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Preconcentration and Determination of Trace Amounts of Aromatic Compounds in Non-alcoholic Beer Samples by In-syringe Dispersive liquid–liquid Microextraction

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Abstract

In this paper, a new simple and effective method based on dispersive liquid–liquid microextraction procedure is proposed for rapid and simultaneous separation and preconcentration of ultra trace amounts of benzene, toluene, ethyl benzene and xylenes (BTEX) in non-alcoholic Beer samples. In this experiment, a glass syringe was used as extraction unit. With this simple configuration, the centrifugation step, which is a time consuming step was eliminated and also the possibility of using solvents with density lighter than water as extractant solvent was provided; and therefore applicability of DLLME will be expanded to a wider range of solvents. The influence of extraction parameters, such as kind and volume of extractant and disperser solvents, volume of sample and pH of the sample solution and its ionic strength were investigated and optimized. The best efficiency of extraction acquired using acetone and nonanol as dispersive and extraction solvents respectively. Under the optimum condition, the proposed method provided a linear range (10-1300 $\mu g.L^{-1}$) with correlation coefficient (R^2) of 0.998, and relative recovery of 97.3-101.5%. The limit of detection was in the range of 2.0-2.8 $\mu g.L^{-1}$. At the end, the proposed micro extraction method was successfully applied for the determination of BTEX compounds in a few real non-alcoholic Beer samples.

Keywords: *Syringe dispersive, Liquid-liquid microextraction, BTEX, Non-alcoholic beer.*

Introduction

Determination of Benzene aromatic compounds have been of great interest for analytical chemists. Benzene is a chemical used for the production of many industrial compounds such as styrene, phenol, cyclohexane,

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Aniline, alkylbenzene, and chlorobenzene [1]. Replacing a hydrogen in benzene cycle with other groups such as methyl or ethyl, leads to formation of new compounds like toluene (methyl benzene), xylenes (dimethyl benzene), and ethyl benzene; these group of compounds are known as benzene aromatic compounds or BTEX. Benzene is a chemical that can cause serious complications of chronic for human. Vapor pressure of Benzene at room temperature and atmospheric pressure is sufficient enough to cause respiratory risk. Benzene exposure for a long time or repeatedly in a short time can cause serious damage to the blood-forming elements and in serious cases it may lead to Leukemia [2,3]. BTEX compounds can enter body through eating (consuming contaminated water and BTEX), breathing contaminated air, and absorption through skin [4]. On the other hand, benzene is formed in low levels in beverages containing preservatives and additives. Benzoate salts, used in some beverages as antimicrobial agents, could react with ascorbic acid (vitamin C) in the presence of light and high temperatures to produce benzene. Ascorbic acid is a natural compound found in some foods and usually it's added to foods and drinks as a vitamin or antioxidant. Benzene can also enter into beverages through some packaging materials [5].

Since the matrix of most samples are complex and the amount of the analytes in samples are not within the limit of detection of analytical instruments, sample preparation plays an important role in identifying and measuring these analytes. Sample preparation is the most important and time consuming step in analysis process. This step is more important when analysis is focused on isolation and/or determination and evaluation of trace amounts of analytes in complex matrix. Since BTEX compounds are widely used in various industries and due to their high toxicity, determination of these compounds even in low concentrations is very important; therefore many preparation and separation methods have been proposed. Determination of BTEX compounds is usually done with gas chromatography with flame ionization detector (GC-FID). Liquid-liquid extraction (LLE) and solid phase extraction (SPE) are usually applied for extraction and preconcentration of BTEX. LEE is time consuming and tedious and requires large volume of organic solvents which are toxic and expensive. SPE uses much less solvent than LLE, but in SPE there is a need for column preparation and it is an expensive method. Recently, liquid phase microextraction (LPME) has been introduced as an available alternative to traditional methods of extraction and sample preparation of organic compounds. LPME is fast, easy, inexpensive, and since it requires very small amount of organic solvent, the exposure to organic solvents is reduced. Also, due to the high ratio of sample solution to extraction phase, usually high concentration factor for the analyte

of interest is obtained. Many LPME technique have been proposed, including Head space-Single drop microextraction (HS-SDME) [6], Direct immersion-Single drop micro extraction (DI-SDME) [7], and Dispersive liquid-liquid microextraction (DLLME) [8].

