

Measurement of benzene, toluene, xylene in small biological samples by syringe equilibrium / headspace gas chromatography

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Abstract

Background: Benzene, toluene, and xylene (BTX) are ubiquitous in the community environment. However, workers who handle them are concerned about adverse effects such as neurotoxicity and carcinogenicity. On the other hand, recently, in a general indoor environment, low-concentration BTX exposure due to release from a house has attracted attention as a cause of sick building syndrome and multiple chemical sensitivity. In order to understand the exposure status of BTX, it is effective to measure the unchanged BTX in biological samples such as blood and urine. However, the burden on the subject when collecting samples cannot be ignored.

Method: To generalize BTX measurement of biological samples in the field, the author has developed a syringe equilibrium / headspace gas chromatography using a small amount of biological sample and Portable GC and analytical accuracy was tested. Also, considering the case of bringing small samples back to the laboratory for analysis, ways to store biological samples containing BTX for extended periods of time was determined. And BTX contamination survey of newly built condominiums was conducted to verify the practicality.

Result: The reproducibility of syringe equilibrium / headspace gas chromatography with standard -added blood. In all BTX, the relative standard deviation was found to be within 10%, and according to the internal standard method, the relative standard deviation was found to be within 5%. BTX contamination survey of newly built condominiums completed successfully. Benzene and toluene were detected in the urine of the resident, but only toluene was detected indoors.

Conclusion: This method and the use of a portable GC with a photoionization detector enable assessment from microexposure in the living environment to occupational exposure levels with 50 µl microblood, reducing the burden on the subject. In addition, a long-term storage method of biological samples containing BTX, was developed to consider the case of bringing small samples back to the laboratory for analysis.

Keywords: Blood BTX; Headspace gas chromatography; Syringe equilibrium; Portable GC; PID detector

1. Introduction

Benzene, toluene, and xylenes (BTX) are ubiquitous in the community environment. However, workers who handle them are concerned about adverse effects such as neurotoxicity and carcinogenicity [1-3] In addition, low-concentration BTX exposure due to release from a house has attracted attention as a cause of sick building syndrome and multiple chemical sensitivity [4, 5]. Biological monitoring methods are effective in assessing the effects of BTX exposure, and urinary metabolites are the primary measure of biological exposure [6]. However, it has been pointed out that evaluation by urinary metabolites makes it difficult to eliminate the effects of food intake, etc [7, 8], and does not

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sufficiently reflect BTX exposure at extremely low pollution levels such as the general environment [9-12]. Therefore, headspace gas chromatography for unchanged BTX in blood and urine is useful [13-17]. This is a very specific indicator of exposure and is a good reflection of exposure to low concentrations. However, in order to generalize the collection of biological fluids, it is necessary to reduce the complexity of sample collection work and the burden on the subject. The author has developed a syringe equilibrium / headspace gas chromatography using a small amount of biological sample such as earlobe blood with the aim of generalizing BTX measurement of biological samples in the field. It is easy to sample and has a relatively low burden on the subject. In addition, the author considered ways to store biological samples containing BTX for extended periods of time, considering the case of bringing small samples back to the laboratory for analysis.

2. Material and methods

2.1. Chemicals and experimental materials

Analytical grade BTX and methanol from Wako pure chemical industries Ltd (Osaka, Japan). an airtight syringe (2.5ml, Hamilton), glass wool, Teflon-capped vial (GL Science) were applied for the experiment. Blood and urine samples were obtained from volunteers with informed consent.

2.2. Analytical standards preparation

A methanol solution containing the 10000 ppm (v/v) of BTX was prepared by diluting 1.0ml of each to 100ml. This solution was maintained at 4°C, and was diluted 5 to 5000 times in methanol as the daily standards. A daily standard aliquot (10 μ l) was added to the 10 ml blood sample to bring the final concentration of benzene, toluene and xylenes to 2-2000 ppb (v / v) (about 1.75-1750 ng / ml).

