Optimization of Headspace Solid Phase Microextraction Procedure for Trace Analysis of Toluene

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This study describes optimization of headspace solid phase microextraction followed by gas chromatography equipped with a flame ionization detector for toluene at trace level in spiked urine. The parameters affecting the extraction and gas chromatographic determination of analytes were studied: extraction time and temperature; desorption time and temperature; addition of NaCl; and pH, volume and agitation of the sample. Optimized headspace extraction was carried out at 30 °C for 6 min in the presence of 0.2 g·m^{-1} of NaCl in the sample solution. Also, sample volume and sample pH were optimized at 5 ml and 7 (neutral pH), respectively. Desorption of the analytes was carried out at 250 °C for 60 s. The optimized procedure was validated with 3 different pools of spiked urine; it showed good reproducibility over 6 consecutive days and 6 within-day experiments. The study also determined the accuracy, linearity and detection limits of this method.

headspace   solid phase microextraction   gas chromatography   toluene

1. INTRODUCTION

Due to increasing concern about toxic substances such as toluene and its analogs in the environment and work places, it is becoming more important to monitor such chemicals to evaluate risk hazards and potential problems caused by exposure to toxic compounds [1, 2]. Toluene is an important industrial compound with widespread usage; it is present in mineral oil and in many combustion processes that cause environmental and industrial pollution [2]. Occupational and environmental exposure to toluene occurs mainly via inhalation, though it is absorbable via skin at low levels.

Because it also has irritating properties, thermal exposure to toluene must be avoided as much as possible. Generally, toluene is an inhibiting agent for the central nervous system, causing anesthetic and narcotic disorders at high concentrations [3]. Toluene is a popular solvent in the production of benzene. Moreover, toluene can be used for providing banzaldehyde, benzoic acid, phenol and 3-nitrotoluene. Toluene is widely used in the chemical, petrochemical, rubber, paint and pharmaceutical industries [4].

In isolating organic compounds from an aqueous solution, sample pretreatment is the most challenging and time-consuming step in the

analytical procedure [5]. Traditionally, liquid–liquid [6] and solid phase extraction [7, 8, 9, 10, 11, 12] have been commonly used to extract compounds from aqueous matrices.

In evaluating occupational exposure to toluene, urinary hippuric acid (U-HA) is monitored. However, food-derived HA is present in urine even in the general population [13, 14] and the upper normal limit of U-HA is ~0.6 g/g of creatinine [15]. This corresponds to ~40 mg·L^{-1} toluene in the air [10]. On the other hand, the occupational exposure level–time weighted average of toluene recommended by the American Conference of Governmental Industrial Hygienists (ACGIH) is now 50 mg·L^{-1} [16]. Thus, evaluation of exposure to toluene by examining U-HA can be a problem. Because toluene is not detected in unexposed people, urinary toluene was considered as a biological index that could replace HA to eliminate those interferences. However, compared with HA, which is expressed in grams per litre, the amount of toluene is very small (micrograms per litre), so a more sensitive method for biological monitoring of toluene was necessary. Therefore solid phase microextraction (SPME) was optimized for trace measurements of urinary toluene.

SPME, developed by Pawliszyn [17], has been successfully used in extracting volatile and other organic compounds from water, solid and air samples [13]. The principle behind SPME is the partitioning of analytes between the sample matrix and the extraction medium with subsequent sorption onto a liquid polymeric coating, which is supported on a fused silica fibre. Adsorption efficiency depends mainly on the retention characteristics of the selected sorbent for the type of organic compounds being sorbed [18]. This technique uses a thin polymer film coating to extract analytes from aqueous or gaseous samples. Then, the fibre is inserted directly into the injector of a gas chromatography system and the extracted analytes are thermally desorbed and analysed. SPME can integrate sampling, extraction, preconcentration and sample introduction into a single step. Nowadays, among the most recommended techniques,

SPME is employed for the extraction and preconcentration of volatile and semivolatile compounds at trace levels in a variety of matrices [19]. The introduction of new polymeric fibres, the development of new experimental configurations and the improvement of automatic devices will undoubtedly lead to the application of SPME in different areas of chemical analysis. The aim of this study was to establish a practical, fast, inexpensive and selective method for trace analysis of urinary toluene using SPME to evaluate environmental and occupational exposure.

