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Determination of substituted benzenes in water samples by fiber-in-tube liquid phase microextraction coupled with gas chromatography

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Abstract

A method for determination of toluene, ethylbenzene, *p*-xylene, *o*-xylene, 1,3,5-trimethylbenzene and 1,2,4-trimethylbenzene in water samples was developed by a fiber-in-tube liquid phase microextraction technique (fiber-in-tube LPME) coupled with GC-flame ionization detector (FID). The method used a tube packed with polytetrafluoroethylene (PTFE) fibers as an extraction medium, improving the stableness of the solvent and the performance of extraction. Certain amounts of curled PTFE fibers were packed into a section of PTFE tube. Because the fibers were curled, they formed network structure in the tube. The fiber packed tube was firstly immersed into organic solvent to be filled with organic solvent and then was exposing to an aqueous solution to extract the target compounds. The extract was then retracted by a conventional GC microsyringe and analyzed by GC-FID. Extraction of the analytes in 8 ml aqueous solution for 15 min yielded enrichment factors of 224–361. The precision (R.S.D., $n = 5$) was 3.6–8.1% for peak area. The limit of detection (LOD, S/N = 3) for the six substituted benzenes were in the range of 0.3–5.0 $\mu g\,l^{-1}$.

Keywords: Liquid phase microextraction; Substituted benzenes; Gas chromatography

1. Introduction

Liquid–liquid extraction (LLE) is widely used and prescribed in many standard analytical methods [1], but it is a time-consuming, generally labor-intensive operation and requires use of large amounts of toxic and expensive high-purity organic solvent. To overcome these drawbacks, many new techniques, such as solid phase extraction (SPE) [2,3], supercritical fluid extraction (SFE) [4,5], solid phase microextraction (SPME) [6–8] and liquid phase microextraction (LPME), have been developed as an alternative to traditional LLE for sample preparation [9,10].

SPME is a solvent-free sample preparation technique introduced by Arthur and Pawliszyn [6] and has been used increasingly over the past decade. In its classical format, a thin coating attached to the surface of a solid support (fiber) is employed as the extractant phase [7]. Then, the fiber is exposed to an aqueous solution or the headspace of the samples to absorb the analytes. The analytes sorbed on the coating are then desorbed by thermal desorption for the analysis. In recent years, in-tube SPME has also been developed [11–13]. In-tube SPME generally uses an open tubular fused-silica capillary column as the SPME device instead of SPME fiber and organic solvent to desorb the sorbed compounds. More than a decade after its introduction, the main problems commonly encountered with SPME include the limited lifetime of the SPME fibers, their relatively fragile nature and the possibility of carry-over between analyses.

Liquid phase microextraction (LPME) is another type of microextraction mode. Compared with SPME, LPME had not the limitation of fiber connatural properties and reduced the possibility of carry-over. LPME has several different operational ways, such as a drop LPME [14,15], hollow fiber protected LPME including static and dynamic modes [16,17] and liquid–liquid–liquid microextraction (LLLME) [18,19]. A drop LPME was performed by suspending a microliter drop of organic solvent on the tip of either a Teflon rod or the

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needle tip of a microsyringe immersed in the stirred aqueous solution. The major problem of a drop LPME is that the microdrop suspended on the needle of microsyringe is easily dislodged by the stirred aqueous sample. The use of a hollow fiber (usually polypropylene hollow fiber) was introduced into liquid phase microextraction. The hollow fiber unit served as the "holder" and "protector" of organic solvent and increased the extraction efficiency owing to its porous property. In LLLME, the analyte was extracted through a thin phase of organic solvent inside the pores of a polypropylene hollow fiber and finally into an acceptor solution inside the hollow fiber or within the syringe.

More recently, a novel miniaturized sample preparation method, fiber-in-tube solid phase extraction (FIT-SPE) or fiber-in-tube solid phase microextraction, has been developed by Jinno and co-workers [20,21]. Wires or fibers were longitudinally packed into a short capillary as the extraction adsorption medium. FIT-SPE has been on-line coupled to microcolumn separations.

