IRAN J ANAL CHEM 1 (2014) 20-28

Iranian Journal of Analytical Chemistry

Journal home page: http://ijac.journals.pnu.ac.ir

# Development of Homogeneous Liquid-Liquid Microextraction via Flotation Assistance Method for Determination of Benzene, Toluene, Xylene and Styrene in Water Samples

Majid Haji Hosseini[a,*], Mohammad Reza Rezaei[b], Mohammad Rezaee[a], Peyman Asaadi[b], Khatereh Ashtari[b]

[a]*Nuclear Fuel Cycle Research School, Nuclear Science & Technology Research, Atomic Energy Organization of Iran, Tehran, Iran*
[b]*Departmant of Chemistry, Azad University of Saveh, Saveh, Iran*
[*]E-mail: Majid2_haji@yahoo.com

Received 9 December 2013; Received in revised form 5 Juauanry 2014; Accepted 27 February2014, Online published: 30 February 2014

**ABSTRACT**
This study propose a new analytical protocol for the determination of benzene, xylene, toluene and styrene in water samples using homogeneous liquid-liquid microextraction via flotation assistance (HLLME-FA) technique followed by gas chromatography-mass spectrometry (GC-MS). In this research, a special extraction cell was designed to facilitate collection of the low-density solvent extraction. No centrifugation was required in this procedure. The water sample solution was added into the extraction cell which contained an appropriate mixture of extraction and homogeneous solvents. By using air flotation, the organic solvent was collected at the conical part of the designed cell. The effects of different variables on the efficiency of the extraction were studied simultaneously using experimental design. Response surface methodology was applied to investigation the optimum conditions of each variable. Using optimized variables in the extraction process, for all target analytes, the detection limits, the precisions and the linearity of the method were found in the range of 0.8-8.2 ng $mL^{-1}$, 3.09-7.96% (RSD, n=4) and 1-100 ng $mL^{-1}$, respectively. The headspace method was used for the accuracy of comparison. The performance of the method was evaluated for extraction and determination of analytes in water samples and satisfactory results were obtained (RSD ≤10.06 %).

**KEYWORDS:** Homogeneous liquid-liquid microextraction; Flotation assistance; BTEX; Gas chromatography; Mass spectrometry; Water samples.

## 1. INTRODUCTION

Mono-ring aromatic hydrocarbons are widely distributed in the environment and can also be present in foods either naturally or as contaminants. Amongst them, BTEXs [benzene, toluene, ethyl benzene, the three xylene isomers (ortho, meta and para) and styrene (also called vinyl benzene)] are a subclass of volatile organic compounds (VOCs) with boiling points between 80 and 150 ºC. This group of VOCs is found in petroleum derivatives, such as gasoline and diesel fuel. These compounds enter the environment from different sources such as combustion products of wood and fuel, industrial paints, adhesives, degreasing agents and aerosols. BTEX can cause adverse health effects such as chromosome aberrations, cancer and damage to the liver, kidneys, eyes and central nervous system [1]. Due to the toxic properties of these compounds, development of specific analytical procedure for the analysis of these compounds in the various matrices is of great importance. Analysis of BTEX compounds is usually carried out by gas chromatography-flame ionization detection system (GC-FID). Since the matrices of environmental samples are often complex, sample preparation plays an important role in the determination of these species [2-9]. The purpose of the sample preparation step is extraction of analyte from the matrix sample into a form that is pre-purified, concentrated and compatible with the analytical system. There is several sample preparation techniques described in the literatures for the determination of these contaminants. These techniques include solid-phase microextraction (SPME) [10], ultrasound-assisted dispersive liquid-liquid microextraction (DLLME) [11] and headspace-gas chromatography/mass spectrometry [12]. However, SPME is also expensive, its fiber is fragile and has limited life time and sample carry-over can be a problem. Also, the main disadvantage of DLLME is that the extraction solvent is generally limited to solvents of density higher than water in order to be sedimented by centrifugation.

In the previous study [13], we developed the homogeneous liquid-liquid microextraction via flotation assistance (HLLME-FA) method for the determination of polycyclic aromatic hydrocarbons (PAHs) in soil samples. In this work, HLLME-FA was developed for the fast, simple and efficient determination of benzene, toluene, xylene and styrene in water samples followed by gas chromatography-mass spectrometry detector (GC/MS). In this procedure, similar to dispersive liquid-liquid microextraction (DLLME) [14-17] and homogeneous liquid-liquid extraction (HLLE) [18-21] a mixture of extraction solvent and disperser-homogeneous solvent is injected into an aqueous sample to form in the initial state a homogeneous solution and then form an emulsion consisting of fine droplets of the extraction solvent, dispersive solvent and water. The main disadvantage of HLLE and DLLME is that the extraction solvent is generally limited to the solvent with a density higher than that of water in order to be sedimented by centrifugation; typically chlorinated solvents such as chlorobenzene, chloroform and carbon tetrachloride, all of which are potentially toxic to human and environment. In addition, the use of high density solvents as extractants restricts wider applicability of DLLME and HLLE due to more limited choices since the number of low-density solvents is more available than high-density ones. In recent years, to overcome this limitation, in several studies, the application of low density solvents in DLLME has been reported [22-25]. Here, a special extraction cell was designed to facilitate collection of the low-density solvent extraction. No centrifugation was required in this procedure. By using air flotation, the organic solvent was collected at the conical part of the designed cell. The most effective variables on the HLLME-FA could be considered as homogeneous solvent volume, ionic strength, type and volume of extractant solvent and time of extraction. The selected variables were optimized by central composite design (CCD).

