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Determination of benzene and alkylated benzenes in ambient and exhaled air by microwave desorption coupled with gas chromatography–mass spectrometry

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Abstract

A sensitive and rapid method for the determination of benzene, toluene, ethylbenzene and the xylenes (BTX) in ambient and exhaled air is described. After sampling of 1–4 l of ambient or exhaled air on graphitized charcoal tubes, the analytes were thermally desorbed by a microwave device coupled to a gas chromatograph interfaced to a mass spectrometer (GC–MS). The detection limits for benzene, toluene, ethylbenzene *m*- + *p*-xylene and *o*-xylene were 1.6, 3.0, 0.2, 1.4 and 0.4 $\mu g/m^3$, respectively, when 1 l of air is sampled. In five smoking households a mean benzene concentration of 13.4 (range: 4.2–18.1) $\mu g/m^3$ was measured. In five homes without smokers, the average concentration of benzene was 8.8 (5.5–12.8) $\mu g/m^3$. The mean benzene concentrations in the exhalates were 50.2 (19.1–98.0) and 3.9 (0.5–8.8) $\mu g/m^3$ for ten smokers and ten non-smokers, respectively. The method was found to be suitable for the determination of environmental BTX exposure in field studies.

Keywords: Sample preparation; Air analysis; Environmental analysis; Desorption; Bezene; Alkylbenzenes

1. Introduction

Benzene, toluene, ethylbenzene and xylenes (BTX) are among the most important volatile aromatic compounds of commercial and environmental significance [1]. Benzene is classified as a human carcinogen [2] and is a risk factor for leukaemia and lymphomas [3,4]. Therefore, regulatory limits have been introduced for occupational exposure to benzene in most developed industrial countries. An upper limit concentration for workplaces of 8 mg/m^3 has been established in Germany [5]. Environmental concentrations of benzene in both indoor and outdoor air are at least 2–3 orders of magnitude lower than those in exposed workplaces. The most important source for environmental benzene is automobile traffic exhaust [6]. In contrast to BTX determinations at workplaces [7,8], analysis of BTX in non-occupational environments and human breath requires preconcentration prior to determination. A widely used technique is the collection of ambient or exhaled air on a porous polymer adsorbent, followed by thermal desorp-

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tion (with intermediate cryo-focusing) into a gas chromatograph (GC) with mass-selective or flame-ionization detection [9–11].

The aim of this work was to develop a sensitive and rapid method for the determination of environmental levels of BTX in ambient and exhaled air. The suitability of the method was tested by determination of BTX in indoor air of homes occupied by smokers and non-smokers as well as in exhaled air samples from smokers and non-smokers.

2. Experimental

2.1. Determination of BTX by microwave desorption GC–MS

A microwave desorption device (MW-1, Rektorik, Geneva, Switzerland) [12] was coupled via a heated interface to a GC–MS system (Hewlett-Packard, Model 5890 with MSD 5970). The heated interface was equipped with a splitter for use with a capillary column [13]. The voltage for the microwave desorber was stabilized with a magnetic constant voltage transformer (Transtec tor F100, APICAL/SA Elektronik, Schwerzenbach, Switzerland). Desorption conditions were as follows: Interface temperature, 140°C; backflush delay, 10 s; bypass delay, 6 s; desorption time, 7 s; desorption energy, 0 to 3 (arbitrary units). Analytes were separated on a 30 m × 0.25 mm I.D. J&W DB5.625, 1-μm film, fused-silica capillary column (Fisons, Loughborough, UK, Code: 122-5633) by using helium as a carrier gas with a head pressure of 1 bar and a split of 25 ml/min. The temperatures for the injection port and the transfer line were 220 and 280°C, respectively. The GC oven was held at 70°C for 2 min, after which the MS was switched on and the GC oven programmed to 220°C at a rate of 50°C/min. The oven temperature was held at 220°C for 3 min in order to purge the column of late-eluting materials. The mass spectrometer was operated in the selected-ion monitoring mode (SIM) (ion-range: 78–106) and was tuned by using perfluorotributylamine. The following ions (m/z) were selected for detection: Benzene: 78; toluene: 91, 92; ethylbenzene: 91, 106; *m*-, *o*-, *p*-xylene: 91, 92, 106.

