A combined micro solid phase-single drop microextraction approach for trace enrichment of volatile organic compounds

Habib Bagheri[1]*, Maryam Zare[1], Hamed Piri-Moghadam[1], Ali Es-haghi[2]

[1]Environmental and Bio-Analytical Laboratories, Department of Chemistry, Sharif University of Technology, P.O. Box 11365-9516, Tehran-Iran

[2]Razi Vaccine & Serum Research Institute, P.O. Box 31975/148, Karaj- Iran

* Corresponding author:

Email: Bagheri@sharif.edu, Tel.: +98-21-66165316 (H. Bagheri)

Abstract

An attempt was made to combine μ-solid phase extraction and headspace single drop microextraction techniques and use their advantages for trace determination of some volatile organic compounds in aqueous samples. After performing the two-step preconcentration approach the desired analytes were determined by gas chromatography- mass spectrometry. A resorcinol-formaldehyde-based xerogel was used as the extracting medium in μ-solid phase extraction (μ-SPE) method. Then the extracted BTEX, was eluted with a rather large amount of methanol. To remove the laborious process including the solvent evaporation and further reconstitution, which usually accompanying with loss of analytes and accuracy, headspace single drop microextraction (HS-SDME) was employed as the second orthogonal enrichment technique. The combination of theses two techniques led to the enrichment factors ranged from 118 to 504 for the extracted BTEX indicating the enhanced capability of the method towards the aromatic compounds. Under the optimum conditions, the limit of detection (LOD) varied from 0.03 to 0.1 µg L^{-1} and the linearity for the analytes was in the range

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of 0.1-100 µg L^{-1}, while the RSD% values (n = 5) were all satisfactory at two concentration levels of 0.1 and 100 µg L^{-1}. The applicability of the developed µ-SPE/HSSDME/GC-MS method was examined by analyzing river and paddy water samples and the acceptable relative recovery percentages were obtained.

Keyword: µ-solid phase extraction, Headspace single drop microextraction, Resorcinol-Formaldehyde xerogel, Gas chromatography-mass spectrometry.

1. Introduction

Solid phase extraction (SPE), nowadays, is widely used for environmental sample pretreatment due to its high recovery, appropriate extraction time, high enrichment factor and ease of automation [1]. The principle of SPE is similar to the conventional liquid-liquid extraction (LLE), in which solutes are partitioned between a liquid sample and a solid sorbent [2]. In spite of wide applications of SPE with different types of sorbents, it requires highly pure organic solvents which are mostly toxic and expensive. Recently, a miniaturized version of this technique, μ-SPE, was developed for microextraction of some environmental pollutants [3]. Compared to other pretreatment techniques, this method has important advantages such as it only consumes a small amount of solvents and sorbents [4]. The μ-SPE methodology, in principal, is quite similar to SPE while smaller amounts of sorbent and sample are required. The appropriate selection of μ-SPE sorbent is so important since it results in improving the extraction efficiency through interaction of the selected sorbent and analyte of interest. Recently, numerous carbon nanomaterials including fullerenes [5], carbon nanotubes (CNTs) [6] carbon nanohorn [7], and carbon nanocones/disks [7] have been widely use as adsorbent in SPE techniques due to their large specific surface areas, high adsorption capacity and good chemical and thermal stability [1]. Silica is an interesting media media, as it does not swell or strain, has good mechanical strength. However the mechanical, thermal and chemical properties of silica are great, but lack of appropriate interactions, make its usage limited for extraction purposes. To increase its applicability in extraction media, sol-gel technology is usually used to prepare inorganic-organic material [8]. So, combination of properties of silica material with organic compounds, interaction with analytes via van der waals, dipole-dipole, л-л interactions and hydrogen bonding, expected to enhance the extraction efficiency of the prepared silica-based sorbents.

Addition of large amount of organic solvent to have a complete desorption of extracted analytes from the solid phase is one of the drawbacks of off-line μ-SPE technique. Addition of organic solvent is

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caused to dilution of the already concentrated analytes on the sorbent. Therefore, another step is needed to reconcentrate analytes of interest. Solvent evaporation was already used to remove the excess of organic solvent followed by reconstitution of desired analytes in the small amount of an appropriate solvent [9,10]. However is an applicable way, but it is laborious and time consuming while loss of analytes and lack of accuracy are other problems associated with solvent evaporation.

