Short Communication

DETERMINATION OF BENZENE IN URINE BY STATIC HEADSPACE GAS CHROMATOGRAPHY

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This paper describes the application of static headspace gas chromatography in determining benzene in urine. The method was analytically validated for sensitivity (DL=42 ng/l), repeatability (RSD=3% and 4%), and accuracy (71%), and was applied in measuring urine benzene in nonsmokers (N=14) and smokers (N=18). All urine samples had measurable benzene concentrations. The method proved sensitive enough to establish a significant statistical difference (P<0.000614) in urine benzene concentrations between smokers (mean=760; range =181–1869 ng/l) and nonsmokers (mean=214; range=61–515 ng/l).

KEY WORDS: *analytical validation, nonsmokers, smokers*

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 (IARC) (1). General population is more or less permanently exposed to this widespread pollutant (2). Nonsmokers are largely exposed to benzene from car exhausts or petrol vapour. The main source of benzene exposure for smokers is the cigarette smoke. Although the health risk of environmental benzene exposure seems low, the significance of a lifetime absorption of low levels of benzene, including the potential effects of interaction with other substances *in vivo*, is unknown. Individual exposure to benzene can be evaluated by the measurement of benzene in biological samples, that is, by biomonitoring. Benzene is usually analysed in blood or exhaled breath (3). The quantitation of benzene concentration in urine has recently become possible thanks to the development of new analytical techniques with high sensitivity (4). These are required because less than 0.1% of the total uptake of benzene is excreted unmetabolised in urine (2). Urinary benzene is a promising biomarker because it is specific and reflects air exposure even at low levels (5). Generally, benzene analysis has involved either static (6, 7) or dynamic (8, 9) headspace sampling, followed by gas chromatographic separation and photoionisation (6, 7) or flame ionisation detection (7, 9). Headspace sampling is an indirect method for determining volatile analytes in a liquid or solid sample in the vapour phase. If the vapour phase has been equilibrated with the sample, this technique is referred to as static. If the vapour phase has been purged from the sample (and there is no equilibrium) and subsequently adsorbed on a cryogenic trap or microfibre and thermally desorbed, this technique is called dynamic.

This paper describes static headspace gas chromatography applied to determine urine benzene concentrations in nonsmokers and smokers.

MATERIALS, METHOD, AND SUBJECTS

Materials

Benzene, methanol and *n*–hexane were analytical–grade–purity products of Merck (Darmstadt, Germany). The standard reference material for benzene MISA group 17 non–halogenated mix (Cat. no. 4–8133),

containing 2000 μg/ml of benzene in methanol, was purchased from Supelco (Bellefonte, PA, USA). Six millilitre headspace vials with aluminium crimp caps with liners, hand crimper, and decapper were obtained from Varian (Harbor City, USA).

Standard preparation

The benzene stock solution was prepared by diluting 3 μl (2.64 mg) of benzene to a volume of 10 ml of methanol. Forty μl of the stock solution was further diluted with methanol to 2 ml and labelled as standard A. Working standards were prepared by spiking known amounts of the standard A into deionised, boiled, and cooled water. Hundred μl of working standards were added to 1.9 ml of blank urine. Since almost every urine sample has a measurable benzene concentration, blank urine was prepared by purging it with nitrogen for 30 min at 40 °C. The final concentrations of benzene in urine ranged from 148 to 3549 ng/l. The stock solution and the standard A were kept at –20 °C, and working standards were freshly prepared every day.

Gas chromatographic conditions

To measure benzene, we used a Varian 3800 gas chromatograph with photoionisation detector (PID) and a 30 m, 0.53 mm I.D., 3.0 μm film thickness VOCOL (6% cyanopropylphenyl 94% dimethyl polysiloxane) capillary column. Helium was the carrier gas (139.5 kPa) with the flow of 40.3 ml/min, and hydrogen was the sweep gas with the flow of 30 ml/min. The initial oven temperature was 50 °C for 2.5 min, then it gradually increased 10 °C/min until it reached and remained 180 °C for four min. The injector and detector temperatures were 120 °C and 180 °C, respectively.