2. MATERIAL AND METHODS

2.1. Reagents and Chemicals

Toluene as a standard was obtained from Aldrich (Germany). Methanol (GC grade), sodium chloride (NaCl) and standard buffered solutions at three pH values (4.00 ± 0.02, 7.00 ± 0.02 and 9.00 ± 0.02) were obtained from Merck (Germany). The stock solution of toluene was prepared at a concentration of 0.1 μg·ml^{-1} in methanol. The model solution containing the required amount of the analyte (0–500 ng·ml^{-1}) was prepared daily by diluting the standard solution with double distilled water to study extraction performance under different conditions. The stock and working standard solutions were stored at 4 °C. To define the effects of real matrices on extraction performance, spiked urine (5 ml of unexposed persons' urine with toluene, 5 ml of a standard methanolic solution of toluene at each concentration and 40 ml of double distilled water) was added.

2.2. Apparatus

Laboratory-made platinum fibres coated with with polypyrrole with a film thickness of 16 μm were prepared by a research team in the Faculty of Chemistry of Tarbiat Modarres University, Tehran, Iran [19]. An SPME fibre holder for manual sampling was purchased from Azar Electrode, Ourumieh (Iran). Ten-millilitre vials were obtained from Supelco (Canada). A digital

pH meter from Hanna (Singapore), was used for pH adjustment. The amount of reagents was measured with a CP 225D Sartorius balance (Germany) for milligram, or lower quantities. An RH-B-KT/CS2 hot plate stirrer (Germany) was used to agitate aqueous samples. The 8 × 3.14 mm stir bars used to mix aqueous samples were from Fisher Scientific (Canada). The GC apparatus used in this study was form Varian (USA).

2.3. Chromatographic Conditions

Gas chromatography equipped with a flame ionization detector (GC-FID) was operated under the following conditions: the analytical column, CP Sil8, 50 m × 0.53 mm I.D., 0.25 μm (film thickness); carrier gas, He (99.999%), flow rate 10 ml·min^{-1}; make up gas, N$_2$, flow rate 25 ml·min^{-1}. The injection port and detector were operated at 250 and 280 °C, respectively. Fibre was introduced into the chromatographic column using splitless injection. The GC split valve was closed for 5 min. The detector gases flow rates were 300 ml·min^{-1} of air and 30 ml·min^{-1} of hydrogen. The separation of toluene on GC-FID was performed by a temperature program as follows: 65 °C for 5 min, increased to 280 °C at a rate of 30 °C·min^{-1} and held at 280 °C for 10 min.

2.4. Headspace Extraction Procedure

Five millilitres of an aqueous solution spiked with toluene was extracted with polypyrrole fibre using headspace solid phase microextraction (HS-SPME). Polypyrrole fibre housed in a manual SPME holder was used. The fibre was conditioned prior to use by being inserted into the GC injection port for 3 hrs at 200, 250 and 300 °C for each hour, respectively. Water (5 ml) containing the target analyte was transferred to a 10-ml glass vial with a polytetrafluoroethene silicon septum. After NaCl and a magnetic stir bar were added, the vial was tightly sealed with an aluminum cap to prevent sample loss due to evaporation. During the extraction process, the vials were heated by using a hot plate accommodated in

a glass beaker contained some water. Thus, the samples were heated indirectly, with temperature controlled with a thermometer. The polypyrrole fibre was exposed to the headspace over the stirring liquid sample for 10 min. After the sampling step, the fibre was withdrawn into the needle and removed from the vial. The fibre was then immediately inserted into the injection port of the GC. To use SPME to measure spiked urinary toluene, the compound was extracted with a laboratory-made fibre with an absorbent from the headspace of the samples and measured with GC. In this study, the extraction conditions (extraction time and temperature; addition of NaCl; pH, concentration, volume and agitation of the sample), reproducibility and linearity of the calibration curve of HS-SPME were optimized.