2.3. Analytical procedures and instruments

The analysis procedure is as follows: A 50 μ l biological sample was placed in an airtight syringe containing 100 mg of glass wool as a sample trapper and held at room temperature (22-24 °C) for 30 minutes. Then gas phase was manually injected into the gas chromatograph (GC) (Figure 1). Laboratory GC analysis was performed using Shimadzu GC8A model equipped with a hydrogen flame ionization detector. Analytical conditions were as follows: column: DB-WAX (0.53mmID×30m), column temperature: 100 °C, detector: FID, detector temperature: 150 °C, carrier gas: N₂, injection: direct injection mode. GC analysis in the field was performed using Voyager (Perkin Elmer -Photovac), a portable GC equipped with a photoionization detector. Analytical conditions were as follows: column: Supercowax 10 (polyethylene glycol, 0.53mmID×20m), column temperature: 50 °C, detector: PID (10.6 eV), detector temperature: 50 °C, carrier gas: N₂, injection: direct injection mode.

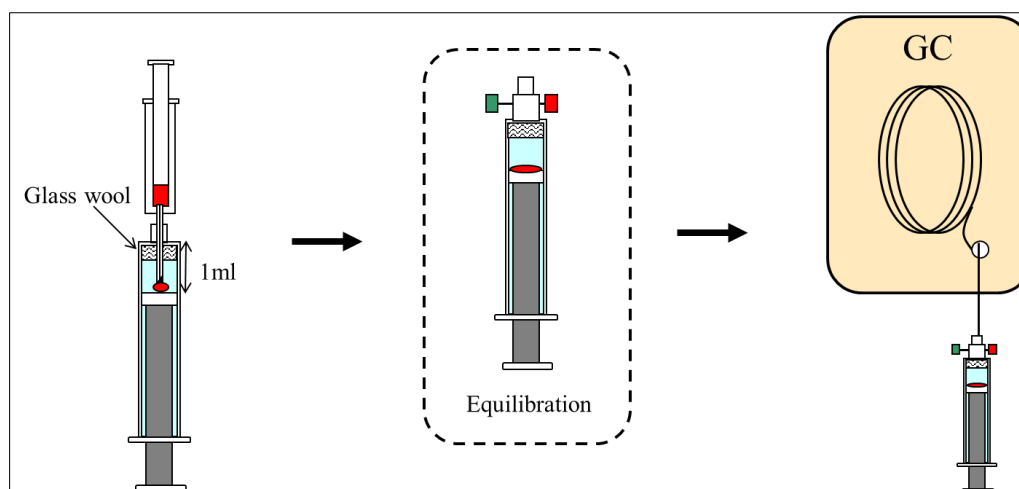


Figure 1 Analytical procedure of syringe equilibrium / headspace gas chromatography

2.4. Examination of small biological sample storage

50 μ l of blood containing 2000 ppb (v / v) of BTX was injected into the bottom of a small test tube containing 50 μ l of saline and frozen (Figure 2). Changes in BTX concentration after freezing were measured up to 7 days later.

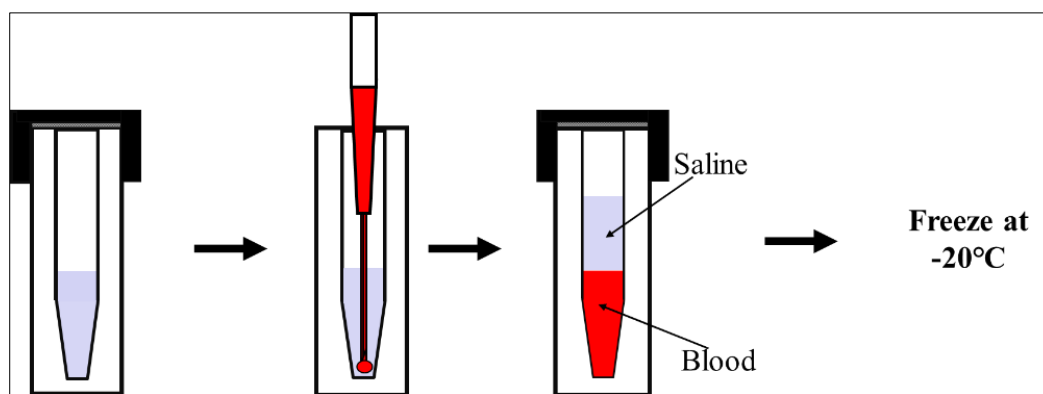


Figure 2 Storage procedure of blood sample containing BTX

2.5. BTX contamination survey of newly built condominiums

Upon request, a BTX contamination survey of newly built condominiums was conducted. The air in the house was collected in a Teflon bag (GL Science) and analyzed using a portable GC along with the urine provided by the resident. In addition, Formaldehyde in the air was also trapped in the Sep-Pak DNPH-Silica Cartridge (Waters). DNPH derivatives were later analyzed by HPLC (TOSOH).

