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Determination of Formaldehyde in Urine by Headspace Gas Chromatography

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Abstract Formaldehyde is a carcinogen to which humans are exposed daily, but few methods are available to quantify formaldehyde in biological samples. We developed a simple, sensitive and rapid technique for the quantification of formaldehyde in urine by derivatization with *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine, using a headspace sampler coupled to a gas chromatograph equipped with an electron capture detector. The detection limit was 1.08 μg/L. The overall recovery of formaldehyde spiked in urine was 99%. The concentration of formaldehyde in urine obtained from healthy volunteers ranged from 56.85 to 144.57 μg/L. This method can be used successfully to measure formaldehyde in urine.

Keywords Formaldehyde · Headspace analysis · Gas chromatography · Human urine · *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine

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Formaldehyde is produced industrially in large quantities and widely used in plastics and resins, and as a preservative. Exposure to formaldehyde causes various symptoms, such as irritation to the eyes and respiratory tract and headache (WHO 1989). In addition, formaldehyde has been classified as a Group 1 human carcinogen (IARC 2004). Formaldehyde is also a product of normal metabolic pathways. Determination of formaldehyde concentrations in urine may be an important indicator of exposure to this chemical.

Among the methods used to measure aldehydes are high performance liquid chromatography (de Andrade et al. 1999; Luo et al. 2001), gas chromatography (Ohata et al. 1997), selected ion flow tube mass spectrometry (Spanel et al. 1999), and radiometric method (Szarvas et al. 1986). Recently, a method using headspace gas chromatography mass spectrometry (Deng and Zhang 2004; Hada et al. 2000) was developed to measure aldehydes. Few of these methods, however, have been applied to the measurement of formaldehyde in biological samples (de Andrade et al. 1999; Luo et al. 2001; Spanel et al. 1999; Szarvas et al. 1986). Aldehyde concentrations in biological samples have been measured following derivatization with *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine (PFBHA), due to the ease of oxime formation over a wide pH range and the solubility of PFBHA in aqueous

solutions (Cancilla and Que Hee 1992). We utilized this reaction to develop a simple, sensitive and rapid method to measure formaldehyde in biological samples. Formaldehyde was derivatized with PFBHA to the *O*-(2,3,4,5,6-pentafluorobenzyl)-formaldoxime (PFBHA-formaldoxime) and analyzed by headspace gas chromatography. This method is simpler, reduces preparation time and contamination, and does not require solvents. The method was applied to the measurement of formaldehyde concentration in human urine.

Materials and Methods

Formaldehyde standard stock solution (1 mg/mL in methanol), PFBHA hydrochloride and methanol (analytical grade) were purchased from Kanto Chemical (Tokyo, Japan), and PFBHA-formaldoxime (99.6%) was obtained from Hayashi Pure Chemical (Osaka, Japan). Commercial mineral water (Acqua Panna, Nestle, Vevey, Switzerland), which has negligible concentrations of formaldehyde, was used as blank water. PFBHA solution was prepared by diluting PFBHA-hydrochloride in blank water to a concentration of 0.1%.

Formaldehyde determinations were performed on a Hewlett–Packard 7694 headspace sampler coupled to a Hewlett–Packard 5890 Series II gas chromatograph (GC) equipped with an electron capture detector (Hewlett–Packard, Avondale, PA, USA). The column was a 30 m × 0.53 mm ID DB-5 capillary column with 1.5 μm film thickness (J&W Scientific, Folsom, CA, USA). Injection was in a split mode with helium as carrier gas. The split rate was 10:1 with a column flow rate of 5.0 mL/min. The injection port and detector temperatures were maintained at 250 and 300°C, respectively. The temperature program of the column consisted of holding at an initial temperature of 80°C for 1 min, increasing to 125°C at 5°C/min, holding at 125°C for 1 min, and increasing to 280°C at 15°C/min. The transfer line and loop temperature of the HS were maintained at 200 and 150°C, respectively. The injection volume was 1 mL.

One milliliter standard solutions or urine samples were placed in 20-mL headspace vials without pre-filtration. To each was added 50 μL PFBHA solution, and each vial was immediately sealed with a Teflon-coated silicone septum. The samples were mixed, incubated at room temperature for 4 h, and heated at 60°C for 30 min in the headspace bath to reach gas–liquid equilibration. The samples underwent automatic pressurization, venting and injection of the vapor phase. For some urine samples, analyte concentrations were corrected for creatinine concentration or a specific gravity of 1.016.

