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Journal of Chromatography A, 977 (2002) 143–153

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Analytical characteristics of the determination of benzene, toluene, ethylbenzene and xylenes in water by headspace solvent microextraction

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Received 9 July 2002; received in revised form 6 September 2002; accepted 6 September 2002

Abstract

Headspace solvent microextraction (HSM) is a novel method of sample preparation for chromatographic analysis. It involves exposing a microdrop of high-boiling point organic solvent extruded from the needle tip of a gas chromatographic syringe to the headspace above a sample. Volatile organic compounds are extracted and concentrated in the microdrop. Next, the microdrop is retracted into the microsyringe and injected directly into the chromatograph. HSM has a number of advantages, including renewable drop (no sample carryover), low cost, simplicity and ease of use, short time of analysis, high sensitivity and low detection limits, good precision, minimal solvent use, and no need for instrument modification. This paper presents analytical characteristics of HSM as applied to the determination of benzene, toluene, ethylbenzene and xylenes in water.

Keywords: Headspace analysis; Solvent extraction; Extraction methods; Water analysis; Benzene; Toluene; Ethylbenzene; Xylenes

1. Introduction

The most difficult and time-consuming step in the determination of organic pollutants in environmental samples is extraction of the analytes from the matrix. Several methods are used to accomplish this task, including gas phase extraction, liquid extraction, and solid extraction.

Headspace sampling has been widely used to analyze volatile compounds [1,2] because the extracting phase (air) is compatible with gas chromatographs. Static headspace sampling is probably the simplest and the most frequently applied solvent-free sample preparation technique, particularly in field analysis. Static headspace cannot achieve exhaustive extraction, except in the case of very volatile gases, and therefore requires very careful calibration. Techniques such as purge-and-trap [1,2], supercritical fluid extraction (SFE) [3], membrane extraction [4] and solid-phase microextraction [5] are commonly used to collect measurable amounts of analytes; however, they require a specialized and/or expensive apparatus and also in the latter two cases some type of solid or polymeric sorbent to collect the analytes.

Recently, solvent microextraction has attracted

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0021-9673/02/$ – see front matter
PII: S0021-9673(02)01422-X

increasing attention. In 1996, Liu and Dasgupta [6] introduced a unique automated liquid–liquid microextraction and detection system using a single, microliter-volume, organic drop and a LED based absorbance detector. Also in 1996, Jeannot and Cantwell [7] developed a liquid–liquid microextraction system in which solvent microextraction was achieved into a single drop (8 μl) of a water-immiscible organic solvent. A disadvantage of this approach was that it required two different apparatuses for extraction and injection. Improvement was made to the original method by using a microsyringe as the solvent holder [8]. The analytes were extracted into a 1-μl solvent drop at the tip of the syringe needle [8,9]. Alternative microextraction modes, static and dynamic, were described by He, Wang and co-workers [10,11]. In static solvent microextraction, the organic drop was exposed to a static aqueous sample solution. The analyte in the aqueous phase was transferred to the organic drop by diffusion. Static solvent microextraction provided some enrichment (~12-fold for chlorobenzenes), good reproducibility (RSD 9.7%) and simplicity.

Dynamic solvent microextraction was performed between microliters of aqueous sample and microliters of extraction agent, by repetitively pulling and pushing the plunger within the glass barrel of a microsyringe. Compared to static solvent microextraction, dynamic solvent microextraction provided higher (~27-fold) enrichment within much shorter extraction time (~3 min), but relatively poorer precision (RSD 12.8%), primarily due to repeated manual manipulation. In addition to the references previously cited, solvent microextraction has also been used to extract organochlorine pollutants from aqueous samples [12–15], nitroaromatic explosives from water samples [16,17], and cocaine and cocaine metabolites from urine [18].

Liu and Lee recently have also reported a novel solvent microextraction technique, which the authors call continuous-flow microextraction (CFME) [19]. In this technique, a 0.5-ml glass chamber is used, in which an organic microdrop is held at the outlet tip of a polyether ether ketone (PEEK) connecting tubing, which is immersed in a continuously flowing sample solution and acts as the fluid delivery duct and as a solvent holder. Under optimum conditions, CFME permits enrichment factors in excess of 1000 for dilute analytes. These large enrichment factors result in very low detection limits and small volumes of samples required (3 ml or less).

Both static and dynamic solvent microextraction techniques as well as CFME suffer from two major disadvantages:

1. the methods can only be used for liquid samples;
2. high molecular mass and other nonvolatile interferences will also be extracted by the microdrop.