Subjects

Benzene was determined in 32 urine samples from healthy adults without any known occupational benzene exposure. The subjects were smokers (N=18) and nonsmokers (N=14).

Urine sampling

The first urine samples were collected in the morning in clean 80–ml bottles, completely filled to avoid loss to the headspace. The bottles were kept in a refrigerator (4 °C). As a rule, the urine samples were analysed the same day when collected. The measurements included the creatinine content in all samples.

Urine analysis

Two ml of urine were pipetted into a 6–ml headspace vial and immediately sealed with an aluminium crimp cap. The vials were gently stirred and incubated at 60 °C for 60 min in a thermostat. After achieving partial pressure equilibration, 0.5 ml of the headspace air was injected into the gas chromatograph.

RESULTS AND DISCUSSION

One of the main advantages of the headspace technique is the elimination of interference from the biological matrix. Static headspace analysis is based on an equilibrium between two phases, the liquid (or solid) phase – that is, the sample – and the gas phase above it. After equilibration, the gas phase (vapour), which is richer in volatile compounds than the liquid, is analysed by gas chromatography and the initial liquid phase concentration is determined by the measurement of the equilibrated vapour phase concentration. Therefore, it is evident that the incubation temperature and the equilibration time are very important parameters in static headspace analysis. Figure 1 shows the benzene peak area at the incubation temperatures of 60, 70, and 80 °C. As the incubation temperature increased, so did the peak area. The reason is that the partition coefficient decreases with the increased temperature, which means that the sensitivity of headspace gas chromatography is better at elevated temperatures (10). Although the maximal response was at 80 °C (Figure 1, equation 3) the intercepts of the calibration curves at 70 and 80 °C were too high, which could yield wrong results at low benzene concentrations. Furthermore, at higher incubation temperatures, vapour may condensate before being injected onto the column. This is why we chose the incubation temperature of 60 °C for the analyses. Headspace benzene reached equilibrium at 60 °C in 60 min, and it was the incubation time we used in the analysis.

The calibration curve was linear over the concentration range of 148–3549 ng/l with a correlation coefficient of 0.9993. It was not easy to obtain linear response over the concentration range. Linearity was achieved only at helium pressure of 139.5 kPa (20 psi), and any decrease (125.6, 111.6, and 83.7 kPa) did not yield satisfactory results. The flow of helium was as high as 40.3 ml/min, although the recommended flow for 0.53 mm I.D. column was 2.7ml/min. The likely reason for this difference is that