Results and Discussion

To optimize this headspace method, several parameters must be controlled, including vial heating temperature and time, and derivatization reaction temperature and time.

In this method, peak response of analyte is proportional to vial heating temperature. Because the matrix of the sample is water or urine, however, overpressure problems can occur while the vials are kept in the vial heating bath at temperatures higher than 80°C. We therefore maintained the vial heating bath temperature at 60°C and assessed the effect of sample equilibration time using a PFBHA-formaldoxime standard solution, prepared by diluting PFBHA-formaldoxime in methanol to 1 mg/mL. To each 20-mL headspace vial containing 1.05 mL blank water was added 1 μL PFBHA-formaldoxime standard solution, the vials were equilibrated for 0–50 min in the vial heating bath at 60°C, and the GC responses of PFBHA-formaldoxime were assessed. Since 20 min at 60°C was required to complete gas-liquid equilibration of formaldehyde (Fig. 1),

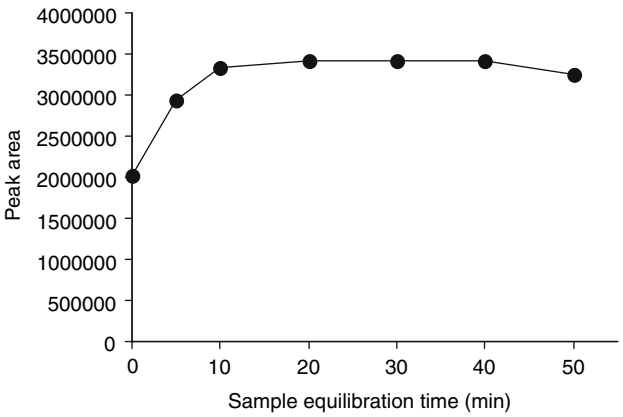

Fig. 1 Effect of sample equilibration time

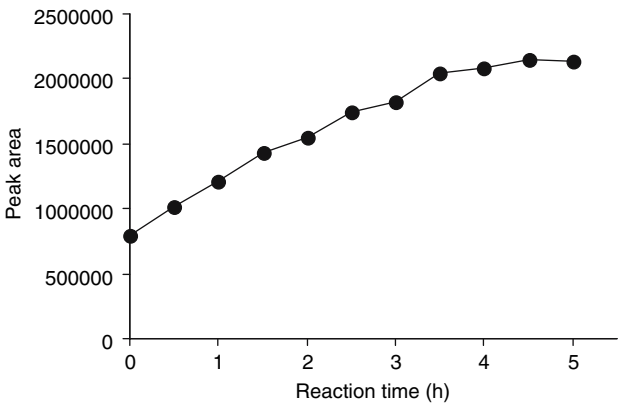

Fig. 2 Effect of reaction time

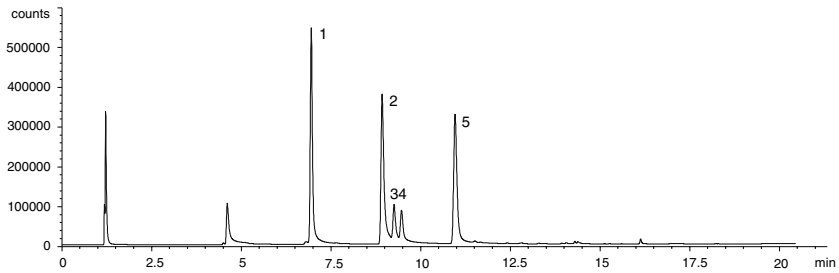

Fig. 3 Chromatograms of the urine of healthy volunteer, containing 79.30 μg/L formaldehyde. *Peak 1* PFBHA-formaldoxime, *Peak 2* PFBHA, *Peak 3* PFBHA-acetaldoxime (syn), *Peak 4* PFBHA-acetaldoxime (anti), *Peak 5* PFBHA-acetonealdoxime

Table 1 Recovery of formaldehyde spiked in urine

Spiked[a] (ng)	Found (ng)	Recovered (ng, mean ± SD, n = 3)	Recovery (%)
None	66.7	–	–
50	113.5	46.8 ± 8.1	94
100	175.6	108.9 ± 1.1	109
200	256.2	189.5 ± 5.8	95
Overall mean	–	–	99

[a] A 1 mL urine sample from a normal adult was spiked with a standard solution of formaldehyde

we selected a sample equilibration time of 30 min to ensure the completeness of gas-liquid equilibration.

