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QUANTITATIVE EVALUATION OF SOME VOLATILE AROMATIC HYDROCARBONS FROM WATER USING A THREE PHASE EXTRACTION SYSTEM AND GAS CHROMATOGRAPHY ANALYSIS*

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ABSTRACT. The analysis of benzene, toluene and xylene by microextraction into a three phase extraction system (liquid-liquid-solid) followed by gas chromatography is described. The analytes are extracted very fast into a very small volume of organic solvent and simultaneously into a solid sorbent. The efficiency of different solvents (dichloromethane, chloroform, carbon tetrachloride, *n*-hexane and cyclohexane) added to the sample in different volumes for liquid microextraction was evaluated. The best results were obtained by using dichloromethane. The extracted quantity in the three phase system was significantly higher than the usual two phase liquid-solid microextraction. The RSD was between 11-12%, the linearity was very good (over 1 magnitude order from 5 ng/mL to 6 µg/mL) for all three compounds, the limits of detection (S/N = 3) were in the ppb range and the recoveries between 84-96%. The working conditions (extraction time, temperature, solvent, solvent volume, stirring rate) were optimized. The optimum conditions for the extraction of all three analytes were experimentally established for 200 µL dichloromethane as organic phase, 25°C, at a stirring rate of 400 rpm and 5 minutes extraction time. The developed method was applied for the analysis of purposed compounds in wastewater samples.

Keywords: *liquid-solid microextraction, liquid-liquid microextraction, liquid-liquid-solid microextraction, volatile aromatic hydrocarbons, gas chromatography*

INTRODUCTION

Benzene, toluene and xylenes are some of the most important and frequent water contaminants. They appear in water due to various causes but especially from industrial wastewaters. These compounds are used in large amounts as precursors in the chemical industry and as solvents. They are also present in fuels [1].

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Aromatic hydrocarbons are considered highly toxic, carcinogenic and dangerous for the environment [1-7]. The toxicity of volatile aromatic hydrocarbons is caused mainly by the degradation products, in most cases phenol derivatives which are toxic at the membrane level. The metabolism of benzene, toluene and xylenes is dose-dependent and usually very extensive at dose level that do not saturate the first metabolic step of each compound which includes P450 cytochrome and mixed function oxidases [1, 2].

Benzene affects mainly the hepatic functions and inhibits the activity of dehydrogenase and phosphatase [2]. It is converted mainly to phenol by the mixed-function oxidase system, primarily in the liver, but also in bone marrow [3]. Benzene toxicology differs from that of other solvents because it is a myelotoxin which affects the blood forming organs (e.g. marrow). The hematological effects caused when the person is chronically exposed to benzene and depend mainly upon the sensibility of the exposed person. In general the hemoglobin level is significantly decreased (in some cases 50% from the normal value) and leukocyte counts are modified [1].

The substituted volatile aromatic hydrocarbons are considered less toxic than benzene. Toluene affects the central nervous system causing dizziness, headaches and fatigue even at small concentration (e.g. 200 ppm for 8 hours) [1]. The small quantity of these compounds, often in traces makes their analysis from environmental matrixes very difficult and often losses occur during sample preparation. For this reason sensible methods for the determination of trace levels of benzene, toluene and xylene (BTX) have been developed during the last decades.

Gas chromatography is considered the most sensitive technique for the analysis of the aromatic hydrocarbons. For gas chromatography analysis the separation from sample matrix and concentration of these compounds has to be performed in order to obtain concentrations over the detection limit.

The classical liquid-liquid extraction is not suitable for the extraction of trace levels of aromatic hydrocarbons; it is time consuming, high amounts of toxic solvents have to be used and most of the analytes can be lost during the extraction procedures.

Several extraction techniques have been developed for the separation and concentration of analytes: liquid-liquid microextraction (single drop extraction), liquid-liquid-liquid microextraction (three phase single drop extraction) [8-10], solid phase extraction [11, 12] and solid phase microextraction [13-20]. The liquid-liquid microextraction and the liquid-liquid-liquid microextraction known also as two respectively three phase single drop extraction techniques have very good enrichment factors and were successfully applied for the microanalysis but they have the disadvantage of the possible dispersion of the extraction drop. Also the maximum volume of solvent which can be used is limited (4 μL) [10, 18].

