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# Headspace solid-phase microextraction for the determination of benzene, toluene, ethylbenzene and xylenes in urine

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**Abstract**

A method for the determination of benzene, toluene, ethylbenzene and xylenes (BTEX) in urine of people exposed to these airborne pollutants present in the living environment, has been described. Solid-phase microextraction has been used for sampling BTEX from the headspace of urine and gas chromatography–mass spectrometry has been applied for the selective analysis of chemicals. The method has the following features: small volume of urine (2 ml) needed, linearity in the range of interest (from the limit of detection up to 5000 ng/l) with coefficient of correlation $\geq$0.998, limit of detection in the range 12–34 ng/l, good repeatability (coefficient of variation 2–7%), high specificity. The stability of the urine sample during storage (−20°C) was evaluated: BTEX remained stable for up to 2 months. The assay has been successfully applied to the biological monitoring of two subjects environmentally exposed to airborne BTEX in an urban area. 

*Keywords:* Benzene; Toluene; Ethylbenzene; Xylene

## 1. Introduction

Headspace solid-phase microextraction (HS-SPME), first published in 1993 by Zhang and Pawliszyn [1], is a modification of SPME [2–4] in which a fused-silica fiber coated with a thin layer of a selective coating is used to trap and concentrate volatile substances directly from the HS of samples for chromatographic analysis. The technique joins the positive features of SPME, such as simplicity, low cost, versatility, precision, selectivity, no solvent use, with the possibility of application to more complex samples which contain solid or high-molecular-mass material such as soil, sludge, food and biological specimens [5–9].

In comparison with traditional HS or purge-and-trap technique, HS-SPME selectively enriches the organic volatiles on the fiber by a suitable coating, the small volume of the fiber enables the flash desorption of the analytes directly in the GC injection chamber, and, using suitable SPME inlet sleeve, no peak broadening is observed, whereas in the case of traditional HS, a cooled trap before the injection chamber is often needed to achieve the analogous result.

Benzene, toluene, ethylbenzene, *m*-, *p*- and *o*-xylene (BTEX) are important industrial chemicals

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used world wide for plastic, as chemical intermediates and solvents. Recently they have acquired great relevance as ubiquitous pollutants of the outdoor and indoor human environment, as two relevant sources of these compounds are vehicular traffic and tobacco smoking [10–13]. Due to the toxicological properties of benzene [14,15], and more in general of these chemicals, there is an interest in the development of specific analytical procedures to measure the exposure to these aromatic hydrocarbons in biological fluids [16–22]. Moreover, the biological monitoring of people exposed to environmental concentrations is the first step toward the evaluation of the toxicological effect of BTEX in the general population.

This study was an extension of the evaluation of HS-SPME as a sampling technique for the determination of BTEX in urine. The aim of the work was to set up an analytical procedure for the biological monitoring of people exposed to low concentration of airborne pollutants (few to hundreds of $\mu g/m^3$) that saves time and labour compared to traditional HS and purge-and-trap methods.

The feasibility of the assay was applied to study two subjects exposed to BTEX as ubiquitous pollutants of urban areas.

## 2. Experimental

### *2.1. General*

#### *2.1.1. Chemicals and standard preparation*

Standard solutions of benzene, toluene, ethylbenzene, *m*-xylene, and *o*-xylene [2000 μg/ml in methanol (MeOH), standards Environmental Protection Agency (EPA)], MeOH (purge-and-trap grade); benzene-$d_6$ (>99.96 atom% D), toluene-$d_8$ (>99.95 atom% D), and *p*-xylene-$d_{10}$ (>99 atom% D), were purchased from Aldrich–Sigma (Milan, Italy). Sodium chloride (NaCl) (reagent for analysis (RPE) grade) was purchased from Carlo Erba (Milan, Italy).

Carbon disulfide ($CS_2$), benzene free, (Fluka, Milan, Italy) and fluorobenzene (Aldrich–Sigma) were used for analysis of BTEX in the environmental samples.

From the standard solutions of the single aromatic hydrocarbons, stock solutions were prepared containing 20, 5, 2.5, 1, 0.5, 0.25 and 0.125 μg/ml of each chemical in MeOH. From the pure deuterated compounds, the internal standards solution was prepared containing 4.75, 4.71 and 4.74 μg/ml of benzene-$d_6$, toluene-$d_8$ and *p*-xylene-$d_{10}$, respectively, in MeOH. The stock solutions and the internal standards solution, kept at −20°C, remained stable, at least, for 6 months. NaCl was kept in an oven at 200°C overnight before use.

#### *2.1.2. Equipment*

SPME fiber [fused-silica fiber 10 mm long, coated with a 100 μm film thick layer of polydimethylsiloxane (PDMS)] and fiber holder for manual use were purchased from Supelco (Milan, Italy).