## 2. EXPERIMENTAL

### *2.1. Reagents and materials*

Benzene, toluene, xylene and styrene were purchased from Merck (Darmstadt, Germany). Stock solution of compounds (10 µg mL$^{-1}$) was prepared by dissolving calculated amounts of each in methanol. Stock solution was stored at 4 ºC in the refrigerator. For the calibration curves standard aqueous samples containing 1.55 mol L$^{-1}$ NaCl were prepared from dilution of stock solution in the range of 1-100 µg L$^{-1}$ in the doubly distilled water. All other chemicals and solvents were analytical grade or better. All the solvent and real samples were filtered through 0.45 µm membrane to eliminate particulate matter before analysis. All experiments were carried out at room temperature (25± 0.5 ºC).

### *2.2. Instrumentation*

### *2.2.1. GC-MS*

The determination process of compounds took place by using a gas chromatograph (GC, Agilent 78090N, Agilent Technologies, Palo Alto, CA, USA) equipped with MS (Agilent 7890N, Agilent Technologies, Palo Alto, CA, USA) equipped with MS (Agilent 5975, Agilent Technologies, Foster City, CA, USA). The features and operating conditions of GC-MS system were as follows: GC, equipped with split/splitless injector with split ratio 20:1 and 200 ºC temperature, DB-5 MS 5% phenylmethyl siloxane fused silica capillary colum (30m length, 0.25 mm i.d. and 0.25 µm film thickness) and helium (purity 99.999%) as carrier gas at constant flow-rate of 1 mL min$^{-1}$. The temperature of the ion source and MS transfer line were adjusted at 230 and 280 ºC, respectively. The GC oven temperature was as follows: initial temperature 40 ºC for 6 min, 3 ºC min$^{-1}$ to 90 ºC for 5 min, 10 ºC min$^{-1}$ to 140 ºC, hold at 140 ºC for 1 min. The sample (0.5 µL) were injected by means of a 5 µL micro-syringe (Hamilton, Reno, NV) in split mode. Electron impact ionization (EIS) source was operated at 70 ev. A filament current of 50 µA and multiplier voltage of 1450 v were used. The mass spectrometer was calibrated as needed with perfluorotributylamine (PFTBA). Chemstation software (G1701EA Ver. E.02.01.1177) was employed for automated analysis and data acquisition. Specific SIM ions were recorded for each compound analyzed (the ion at m/z 78, 91, 104 and 106 was selected as the ion fragment for benzene, toluene, styrene and xylene).

### *2.2.2. HS-GC-MS determination of compounds in real samples*

The real samples initially were filtered through a 0.45 µm membrane to eliminate particulate matter before analysis. 5 ml from the real samples were injected into a 20 mL Head Space (HS) glass vial with a PTFE- encapsulated magnetic stirring bar and was immediately sealed with a PTFE- silicone septum. The HS vial containing the sample was placed in the CombiPal autosampler furnished with a heating module for automated unattended heating by stirring (700 rpm) for 10 min at 80 ºC in order to ensure the equilibration between the gas phase and the sample. An aliquot of the gas phase above the sample (1 mL) was automatically injected into the GC-MS system. During GC-MS analysis, specific SIM ions were recorded for each target compound.

### *2.3. HLLME-FA*

Fig. 1 shows the schematic procedure of the proposed method. A mixture of 0.5 mL methanol (homogeneous solvent) and 150 µL dodecane (extraction solvent) were added to the home-designed microextraction cell (Fig. 1-1). A volume of 22 mL of saline aqueous sample solution was injected into the microextraction cell by syringe, rapidly (Fig. 1-2). In this step, in the initial state of injection, a homogeneous solution was formed and then with the continuation of injection an emulsion consisting of fine droplets of the extraction solvent were formed (Fig. 1-3). After about 10 min, by using air flotation, the organic solvent was collected on the top of the solution (Fig. 1-4). After separation of the two phases, a few volumes of distilled water were added into the glass tube on the side of the cell (Fig. 1-5). The floated organic solvent was raised into the conical part of the cell. 0.5 µL of the collected organic solvent was injected into GC-MS instrument.

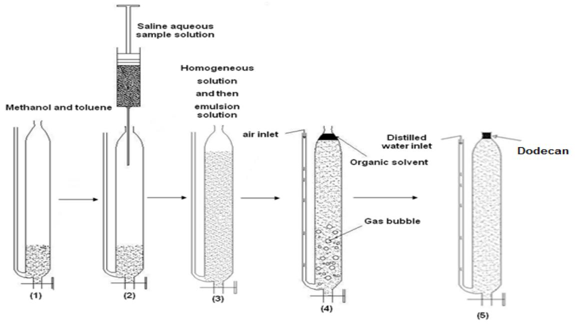

**Fig. 1.** Schematic of HLLME-FA procedure.

### *2.4. Optimization strategy*

There are several factors such as homogeneous solvent volume, extraction solvent volume, ionic strength and extraction time which may affect the extraction process. In order to find the optimum conditions of HLLME-FA in extraction of benzene, toluene, xylene and styrene from water samples and also to investigate the probable interaction between variables, response surface methodology (RSM), based on rotatable face center-central composite design (FCCD) was used. The design was build up of four factors at 3 levels (Table 1) with 8 axial points (α=1).

