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Dispersive liquid–liquid microextraction for determination of volatile aromatic hydrocarbons in water

Edita Pusvaškienė,

Aliona Jurkina,

Vida Vičkačkaitė*

Department of Analytical and Environmental Chemistry, Vilnius University, Naugarduko 24, LT-03225 Vilnius, Lithuania

Dispersive liquid–liquid microextraction is suggested for volatile aromatic hydrocarbon sampling and preconcentration. The effects of extraction solvent type, extraction and disperser solvent volume, extraction time and ionic strength of the solution were investigated. Carbon tetrachloride containing *n*-octane as the internal standard was used as an extracting solvent, and acetone was used as a disperser solvent. The calibration graphs were linear up to 2 mg ml^{-1}, the correlation coefficients were 0.998–0.999, the enrichment factors were from 144 for toluene to 239 for *o*-xylene, and the detection limits were 0.40, 0.35 and 0.33 μg l^{-1} for toluene, ethylbenzene and *o*-xylene, respectively. Repeatabilities of the results were acceptable, with relative standard deviations up to 11.7%. A possibility to apply the proposed method for volatile aromatic hydrocarbon determination in water samples was demonstrated.

Key words: dispersive liquid–liquid microextraction, gas chromatography, volatile aromatic hydrocarbons, water samples

INTRODUCTION

Volatile aromatic hydrocarbons are widely spread in the environment. Benzene, toluene, ethylbenzene and xylenes (BTEX) are used as solvents and precursors for many plastics, are found in oil and oil products and released into the environment from petroleum refining industries [1]. BTEX seriously affect human health. Short-term effects include headache, fatigue, nervous system disorders, immune system depression, anemia; long-term effects are chromosome aberrations, cancer, spasms, damage to the liver, kidney, eyes and central nervous system [1]. Thus, for BTEX control in all fields of interest, precise and accurate analytical techniques are necessary.

Sample preparation is an essential step in chemical analysis. Traditional sample preparation techniques such as liquid–liquid extraction and solid phase extraction are time-consuming and use large amounts of toxic organic solvents which are dangerous to human health and to the environment. Because of these disadvantages, development of new fast, inexpensive, environmentally friendly and easy to use microextraction techniques gain a growing interest.

Liquid phase microextraction (LPME) has been first introduced in 1996 [2] as a miniaturised version of liquid–liquid extraction. Up to now, several LPME methods have been developed [3–5], dispersive liquid–liquid microextraction (DLLME) being the newest one.

The DLLME method was suggested in 2006 by Assadi et al. [6]. The method is based on the ternary component solvent system. A mixture of a water-immiscible extraction solvent dissolved in a water-miscible disperser solvent is injected rapidly into an aqueous sample. A cloudy solution formed consists of fine droplets of the extraction solvent dispersed into aqueous phase. Due to the considerably large surface area between the extraction solvent and the aqueous sample, the extraction of the analytes is achieved quickly. Then centrifugation takes place, and the extraction solvent with the analytes is sedimented and analysed by an appropriate method.

DLLME is simple to operate, and is an especially rapid, inexpensive extraction method with high preconcentration factors and low sample volume requirements. DLLME was applied for preconcentration of polyaromatic hydrocarbons [6], organophosphorus pesticides [7], phenols [8], chlorophenols [9], phthalate esters [10, 11], triazine herbicides [12]. Simultaneous DLLME and derivatization was suggested for chlorophenols [13], anilines [14] and fatty acids [15].

In the present study, the DLLME method has been investigated for the determination of volatile aromatic hydrocarbons in aqueous solutions.

* Corresponding author. E-mail: vida.vickackaite@chf.vu.lt

EXPERIMENTAL

Reagents

Benzene (≥99.8%), toluene (≥99.9%), ethylbenzene (≥99.5%), *o*-xylene (≥99%) and carbon tetrachloride (≥99.5%) were purchased from Merck (Hohenbrunn, Germany). *n*-Octane (C_8H_{18}) (≥98%), acetone (≥99.9%), dichloromethane (CH_2Cl_2) (≥99.5%), chloroform ($CHCl_3$) (≥99%), chlorobenzene (C_6H_5Cl) (≥99%), bromobenzene (C_6H_5Br) (≥99%) and 1,2-dichlorobenzene ($C_6H_4Cl_2$) (≥99%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). NaCl (analytical grade) was purchased from "Reachim" (Ukraine).