A gas chromatograph HP 5890 Series II, interfaced with the mass detector HP 5972 operating in the electron impact (EI) mode was obtained from Hewlett-Packard (Cernusco sul Naviglio, Italy). The gas chromatograph was equipped with a split-splitless injector (0.75 mm I.D. inlet liner for SPME, Supelco), operating in the splitless mode, and a DB1 J&W column (60 m length, 0.25 I.D., 1 μm film thickness, Supelco).

For sample storage and analysis clear glass vials were employed (7.9 ml effective volume); vials were closed with 20 mm butyl rubber lined with PTFE septa and crimped with holed aluminium seals (Supelco). Before use vials and septa were heated overnight at 200 and 80°C, respectively; vials were additionally cleaned by washing with ultrapure helium.

For environmental sampling were used passive diffusive personal samplers OV M2 Badge, Trace Air (Gillian, Italy). Analysis was performed with a GC/flame-ionisation system (FID) 8100 Perkin Elmer (Monza, Italy), equipped with a split-splitless injector, operating in the splitless mode, and a DB1 J&W column (30 m length, 0.25 I.D., 1 μm film thickness, Supelco).

#### *2.1.3. Sample collection*

The samples were obtained from students and staff of the Department of Occupational Medicine, University of Milan. The spot urine specimens were collected, directly from the donors, in 50 ml polyethylene tubes. About 7 ml of urine were immediately transferred, by means of a disposable syringe, into the presealed storage vial. To allow the transfer of urine an additional needle was inserted in the

septa during the injection of the sample in the vial. The specimens were frozen and delivered to the laboratory, where they were kept at −20°C until analysis.

#### 2.1.4. Sample preparation

The urine samples were left at room temperature until completly thawed. After gentle mixing, 2 ml of urine were transferred, by means of a syringe, into the open analysis vial, containing 1 g of NaCl. The amount of NaCl added was in excess to saturate the aqueous solution. The analysis vial was sealed and 0.5 μl of the internal standard solution was added to the sample to the final concentration of 1187, 1177 and 1185 ng/l for benzene-$d_6$, toluene-$d_8$ and *p*-xylene-$d_{10}$, respectively.

### 2.2. HS-SPME

The sample was heated at 40°C in a dryblock, and then kept at this temperature for, at least, 30 min to allow the analytes to reach the equilibrium between gas and aqueous solution. The analytes were sampled from the HS by means of the SPME fiber. The fiber is housed in a stainless steel needle which allows for penetration of the septum of the vial cap and the septum in the GC injector. For sampling the fiber was pushed out and exposed directly to the headspace above the sample for 15 min. At the end of the sampling time the fiber was pulled in the stainless steel needle and inserted into the GC injector.

### 2.3. Gas chromatography–mass spectrometry

After insertion into the injector, the SPME fiber was pushed out of the needle and thermally desorbed for 3 min at 200°C into the GC column. The oven temperature was kept at 50°C during the injection (3 min), then the temperature was increased to 160°C at 8 C°/min. Late-eluting compounds were removed by increasing the temperature to 260°C at 50 C°/min and maintaining this temperature for 4 min. Helium was used as the carrier gas at 1 ml/min, constant flow. The total run time was 32 min.

The MS detector, with the source kept at 178°C, was operating in EI mode (70 eV) with the selected ion monitoring mode. The delay time was 7 min; the dwell time was 100 ms. From 7 to 9 min the mass spectrometer was focused at $m/z$ 78 ($[M]^+$ molecular ion, benzene) and $m/z$ 84 ($[M]^+$ benzene-$d_6$), from 9 to 16 min the mass spectrometer was focused at $m/z$ 91 ($[M\text{-}H]^+$ toluene, $[M\text{-}Me]^+$ ethylbenzene and xylenes), and $m/z$ 98 ($[M\text{-}D]^+$ toluene-$d_8$ and $[M\text{-}Me]^+$ *p*-xylene-$d_{10}$). Quantification was based on the ratio between the chromatographic peak area of the analyte and the relative internal standard. Benzene-$d_6$ and toluene-$d_8$ were used as internal standards for benzene and toluene respectively, *p*-xylene-$d_{10}$ was used as internal standard either for ethylbenzene and xylenes. Approximate retention times were as follow: benzene=8.6 min, benzene-$d_6$=8.5 min; toluene=11.4 min, toluene-$d_8$=11.3 min; ethylbenzene=14.0 min, (*m*+*p*)-xylene=14.2 min, *o*-xylene=14.8 min, *p*-xylene-$d_{10}$=14.1 min. The *m*- and *p*-xylene isomers were not resolved on the chromatographic column described above (see Fig. 1).

### 2.4. Calibration, limit of detection (LOD) and repeatability

A pool of urine from nonsmoking, nonoccupationally exposed donors was used for calibration and estimation of the repeatability of the assay. For calibration, the samples were prepared as above (see Section 2.1.4) and spiked with 1 μl of each stock solution containing BTEX. Seven calibration samples (5000, 1250, 625, 250, 125, 62.5, 31.2 ng/l of each BTEX) and a blank were obtained. Samples were run as described in Sections 2.2 and 2.3. Least-squares linear regression analysis was used to estimate slopes ($m$) and intercepts ($b$) of calibration curves $y=mx+b$, where $y$ is the ratio between the chromatographic area of the analyte and the relative internal standard, and $x$ is the urinary concentration of the analyte (ng/l). The LOD of the assay, for each aromatic hydrocarbon, was calculated according to the expression:

$$\text{LOD} = (3\,\text{SE}_b + b)/m$$

where $\text{SE}_b$ is the standard error of the intercept [23].