To minimize possible loss or contamination of formaldehyde in urine samples, we performed the derivatization procedure on-site without addition of salt and brought the samples to the analytical laboratory (Ikeda 1999). We therefore chose room temperature as the reaction temperature and estimated the effect of reaction time using a formaldehyde standard solution, prepared by diluting formaldehyde standard stock solution in blank water to 100 μg/L. The samples were analyzed, and the completeness of derivatization was assessed at 0–5 h by determining the GC responses of PFBHA-formaldoxime at room temperature. Since 3.5 h at room temperature was required to complete derivatization of formaldehyde (Fig. 2), we chose a reaction time of 4 h to ensure the completeness of derivatization.

Parameters such as linearity, limit of detection and precision were calculated at the optimum conditions of this method. The working standard solutions were prepared from formaldehyde standard stock solution by dilution with blank water. Linearity was determined by plotting the calibration curve of the peak area versus the concentration of standards. A standard calibration curve was plotted for concentrations from 10 to 500 μg/L. This five-point calibration curve had good linearity in this range, with a correlation coefficient of 0.999.

The blank water used for this study contained a small quantity of formaldehyde. Therefore, based on the calibration curve, the limit of detection (LOD) and the limit of quantitation (LOQ) were determined. LOD is the analyte concentration giving a peak area equal to the blank peak area, plus three standard deviations, and LOQ is the analyte concentration giving a peak area equal to the blank peak area, plus 10 standard deviations. LOD and LOQ were 1.08 and 3.60 μg/L, respectively.

Table 2 Formaldehyde concentration in the urine of healthy volunteers

Sex	Number	Observed values				Values corrected for			
						Creatinine		Specific gravity (1.016)	
		Median[a]	Range[a]	AM[a] (ASD[a])	GM[a] (GSD[b])	AM[c] (ASD[c])	GM[c] (GSD[b])	AM[a] (ASD[a])	GM[a] (GSD[b])
Men	9	62.10	56.85–70.57	91.65 (23.48)	89.27 (1.27)	103.35 (80.08)	82.02 (2.03)	90.32 (52.46)	79.83 (1.66)
Women	4	85.25	60.84–144.57	62.90 (6.09)	62.69 (1.10)	87.62 (96.46)	60.96 (2.47)	69.63 (55.05)	57.61 (1.95)
Total	13	79.30	56.85–144.57	82.80 (23.82)	80.07 (1.30)	98.51 (81.60)	74.86 (2.11)	83.96 (51.88)	72.21 (1.74)

AM arithmetic mean, *ASD* arithmetic standard deviation, *GM* geometric mean, *GSD* geometric standard deviation

[a] μg/L

[b] Dimensionless

[c] μg/g creatinine

The precision of this method was evaluated by analyzing five samples (100 μg/L) on the same day (repeatability) and five samples on five different days (reproducibility). The repeatability and reproducibility were 3.1% and 5.5%, respectively.

A typical chromatogram of a urine sample is shown in Fig. 3. In order to identify the chromatographic peaks, electric ionization (70 eV) mass spectra were obtained with a mass spectrometer. PFBHA derivatives of formaldehyde (Peak 1), acetaldehyde (Peaks 3, 4) and acetone (Peak 5) were detected, because the electric ionization mass spectra of peaks had a base fragment ion of [C6F5–CH2]+ at *m*/*z* 181 for each peak, and a molecular ion at *m*/*z* 225 for peak 1, 239 for peak 3 and 4, 253 for peak 5, respectively. Minimal peak tailing is observed, but it did not interfere with the quantitation of PFBHA-formaldoxime. To determine the applicability of this procedure to biological samples, we performed a recovery study. Various amounts of formaldehyde were added to urine from a normal adult human, and recovery was determined by comparing the formaldehyde concentrations in spiked urine samples with those of water standards subjected to the same procedure. Background concentrations of urine were taken into account in the calculations. We found that the overall recovery of formaldehyde in urine was 99% (Table 1), indicating that water and urine behave similarly in this procedure and that the urine matrix does not influence the analysis. This result also shows that distilled water is a good solvating media for calibration standards.

Urine samples were collected from 13 volunteers (9 men and 4 women), aged 24 to 50 years (mean: 35.5 years; standard deviation: 7.9 years) and the background concentrations of formaldehyde were determined. The results were calculated using two separate assumptions of normal and log-normal distribution prior to and after correction for creatinine concentration and urinary specific gravity, respectively (Table 2). Formaldehyde was measurable in all 13 urine samples and ranged from 56.85 to 144.57 μg/L.

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