These drawbacks can be eliminated if solvent microextraction is performed in the headspace mode. Headspace solvent microextraction (HSM) is a sample preparation technique, in which a microdrop of high-boiling point organic solvent extruded from the needle tip of a gas chromatographic microsyringe is exposed to the headspace above a sample. Volatile compounds are extracted and concentrated in the microdrop. Next, the microdrop is retracted into the microsyringe and injected directly into the gas chromatograph for analysis. Typical microdrop volumes vary from 0.5 to 2.0 μl. HSM integrates sampling, extraction, concentration and sample introduction into a single step. The technique was first reported in 2000 [20], and recently Theis et al. [21] described some fundamental aspects of HSM, including detailed kinetic studies of a model system, in which 1-octanol was used as a solvent, and benzene, toluene, ethylbenzene, and *o*-xylene (BTEX) were the analytes. HSM was also used by Vickackaite et al. [22] to determine alcohols in beer.

The objective of the present study was to investigate the applicability of HSM to the determination of volatile organic compounds in aqueous matrices, studying the effect of several variables on the method performance, and developing analytical characteristics of headspace solvent microextraction. The solvents used included 1-octanol and *n*-hexadecane, and the analytes were benzene, toluene, ethylbenzene, and *o*-, *m*-, and *p*-xylene (BTEX). The BTEX chemicals are considered one of the major causes of environmental pollution because of widespread occurrences of leakage from underground gasoline storage tanks and spills.

2. Experimental

2.1. Apparatus

The extraction procedure was carried out using a

Hamilton 701 10-μl microsyringe (Hamilton, Reno, NV, USA). Samples were stirred either in 2.0-ml Target DP glass vials containing PTFE-lined septa (Greenwood Environmental Supply, Dunellen, NJ, USA) or in 40-ml screw top vials (Supelco, Bellefonte, PA, USA) using a Thermolyne Mirak ceramic top digital stirrer (Fisher, Pittsburgh, PA, USA). The syringe was clamped in a fixed position relative to the vial in order to keep the position of the needle tip constant relative to the headspace. In some experiments, the vial was heated to 50 °C in a Multi-Temp-Blok (Lab-Line Instruments, Melrose Park, IL, USA).

A Hewlett-Packard 6890 gas chromatograph equipped with a 30 m×0.25 mm, 1.0 μm HP-5 capillary column and flame ionization detection (FID) system (Hewlett-Packard, Palo Alto, CA, USA) was used for all analyses. The injector and detector temperatures were 250 and 300 °C, respectively. The inlet was operated in split mode with a split ratio of 10:1 and an on-column flow-rate of carrier gas (helium) 2.0 ml/min. The GC oven temperature was programmed as follows: 35 °C for 5 min, then raised to 135 °C at 10 °C/min, and to 260 °C at 25 °C/min, then held at 260 °C for 5 min.

2.2. Reagents

Reagent grade benzene (b.p. 80.1 °C), toluene (b.p. 110.6 °C), ethylbenzene (b.p. 136.2 °C), and *o*-, *m*-, and *p*-xylene (boiling points 144, 139.1 and 138.3 °C, respectively) (Aldrich, Milwaukee, WI, USA) were used as received. 1-octanol (99+%, HPLC grade) and *n*-hexadecane (99+%, anhydrous) (Aldrich) were further purified by vacuum distillation at 20 mmHg to remove residual volatile contaminants, stored in 0.5-ml batches in the freezer and used as needed (1 mmHg=133.322 Pa). Avoid skin and eye contact and breathing vapors, especially with benzene, which is a known carcinogen.

2.3. Extraction procedure

A stock solution of BTEX components was prepared in methanol and contained 870 ppm of each analyte. To ensure stability of the solution, it was stored in a refrigerator at 4 °C and brought to ambient temperature just prior to use. Fresh stock solutions were prepared every 2 weeks. Standard solutions of BTEX at the concentration level of interest were prepared daily by spiking deionized water. In some cases, ethyl acetate (0.4%,v/v) was added to samples as an internal standard. The BTEX concentrations ranged from 8.7 ppb to 870 ppb. A 1.5-ml of the mixture was pipetted into the 2-ml vial with a stir bar. In case of 40-ml vials, 20.0 ml of the mixture was used. In some cases, the solution was not stirred. The Hamilton 701 syringe was rinsed and primed at least 10 times with the solvent. After the uptake of a known volume of solvent, the needle was used to puncture the vial septum, and the syringe was clamped in such a way that the position of the needle in the headspace was constant. The syringe plunger was then depressed to expose the drop to the analytes present in the headspace. The solution was either unstirred or stirred at 1200 rpm for various times. After prescribed time the drop was retracted into the microsyringe and injected into the GC. The analytical signal was either the peak area or the peak area ratio of the analyte to the internal standard.

3. Results and discussion

This study explored the applicability of HSM to the analysis of volatile organic compounds in aqueous matrices. The effect of a number of variables, including the type of solvent, drop size, time of extraction, temperature, and stirring, on the sensitivity of the method was examined. Performance criteria being evaluated included sensitivity and detection limit, precision, and linearity.