3. Results

3.1. Analytical performance by syringe equilibrium / headspace gas chromatography

As shown in Figure 3, the gas phase BTX concentration in the syringe remained stable for 20-60 minutes. The calibration curves for BTX in blood at the range of 100-600ppb (v/v) (87.5-525 ng/ml) showed good linearity with determination coefficient (R^2) of 0.987-1.000 (Figure 4). Table 1 shows the reproducibility of syringe equilibrium / headspace gas chromatography with standard -added blood. In all BTX, the relative standard deviation was found to be within 10%, and according to the internal standard method, the relative standard deviation was found to be within 5%.

As shown in Figure 5- (a), 0.18 ppb (v/v) (0.16 ng/ml) of benzene was detected in 50 μ l of blood of a person living in a general environment by the syringe equilibrium / headspace gas chromatography and the minimum limit of detection (N/S=3) was estimated to be 0.06ppb (v/v) (0.05 ng/ml) by the concentration in blood.

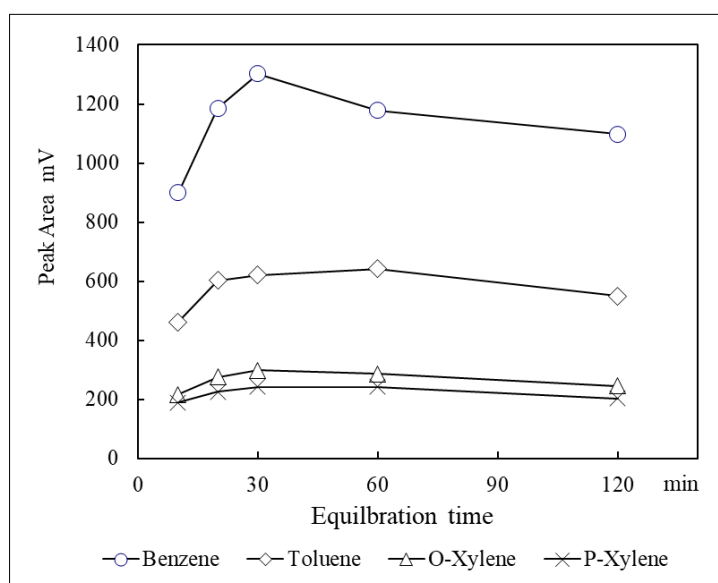


Figure 3 Changes of the gas phase BTX concentration in the syringe

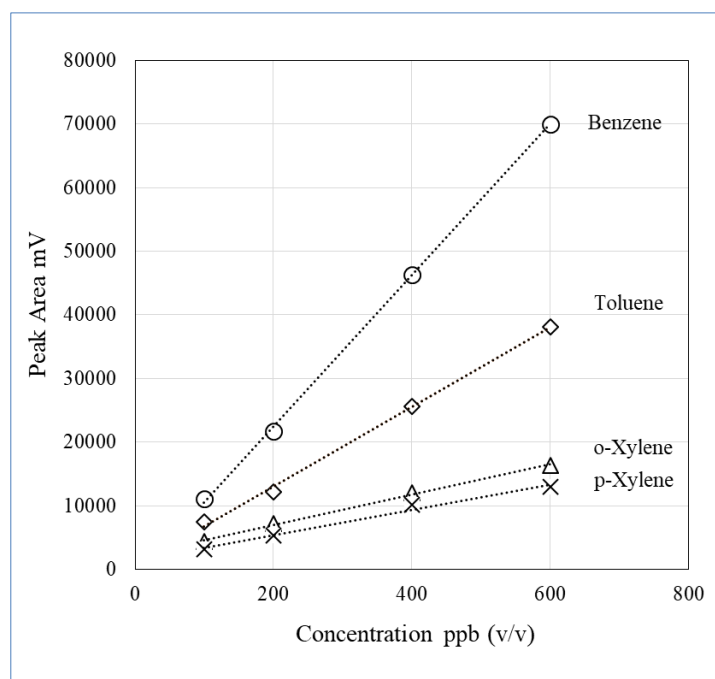


Figure 4 Calibration curves for BTX in blood by syringe equilibrium / headspace gas chromatography

Table 1 Reproducibility of syringe equilibrium / headspace gas chromatography with standard -added blood

