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Short communication

Simultaneous determination of benzene, toluene, ethylbenzene, and xylenes in urine by thermal desorption–gas chromatography

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Abstract

The determination of metabolites of benzene, toluene, ethylbenzene, and xylenes in urine has been used to assess human exposure to these compounds. The analyses of urine samples for these metabolites are tedious and time consuming. The determination of unmetabolized individual compounds in urine has been studied previously with some success. A simultaneous determination of several unmetabolized VOC compounds in urine by thermal desorption–gas chromatography was conducted to assess the exposure of smokers and nonsmokers to these compounds. The method of thermal desorption–GC was sensitive enough to detect a significant difference in exposure levels due to the contribution of light smoking in the environmentally-exposed group. © 1998 Elsevier Science B.V.

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1. Introduction

Benzene, toluene, ethylbenzene, and xylenes are compounds found in solvents used in various industries. These compounds are also major components of gasoline used in motor vehicles. In addition to the fugitive emission of these compounds from the use of gasoline, they are reported to be the major compounds emitted from motor vehicle exhaust. Due to the tremendous growth of the number of motor vehicles in use in metropolitan areas of Taiwan, pollution of ambient air due to these compounds and exposure of motorists to these compounds have been the subjects of intense study in recent years [1,2].

To assess the impact of human exposure to these compounds, the concentration of their metabolites in urine must be determined [3,4]. Unfortunately, the analysis of urine for these metabolites can be very tedious and time consuming. Furthermore, in most cases, only one metabolite can be determined per analytical run. Methods for the simultaneous determination of these metabolites in urine have been developed [5,6]. However, there is still the need to develop an alternative method to determine unmetabolized compounds directly [7,8]. Recently, there have been reports of the successful determination of single unmetabolized compounds instead of their respective metabolites in urine. For example, Ghittori et al. [9] reported using urinary excretion of unmetabolized benzene as an indicator of benzene exposure in an occupational environment. Kok and Ong [10] also reported the use of headspace GC with photoionization detection to study benzene levels in blood and urine, for low-level nonoccupational expo-

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sure. Periago et al. [11] reported the use of the purge-and-trap method for the determination of styrene in urine. These methods determined only one compound at a time, according to the descriptions reported in their articles.

In a recent study, thermal desorption–gas chromatography was used to determine VOC compounds in indoor air [12]. Major aromatic VOC compounds including benzene, toluene, ethylbenzene, and xylenes were determined simultaneously. This kind of application was also reported by Riedel et al. [13] in a study of benzene and alkyl benzenes in ambient and exhaled air using microwave desorption coupled with gas chromatography–mass spectroscopy. The same method certainly could be applied equally well in the determination of VOC compounds in urine. The use of this method could save a significant amount of time if these unmetabolized VOC compounds can be determined simultaneously for large-scale screenings in either environmental or occupational exposure studies. Thus, it was no surprise that a general article was reported by Dunemann and Hajimiragha [14] using purge-and-trap gas chromatography–mass spectroscopy to determine VOCs in body fluids and environmental samples. In this paper, the concentrations of benzene, toluene, ethylbenzene, and xylenes in the urine of smokers and nonsmokers were determined simultaneously using thermal desorption–gas chromatography. For the remainder of this article, benzene, toluene, ethylbenzene, and xylenes will be collectively called VOC compounds for convenience.

2. Experimental

Twenty three subjects including 12 smokers and 11 nonsmokers participated in this study. More subjects for both groups will be added in the future to extend the study population. These subjects were male graduate students of about 25 years old. They were about 165~175 cm tall. Their weights were around 60~70 kg. Their living quarters were in the campus and the living habits including physical work and sport activities were quite similar. They had no known history of occupational exposure to the compounds of interest. Smokers were subjects who smoked fewer than 10 cigarettes a day. Other details of smoking habits, such as the brand or the content of tar of the cigarette and the time of a cigarette smoked last before collection of urine sample, were not included this time. However, these information will be added next time to provide a more comprehensive understanding about the effect of smoking.

The timing of urine collection was studied on two subjects. Concentration of VOC compounds in urine was found to be variable during the day. A higher concentration was determined for the first urine after waking-up and the last urine before going to sleep. The highest concentration was about twice the lowest one. The first urine of the day, instead of the urine collected over a 24-h period, was therefore collected from these subjects to ensure a high enough concentration for detection by this method. The volume and specific gravity of the urine were determined as soon as the sample was received from the subject. Creatinine concentration in urine was also determined for each sample [15]. Each urine sample was later separated into aliquots of 20 ml for storage in the refrigerator ($-20^{\circ}C$). The urine samples were thawed to room temperature before the determination of VOC compounds.