The simple two phase liquid-solid microextraction is a solvent free technique which consists in the extraction of the analytes on a sorbent (usually a polymer coating on a silica rod) and the direct desorption into the gas chromatograph. This technique is also applied in the analysis of trace level compounds but the extracted quantities are very low because of the small quantity of sorbent available on the fiber [13].

Several methods were developed to increase the sorbent layer. One of these methods uses a wall coated needle (with the advantage that there is no fiber which could be broken) but a desorption gas flow is necessary [19]. Another technique is the “stir bar sorptive extraction” which uses a magnetic stirring bar covered with sorbent [20]. This technique has the advantage of high extraction volume because of the high quantity of sorbent available but has the disadvantage that the injection port of the gas chromatograph has to be modified.

In order to increase the extracted quantity of analytes and to overcome the disadvantages of the above mentioned techniques, we developed a liquid-liquid microextraction in small drops simultaneously with a solid-liquid microextraction. Actually we used a three phase liquid-liquid-solid microextraction system.

RESULTS AND DISCUSSION

Influence of the solvent on the extracted quantity

The volume of the organic solvent added to the sample is very important. If the volume of organic solvent added to the water sample is lower than its solubility limit, no extraction improvement was noticed (on the contrary: a competition between the analyte and the added organic solvent for the sorbent will occur and the extracted quantity will decrease significantly). When volume of the solvent added to the water sample is higher than the solubility limit, a three phase system will be formed. The analytes will be concentrated in the organic phase and than transferred to the sorbent. When a high volume of organic solvent is used, the analytes will be diluted in the organic phase and the extraction efficiency will decrease.

Figure 1 shows the influence of the quantity of organic solvent added to the sample.

At high volumes of solvent (over 600 µL in case of dichloromethane and over 200 µL for carbon tetrachloride, *n*-hexane and cyclohexane) the polydimethylsiloxane fiber will be supersaturated with organic solvent, and a significant loss of extraction efficiency will occur. At 12 mL water sample containing the analytes at a concentration of 5 µg/mL each, different solvents were added in different volumes (200 µL dichloromethane, 20 µL chloroform, 20 µL carbon tetrachloride, 20 µL *n*-hexane and 20 µL cyclohexane).

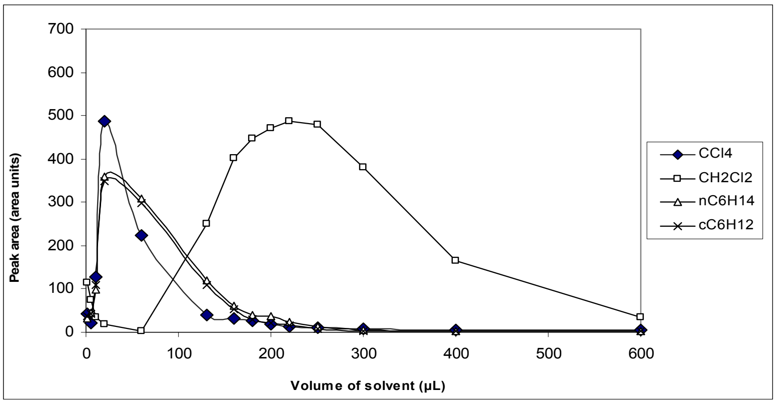

Figure 1. Influence of solvent volume upon extracted quantity of *o*-xylene. All extractions were performed at 25°C, 500 rpm using 12 mL of water sample containing 5 µg/mL of *o*-xylene.

Figures 2-4 show the influence of the volume of organic solvent in the in the liquid-liquid-solid microextraction.

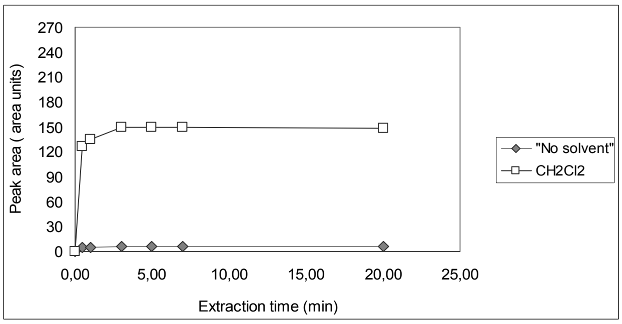

Figure 2. Three phase extraction of benzene (5 µg/mL concentration, 400 rpm, 25°C).