In the case of extraction of organic compounds from the headspace over aqueous samples, the amount of the analyte n extracted by the microdrop at equilibrium is described by the following equation, which is analogous to the equation describing the amount of analyte extracted by the SPME fiber in three-phase systems [5,23]:

$$n = \frac{K_{\mathrm{odw}} V_{\mathrm{d}} C_0 V_{\mathrm{s}}}{K_{\mathrm{odw}} V_{\mathrm{d}} + K_{\mathrm{hs}} V_{\mathrm{h}} + V_{\mathrm{s}}} \quad (1)$$

where K_{odw} and K_{hs} are the organic drop–water and the gas–sample (water) distribution constants, respectively, C_0 is the initial concentration of the analyte in the matrix, and V_{d}, V_{s}, and V_{h} are the volumes of the drop, the sample, and the headspace,

respectively. The equation states, as expected from the equilibrium conditions, that the amount of analyte extracted is independent of the location of the drop in the system. It may be placed in the headspace or directly in the sample as long as the volume of the drop, headspace, and sample are kept constant.

If we assume that the vial containing sample is fully filled with the aqueous matrix (no headspace), the term $K_{hs}V_h$ in the denominator, which is related to the capacity ($C_h^{\infty}V_h$) of the headspace, can be eliminated resulting in [5,23]:

$$n = \frac{K_{odw}V_dC_0V_s}{K_{odw}V_d + V_s} \quad (2)$$

Both equations describe the mass absorbed by the microdrop after equilibrium has been reached. For most analytes K_{hs} is relatively small (e.g. benzene has a K_{hs} value of 0.224) and sampling from the headspace will not affect the mass absorbed by the drop if the volume of the headspace is much lower than that of the aqueous solution ($V_h \ll V_s$). The detection limits of headspace solvent microextraction are therefore expected to be very similar to those of direct solvent microextraction for these conditions.

The amount of analyte present at equilibrium in the headspace over aqueous samples is described by the following equation:

$$n = \frac{K_{hs}V_hC_0V_s}{K_{hs}V_h + V_s} \quad (3)$$

An inspection of Eqs. (1) and (3) allows us to compare sensitivities of direct headspace analysis and headspace solvent microextraction. As long as sample volume V_s is much greater than the products $K_{hs}V_h$ and $K_{odw}V_d$, the sensitivity of HSM will be higher than the sensitivity of direct headspace analysis by the factor $(K_{odw}V_d)/(K_{hs}V_h)$. Several factors affect the amount of analytes extracted, and thus the sensitivity of HSM: the volume of the drop (V_d), the solvent and analyte characteristics (K_{odw} and K_{hs}) sample and headspace volumes and the temperature of absorption. As indicated by Eq. (1), the amount of analyte extracted by the microdrop is related to the volume of the drop, and the sensitivity improves as the volume of the drop increases. Because matrix, headspace and solvent drop compete for analytes, the affinity of the solvent for target analytes is crucial in HSM sampling. For example, nonpolar compounds are more likely to be extracted by nonpolar solvents and vice versa.

For analytes with low boiling points, direct headspace analysis may have sensitivity comparable to that of headspace solvent microextraction. As the analyte volatility decreases, however, HSM tends to have superior sensitivity. For example, let us consider 20 ml of an aqueous solution of benzene (boiling point 80.1 °C) with an initial concentration of 100 μg/l, which was equilibrated at 25 °C with 20 ml of headspace. Using a K_{hs} value of 0.224 [21], it follows from Eq. (3) that the amount of benzene in the headspace is 366 ng. Assuming that a realistic injection volume for GC is 1 ml of the headspace, it follows that the amount of benzene injected would be 18.3 ng. If headspace solvent microextraction with 1-octanol is applied to the same system, one can calculate from Eq. (1), using a K_{ow} value of 135 [21], that a 2-μl microdrop is required to extract a similar amount of the analyte (22 ng). Similar calculations for *o*-xylene (boiling point 144 °C) reveal that the equilibrium concentration of the analyte in the headspace is now 15.7 ng/ml, but 1 μl of 1-octanol at equilibrium contains 105.4 ng of *o*-xylene.

3.1. Syringe requirements

Sampling repeatability in HSM requires the use of the proper syringe needle for the extraction. The needle should have a minimum dead volume (26s gauge) and a no. 2 point style beveled tip. The ability to withdraw the microdrop into the syringe after sampling is crucial and depends on the shape of the needle tip and flat surface area which the drop can adhere to. The large surface area of a standard concave beveled Hamilton no. 2 style needle allows more than 95% of the microdrop to be withdrawn. The flat bevel used on some SGE syringes results in some of the microdrop wicking onto the outer surface of the needle. Thus, this style allows only 60–70% of the microdrop to be withdrawn. The conical style of syringe needle tips popular with GC autosamplers has almost no surface at the end of the needle to which the microdrop could adhere and, consequently, almost no microdrop is retracted into the syringe barrel. Finally, for the most precise work,

the same syringe should be used for all sampling during a series of analyses, since each needle varies slightly in its absolute dead volume.

