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SIMPLE METHOD FOR ANALYSIS OF UNMETABOLIZED BTEX IN URINE SAMPLES

Ján Hrivňák[1] and Eva Kráľovičová[2*]

*[1]Expertise & Analytical Services, Astrová 46, 821 01 Bratislava, Slovak Republic, [2]Regional Authority of Public Health Service, Bratislava, Slovak Republic, Ružinovká 8, 820 09 Bratislava, Slovak Republic, * corresponding author: e-mail: ba.kralovicova@uvzsr.sk*

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Abstract

A rapid and simple method for quantitative analysis of benzene, toluene, ethylbenzene and (o-, m-, p-) xylenes (BTEX) in urine using headspace solid-phase microcolumn extraction (HS-SPMCE) and gas chromatography with flame ionization detection (GC-FID) is described. Only 2 ml of sample are needed for analysis. The sample treatment is carried out in a 20 ml all glass syringe.
The method provides a good repeatability (relative standard deviations (s_r) ranging 3.1 – 5.2%) and repeatability (correlation coefficient (r^2) ranging 0.9981 – 0.9993). The limits of detection (LOD) and quantification (LOQ) were 60 – 71 ng/l and 385 – 412 ng/l, respectively.

Key words: BTEX; solid-phase microcolumn extraction; SPMCE; urine

1. INTRODUCTION

Benzene is one of the toxic air pollutants released from automobile fuels, exhaust gases, tobacco smoke etc. Since benzene, toluene, ethylbenzene and (o-, m-, p-) xylenes (BTEX) are used widely in organic synthesis as solvents and materials and are contained in petrochemicals, including automobile gasoline, fuel oil and solvents, they are ubiquitous environmental contaminants. Among them benzene is a carcinogenic substance classified into Group 1 carcinogen (carcinogenic to humans) [1].

Unmetabolized urinary benzene is a promising biomarker because it is specific and reflects air exposure even at low levels [2]. Generally, benzene and BTEX analysis has involved either static [3] or dynamic [4] headspace sampling, followed by gas chromatographic separation and flame ionization detector (GC-FID) [5].

In this study a simple static headspace technique for analysis of BTEX in urine samples was developed using an all glass syringe for the sample treatment. Solid-phase microcolumn extraction (SPMCE) for collection of analytes and thermal desorption in the modified GC inlet were used [5-10].

2. EXPERIMENTAL

2.1 Chemicals and materials

Benzene and toluene were obtained from Aldrich (Milwaukee, WI, USA), ethylbenzene from Merck (Schuchardt, Germany), p-xylene and o-xylene from Supelco (Bellefonte, PA, USA) and Tenax TA (60-80 mesh) from Alltech (Deerfield, IL, USA). HPLC grade methanol and anhydrous sodium sulphate (Na_2SO_4, p.a.) were obtained from Merck (Darmstadt, Germany). The all-glass, gas-tight syringe of 20 ml volume was obtained from Poulten & Graf (Wertheim, Germany) and the microcolumn packed with 15 mg of 60 – 80 mesh Tenax TA from Alltech (Deerfield, IL, USA).

2.2 Instrumentation and chromatographic conditions

Analyses were carried out on a GC 8000 Top Series, CE Instruments (Rodano-Milano, Italy) provided with a computer program (Shimadzu, Class-VP.2, SP1) for data acquisition. The chromatograph was equipped with a flame ionization detector 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 oven temperature program was as follows: initial isothermal temperature 30°C (1 min), then increased from 30 to 180°C at a rate of 5°C/min, and then ramped to 230°C (5 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.

3. RESULTS AND DISCUSSION

The static headspace method enables the maximum concentration of analytes to be obtained in the minimum volume of gaseous phase in a relatively short time, 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. In order to be able to analyse a small volume of urine samples, we decided to use a syringe for the sample treatment. The sample of 2 ml volume containing 0,4 g of Na_2SO_4 was placed in a 20 ml all glass syringe, the syringe with the piston at a position of 20 ml mark was closed and vigorously shaken for 20 seconds at 23±1°C. After 4 – 5 min to allow the analytes to come to equilibrium the droplets from the neck were removed by a thin strip of filter paper, the microcolumn connected to the syringe, and the headspace of 15.0 ml carefully aspirated at a rate of 10 ml/min. The loaded microcolumn was transferred to a modified GC inlet 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. Modification of the inlet is described in our previous study [5].

One of the main advantages of the headspace technique is the elimination of interferences from the biological matrix and very high boiling compounds. We chose the equilibrium temperature 23±1°C for the analysis because at higher temperatures vapour might condense in the microcolumn. All analytical conditions in this study are in agreement with the conditions applied in our previous publications [5-10]. Among the tested columns, the best separations of analytes from compounds inherently present in urine were achieved on the HP-5 column.

Rather serious problem was to obtain analytes-free model urine samples for quantitative analysis. In "unexposed" urine samples just BTEX compounds were present at detectable levels. Given that practically at the same concentration of Na_2SO_4 there are no differences in the response between the water and urine model samples [3], we have used water instead of urine for preparation calibration mixtures. Five calibration mixtures of concentration 2000, 1000, 500, 250 and 125 ng/l of each analyte were used. Each and every mixture was analysed three times. Good linearity was achieved for all the compounds studied. Correlation coefficients (r^2) were in the range of 0.9981 - 0.9993. The repeatability of the method was determined on the model mixture 500 ng/l (n=5). The obtained relative standard deviations (s_r) in range of 3.1 – 5.2 % indicate a good repeatability of the method. The limits of detection (LOD) and quantification (LOQ) were 60 - 71 ng/l and 385 - 412 ng/l, respectively.

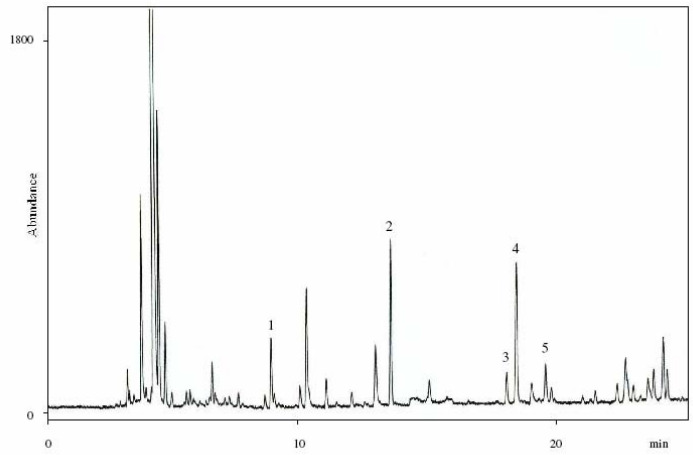

Figure 1. Chromatogram of exposed urine sample. Peaks: 1: benzene (1.07 µg/l), 2: toluene (2.24 µg/l), 3: ethylbenzene (0.49 µg/l), 4: m+p-xylene (1.68 µg/l), 5: o-xylene (0.61µg/l).

The chromatogram in Fig.1 shows an example of analysis of an "exposed" urine sample. The sample of urine was obtained from a man who was an auto-mechanic exposed to atmospheric BTEX.

The presented results demonstrate a possible usage of the HS-SPMCE method in urine analysis. The proposed method is simple, inexpensive and gives reproducible assays of unmetabolized BTEX in urine samples of small volume.

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