2.5. Statistical Analysis

Statistical analyses were performed using SPSS/ PC version 11.5. Therefore, data obtained from this study were analysed using analysis of variance with Tukey's multiple comparisons. Comparisons were considered statistically significant when $p < .05$. Also, method reproducibility of the optimized procedure was performed with within-day (6 times a day) and day-to-day (for 6 consecutive days) assessments. In this experiment, 50-ml spiked urine samples of toluene at low, medium and high concentrations were used for extraction followed by GC-FID determination.

3. RESULTS

3.1. Optimization of Chromatographic Analysis

3.1.1. Desorption temperature

Temperature can reduce desorption time and carry-over, i.e., contamination by material remaining from previous analyses; however, thermal degradation of fibres limits elevation of desorption temperature. The high thermal stability of polypyrrole fibres made increasing

desorption temperature possible; therefore, the efficiency of thermal desorption improved. In this way, complete desorption of thermally stable toluene could be achieved. Desorption in the GC injection port was measured in the temperature range of 200–300 °C. A symmetrical peak was achieved at 250 °C (Figure 1). So, this temperature was selected as optimum.

3.1.2. Desorption time

Desorption time in the GC injection port was measured at optimized desorption temperature

(a)

(b)
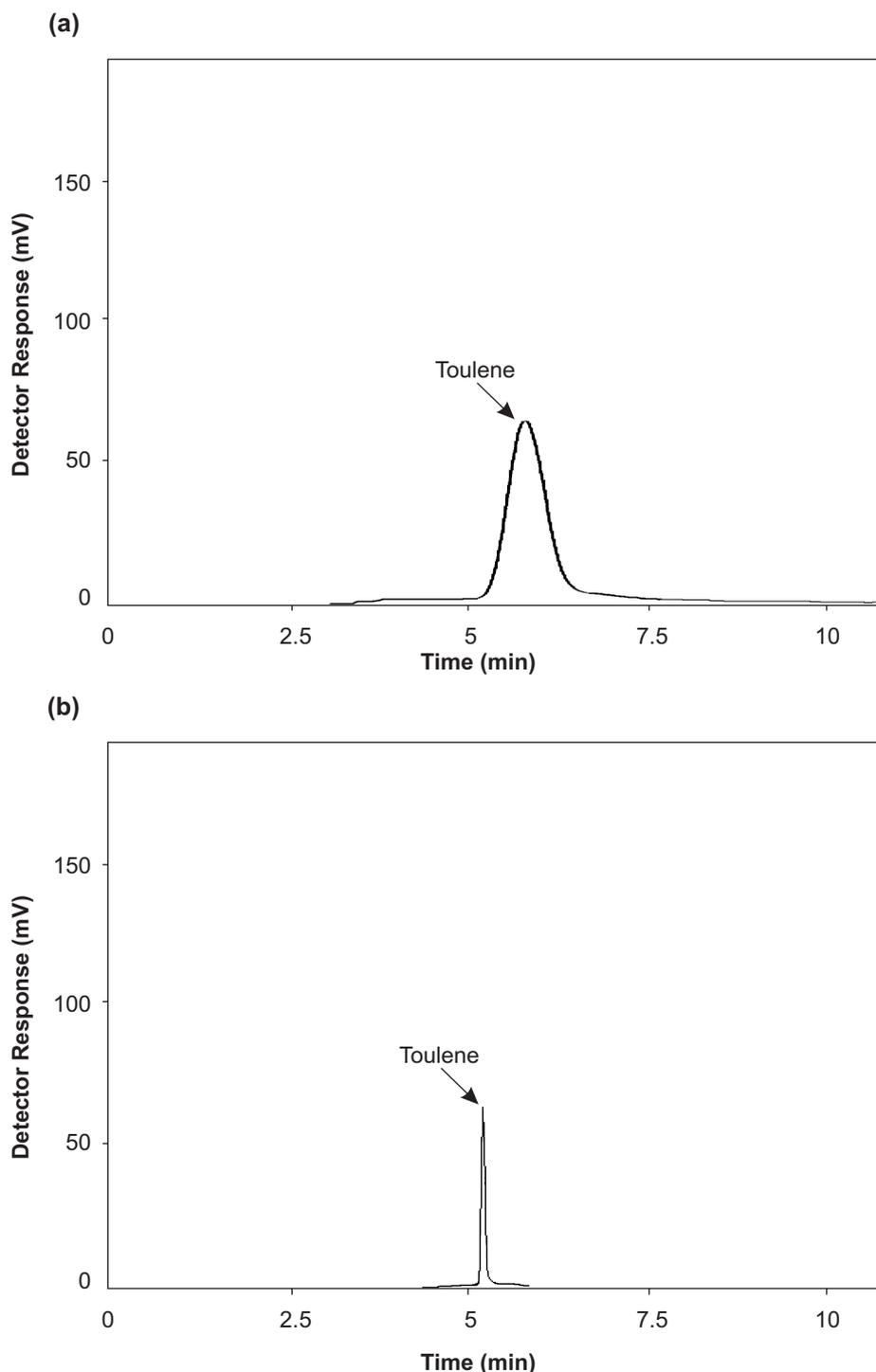
Figure 1. The effect of desorption temperature on chromatogram form: (a) 300 °C, (b) 250 °C.
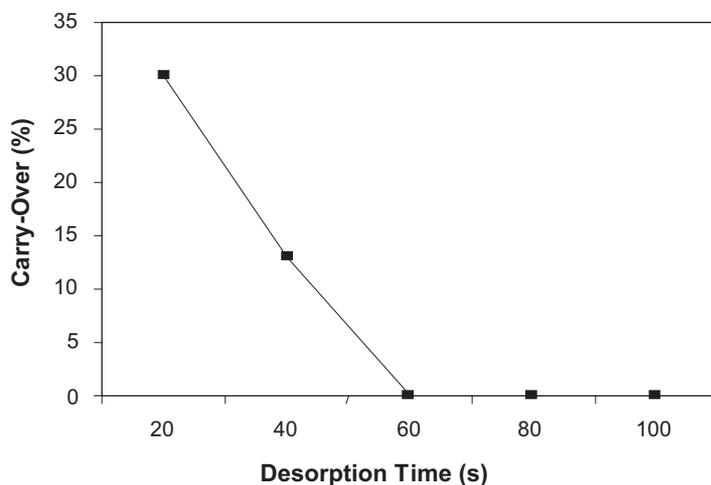
Figure 2. The effect of desorption time on carry-over of toluene at optimized desorption temperature (250 °C).
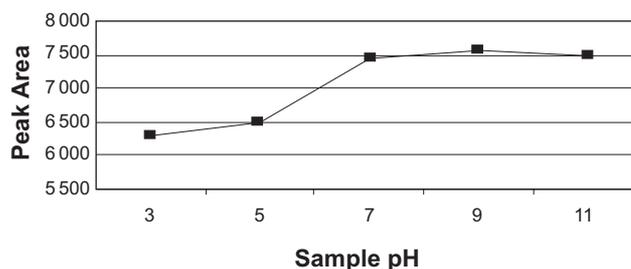
Figure 3. The effect of sample pH on extraction efficiency.

(250 °C) for 20–100 s. Figure 2 illustrates the dependence of carry-over of toluene on desorption time. From these results it is apparent that desorption of toluene was complete after 1 min at 250 °C.