The repeatability of the assay (as coefficient of variation, C.V.%) was estimated by repeated analysis of samples of urine (six for each concentration) spiked with BTEX each at the concentration of 125, 500 and 1250 ng/l.

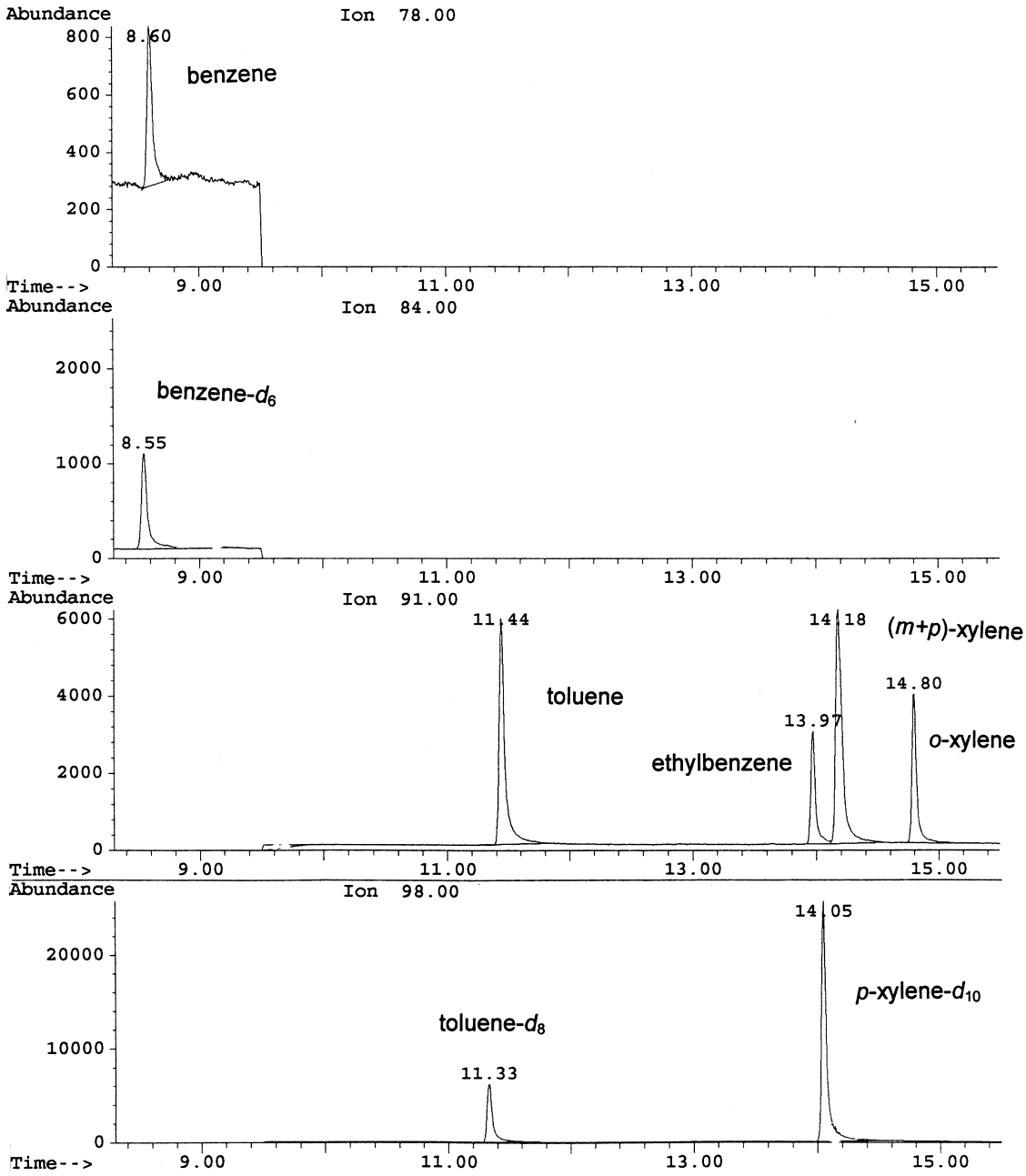


Fig. 1. The single ion mass-chromatograms of a urine sample from a nonsmoking subject. The concentration of benzene, toluene, ethylbenzene, (*m*+*p*)-xylene and *o*-xylene in the sample is 92, 150, 23, 78, 34 ng/l, respectively.

