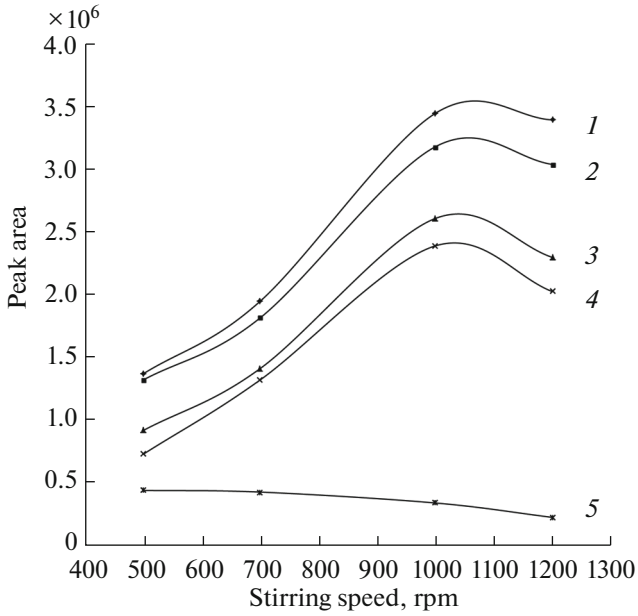

Fig. 3. Influence of stirring speed on the relative peak area of chlorobenzenes; 50 μg/L concentration level; 15 mL aqueous sample in 20 mL glass vial; 24°C; extraction time 10 min; acceptor (toluene) volume 15 μL; injection volume 2 μL; *1*, 1,3-DCB; *2,* 1,4-DCB; *3*, 1,2-DCB; *4*, 1,2,4-TCB; *5*, 1,2,4,5-TeCB.

Stirring rate. Agitation of the aqueous phase can lead to decreased extraction time and increase the extraction efficiency. Stirring the aqueous sample results in a degree of convection of the headspace and faster mass transfer of the analyte from the surface into the bulk of the drop [14]. Therefore, increasing the speed of sample agitation is expected to enhance the rate of extraction of all target analytes.

In a series of experiments, the effect of sample agitation speed on extraction efficiency was investigated. A 15 μL toluene drop was used each time to extract for 10 min, at 24°C, water samples containing 50 μg/L of all target analytes and stirred at different agitation rates (namely: 500, 700, 1000 and 1200 rpm). The peak areas obtained from different analytes under different stirring rate are shown in Fig. 3. As expected, most of the analytes (1,2-DCB, 1,3-DCB, 1,4-DCB, 1,2,4-TCB) showed an upward trend with increasing stirring speed except for 1,2,4,5-TeCB, which did not show much difference under different stirring speed. This may be due to the lower vapour pressure of 1,2,4,5-CB compared with the other CBs. For the first four CBs, results revealed that the extraction effect reached maximum at 1000 rpm and then decreased slightly at 1200 rpm (maximum speed of the magnetic stirrer). The decreased efficiency at 1200 rpm may result from the decreased amount of the extractant. Based on these observations, stirring rate of 1000 rpm was selected for further experiments.

Extraction time. In general, the amount of analyte transfer into the microdrop is expected to increase with increasing its exposure time to the headspace of the stirred sample solution. However, the HS-SDME is not an exhaustive extraction method and the analyte is partitioned between the bulk aqueous phase, the headspace, and the microdrop. Thus, the amount of analyte transferred into the microdrop can reaches its maximum when this equilibrium is established [15].

