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Short communication

Quantitative analysis of benzene, toluene, and xylenes in urine by means of headspace solid-phase microextraction

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Abstract

A simple method for benzene, toluene, and xylenes (BTX) quantitative analyses in human urine was developed, using headspace solid-phase microextraction (HS-SPME) and gas chromatography coupled to mass spectrometry detection in the single ion monitoring mode. The developed method is solventless, non-invasive, requires small volume of sample (1 ml), shows high selectivity, sensitivity, repeatability, and linearity (correlation coefficients >0.998), providing a useful alternative to assess human exposure to BTX compounds due to occupational reasons or eventual exposure to organic solvents. Detection limit varies from 0.28 to 0.5 ppb (v/v).

Keywords: Headspace analysis; Benzene; Toluene; Xylenes; Volatile organic compounds

1. Introduction

Organic solvents have been historically used in different areas of chemistry. Increasing research on the toxicity of solvents and their carcinogenic effects reduced undiscriminated use during the last years [1–3]. The quantitative determination of organic solvents in human fluids is of importance as some of them are highly volatile and hazardous to human health, even when present in low concentrations [1–3].

Biomonitoring of human contamination is related to the determination of the individual exposure to the hazardous material. This allows the determination of the individual health risk and not only the measurement of the target analytes concentration in the environment [4,5]. Solvents like benzene, toluene and xylenes (BTX) have been a constant concern for society, as they promote damages to the central nervous system, when inhaled [2,3]. Solvent inhalation is usually occupational and not intentional, requiring severe preventive control of solvent use in industrial or commercial sites.

Several research works on the monitoring of exposure to BTX compounds [6–11] and their effects on human health have been reported [12–14]. However, the analysis of these solvents in body fluids is difficult to accomplish, once they are metabolized and transformed in their respective metabolites [15,16].

One way to measure the human contamination with BTX is to test urine for specific metabolites, monitoring their internal dosage. *trans–trans*-Muconic, hippuric and methylhippuric acid, (*S*)-phenyl-mercapturic and (*S*)-benzyl-mercapturic acid can be used for this purpose. Some of the main disadvantages of these procedures are the presence of the same biomarkers from extraneous sources [4,5] and the long time they consume to be accomplished. These urinary metabolites are influenced by several factors, such as age, alcohol and smoking habit, and the amount found in different individuals shows a significant variation, depending on environmental factors and personal characteristics [17,18]. Fast analyses are usually necessary in this field, as results are associated with urgent health aspects. In view of that, in the last years, research on method development has been focused on volatile non-metabolized products in biological fluids, such as urine or blood, in order to avoid time consuming metabolites determination [19,30–32].

The classical methods make use of the extraction of BTX (or their metabolites) from blood or urine samples [22] using methods, such as solvent extraction, and headspace techniques. Disadvantages of solvent extraction are the presence of the solvent peak, and the environmental and occupational risks posed by solvent manipulation. Headspace techniques

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require either the use of solvents or of expensive equipment for elution of the target compounds, even though they provide a high reproducibility for the analysis of volatile organic compounds (VOCS) [20,21,22].

Solid-phase microextraction (SPME) has proved to be an excellent method for sampling and analysis of volatile and semi-volatile organic compounds, including organic solvents [23–25]. It is a simple, fast and solvent free extraction technique. It also allows a high number of chromatographic injections with only one fiber, reducing its cost [26–28]. It has recently been used to compare toluene as a biomarker in blood and urine [30], and for determination of toluene in glue-sniffer's blood and urine samples [31].

The present work aims to develop a fast, simple, and non-invasive method for quantitative analysis of benzene, toluene and xylene in urine samples, using headspace solid-phase microextraction (HS-SPME) followed by gas chromatography coupled to mass spectrometry detection (GC–MS). The proposed method is intended to assess human exposure to these compounds.

2. Experimental

All solvents were of analytical grade and distilled prior to use (Merck, Darmstadt, Germany). Double-distilled water was used. After thorough cleansing of glass material, it was placed in an oven for 30 min at 250 °C. Volumetric glass material was washed with detergent followed by rinsing with water and acetone, and left to dryness at room temperature.

BTX contaminated urine samples were kindly supplied by workers from an ink industrial site, and urine blanks by a non-exposed volunteer. A non-contaminated urine sample was employed to make the BTX stock solution (100 $\mu l\,l^{-1}$).

Due to the high volatility of the analytes, the stock solution was prepared in the beginning of each extraction and analysis procedure and kept at 4 °C for not more than 2 days. Spiked solutions (from 3.0 ppm to 0.5 ppb, v/v) were prepared by dilution of the stock solution. Analyses were performed in triplicate. Repeatability of the analytical method was calculated on the basis of the results obtained from six samples of the 10 ppb (v/v) spiked standard solution.