### *2.5. Environmental and biological monitoring of benzene*

Two volunteers, a man (24 years old, subject A) and a woman (33 years old, subject B), from the Department of Occupational Medicine of Milan, both nonsmokers and nonoccupationally exposed, were continuously monitored for 5 and 7 days, respectively, to evaluate their exposure to BTEX in urban areas by environmental and biological monitoring.

*2.5.1. Environmental collection and analysis*

Airborne BTEX were collected by means of passive diffusive personal samplers worn by the subjects in the respiratory zone. Subject A worn the personal samplers during all the experiment (5 days), while subject B worn the personal samplers from day 2 to day 6 of the experiment (5 days). Each personal sampler was used for 24 h, beginning from 7.00 a.m.

BTEX were desorbed from the coconut shell coal strip, using $CS_2$ (2 ml) as solvent, by mechanic agitation (30 min), and analysed in the presence of fluorobenzene as internal standard, by injection in GC–FID. The oven temperature was kept at 40°C for 10 min, then the temperature was increased to 165°C at 8 C°/min. Late-eluting compounds were removed by increasing the temperature to 250°C at 30 C°/min and maintaining this temperature for 2 min. Helium was used as the carrier gas at 1 ml/min, constant flow.

*2.5.2. Biological collection and analysis*

The subjects (A and B) collected spot samples (20 and 41, respectively) of all their micturitions during the whole time of the experiment (see Section 2.1.3). The specimens were analysed for BTEX following the HS-SPME GC–MS procedure.

## 3. Results

### *3.1. Chromatographic separation*

The single ion mass-chromatograms shown in Fig. 1 correspond to a urine sample from a nonsmoking subject. The analytes are univocally assigned based on their retention time and mass to charge ratio. The concentration of benzene, toluene, ethylbenzene, (*m*+*p*)-xylene and *o*-xylene in the sample is 92, 150, 23, 78, 34 ng/l, respectively.

### *3.2. Optimisation of sampling conditions*

The equilibration kinetics for BTEX at 40°C in the two- and the three phase systems (fiber–urine and fiber–headspace–urine, respectively) were studied. Four sets (A, B, C and D) of 24 urine samples, spiked with BTEX each at the concentration of 1250 ng/l, were prepared; each sample of sets B and D was added with saturating amount of NaCl (1 g). Set A and B were sampled in the HS mode, while set C and D were sampled by direct immersion of the fiber in urine. Analysis in each set was performed, in triplicate, using different sampling time: 30 s, 1, 2, 5, 10, 15, 30 and 60 min. Typical curves, obtained from set B, are shown in Fig. 2: the chromatographic signal increased with time until the equilibrium among the phases was reached. The time to reach the equilibrium was longer for higher boiling point compounds. The equilibration kinetics of the internal standards were quite comparable to those of the corresponding aromatic compounds. The results of this experiment are summarised in Table 1 where, for each set of samples are given: the relative intensity of the chromatographic signal (average signal in the set vs. average signal in set D, considered as reference), the sampling time (time that allows the signal to be, at least, the 90% of the maximum value [4], evaluated visually), and the repeatability (as C.V.%) of the ratio between the signals of analyte and internal standard, within the set. Lowest signal intensity was found for set A (HS sampling, no NaCl added), while roughly double signal intensity was found either for addition of salt (set B) or for sampling by direct immersion (set C); the highest signal intensity was found when urine was sampled by direct immersion in the presence of NaCl (set D). Sampling time was longer for direct immersion than for HS sampling (set C, D vs. set A, B) and in the presence of salt. The repeatability of the analyte to the internal standard ratio within the set was higher (lower C.V.%) in set B and C (6 and 7% as average value) than in set A and D (18% as average value for both sets).

When analogous experiment was performed at higher temperatures, different curves describing the kinetics were found: at 80°C an increase of the chromatographic signal was observed with time: within 60 min the system did not reach the equilibrium. Furthermore the average intensity of the chromatographic signals, calculated within 20 min of sampling time, was significantly reduced: at 60 and 80°C the peaks area decreased to 50–55% and to 25–35%, respectively, of their values at 40°C.

Based on the results of this experiment, and additionally considering that HS is cleaner than direct immersion sampling, so that longer lifetime of SPME fiber is expected, the following sampling