A standard stock solution containing 1 mg ml^{-1} of benzene, toluene, ethylbenzene and *o*-xylene was prepared in acetone. The stock solution was stored refrigerated at +4 °C. Working standard solutions were prepared daily by diluting the stock standard solution with distilled water to the required concentrations.

DLLME procedure

Eight millilitres of an aqueous solution of BTEX was placed in a 12 ml screwcape glass tube; 0.5 ml of a solution containing 0.485 ml of acetone (as disperser solvent) and 15 µl of carbon tetrachloride (as extraction solvent) with *n*-octane as an internal standard (1 µg ml^{-1}) were rapidly injected using a 1 ml syringe. A cloudy solution formed was centrifuged in a Boeco S-8 centrifuge (Germany) for 2 min at 5000 rpm. The sedimented phase was taken with a 10 µl microsyringe (Hamilton, Reno, NV, USA) and injected into a gas chromatograph.

GC analysis

Extracted analytes were detected using gas chromatography. Gas chromatography was carried out in a Varian 3400 (Palo Alto, CA, USA) gas chromatograph equipped with a flame ionisation detector coupled with an SP4290 integrator (Spectra-Physics San Jose, USA) and an Equity™-5 fused silica capillary column (30 m × 0.53 mm, 1.5 µm film thickness) supplied by Supelco (Bellefonte, PA, USA). A splittless injection mode was used. The injector and detector temperature was 280 °C. The oven temperature was programmed: it was initially set at 35 °C for 5 min, then gradually ramped to 100 °C (6 °C min^{-1}), to 150 °C (50 °C min^{-1}) and kept for 3 min. The following gas flow rates were used: carrier (nitrogen) 10, make-up gas (nitrogen) 20, hydrogen 30 and air 300 ml min^{-1}; 1 µL of the extract was injected for GC analysis.

RESULTS AND DISCUSSION

For an efficient performance of the extraction, several parameters that influence the extraction efficiency were studied and optimized. Those parameters were the nature and the volume of the extraction solvent and of the disperser solvent, the extraction time, and the ionic strength of the solution.

Extraction solvent

The first step in the method development was to select a proper extraction solvent. An extraction solvent should satisfy several requirements: it should demonstrate a good extraction capability of the compounds of interest; it should have a higher density than water; its solubility in water should be low. In addition, in the case of subsequent gas chromatographic analysis, the solvent should demonstrate good gas chromatography behaviour and it should be soluble in the disperser reagent.

In order to select an extraction solvent, 40 µl of extraction solvent was mixed with 500 µl of acetone, and the obtained solution was rapidly injected into an aqueous solution containing 1 µg ml^{-1} of BTEX. The mixture was centrifuged for 5 min. and the sedimented phase was injected into the GC for analysis. Several potential extraction solvents – carbon tetrachloride, chloroform, chlorobenzene, bromobenzene and 1,2-dichlorobenzene – were examined. The physical properties of the selected organic solvents are presented in Table 1.

Table 1. **Physical properties of extraction solvents**

Solvent	Boiling point, °C	Density, g ml^{-1}	Water solubility, g l^{-1}
CH_2Cl_2	40	1.33	13
$CHCl_3$	62	1.48	8
CCl_4	76.5	1.59	0.8
C_6H_5Cl	132	1.11	0.5
C_6H_5Br	153	1.50	0.4
$C_6H_4Cl_2$	180	1.30	0.15

Dichloromethane and chloroform were rejected immediately as they resulted in a high water solubility (13 and 8 g l^{-1}, respectively) and did not form a separate phase in the aqueous solution. The retention time of chlorobenzene was very close to that of benzene. Moreover, as the solvent peak was very broad, it also interfered with toluene determination. Bromobenzene and dichlorobenzene were not suitable for BTEX extraction because of the presence of a significant quantity of benzene in the solvents. Thus, carbon tetrachloride was the only solvent suitable for the DLLME of BTEX. However, even in the case of carbon tetrachloride, small concentrations of benzene could not be determined as carbon tetrachloride retention time was also close to that of benzene. Thus, for our further work, three analytes – toluene, ethylbenzene and *o*-xylene – were selected.

The effect of extraction solvent volume on the analytical signal was studied in the range of 10–50 µl. For all the analytes, an increase in the extraction solvent volume led to lower peak areas (Fig. 1). However, 10 µl of carbon tetrachloride resulted in a too small and difficult to handle volume of the sedimented phase. Thus, 15 µl of carbon tetrachloride was determined to be the optimal extraction solvent volume.