An aqueous emulsion (30%) of antifoam A concentrate (A-5758) from Sigma Chemical (St. Louis, MO, USA) was added to the urine sample to suppress the formation of foam in the urine. The external standard solution, a VOC Mix No.2 (containing 13 compounds; benzene, toluene, ethylbenzene, *m*-xylene, styrene, bromobenzene, 1,3,5-trimethylbenzene, 1,2,4-trimethylbenzene, *p*-isopropyltoluene, *n*-butylbenzene, 1,2,4-trichlorobenzene, naphthalene and 1,3,5-trichlorobenzene) purchased from Supelco, was used for qualitative and quantitative analysis. Solid adsorbent Carbotrap C, mesh 20/40 (cat. No. 2-0309), from Supelco was packed into the thermal desorption tube to trap VOC compounds purged from urine. The thermal desorption tube, a stainless steel tube (L427-0123), 1/4× 3.5 in., (packed with 450 mg adsorbent), purchased from Perkin Elmer was used for the desorption unit.

A thermal dynamic stripper (TDS), model 1000, from Supelco was used to purge VOC compounds from urine to the solid adsorbent in the desorption tube. The amount of urine used for each purging was 20 ml. Pure nitrogen gas was used for purging VOC

compounds. The flow-rate of the purging gas was 50 ml/min. The duration of the purge was 10 min. The oven housing of the TDS was maintained at 50°C for the entire purging procedure. An automatic thermal desorption system, ATD-400 from Perkin Elmer and a gas chromatograph (GC) 5890 Series II, equipped with a FID detector, from Hewlett–Packard were used for the determination of VOC compounds. The GC–MS 5988A system from Hewlett–Packard was used for compound identification. The column used for GC was a DB-624 capillary column, 30 m×0.53 mm I.D., 3.0-μm film thickness, purchased from J&W Scientific. The same type of column with different dimensions, 30 m×0.32 mm I.D., 0.5-μm film thickness, was used for the GC–MS system.

The operating conditions for the thermal desorption system, gas chromatograph, and mass spectrometer were as follows. The temperature of the desorption chamber was kept at 350°C for a total of 10 min to desorb all VOC compounds. The flow-rate of desorption was 50 ml/min. The carrier gas for the analytical column was nitrogen at a flow-rate of 5 ml/min. The temperatures of the GC injector and FID detector were set at 200 and 250°C, respectively. The column oven was programmed with the initial temperature at 40°C for 10 min, then the temperature was increased 5°C/min to 200°C, then it was increased by 20°C/min until the temperature reached 240°C and was held for 10 min at 240°C. Compounds were identified using the external standard solutions, VOC Mix No.2. The HP 5988A GC–MS system was also used to further confirm these compounds. The GC–MS system was operated in the electron-impact mode at 70 eV. The column oven temperature was programmed to be at 40°C for 3 min, then to increase at 6°C/min until 160°C was reached, and at 20°C/min until 240°C was reached, then to be held for 5 min at 240°C. The MS ChemStation 59970 with a NIST library database (NBS49K.L.) was used for compound identification.

3. Results and discussion

In Fig. 1, typical chromatograms from the analysis of a standard solution and urine samples are shown with peaks numbered from 1 to 5 for benzene, toluene, ethylbenzene, *m*-xylene, and *o*-xylene, respectively. These compounds were very well-resolved. Their identity was confirmed by GC and GC–MS analysis of a standard solution of VOC Mix No. 2. The quantitative analysis of urine samples was carried out using the same standard solution. *p*-Xylene and *m*-xylene were eluted together under the experimental conditions used in this analysis. The concentrations of *p*- and *m*-xylene were therefore calculated together and given as the concentration of *m*-xylene. *o*-Xylene was eluted together with styrene under similar conditions. Concentrations of *m*-, *o*- and *p*-xylene were later calculated together as that of xylenes for convenience of discussion in the following sections.