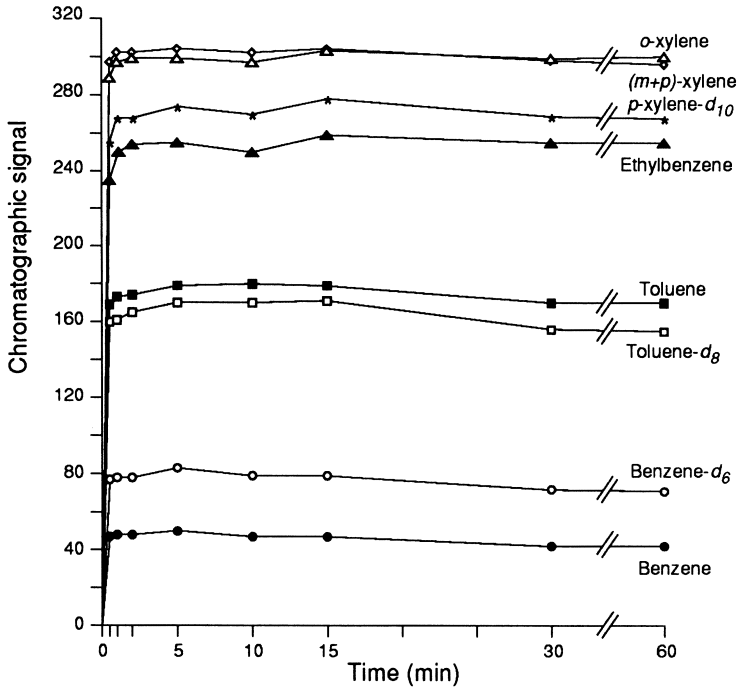


Fig. 2. Equilibration kinetics at 40°C of BTEX in the three-phase system PDMS fiber–HS–urine.

Table 1
Summary of results of the equilibration kinetics study, performed at 40°C on four different sample sets (A, B, C and D) to study the effect of sampling mode (HS vs. direct immersion) and of addition of saturating amount of NaCl to urine

| Set (24 samples) | | Benzene | Benzene-$d_6$ | Toluene | Toluene-$d_8$ | Ethylbenzene | ($m+p$)-Xylene | $o$-Xylene | $p$-Xylene-$d_{10}$ |
|---|---|---|---|---|---|---|---|---|---|
| A (HS) | Relative intensity[a] | 0.26 | 0.22 | 0.36 | 0.28 | 0.43 | 0.42 | 0.41 | 0.37 |
| | Sampling time[b] (min) | 1 | 1 | 2 | 2 | 5 | 5 | 5 | 5 |
| | Repeatability[c] (C.V.%) | 17 | – | 6 | – | 25 | 13 | 26 | – |
| B (HS+NaCl) | Relative intensity[a] | 0.53 | 0.48 | 0.71 | 0.61 | 0.71 | 0.66 | 0.80 | 0.66 |
| | Sampling time[b] (min) | 2 | 2 | 10 | 10 | 15 | 15 | 15 | 15 |
| | Repeatability[c] (C.V.%) | 6 | – | 7 | – | 5 | 4 | 6 | – |
| C (direct immersion) | Relative intensity[a] | 0.49 | 0.38 | 0.52 | 0.46 | 0.74 | 0.70 | 0.64 | 0.64 |
| | Sampling time[b] (min) | 5 | 5 | 10 | 10 | 15 | 15 | 15 | 15 |
| | Repeatability[c] (C.V.%) | 7 | – | 7 | – | 7 | 7 | 8 | – |
| D (direct immersion +NaCl) | Relative intensity[a] | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | Sampling time[b] (min) | 10 | 10 | 15 | 15 | 25 | 25 | 25 | 25 |
| | Repeatability[c] (C.V.%) | 19 | – | 18 | – | 19 | 19 | 16 | – |

[a] Referred to set D.
[b] Time that allows the signal to be, at least, 90% of the maximum value.
[c] Repeatability of the analyte to internal standard ratio within the set.

Table 2
Extraction efficiency % and log $K_{F/W}$ of BTEX from urine. Extraction efficiency was calculated comparing HS-SPME (sampling at 40°C for 15 min), vs. the direct injection of liquid sample in the splitless mode

| Concentration (ng/l) | Extraction efficiency % | | | | |
|---|---|---|---|---|---|
| | Benzene | Toluene | Ethylbenzene | (*m*+*p*)-Xylene | *o*-Xylene |
| 125 | 4.6 | 6.4 | 11.7 | 10.8 | 14.1 |
| 500 | 4.4 | 6.3 | 11.6 | 12.7 | 14.6 |
| 1250 | 4.3 | 6.5 | 12.0 | 14.0 | 15.1 |
| Average | 4.4 | 6.4 | 11.8 | 12.5 | 14.6 |
| Log $K_{F/W}$ | 2.15 | 2.32 | 2.61 | 2.64 | 2.71 |

conditions were selected: HS sampling at 40°C for 15 min, using urine samples saturated with NaCl.

### 3.3. Extraction efficiency

For estimation of the extraction efficiency, HS-SPME was compared with the direct injection of liquid samples in the splitless mode, considered as the technique of reference. Urine samples were spiked with 1250, 500 and 125 ng/l of each BTEX, sampled by HS-SPME at 40°C for 15 min and analysed in GC–MS. The peak areas resulting from these analysis were compared with those obtained by splitless syringe injection of the same amount of BTEX in $CS_2$, followed by GC–MS analysis. The distribution coefficient fiber/water, $K_{F/W}$ was calculated from the average extraction efficiency value, the known urine volume (2 ml) and the calculated fiber coating volume ($6.6\times10^{-4}$ ml). The extraction efficiency values at the different concentration and the log $K_{F/W}$ values are reported in Table 2. It was noticed that, for each BTEX, the extraction efficiency was very similar at the different concentrations, according to a linear response of the SPME fiber in the investigated range. Among the different substances, extraction efficiency increases with the boiling point, passing from benzene (4.4%) to *o*-xylene (14.6%).