The purpose of this work was to develop a fiber-in-tube LPME technique coupled with GC-FID for determination of toluene, ethylbenzene, *p*-xylene, *o*-xylene, 1,3,5-trimethylbenzene (1,3,5-TMB) and 1,2,4-trimethylbenzene (1,2,4-TMB) in water samples. In the developed fiber-in-tube LPME technique, a polytetrafluoroethylene (PTFE) tube packed with PTFE fibers was used as an extraction medium to increase the stableness of solvent and the adsorption ability of PTFE fibers was employed to improve the microextraction performance. The effects of fiber quantity, extraction time, agitation and addition of salt were investigated in detail.

2. Experimental

2.1. Materials and chemicals

PTFE fibers (20 μm diameter, curled, Tongchuang Co., Beijing, China) and PTFE tube with 2 mm i.d. and 3 mm o.d. (Tianjin 9th Factory for Plastics, Tianjin, China) were employed for the fiber-in-tube LPME. All chemicals used were of analytical grade. Doubly deionized water (DDW, 18.2 M Ω cm^{-1}) was obtained from a WaterPro Water Purification System (Labconco Corporation, Kansas City, MO, USA). Stock solutions of toluene, ethylbenzene, *p*-xylene, *o*-xylene, 1,3,5-TMB and 1,2,4-TMB (4000 mg l^{-1}) were prepared in methanol. Working standard solutions were prepared by diluting the standard solutions with DDW just before use.

2.2. Instrumentation

A Shimadzu GC-9A system equipped with a flame ionization detector (FID) was used for all experiments. Separations were performed on a 25 m long × 0.24 mm i.d. capillary column (SE-52, Shimadzu, Japan). 99.99% nitrogen (BOC Gases Co. Ltd., Tianjin, China) was used as carrier gas at a flow rate of 50 ml min^{-1}. All injections were made in the split mode. The detector temperature was set at 200 °C. The following temperature program was employed: 40 °C for 3 min, 2 °C min^{-1} to 60 °C, held for 4 min. A Model 85-1 stir plate (Jintan Instruments Co. Ltd., Jintan, Jiangsu) and a Teflon-coated stir bar (9.9 mm × 5.9 mm × 5 mm) were used for agitation.

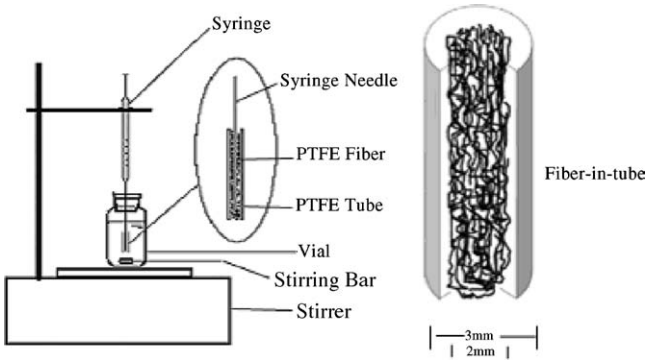

Fig. 1. Schematic setup for the proposed fiber-in-tube LPME system.

2.3. Extraction procedures

The fiber-in-tube LPME device is illustrated in Fig. 1. Eight millilitres of sample solution was filled into a 10 ml vial with a silicon septum and then a stir bar was placed into the vial. A conventional 10 μl microsyringe (Shanghai Gaoxing Glassware Instruments, Shanghai, China) designed for GC was adopted to support the tube. The PTFE fibers were sonicated for 2 min in acetone to remove contaminants before use. Certain amounts of curled PTFE fibers were packed into the tube using a nipper designed for GC. Then the fiber packed tube was immersed in hexane for a few seconds. It could be observed that hexane immediately permeated the fiber when the fiber packed tube just touched the solvent. The microsyringe needle pierced the silicon septum and was inserted into the fiber packed tube, which was subsequently placed into the aqueous sample. The magnetic stirrer was switched on to start the extraction. After a period of time, the solvent in the fiber packed tube was retracted into the syringe. A 2 μl aliquot of the extract was injected into the GC for analysis. The PTFE fibers are either disposable for one extraction or used for repetitive extractions after washing with acetone.