**Table 1.** The experimental variables and levels of the FCCD.

| Factor | level | | |
|---|---|---|---|
| | Low | Center | High |
| A: Sodium Chlorid Concentration (mol/L) | 0.1 | 1.55 | 3 |
| B: Volume of Methanol (ml) | 0.5 | 1.5 | 2.5 |
| C: Volume of Dodecan (μl) | 50 | 150 | 250 |
| D: Time of extraction (min) | 1 | 5.5 | 10 |

In order to obtain an estimation of the experimental error, 6 replica at the center point was applied. The design was rotatable; this mean that the design had points which were equidistant from the center. This procedure led to 30 experiments (Table 2), where the experimental response data were analyzed by a regression procedure based on the RSM [26]. Composit design is well known as a useful method to describe the curvature dependency of a typical response to its variables, which is needed to explain a non-linear variation behavior. The model which can be fitted to a composite design is an empirical function, determined from the statistical correlation suitability of the observed responses and the experimental factors. For this purpose, a second-order polynominal model equation is usually used [27, 28]:

$$Y= a_0 + a_1x_1 + a_2x_2 + a_3x_3 + a_4x_4 + a_{11}x_1^2 + a_{22}x_2^2 + a_{33}x_3^2 + a_{44}x_4^2 + a_{12}x_1x_2 + a_{13}x_1x_3 + a_{14}x_1x_4 + a_{23}x_2x_3 + a_{24}x_2x_4 + a_{34}x_3x_4 \qquad \text{(Eq. 1)}$$

in which Y is the predicted response. Response here is the peak area (PE) of analyte. $X_1$, $X_2$, $X_3$ and $X_4$ are the independent variables or the experimental factors. The linear coefficients $a_1$–$a_4$ express the linear effect of each variable; the $a_{11}$, $a_{22}$, $a_{33}$ and $a_{44}$ coefficients express the quadratic effects; $a_{12}$–$a_{14}$, $a_{23}$–$a_{24}$ and $a_{34}$, coefficients express interactive effects between the variables and $a_0$ is a constant corresponding to the central point of experimental variables. The statistical design, data analysis and various plots were obtained by using Minitab statistical software.

Dodecane has the highest extraction efficiency.

**Table 2.** Design table showing the randomized run order of the experiment, and the uncoded values of the different variables in the experimental design for determination of the modeled response (Eq. 1).

| Number | Salt Concentration (mol $L^{-1}$) | Volume of Methanol (ml) | Volume of Dodecan (μl) | Time (min) |
|---|---|---|---|---|
| 1 | 0.1 | 0.5 | 50 | 1 |
| 2 | 3 | 2.5 | 50 | 1 |
| 3 | 1.55 | 2.5 | 150 | 5.5 |
| 4 | 0.1 | 0.5 | 250 | 1 |
| 5 | 0.1 | 0.5 | 50 | 10 |
| 6 | 0.1 | 2.5 | 50 | 10 |
| 7 | 3 | 0.5 | 50 | 1 |
| 8 | 3 | 0.5 | 250 | 1 |
| 9 | 1.55 | 1.5 | 150 | 5.5 |
| 10 | 1.55 | 1.5 | 150 | 5.5 |
| 11 | 1.55 | 1.5 | 150 | 5.5 |
| 12 | 0.1 | 2.5 | 50 | 1 |
| 13 | 3 | 2.5 | 250 | 1 |
| 14 | 0.1 | 2.5 | 250 | 1 |
| 15 | 3 | 2.5 | 250 | 10 |
| 16 | 1.55 | 1.5 | 150 | 5.5 |
| 17 | 1.55 | 1.5 | 150 | 10 |
| 18 | 3 | 1.5 | 150 | 5.5 |
| 19 | 1.55 | 1.5 | 50 | 5.5 |
| 20 | 1.55 | 1.5 | 150 | 5.5 |
| 21 | 1.55 | 1.5 | 150 | 1 |
| 22 | 1.55 | 0.5 | 150 | 5.5 |
| 23 | 3 | 2.5 | 50 | 10 |
| 24 | 3 | 0.5 | 50 | 10 |
| 25 | 1.55 | 1.5 | 250 | 5.5 |
| 26 | 3 | 0.5 | 250 | 10 |
| 27 | 1.55 | 1.5 | 150 | 5.5 |
| 28 | 0.1 | 0.5 | 250 | 10 |
| 29 | 0.1 | 2.5 | 250 | 10 |
| 30 | 0.1 | 1.5 | 150 | 5.5 |

## 3. RESULT AND DISCUSSION

### *3.1. Selection of extraction and homogeneous solvent*

Selection of an appropriate extraction solvent is of great importance to optimize the HLLME-FA. Three different low density solvents (n-hexane, n-decane and dodecane) were tested for this purpose.

Miscibility of homogeneous solvent in the extraction solvent and aqueous phase is the main point for selection of a homogeneous solvent. Therefore, methanol was selected for this purpose.