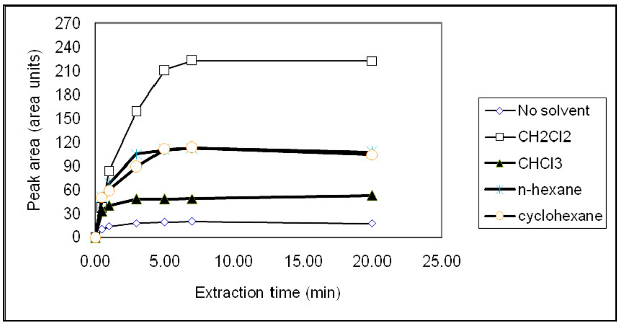

Figure 3. Three phase extraction of toluene (5 µg/mL concentration, 400 rpm, 25°C).

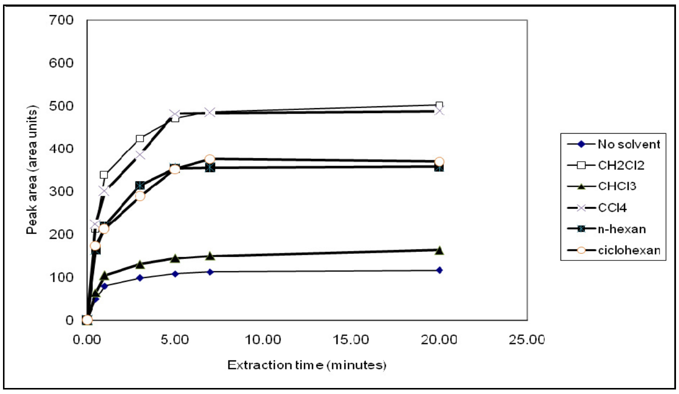

Figure 4. Three phase extraction of *o*-xylene (5 µg/mL concentration, 400 rpm, 25°C).

Table 1. Retention time of analytes and solvents at oven temperature 50°C, isothermal elution, splitless mode, nitrogen flow rate of 1 mL/min.

No.	Compound	Retention time (min)
1.	Benzene	3.11
2.	Toluene	4.92
3.	*o*-Xylene	9.32
4.	Dichloromethane	2.42
5.	Chloroform	2.66
6.	Carbon Tetrachloride	3.09
7.	*n*-Hexane	3.12
8.	Cyclohexane	3.13

Benzene, carbon tetrachloride and cyclohexane could not be separated in the conditions described above because the solvent peak overlaps the benzene peak. Benzene, carbon tetrachloride, *n*-hexane and cyclohexane have very close retention times, as it is shown in Table 1.

Chlorinated hydrocarbons have higher affinity for lipophilic compounds than aliphatic hydrocarbons like *n*-hexane and cyclohexane [21].

For this reason, the extracted quantity of analyte is higher when using a chlorinated solvent, compared to aliphatic hydrocarbons. In the optimum conditions (200 µL dichloromethane for 12 mL sample volumes, at 25°C, 400 rpm and 5 µg/mL concentration) the extracted quantity for benzene is 6 times higher, for toluene 11 times higher and for *o*-xylene 16 times higher with the three phase liquid-liquid-solid microextraction system compared with the simple two phase liquid-solid microextraction.

The 100 µm non-bonded polydimethylsiloxane fiber is not adequate for this method. In the presence of solvents it swells and there is the possibility that the coating is stripped off when it is retracted into the protection needle. Also the amount of solvent extracted is very high and the desorption process is not constant due to the diffusion of solvent in the polydimethylsiloxane coating and the results are not reproducible.

Influence of the stirring rate, temperature and salt addition

The three phase extraction of benzene and toluene is an equilibrium process and the parameters of this equilibrium depend on the analyte, solvent used as the third phase, type of solid phase (sorbent), amount of solvent, temperature, volatility, agitation. The time needed for the equilibrium to be reached can be determined from the extraction curve.