3. Results and discussion

3.1. Choice of organic solvent

The organic solvent used is crucial to the performance of the present fiber-in-tube LPME. The choice of the organic solvent should consider following factors. First, the solvent must have good affinity for target compounds and be compatible with the PTFE fiber. Second, it should have a low

Table 1
The EF for the target analytes obtained with different solvents (octanol, chloroform and hexane)

Analytes	Octanol	Chloroform	Hexane
Toluene	24	116	361
Ethylbenzene	19	230	290
p-Xylene	13	90	348
o-Xylene	12	79	293
1,3,5-TMB	14	60	224
1,2,4-TMB	17	63	270

solubility in water so as to prevent dissolution into the aqueous phase. Finally, the organic solvent should have excellent gas chromatographic behavior. On the basis of these considerations, chloroform, *n*-octanol and hexane were tested in preliminary experiments. The enrichment factor (EF) for the analyte, which was defined as the ratio of the final concentration of the analyte in organic phase to its initial concentration in aqueous phase, was significantly different for the respective solvents (Table 1). Among the solvents studied hexane gave the highest EF for target analytes and therefore was employed for all further extractions.

3.2. Fiber and its quantity

Three kinds of fiber, namely acetate fiber, fiberglass and PTFE fiber were tested for the fiber-in-tube LPME. Acetate fiber was easily dissolved in hexane, and so was not suitable for the present fiber-in-tube LPME. The PTFE fiber was found to give higher enrichment factor probably because PTFE fiber had better hydrophobicity than fiberglass. So PTFE fiber was used for subsequent experiments. To investigate the effect of fiber quantity on the extraction, the PTFE tube (10 mm long × 2.0 mm i.d.) was packed with various quantities of fiber and utilized for the microextraction of 8 ml sample solution. Results shown in Fig. 2 indicate that the signal intensities of the analytes increased with the fiber quantity and reached a maximum when the tube was full of 28 mg fibers. Since the inner volume of the tube was constant, the volume of solvent decreased with the increase of fiber quantity, resulting in the decrease of the phase ratio and the increase of final organic phase concentrations. The increase of fiber quantity perhaps had positive effect. It needed further investigations. When the PTFE tube (10 mm long × 2.0 mm i.d.) was packed with 14, 21, 24 and 28 mg PTFE fibers, respectively, the volumes of hexane were 70, 43, 31 and 16 μl, respectively. For further experiments an amount of 28 mg PTFE fiber was used.

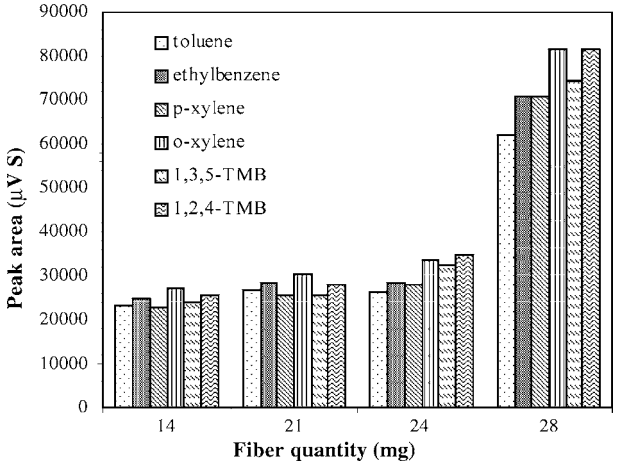

Fig. 2. Effect of fiber quantity on the present fiber-in-tube LPME for 16 mg l^{-1} substituted benzenes with 15 min extraction.

3.3. Tube length

Various lengths (10, 15 and 20 mm) of the PTFE tubing packed with the same quantity of PTFE fiber (28 mg) were tested to investigate the effect of tube length on the fiber-in-tube microextraction. It was found that a 10 mm long tube gave the highest EF (Fig. 3). The volume of solvent decreased with the decrease of tube length and thus decreased the phase ratio and increased final organic phase concentrations. For all the rest of this work, a 10 mm long × 2.0 mm i.d. PTFE tubing was employed for the present fiber-in-tube LPME.

