Iranian J Publ Health, Vol. 38, No.4, 2009, pp.103-110

Original Article

Measurement of Chloroform in Swimming Pools' Waters and Swimmers' Blood

S Shegefti, *H Sereshti, S Samadi

School of Chemistry, University College of Science, University of Tehran, Iran

(Received 14 Apr 2009; accepted 20 Jul 2009)

Abstract

Background: A new microextraction method named dispersive liquid-liquid microextraction (DLLME) for determination of chloroform in pool water and blood of swimmers after swimming is described.

Methods: This method was performed based on coupling dispersive liquid-liquid microextraction (DLLME) with gas chromatography-mass spectrometry (GC-MS). Methanol and trichloroethylenes were used as the disperser solvent and the extraction solvents, respectively. The volumes of these solvents were optimized for pool water by central composite design. The study involved three indoor swimming pools and nine swimmers.

Results: Chloroform concentration of pool water was 118-135 μg L^{-1} and of blood ranged from 1.26 to 1.66 μg L^{-1}.

Conclusion: Indoor swimming pools are closed environments presenting detectable levels of trihalomethanes (THMs). Chloroform ($CHCl_3$) is the most represented THMs. Therefore, the presence of $CHCl_3$ may be considered representative of the THMs. The new method DLLME was applied for determination of $CHCl_3$ in pool water and blood of swimmers after swimming inside the indoor swimming pool. The method was optimized by experimental design. Chloroform concentrations in the specified pool waters were 135, 124, 118 μg L^{-1}.

Keywords: *Dispersive liquid-liquid microextraction, Chloroform, Swimming pool, Gas chromatography-mass spectrometry, Chemometrics*

Introduction

Swimming and bathing are popular and healthy leisure activities. Pool waters are recycled for a long period of time and are continuously polluted by swimmers who are sources of organic compounds and microorganisms. Therefore, continuous pool water disinfection is needed to minimize the risk of microbiological pollution and to avoid outbreaks of waterborne diseases. Chlorine-based disinfection compounds are commonly used in the disinfection of swimming pool water (1).

Since 1974, some chlorination by-products have been discovered in chlorinated water as a result of reaction between the disinfectant and organic contaminants in the water. They are mainly trihalomethanes (THMs) including chloroform, bromodichloromethane, dibromochloromethane, bromoform and other volatile and non-volatile compounds. Chloroform generally occurs at the greatest concentrations (2). Being highly volatile, chloroform can be found in the airspace over the water. Therefore, it can be taken up by swimmers over the skin, by swallowing of water, or by inhalation from air (2, 3).

As chloroform is a toxic and possibly carcinogenic substance, and prevalent among THMs, most studies on environmental and biological monitoring of THMs exposure in indoor swimming pools have considered chloroform exposure representative of total exposure to chlorination by-products (1).

A number of analytical methods have been reported for the analysis of THMs in water such as direct aqueous injection (4, 5), liquid–liquid extraction (LLE) (6), headspace technique (7, 8), purge and trap technique (9,10), liquid-phase microextraction (LPME) technique (11, 12) and solid-phase microextraction (SPME) technique (13-15). Direct aqueous injection has problems with column stability and critical temperatures for column and injector. Conventional liquid-liquid extraction

***Corresponding author:** Tel: +98 21 61113632, Fax: +98 21 66495291, E- mail: shegefti_85@khayam.ut.ac.ir

(LLE) is laborious and time-consuming, expensive, and apt to form emulsions; it requires the evaporation of large volumes of solvents and the disposal of toxic or flammable chemicals. The fact that in headspace method an aliquot of vapor with only a portion of the total volatile samples is used, leads to concern about sensitivity. Purge and trap is more time consuming technique and requires special instrument (14). Liquid-phase microextraction (LPME) was developed as a solvent-minimized sample pretreatment method, which uses very little toxic organic solvents (16, 17). However, some disadvantages of this method are fast stirring which may cause break up the organic solvent drop and air bubble formation (18); it is time-consuming and in most cases equilibrium is not attained even after a along time (17). For SPME, despite that it combines extraction and preconcentration in one step, difficulties in automation, sample stirring, temperature control and fiber aging, limited fiber life, insufficient diversity of commercially available fiber coatings, fiber breakage, stationary-phase bleeding, competitive absorption, and the relatively high cost of fibers, have been reported by users of SPME (19,20).
Recently, Assadi and coworkers have developed a simple and rapid preconcentration and microextraction method, dispersive liquid-liquid microextraction (DLLME) (21, 22).
This method consists of two steps: (a) injection of the mixture of extraction and disperser solvents into aqueous sample solution. (b) The centrifugation of cloudy solution. Determination of analytes in organic phase can be performed by GC-MS (23).
The aim of this study was to introduce DLLME combined with GC-MS for determination of $CHCl_3$ in the blood of the swimmers after swimming inside the indoor swimming pool. A central composite design (CCD) was used to optimize the method.