Compounds	Blood content (ppb (v/v))	Relative standard deviation (%)*	
		Peak area	Peak area ratio**
Benzene	80	8.3	4.1
Benzene	400	5.4	2.8
Benzene	800	3.2	2.5
Toluene	800	6.6	3.5
o-Xylene	800	7.1	3.6
p-Xylene	800	6.6	4.5

*n=5, **internal standard: chloroform 2000 ppb

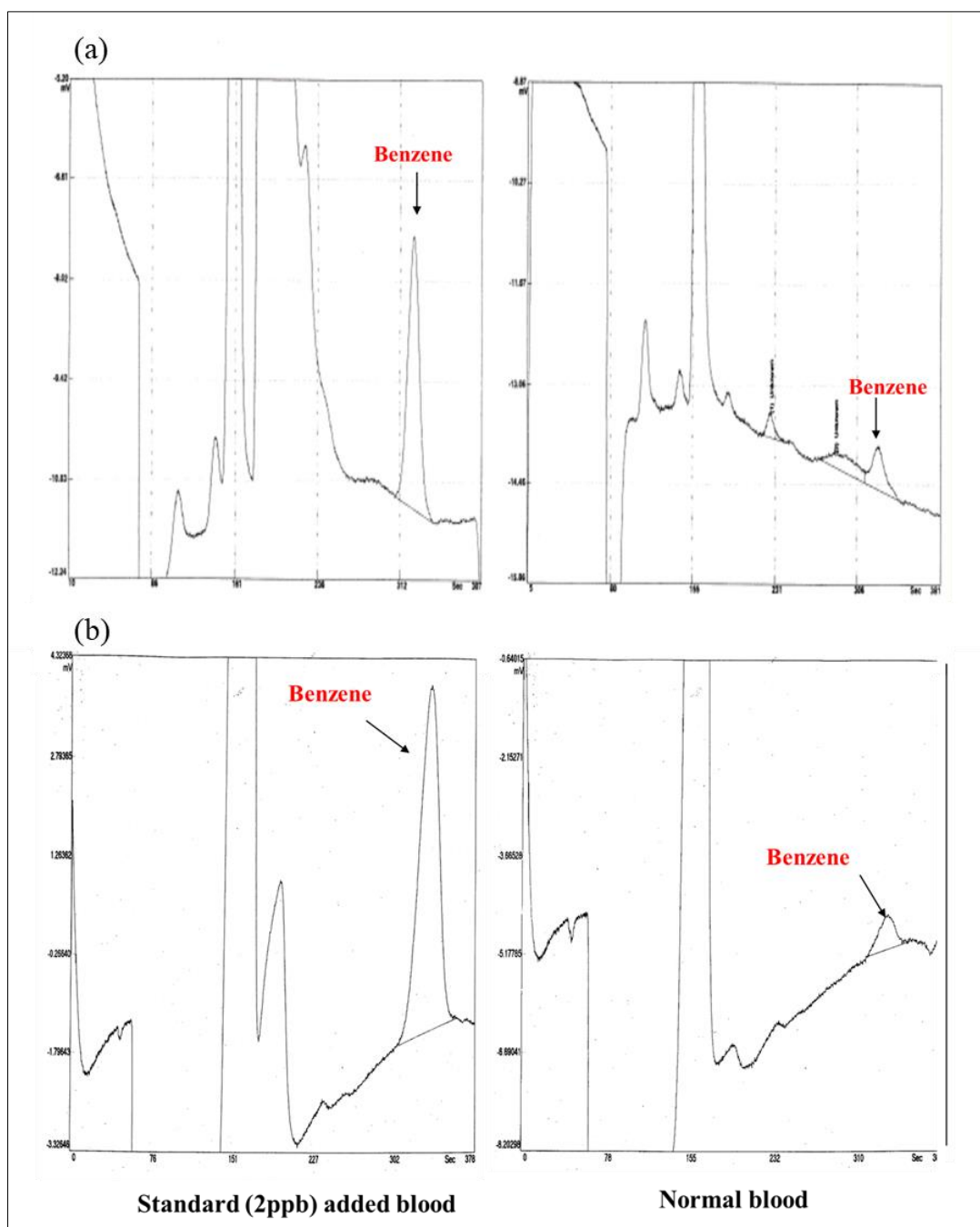


Figure 5 Measurement of blood benzene of a person living in a general environment. (a) Syringe equilibrium / headspace gas chromatography. (b) Vial equilibrium / headspace gas chromatography (7.5 ml vial, 2.0ml blood)

3.2. Examination of small biological sample storage

Figure 6 shows changes in benzene content under several storage conditions of standard added blood. Blood benzene frozen after sealed with saline was stable until 7 days. As shown in Figure 7, toluene and xylenes were stable about 3 storage days.