3.2. Optimization of SPME

3.2.1. Sample pH

Theoretically, manipulation of sample pH value of the matrix can change the solubility of analytes in water, thus affecting their extraction efficiency. So, in this experiment, 5-ml samples of pH value of 3, 5, 7, 9 and 11 were used. Figure 3 shows the influence of sample pH on extraction efficiency. The results showed that increasing sample pH to 7 improved extraction efficiency; however, recovery of toluene did not improve when sample pH increased to over 7. Therefore, neutral pH (pH = 7) was selected as optimum.

3.2.2. Sample volume

To evaluate the effect of the volume of the sample on the efficiency of HS-SPME, a set of experiments was carried out. The total volume of the vial (the sample plus headspace) was 10 ml, the volume of samples ranged from 2 to 6 ml. The samples were extracted at optimized pH for 10 min (Figure 4). The results showed that increasing volume to 5 ml increased extraction efficiency; afterwards, there was no significant effect on extraction recovery.
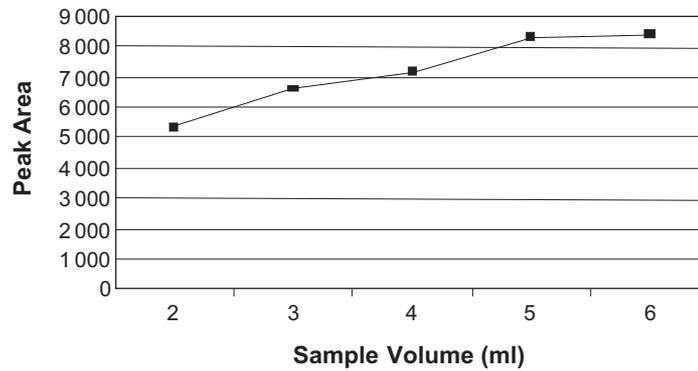
Figure 4. The effect of sample volume on extraction efficiency.

3.2.3. Extraction temperature

To investigate the influence of temperature on extraction efficiency, the effect of five extraction temperatures (20, 25, 30, 40 and 50 °C) was investigated. The highest extraction efficiency was obtained at 30 °C; there was no significant increase afterwards (Figure 5). So, this temperature was chosen as optimum.
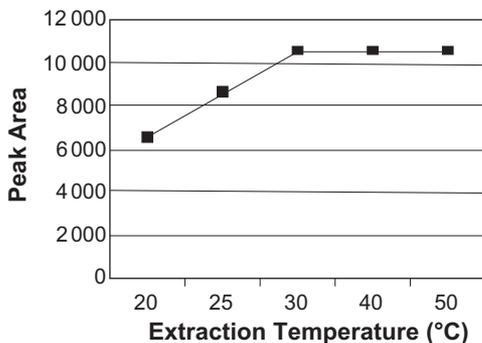
Figure 5. The effect of extraction temperature on extraction efficiency.

3.2.4. NaCl

To investigate how addition of NaCl influenced extraction efficiency, 0–0.4 $g \cdot ml^{-1}$ of NaCl was added to the samples; after complete mixing, extraction took place. The obtained results showed that adding up to 0.2 $g \cdot ml^{-1}$ of NaCl to the solution improved extraction efficiency. As for amounts of NaCl greater than 0.2 $g \cdot ml^{-1}$, extraction efficiency reached a plateau and remained constant (Figure 6), this amount was selected as optimum.