### *3.2. Response surface methodology*

In order to obtain a high recovery and enrichment factor, the effect of different parameters such as the volume of extraction and homogeneous solvents, salt amount and extraction time were analyzed. Response surface methodology including face-center central composite approach was utilized to find the optimum values of effective variables involved in the system. Since all analytes showed similar results, thus only the result of styrene was selected as a representative example of the analyte responses. The peak area (PE) of benzene, toluene, xylene, styrene and the randomized experimental design (RED) is presented in Table 3. The coefficients of the empirical model equation (1) and their statistical characteristics were evaluated (Table 4).

The results show that the factors affect the response in the following order: volume of methanol > amount of salt in aqueous solution > time of extraction > volume of dodecane. Concentration of salt in water sample has a positive effect but time of extraction, volume of dodecane and volume of methanol has negative effects. It can be seen that by increasing of concentration of NaCl, the efficiency of extraction increases which is because of salting out effect [29]. In these experiments, extraction time is the interval between beginning of the dispersion and its end just before air flotation. The results show that the extraction time has a negative effect on the extraction efficiency of analytes. By increasing the volume of dodecane, the concentration of analyte in the extraction phase is decreased which can be related to the increasing volume of collected organic solvent consequently increasing in the volume of dodecane has a negative effect. With increasing of homogeneous solvent volume (methanol) the responses are decreased; that is because of the increasing lipophilic characteristic in aqueous sample solution and decreasing distribution constant.

The estimated value of the determination coefficient ($R^2$), expressed as a percentage, indicates that the model fits 93.1% of the experimental raw data. The quality of the regression, estimated by the analysis of variance (ANOVA) is shown in Table 5. If the model is a good predictor of the experimental data, the computed F-value would be higher. Generally, the P value in ANOVA table is an appropriate tool to check the significance of each variable. This information is necessary to explain the correlation of the mutual interaction between the factors. The smaller the magnitude of the *P*, the more significant is the corresponding coefficient. The P-values in Table 5 reveal that some of the linear and square and interaction terms in model (equation 1) at the 95% confidence level of significant (P-value<0.05). Table 5 also shows that, in the model, the resulting 'lack of fit F-value' (=0.525) is also not very significant.

**Table 3.** Design table showing the randomized run order of the experiment and responses of experiments. Concentration of BTXs in water samples 50 μg $L^{-1}$.

| RED | Benzene (PA) | Toluene (PA) | Xylene (PA) | Styrene (PA) |
|---|---|---|---|---|
| 1 | 1537065 | 2954506 | 5335423 | 3707022 |
| 2 | 2171506 | 3901676 | 6754901 | 5505576 |
| 3 | 1708086 | 3021739 | 5608612 | 3866007 |
| 4 | 1117789 | 1820028 | 3246144 | 2236480 |
| 5 | 1878754 | 3068933 | 6362265 | 3361683 |
| 6 | 902472 | 1657416 | 3636351 | 1730432 |
| 7 | 882615 | 1769279 | 3310175 | 2948777 |
| 8 | 847038 | 1777461 | 2909286 | 2312298 |
| 9 | 2518221 | 3887127 | 1875165 | 1670899 |
| 10 | 2321870 | 3876926 | 2180653 | 2177061 |
| 11 | 2410202 | 3583017 | 1970717 | 1872031 |
| 12 | 1920453 | 4000781 | 6204351 | 4293170 |
| 13 | 1921322 | 4196697 | 7058427 | 5811754 |
| 14 | 1913349 | 3060081 | 4454551 | 3316663 |
| 15 | 1670576 | 2283545 | 3256359 | 2472310 |
| 16 | 1940374 | 3475816 | 3661849 | 2831424 |
| 17 | 1513296 | 2263004 | 3247073 | 2339028 |
| 18 | 1574275 | 2228469 | 2968589 | 2580510 |
| 19 | 1159992 | 1983836 | 3169924 | 2568862 |
| 20 | 2177205 | 3147318 | 2709620 | 2288476 |
| 21 | 1881171 | 3416213 | 5266383 | 4194822 |
| 22 | 1720841 | 2728150 | 3964367 | 2975420 |
| 23 | 1205549 | 1814527 | 2884246 | 2305518 |
| 24 | 1456111 | 2151747 | 3057574 | 2715223 |
| 25 | 1673993 | 2313159 | 3100613 | 2397324 |
| 26 | 1311130 | 1637935 | 2122368 | 1819245 |
| 27 | 1782455 | 2695801 | 2847671 | 2278283 |
| 28 | 958732 | 1365783 | 1896303 | 1459662 |
| 29 | 1651173 | 1414803 | 1704693 | 1387903 |
| 30 | 1614252 | 2504120 | 2002186 | 1510513 |

Fig. 2 (a-f) shows the various three-dimensional plots of the response surface model. These plots are useful to visualize the generated response surface by the model. A point of maximum response could visually be determined from Fig.2 (a-f). As can be seen, the maximum point on the response surfaces has been located in the following values of variables: volume of dodecane = 150 μL, volume of methanol=0.5 mL, time of extraction=10 min and concentration of NaCl=1.55 mol $L^{-1}$.