Single drop microextraction (SDME) is a miniaturized modification of the classical solvent extraction. This extraction approach, in combination with GC, has been shown to be quite effective for extraction and determination of different analytes [11-22]. It is fast, inexpensive, and due to the need for small volumes of solvent, there is minimal exposure to toxic organic solvents. SDME needs only common laboratory equipment and does not suffer from carryover between extractions that may be experienced using SPME [23].

In this project, to overcome the mention problems, HS-SDME was employed to re-concentrate the eluted analytes from the μ-SPE cartridge. Sol-gel technology was used to prepare organic-inorganic sorbent, resorcinol-formaldehyde-xerogel, and functionalize the silica sorbent. This new combination led to significant extraction efficiency, E.F. 118-504, as well as good repeatability. The results obtained shown that the developed approach, μ-SPE/HS-SDME is as an efficient technique that offers the advantages such as simplicity, low disposal costs, high enrichment factor, sample clean-up and good repeatability. It was successfully applied for analysis of BTEX in real water samples and good relative recoveries obtained for the spiked river and paddy samples.

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2. Experimental

2.1. *Chemicals and reagents*

Tetramethoxysilane, trifouroacetic acid (TFA), methanol, resorcinol, formaldehyde 37% w/w, sodium carbonate, 1-octanol and n-hexadecane were analytical grades and purchased from Merck (Darmstadt, Germany). A mixture of BTEX consists of benzene, toluene, ethylbenzen, m-xylene and o-xylene in analytical grades which was purchased from Merck and prepared in methanol. A stock solution of 8000 mg L^{-1} of BTEX was prepared. More diluted solutions were prepared daily using this stock solution2.2.

2.2. *Apparatus*

An Agilent 6820 (Shanghai, China) gas chromatograph with a split–splitless injection port and a flame ionization detector was used to determine the optimized extraction conditions. Separation of analytes was carried out on an HP-1MS 30 m, 0.53 mm with a 1.5 μm thickness capillary column. The carrier gas was nitrogen (99.999%) at a flow-rate of 2.5 mL min^{-1}. The gas chromatograph was operated in the split mode and the split valve was opened after 1 min. The GC column temperature was programmed at 30°C for 5 min, increased to 80°C at a rate of 5°C min^{-1} and kept in this temperature for 2 minutes, then raised to 250°C at rate of 40°C min^{-1} and kept at this temperature for 5 minutes. The injector and detector temperatures were set at 220 and 290°C, respectively. For quantitative analysis, a Hewlett-Packard (HP, PaloAlto, CA, USA) HP 6890 plus series GC equipped with a split–splitless injector and a HP 5973 mass-selective detector system were used. The MS was operated in the EI mode (70 eV). Helium (99.999%) was employed as a carrier gas at the constant

flow rate of 1.0 mL min^{-1}. The gas chromatograph was operated in the split mode and the split valve was kept closed for 1 min.

The separation of analytes was performed on a 30 m × 0.25 mm i.d. fused-silica capillary column coated with a 0.25 µm bonded film of TRB-5MS (Teknokroma, Barcelona, Spain). The GC column temperature was programmed at 35°C for 3 min, increased to 80°C at a rate of 5°C min^{-1}, then raised to 280°C at 120°C min^{-1} and kept at this temperature for 5 min. The injector temperature was set at 270. The GC-MS interface, ion source and quadrupole temperatures were set at 280, 230 and 150°C, respectively. The detection method was programmed for SIM considering characteristic ions for each compound. At a dwell time of 100 ms, three sets of characteristic ions at m/z values of 77 and 78 within 1.5-3 min, 91 and 92 within 3-6 min and 91 and 106 after 6 min were selected for GC-MS analysis of benzene, toluene and ethyl-benzene, m-xylene, o-xylene, respectively.