Accuracy and reproducibility of the organic drop volume was determined by weighing the microdrop on an analytical balance. The error in the drop volume was less than 5% for all the volumes used, and the relative standard deviation based on 7 measurements was less than 5%.

3.2. Solvent selection

In initial investigations, 1-octanol was selected as the extracting solvent because of its very low vapor pressure, good solubility of a large number of organic compounds, and the ready availability of octanol–water partition coefficients for a large number of organic environmental pollutants. However, it was established that despite careful purification of 1-octanol, the solvent still contained a minor amount of impurities, which interfered with the determination of very low concentrations of BTEX components (below a 100 ppb level) when using a flame ionization detector. 1-Octanol can still be used as a HSM solvent in the determination of BTEX if mass spectrometric detection in selected-ion monitoring mode is employed [21]. *n*-Hexadecane was found to provide similar extraction efficiency to 1-octanol (defined as the amount of analytes transferred to the microdrop) for the BTEX chemicals, while having fewer impurities that could interfere with this analysis (see Fig. 1a and b). In light of these results, *n*-hexadecane was chosen as the extraction solvent for the remainder of the study. Any solvent used in headspace solvent microextraction will elute after the analytes; this is a consequence of the need for a solvent with a high boiling point (and, therefore, a low vapor pressure) so as to minimize the evaporation of the drop during the extraction. *n*-Hexadecane has a high boiling point (287 °C) and a very low vapor pressure (0.00143 mmHg at 25 °C).

3.3. Organic drop volume

The experiment was conducted to study the effect of organic drop volume on the analytical signal. Theoretical relationship between the amount of analyte extracted and the organic drop volume for

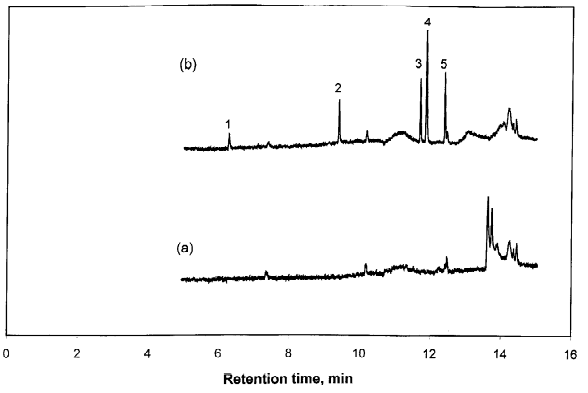

Fig. 1. FID chromatograms for (a) laboratory blank (sample of deionized water extracted into 1 μl of *n*-hexadecane) and (b) 8.7 ppb solution of benzene (peak 1), toluene (peak 2), ethylbenzene (peak 3), *m*, *p*-xylene (peak 4), and *o*-xylene (peak 5) extracted using headspace solvent microextraction. Extraction conditions: drop volume: 1 μl; extraction time: 6 min; temperature: 23 °C; stirring rate: 1200 rpm; sample volume: 1.5 ml; headspace volume: 0.5 ml.

the three-phase system is described by Eq. (1). It follows from Eq. (1) that the amount of analyte extracted (and thus the analytical signal) depends not only on V_d, but also on the volumes of headspace and sample. Fig. 2 illustrates the theoretical dependence of the amount of analyte extracted on the drop volume of 1-octanol for two analytes (benzene and *o*-xylene) and two different vial sizes: one 2-ml (1.5 ml of sample and 0.5 ml of headspace) and one 40-ml (20 ml of sample and 20 ml of headspace). It is apparent from Fig. 2 that for analytes with low boiling points (benzene) and therefore small K_{odw} values, the relationship between the amount of analyte extracted and the microdrop volume is almost linear and does not significantly depend on the sample and headspace volumes. In contrast, for *o*-xylene which has a large K_{odw} value (1320 [21]), the mass of analyte extracted strongly depends not only on the microdrop volume, but also on the vial size and, hence, the sample and headspace volumes. This is due to the fact that for small sample volumes and large K_{odw} values, the analyte may be depleted from the aqueous sample. For example, when 1.5 ml of a 100 μg/l aqueous *o*-xylene solution is placed in a 2.0-ml vial and exposed to a 1.0-μl drop of 1-octanol, the amount of the analyte extracted into the microdrop is 68 ng, which is 45% of the initial