### 3.4. Calibration, LOD and repeatability of the assay

Table 3 summarises the regression data for the calibration curves and the calculated LOD for the determination of BTEX in urine. The resulting calibration curves were linear, in the investigated range (LOD—5000 ng/l), for all the BTEX, with a correlation coefficient $\geq$0.998. The LOD for benzene, toluene, ethylbenzene, (*m*+*p*)-xylene and *o*-xylene were: 25, 34, 12, 23 and 15 ng/l, respectively. The overall repeatability of the assay, estimated by the coefficient of variation (C.V.%) calculated repeating six samples for each concentration, resulted in the range 2–7% for the different BTEX

Table 3
Summary of the linear regression data for the calibration curves and LOD for the determination of BTEX in urine

| | Benzene | Toluene | Ethylbenzene | (*m*+*p*)-Xylene | *o*-Xylene |
|---|---|---|---|---|---|
| *Parameters of linear regression* | | | | | |
| Dynamic range (ng/l) | 0–5000 | 0–5000 | 0–5000 | 0–5000 | 0–5000 |
| $m\pm SE_m$ [a] | $9.9\times10^{-4}\pm9.8\times10^{-6}$ | $1.4\times10^{-3}\pm9.6\times10^{-6}$ | $1.5\times10^{-3}\pm2.2\times10^{-5}$ | $1.4\times10^{-3}\pm1.4\times10^{-5}$ | $1.5\times10^{-3}\pm1.7\times10^{-5}$ |
| $b\pm SE_b$ [b] | $9.7\times10^{-3}\pm5.0\times10^{-3}$ | $3.4\times10^{-2}\pm4.5\times10^{-3}$ | $-2.6\times10^{-2}1.4\times10^{-2}$ | $1.7\times10^{-2}\pm5.1\times10^{-3}$ | $7.0\times10^{-4}\pm7.3\times10^{-3}$ |
| Number of datapoints | 24 | 24 | 24 | 24 | 24 |
| *r* | 0.999 | 0.999 | 0.998 | 0.999 | 0.999 |
| *Detection limit* | | | | | |
| LOD (ng/l) | 25 | 34 | 12 | 23 | 15 |

[a] Slope and relative standard error.
[b] Intercept and relative standard error.

Table 4
Repeatability, as C.V.%, of BTEX in urine, at different concentrations. Determinations were performed in one-day experiment repeating six times for each concentration

| Concentration (ng/l) | *N* determinations | Repeatability as C.V.% | | | | |
|---|---|---|---|---|---|---|
| | | Benzene | Toluene | Ethylbenzene | (*m*+*p*)-Xylene | *o*-Xylene |
| 125 | 6 | 6 | 9 | 3 | 5 | 2 |
| 500 | 6 | 2 | 8 | 1 | 3 | 2 |
| 1250 | 6 | 1 | 2 | 1 | 1 | 1 |
| Average | | 3 | 7 | 2 | 3 | 2 |

(Table 4); as expected, the repeatability increases with the concentration.

### *3.5. Stability of BTEX in urine*

The stability of BTEX in the specimen stored at −20°C was studied with an experiment involving three subjects, a nonsmoker, a light-smoker (7 cigarettes/day) and a strong-smoker (20 cigarettes/day) that furnished a spot sample (≅100 ml) of their urine. The urine specimens were immediately partitioned into storage vials and stored at −20°C until analysis. Analysis was performed, in double, at different times: zero (day of sample collection), and after 2, 4, 7, 11, 16, 23, 30 and 60 days. The results were analogous for the three subjects and for each BTEX: little fluctuations around the mean value were observed, but no decrease of concentration with time. It was concluded that all the chemicals in the specimens are stable in the storage condition (−20°C) for up to 2 months. In Table 5 the mean concentration and the repeatability (as C.V.%) of the BTEX for the three subjects are reported, considering all the determinations performed along the 2 months. The concentrations of BTEX increased with the number of smoked cigarettes, in particularly average benzene concentration was 123, 347 and 441 ng/l urine for the nonsmoker, light-smoker and heavy-smoker, respectively. The C.V.% was ≤23% and can be considered as the overall day-to-day precision, including influence of storage and inter-operator variability.