2.3. Preparation of resorcinol formaldehyde-xerogel sorbent

2.3.1. *Preparation of resorcinol formaldehyde polymer*

1.000 g of resorcinol was mixed with 2.753 g formaldehyde and 5.160 mg sodium carbonate and 1.265 mg distilled water in 10 mL vial. This mixture was stirred at maximum rate with a stirrer. After 7 hours this polymer was used for synthesis of RF-xerogel.

2.3.2. . *Preparation of resorcinol formaldehyde -xerogel*

For preparation of sol solution the following procedure was utilized: the amount of 200 µL RF (polymer) was weighted in ependorf microtube. Then a volume of 200µl tetramethxysilane (precursor) and 2µl of TFA (acid catalyst with purity of 1%) were added to the above ependorf microtube, respectively and mixed with ultrasonic for 15 minutes. Then the solution was kept for 3 hours in room

temperature, 12 hours in the dedicator and 3 hours in the oven at 200°C. The xerogel then was

grounded into the powder and sieved to 90-125 μm.

2.4. μ-SPE/HS-SDME/GC-MS analysis

At first, 25 mg of the prepared xerogel with particle size between 90-125 μm was inserted between two

20 μm frits and fitted in μ-SPE cartridge (1.5-mL syringe with 1 cm diameter). Then 100 mL distilled

water spiked at 2 mg L^{-1} of BTEX was pumped and cycled by a peristaltic pump. Desorption process

was performed with 1mL methanol with the flow rate of 1.3 mL min^{-1} (Fig. 2). Memory effect was also

carefully checked by extraction-determination of a blank sample. After performing the desorption

process, the sorbent was washed by 1 mL methanol and this was subjected to SDME. The analysis of

the micro drop by GC revealed that there is no sign of BTEX, indicating the absence of the memory

effect. Then, to perform the HS-SDME technique, the desorbed solution was heated in 45°C for 5

minutes and then stirred with maximum speed. 2μL of 1-octanol was used for HS/SDME for 10 min. It

was finally injected into the GC/FID. All preliminary and optimization experiments were accomplished

with GC/FID. Fig. 2 shows the schematic diagram of the process.

3. Results and discussion

3.1. Optimization

After achieving acceptable preliminary results from the hyphenated μ-SPE-HS-SDME method, several

influential parameters including the sample flow rate, the sample volume, the μ-SPE extraction time,

the SDME extraction temperature and time, were considered to be optimized. The chromatographic

peak area of each individual analyte was used to assess the comparative extraction efficiency under

different conditions. GC-FID was used to perform the optimization procedure using 100 mL distilled water spiked at 2 mg L^{-1} of BTEX.

As μ-SPE is a non-exhaustive extraction technique, the sample flow rate was firstly optimized to increase the extraction efficiency and therefore reduce the extraction time. The sample flow rate was tested in the range of 1.3-33.3 mL min^{-1}. The extraction efficiency was increased at higher flow rates (as the consequence of more cycles), which is due to the higher mass transfer of the analytes from the aqueous sample to the solid phase. A flow rate of 27.3 mL min^{-1} was selected as the optimum amount for further analysis. A sample volume range of 10-300 mL was also considered for optimization. As Fig. 4 shows the extraction efficiencies were improved as the sample volume was increased up to 100 mL and then the equilibrium between two phases could be observed.

The extraction time is also a paramount parameter affecting the extraction efficiency. So, an extraction time from 5 to 40 was investigated and according to Fig. 5, after 5 min the equilibrium was obtained indicating the dynamic nature of extraction system in which the analytes are continuously exposed to the solid phase surface.

Two further parameters were also optimized for the developed HS-SDME. At first, the methanol desorption solution including BTEX should be heated in order to drive them into the headspace. A temperature range of 20-50 °C was investigated and according to Fig. 6, the temperature of 50°C was selected as the optimum temperature. At higher temperatures, the extraction efficiency is also increased since the BTEX components could easily and rapidly evaporate and penetrate into the headspace.

3.1.5. HS-SDME time

After heating the desorbing methanol at 50°C for 5 minutes, it was stirred under maximum stirring efficiency. Then 2-μL of 1-octanol was inserted in headspace of the solution by a GC syringe. The HS-SDME extraction time was optimized from 2 to 15 min. The extraction efficiencies were improved as the extraction time was increased up to 10 min.