Materials and Methods

Chemicals and standards

Chlorobenzene, tetrachloroethylene, trichloroethylene, carbon disulfide, dichloromethane, acetonitrile, ethanol, methanol, chloroform, with the purity higher than 99% and perchloric acid 72% were supplied by Merck chemical company (Merck, Darmstadt, Germany). A chloroform stock standard solution was prepared at concentration of 1000 mg L^{-1} in methanol and stored at 4 °C. Intermediate standard solutions were prepared by diluting the stock solution in methanol. Working standards were prepared at the μg L^{-1} level by spiking known amounts of the intermediate standards into bidistilled water and blank serum samples.

Instrumentation

Solvent selection and optimization experiments were carried out on a Shimadzu-17A gas chromatograph equipped with a flame ionization detector (FID) and a DBP-5 capillary fused silica column (25 m; 0.25 mm I.D.; 0.22 μm film thickness; methyl 5% phenyl polysiloxane). The oven temperature was held at 50 ºC for 10 min. Other operating conditions were as follows: carrier gas, He (99.999%); inlet pressure, 72 kPa; with a linear velocity of 20 cm/s; injector temperature, 200 ºC; detector temperature, 250 ºC; split ratio, 1:20.
GC-MS analyses were performed on a HP-6890 GC system coupled with a 5973 network mass selective detector and equipped with a HP5-MS capillary fused silica column (60 m; 0.25 mm I.D.; 0.25 μm film thickness; methyl 5% phenyl polysiloxane). The oven temperature program initiated at 50 ºC, held for 10 min. Other operating conditions were as follows: carrier gas, He (99.999%); with a flow rate of 1 mL/min; injector temperature, 250 ºC; split ratio, 1:20. Mass spectra were taken at 70 eV. Mass range was from m/z 20–500 amu. Both injections into GC-FID and GC-MS were carried out using one 1μl microsyringe model Hamilton 7001. Centrifuges were performed by Hermle Z 200 A centrifuge instrument.

Blood and water samples

Data were collected under regular pool conditions (without additional contamination) at three swimming pools in Tehran, Iran. Water samples were collected at a depth of near the edge of the pool in amber glass vials and stored at 4 °C. The blood samples were kindly supplied by nine swimmers

in three indoor swimming pools. Samples were collected 15-20 min after the bath activity by a clinical analysis laboratory. About 2 ml serum was obtained form each sample and stored at -20 °C. The frozen samples were left at room temperature until completely thawed. After gentle mixing, serum was transferred into the analysis vials.

Dispersive liquid-liquid microextraction procedure

For pool water samples, 1 ml of each sample was placed in a 10 ml screw cap glass tube with conic bottom, and 0.39 ml of methanol (as disperser solvent) containing 20 µl trichloroethylene (as extraction solvent) was injected rapidly into each sample solution using a 1.0 ml syringe. The mixture was centrifuged for 3 min at 4500 rpm using the centrifuge. The dispersed fine particles of extraction solvent separated and settled at the bottom of conical tube. 0.5 µl of the separated phase was removed using a 1.0 µl-micro syringe and injected into the GC-MS.