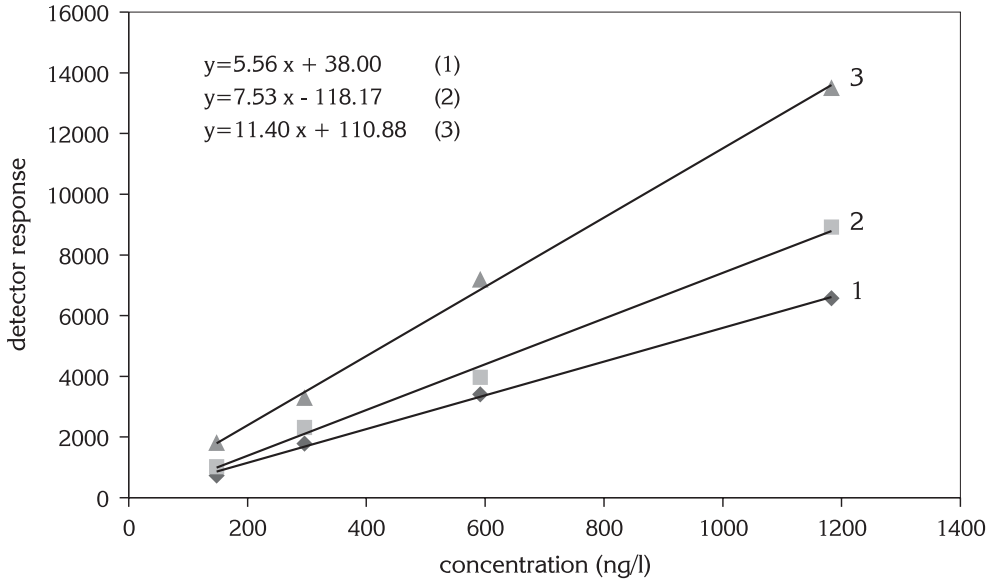

Figure 1 *Effect of incubation temperature on the chromatographic peak areas and regression equations at 60 °C (1), 70 °C (2), and 80 °C (3)*

we used vapour injection, and the recommended flow refers to liquid analysis.

Relative standard deviations (RSD) from benzene urine concentrations of 147.9 ng/l (N=9) and 1183.1 ng/l (N=5) were 2.78% and 3.63%, respectively. The detection limit (DL) for benzene was calculated according to the formula (11): DL=(3 SE_b+b)/m, where SE_b is the standard error of the intercept, b is the intercept and m is slope of calibration curve $y=mx+b$. The calculated DL was 42 ng/l. There are but a few papers published on benzene determination in urine, and they report a different detection limits. These differences depend on the technique and detector used. Generally, the dynamic headspace technique (purge and trap) increases the fraction of volatile analytes in the specimen that is injected in gas chromatograph decreasing, in turn, the detection limit. The detection limit of static headspace and PID achieved by *Kok and Ong* (12) was 40 ng/l. The detection limit achieved by *Ghittori and co–workers* (13), who used dynamic headspace and FID 50 ng/l, and by *Ljungkvist and co–workers* (4), who used dynamic headspace and mass selective detector, was 7 ng/l.

The trueness of the method was obtained by analysing the urine samples spiked with the standard reference material for benzene. The recovery of the five samples with the benzene concentration of 609.5 ng/l was 71%. The average recoveries achieved for dynamic headspace by *Ghittori and co–workers* (13) were in the range of 80–84%, whereas *Hung and co–workers* (14) obtained a 64% recovery.

Benzene was measured in urine of two groups of subjects: nonsmokers and smokers (Table 1). It is known that benzene in human urine is not the result of normal physiological metabolism, and the finding of measurable concentrations of benzene in urine reflects exposure to this compound. The mean benzene urine concentration was 214 (range 61–515 ng/l) in nonsmokers and 760 ng/l (range 181–1869 ng/l) in smokers. The difference between the two groups was statistical significant (t=3.826; P<0.000614). Figure 2 shows gas chromatograms of the urine samples.

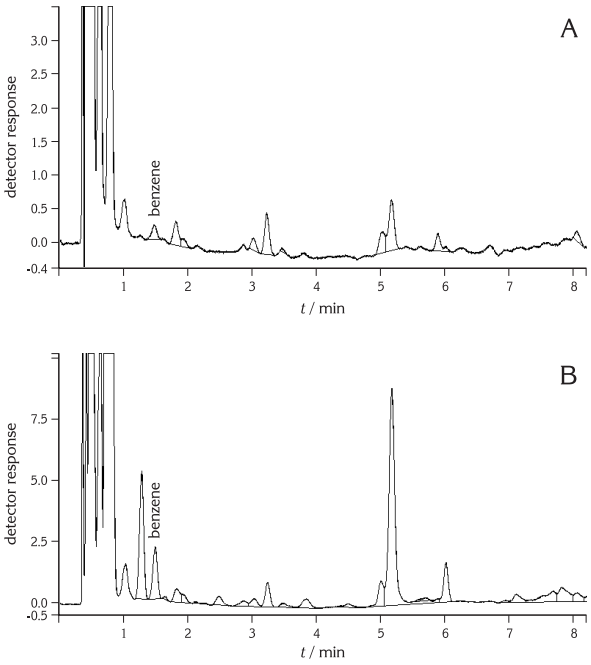

Figure 2 *Chromatograms of A) urine sample from a nonsmoker with 65 ng/l of benzene, and B) urine sample from a smoker with 760 ng/l of benzene*