The organic solvent used for HS-SDME, 1-octanol, contained a minor amount of impurities of BTEX, which interfered with the determination of trace concentrations of BTEX components (below 100 μg L^{-1} level), which is consistent with the literature revealing that it is impossible to trace analysis of BTEX with 1-octanol in SDME [24]. To overcome the problem, n-hexadecane was found to provide similar extraction efficiency for the BTEX, with no impurities of BTEX [24]. So, n-hexadecane was chosen as the extraction solvent for analysis of BTEX for validation of the method by GC-MS.

3.2. Method validation

The method validation was performed by determination of the target analytes based on the optimized conditions, using a sampling flow rate of 27.3 mL min^{-1}, a loading time of 5 min and a sample volume of 100 mL for μ-SPE process while a temperature of 50°C for 5 minutes and headspace extraction time of 10 min were selected for HS-SDME method. The distilled water spiked with BTEX was used to evaluate the precision of the measurements, LOD, LOQ, the dynamic range and selectivity of the method. Comparison of the chromatograms obtained before (direct injection) and after μ-SPE/HS-SDME/GC-MS revealed are indicative of achieving high enrichment factors, ranging from 118 to 504. The linearity for the analytes was in the range of 0.1-100 μg L^{-1}. The regression coefficients obtained for all analytes were satisfactory ($R^2 > 0.99$). The values of LOD (S/N = 3/1) were in the range of 0.03-0.1 μg L^{-1} and LOQ (S/N = 10/1) 0.10-0.25 μg L^{-1}. The precision of the method was determined by performing two consecutive extractions from the aqueous solutions at two

concentration levels. The standard deviations of the peak area of analyte, spiked at the concentration levels of 0.1 and 100 μg L^{-1}, were satisfactory (Table 1). Zayandeh rood river and paddy water samples were chosen to evaluate the applicability of the developed method to the real samples and to investigate the matrix effect. No peaks were observed at the desired retention time of analytes, so they were spiked at concentration level of 100 μg L^{-1}. The obtained results proved the robustness of the developed sorbent in real sample analysis (Table 2). The performance of the developed method is compared with other relevant works is shown in Table 3. As it is shown the matrix effect of paddy water is more than the surface water.

4. Conclusion

The hyphenation of μ-SPE with HS-SDME is implemented for pre-concentration and trace quantification of some organic compounds in water samples. The strategy to apply these two wide spread extraction techniques is proved to be an efficient approach which leads to high enrichment factors. The RF polymer was chemically bonded to the silica structure within the sol-gel process. According to functional groups in RF polymer, it has the ability of dipole-dipole interactions with polar compounds and π-π interaction with aromatic hydrocarbons during the first step of extraction. Moreover, application of HS-SDME prevented any need for solvent evaporation after μ-SPE which usually accompanies with the loss of analytes. Besides of higher accuracy of the method, the subsequent use of HS-SDME after μ-SPE led to enhanced enrichment factors.

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Figures caption

Figure. 1. Synthesis process and structure of RF polymer.

Figure. 2. Schematic of the extraction process.

Figure. 3. A) Effect of sample flow rate on extraction efficiency. Extractions were performed from 100-mL aqueous sample solution for 20 min spiked with BTEX at 2 mg L^{-1}. The HS-SDME temperature and time was 45°C and 10 min respectively.

B) Effect of sample volume on extraction efficiency. Extractions were performed from aqueous sample solutions for 20min spiked with BTEX at 2 mg L^{-1} with flow rate of 27.3 mL min^{-1}. The HS-SDME temperature and time was 45°C and 10 min respectively.

C) μ-SPE time profiles of BTEX from aqueous samples. Extractions were performed from a 100 mL aqueous sample solution spiked with each BTEX at 2 mg L^{-1}, with flow rate of 27.3 mL min^{-1}. The HS-SDME temperature and time was 45°C and 10 min respectively.

Figure. 4. A) Effect of HS-SDME temperature on extraction efficiency. Extraction condition as follows: extraction of 100 ml sample for 5min spiked with 2 mg L^{-1} BTEX, with flow rate of 27.3 mL min^{-1}. The HS-SDME time was 10 min.