DLLME, introduced in 2006 by Rezaei et al, is based on the extension of contact surface between two liquid phases [9]. This microextraction technique, is quick and easy and due to its high performance, a variety of DLLME methods have been developed such as Cold-induced aggregation dispersive liquid-liquid microextraction (CIA-DLLME) [10], Ultrasound assisted-Dispersive liquid-liquid microextraction (UA- DLLME) [11] and Air-assisted liquid-liquid microextraction (AA-DLLME) [12]. All of these methods require the use of a centrifuge to separate the extracted phase from aqueous phase. In this paper one step in syringe-dispersive liquid-liquid microextraction (IS-DLLME) based on DLLME is proposed for separation and preconcentration of BTEX in non-alcoholic beer as a simple and effective method. In this technique, analyte extraction is carried out in an ordinary glass syringe as an extractor unit [13]. By applying this simple strategy, centrifuge step which is a time consuming step is eliminated completely and the use of extractant solvents is extended to solvent with lower density than water; therefore, potential of DLLME application is extended to a wider range of extractant solvents.

Experimental

Chemicals and standards

All chemical used in this research were of analytical grade. Benzene, toluene, ethylbenzene, and xylene isomers were purchased from Fluka (Switzerland). The other chemicals were obtained from Merck (Germany). Stock standard solutions containing 1000 $mg.L^{-1}$ of each of BTEX compounds were prepared in methanol. Working solutions were prepared freshly every day by sequentially diluting the intermediate solutions.

Preparation of real sample

Since non-alcoholic beer samples, Istak (Arpanoush), contain high level of gas, degassing of sample was necessary. Therefore samples were transferred to a beaker and were stirred for an hour by a magnetic stirrer, and then IS-DLLME was applied on them.

GC analysis for IS-DLLME

Separation and detection of BTEX compounds were carried out using a Varian 3400 gas chromatograph system (USA) equipped with a flame ionization detector (FID) and a CBPS fused silica capillary column (25 m×0.25 mm i.d., 3 μm film thickness). The injection port was operated at splitless mode and nitrogen was employed as carrier gas at a constant flow rate of 1.0 $mL.min^{-1}$. The temperature of injector and detector were set as 250°C. The oven temperature program was: 60°C, held for 4 min;

rating 3°C.min^{-1} to 80°C; rating 30°C.min^{-1} to a final temperature of 230°C and held for 5 min.

Microextraction procedure

In this method a simple 10 mL glass syringe was utilized as the extraction unit. First a sample solution containing appropriate amount of BETX was prepared in a 10 mL measuring flask and then it was transferred into the glass syringe. 30 µL of nonanol (extractant solvent) and 700 µL of acetone were mixed separately into a vial as the binary solution. This binary solution was rapidly injected into the sample with the use of micropipette which rapidly leads to formation of a turbid solution consisting of tiny droplets of extractant solvent by dispersion in aqueous solution. After a few seconds due to lower density of extractant, a droplet of extractant phase forms at top of the sample solution. The plunger of the syringe is slowly moved up to lead the extractant droplet to the tip of syringe. 1 µL of extractant is easily removed with a 10 µL syringe and injected directly to GC (figure 1).

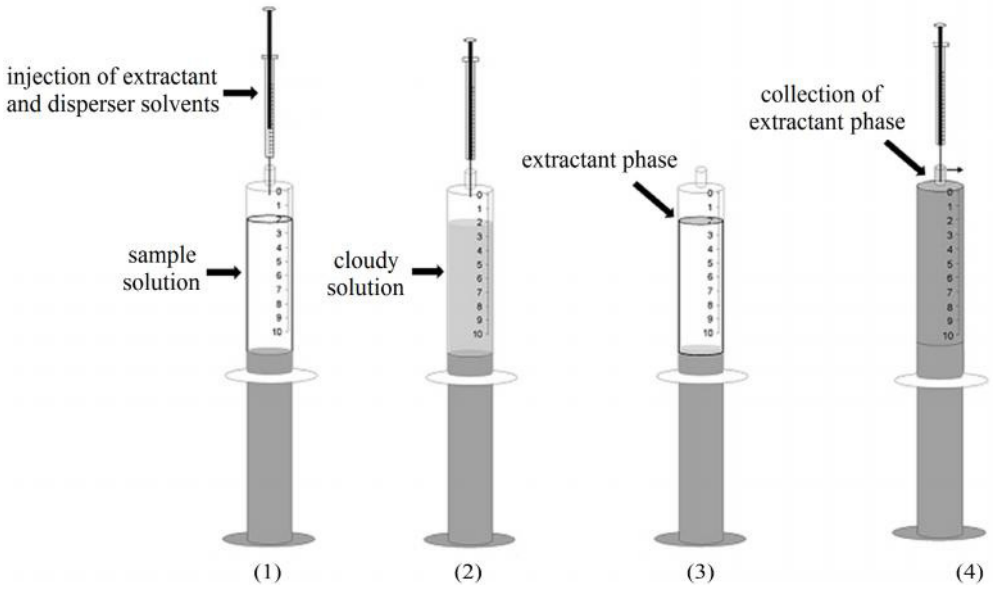

Figure 1. One step in syringe dispersive liquid-liquid microextraction process: (1) injection of extractant and disperser solvent into sample solution, (2) formation of cloudy solution containing tiny droplets of extractant, (3) extractant phase formed on the surface of aqueous sample solution, and (4) collection of extractant phase from the tip of the syringe.