Because of the different and complicated matrix of blood, for blood samples, 1 ml of serum was diluted with 4 ml deionized water. 1 ml of diluted solution was placed in a 10 ml closed vessel (centrifuge tube with cover). Then 0.5 ml perchloric acid was added for deproteinization of serum (24) and centrifuged for 3 min at 4500 rpm. The supernatant was collected in another centrifuge tube with conic bottom. Then, 0.3 ml of methanol (disperser solvent) containing 50 µl trichloroethylene (extraction solvent) was injected rapidly into each sample tube using a 1.0 ml syringe. The mixture was centrifuged for 3 min at 4500 rpm using the centrifuge. Finally, 0.5 µl of the separated phase was removed using a 1.0 µl microsyringe and injected into the GC-MS.

Results

The peak area as the extraction efficiency for each solvent was presented in Fig. 1. The data indicated that trichloroethylene gave the best efficiency. Therefore, it was selected as the extraction solvent.

Table 1 shows the factors and the corresponding symbols and levels. The CCD with two blocks, including the factors, their levels, and the result from each run, is shown in Table 2.

Various statistical data (sum of squares, degrees of freedom, F and *P* values) were calculated (Table 3). Experimental results and the predicted values obtained using model (Eq. (1)) are given in Fig. 2. Fig. 2 shows that in the range of 0.30-0.50 ml of disperser solvent volume, at first the extraction efficiency increases and then decreases by increasing the volume of disperser. It seems at a low volume of methanol, cloudy state is not formed well, thereby, the response decreases. Finally, optimum conditions obtained using optimization mode of software package, Design-Expert 7.1.3 for further examinations (Table 4). As can be seen in Table 4, there was a good agreement between the calculated and experimental responses.

Analysis of real samples

Pool water samples

The optimized DLLME method was applied to the determination of chloroform in pool water samples. A typical GC-MS chromatogram of a sample from the pool is given in Fig. 3. The concentrations of chloroform found in pool water were summarized in Table 5. Calibration was performed by the external standard method.

Discussion

The main point for selection of disperser solvent is its miscibility in the organic phase (extraction solvent) and aqueous sample solution. Acetonitrile, ethanol, and methanol were assayed for this purpose. The results showed that, by using methanol as disperser solvent, the maximum efficiency was obtained (Fig. 1). Samples were analyzed by GC-FID.

Optimization of DLLME using central composite design

The experimental design techniques commonly used for process analysis and modeling are the full factorial, partial factorial and central composite design. A full factorial requires at mul-

tilevel many experiments. A partial factorial design requires fewer experiments than a full factorial. The central composite design gives almost as much information as a multilevel factorial, requires much fewer experiments than a full factorial and has been shown to be sufficient to describe the majority of steady-state process responses (25). CCD consists of a 2^f (2^2=4) factorial runs, $2f$ (2×2=4) axial or star runs and n center runs. Eight replicates at the center point of the design permit to calculate the experimental error of the process (26). The a value (axial spacing) is fixed at 1.414 to enable orthogonality and rotatability of the design. Therefore, the complete design consisted of 16 randomly-performed experiments in order to minimize the effect of uncontrolled variables.

The statistical significance of the quadratic model predicted was evaluated by the analysis of variance (ANOVA) technique. The ANOVA consists in determining which of the factors significantly affect the response variables in study, using a Fisher's statistical test (*F*-test). Effects with less than 95% of significance that is, effects with a *P*-value higher than 0.05, were discarded and pooled into the error term (often called residual error) and a new analysis of variance was performed for the reduced model. The significance of the model can be evaluated by considering either the *F*-values or the *P*-values of the model and of the lack of fit (27). The Model *F*-value of 21.35 implies the model is significant. There is only a 0.01% chance that a "Model *F*-Value" this large could occur due to noise. The "Lack of Fit *F*-value" of 3.36 implies the Lack of Fit is not significant relative to the pure error. There is a 9.62% chance that a "Lack of Fit *F*-value" this large could occur due to noise. As shown in Table 3, effects of E, D and D^2 terms were statistically significant, whereas the blocks were insignificant.