3.4. Agitation

Sample stirring is routinely applied to accelerate the extraction kinetics. The aqueous sample solution was extracted at 0, 200, 400, 600, 800, 1000 and 1200 rpm, respectively. As expected, increasing the stirring speed up to 800 rpm accelerated the extraction. However, violent stirring (>800 rpm) resulted in massive air bubbles and decreased the enrichment factors. Therefore, a 600 rpm setting was selected for the subsequent experiments.

3.5. Salt effect

The effect of adding salt to the donor solution prior to extraction has been widely investigated [1]. Depending on the target ananlytes, an increase in the ionic strength of aque-

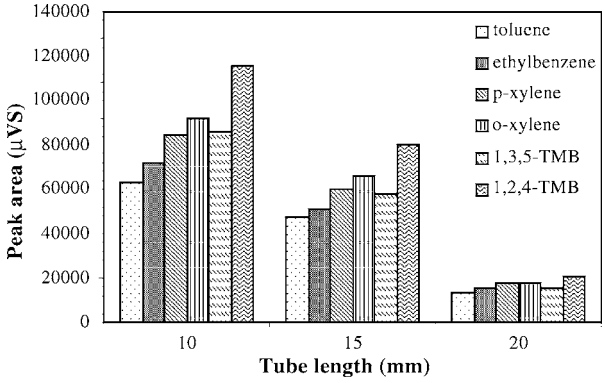

Fig. 3. Effects of the length of the PTFE tubing on the present fiber-in-tube LPME for 16 mg l^{-1} substituted benzenes with 15 min extraction.

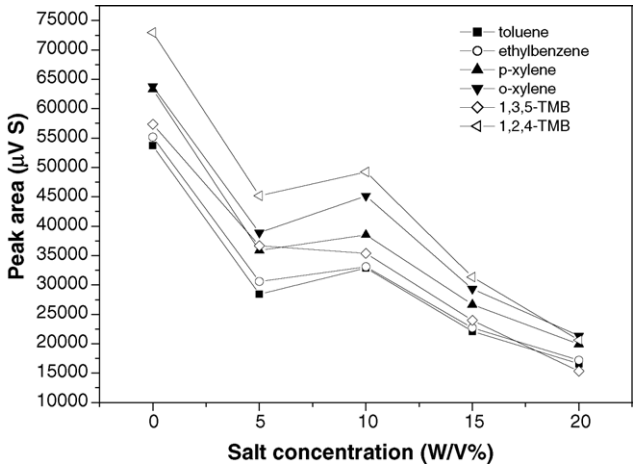

Fig. 4. Effect of salt concentration on the present fiber-in-tube LPME for 8 ml solution containing 16 mg l^{-1} of each substituted benzenes with 15 min extraction.

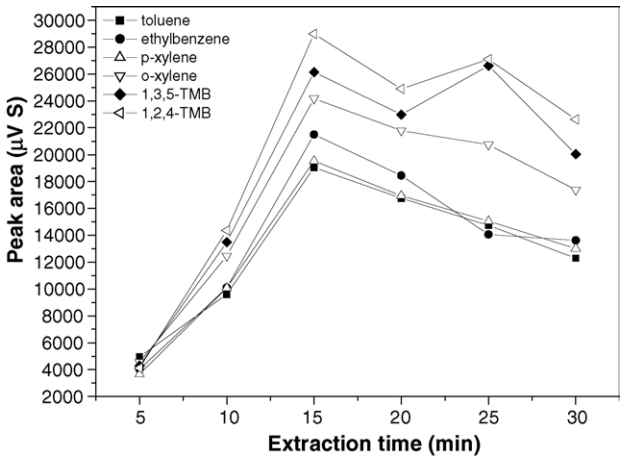

Fig. 5. Effect of extraction time on the present fiber-in-tube LPME for 16 mg l^{-1} substituted benzenes.

ous solution may have various effects on extraction: it may enhance [17,22], not influence [23,24] or limit extraction [17,25].

To investigate the salt effect on the present fiber-in-tube LPME, the extraction was performed with 8 ml sample solution containing various concentrations of NaCl (0, 5, 10, 15 and 20%). The enrichment factors decreased with increasing salt concentration in the aqueous sample (Fig. 4). The result indicated that in present fiber-in-tube LPME the addition of salt limited the extraction. It was assumed similar to a drop LPME [26], that apart from the salting-out effect, the presence of salt caused a second effect, adverse for the extraction, whereby the physical properties of the extraction film were changed, reducing the diffusion rates of analytes into the solvent. So, no salt was added to the sample solution in further extractions.