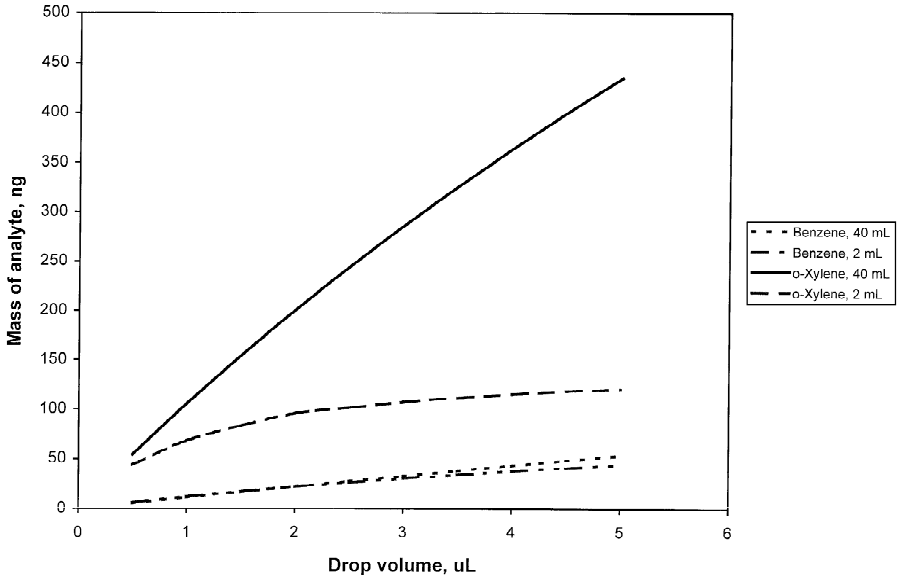

Fig. 2. Theoretical dependence of mass of analyte extracted on volume of 1-octanol for benzene and *o*-xylene for two different vial sizes: 2.0 ml (1.5 ml of sample and 0.5 ml of headspace) and 40 ml (20 ml of sample and 20 ml of headspace).

amount in the sample. On the other hand, a 1-μl microdrop of 1-octanol will extract only about 5% of the same analyte from a 40-ml vial containing 20 ml of the sample and 20 ml of the headspace. In practice, the vial size selected should be a compromise between the sensitivity, equilibration time, and accuracy of analysis. Using large vials will result in higher sensitivity and shorter equilibration times, whereas small vials will minimize such sources of errors as losses of analytes due to absorption by exposed parts of silicone rubber septa and adsorption on glass.

In the present work, the effect of drop volume on the analytical signal (peak area) was studied for two vial sizes: 2.0 ml and 40 ml. In the former case, the *n*-hexadecane drop was exposed to 0.5 ml headspace over 1.5 ml of a 87 ppb aqueous BTEX solution stirred at 1200 rpm for 6 min prior to injection for GC analysis. Fig. 3 shows that in this case the shape of the curves is similar to the theoretical dependence shown in Fig. 2. A decrease in the analytical signal observed for all the analytes except for benzene for drop volumes of 2.5 μl compared to smaller volumes is probably attributable to insufficient equilibration time, since in this case a significant portion of the analyte has to be transported to the microdrop from the liquid sample and not just from the headspace, and also mass transfer of the analytes into the bulk of the drop represents a slow step in the overall extraction process [21]. Fig. 3b illustrates the dependence of analytical signal on drop volume for a 20 ppb aqueous toluene solution stirred at 1200 rpm. In this case, 1-octanol was used as a solvent; the vial size was 40 ml (20 ml sample and 20 ml headspace), and the extraction time was 20 min. Under these conditions, sample and headspace volumes were large enough and the extraction time long enough to be close to equilibrium conditions as reflected by the shape of the curve. When drop size exceeded 3 μl, the microdrop tended to creep up along the outside of the needle, and could not be completely withdrawn back into the syringe. Although a larger organic drop gives an improved signal, its manipulation is more elaborate and less reliable. Further, large injection volumes result in more extensive band broadening in capillary GC. Taking these factors into consideration, in the following investigations the drop volume was fixed at 1.0 μl.