The calibration was carried out by the analysis of urine samples spiked with 1, 5, 10, 20 and 40 ng/ml

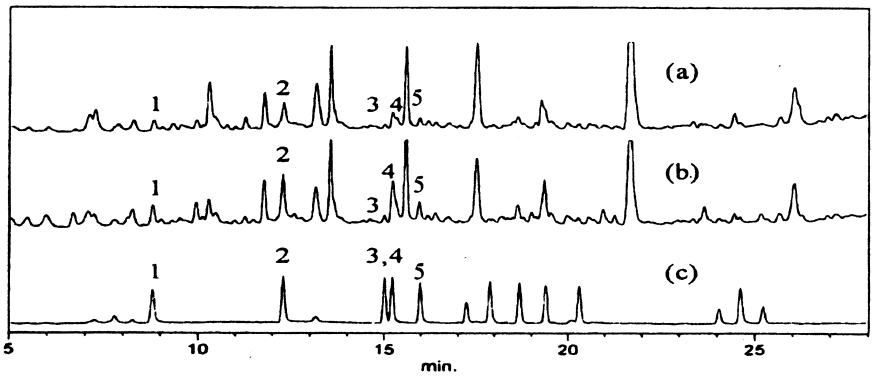

Fig. 1. Gas chromatogram of urine samples. (a) Nonsmoker; (b) smoker; (c) standard solution. Peaks: 1=benzene; 2=toluene; 3=ethylbenzene; 4=*m*-xylene; 5=*o*-xylene.

Table 1
Concentrations of VOC compounds in urine (ng/ml)

No.	Benzene		Toluene		Ethylbenzene		Xylenes	
	Smoker	Nonsmoker	Smoker	Nonsmoker	Smoker	Nonsmoker	Smoker	Nonsmoker
1	0.194	0.172	0.658	0.487	0.141	0.087	0.563	0.291
2	0.324	0.157	0.693	0.667	0.127	0.094	0.823	0.348
3	0.519	0.161	1.415	0.545	0.170	0.119	0.940	0.433
4	0.714	0.214	1.140	0.544	0.178	0.096	0.619	0.449
5	0.394	0.229	0.748	0.575	0.129	0.103	1.215	0.389
6	0.251	0.168	0.766	0.916	0.124	0.150	0.494	0.695
7	0.707	0.139	0.879	0.769	0.134	0.147	0.446	0.866
8	0.363	0.217	4.251	1.553	0.290	0.143	2.439	0.495
9	0.357	0.175	2.216	0.793	0.280	0.104	2.382	0.415
10	0.197	0.201	1.155	0.805	0.133	0.090	0.875	0.504
11	0.973	0.178	1.320	0.875	0.159	0.083	0.954	0.400
12	0.339	(n.a.)	0.995	(n.a.)	0.096	(n.a.)	0.495	(n.a.)

(n.a.): not available.

of standard solution. The correlation coefficient was better than 0.995 for all VOC compounds in the concentration range. The percentage recovery of these compounds, done by analyzing a 10 ng/ml spiked urine sample, was 64.2%, 58.6%, 90.3%, 83.4%, and 80.1% for benzene, toluene, ethylbenzene, *m*-xylene, and *o*-xylene, respectively. The average relative standard deviation of seven analyses of 10 ng/ml spiked urine sample was 0.1% for retention time and 7.3% for the integrated area of the compound peaks. The method detection limits for these compounds were 0.015, 0.008, 0.011, 0.050 and 0.02 ng/ml. The effects of prolonged storage of these urine samples were minimal. Most of the samples lasted for two weeks without substantial loss of VOC compounds. The thermal dynamic stripper unit used for purging VOC compounds from the urine sample provided an off-line sample treatment step which could prevent the occurrence of a flame-out phenomenon of the FID detector due to the large amount of water vapor purged from the urine samples.

Creatinine concentration was determined for each urine sample. Results showed that there was no need for creatinine correction of VOC compounds in urine. This is in agreement with the fact that VOC compounds are eliminated in the kidney by filtration. Therefore, concentrations of VOC compounds in urine reported in this study were without correction for creatinine. The specific gravity of urine was also determined for each sample. VOC concentrations corrected for the specific gravity in urine provided no further meaningful results.