2.2. Calibration

A certified gas standard (Messer-Griesheim, Duisburg, Germany) containing 0.24 vpm (83.6 $\mu g/m^3$) of benzene, 0.023 vpm (94.5 $\mu g/m^3$) of toluene, 0.017 vpm (80.5 $\mu g/m^3$) of ethylbenzene and 0.016 vpm (75.7 $\mu g/m^3$) of *p*-xylene at 0°C and 1013 mbar was used for calibration. The adsorption tubes (Analyt, Müllheim Germany; Code, 47754; diameter, 6 mm; length, 95 mm; containing approximately 70 mg of graphitized charcoal) were loaded with defined amounts of BTX by using the device shown in Fig. 1. The gas standard was conducted through a 2-l round-bottom flask with two necks, equipped with an insert for a gas washing bottle (flow-rate: approximately 0.3 l/min). After 30 min the pump P was switched on and the valve V was opened. The gas standard was drawn through the adsorption tube A at a flow-rate of approximately 0.1 l/min (controlled by flowmeter F1). Sampling times of 30 s to 10 min were applied, in order to reach concentrations of 5 to 100 ng/tube. The trapped compounds were desorbed by the microwave device and determined by GC–MS as described above. Completeness of the desorption was controlled by subsequent desorption runs combined with GC–MS analysis. To control for breakthrough of BTX during sampling, a second adsorption tube was connected as a back-up tube and 0.7 l (approximately 50 ng of each com-

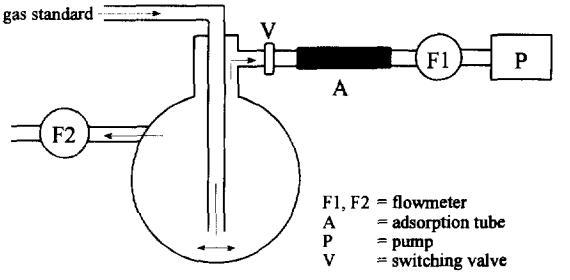

Fig. 1. Device for loading adsorption tubes with defined amounts of analytes for calibration. The standard gas contained benzene, toluene, ethylbenzene and *p*-xylene.

pound) of the gas standard was collected with a flow-rate of 0.3 l/min.

2.3. Preparation of the adsorption tubes

Before sampling, the charcoal-filled adsorption tubes were desorbed three times with the microwave desorber in the "purge" mode. The cleaned adsorption tubes were stored in sealed glass tubes at room temperature in Teflon or aluminium boxes coated with charcoal filter pads.

2.4. Sampling of ambient air

Indoor air samples were collected for 3 h in the living rooms of homes with and without smokers during the late afternoon or evening. The air was drawn through an adsorption tube by means of a micro-pump (NMP30 KNDC, KNF Neuberger, Schallstadt, Germany) at a flow-rate of approximately 20 ml/min. In total, 3 to 4 l of ambient air was collected. In order to avoid contamination, the adsorption tubes were stored as described above. Samples were analyzed within 72 h after collection.

2.5. Sampling of exhaled air

The apparatus for sampling of exhaled air is shown in Fig. 2. The device is a modification of that described by Raymer et al. [14]. The subjects (ten non-smokers, aged 4–65 years and ten smokers, aged 20–60 years) wore nose clips and breathed normally through the mouthpiece (M) for 2.5 min. The inhaled air was drawn through two charcoal filter cartridges (diameter, 70 mm; length, 120 mm) to remove ambient BTX and other volatile organic compounds (VOCs). The exhaled air was led into the Teflon tube T (diameter, 14 mm; length, 5 m). Thirty seconds after starting to breath through the mouthpiece, the sampling valve (V3) was opened and the two sampling pumps (P) (NMP50 KNDC, KNF Neuberger, Schallstadt, Germany) were switched on. The exhaled breath was drawn for 2 min through the two adsorption tubes (A) filled with graphitized charcoal at a flow-rate of 0.5 to 1 l/min. The sampling tubes were stored as describe above. In order to demonstrate that predominantly end-expiratory air was sampled, the concentrations of carbon dioxide (CO_2) and oxygen (O_2) in exhaled air at the sampling site (A) were measured by replacing the charcoal tubes (A) and the pumps (P) with a Multiwarn CO_2/O_2 monitor (Dräger, Lübeck), which was operated in the active sampling mode with a flow-rate of 2 l/min. After each sampling, the device was cleaned by sealing the mouthpiece (M) site and connecting a vacuum pump to the open end of the Teflon tube. In addition, the breathing valve (V1/V2) is heated by means of an infrared lamp (300 W) to remove humidity and volatile compounds. Air was pumped through the device for 5 min after each sampling and for several hours after each day of sampling. In order to check for background contamination of the sampling device, samples were taken during the described cleaning procedure. Under these conditions, benzene, ethylbenzene and *o*-xylene were below the detection limit, whereas 1–2 $\mu g/m^3$ of toluene and *m*-/*p*-xylene were occasionally found.