3.4. Effect of temperature

In headspace mode sampling, the analytes need to

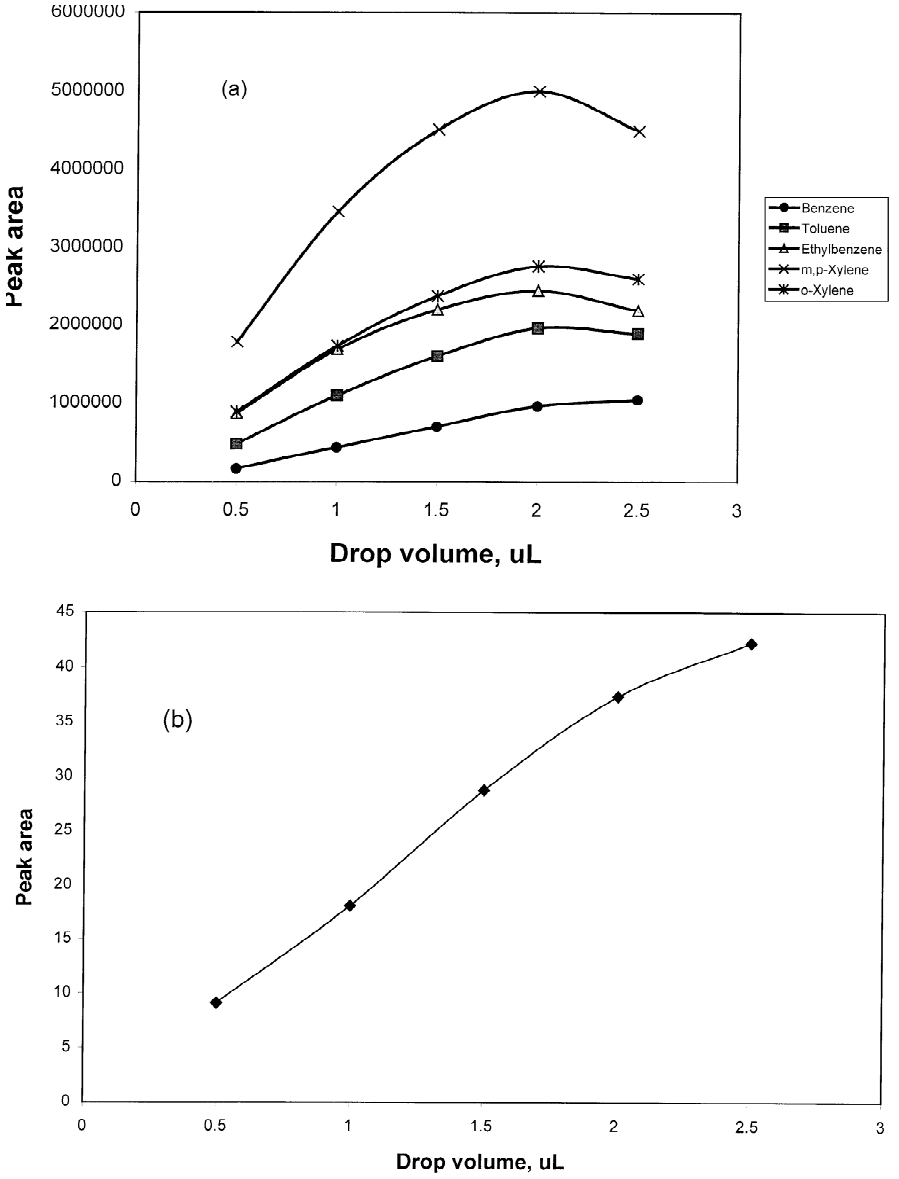

Fig. 3. Effect of organic drop volume on the analytical signal in HSM. (a) 87 ppb aqueous BTEX solution, stirring rate 1200 rpm, headspace volume 0.5 ml, sample volume 1.5 ml, extraction time 6 min. (b) 20 ppb aqueous toluene solution, stirring rate 1200 rpm, headspace volume 20 ml, sample volume 20 ml, extraction time 20 min.

be transported through the barrier of air before they reach the drop. The choice of sampling mode has a significant impact on extraction kinetics. When the microdrop is in the headspace, analytes are removed from the headspace first, followed by indirect extraction from the matrix. Therefore, volatile analytes are extracted faster than semivolatiles, since they are at a higher concentration in the headspace, which contributes to faster mass transport rates through the headspace. Temperature has a significant effect on both the kinetics and the thermodynamics of the sorption process. Temperature affects the kinetics of

sorption in the microdrop by determining the vapor pressure of analytes and diffusion coefficient values in all three phases. In fact, the equilibration times for volatiles are shorter in the headspace solvent microextraction mode than for direct extraction under similar conditions. This outcome is produced by two factors: a substantial portion of analytes is in the headspace prior to extraction, and diffusion coefficients in the gaseous phase are typically four orders of magnitude larger than in liquid media [5].

If exhaustive extraction is not achieved by HSM, the amount of analytes absorbed in the drop also depends upon the temperature. The amount of analytes absorbed by the drop increases when the extraction temperature drops, because the process of analyte absorption in the microdrop is exothermic. The temperature effect occurs because the partition coefficient between the organic solvent and the gaseous phase is temperature dependent. If a low microdrop temperature is maintained during sampling, sensitivity should significantly increase.