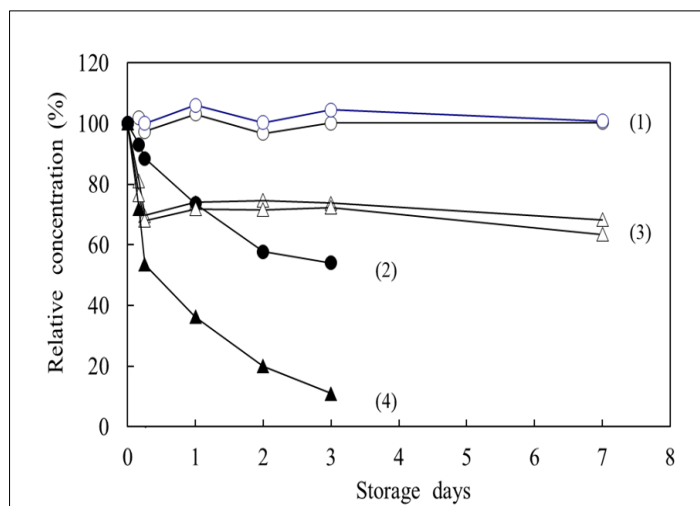


Figure 6 Relative concentration of benzene in blood after storage procedure. 50 µl of blood containing 2000 ppb (v / v) of benzene was maintained under the following conditions: (1) Seal with 50 µl saline and freeze at -20 ° C, (2) Seal with 50 µl saline and keep at 4 ° C, (3) Freeze at -20 ° C without sealing, (4) Keep at 4 ° C without sealing

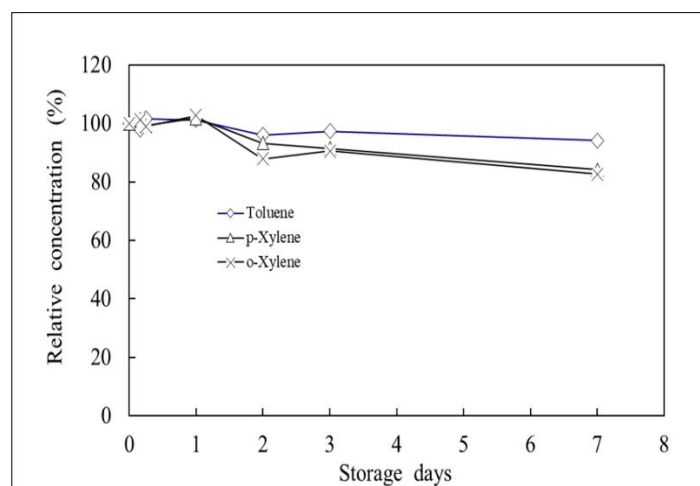


Figure 7 Relative concentration of toluene and xylenes in blood after storage procedure. 50 µl of blood containing 2000 ppb (v / v) of toluene and xylenes was sealed with 50 µl of saline and frozen at -20 ° C

3.3. BTX contamination survey of newly built condominium

As shown in Table 2, formaldehyde was detected in 70-80 µg/m³ and toluene was detected in about 100 µg/m³ in each room of the newly built condominium. But no benzene or xylene was detected. As shown in Figure 8, 0.15ppb (v/v) (0.13 ng/ml) of benzene and 0.21ppb (v/v) (0.18 ng/ml) of toluene were detected in the urine provided by the resident.

Table 2 Indoor air concentration of volatile organic compounds

	µg/m ³			
	Formaldehyde	Toluene	Xylene	Benzene
Room A	73.6	89.9	BDL	BDL
Room B	79.9	108.5	BDL	BDL
Dining room	BDL	85.4	BDL	BDL

BDL, below the detection limit.