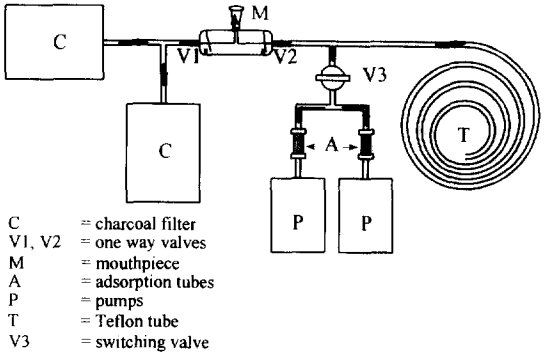

Fig. 2. Apparatus for sampling of exhaled air on two adsorption tubes (A) for subsequent analysis for benzene, toluene, ethylbenzene and xylenes (BTX).

3. Results and discussion

No break-through of benzene, toluene, ethylbenzene and *p*-xylene was observed when the adsorption tubes were loaded with about 50

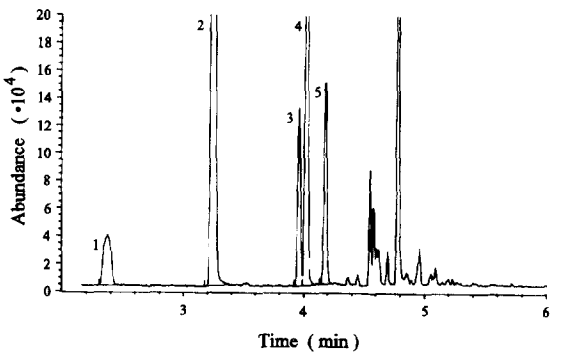

Fig. 3. Gas chromatogram of an ambient air sample. Peaks: 1 = benzene, 2 = toluene, 3 = ethylbenzene, 4 = *m*-/*p*-xylene, 5 = *o*-xylene.

ng of each of the compounds at a flow-rate of 0.3 l/min.

A linear relationship between peak area and concentration of benzene, toluene, ethylbenzene and *p*-xylene was obtained over the range of 2.5 to 100 ng/tube. The coefficients of correlation were 0.98, 0.98, 0.97 and 0.96 for benzene, toluene, ethylbenzene and *p*-xylene, respectively. Blank samples were run at regular intervals to control for background contaminations. Average background levels under our test conditions were 0.3 ± 0.6 $\mu g/m^3$ for benzene, 1.4 ± 0.6 $\mu g/m^3$ for toluene, 0.1 ± 0.1 $\mu g/m^3$ for ethylbenzene, 0.4 ± 0.5 $\mu g/m^3$ for *m*- + *p*-xylene and 0.2 ± 0.1 $\mu g/m^3$ for *o*-xylene. Limits of detection of 1.6 ng/tube for benzene, 3.0 ng/tube for toluene, 0.2 ng/tube for ethylbenzene, 1.4 ng/tube for *m*- + *p*-xylene and 0.4 ng/tube for *o*-xylene were calculated from the variation of the background levels.

A typical chromatogram of an ambient indoor air sample from a non-smoking environment is shown in Fig. 3. A separation of *m*- and *p*-xylene (peak 4) was not achieved under the chromatographic conditions applied. The concentration of *o*-xylene was calculated using the calibration curve for *p*-xylene. The results of the indoor air measurements in five non-smoking homes and five smoking homes are shown in Table 1. The differences between the two types of homes were not statistically significant, probably due to the limited number of households investigated. The coefficients of variation for duplicate samples were on average 25.5% for benzene, 16.9% for toluene, 18.4% for ethylbenzene, 19.7% for *m*- + *p*-xylene and 24.0% for *o*-xylene. The observed indoor air concentrations are comparable to those found in other studies. In a German study

Table 1
Indoor air concentrations ($\mu g/m^3$) of benzene, toluene, ethylbenzene, *m*- + *p*-xylene and *o*-xylene in 10 households (5 households with smokers, 5 households without smokers)

