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Short Communication

Analysis of Unmetabolized VOCs in Urine by Headspace Solid-Phase Microcolumn Extraction

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Exposure to high concentrations of volatile organic compounds (VOCs) can lead to adverse health effects[1)]. Among VOCs, benzene is a haematotoxic substance that can induce acute myeloid leukaemia in occupational exposure, and has been classified as a carcinogen in humans by the International Agency for Research on Cancer[2)]. Other VOCs, trihalomethanes (chloroform, bromodichloromethane and dibromochloromethane) were detected in the urine of all persons attending a swimming pool, where the water was treated with chlorine[3)]. Tetrachloroethylene in urine of drycleaners has been used as biologic index of low-level exposure[4)]. A significant correlation was found between the environmental trichloroethylene concentration and urinary trichloroethylene concentration[5)]. BTEX (benzene, toluene, ethylbenzene and isomeric xylenes) have attained great relevance as ubiquitous pollutants of outdoor human environments and analysis of urine has been used for the evaluation of environmental exposures to these compounds[6)]. Similarly, styrene analysis in urine is a useful biological indicator of exposed workers[7)].

Generally, VOCs analyses involve either static or dynamic headspace (HS) sampling, followed by gas chromatographic separation and flame ionization (GC-FID) or mass spectrometric detection (GC-MS). In the majority of the newly developed HS sample preparation techniques, the recovery of volatiles is based on sorption in a solid phase[8, 9)]. Among them, equilibrium sampling techniques such as HS solid-phase microextraction[10)], HS sorptive extraction[11, 12)], and a non-equilibrium sampling technique, HS solid-phase dynamic extraction, are available[8)].

In this study we have used the recently developed sampling technique, solid-phase microcolumn extraction (SPMCE)[13–17)], for the analysis of BTEX, styrene, chloroform, trichloroethylene and tetrachloroethylene in urine using GC-FID.

Materials and Methods

Chemicals

HPLC grade methanol and anhydrous sodium sulfate (Na_2SO_4, p.a.) were obtained from Merck (Darmstadt, Germany). Chloroform, trichloroethylene and tetrachloroethylene were obtained from Acros Organics (Geel, Belgium), benzene and toluene from Aldrich (Milwaukee, WI, USA), ethylbenzene and styrene from Merck (Schuchardt, Germany), *p*-xylene, *o*-xylene from Supelco (Bellefonte, PA, USA), and Tenax TA was purchased from Alltech (Deerfield, IL,USA).

Instrumentation and chromatographic conditions

For the analysis, a 20-m*l* aliquot of the urine sample was quickly transferred (by means of a graduated cylinder) into a 100-m*l* volumetric flask containing 4 g Na_2SO_4 and the flask was vigorously shaken by hand (approximately 0.5 min) at room temperature (23 ± 1°C). After 10–15 min equilibration, the flask was capped with aluminum foil. The foil was pierced with a narrow glass tube connected (with a short piece of flexible tubing, glass to glass) to a 10-m*l* all-glass syringe (Poulten & Graf, Wertheim, Germany) and 10 m*l* of headspace from a distance of about 1 cm above the liquid phase was withdrawn. The headspace content of the syringe was pressed through the microcolumn (1.5 mm I.D., packed with 15 mg of 60–80 mesh Tenax TA) at a flow rate of 2–3 m*l*/min. The loaded microcolumn was transferred to a modified GC inlet (Fig. 1) and the trapped analytes were desorbed at 10 kPa by heating the microcolumn for 1 min at 230°C. After the desorption, the carrier gas pressure was increased to 60 kPa and the temperature program was started. Analyses were carried out on a GC 8000 Top Series, CE Instruments (Rodano-Milano, Italy) and a computer program (Shimadzu, Class-VP.2, SP1) was used for data acquisition. The chromatograph was equipped with FID and a fused silica HP-5 capillary column of 50 m length × 0.32 mm I.D. and 0.52 μm film thickness (Hewlett Packard, Palo Alto, CA, USA). The chromatographic elution was temperature programmed as follows: isothermal at 30°C (1 min), then increased from 30 to 150°C at a rate of 5°C/min, and then ramped to 230°C (10 min) at a rate of 20°C/min (to exhaust the high boiling compounds from the column). The temperature of the inlet chamber was 230°C and helium was used as a carrier gas.