B) Effect of HS-SDME time on extraction efficiency. Extraction condition as follows: extraction of 100 ml sample for 5 min spiked with 2 mg L^{-1} BTEX, with flow rate of 27.3 mL min^{-1}. The HS-SDME temperature was 45°C.

Fig. 1
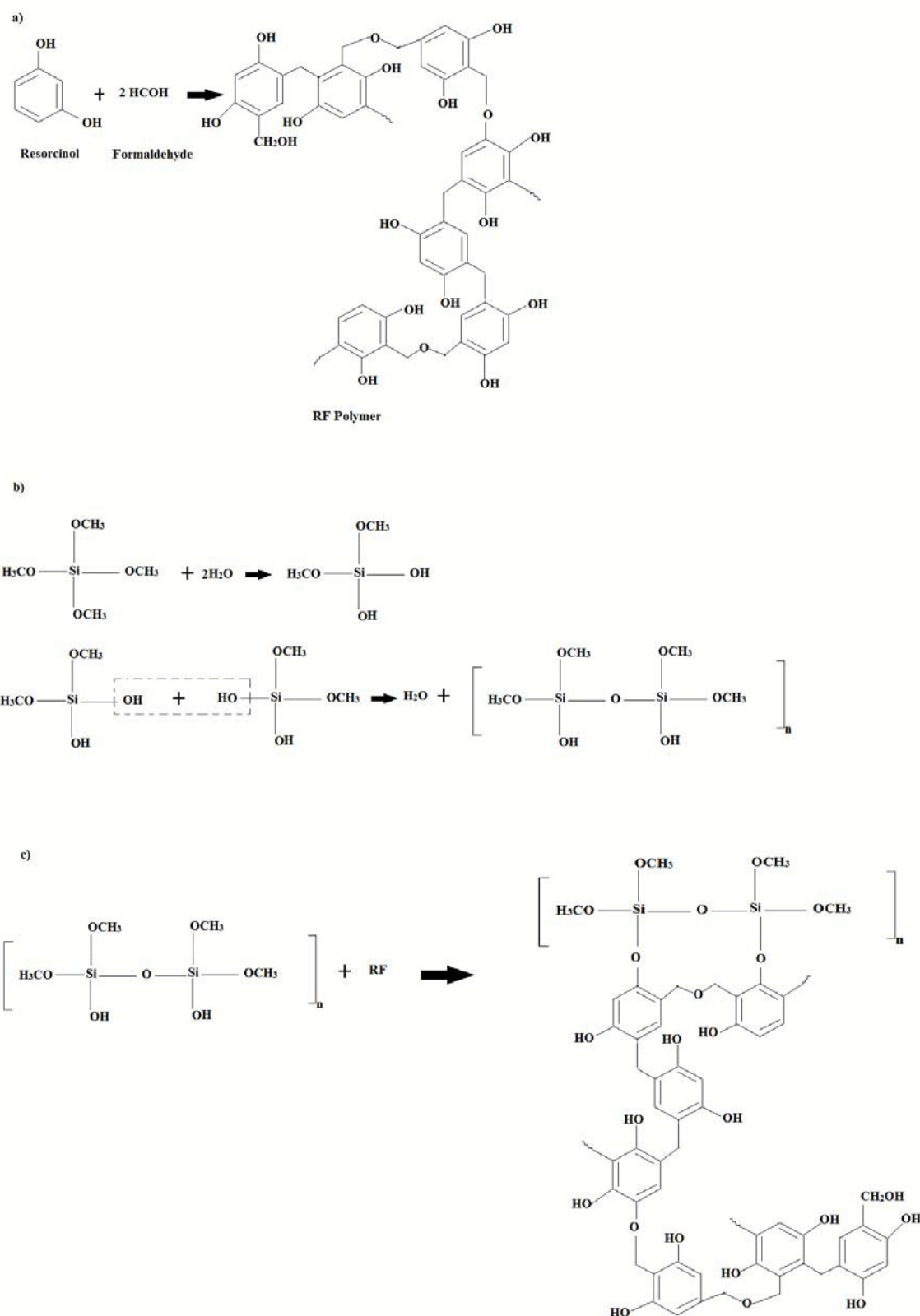
Fig. 2

RF Xerogel

Peristaltic pump

Desorption process

Adsorption process

GC

Fig3.
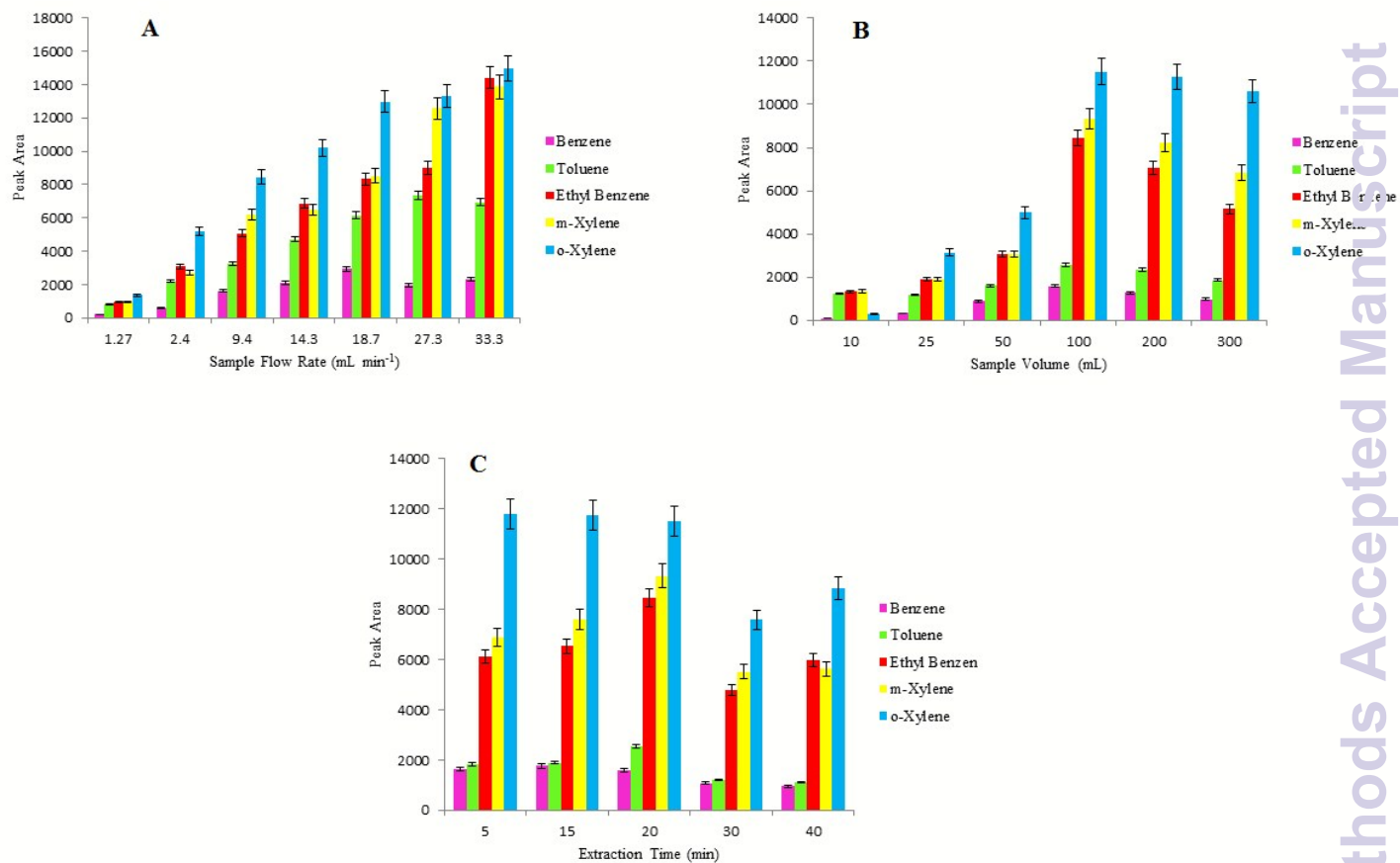
Fig.4
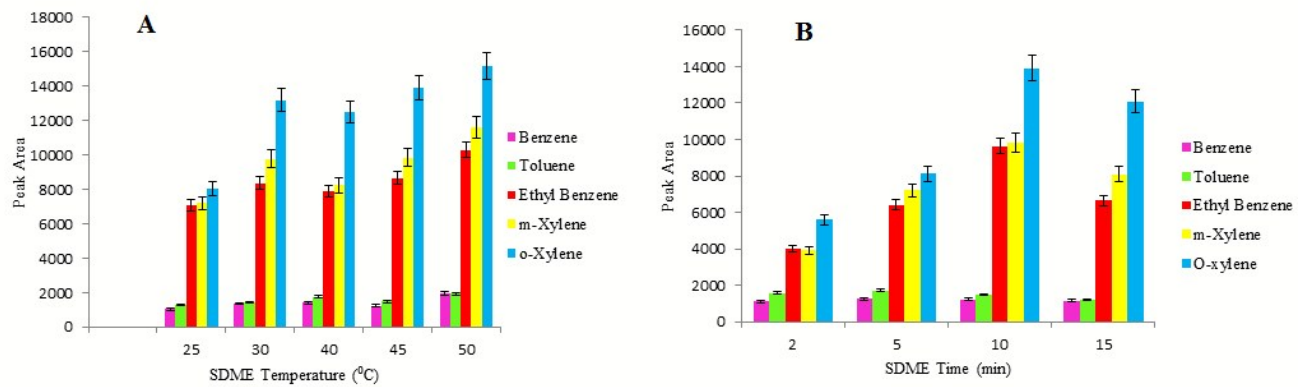
Table 1