Result and discussion

In order to obtain the maximal extraction efficiency, important experimental parameters which can potentially influence the enrichment performance, such as kind and volume of extractant and disperser solvents, volume of sample, time of the extraction and etc. have been investigated in detail for proposed method. The uni-variant method was used to simplify the optimization procedure. A series of experiments were designed for this goal. All optimizations were applied on sample solutions

containing 500 μg.L^{-1} and numbers of replicates of analysis were at least 3 for each experiment.

Selection of extractant solvent

Selection of an appropriate extraction solvent is of great importance in all DLLME processes. The primary requirements of an adequate extraction solvent for the proposed DLLME methods are: low solubility in water, being less dense than water, and high extraction capability for the analytes of interest. Moreover, low level of toxicity and good chromatographic behavior are other desirable properties. For this purpose, heptanol, octanol, nonanol, hexadecane, and cyclohexadecane were selected and compared for their demonstrated capability of extracting BTEX. Experiments showed that, extraction efficiency of nonanol is higher than other solvents. Hence, nonanol was selected as the extraction solvent for IS-DLLME procedure.

Selection of disperser solvent

In DLLME it is necessary that the extractant solvent is dispersed as very fine droplets into the aqueous sample in order to obtain a very high amount of contact area and achieve fast migration of analytes from aqueous sample into the extraction phase. This purpose is achieved with utilization of disperser solvent. Disperser solvent must be miscible in both extractant solvent (organic phase) and sample solution (aqueous phase). Therefore acetone, methanol and ethanol were chosen and the effect of these solvents on the extraction efficiency of DLLME was investigated accurately. 500 μL of each disperser solvent containing 50 μL nonanol as the extractant solvent were used as the binary solution for extraction. The maximum extraction efficiency was obtained by using acetone as a disperser solvent (figure 2). Therefore, acetone was selected for further experiments as disperser solvent.

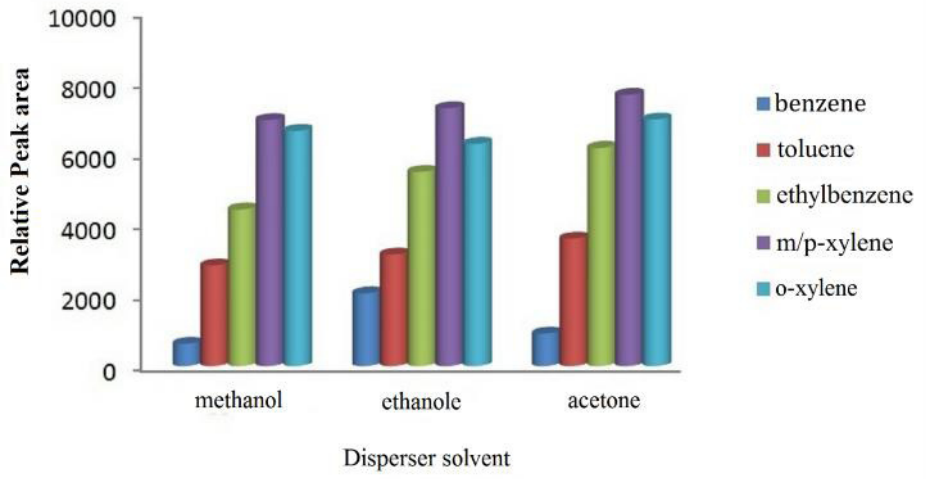

Figure 2. Effect of kind of disperser solvent on extraction efficiency of BTEX by IS-DLLME.

Effect of the extraction solvent volume

Volume of the extraction solvent used have direct affect on volume of the organic phase collected above the aqueous phase, repeatability of results, and extraction efficiency. Therefore, extraction solvent volume was studied for the proposed method. For this purpose different volumes of nonanol, in the range of 30 to 90 µL, were studied. In volume lower than 30 µL the obtained extractant phase was very small and removal of 1µL of it was very difficult. And after 30 µL there was a decrease in signal of analytes, thus 30 µL of nonanol was chosen as the volume of extractant solvent for the IS-DLLME.