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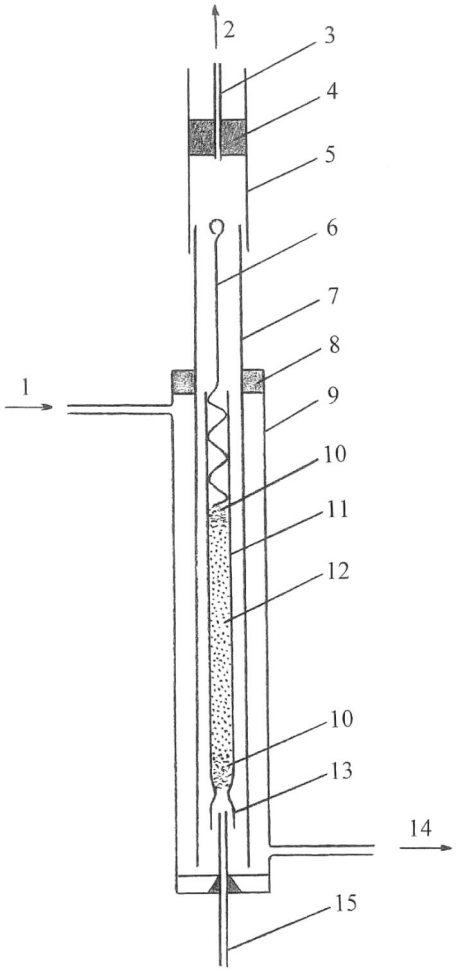

Fig. 1. Schematic diagram of the modified GC inlet: (1) carrier gas, (2) septum purge (3–5 ml/min), (3) capillary restrictor, (4) silicone septum, (5) silicone tubing, (6) wire eye (for manipulation), (7) glass liner, (8) silicone septum, (9) inlet body, (10) glass wool, (11) microcolumn, (12) Tenax TA (60–80 mesh), (13) gas tight connection, (14) to split valve (5–8 ml/min) and (15) capillary column.

Results and Discussion

One of the main advantages of the headspace technique is the elimination of interference from the biological matrix and very high boiling compounds. We chose an equilibrium temperature 23 ± 1°C for the analysis, because at higher temperatures vapor might condense in the microcolumn. The gaseous phase after equilibration is stable for up to 1 h. Through studying the dependence of desorption temperature on recoveries of analytes from the microcolumn we have found the optimum temperature of 230°C and the desorption time was optimized to 1 min. The applied carrier gas pressure during the desorption was optimized to 10 kPa. All these conditions are in agreement with the conditions applied in our previous works[13–16].

The main advantages of the thermal desorption in a GC inlet are a low dead volume of the microcolumn and there is no need for connection tubing as in the case of a thermal desorption unit. The distance between the adsorbent in the microcolumn and the head of the capillary column is only 10 mm, which results in symmetrical, well separated peaks of the compounds eluted, even at the beginning of the analysis run. The memory effect usually caused by the purge and trap method is also avoided. The static headspace method enables the maximum concentration of analytes to be obtained in the minimum volume of gaseous phase, while in dynamic methods the analytes are diluted with large amounts of carrier gas, resulting in the use of large amounts of adsorbent in the trap column. Due to the low dead volume, the microcolumn is practically a part of the capillary column, which means that there is no need for cryofocusation or use of subambient temperatures. The amount of adsorbent in commercially available thermal desorption units are usually in grams; in many cases it is preferable to use only miligram amounts of adsorbent. During the analysis run, the microcolumn remains in the GC inlet, so there is no need of a unit for conditioning the adsorbent. The microcolumn makes it possible to analyse hundreds of samples without any distortion. It is possible to reverse the modified GC inlet to its initial split/splitless status in a few minutes. This may be useful for laboratories that lack appropriate apparatus.

The main problem during the elaboration of the method using a non-selective FID detector was to find a suitable capillary column for the separation of urine compounds from the peaks of analytes. The best separations among the tested columns were achieved on the HP-5 column. Another problem was to obtain analytes-free model urine samples for quantitative evaluation of the method. The detectable analytes in unexposed urine samples were obviously benzene and traces of aromates. Therefore we preferred the standard addition method for calibration. The added concentrations of each analyte in urine model mixtures were 2,000, 1,500, 1,000, 500, 250 and 125 ng/*l*. Each and every mixture was analyzed three times while the mixture of 500 ng/*l* was analyzed five times.