Disperser solvent volume

The main selection criterion of the disperser solvent for DLLME is its miscibility with extraction solvent and aqueous

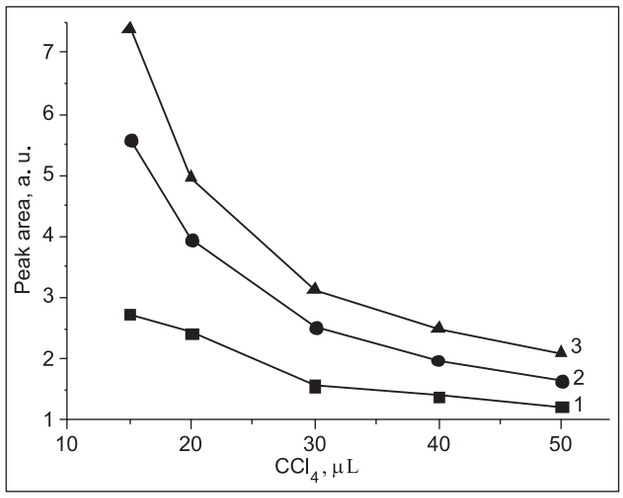

Fig. 1. Effect of DLLME solvent volume on the peak area of (1) toluene, (2) ethylbenzene and (3) *o*-xylene. Concentration of each analyte is 1 μg ml^{-1}, acetone volume 500 μl, centrifugation 2 min

phase [6]. As the disperser solvent must be miscible with both the organic and aqueous phases, the choice of it is rather limited. In most of the publications concerning DLLME, acetone, acetonitrile and methanol were examined as disperser solvents [6, 8, 11–17] and it was demonstrated that the recovery variations using different disperser solvents were not remarkable. Referring to the data and considering its low toxicity and cost, acetone was selected as a disperser solvent for our work.

Different acetone volumes (0.1–1.5 ml) were used to maintain the constant quantity of the extraction solvent carbon tetrachloride (15 μl). With an increase in acetone volume, peak areas initially increased because at a low acetone volume the cloudy state was not stable, and probably this caused an incomplete extraction. On the other hand, with the further increase in acetone volume, analyte peak areas began to decrease (Fig. 2). Probably because of a significant quantity of acetone in the aqueous phase, the partition of the analytes between the extraction solvent and the aqueous phase decreased. Hence, 0.4–0.9 ml acetone volume was the optimum. In order to have a convenient 0.5 ml acetone–CCl_4 mixture volume for the injection and considering that the optimum CCl_4 volume is 15 μl, acetone volume of 0.485 ml was selected for the further work.

Extraction time

For DLLME, extraction time is defined as the time between the injection of a mixture of disperser and extraction solvents and the centrifuge step. The DLLME extraction time up to 30 min was investigated. Peak area variations at different extraction times were not significant. Evidently, the surface area between the aqueous and organic phases was large, and 20–30 seconds (time between the injection and the beginning of centrifugation) were sufficient for the extraction.

Effect of ionic strength

The addition of salt to the aqueous sample solution is widely used to enhance the extraction of analytes as it generally decreases the solubility of organic compounds in water. In order to examine salt influence on DLLME of the analytes of interest, the extraction was performed in the presence of different concentrations of NaCl (from saltless up to saturation). The results presented in Fig. 3 demonstrate that the extraction efficiency decreases with increasing the concentration of NaCl, probably because dissolved NaCl might change the physical properties of the Nernst diffusion film of the droplets and thus impede the extraction. Moreover, addition of big quantities of salt was unacceptable as after injection of the extraction–disperser solvent mixture a saturation of the aqueous solution with the salt is reached. Thus, after centrifugation,

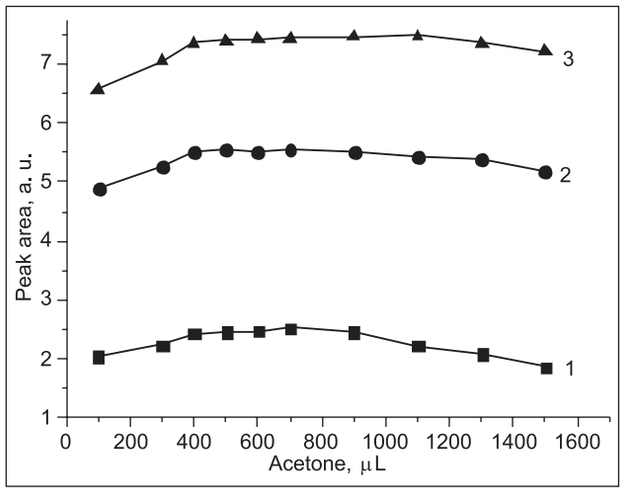

Fig. 2. Effect of DLLME disperser solvent volume on the peak area of (1) toluene, (2) ethylbenzene and (3) *o*-xylene. Concentration of each analyte is 1 μg ml^{-1}, carbon tetrachloride volume 15 μl, centrifugation 2 min