Table 1: Factor levels used in the central composite design

Factor	Symbol	Levels				
		-a	-1	0	+1	+a
Volume of extraction solvent (µl)	E	16	20	30	40	44
Volume of disperser solvent (mL)	D	0.26	0.30	0.40	0.50	0.54

Table 2: Design matrix and responses for the central composite design

Run	Block	E	D	Response
1	1	0	0	71
2	1	+1	-1	63
3	1	0	0	69
4	1	0	0	74
5	1	+1	+1	52
6	1	0	0	68
7	1	-1	-1	79
8	1	-1	+1	75
9	2	0	0	75
10	2	0	0	68
11	2	+1.414	0	49
12	2	0	0	73
13	2	-1.414	0	87
14	2	0	0	70
15	2	0	+1.414	49
16	2	0	-1.414	58

Extraction conditions: 1 ml standard solution of chloroform; centrifuge for 3 min at 4500 rpm; 0.5 µl injection volume to GC-FID.

Table 3: Analysis of variance table (ANOVA) for response surface quadratic model

Source	Sum of squares	df [a]	Mean square	F value [b]	*P*-value prob > F [c]	Significance
Block	30.25	1	30.25	2.03	0.1880	not significant
Model	1589.70	5	317.94	21.35	< 0.0001	significant
E	1075.09	1	1075.09	72.18	< 0.0001	
D	96.10	1	96.10	6.45	0.0317	
ED	12.25	1	12.25	0.82	0.3881	
E^2	0.12	1	0.12	8.392×10^{-3}	0.9290	
D^2	406.12	1	406.12	27.27	0.0005	
Residual	134.05	9	14.89			
Lack of fit [d]	84.05	3	28.02	3.36	0.0962	not significant
Pure error	50.00	6	8.33			
Cor total	1754.00	15				

[a] The degrees of freedom.

[b] The F Value for a term is the test for comparing the variance associated with that term with the residual variance. It is the Mean Square for the term divided by the Mean Square for the Residual.

[c] The probability value associated with the F Value.

[d] The portion of the residual SS that is due to the model not fitting the data.

Table 4: Optimum response and the corresponding levels

E(µl)	D(mL)	Optimum response	Experimental response [a]	RSD% [b]
20	0.39	83	80	3.84

[a] Mean value of three measurements.
[b] Relative standard deviation of 8 measurements.

Table 5: Chloroform values in water samples of three indoor swimming pools and blood samples of nine swimmers after swimming

Water (µg L^{-1})		Blood (µg L^{-1})			
Subject	**Mean (%RSD)[a]**	**Subject**	**Mean (%RSD)[a]**	**Subject**	**Mean (%RSD)[a]**
Pool 1	135 (6.3)	Swimmer 1	1.26 (8.5)	Swimmer 6	1.37 (1.3)
Pool 2	124 (2.9)	Swimmer 2	1.33 (1.4)	Swimmer 7	1.30 (4.1)
Pool 3	118 (5.1)	Swimmer 3	1.66 (2.5)	Swimmer 8	1.66 (2.9)
		Swimmer 4	1.42 (4.0)	Swimmer 9	1.64 (5.6)
		Swimmer 5	1.52 (4.4)		

[a] Relative standard deviation, n=3.

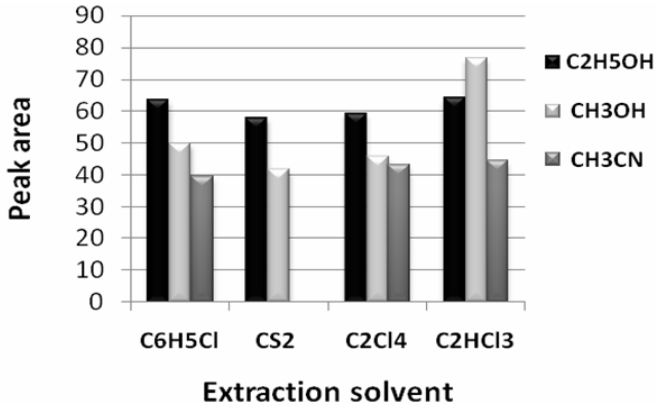

Fig. 1: Effect of various extraction and disperser solvents on the extraction efficiency. Extraction conditions: sample volume, 1 mL; dispersive solvent volume, 0.3 mL; extraction solvent, 20 µL. Two phase system was not observed by carbon disulfide and acetonitrile.