	Benzene	Toluene	Ethylbenzene	*m*- + *p*-Xylene	*o*-Xylene
Households without smokers ($n = 5$)					
1	5.9	40.6	2.8	7.9	3.1
2	9.4	48.0	5.3	15.2	5.0
3	12.8	58.6	6.0	18.8	5.6
4	5.5	28.1	3.1	10.4	3.1
5	10.1	84.5	7.3	26.9	6.3
Mean	8.8	51.9	4.9	15.9	4.6
Households with smokers ($n = 5$)					
6	4.2	16.0	1.8	5.7	1.5
7	14.5	99.3	9.0	19.9	5.6
8	18.1	122.6	7.5	21.1	5.3
9	15.4	74.1	5.6	19.8	6.2
10	14.6	41.1	4.9	14.2	4.6
Mean	13.4	70.6	5.8	16.1	4.6

of 230 homes (approximately the same number of homes of smokers and non-smokers), median indoor air benzene concentrations of 6.9 $\mu g/m^3$ for homes of non-smokers and 9.3 $\mu g/m^3$ for homes with smokers have been reported [15]. In 164 nonsmoking homes in the US in autumn and winter, the median levels of benzene, ethylbenzene, *m*- + *p*-xylene and *o*-xylene amounted to 9.2, 5.1, 13 and 5.2 $\mu g/m^3$, respectively [16]. The corresponding concentrations for 345 US smoking homes were 16, 8.3, 20 and 7.1 $\mu g/m^3$. The levels in spring and summer were reported to be significantly lower. In a personal monitoring study of non-working British women, median benzene concentrations of 10.4 $\mu g/m^3$ for women from non-smoking households and 13.4 $\mu g/m^3$ for women from smoking households were found [17]. Levels of volatile organic compounds measured in a modern air-conditioned building showed median benzene concentrations of 8 $\mu g/m^3$ (3–49 $\mu g/m^3$) and 10 $\mu g/m^3$ (3–31 $\mu g/m^3$) and toluene concentrations of 36 $\mu g/m^3$ (10–292 $\mu g/m^3$) and 21 $\mu g/m^3$ (7–65 $\mu g/m^3$) in the air of smokers' and non-smokers' offices, respectively [18].

Fig. 4 shows the changes in CO_2 and O_2 concentrations during the sampling procedure of exhaled air. On inhalation, valve V2 is closed and the pumps (P) draw end-expiratory air from the Teflon tube (Fig. 1). During the inhalation period, the maximum CO_2 concentration and the minimum O_2 concentration are reached. On exhalation, valve V1 is closed and valve V2 is opened, resulting in the determination of the lowest CO_2 and highest O_2 concentration in exhaled air from the upper respiratory tract. Fig. 4 also shows that very deep and slow breathing (frequency: 3–4 breaths per min) does not lead to a "break-through" of ambient air from the open (downstream) end of the Teflon tube during inhalation. Exhaled breath samples from ten non-smokers and ten smokers were analyzed for BTX (Table 2). Gas chromatograms of exhalate samples were similar to those of ambient air. Duplicate samples were obtained and analyzed from each subject. The coefficients of variation were 10.6, 8.9, 12.3, 14.9 and 12.8% for benzene, toluene, ethylbenzene, *m*- + *p*-xylene and *o*-xylene, respectively. BTX concentrations in smokers' exhalate were significantly higher than those in non-smokers. Storaging the samples for 6 days did not affect the results. Our findings on volatile aromatic compounds in exhaled air of smokers and non-smokers are in fair agreement with those from other studies. Brugnone et al. [19] reported smaller benzene levels for smokers (32.5 $\mu g/m^3$) but higher benzene concentrations for non-smokers (7.8 $\mu g/m^3$) compared to our findings (Table 2). In a US study [16], breath concentrations of benzene, ethylbenzene, *m*- + *p*-xylene and *o*-xylene in 322 non-smokers were 2.5, 0.8, 2.1 and 0.8 $\mu g/m^3$, respectively, whereas the corresponding levels for 198 smokers amounted to 16, 2.6, 5.5 and 1.6 $\mu g/m^3$.

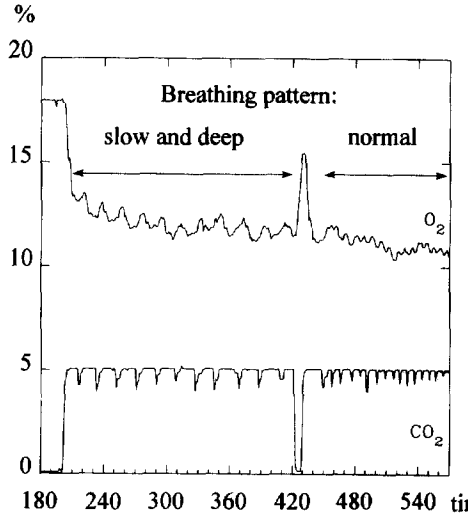

Fig. 4. Concentrations of oxygen (O_2) and carbon dioxide (CO_2) at the sampling site of the exhalate apparatus during normal as well as slow and deep breathing.