Concentrations of benzene, toluene, ethylbenzene, and xylenes in urine of smokers and nonsmokers are given in Table 1. Mean, minimum and maximum concentrations of these compounds are also given in Table 2. Concentrations of *m*-, *o*- and *p*-xylene were

Table 2
Mean, minimum and maximum concentration (ng/ml) of VOC compounds in urine

	Benzene		Toluene		Ethylbenzene		Xylenes	
	Smoker	Nonsmoker	Smoker	Nonsmoker	Smoker	Nonsmoker	Smoker	Nonsmoker
Mean	0.444	0.183	1.353	0.775	0.163	0.111	1.020	0.480
Min.	0.194	0.139	0.658	0.487	0.096	0.083	0.446	0.291
Max.	0.973	0.229	4.251	1.553	0.290	0.150	2.439	0.866
P-value	0.0030		0.0805		0.0146		0.0214	

combined together as that of xylenes. Two groups of subjects were studied, one smoking group of 12 persons and one nonsmoking group of 11 persons. In the case of benzene exposure, we found that the concentration of benzene ranged from 0.194 to 0.973 ng/ml for smokers and from 0.139 to 0.229 ng/ml for nonsmokers. The mean concentration was 0.444 and 0.183 ng/ml for smokers and nonsmokers, respectively. The concentration of toluene in urine was found to range from 0.658 to 4.251 ng/ml for smokers and from 0.487 to 1.553 ng/ml for nonsmokers. The mean concentration for the two groups was 1.353 and 0.775 ng/ml. The concentration of ethylbenzene in urine was found to range from 0.096 to 0.290 ng/ml for smokers, and from 0.083 to 0.150 ng/ml for nonsmokers. The mean concentration was 0.163 and 0.111 ng/ml, for smokers and nonsmokers respectively. Lastly, the concentration of xylenes in urine was found to range from 0.446 to 2.439 ng/ml, and from 0.291 to 0.866 ng/ml, for smokers and nonsmokers. The mean concentration was 1.020 and 0.480 ng/ml, for the two groups respectively.

A student *t*-test was performed for these two sets of results from smokers and nonsmokers. The *P*-value determined for the two groups is given in Table 2. There was a significant difference ($P = 0.0030 < 0.05$) between benzene concentrations in urine for the smoking and nonsmoking groups. There was, however, no significant difference ($P = 0.0805 > 0.05$) between toluene concentrations in urine for smokers and nonsmokers. The *P*-value was found to be 0.0146 and 0.0214 ($P < 0.05$), respectively for ethylbenzene and xylenes in urine. The difference was therefore significant between concentrations of ethylbenzene and xylenes in the urine of smokers and nonsmokers.

Overall results from this study provided a mean urine concentration of 0.183, 0.775, 0.111, and 0.480 ng/ml of nonsmokers for benzene, toluene, ethylbenzene, and xylenes, respectively. These concentrations can be used as reference values for the environmental exposure to VOC compounds. Mean concentration of 0.444, 1.353, 0.163, and 1.020 ng/ml of these compounds in urine was determined for smokers. Cigarette smoke has been reported to contain benzene, toluene, ethylbenzene and xylenes [16]. VOC compounds in urine are about two times higher for smokers than for nonsmokers in a low-level environmental exposure situation. A student *t*-test for these results showed a significant difference between concentrations of benzene, ethylbenzene and xylenes in urine samples of smokers and nonsmokers. The difference, however, was not significant in the case of toluene exposure due to some effects from smoking, as reported in the literature [17]. Thus, the contribution of these compounds except for toluene, from cigarette smoke to their concentrations in the urine of smokers has been shown to be significant. The discriminative power of this method will be further demonstrated in the screening study of groups such as gas station attendants, policemen patrolling outdoors and other occupationally exposed subjects.

4. Conclusions

The concentrations of VOC compounds determined from this low-level environmental exposure study provided reference values of these compounds in urine. Light smoking (fewer than 10 cigarettes a day) has a significant contribution to the concentration of these compounds (except for toluene) in the urine of smokers. The determination of unmetabolized compounds in urine requires no creatinine correction, unlike in the determination of metabolites in urine. The analysis is therefore much simpler in the treatment of urine samples. This method can determine several compounds simultaneously instead of only one compound at a time. Also, the use of an automatic thermal desorption unit provided a faster urine sample analysis. The method used in this study was sensitive enough to discern the difference between VOC concentrations in the urine of smokers and nonsmokers. Thus, this method is easy, fast, sensitive and reliable. It is a good substitute for the metabolite determination method. Currently, this method is being applied to the study of occupationally-exposed groups.

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