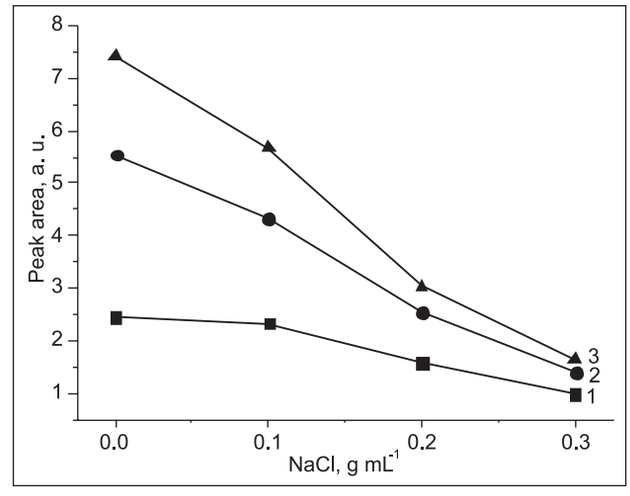

Fig. 3. Effect of NaCl content on the peak area of (1) toluene, (2) ethylbenzene and (3) *o*-xylene. Concentration of each analyte is 1 μg ml^{-1}, carbon tetrachloride volume 15 μl, acetone volume 485 μl, centrifugation 2 min

the sedimented phase contained not only the extraction solvent but also the salt. Assuming this, in further experiments no salt was added to the samples.

Validation of the method

The quality parameters of the proposed method, such as linearity, limits of detection, enrichment factors and repeatabilities, were calculated under the optimized extraction conditions. However, before that, in order to improve repeatability, *n*-octane (1 μg ml^{-1}) had been added to the extraction solvent as an internal standard.

For the enrichment factor calculation, three replicate extractions were performed in the optimal conditions from an aqueous solution containing 2 μg ml^{-1} of each analyte. The enrichment factor was calculated as a ratio of the final analyte concentration in the extraction solution and its concentration in the original solution. The actual concentration of each extracted analyte was calculated from the calibration curves. The enrichment factors were similar for ethylbenzene and *o*-xylene and somewhat lower for toluene (Table 2). This can be explained by a higher toluene water solubility.

Table 2. **Enrichment factors, detection limits and repeatabilities**

Analyte	Enrichment factor	Detection limit, μg l^{-1}	RSD, % (n = 5)	
			1 μg ml^{-1}	0.1 μg ml^{-1}
Toluene	144	0.4	5.0	8.5
Ethylbenzene	224	0.35	9.6	9.6
o-Xylene	239	0.33	7.5	11.7

The calibration curves were drawn with three replicate direct injections with 10 calibration points. The linear ranges for all the analytes were up to 2 mg ml^{-1}. The correlation coefficients were 0.998–0.999. The detection limits, defined as three times of base-line noise, are presented in Table 2. The repeatabilities were determined by five repetition analysis for two concentrations of BTEX. Relative standard deviations (RSDs) were calculated and are summarized in Table 2. These data show that the repeatability of the method is satisfactory.

Application

The method was applied for water samples analysis. Osmosis-cleaned tap water (AB "Vilniaus degtinė"), petrol station wastewater and wastewater cleaned from dye residue ("UAB Baltik vairas") were analysed without any pretreatment.

The osmosis-cleaned water did not contain the analytes of interest even if polluted with unidentified compounds (Fig. 4a). Petrol station wastewater was rather polluted (Fig. 4b) and contained the analytes of interest. The concentrations of the analytes were calculated using the standard addition method and were determined to be 0.25, 0.20 and 0.16 for toluene, ethylbenzene and *o*-xylene, respectively.

Unfortunately, the method was inappropriate for the extraction of "UAB Baltik vairas" wastewater because after injection a mixture of extraction-disperser solvent solid sediments formed. Thus, after centrifugation, the extraction phase was mixed with solid particles, and it was impossible to collect an extraction solvent with the analytes.