To test this hypothesis, 1.5 ml of an 87 ppb BTEX solution was extracted in triplicate at 23 °C (ambient temperature) and at 50 °C under identical conditions (90 min extraction, unstirred solution, 1 μl drop volume). It was found that, as expected, the amounts of BTEX components extracted at 23 °C were larger than those extracted at 50 °C by a factor of 1.7–1.4, the former value corresponding to benzene and the latter to *o*-xylene. Consequently, all other experiments were carried out at 23 °C. It should be pointed out, however, that when using HSM for the analysis of semivolatiles, it might be advantageous to heat the sample to increase extraction rates, because for the analytes with higher boiling points the *n*-hexadecane–headspace distribution constant should be large enough to enable extraction of sufficient amounts of the analytes even at elevated temperatures.

3.5. Extraction time

The extraction–time profiles were investigated by monitoring the variation of analytical signal with exposure time under the following conditions: BTEX concentration: 87 ppb, solution stirred at 1200 rpm, solution temperature 23 °C, and 1 μl drop volume. In general, the amount of BTEX components extracted into *n*-hexadecane increased with extraction time. Fig. 4 shows that the analytical signal increased in the range 1–8 min for higher boiling compounds (xylenes, ethylbenzene), while it remained practically constant for benzene and toluene. This rapid initial increase in the amount of analyte extracted followed by a much slower increase lasting a long time

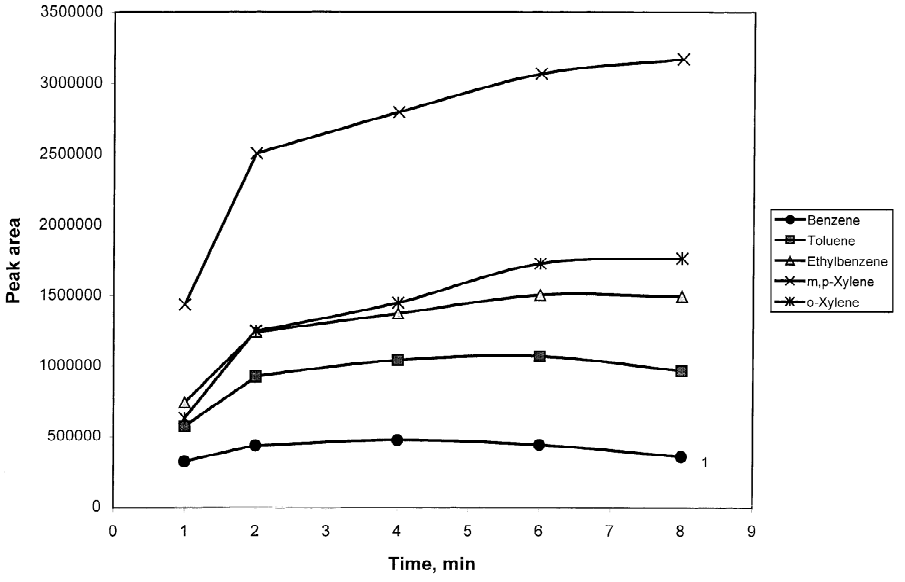

Fig. 4. Plot of peak areas of BTEX components versus extraction time. For extraction conditions, see Fig. 1.

reflects the processes taking place in the system [23]. The first stage corresponds to analyte extraction from the headspace only. As soon as the headspace concentration of the analyte falls below the equilibrium value with respect to the aqueous phase, analyte molecules begin to diffuse from the aqueous phase to the gaseous phase, which is a rate-determining step. Since it is not practical to wait for equilibrium to occur, the extraction time should be just long enough for the extraction rate to slow down for improved precision. For the remainder of the experiments, an exposure time of 6.0 min was selected. As demonstrated by Theis et al. [21], the overall extraction rate has two rate-determining steps: aqueous-phase mass transfer and diffusion of solutes into the extracting solvent. Although the diffusion of BTEX components into the extracting solvent cannot be easily enhanced in practice, the aqueous-phase mass transfer can be improved by stirring the solution.

3.6. Stirring rate

The effect of stirring on the extraction of BTEX components was studied next. Four sets of experiments were carried out in replicate. In the first experiment, 87 ppb standard BTEX solutions (1.5 ml) were not stirred. They were equilibrated in 2.0-ml vials with the headspace for 1 h at 23 °C, followed by 4-min extraction into 1 μl of *n*-hexadecane and GC analysis. In the second experiment, the solutions were also unstirred, but in this case the extraction into the microdrop was performed for 1 h immediately following the transfer of the solutions into the 2.0-ml vial. In the third experiment, the solutions were equilibrated with the headspace for 1 h with stirring at 1200 rpm, followed by 4-min extraction into 1 μl of *n*-hexadecane. Finally, in the last set of experiments, the solutions were extracted immediately after transfer into the vial for 1 h while being stirred at 1200 rpm. The results are shown in Table 1. It follows from the data in Table 1 that for lower boiling components, such as benzene, which have larger diffusion coefficients, equilibrium is established between the microdrop and the sample solution when the sample is extracted for 1 h for both stirred and unstirred solutions. For higher boiling components, the extraction efficiency was highest for the solutions that were extracted for 1 h with stirring. The extraction efficiencies were similar for the solutions that were extracted for 1 h without stirring and for the solutions that were equilibrated for 1 h with stirring, followed by 4-min extraction. These results support the notion of two rate-determining steps in the overall extraction rate: aqueous-phase mass transfer and diffusion of solutes into the extracting solvent. In practical terms it follows from this discussion that when using headspace solvent microextraction, it is not necessary to wait for the system to come to equilibrium. After 6 min, the rate of extraction becomes slow due to slow mass transfer into *n*-hexadecane, so if the sample solution is stirred at 1200 rpm, enough analytes have been accumulated in the microdrop to allow a sensitive determination of BTEX compounds.