3.6. Extraction time

For method optimization, it is important to establish the extraction time profiles of target analytes so as to configure optimal extraction time. Extractions were performed in a period of 5, 10, 15, 20, 25 and 30 min, respectively, while the other parameters remained the same. The results shown in Fig. 5 demonstrated that all target compounds gave a similar trend. All the analytes studied gained the largest peak areas in a period of 15 min and then the peak areas decreased slightly with the increase of extraction time except 1,3,5-TMB and 1,2,4-TMB. So a period of 15 min was used for the subsequent experiments. The optimized parameters of developed fiber-in-tube LPME were summarized in Table 2.

Table 2
The parameters of the developed fiber-in-tube LPME

Fiber quantity (mg)	28 (PTFE fibers)
Tube length (mm)	10 (2 mm i.d. and 3 mm o.d.)
Organic solvent	Hexane (16 μl)
Sample volume (ml)	8
Stirring rate (rpm)	600
Extraction time (min)	15
Added salt concentration (%, w/v)	0

3.7. Analytical performance of the developed fiber-in-tube LPME

The characteristic data for the performance of the fiber-in-tube LPME are summarized in Table 3. Eight millilitres solution containing 50 μg l^{-1} of each compound submitted to the fiber-in-tube LPME for evaluating the limit of detection (LOD, S/N = 3). The reproducibility study was car-

Table 3
Comparison of the fiber-in-tube LPME with a drop LPME

Analytes	EF		LOD (μg l^{-1})		R.S.D.s (n = 5) (%)[a]	
	Fiber-in-tube LPME	A drop LPME	Fiber-in-tube LPME	A drop LPME[b]	Fiber-in-tube LPME	A drop LPME
Toluene	361	78	1.6	7.2	6.7	11.1
Ethylbenzene	290	60	1.0	12.7	3.7	10.6
p-Xylene	348	69	0.8	9.0	3.6	12.2
o-Xylene	293	54	0.3	8.2	4.2	11.6
1,3,5-TMB	224	38	5.0	16.5	8.1	11.5
1,2,4-TMB	270	39	4.5	16.1	6.5	11.2

[a] Water samples spiked at 16 mg l^{-1} for each compound.
[b] Calculated from 50 μg l^{-1} spiked level, S/N = 3.

Table 4
The concentration (Conc.) of substituted benzenes (mean ± σ, $n = 3$) (μg l^{-1}) found in water samples and recoveries (Rec., %) of spiked analytes at 500 μg l^{-1} in water samples obtained by the fiber-in-tube microextraction and a drop LPME

Analytes	River water 1				Wastewater 1				Wastewater 2			
	Fiber-in-tube LPME		A drop LPME		Fiber-in-tube LPME		A drop LPME		Fiber-in-tube LPME		A drop LPME	
	Conc.	Rec.	Conc.	Rec.	Conc.	Rec.	Conc.	Rec.	Conc.	Rec.	Conc.	Rec.
Toluene	nd[a]	90	nd	96	nd	95	nd	98	nd	86	nd	94
Ethylbenzene	nd	86	nd	108	nd	99	nd	96	40 ± 5	93	47 ± 7	84
p-Xylene	nd	91	nd	103	58 ± 5	93	55 ± 19	96	81 ± 6	84	97 ± 15	88
o-Xylene	nd	94	nd	107	47 ± 4	99	53 ± 14	106	120 ± 16	90	146 ± 17	86
1,3,5-TMB	nd	101	nd	99	nd	96	nd	101	nd	89	nd	94
1,2,4-TMB	nd	101	nd	97	nd	97	nd	99	nd	87	nd	86

[a] Not detected.

ried out by extracting aqueous sample (16 mg l^{-1} of each compound) using five freshly prepared 10 mm long tubes packed with identical quantity of fibers (28 mg). The linear calibration ranges for target analytes were in the range of 0.005–20 mg l^{-1} and the linear squared regression coefficients (r^2) of the calibration functions ranged from 0.9982 to 0.9991.