A chromatogram of an unexposed urine sample is shown in Fig. 2A and a chromatogram of urine spiked with 500 ng/*l* of each analyte is shown in Fig. 2B. The performance characteristics of the method are presented in Table 1. Calibration curves of the area versus the concentration were plotted in the range from 125 to 2,000 ng/*l* (n=6). Good linearity was achieved for all the compounds studied, and correlation coefficients (r^2) were 0.9969–0.9995. The repeatability of the method was investigated by analysing the analytes at the concentration of 500 ng/*l* (n=5). The obtained relative standard

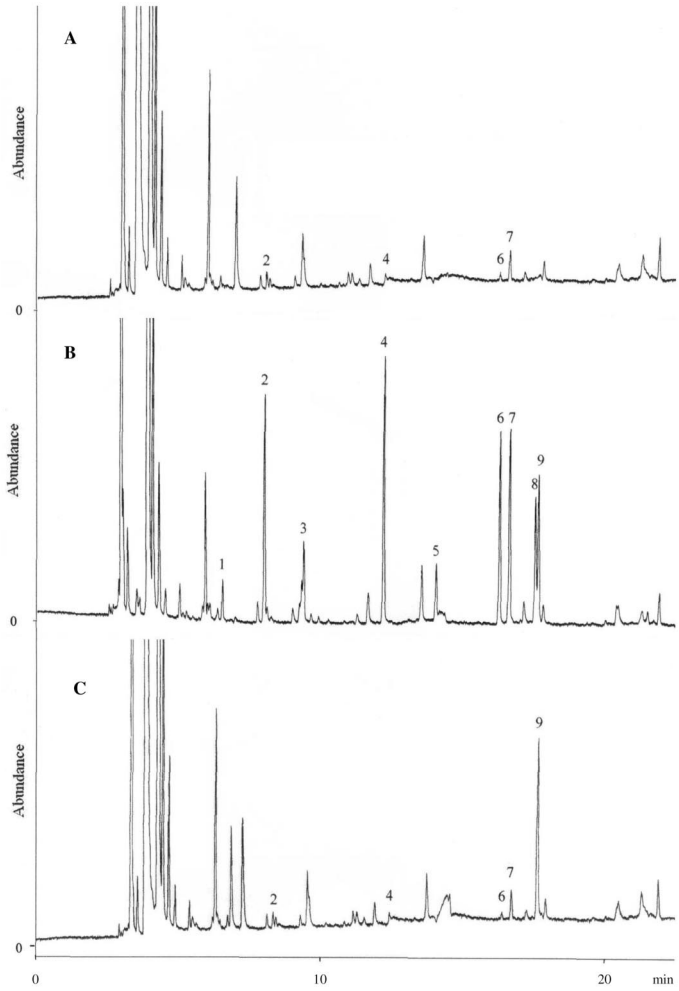

Fig. 2. Chromatogram of: (A) unexposed urine sample, (B) unexposed urine sample spiked with 500 ng/*l* of each compound, (C) exposed urine sample (562 ng/*l* of styrene). Peaks: (1) chloroform, (2) benzene, (3) trichloroethylene, (4) toluene, (5) tetrachloroethylene, (6) ethylbenzene, (7) *p*-xylene, (8) *o*-xylene, (9) styrene.

Table 1. Linearity, limits of detection (LOD), limits of quantification (LOQ) and repeatability of analyses for investigated analytes in spiked unexposed urine samples

Compound	Slope	Intercept	r^2	LOD [ng/*l*]	LOQ [ng/*l*]	s (n=5)	s_r [%]
						500 ng/*l*	
Chloroform	0.076	1.15	0.9969	59	394	3.0	7.3
Benzene	0.770	–9.73	0.9991	32	234	13.7	3.6
Trichloroethylene	0.278	–1.04	0.9973	55	387	6.5	4.6
Toluene	0.904	–0.83	0.9995	25	171	8.1	1.8
Tetrachloroethylene	0.189	–0.56	0.9980	48	334	2.1	2.1
Ethylbenzene	0.718	–4.92	0.9982	45	318	11.4	3.2
p-Xylene	0.664	8.75	0.9991	32	211	9.8	2.8
o-Xylene	0.520	–12.47	0.9992	30	230	8.9	3.7
Styrene	0.565	–1.69	0.9993	28	197	12.6	4.5

r^2=correlation coefficient, LOD=limit of detection, LOQ=limit of quantification, s=standard deviation, s_r=relative standard deviation

deviations (s_r) in the range of 1.8–7.3 indicate good repeatability of the method. The limits of detection (LOD) and quantification (LOQ) calculated by Adstat Calibration Program (TriloByte, Czech Republic) show good sensitivities for BTEX and styrene, when a non-selective FID is used, however, better sensitivities for chlorinated compounds can be obtained using ECD or MS detectors.

The chromatogram in Fig. 2C shows an example of an exposed urine sample analysis. The sample of urine was obtained from a worker who was laminating plastics and was exposed to atmospheric styrene. In this sample 562 ng/*l* of styrene was determined.

The presented results demonstrate the possibility of usage of the HS-SPMCE method in urine analysis. The proposed method is simple, inexpensive and enables reproducible results of analyses of unmetabolized VOCs in urine samples.

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