3.2.5. Sample agitation

Extraction efficiency was investigated on the basis of 5 ml of model solutions containing 100 $\mu g \cdot L^{-1}$ of toluene at stirring speeds of 200–1000 rpm (Figure 7). The results showed that extraction efficiency increased with stirring speed, and for speeds greater than 600 rpm, the extraction profile
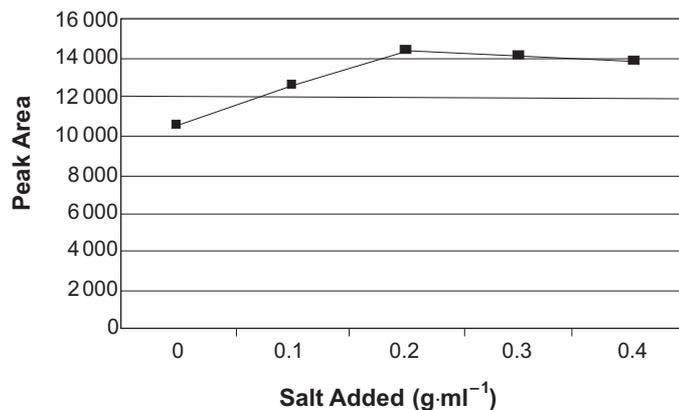
Figure 6. The effect of added NaCl on extraction efficiency.
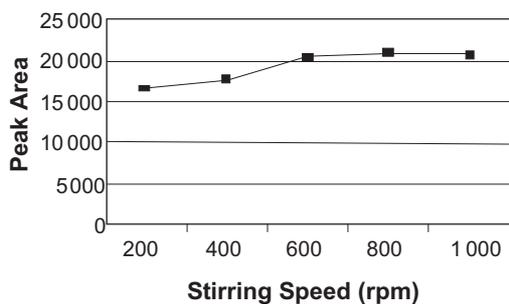
Figure 7. The effect of stirring speed on extraction efficiency.

was flat. Therefore, in the next experiments, the solutions were stirred at 600 rpm.

3.2.6. Extraction time

To optimize extraction time, the fibre was exposed to the gaseous sample in the headspace area for 2, 4, 6, 8 and 10 min. Then, the fibre was retracted into the needle, transferred into the injection port of GC and the thermally desorbed analyte was determined. Extraction equilibrium was established after 6 min (Figure 8). Therefore, this extraction time was chosen for further experiments.

3.2.7. Sample concentration

After optimizing parameters affecting on extraction efficiency of toluene, a range of concentrations of the analyte of 50–400 μg·L^{-1} was prepared to define the influence of sample concentration on extraction efficiency. When concentration changed, the peak area increased, illustrating linear sensitivity of the method (Figure 9).

Figure 9. The effect of sample concentration on extraction efficiency.

3.2.8. Reproducibility assessment of the optimized method

Performance of the optimized SPME technique for determining toluene in urine was evaluated using a spiked urine sample as this matrix may contain some interference compounds similar to a real sample. Six 50-ml samples of urine spiked with toluene were prepared at concentrations of 0, 50, 100, 200, 300 and 400 μg·L^{-1} and were individually extracted for calibration curves. Linear standard curves in the 50–400 μg·L^{-1} range were obtained each day for 6 consecutive days. They showed satisfactory linearity with correlation coefficients of .999 or greater. Three 50-ml spiked samples of 50 (low), 200 (medium) and 400 (high) μg·L^{-1} were extracted and analysed each day for 6 consecutive days to estimate day-to-day reproducibility. The optimized extraction protocol was followed along with optimum chromatographic conditions.

Figure 8. The effect of time on extraction efficiency.

TABLE 1. Day-to-Day (D-Day) and Within-Day (W-Day) Reproducibility of the Method in Urine

| Statistical Data | Concentration Added ($\mu g \cdot L^{-1}$) | | | | | |
| | 50 | | 200 | | 400 | |
	D-Day	W-Day	D-Day	W-Day	D-Day	W-Day
M	48.78	48.87	198.66	197	398.07	396.73
SD	5.14	1.78	4.15	2.96	4.52	4.89
%CV	10.53	3.64	2.08	1.5	1.13	1.23

Notes. Sample volume: 50 ml, *N* = 6; *CV*—coefficient of variation.