Effect of the disperser solvent volume

To find out the best volume of disperser solvent, various volume of acetone in the range of 500 to 1000 µL containing 30 µL of nonanol were investigated. In the volume from 500 to 700 µL the efficiency of extraction increased and then it decreased so 700 µL was chosen as the optimized volume for IS -DLLME procedure (figure 3).

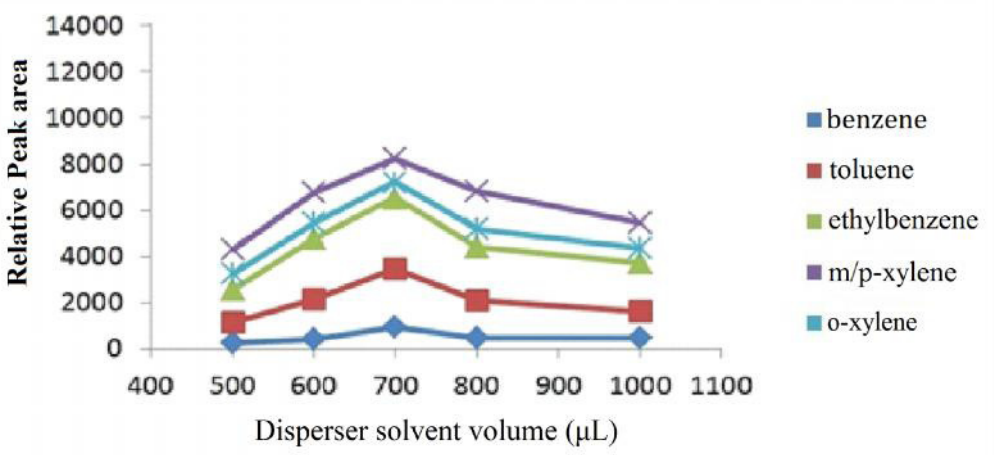

Figure 3. Effect of volume of disperser solvent on extraction efficiency of BTEX by IS-DLLME.

Effect of sample volume

Sample volume can affect disperse ability of binary solution and therefore affect the efficiency of extraction. Various sample volumes were investigated in the range of 5-11 mL and IS-DLLME was applied on them. It was observed that in volume more than 10 mL, the amount of extractant solvent collected on surface of sample solution was reduced because of its solubility in larger volume of water. Therefore 10 mL was chosen for the solvent volume.

Effect of ionic strength

Different sample solution containing various concentration of NaCl (0-1.4%) were prepared to study the effect of ionic strength on proposed IS-DLLME method. The effect of ionic strength

on efficiency of extraction can be explained by the fact that water molecules form hydration spheres around the salt ions. These hydration spheres reduce the concentration of available water to dissolve analyte molecules; hence, it was expected that this would drive additional analytes into the extraction phase. As shown in figure 4, the highest efficiency were obtained when concentration of salt was 0.5%.

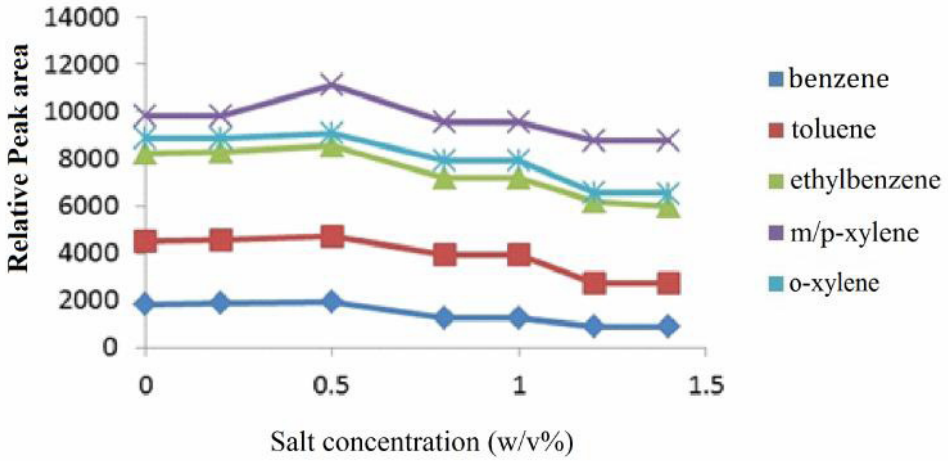

Figure 4. Effect of salt concentration on extraction efficiency of BTEX by IS-DLLME.