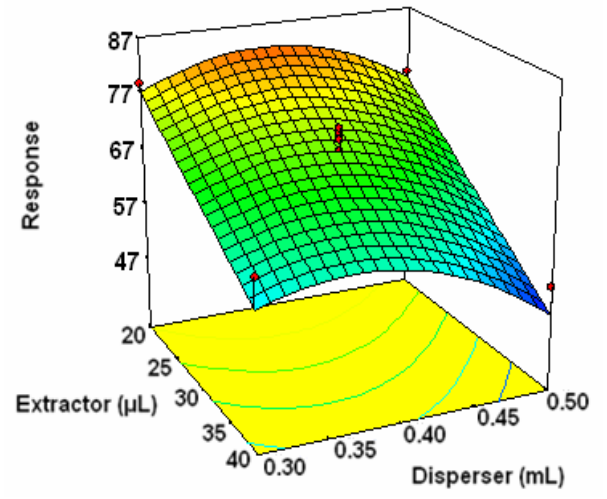

Fig. 2: Response surface for endosulfan extraction

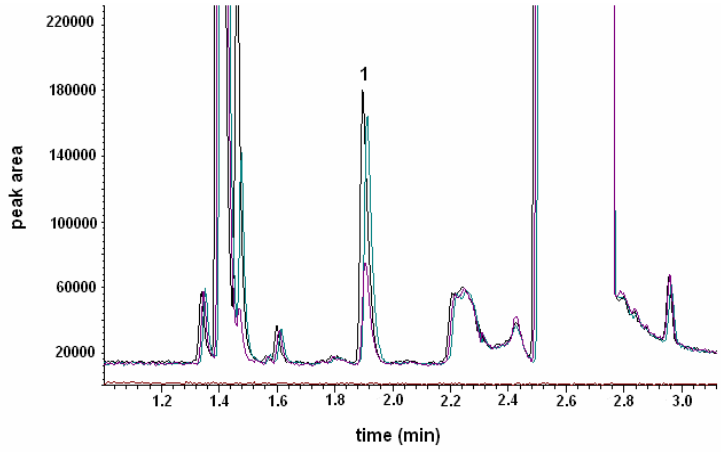

Fig. 3: A typical GC-MS chromatogram of a sample from the pool. (1) chloroform

Acknowledgements

This research was funded by the Deputy for Research of University of Tehran. The authors declare that they have no conflicts of interest.

References

1. Fantuzzi G, Righi E, Predieri G, Ceppelli G, Gobba F, Aggazzotti G (2001). Occupational exposure to trihalomethanes in indoor swimming pools. *Sci Total Environ,* 264: 257-65.
2. Erdinger L, Kühn KP, Krisch F, Feldhues R, Fröbel T, Nohynek B, Gabrio T (2004). Pathways of trihaomethan uptake in swimming pools. *Int J Hyg Environ Health*, 207: 571-75.
3. Aggazzotti G, Fantuzzi G, Righi E, Predieri G (1998). Blood and breath analyses as biological indicators of exposure to trihalomethanes in indoor swimming pools. *Sci Total Environ,* 217: 155-63.
4. Biziulk M, Namieśnik J, Czerwiński J, Gorlo D, Makuch B, Janicki W, Polkowska Z, Wolska L (1996). Occurrence and determination of organic pollutants in tap and surface waters of the Gdańsk district. *J Chromatogr A,* 733: 171-83.
5. Wolska L, Olszewska C, Turska M, Zygmunt B, Namieśnik J (1998). Volatile and semivolatile organo-halogen traces analysis in surface water by direct aqueous injection GC-ECD. *Chemosphere,* 37(13): 2645-51.
6. Nikolaou AD, Lekkas TD, Golfinopoulos SK, Kostopoulou MN (2002). Application of different analytical methods for determination of volatile chlorination by-products in drinking water. *Talanta,* 56: 717-26.
7. Golfinopoulos SK, Lekkas TD, Nokolaou AD (2001). Comparison of methods for determination of volatile organic compounds in drinking water. *Chemosphere*, 45: 275-84.
8. Kuivinen J, Johnsson H (1999). Determination of trihalomethanes and some chlorinated solvents in drinking water by headspace technique with capillary column gas-chromatography. *Water Res,* 33(5): 1201-8.
9. Zoccolillo L, Amendola L, Cafaro C, Insogna S (2005). Improved analysis of volatile halogenated hydrocarbons in water by purge-and-trap with gas chromatography and mass spectrometric detection. *J Chromatogr A*, 1077: 181-7.
10. Allonier AS, Khalanski M, Bermond A, Camel V (2000). Determination of trihalomethanes in chlorinated sea water samples using a purge-and-trap system coupled to gas chromatography. *Talanta*, 51: 467-77.
11. Zhao RS, Lao WJ, Xu XB (2004). Headspace liquid-phase microextraction of trihalomethanes in drinking water and their gas chromatographic determination. *Talanta*, 62: 751-6.
12. Tor A, Aydin ME (2006). Application of liquid-phase microextraction to the analysis of trihalomethanes in water. *Anal Chim Acta*, 575: 138-43.
13. Stack MA, Fitzgerald G, OConnell S, James KJ (2000). Measurement of trihalomethanes in potable and recreational waters using solid phase micro extraction with gas chromatography-mass spectrometry. *Chemosphere*, 41: 1821-26.
14. Cho DH, Kong SH, Oh SG (2003). Analysis of trihalomethanes in drinking water using headspace-SPME technique with gas chromatography. *Water Res*, 37: 402-8.
15. San Juan PM, Carrillo JD, Tena MT (2007). Fibre selection based on an overall analytical feature comparison for the solid-phase microextraction of trihalomethanes from drinking water. *J Chromatogr A*, 1139: 27-35.
16. Lambropoulou DA, Psillakis E, Albanis TA, Kalogerakis N (2004). Single-drop microextraction for the analysis of organophosphorous insecticides in water. *Anal Chim Acta,* 516: 205-11.