Table 1 *Benzene urine levels in nonsmokers (N=14) and smokers (N=18)*

	Benzene mass concentration (ng/l)		Benzene mass fraction (ng/g creatinine)	
	smokers	nonsmokers	smokers	nonsmokers
Mean±SD	760±515	214±153	558±451	151±122
Median	607	163	433	108
Minimum	181	61	112	46
Maximum	1869	515	1712	379

Table 2 *Literature data on benzene urine levels in nonsmokers and smokers*

	Benzene mass concentration (ng/l)				
	Mean±SD	Median	Minimum	Maximum	Reference
Nonsmokers (N=25)	116±73	113	50	345	(12)
Nonsmokers (N=20)	139±42				(13)
Nonsmokers (N=11)	183		139	229	(14)
Nonsmokers (N=23)	116±172	74	27	878	(15)
Smokers (N=50)	405±270	348	111	1451	(12)
Smokers (N=20)	942±550				(13)
Smokers (N=12)	444		194	973	(14)
Smokers (N=18)	191±486	56	27	2070	(15)

Available literature data on benzene concentrations in urine are shown in Table 2. Benzene urine concentrations show a wide range in both nonsmokers and smokers. Our results for nonsmokers are generally higher than the corresponding results in other studies, whereas our results for smokers are comparable to *Ghittori and co–workers* (13). The smokers in our study smoked more than 25 cigarettes a day, in the study of *Ghittori and co–workers* (13) more than 20, and in *Kok's and Ong's* (12) study 90% of the smokers smoked less than 20 cigarettes.

We analysed creatinine as a correction for urine dilution. However, the equilibration of partial pressures in urine and plasma suggests that benzene is eliminated from the kidney by diffusion (16). As a result, the urine/blood concentration ratio equals the urine/blood distribution coefficient, and the concentration of benzene in urine is independent of urine output. Similar benzene concentration ranges in smokers and nonsmokers, expressed as ng/l and ng/g creatinine in Table 1, show that there is no need for creatinine correction of benzene in urine, which is consistent with findings published elsewhere (13–16).

Very low benzene concentrations in urine require special attention in all steps of specimen handling. Namely, benzene is volatile and can easily disappear. Furthermore, as it is ubiquitous, it may contaminate the sample.

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Sažetak

ODREĐIVANJE BENZENA U URINU PLINSKOKROMATOGRAFSKOM TEHNIKOM ANALIZE PARA IZNAD OTOPINE

Opisana je metoda za određivanje benzena u urinu plinskokromatografskom tehnikom analize para iznad otopine ("headspace"). Metoda je analitički vrednovana s obzirom na osjetljivost (granica detekcije = 42 ng/l), ponovljivost (RSD = 3 i 4%) i točnost (71%). Primijenjena je u analizi benzena u urinu nepušača (N=14) i pušača (N=18). Benzen je bio mjerljiv u svim uzorcima urina. Dobivena je statistički značajna razlika ($p<0.000614$) između koncentracija benzena u urinu pušača (x=760; raspon=181–1869 ng/l) i nepušača (x=214; raspon=61–515 ng/l). Benzen ne dolazi u urin normalnim fiziološkim procesima; u nepušača uglavnom potječe iz ispušnih plinova automobila i izgaranja benzina, a u pušača je glavni izvor izloženosti benzenu dim cigarete.

KLJUČNE RIJEČI: *analitičko vrednovanje, nepušači, pušači*

REQUESTS FOR REPRINTS:

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