The measurement of toluene on each day of the analysis was calculated by comparing it with the extracted calibration curve generated on that day. Day-to-day variation or reproducibility of the method was assessed by calculating the mean, standard deviation and the coefficient of variation (*CV*) (Table 1). As indicated by *%CV*, the precision of the optimized method was high enough. The coefficient of variation at 200 and 400 $\mu g \cdot L^{-1}$ showed higher precision. Experiments were also undertaken to assess within-day reproducibility. To perform this assessment, the same concentration levels of 50, 200 and 400 $\mu g \cdot L^{-1}$ were used. The experiments were preformed on the first 3 consecutive days (on each day, one concentration was extracted and analysed 6 times). The within-day reproducibility of the method was evaluated in the same manner as day-to-day evaluation. The results are shown in Table 1. The obtained *%CV* indicates that the method was much more precise at medium and high concentrations.

4. DISCUSSION

The aim of this research was to establish a method of determining urinary toluene to evaluate environmental and occupational exposure using HS-SPME. Therefore, it was necessary to optimize parameters affecting the extraction of the analytes by testing the effect of each parameter at different ranges. The pH of the sample was the main parameter which could theoretically affect extraction efficiency. The ionic strength of the analytes could be changed by increasing or decreasing pH. To investigate the role of sample pH on the extraction of toluene with HS-SPME, a range of pH of 3–11

was tested on standard solutions. To change the pH of a sample, standard buffers of NaOH and HCl were used. Figure 3 shows that pH up to 7 increased the extraction of the analyte, then reached a plateau and then remained constant. Theoretically, it seems that toluene by itself was a pH-independent solution and at low pH (2–7), the ionic environment of matrix could push toluene to be moved out inside of sample headspace and then the equilibrium was reached. So, neutral pH (pH = 7) was chosen as optimized sample pH for further experiments.

Since toluene is a volatile organic compound, it can easily exist in the headspace of the sample. On the other hand, there is a direct relationship between the volume of the aqueous solution and the gaseous phase. So, it is obvious that, in a constant vial, by increasing the volume of sample in aqueous phase, the volume of sample in the gaseous phase is decreased. Thereby, the amount of the analyte increases in the gaseous phase and extraction takes place in a concentrated phase. For optimizing the volume of the sample, since the size of headspace vials was 10 ml, 2–6 ml of the sample of was tested. Figure 4 shows that by increasing the volume of the sample to 5 ml, the amount of the analyte extracted can be increased; however, there was no significant difference (α < .05) between the extraction efficiency afterward. So, 5 ml was selected as optimized volume and was used in further experiments.

Temperature is another parameter that can affect the extraction of the analyte in HS-SPME. By raising temperature, the partition constant of the analyte is increased in favour of the gaseous sample; therefore, partitioning of the analytes between the sample and headspace can reach equilibrium more quickly. Therefore, the analyte can exist in the headspace of the

sample and can be extracted by the SPME fibre. Theoretically, increasing temperature causes an increase in the amount of the analyte extracted. In this study, temperature of 20–50 °C was tested to evaluate the effect of temperature on extraction efficiency. The obtained results indicated that temperature up to 30 °C could increase the amount of the analyte extracted, where it reached a plateau and remained constant. No significant effect was seen at higher temperatures (Figure 5). However, at higher temperatures, some extra peaks on the chromatographic analyses were seen; they resulted from fibre materials.

NaCl is also a parameter that can affect extraction efficiency, because in its presence, the partition constant of the analyte can change and, therefore, allow more analyte to exist in the headspace of the sample. In fact, the solubility of the analyte decreases when NaCl is added. To study the effect of extra NaCl on the extraction efficiency of toluene, 0–0.4 $g \cdot ml^{-1}$ of NaCl was added to 5 ml of the sample solution, and after complete mixing, the extraction and analysis were performed. Figure 6 shows that by adding up to 0.2 $g \cdot ml^{-1}$ of NaCl, the amount of the extracted analyte increased; then, extraction remained constant. However, there was no significant difference ($\alpha < .05$) between 0.2, 0.3 and 0.4 $g \cdot ml^{-1}$. So, 0.2 $g \cdot ml^{-1}$ was chosen as the optimum amount of NaCl to add.