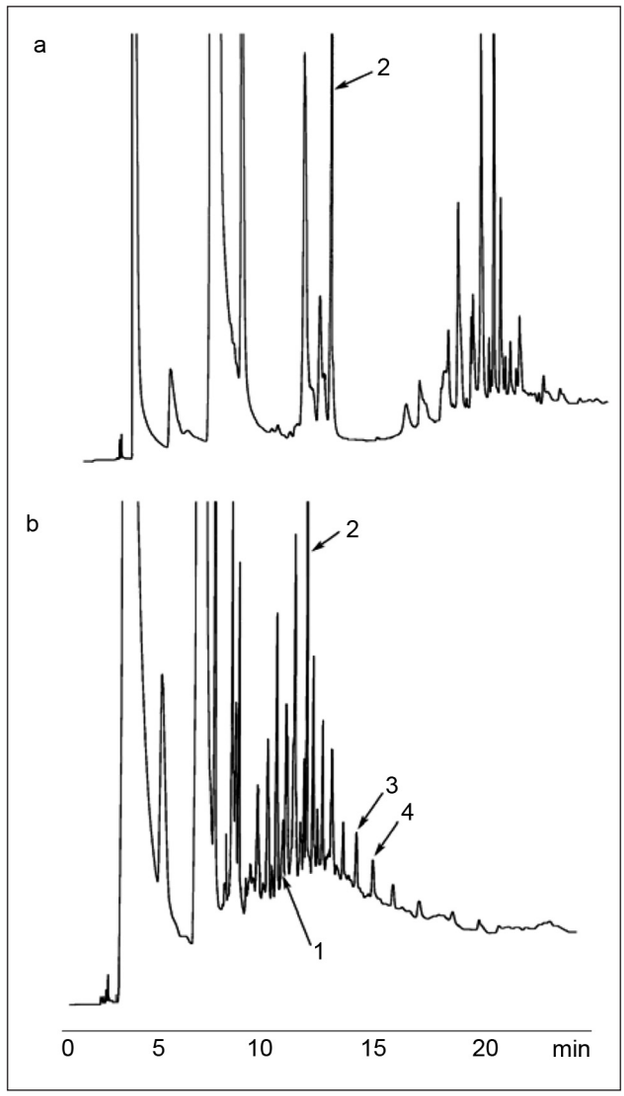

Fig. 4. Chromatograms of osmosis cleaned water (a) and petrol station waste water: (1) toluene, (2) internal standard *n*-octane (1 μg ml^{-1}), (3) ethylbenzene and (4) *o*-xylene. Carbon tetrachloride volume 15 μl, acetone volume 485 μl, centrifugation 2 min. For GC conditions, see Experimental

CONCLUSIONS

The paper describes the use of the dispersive liquid–liquid microextraction technique for volatile aromatic hydrocarbon sampling and preconcentration. The proposed method provides high enrichment factors, is particularly time-saving, environmentally friendly, precise, reproducible and linear over a broad concentration range. Only 15 microlitres of the extracting solvent are used for the extraction. The technique is compatible with gas chromatographic analysis and was successfully applied for benzene, toluene and *o*-xylene determination in water samples.

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DISPERSINĖ SKYSČIŲ–SKYSČIŲ MIKROEKSTRAKCIJA LAKIESIEMS AROMATINIAMS ANGLIAVANDENILIAMS VANDENYJE NUSTATYTI

S a n t r a u k a

Lakiųjų aromatinių angliavandenilių sukoncentravimui iš vandens mėginių pasiūlytas dispersinės skysčių–skysčių mikroekstrakcijos metodas. Ištirta ekstrahento kilmės ir tūrio, disperguojančiojo tirpiklio tūrio, ekstrakcijos trukmės ir tirpalo joninės jėgos įtaka ekstrakcijos efektyvumui. Ekstrahentu pasirinktas anglies tetrachloridas, disperguojančiuoju tirpikliu – acetonas, vidiniu standartu – *n*-oktanas. Kalibracinės kreivės tiesinės iki 2 mg ml^{-1} analičių koncentracijos, koreliacijos koeficientai 0,998–0,999, sukoncentravimo laipsnis nuo 144 (tolueno) iki 239 (*o*-ksileno), aptikimo ribos 0,40 μg l^{-1} (tolueno), 0,35 μg l^{-1} (etilbenzeno) ir 0,33 μg l^{-1} (*o*-ksileno), santykiniai standartiniai nuokrypiai ne didesni kaip 11,7 %. Parodyta galimybė pritaikyti šį metodą lakiesiems aromatiniams angliavandeniliams nustatyti vandens mėginiuose.