Before the equilibrium is reached, the extracted quantity increases almost exponentially with the extraction times in case of liquid-liquid-solid microextraction. After the equilibrium is reached, the extracted quantity will not be significantly higher with the increase of the extraction time.

We established experimentally that after 5 minutes the extracted quantity of analytes will not increase significantly (see Figures 1-3). All the extractions were carried out at 5 minutes extraction time.

The stirring of the sample improves the mass transfer from the sample (first liquid phase) into the second phase (organic solvent) and third phase (the sorbent).

When solvents with lower density than water are used (like *n*-hexane and cyclohexane) a higher stirring rate does not lead to a significant increase in extraction efficiency. At a high stirring rate (over 600 rpm) a decrease of extracted quantity can be observed, probably because the dispersion of the solvent in the aqueous sample occurs. As a consequence, the amount of solvent extracted with the fiber is lower and also the extracted quantity of analytes decreases.

For the solvents with higher density than water (like dichloromethane and chloroform) the stirring rate is very important. The increase of stirring rate leads to an increase of extracted quantity of analytes because their mass transfer from water sample into the organic phase is higher. Also the high density solvent is dispersed into the water sample and has easier access to the fiber which is positioned on the upper side of the sample vial.

However the stirring rate cannot be increased over 800 rpm because a turbulent flow occurs in the vial and produces the breaking of the silica fiber which is very sensitive.

The maximum acceptable stirring rate was 400 rpm. Increasing the stirring rate over this value does not significantly increase the extracted quantity of analytes.

Figure 5 shows the influence of the stirring rate upon the extracted quantity of analytes.

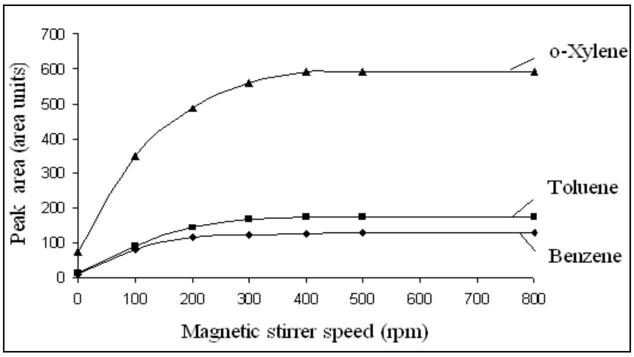

Figure 5. Influence of the stirring rate upon the extracted quantity of benzene, toluene and *o*-xylene extracted with 200 µL of dichloromethane at 25°C, with a cylindrical-shaped stirring bar (1.5 cm x 0.5 cm) in 14 mL sample vials containing 12 mL sample volume and 5 µg/mL concentration for each analyte.

The temperature is very important for the extraction of these volatile analytes and it is important to keep it constant. Increasing the temperature will decrease the extracted volume because of the volatilization of the analytes. The optimum temperature was found to be 25°C.

The addition of NaCl to the samples will decrease the extracted quantity of benzene and increase the one of toluene and *o*-xylene. Addition of all other salts ($NaHCO_3$, $FeSO_4 \cdot 7H_2O$, $CaCO_3$) will slightly decrease the extracted quantity.

At low concentrations the salt effect can be neglected. The decrease respectively the increase of the extracted quantity of analytes by salt addition in a concentration range from 1-100 mg/mL is not significantly higher than the relative standard deviation of the method.

Calcium carbonate is not soluble in water over 14 mg/L. In this case the saturated solution of calcium carbonate was tested. If solid particles of insoluble salt are present, the extracted quantity of analytes decreases significantly, probably because a part of analytes are adsorbed on the surface of the solid particles.

These results are in accordance with the general observation that the addition of salts enhances the extraction of polar compounds while for the nonpolar compounds the influence is insignificant [18].

Quantitative determination of BTX from wastewater

Quantitative evaluation was performed by calibration curve method. As external standard we used the analyte itself by performing extractions at the optimum conditions at different concentrations. The linearity range of the method was verified by extracting analytes at different concentrations.