17. Ahmadi F, Assadi Y, Milani Hosseini SMR, Rezaee M (2006). Determination of organophosphorus pesticides in water samples by single drop microextraction and gas chromatography-flame photometric detector. *J Chromatogr A,* 1101: 307-12.
18. Shen G, Lee HK (2002). Hollow fiber-Protected liquid-phase microextraction of triazine herbicides. *Anal Chem,* 74(3): 648-54.
19. Vora-adisak N, Varanusupakul P (2006). A simple supported liquid hollow fiber membrane microextraction for sample preparation of trihalomethanes in water samples. *J Chromatogr A,* 1121: 236-41.
20. Stashenko EE, Martinez JR (2007). Sampling volatile compounds from natural products with headspace/solid-phase micro-extraction. *J Biochem Biophys Methods,* 70: 235-42.
21. Rezaee M, Assadi Y, Milani Hosseini MR, Aghaee E, Ahmadi F, Berijani S (2006). Determination of organic compounds in water using dispersive liquid–liquid microextraction. *J Chromatogr A,* 1116: 1-9.
22. Berijani S, Assadi Y, Anbia M, Milani Hosseini MR, Aghaee E (2006). Dispersive liquid-liquid microextraction combined with gas chromatography-flame photometric detection very simple, rapid and sensitive method for the determination of organophosphorus pesticides in water. *J Chromatogr A*, 1123: 1-9.
23. Rahnama Kozani R, Assadi Y, Shemirani F, Milani Hosseini MR, Jamali MR (2007). Part-per-trillion determination of chlorobenzenes in water using dispersive liquid–liquid microextraction combined gas chromatography-electron capture detection. *Talanta*, 72: 387-93.
24. Bohrer D, do Nascimento PC, Pomblum SG (1998). Deproteinization of blood serum by acid treatment and microwave irradiation for the determination of aluminium by electrothermal atomic absorption spectrometry. *J Anal At Spectrom*, 13: 635-39.
25. Zhang X, Wang R, Yang X, Yu J (2007). Central composite experimental design applied to the catalytic aromatization of isophorone to 3, 5-xylenol. *Chemom Intell Lab Syst*, 89: 45-50.
26. Armenta S, Garrigues S, de la Guardia M (2006). Optimization of transmission near infrared spectrometry procedures for quality control of pesticide formulations. *Anal Chim Acta,* 571: 288-97.
27. Rosa PAJ, Azevedo AM, Aires-Barros MR (2007). Application of central composite design to the optimisation of aqueous two-phase extraction of human antibodies. *J Chromatogr A,* 1141: 50-60.