With the described microwave desorption GC–MS method, BTX concentrations in ambient and exhaled air can be rapidly determined in the low $\mu g/m^3$ range, relevant for environmental exposures. Compared to solvent extraction methods [7,8], a higher sensitivity is achieved by instantaneous microwave desorption of the whole sample from the charcoal sampling tube directly into the GC–MS system. In contrast to conventional thermal desorption methods [9,20], no intermediate cryo-focusing step is necessary.

Table 2
Exhaled air concentrations ($\mu g/m^3$) of benzene, toluene, ethylbenzene, *m*- + *p*-xylene and *o*-xylene in 10 non-smokers and 10 smokers

	Cigarettes/d	Benzene	Toluene	Ethylbenzene	*m*- + *p*-Xylene	*o*-Xylene
Non-smokers ($n = 10$)						
1	–	1.2	9.6	nd	nd	nd
2	–	3.6	11.3	nd	0.4	nd
3	–	0.5	5.1	nd	1.5	nd
4	–	3.0	6.0	nd	0.8	nd
5	–	0.6	nd	nd	nd	nd
6	–	7.8	21.4	0.9	2.7	nd
7	–	8.8	19.4	0.9	2.8	nd
8	–	5.8	15.4	0.5	1.9	nd
9	–	4.5	18.3	0.8	2.6	nd
10	–	2.9	15.4	nd	1.2	nd
Mean		3.9	12.2	0.3	1.4	–
Smokers ($n = 10$)						
11	30	54.5	100.6	8.7	26.8	7.0
12	30	24.8	42.0	2.6	6.8	1.2
13	3[a]	67.6	141.6	12.2	27.2	4.7
14	20	19.1	35.5	1.0	3.0	nd
15	48	34.2	51.5	3.7	9.4	2.0
16	20	55.1	74.3	4.0	8.8	1.3
17	15	43.0	54.7	3.2	6.5	1.3
18	40	98.0	120.6	13.0	27.1	5.9
19	50	71.3	99.9	7.0	13.6	3.2
20	20	34.6	53.9	3.4	7.5	1.2
Mean		50.2[b]	77.5[b]	5.9[b]	13.7[b]	2.8[b]

nd = not detectable.
[a] Cigars/d.
[b] Statistical significance of difference between smokers and non-smokers according to Mann–Whitney rank sum test: $p < 0.001$.

Analyzing BTX with our method takes approximately 30 min/sample. A disadvantage, as with all thermal desorption procedures, is that only a single GC determination can be performed with one sample. It is therefore advisable to collect several samples in parallel for multiple analysis. According to our experience, the adsorption tubes can be reused up to 20 times after cleaning them with the microwave device in the "purge mode".

The microwave desorption GC–MS method is particularly applicable to BTX breath analysis. Two major obstacles in trace analysis of breath are the limited volume of exhaled air available under field conditions and the high humidity of exhaled air. Both problems are adequately solved by the method described. Since the detection limit for benzene, the compound of major interest, is 1.6 ng/tube, a sample volume of 2 l is sufficient for the breath analysis of subjects exposed to ambient air benzene. In a previous investigation of BTX in exhaled air [21] using a solvent extraction technique [7], 30–40 l of exhaled air had to be sampled over 10 min in Tedlar bags [22]. Applying the modified breath sampling technique combined with the microwave desorption GC–MS method reduces the sampling time to 2 min. Furthermore, the exha-

late sampling device (Fig. 2) is portable and can be used in field studies on BTX determinations in exhaled air.

4. Conclusion

In conclusion, the described microwave desorption GC–MS technique is a fast and sensitive method for the determination of BTX. Its suitability for the determination of BTX in the low $\mu g/m^3$ range has been demonstrated by indoor air measurements in homes of non-smokers and smokers. Since a sampling volume of 1 to 2 l air is sufficient and there is no interference by high humidity, the method is particularly suitable for BTX breath analysis under field sampling conditions. The presented sampling techniques and analytical methods are presently being applied in field studies for assessing the exposure to BTX and other VOCs from environmental sources.

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