For this purpose, a stock solution of 6000 ng/mL was prepared. This stock solution was then diluted to desired concentrations (12 times by adding 1 mL concentrated solution to 11 mL of ultrapure water obtaining a concentration of 500 ng/L of each analyte. The second solution containing 500 ng/L analyte was then diluted 1:10 and 1:100 obtaining solutions of 50 ng/mL and 5 ng/mL for each analyte).

The extractions were performed in 14 mL extraction vials containing 12 mL sample volume. For each extraction the optimum conditions were used, namely: dichloromethane as the second phase, 200 µL solvent volume, 400 rpm, at 25°C and 5 minutes extraction time.

The linearity of the method was very good, from 5 ng/mL to 6 µg/mL (over 1 magnitude order). The linear equation, regression coefficients, relative standard deviations and limit of detections are shown in Table 2.

In order to check the recovery of each analyte, a tap water sample was analyzed. To this sample each analyte was added in a concentration of 1 µg/mL and 5 µg/mL (by adding 0.25 mg and 1.25 mg to 250 mL water corresponding to 0.28 µL and 1.44 µL of each analyte according to their density). The results are presented in the table below.

Table 2. Linearity range, equations, regression coefficients, RSD and detection limits of the liquid-liquid-solid microextraction system.

Compound	Linearity range (ng mL^{-1})	Calibration curve	Correlation coefficient	RSD (%) n = 5	LOD (ng/mL) 3 phase extraction	LOD (ng/mL) 2 phase extraction
Benzene	5-6000	y=225.79x	0.9955	12.09	2.48	17.82
Toluene	5-6000	y=297.05x	0.9920	11.92	0.53	6.21
o-Xylene	5-6000	y=1172.3x	0.9902	12.05	0.08	2.29

Table 3. Recovered quantities of analytes added to tap water using three phase liquid-liquid-solid microextraction.

Sample	Compound	Spiked quantity (µg/mL)	Found quantity (µg/mL)	Recovery (%)
Tap water	Benzene	1.00	0.89	88.89
		5.00	4.22	84.44
	Toluene	1.00	0.98	97.93
		5.00	4.55	90.93
	o-Xylene	1.00	0.93	92.61
		5.00	4.85	97.02

Application of the method

This method can be successfully applied to the analysis of benzene, toluene and *o*-xylene from wastewater. This wastewater was taken from the canalization system before entering the sewage facility.

A sample of wastewater from a petroleum storage facility was analyzed using the two phase extraction system and the three phase extraction system.

Because this sample contains a complex mixture of hydrocarbons the experimental conditions were modified in order to elute also the heavy components. Details about the gas chromatographic conditions are presented below.