Sample agitation is another important parameter that has to be optimized. It can increase extraction efficiency, like temperature explained earlier. Therefore, to optimize sample agitation, the speed of 200–1 000 rpm was tested using a magnetic stir bar. Figure 7 shows that increase of agitation to 600 rpm, increased extraction efficiency; then, there was no more significant effect ($\alpha < .05$) on the amount of extracted analyte. So, 600 rpm was chosen as an optimum agitation of the sample.

The effect of extraction time of 2–10 min on extraction efficiency with the SPME fibre indicated that in all experiments after 6 min there was an equilibrium of the analyte between the fibre coating and the headspace of the sample; up to 6 min there was no significant effect ($\alpha < .05$) (Figure 8).

Finally, to evaluate the effect of sample concentration on extraction efficiency, 50–400 $\mu g \cdot L^{-1}$ of sample concentration was prepared. Extraction and analysis was performed with the other optimized parameters. Figure 9 shows that in all experiments a direct proportion was seen between the analyte in the sample and the analyte adsorbed by the SPME fibre. This relationship was linear ($r^2 > .99$). It means that in the SPME sample concentration cannot affect extraction efficiency; in other words, extraction time can be decreased through an increase in the amount of analyte in a certain volume. Therefore, extraction was efficient in the range of the examined concentrations. However, since SPME is a preferred method for low-concentration extraction, 100 $\mu g \cdot L^{-1}$ was chosen.

Finally, a preliminary validation of the possible use of the optimized method for determining toluene in urine was performed, using spiked samples and standards. The day-to-day and within-day relative standard deviations of the method were investigated by spiking urine sample with toluene. Linear standard curves (extracted) of 50 (low), 200 (medium) and 400 (high) $\mu g \cdot L^{-1}$ were obtained every day ($n = 6$) with a correlation coefficient of .999 or greater (Table 1). Table 1 shows that at 50 $\mu g \cdot L^{-1}$, %CV was higher at 200 and 400 $\mu g \cdot L^{-1}$. However, the optimized method was sensitive enough for all concentrations. It is worth mentioning that in this study the selected concentrations were rather low (at the $\mu g \cdot L^{-1}$ level) and that excellent linearity (>.999) was obtained for all the applied concentrations. However, trace concentrations of 50 $\mu g \cdot L^{-1}$, close to the limit of detection (30 $\mu g \cdot L^{-1}$), may cause some analyte to be lost from the SPME fibre during handling operation of the syringe (this is probably due to loss of the analyte from the fibre through evaporation when transporting the SPME device from the vial to the injector). Of course, by applying an automated system for injection, analyte loss can be decreased and extraction efficiency can increase. So, the concentration of 100 $\mu g \cdot L^{-1}$ was chosen. This concentration was as low as needed and it had

an appropriate sensitivity considering the limit of detection obtained in this research (30 µg·L^{-1}), and allowed a very sharp and symmetrical peak at all chromatographic analyses.

On the basis of the reported methods [20, 21], for optimizing SPME, authors generally have used commercial fibres such as PDMS to evaluate the extraction efficiency of analyte, while, in this study, a laboratory made fibre was used which is more available and inexpensive than commercial fibres. Moreover, to make an advantage from this study compare to the other studies [22, 23], further experiments of reproducibility of the method were carried out on spiked urine samples to validate the possible use of the optimized SPME for measuring toluene when an environmental study and biological monitoring of worker exposed to such pollutant are required. On the basis of the obtained results, it is concluded that HS-SPME is an appropriate method as well as simple, fast, selective and reliable for determining and evaluating of urinary toluene in environmental and occupational exposures.

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