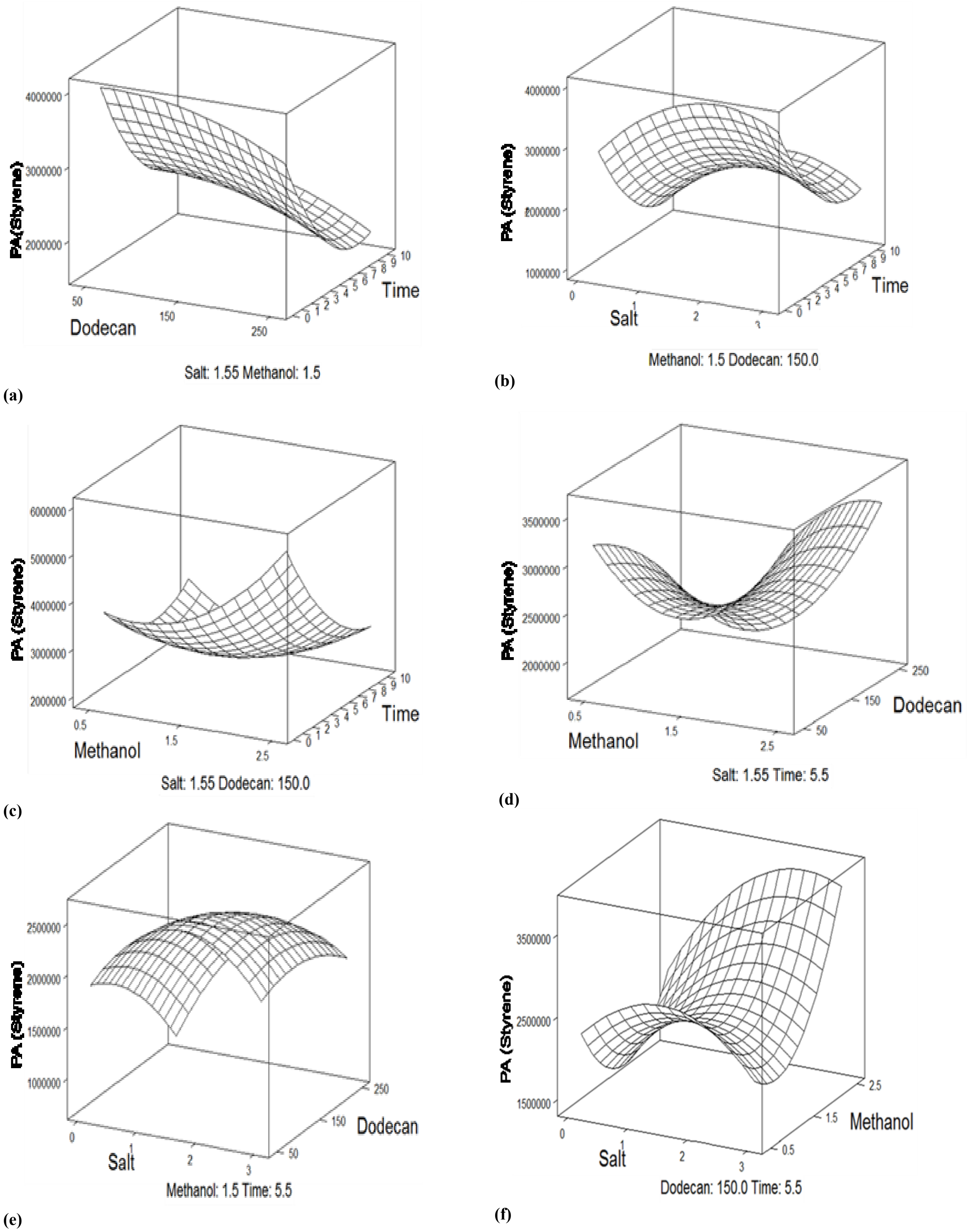

**Fig. 2.** a-f Different three-dimensional response surface plots. The units used in these figures are μL for volume of dodecan, mL for volume of methanol, mine for time of extraction, mol L$^{-1}$ for concentration of salt (NaCl).

**Table 4.** Estimated Regression coefficients for the quadratic response for styrene results (Eq. (1)).

| Term | Coef | SE Coef | T | P |
|---|---|---|---|---|
| Constant | 4859173 | 611339 | 7.948 | 0.000 |
| Salt | 561510 | 411338 | 1.365 | 0.192 |
| Methanol | -1980136 | 788461 | -2.511 | 0.024 |
| Dodecan | -3995 | 7885 | -0.507 | 0.620 |
| Time | -290415 | 147417 | -1.970 | 0.068 |
| Salt*Salt | -293379 | 118975 | -2.466 | 0.026 |
| Methanol*Methanol | 758373 | 250145 | 3.032 | 0.008 |
| Dodecan*Dodecan | -18 | 25 | -0.717 | 0.485 |
| Time*Time | 29856 | 12353 | 2.417 | 0.029 |
| Salt*Methanol | 273116 | 69421 | 3.934 | 0.001 |
| Salt*Dodecan | 1566 | 694 | 2.255 | 0.039 |
| Salt*Time | -15828 | 15427 | -1.026 | 0.321 |
| Methanol*Dodecan | 2537 | 1007 | 2.520 | 0.024 |
| Methanol*Time | -127531 | 22369 | -5.701 | 0.000 |
| Dodecan*Time | -27 | 224 | -0.122 | 0.905 |
| S = 402641 | R-Sq= 93.1 % | R-Sq(adj) =86.6 % | | |