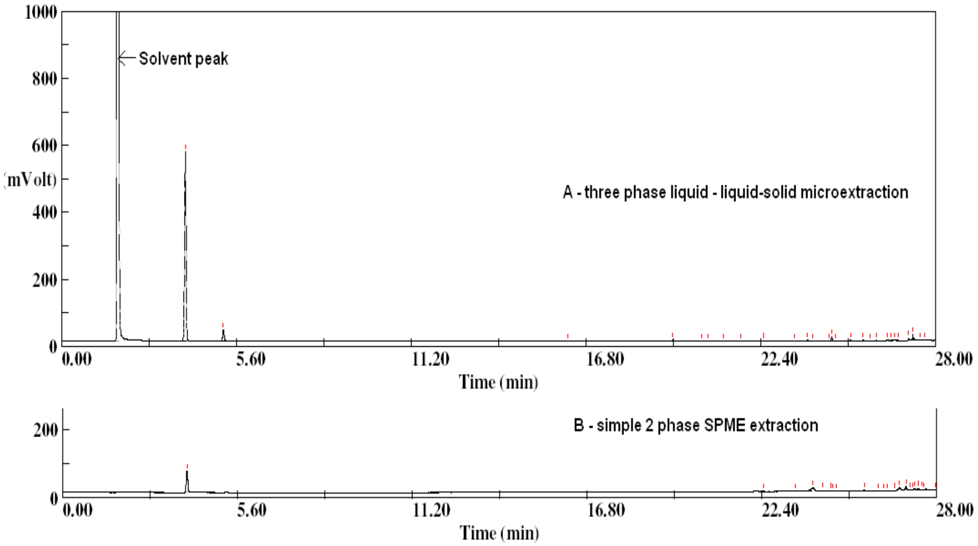

Figure 6. Analysis of a complex sample of hydrocarbons from wastewater from a petroleum storage facility. A: 3 phase liquid-liquid-solid microextraction, B: 2 phase liquid-solid microextraction. Compounds were identified according to the retention time: 1 – benzene, 2- toluene). All the tests were performed at 25°C, at 400 rpm, in 14 mL vials, containing 12 mL sample. Gas chromatographic conditions: injector temperature 220°C, FID detector temperature 250°C, oven temperature 50°C isothermal for 10 minutes followed by temperature gradient of 3°C/min until 250°C, kept 5 minutes at 250°C and then the oven was cooled at 50°C.

Even in case of complex samples, the three phase liquid-liquid-solid microextraction is very efficient compared with the 2 phase liquid-solid microextraction.

Using the three phase extraction system, certain selectivity is achieved for the volatile compounds with low molecular mass, because of the higher affinity of these analytes for the solvent compared with the other, heavier and more polar ones. Choosing the right solvent, the selectivity is achieved.

This method will be tested also for other classes of water contaminants with different polarity and also with other type of solvents beside chlorinated ones and hydrocarbons. These studies will be presented in a following paper.

CONCLUSIONS

This study proved that the three phase liquid-liquid-solid microextraction is a reliable method for the quantification of BTX from wastewater. The optimum extractions were performed at 25°C, using dichloromethane as the third phase (optimum volume 200 µL for 12 mL sample volume), at 400 rpm stirring rate and 5 minutes extraction time.

The salt addition does not influence significantly the extraction procedure.

The linearity of the method is very good, over a wide range of concentrations (from 5 ng/mL to 6 µg/mL). All the peaks were narrow, with an asymmetry at 10% in the range 0.95-1.10.

The peaks of solvent (dichloromethane) and of analytes are separated very well.

EXPERIMENTAL SECTION

Chemicals and Materials

Benzene (99% purity), toluene (99.8% purity), *o*-xylene (reagent grade 97%), calcium carbonate (99% purity), ferrous sulfate heptahydrate (99% purity) and sodium hydrogen carbonate (99.7%) were purchased from Sigma-Aldrich (Steinheim, Germany), dichloromethane (HPLC purity), sodium chloride (analytical grade), *n*-hexane (HPLC purity) were purchased from Riedel de Haën (Seelze, Germany). Cyclohexane (99.7% purity) was purchased from Merck (Darmstadt, Germany). Ultra pure, deionized water was obtained with a Millipore system at 18.2 MΩ·cm.

The stirring of samples was carried out with a Gerhardt magnetic stirrer (2.5 A, 580 V, 230W) at 25°C, with a 1 cm magnet.

The 7 µm thick bonded polydimethylsiloxane coated silica fiber, the 100 µm non-bonded polydimethylsiloxane coated silica fiber and the manual holder were purchased from Supelco (Bellafonte PA, USA).

Standard solutions of 5 µg/mL were prepared by adding 1.25 mg benzene, toluene and *o*-xylene to deionized water to fill up 250 mL glass bottles. Because these quantities are difficult to weigh, the adequate volume was added with a Hamilton syringe, according to the density of each analyte (1.42 µL benzene, 1.44 µL toluene and 1.43 µL of *o*-xylene). The mixture was magnetically stirred at 25°C for quick dissolution for 30 minutes. The concentrated solutions were successively diluted to obtain the desired concentrations.

Dichloromethane (20-800 µL) that was the third phase has been transferred with a micropipette in the 14 mL sample vials containing 12 mL sample volume.

Gas chromatography

All the tests were done on an Ultra Trace Gas Chromatograph from Thermo Finnigan with the following parameters: injection temperature - 220°C, oven temperature - at 50°C isothermal elution, detector temperature - 250°C, hydrogen flow rate - 35 mL/min, air flow rate - 250 mL/min and nitrogen as carrier flow rate - 1 mL/min.

The column used was DB-5 (polydimethylsiloxane with 5% phenyl) having 30 m length, 0.25 mm internal diameter and 0.25 film thickness of the stationary phase.

Quantitative evaluation was done by calibration curve method. The calibration curve was done by plotting the peak area versus concentration of analyte.

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