### *3.6. Environmental and biological monitoring of benzene*

In Table 6 the mean values and S.D. of airborne and urinary BTEX are reported, referring to all the samples collected during the study (five air samples for each subject; 20 and 41 urinary samples for subject A and B, respectively). Fig. 3A and B shows the result of environmental and biological monitoring of benzene; benzene in air, as the time-weighted-average (TWA) value for 24 h, is given in $\mu g/m^3$; benzene in urine, as concentration in the spot sample, is given in ng/l. The average value for benzene excretion during 24 h, calculated as average value of the urinary benzene concentration in the spot samples, and the corresponding TWA airborne benzene concentration was significantly correlated for subject

Table 5
Concentration and repeatability, as C.V.%, of BTEX in a spot sample of urine furnished by each of three subjects with different smoking habits. Specimens were stored at −20°C and the determinations were performed along 2 months

| Subject | *N* determinations | | Urinary BTEX (ng/l) | | | | |
|---|---|---|---|---|---|---|---|
| | | | Benzene | Toluene | Ethylbenzene | (*m*+*p*)-Xylene | *o*-Xylene |
| Non-smoker | 16 | Mean | 123 | 215 | 30 | 108 | 43 |
| | | C.V.% | 15 | 20 | 22 | 23 | 23 |
| Light-smoker | 16 | Mean | 347 | 265 | 38 | 137 | 56 |
| | | C.V.% | 19 | 12 | 14 | 13 | 15 |
| Heavy-smoker | 16 | Mean | 441 | 336 | 57 | 163 | 61 |
| | | C.V.% | 12 | 12 | 16 | 16 | 17 |

Table 6
Statistic values for airborne and urinary BTEX referred to two subjects living in urban areas

| | *N* determinations | Benzene Mean±SD | Toluene Mean±SD | Ethylbenzene Mean±SD | (*m*+*p*)-Xylene Mean±SD | *o*-Xylene Mean±SD |
|---|---|---|---|---|---|---|
| *Subject A* | | | | | | |
| Airborne (μg/m$^3$) | 5 | 11±6 | 30±11 | 4±1 | 17±6 | 6±4 |
| Urinary (ng/l) | 20 | 114±74 | 123±56 | 15±4 | 48±10 | 18±3 |
| *Subject B* | | | | | | |
| Airborne (μg/m$^3$) | 5 | 10±4 | 50±36 | 6±2 | 17±3 | 10±6 |
| Urinary (ng/l) | 41 | 122±45 | 205±59 | 23±4 | 77±17 | 28±5 |

A [correlation coefficient 0.93 (Spearman *r*) and significance 0.02 (*p*-value)], but not for subject B (correlation coefficient 0.80 and significance 0.10).

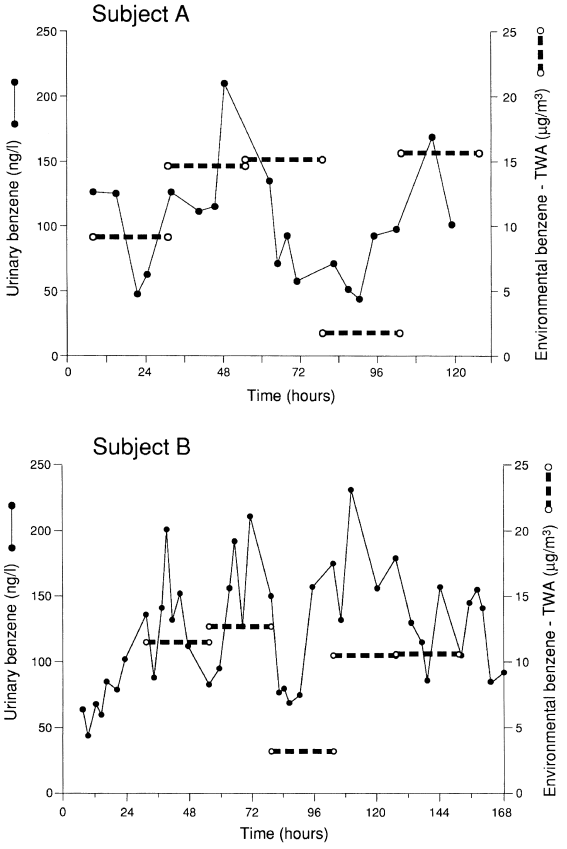


Fig. 3. Biological and environmental monitoring of benzene in two subjects living in an urban area. Black dots represent the urinary concentration of benzene (ng/l) in the spot sample. Dashes represent the airborne benzene concentration (μg/m$^3$), as TWA value during 24 h. **A.** Subject A was monitored for 5 days. **B.** Subject B was monitored for 7 days.

Analogously to benzene, intra- and inter-day fluctuations in the urinary levels of toluene, ethylbenzene and xylenes were observed, but no significance in correlation between environmental and biological exposure values was found.

## 4. Discussion

Unmetabolised BTEX in urine may represent specific indicators for the evaluation of the bioavailability of these chemicals, but measurements of them have been scarcely carried out to date, due to the troublesomeness of the existing analytical procedures. All the methods are based on extraction of the analytes from the HS of sample, followed by GC analysis [17–22]. Recently, some authors [20,22] proposed HS-SPME for sampling hydrocarbons from biological fluids. The first report [20] published a method where toluene, benzene, *n*-butyl acetate, *n*-butanol and *n*-isoamyl acetate were sampled from urine or blood and analysed by GC equipped with a FID system, in the range 1–50 μg/l, for application in forensic toxicology. The second report [22] presented a study where BTEX were measured in urine (40 ml sample volume) obtained from 432 primary school children in Italy and analysed by GC–MS. In this paper the use of SPME for sampling BTEX directly from the HS of urine, for the biological monitoring of the exposure to BTEX present as pollutants in the urban atmosphere (few to hundreds of μg/m$^3$), has been further investigated.