**Table 5.** Analysis of variance for the quadratic response (Eq. (1)).

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| Regression | 14 | 3.27E+13 | 3.27E+13 | 2.34E+12 | 14.41 | 0.000 |
| Linear | 4 | 1.85E+13 | 2.81E+12 | 7.03E+11 | 4.34 | 0.016 |
| Square | 4 | 4.38E+12 | 4.38E+12 | 1.09E+12 | 6.75 | 0.003 |
| Interaction | 6 | 9.81E+12 | 9.81E+12 | 1.63E+12 | 10.08 | 0.000 |
| Residual Error | 15 | 2.43E+12 | 2.43E+12 | 1.62E+11 | | |
| Lack-of-Fit | 10 | 1.63E+12 | 1.63E+12 | 1.63E+11 | 1.02 | 0.525 |
| Pure Error | 5 | 8.00E+11 | 8.00E+11 | 1.60E+11 | | |
| Total | 29 | 3.51E+13 | | | | |

**Table 6.** The characteristic of calibration curves and preconcentration of factors for BTEX

| Analyte | Slope | Intercept | LOD (μg $L^{-1}$) | $r^2$ | DLR (μg $L^{-1}$) | PFs |
|---|---|---|---|---|---|---|
| Benzene | 4055.8 | 16882 | 8.09 | 0.997 | 10-100 | 138 |
| Toluene | 9223.3 | 69366 | 6.96 | 0.998 | 10-100 | 125 |
| Xylene | 18185 | -3773 | 8.23 | 0.998 | 10-100 | 207 |
| Styrene | 10185 | 13504 | 8.32 | 0.999 | 10-100 | 134 |

### *3.3. Evaluation of the method performance*

Using optimum values of variables, regression equation, correlation of determination ($r^2$), dynamic linear ranges (DLRs), limit of detections (LODs) and preconcentrations factors (PFs) were calculated and summarized in Table 6. The LODs were calculated as the analytes concentration equal to three times of the standard deviation of the blank signal divided by the slope of the calibration curve. The reproducibility in peak response was investigated on three replicate experiments under optimized conditions. The PFs were calculated as the ratio of the final concentration of the analyte in the organic phase and its concentration in the original solution concentration 50 μg $L^{-1}$ of each analyte under optimum conditions. A comparison between the proposed method and other methods for the extraction of BTEX are presented in Table 7. The RSD% for the proposed method is comparable with those of both SPME [10] and DLLME [11] methods and better than headspace method [12]. The quantitative results of HLLME-FA is comparable with SPME method and better than those of both DLLME and headspace methods. The comparison indicates that this proposed method needs very short extraction time comparing to the SPME and headspace methods. Also, the main advantages of the proposed method are this novel method does not need centrifugation to separate the organic phase and it is possible to the usage of low-density extraction solvents. The practical suitability of the developed HLLME-FA-GC-MS method was confirmed by the determination of the BTEX in industrial waste water samples collected from tow locations in an oil industrial media. Each sample were divided into three aliquots and analyzed. The results are summarized in Table 8. To determine the relative recoveries of the method, the water samples were spiked with 20 and 40 μg $L^{-1}$ from the BTEX. Fig. 3 shows the chromatogram of the extract BTEX from sample spiking with 20 μg $L^{-1}$ of the

BTEX. For comparison and validation of the obtained results from HLLME-FA-GC-MS, after spiking in the real samples, those were also analyzed with head space-gas chromatography-mass spectrometry (HS-GC-MS). Table 8 shows that the results are in satisfactory agreement.

## 4. CONCLUSION

In this study HLLME-FA-GC-MS as a simple and fast method has been developed for the determination of BTEX in the water samples for first time. The developed method was convenient for the usage of low-density extraction solvents. The new procedure of HLLME-FA is preferred to normal HLLME method which requires centrifugation to separate the organic phase. Air flotation was used to breakup organic solvent in water emulsion and to finish the extraction process.

**Table 7.** Comparison of the proposed method with other extraction methods for determination of the BTEX in water samples.

| Methods | R.S.D.% | Dynamic linear range ($\mu g\ L^{-1}$) | Limit of detection ($\mu g\ L^{-1}$) | Extraction time (min) | Ref. |
|---|---|---|---|---|---|
| SPME-GC-MS | 6.5-11.5 | 0.3-10 | 0.07-0.30 | 30 | [8] |
| Ultrasound-assisted-DLLME-GC-FID | 1.9-5.7 | 5.0-2500 | 0.2-0.3 | 2 | [9] |
| Headspace-GC-MS | 5.2-14.2 | 10-500 | 0.4-7.4 | 30 | [10] |
| HLLME-FA-GC-MS | 3.0-7.9 | 1.0-100 | 0.8-8.2 | 10 | This work |