A comparison of the fiber-in-tube LPME and a drop LPME was made to demonstrate the performance of the proposed fiber-in-tube LPME technique (Table 3). From the work of He and Lee [15] we know that the analytical signal increased with drop volume. In our experiments, when drop size exceeded 3 μl, the hexane drop became more buoyant, crept up along the outside of the needle and could not be retracted back into the syringe. So, a drop LPME was performed by suspending a 3 μl drop of hexane on the needle tip of a microsyringe immersed in 8 ml solution and then a 2 μl aliquot of hexane was injected into the GC for analysis. Obviously, the developed fiber-in-tube LPME offers much larger enrichment factors, lower detection limits and better reproducibility in comparison with a drop LPME.

The fiber-in-tube LPME technique was applied to the determination of the six substituted benzenes in river water and wastewater samples. River water sample was collected from local rivers. Wastewater samples were dye industrial wastewater. Typical chromatograms of the six standard substituted benzenes (500 μg l^{-1}), wastewater 1 and wasterwater 1 spiked with 500 μg l^{-1} of each analyte after extraction by the developed fiber-in-tube microextraction for 15 min were shown in Fig. 6. To demonstrate the accuracy of the developed fiber-in-tube LPME, the concentrations of substituted benzenes in these water samples obtained by the fiber-in-tube LPME and by a drop LPME were compared in Table 4. As shown in Table 4, the two methods gave consistent results.

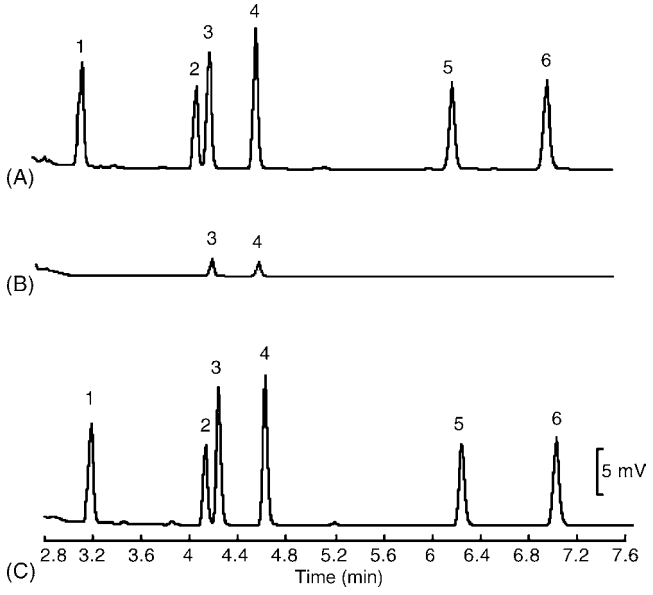

Fig. 6. Typical chromatograms of (A) a mixture standard solution containing 500 μg l^{-1} of toluene, ethylbenzene, *p*-xylene, *o*-xylene, 1,3,5-TMB and 1,2,4-TMB, respectively, (B) wastewater 1 and (C) wastewater 1 spiked with 500 μg l^{-1} of toluene, ethylbenzene, *p*-xylene, *o*-xylene, 1,3,5-TMB and 1,2,4-TMB, respectively with 15 min extraction. *Notes*: (1) toluene; (2) ethylbenzene; (3) *p*-xylene; (4) *o*-xylene; (5) 1,3,5-TMB; (6) 1,2,4-TMB.

4. Conclusions

This work has demonstrated the feasibility of the proposed fiber-in-tube LPME technique in combination with GC-FID for determination of toluene, ethylbenzene, *p*-xylene, *o*-xylene, 1,3,5-trimethylbenzene and 1,2,4-trimethylbenzene in water samples. The fiber-in-tube LPME reduces the extraction time and consumes little organic solvent, which is friendly to the environment. Because the PTFE fiber is disposable, the possibility of carry-over can be avoided by the fiber-in-tube LPME. The low cost of PTFE fiber decreases the expense of each extraction unit. The method provides high enrichment factors and good linearity.

Acknowledgements

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