Analytical data obtained after μ-SPE/HSSDME/GC-MS of spiked distilled water.

Compound	LOD[a]	LOQ[b]	LDR[c]	R^2	EF[d]	EF
	(μg L^{-1})	(μg L^{-1})	(μg L^{-1})	(μg L^{-1})	(SDME)	(μ-SPE+SDME)
Benzene	0.10	0.25	0.25-100	0.9903	92	118
Toluene	0.03	0.10	0.10-100	0.9971	340	450
Ethylbenzene	0.05	0.10	0.10-100	0.9986	279	376
m-Xylene	0.03	0.10	0.10-100	0.9936	284	408
o-Xylene	0.03	0.10	0.10-100	0.9978	320	504

Maximum residue limit (mg L^{-1}): Benzene 0.005, Toluene 1, Ethylbenzene 0.7, Xylene 10 [25]

a) LOD= Limit of Detection

b) LOQ= Limit of Quantitation

c) LDR= Linear Dynamic Range

d) EF= Enrichment Factor

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Table 2

The relative recoveries and RSD % for some real samples spiked at 100 µg L^{-1} level.

Compound	Relative recovery		RSD%	
	Zayandeh Rood	**Paddy water**	**Zayandeh Rood**	**Paddy water**
Benzene	105.2	92.3	7.3	8.4
Toluene	104.9	90.6	5.1	9.1
Ethylbenzen	101.9	91.5	6.2	8.6
m-Xylene	105.7	89.8	5.9	6.7
o-Xylene	105.8	90.8	7.1	10.5

Table 3

Comparison of some other methods used for determination of BTEX compounds.

Instrument	Extraction method	LOD ($\mu g\ L^{-1}$)	LDR ($\mu g\ L^{-1}$)	R	RSD%	Ref.
GC-FID	HS-SDME	0.7-5	-	> 0.999	< 10	[24]
GC-MS	SPME	0.15-0.30	0.30-10	> 0.999	< 17	[26]
GC-MS	SPME	0.75-10	10-25000	> 0.990	< 9	[27]
GC-FID	SPME	0.0004-0.0006	0.0003-20	> 0.989	< 5	[28]
GC-FID	DSDME	0.8-7	10-20000	> 0.998	< 3	[29]
GC-MS	μ-SPE/HS SDME	0.03-0.1	0.1-100	> 0.9903	< 10.5	CW[a]

[a] CW: Current work