Table 1
Effect of stirring and extraction time on the extraction efficiency for BTEX (87 ppb) from aqueous solutions

Target compound	Relative response			
	No stirring[a]	No stirring[b]	Stirred at 1200 rpm[a]	Stirred at 1200 rpm[b]
Benzene	100	140	122	120
Toluene	100	172	162	201
Ethylbenzene	100	196	192	324
m,p-Xylene	100	198	193	351
o-Xylene	100	227	217	395

[a] 1-h equilibration, followed by 4-min extraction.
[b] Immediate extraction for 1 h.

3.7. Quantitative analysis of model compounds

Replicate headspace extractions of a series of BTEX standards in the range 8.7–870 ppb in water were carried out under the optimized conditions (extraction time: 6 min; drop volume: 1 μl; stirring rate: 1200 rpm; temperature: 23 °C) and yielded the calibration plots shown in Table 2. Even though the system was not at equilibrium, the response was linear for all analytes. The slopes of calibration curves, that is the sensitivity of the method, are related to the *n*-hexadecane–water distribution coefficients; for example, the K_{odw} values are practically identical for all three isomers of xylene; therefore, the slope for the calibration plot of equimolar

Table 2
Calibration plot parameters for five aqueous solutions (drop: 1 μl; time: 6 min; stirring rate: 1200 rpm). Three repetitions of each experimental point

Compound	R^2	Calibration equation
Benzene	0.9993	$y = 6487.3x - 58\,951$
Toluene	0.9993	$y = 16\,932x - 168\,286$
Ethylbenzene	0.9991	$y = 26\,159x - 299\,218$
m,p-Xylene	0.9992	$y = 52\,925x - 573\,026$
o-Xylene	0.9994	$y = 26\,158x - 250\,612$

mixture of *m*- and *p*-xylene (52 925) is double that of an equal amount of *o*-xylene (26 158).

The repeatability of the procedure was investigated on eight replicate samples (87 ppb) under the optimized conditions listed above. One set of samples did not contain an internal standard, while the other set contained 0.4% ethyl acetate added as an internal standard. The first set of samples was analyzed in order to determine the overall variability of the procedure, whereas the second set was analyzed to establish the improvement in precision resulting from the use of internal standard. The results of these experiments are shown in Table 3 along with the detection limit for BTEX compounds estimated from the calibration plots. It follows from the data in Table 3 that the precision obtained with manual extractions and injections varies from 6.9 to 9.6% RSD without internal standard down to 2.7–5.9% RSD when using the I.S. The precision of HSM is much improved at higher analyte concentrations: seven replicate determinations of a 1 ppm toluene solution yielded a relative standard deviation of 2.2%. The detection limits for BTEX compounds when using the optimized conditions and flame ionization detection, based on a signal-to-noise ratio (S/N) of 3, were at the low parts-per-billion level, below guidelines established by the US Environmental Protection Agency (EPA) for drinking water [24], except for benzene for which the detection limit was equal to the maximum contaminant level (5 μg/l).

Table 3
Analytical characteristics of the HSM procedure for BTEX

Compound	RSD (%), ($n = 8$)		LOD (μg/l)
	No I.S.	I.S. added[a]	
Benzene	6.9	2.7	5.0
Toluene	7.9	3.5	1.9
Ethylbenzene	9.6	4.9	1.3
m,p-Xylene	9.3	5.9	0.72
o-Xylene	8.5	5.0	1.2

[a] 0.4% by mass of ethyl acetate added to the sample.

3.8. Significance of the technique

The developed technique, headspace solvent microextraction, has a number of advantages, including: (1) renewable drop (no sample carryover); (2) high sensitivity and low detection limit; (3) good precision; (4) wide selection of available solvents; (5) low cost; (6) simplicity and ease of use; (7) minimal solvent use; (8) short preconcentration time; (9) possibility of automation; (10) no conditioning required (as is the case with the fiber in solid-phase microextraction); (11) no need for instrument modification.

Potentially, HSM should find use in the areas of analytical chemistry in which volatile compounds are frequently determined. Those areas include environmental, pharmaceutical, forensic, and food analysis.

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