PDMS fibers (polydimethylsiloxane, 30 μm) were [25,26,29] purchased from Supelco (Oakville, Canada). Extractions were performed at 25 °C, using 20 ml clear glass vials containing 10 ml of urine. Analysis were performed with a Shimadzu QP5050 A GC–MS system, using a 30 m × 0.25 mm, 0.25 μm DB-5 capillary column. Injections were performed in the splitless mode (the split valve opened after 40 s), carrier gas flow (helium) was 1.5 ml/min, and the mass spectrometer was used in the electron impact mode (70 eV). Monitored ions were *m/z*, 78 (benzene) and 91 (toluene and xylenes). For xylenes only the *meta*-isomer was quantified, as it was the most intense signal among the three. Injector and interface temperatures were kept at 250 °C, while the chromatographic column was kept at 50 °C during analyses. PDMS fiber extraction and desorption time were established after several trials using 3.0 ppm (v/v) spike solution [25]. No carryover was found after 2 min fiber desorption time. Even though, the fiber was kept inside the injection port in between analyses to prevent contamination, and carrier gas flow was kept as 1.5 ml/min. Quantitative analysis was performed using the standard addition method [22]. Detection limits (DLS) were calculated using bidistilled water. The noise recorded (area counts) on the retention times of each analyte on the chromatogram was measured 10 times. The detection limit was calculated as three times the standard deviation of these 10 values recorded. The quantitation limit corresponds to approximately three times the DLS value. It was also measured through the analysis of decreasing concentration solutions of the analytes until achieving the least possible detectable peak area.

Recovery was determined comparing the average values obtained from three liquid injections and three SPME and analysis using a 10 ppb standard solution.

3. Results and discussion

The equilibration time between the PDMS coating and the volatile target compounds is shorter than 2 min for all compounds under study, confirming data found in the literature for these compounds from other matrices [26]. A 3 min time was chosen as extraction time. Table 1 shows that correlation coefficients were better than 0.998 for all

Table 1
Figures of merit of the proposed method

Analytes	Recovery (%)	*r*	DL	QL	R.S.D. (%)
Benzene	99.30	0.9989	0.31	1.10	4.71
Toluene	87.38	0.9992	0.50	1.60	2.37
Xylenes	76.79	0.9995	0.28	1.00	2.04

r: correlation coefficient in the range of 0.5–3000 ppb (v/v); DL: detection limit; QL: quantification limit (DL and QL are expressed in ppb (v/v); R.S.D.: relative standard deviation based on six replicate analyses of spike solutions.

Table 2
BTX concentration of (BTX) in the urine samples

Sample	Concentration in ppb (v/v)		
	Benzene	Toluene	Xylenes[a]
1	3.24	13.45	18.75
2	1.37	4.00	3.07
3	4.36	2.10	1.18
4	3.73	2.34	2.10
5	8.06	9.31	16.79
6	6.01	7.12	6.02
7	162.48	174.31	168.42
8	18.80	30.18	29.21

[a] Measured by the *meta*-isomer, which is the most intense peak of the three isomers.

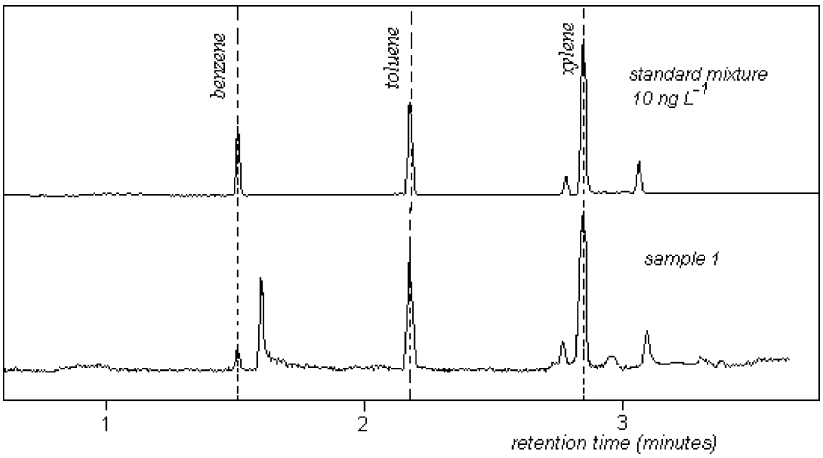

Fig. 1. Comparison with a monitored ion chromatogram (ions *m*/*z* 78 and 91) of a 10 ppb (v/v) urine spiked solution of BTX (A) and one of the contaminated urine samples (B, sample 7).

three target compounds in the concentration range of work. The highest value for relative standard deviation of six replicate analysis of a spiked solution (10 ppb, v/v) was found to be less than 5%, demonstrating a good repeatability. The percentage recovery of BTX compounds; done by analyzing a 10 ppb (v/v) spiked urine sample was the one obtained for xylenes (76.79%), showing a good level of recovery. Low detection levels (0.28–0.50 ppb, v/v) can be used to advantage in the cases of light BTX contamination and health preventive actions.

Eight urine samples were extracted and analyzed using the proposed method. Results are presented in Table 2 and one example of chromatogram of a sample (sample 7) and a standard solution at 10 ppb (v/v) are showed in Fig. 1. These results show the applicability of this method to assess human exposure contamination to solvents in industrial or commercial sites.

4. Conclusions

The developed method is simple, non-invasive, fast, environment-friendly and relatively inexpensive, providing an alternative to assess BTX occupational contamination in workers. The DLS achieved with this method also allow the detection of low concentrations of BTX in urine, providing precautionary information for a preventive action in cases of light contamination. The short time taken (<10 min) for the whole analytical process may contribute to the establishment of convenient routine health management programs in work places where workers are daily exposed to organic solvents as well as forensic studies.

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