Petroleum & Coal
ISSN 1337-7027

Available online at www,vurup,sk/pc
Petroleum & Coal 50 (3), 10-12, (2008)

ANALYSIS OF BENZENE IN EXHALED BREATH BY SOLID-PHASE MICROCOLUMN EXTRACTION

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Received May 14, 2008, accepted September 5, 2008

Abstract
Exhaled breath was aspirated from a distance of 3-5 cm through a thermostated (40 ±1 °C) microcolumn packed with Tenax TA using a gas-tight syringe. The loaded microcolumn was put into a modified inlet of gas chromatograph and desorbed at 230 °C. Calibration curve of benzene was in the range of 0.1-10 ng per microcolumn. Correlation coefficients (r^2) and reproducibility (s_r) were 0.9994 and 2-4 %, respectively. Using the sample volume of 100 ml and flame ionization detector the detection limit for benzene was 0.3μg/m^3.

***Key words**:* benzene; solid phase microcolumn extraction; breath analysis

1. INTRODUCTION

Benzene is one of the world's major commodity chemicals and one from the main air pollutants. Benzene has long been recognized as a carcinogen and recent concern has centred on the effects of continuous exposure to low concentrations of benzene both occupationally and environmentally.

The major sources of benzene exposure are tobacco smoke, automobile service stations, exhaust from motor vehicles, industrial emissions, vapours from products that contain benzene, such as glues, paints, furniture wax and detergents [1]. The analysis of exhaled air has several advantages, since it is non-invasive method applicable to a large number of toxic substances and can be used as a biomarker in biological monitoring of occupational and environmental exposure to chemical agents[2]. It presents the necessity of a sensitive sampling procedure, since the exhaled compounds are at extremely low concentrations, i.e. in the nanomolar range. To improve the sensitivity of the determination of these substances the sample usually has to be concentrated before assay by gas chromatography. The most commonly utilised methods are based on the usage of metallic canisters or plastic bags followed by solid-phase extraction (SPE) on various adsorbents or solid-phase microextraction (SPME) on coated fused silica fibres [3].

In this work for the analysis of benzene in exhaled breath we have used solid-phase microcolumn extraction (SPMCE) followed by thermal desorption of loaded microcolumn in the inlet of gas chromatograph [4-8].

2. EXPERIMENTAL

2.1 Instrumentation and chromatographic conditions

Analyses were carried out on GC 8000 Top Series, CE Instruments (Rodano-Milano, Italy) and the computer program (Shimadzu, Clas-VP, SP1) was used for data acquisition. The chromatograph was equipped with flame ionization detector and fused silica HP-VOC/MS capillary column with 60 m length × 0.32 mm I.D. and 1.8 μm film thickness (Hewlett-Packard, Palo Alto, CA, USA). The chromatographic elution was temperature programmed as follows: isothermal at 30°C for 1 min, then at a rate of 5°C/min to 230°C for 10 min (to remove the high boiling compounds from the column). The temperature of the inlet chamber was 230°C and as a carrier gas helium was used.

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2.2 Sampling procedure

Exhaled breath from the distance of 3-5 cm from the inlet of the microcolumn, filled with 100 mg of 60-80 mesh Tenax TA (Alltech, Deerfield, IL, USA) and thermostated at 40±1°C, was aspirated by means of 30-ml all-glass syringe (Poulten & Graf, Wertheim, Germany) connected to the microcolumn and used to measure the sample volume. After sampling, the microcolumn was put into the modified injection port of gas chromatograph [4-6]. During the desorption (1 min), pressure of the carrier gas was maintained at 10 kPa and then set to 60 kPa up to the end of the chromatographic run.

3. RESULTS AND DISCUSION

Because the temperature of the microcolumn utilized is higher as the ambient temperature (to prevent the condensation of water), the first steps of our work led us to determine the breakthrough volume of the microcolumn at 40±1°C. In order to determine the breakthrough volume for benzene, the microcolumn was always initially loaded with 1 ml of gaseous sample containing 100 ng of benzene and then various volumes of air (30-300 ml) were aspirated through it. From Fig. 1, it can be seen that the maximum sample breath volume before breakthrough is about 100-120 ml. (Breakthrough volume of the same microcolumn at ambient temperature of 20°C was 200 ml [5]).

Calibration and other performance characteristics of the method were obtained by direct injection of benzene solution in n-pentane (0,5 µl) on the Tenax filling of microcolumn using 1 µl syringe. Amounts of benzene for calibration were in the range of 0.1-10 ng per microcolumn. Good linearity was achieved and the obtained correlation coefficient (r^2) was 0.9994. The repeatability of the method was investigated by triplicate analysis of microcolumn loaded with selected amounts of benzene. The obtained relative standard deviations (s_r) in the range of 2-4 % were very satisfactory. The gained results are in agreement with our previous work [5].

As an example, the results from the application of the method are shown in Fig. 2. In Fig. 2A is a peak of 0.05 ng of benzene, which indicates the method detection limit of 0.3 µg/m^3 (using the sample volume of 100 ml). In Fig. 2B is a chromatogram of exhaled breath sample from worker of chemical laboratory. The sample volume of 100 ml was collected as is described in the sampling procedure.

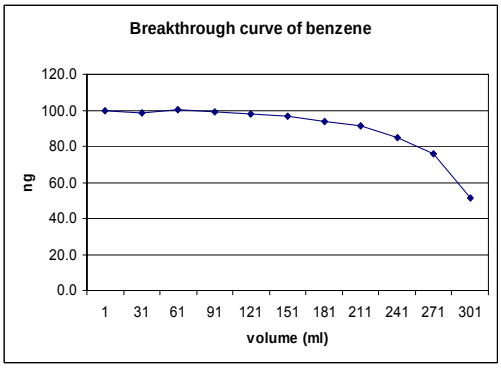

Figure 1. Breakthrough curve of benzene

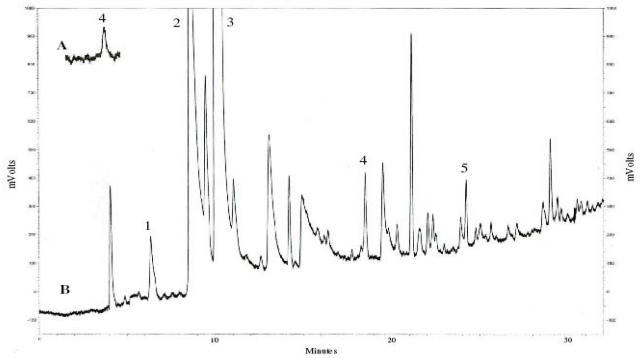

Figure 2. **A:** Peak of benzene at 0.05 ng/microcolumn, **B:** Chromatogram of exhaled breath sample (100 ml), peaks: 1:methanol, 2:ethanol, 3:acetone, 4:benzene (4,7 µg/m^3), 5:toluene.

Sampling of exhaled breath is a crucial issue in the analysis. When using canisters or bags for sample collection, condensation of water vapour on their inner surface may cause problems in the analysis of organic compounds, such is benzene. Except of this, some problems may be caused by possible contamination of sample with compounds coming from various materials, which are in contact with breath, such are mouth piece, face mask, tubing and stop valve. In chromatograms peaks of these compounds may interfere with the peaks of compounds from breath (in breath up to date more than 1000 compounds were identified).

The contactless sampling using the microcolumn thermostated at 40°C can be the solution of the problems concerning the condensation of water vapour and secondary contamination. It was shown that breath sampling is feasible without discomfort or adverse effects. The sampling procedure can be easily automated using CO_2 trigger[9].

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