For the purpose of investigating the equilibrium time of extraction, 15 μL of toluene was exposed for 5, 8, 10, 12 and 15 min separately to extract water samples containing 50 μg/L of all target analytes and stirred at 1000 rpm. From the curves obtained (Fig. 4), it is seen that all the analytes showed an increasing trend from 5 to 10 min, and four kinds of CBs reached the highest point at 15 min. However, a problem that emerged after exposing 10 min was the high RSD values that were higher than 20% for 12 and 15 min, which reflected the bad repeatability of the extraction. It may result from the high volatile properties of chlorobenzenes, since a longer extraction time can result in significant solvent evaporation and make the results unstable. In order to obtain reliable results and decrease the drop evaporation, 10 min sampling time was finally selected. A similar way of selecting extraction time for the HS-SDME extraction of CBs

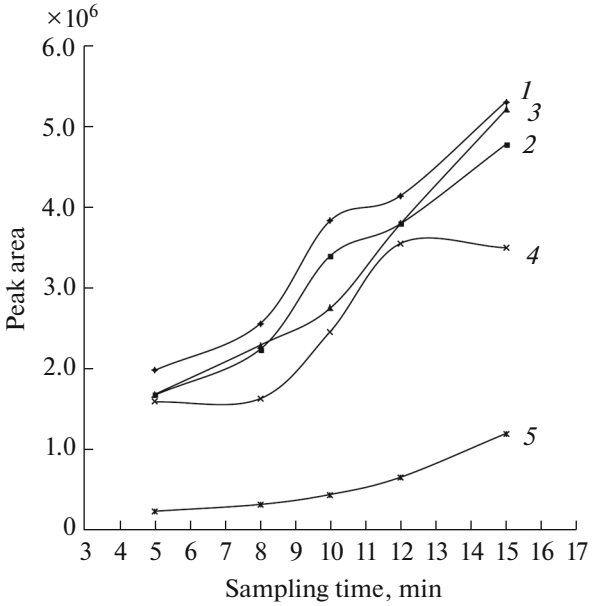

Fig. 4. Influence of sampling time on the relative peak area of chlorobenzenes; 50 μg/L concentration level; 15 mL aqueous sample in 20 mL glass vial; 24°C; 1000 rpm stirring rate; acceptor (toluene) volume 15 μL; injection volume 2 μL; *1*, 1,3-DCB; *2*, 1,4-DCB; *3*, 1,2-DCB; *4*, 1,2,4-TCB; *5*, 1,2,4,5-TeCB.

was also reported [7]. It is worth noting that, for the quantitative analysis, it is not necessary for the analytes to reach the equilibrium, but instead, to only allow a sufficient mass transfer into the microdrop in an exact reproducible extraction time [7].

Overall, the optimized extraction conditions found in the present studies are: a 15 μL toluene solvent exposed for 10 min to the headspace of a 15 mL aqueous sample placed in a 20 mL vial and stirred at 1000 rpm. A typical chromatogram obtained from extraction of 50 μg/L aqueous standard solution using the proposed MS-HS-SDME approach is shown in Fig. 5.

Evaluation of MS-HS-SDME performance. The performance of the proposed method was evaluated by spiking all target analytes in aqueous solution in five concentration levels (0.5, 5, 15, 30 and 50 μg/L) under optimized conditions. The calculated calibration curves give a high level of linearity for all target analytes with correlation coefficient (r^2) ranged between 0.9986 and 0.9945 except for 1,2,4,5-TeCB where the correlation coefficient was 0.9874 (Table 1). Furthermore, the repeatability of the proposed method evaluated by extracting the aqueous samples spiked at 5 μg/L with mixed target analytes and expressed as RSD varied between 4.0 and 6.2%. The limits of detection (LODs) for all target analytes were determined according to published guidelines at a signal-to-noise ratio (S/N) of three [16]. The LODs ranged from 0.01 to 0.05 μg/L except for 1,2,4,5-TeCB (0.11 μg/L) (Table 1) which significantly lower than the maximum allowed levels of CBs in drinking water standards of US EPA [6]. The limit of quantification (LOQ) for all target analytes were evaluated four times that of the LOD according to published guidelines [17] (Table 1).

The applicability of this extraction method to the real samples was investigated for spiked tap waters. Water samples were freshly collected from laboratory, after allowing the water to flow for at least 10 min. As a result of these analyses, tap water blank samples were free of chlorobenzenes contamination. The results are summarized in Table 2 which shows that relative recoveries range from 102 to 121%, and the RSD values range between 3.7 and 16%.