**Table 8.** Determination BTEX in industrial waste water samples by HLLME-FA-GC-MS and HS-GC-MS.

| | | | HLLME- FA- GC-MS | | | HS- GC- MS |
|---|---|---|---|---|---|---|
| Sample | Analyte | $C_{added}$ ($\mu g\ L^{-1}$) | $C_{found}$ | RSD (n=3) | Error % | $C_{found}$ |
| Sample 1 | Benzene | -- | 36.50 | 3.09 | -- | n. A.[a] |
| | | 20 | 49.57 | 2.04 | -12.3 | 56 |
| | Toluene | -- | n. d.[b] | -- | -- | n. A. |
| | | 20 | 23.87 | 6.7 | 19.35 | 23 |
| | Xylene | -- | 21.61 | 7.9 | -- | n. A. |
| | | 20 | 43.16 | 2.7 | 3.7 | 37 |
| | Styrene | -- | n. d. | -- | -- | n. A. |
| | | 20 | 25.12 | 10.1 | 25.6 | 25 |
| Sample 2 | Benzene | -- | 42.05 | 7.84 | -- | n. A. |
| | | 40 | 85.78 | 5.98 | 4.54 | 82 |
| | Toluene | -- | n. d. | -- | -- | n. A. |
| | | 40 | 45.96 | 8.25 | 14.9 | 53 |
| | Xylene | -- | 32.58 | 6.45 | -- | n. A. |
| | | 40 | 78.28 | 9.82 | 7.85 | 85 |
| | Styrene | -- | n. d. | -- | -- | n. A. |
| | | 40 | 49.7 | 4.65 | 24.25 | 54 |

[a] non_analysed

[b] non_detected or below LOD

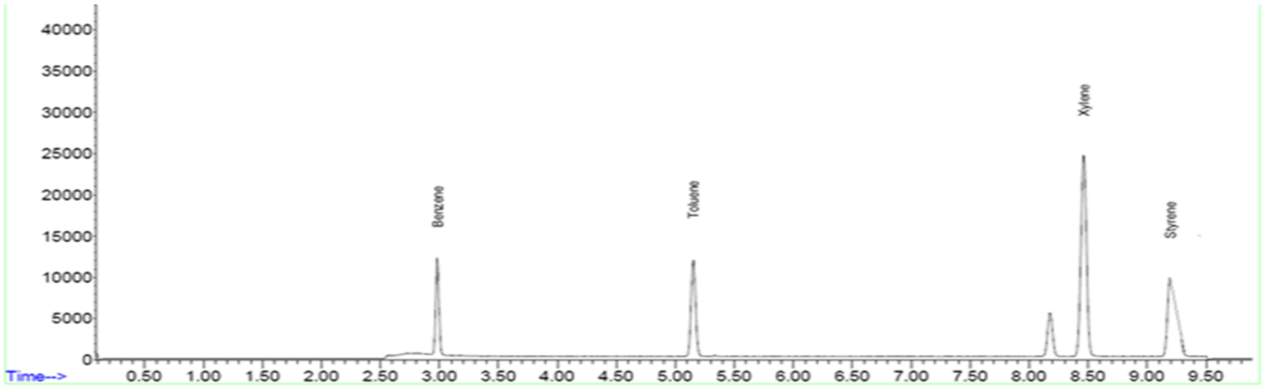

**Fig. 3.** Chromatogram of the extracted BTEX from spiked (20 $\mu gL^{-1}$) real sample. Extraction conditions: homogeneous solvent (methanol) volume, 0.5 mL; extraction solvent (dodecane) volume, 150 µL; concentration of NaCl, 1.55 M; time of extraction, 10min.

## REFERENCES

[1] R.M. Alerici, G.C. Zamproni, R.J. Poppi and M.N. Eberlin, Water solubilization of ethanol and BTEX from gasoline: on-line monitoring by membrane introduction mass spectrometry, *Analyst* 127 (2002) 230-234.

[2] Q. Li, X. Ma, D. Yuan and J. Chen, Evaluation of the solid-phase microexrtraction fiber coated with single walled carbon nanotubes for the determination of benzene, toluene, ethylbenzene, xylenes in aqueous samples, *J. Chromatogr. A* 1217 (2010) 2191-2196.

[3] I. Arambrri, M. Lasa, R. Garcia and E. Millan, Determination of fuel dialkylethers and BTEX in water using headspace solid- phase microextraction and gas chromatography– flame ionization detection, *J. Chromatogr. A* 1033 (2004) 193-203.

[4] J. Ii, C. Deng, W. Shen and X. Zheng, Field analysis of benzene, toluene, ethylbenzene and xylene in water by portable gas chromatography – microflame ionization detector combined with head space solid-phase microextraction, *Talanta* 69 (2006) 894-899.

[5] A. Gaujac, E.S. Emidio, S. Navickiene, S.L.C. Ferreira and H.S. Dorea, Multivariate optimization of a solid phase microextraction head space procedure for the determination of benzene, toluene, ethylbenzene and xylene in effluent samples from a waste treatment plant, *J. Chromatogr. A* 1203 (2008) 99-104.

[6] M. Bahri and M.R. Driss, Development of solid- phase microextraction for the determination of trihalomethanes in drinking water from Bizerte, Tunisia, *Desalination* 250 (2010) 414-417.

[7] H. Prosen and L. Zupanciici-kralj, solid-phase microextraction, *Trends Anal. Chem.* 18 (1999) 272-288.

[8] M. Palit, O. Pardasni, A.K. Gupta and D.K. Dubey, Application of single drop microextraction for analysis of chemical warfare agents and related compounds in water by gas chromatography/mass spectrometry, *Anal. Chem.* 77 (2005) 711-717.

[9] E. Psillakis and N. Kalogerakis, Developments in liquid-phase microextraction, *Trends Anal. Chem*. 22 (2003) 563-574.