Effect of pH of sample solution

To determine the effect of pH on efficiency of IS-DLLME for BTEX, pH of sample solution was set in the range of 4-9 (with help of acetate and ammonia buffer, HCl, and NaOH). Figure 5 illustrates the analytical response of analytes based on pH of sample solution. As can be seen, in pH 7, the best response was recorded for all analytes. Therefore, since pH of non-alcoholic beer (real sample) is near 7, there was no necessity of adding buffer to the sample solution and thus the pH was not set for further experiments.

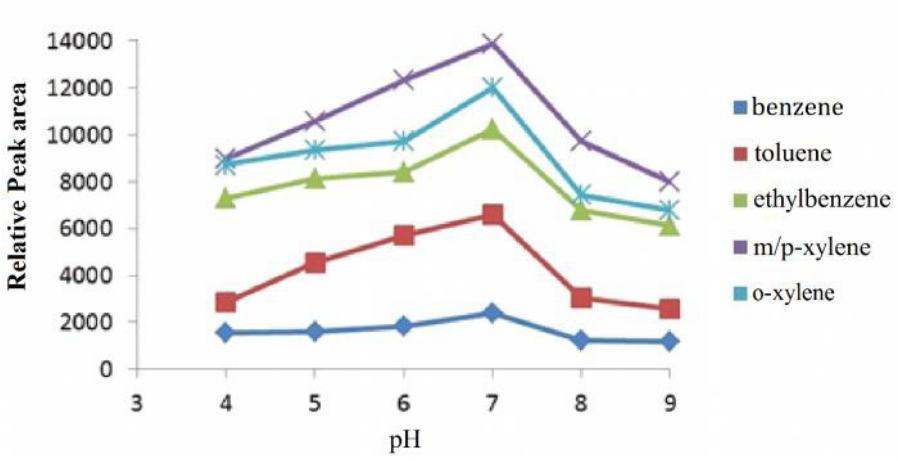

Figure 5. Effect of pH of sample solution on extraction efficiency of BTEX by IS-DLLME.

Figures of merit for IS-DLLME

Quantitative parameters of proposed IS-DLLME method were evaluated by determining of BTEX in spiked aqueous samples. Calibration was performed individually using aqueous standard solutions submitted to the IS-DLLME procedures as described above. Linearity of calibration curve was observed in the range of 10-1300 μg.L^{-1} (R^2= 0.998). The limit of detections (LODs), based on signal to noise ratio of 3, calculated to be in range of 2.0-2.8. The precision of the methods, expressed as relative standard deviation (RSD), obtained by five consecutive aqueous samples of BTEX at the optimized experimental conditions was between 1.05 to 2.92. The enrichment factor, defined as the slope ratio of two calibration curves with microextraction and without microextraction (table 1).

Analysis of real samples

To evaluate the applicability of the proposed methods, it was applied for determination of BTEX compounds in non-alcoholic beer (Istak, Arpanoush). Direct analysis revealed no measurable BTEX. Therefore, samples were spiked with BTEX compounds and used for investigation of matrix effects. The results are presented in Table 1. As can be seen, good recoveries obtained for proposed IS-DLLME method (97.3-101.5%) indicate that the matrix effect was negligible.

Table 1. figures of merit for determination of BTEX by IS-LLME.

Analyte	Linear range (μg.L^{-1})	R^2	LOD (μg.L^{-1})	Enrichment factor	Recovery%[a]
Benzene	10-1300	0.998	2.0	29.0	97.5
Toluene	10-1000	0.998	2.4	24.0	97.3
Ethylbenzene	10-800	0.998	2.0	20.0	97.9
m/p-Xylene	10-1100	0.997	2.5	25.0	101.5
o-Xylene	10-800	0.995	2.8	28.0	97.9

[a] spiked with 500 μg.L^{-1} BTEX compounds

Conclusion

In the present paper one step in syringe liquid-liquid microextraction (IS-DLLME) was studied for preconcentration and determination of ultra-trace amounts of BTEX in non-alcoholic beer samples. The results showed that IS-DLLME exhibit good linearity, precision, enrichment factor, and detection limit for extraction of BETX.

Application of syringe as the extraction unit not only makes the DLLME faster and easier to perform, but also conquers over two problems existing in conventional DLLME. This means that the centrifugation step is

eliminated in IS-DLLME and also solvents with lower density than water can be used in DLLME. More important, this method is fast, simple, sensitive, and inexpensive, and with elimination of centrifuge step, allows sample extraction and preconcentration to be performed in a single step.

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