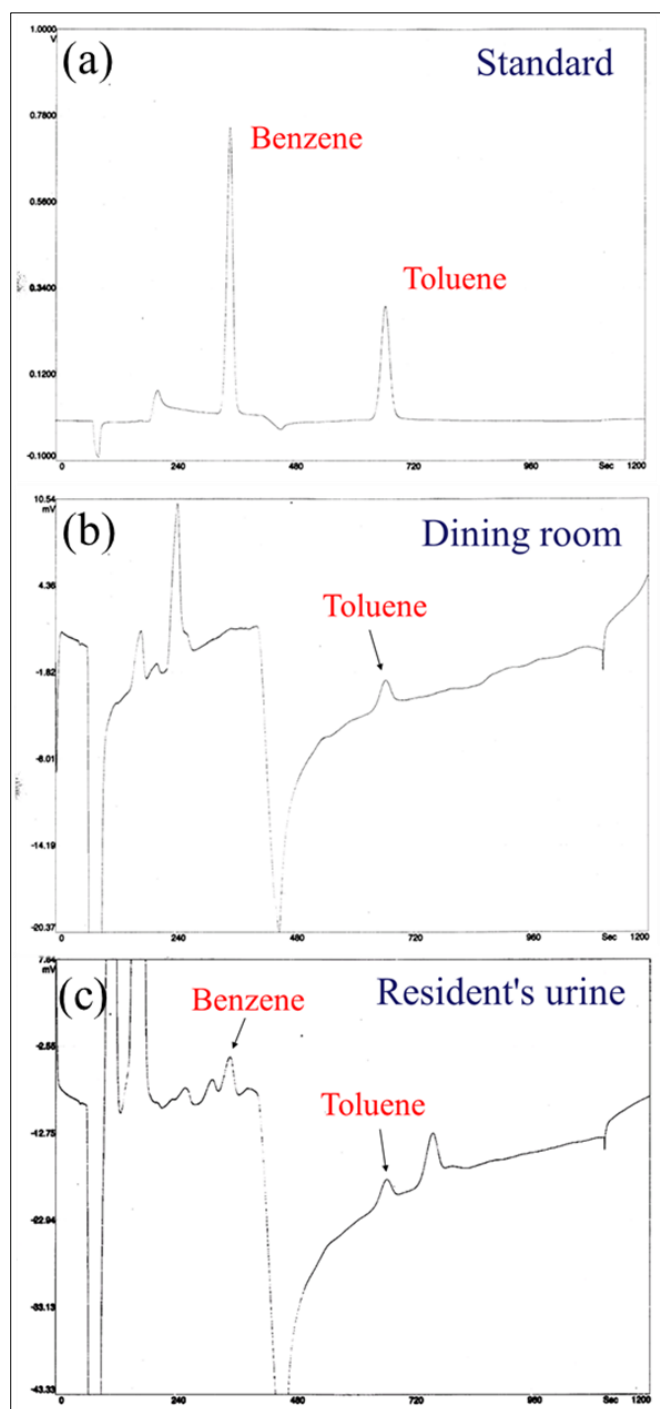


Figure 8 Indoor contamination assay with portable GC

4. Discussion

BTX pollution is ubiquitous in the community environment. People are exposed to BTX from high concentrations in industrial settings to low concentrations in the local indoor and outdoor environment. And there are concerns about the adverse health effects of BTX exposure [19-21]. Therefore, it is useful to develop a simple and rapid BTX exposure monitoring method.

The development of the syringe equilibrium / headspace gas chromatography has made it possible to measure BTX in biological samples without difficulty in the field. In addition, it was found that a portable GC equipped with a PID detector can easily detect micro-contamination exposure of people living in a general living environment. Therefore,

syringe equilibrium / headspace gas chromatography can be applied for exposure assessment from occupational contamination levels to general contamination levels.

A pollution survey of a newly built condominium revealed that formaldehyde was detected at 70-80 $\mu\text{g} / \text{m}^3$ and toluene was detected at about 100 $\mu\text{g} / \text{m}^3$ in each room, which is far below the Indoor Air Guidelines of the Ministry of Health, Labor and Welfare of Japan [22]. In addition, benzene and toluene were detected in the urine of the resident, but only toluene was detected indoors, and benzene was not detected. It is known that benzene is abundant in cigarette smoke and automobile exhaust gas [23-25]. Exposure from other than indoor air is possible.

5. Conclusion

To generalize BTX measurement of biological samples in the field, the author has developed a syringe equilibrium / headspace gas chromatography using a small amount of biological sample such as earlobe blood. This method and the use of a portable GC with a PID detector enable assessment from microexposure in the living environment to occupational exposure levels with 50 μl microblood, reducing the burden on the subject.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

There is no conflict of interest in this work.

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