[10] J.N. Bianchin, G. Nardin, J. Merib, A. Neves Dias, E. Martendal and E. Carasek, Simultaneous determination of polycyclic hydrocarbons and benzene, toluene, ethyl benzene and xylene in water samples using a new sampling strategy combining different extraction modes and temperatures in a single extraction solid-phase microextraction-gas chromatography-mass spectrometry procedure, *J. Chromatogr. A* 1233 (2012) 22-29.

[11] M. Khajeh and F. Musavi Zadeh, Response surface modeling of ultrasound-assisted dispersive liquid-liquid microextraction for determination of benzene, toluene and xylenes in water samples: box-behnken design, *Bull Environ. Contam. Toxicol.* 89 (2012) 38-43.

[12] B. Gilbert-Lopez, J. Robles-Molina, J.F. Garcia-Reyes and A. Molina-Diaz, Rapid determination of BTEXS in olives and olive oil by headspace-gas chromatography/mass spectrometry, *Talanta* 83 (2010) 391-399.

[13] M. Haji Hosseini, M. Rezaee, H.A. Mashayekhi, S. Akbarian, F. Mizani and M.R. Pourjavid, Determination of polycyclic aromatic hydrocarbons in soil samples using flotation-assisted homogeneous liquid-liquid microextraction, *J. Chromatogr. A* 1265 (2012) 52-56.

[14] M. Rezaee, Y. Assadi, M.R. Milani Hosseini, E. Aghee, F. Ahmadi and S. Berijani, Determination of organic compounds in water using dispersive liquid-liquid microextraction*, J. Chromatogr. A* 1116 (2006) 1-9.

[15] M. Rezaee, Y. Yamini and M. Faraji, Evolution of dispersive liquid-liquid microextraction method, *J. Chromatogr. A* 1217 (2010) 2342-2357.

[16] W.C. Tsai and S.D. Huang, Dispersive liquid- liquid microextraction combined with liquid chromatography for the determination of chlorophenoxy acid herbicides in aqueous samples, *J. Chromatogr. A* 1216 (2009) 7846-7850.

[17] M. Garcia-Lopez, I. Rodriguez and R. Cela, Development of a dispersive liquid-liquid microextraction method for organophosphorus flame retardants and plastizicers determination in water samples, *J. Chromatogr. A* 1166 (2007) 9-15.

[18] M.A. Farajzadeh, M. Bahram, S. Zorita and B. G. Mehr, Optimization and application of homogeneous liquid-liquid extraction in preconcentration of copper (II) in a ternary solvent system, *J. Hazard. Mater.* 161 (2009) 1535-1543.

[19] S. Oshite, M. Furukawa and S. Igarashi, Homogeneous liquid- liquid extraction method for the selective spectrofluorimetric determination of trace amounts of tryptophan, *Analyst* 126 (2001) 703-706.

[20] Y. Takagai and S. Igarashi, UV- detection capillary electrophoresis for benzo[a]pyrene following a two- step concentration system using homogeneous liquid-liquid extraction and a sweeping method, *Analyst* 126 (2001) 551-552.

[21] S. Igarashi, A. Takahashi, Y. Ueki and H. Yamaguchi, Homogeneous liquid-liquid extraction followed by X-ray fluorescence spectrometry of a microdroplet on filter-paper for the simultaneous determination of small amounts of metals, *Analyst* 125 (2000) 797-798.

[22] M.A. Farajzadeh, S.E. Seyedi, M.S. Shalamzali and M. Bamorowat, Dispersive liquid- liquid microextraction using extraction solvent lighter than water, *J. Sep. Sci.* 32 (2009) 3191-3200.

[23] A. Saleh, Y.Yamini, M. Faraji, M. Rezaee and M. Ghambarian, Ultrasound-assisted emulsification microextraction method based on applying low density organic solvent followed by gas chromatography analysis for the determination of polycyclic aromatic hydrocarbons in water samples, *J. Chromatogr. A* 1216 (2009) 6673-6679.

[24] M. Leong and S.D. Huang, Dispersive liquid-liquid microextraction method based on solidification of floating organic drop combined with gas chromatography with electron-capture or mass spectrometry, *J. Chromatogr. A* 1211 (2008) 8-12.

[25] Q. Zhou, H. Bai, G. Xie and J. Xiao, Trace determination of organophosphorus pesticides in environmental samples by temperature controlled ionic liquid dispersive liquid-phase microextraction, *J. Chromatogr. A* 1188 (2008) 148-153.

[26] D.C. Montgomery, *Design and Analysis of Experiments.* 5th Ed. Wiley, New York, 2001.

[27] F. Deyhimi, M. Arabieh and L. Parvin, Optimization of the emerson- trinder enzymatic reaction by response surface methodology, *Biocatalysis and Biotransformation* 24 (2006) 263-271.

[28] F. Deyhimi, R.S. Ahangari, M. Arabieh and L. Parvin, Application of response surface methodology for modeling the enzymatic assay of hydrogen peroxide by emerson-trinder reaction using 4- iodophenol, *Intern. J. Environ. Anal. Chem.* 86 (2006) 1151-1163.

[29] A. Hasseine, A.-H. Meniai and M. Korichi, Salting-out effect of single NaCl and KCl on the LLE of the systems (water + toluene + acetone), (water + cyclohexane + 2-propanol) and (water + xylene + methanol), *Desalination* 242 (2009) 264-276.