To maximise the recovery of the aromatic hydrocarbons we chose the 100 μm film thick layer PDMS fiber: in fact this coating has high chemical affinity for BTEX and the film is the thickest among those purchasable. Nevertheless, even after optimisation of

analytical conditions, the extraction efficiency was rather poor, in the range 4.4–14.6% (Table 2). Recently some new coatings have been proposed to improve extraction of BTEX from aqueous samples: Popp and Paschke [24] and Djozan and Assadi [25] claimed high extraction efficiency (up to 90%) using carboxen-PDMS and charcoal coated fibers, achieving LODs in the range of ng/l using GC–FID. The difference in the sampling mechanism, absorption in the case of carboxen and charcoal, and partition in the case of PDMS, could be the reason for the observed difference in extraction efficiency.

Based on the equations describing the equilibrium of the BTEX between fiber and aqueous sample (two-phase system) [1] and among fiber, aqueous sample and HS (three-phase system) [1], the mass adsorbed by the coating during the sampling is expected to be lower in the three- than in the two-phase system. This was confirmed by the experiment performed to optimise the sampling conditions (Table 1), where it was found that HS sampling results in an average loss of signal of about 40% compared to direct immersion sampling. Moreover, based on the knowledge that an increase of the ionic force of aqueous solutions decreases the solubility of hydrocarbons, the addition of saturating amount of NaCl to urine sample was studied. The results of this experiment (Table 1) demonstrate that NaCl improved significantly the extraction efficiency of SPME fiber and that the loss of sensitivity due to HS sampling is compensated by the addition of salt. The highest extraction efficiencies were observed performing direct immersion sampling in the presence of salt, but, in the attempt to protec the fiber from contamination arising from high-molecular-mass substances present in the biological sample, the HS was preferred over direct immersion sampling. No data are available to compare fiber lifetime in the case of direct immersion vs. HS sampling, but, so far, over 500 HS-SPME analyses have been run using the same fiber without apparent loss of performance.

Stirring was effective in shortening equilibration time when SPME sampling was performed by direct immersion of the PDMS fiber in the aqueous phase [2] but when benzene and *o*-xylene were sampled in the HS, only an irrelevant difference in equilibration time was found between static or well agitated aqueous solutions [1]. Based on this observation, in the present work, the effect of stirring on the equilibration time was not evaluated. Besides, though the HS-SPME technique is primarily based on the equilibrium among the involved phases, it can also be performed without reaching the equilibrium by adding suitable internal standards. That is demonstrated by the repeatability (as C.V.%) of the analyte to the internal standard ratio, that, in the selected sampling condition, was $\leq$ 7% for the different BTEX (see Table 1, set B).

When the equilibration kinetics were studied, the curves obtained after sampling from urine at 40°C (Fig. 1) were very similar to those previously described for water samples [26,27]. At higher temperatures a different behaviour was surprisingly found: at 80°C the chromatographic signal increased with time and equilibrium was not reached within 60 min. To better understand the discrepancy in the equilibration kinetics between water and urine samples, further investigations have been undertaken, the result of which are discussed elsewhere [28].

Crucial steps in the definition of a procedure suitable for the biological monitoring of the environmental exposure to BTEX, are the collection and handling of the samples, during which contamination and/or loss of analytes could occur. Due to the importance of storage and analysis vials, the problems of pollution arising from septa and vials has been carefully checked and a suitable procedure has been proposed for their cleaning. For other sources of possible contamination, such as the polyethylene tube and disposable syringe used during sample collection, we assumed that their contribution to the level of BTEX in the sample is negligible, due to the short contact time of these objects with urine. Furthermore, also the possible loss of volatile analytes from urine during sample collection and transfer to the vial was considered negligible, as the operation was performed immediately after micturition. This assumption is supported by the work of Ukai et al.[29] showing that the decrease of the concentration of urinary dichloromethane was insignificant if the transfer to storage vial was performed within 3 min after collection. A quantitative verification of these assumptions will be the object of further investigations.

Finally, the two applications of the assay, i.e., the

biological monitoring of two subjects exposed to environmental pollution and the comparison of three subjects with different smoking habits, showed urinary concentration of BTEX comparable to those previously reported [17–19,22]. The intra- and inter-day fluctuations in the concentration of urinary benzene may indicate analogous variations in the airborne concentration, as those we previously observed in the atmosphere of our city [13]. The significant correlation found between environmental and urinary benzene in one of the two studied subjects, in spite of the low number of available data points, may support this hypothesis.

We conclude that the features of the proposed method, i.e., the good analytical performance, the simplicity of the assay and the low cost of the SPME technique, make this approach very convenient for the biological monitoring of exposure to low levels of BTEX.

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