CONCLUSIONS

This work describes a new analytical method for determining trace level CBs in water samples. The low LOD values and high recoveries show the high sensi-

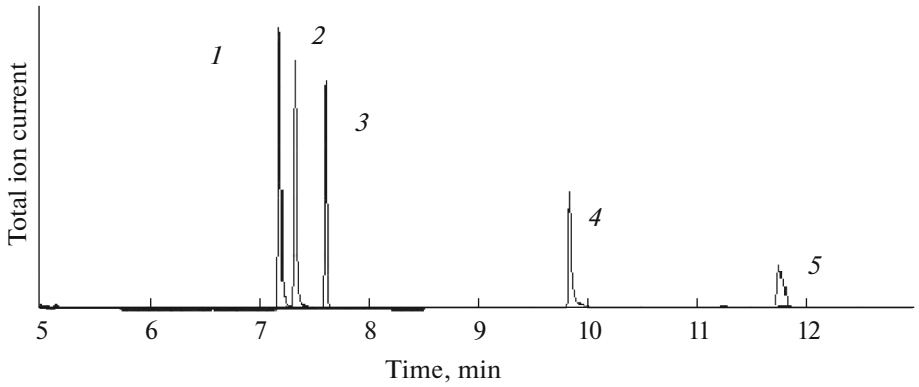

Fig. 5. Total ion current chromatogram obtained from MS-HS-SDME of five chlorobenzenes (50 μg/L) from a spiked nanopure water sample. Peaks: *1*, 1,3-DCB; *2*, 1,4-DCB; *3*, 1,2-DCB; *4*, 1,2,4-TCB; *5*, 1,2,4,5-TeCB. For GC–MS experimental details see GC–MS analysis section.

Table 1. Main method parameters for the extraction of chlorobenzenes from nanopure water samples using the optimized membrane-supported headspace SDME method

Analyte	Correlation coefficient (r^2)[a]	RSD[b], %	LOD[c], μg/L	LOQ[d], μg/L	LOD (EPA8121) [e], μg/L
1,3-DCB	0.9986	6.1	0.02	0.07	0.25
1,4-DCB	0.9987	5.8	0.02	0.09	0.89
1,2-DCB	0.9975	6.2	0.01	0.04	0.27
1,2,4-TCB	0.9945	4.3	0.05	0.20	0.13
1,2,4,5-TeCB	0.9874	4.0	0.11	0.44	0.01

[a]Linear range 0.5–50 μg/L (number of calibration points: 5).
[b]Mean value for three replicate analyses (intra-day, $n = 3$); spiking level 5 μg/L.
[c]LOD_s were calculated for a three signal to noise ratio ($S/N = 3$).
[d]LOQ_s were calculated for four times that of LOD [17].
[e]Data taken from reference [5, 7], and the LOD of EPA method 8121.

Table 2. Determination of chlorobenzenes in spiked tap water ($n = 3$)

Analyte	Added, μg/L	Found, μg/L	Recovery, %	RSD[a], %
1,3-DCB	15.00	16.22	108.1	8.8
1,4-DCB	15.00	15.30	102.0	6.8
1,2-DCB	15.00	15.34	102.3	11
1,2,4-TCB	15.00	15.29	101.9	3.7
1,2,4,5-TeCB	15.00	18.22	121.5	16

[a]RSD, inter-day.

tivity and efficiency of developed method. In general, there are many advantages of this method, including: (1) minimal solvent use and wide selection of available solvents, (2) simplicity and easy use of the apparatus, (3) low cost of apparatus (compared to SPME fibers), (4) short preconcentration time (the whole extraction process only takes 10 min compared with 30 min for the SPME method), (5) high sensitivity and low detection limit, (6) good precision. In fact, this MS-HS-SDME method is developed on the basis of HS-SDME and appears to have similar capabilities in terms of precision and speed of analysis, but this new method appears to offer two distinct advantages over the HS-SDME. First of all, it overcomes the problem of drop instability since the drop hold in the membrane is very stable even in high stirring speed, and the fast stirring rate can enhance the efficiency of extraction. HS-SDME needs very careful operation because of the instability of the single drop, but the newly developed method shows a strong stability of the single-drop making the operation easier.

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