Simple Liquid-Chromatographic Determination of Urinary Coproporphyrin in Workers Exposed to Lead

Katsumaro Tomokuni and Yukio Hiraß

We developed a simple method for the determination of urinary coproporphyrin (CP) in lead workers, using a "high-performance" liquid chromatograph (HPLC) equipped with a fluorescence detector. The detection limit of urinary CP in this method was 5 \( \mu \text{g} \)/L. The working linear range of urinary CP concentration was 5 to 1500 \( \mu \text{g} \)/L. In 41 lead-exposed workers, the urinary CP values obtained by the present HPLC method were well correlated with those obtained by a conventional spectrophotometric method \((r = 0.94)\). The present method is useful for screening workers exposed to lead.

**Additional Keyphrases:** screening ∙ toxicology ∙ environmental hazards ∙ fluorometry ∙ chromatography, reversed-phase

The concentration of coproporphyrin (CP) in urine has been widely used as a measure of the biological effect of lead (1–3). Generally, urinary CP has been determined by the method of Rimington (4) or its modification (5). These conventional methods are based on sample extraction and the spectrophotometric measurement at the peak of the Soret band for CP. Recently, Sakai et al. (6) reported a method for determining urinary CP isomers I and III, using a "high-performance" liquid chromatograph (HPLC) equipped with a spectrofluorometer. This HPLC method seems to be simpler and more accurate than the spectrophotometric method.

We examined this HPLC procedure in regard to the analytical conditions, and our findings led us to modify the HPLC procedure for urinary CP. The results are described here.

**Materials and Methods**

**Urine specimens.** The spot (untimed) urine for lead exposure was collected from 41 workers whose occupational exposure to lead ranged from two to 13 years. "Normal" spot urines were collected from workers with no history of lead exposure. The relative density (specific gravity) of the urines was measured with a refractometer, and the concentration of CP observed was adjusted in relation to that in a urine with a relative density of 1.024.

**Apparatus.** We used a Model LC-6A HPLC equipped with a fluorescence HPLC monitor (RF-530; both from Shimadzu, Ltd., Kyoto, Japan). For comparison with the conventional method, we also used a Hitachi Model 100-40 spectrophotometer (Hitachi, Ltd., Tokyo, Japan).

**Reagents.** All chemicals used were of analytical grade. CP I was purchased from Sigma Chemical Co., St. Louis, MO, and CP III from Porphyrin Products, Logan, UT. Other chemicals were obtained from Wako Pure Chemicals, Osaka, Japan.

The stock solution of CP I (500 \( \mu \text{g} \)/L), prepared by dissolving 5 \( \mu \text{g} \) of CP I in 10 mL of glacial acetic acid, was stable for more than six months at \(-20°C\). To make the working standard of CP I (50 \( \mu \text{g} \)/L), we diluted the stock solution 10-fold with distilled water. This CP I working standard was stable for more than two weeks at 4°C in the dark and for more than 5 h at room temperature. Dilution with acetic acid instead of distilled water did not give a sharp peak for CP on the fluorescence chromatogram. On the other hand, the peak of CP I standard prepared in acetic acid diluted 10-fold with water was almost the same as that prepared in twofold-diluted acetic acid.

**Procedure.** To prepare the sample for HPLC analysis, we mixed 0.5 mL of glacial acetic acid with 0.5 mL of urine, and let this stand for at least 1 h at room temperature. HPLC analysis was carried out according to the analytical conditions shown in Table 1. The urinary CP (\( \mu \text{g} \)/L) was expressed as the sum of the fluorescence intensity (FI) of CP I and III (CP isomers), calculated by the following formula:

\[
\frac{\text{FI CP I} + \text{FI CP III}}{\text{FI CP I std (50 \mu\text{g}/L)}} \times 50 \times 2
\]

The fluorescence intensity of the CP I standard was almost equal to that of the CP III standard. The working linear range for CP concentration in urine was 5 to 1500 \( \mu \text{g} \)/L.

For comparison we used the conventional spectrophotometric procedure of Soulsby and Smith (5) for urinary CP analysis.

**Results and Discussion**

**Chromatography of urinary CP.** The chromatograms of urinary CP I and III by the fluorescence HPLC method are shown in Figure 1.

Urine CP I and III were completely separated from

<table>
<thead>
<tr>
<th>Table 1. Analytical Conditions for HPLC Measurement of Urinary CP</th>
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<tr>
<td><strong>Column</strong></td>
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<tr>
<td>150 × 6.0 mm, 5-µm particle size</td>
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<tr>
<td><strong>Detector</strong></td>
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<tr>
<td>Excitation wavelength 400 nm</td>
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<tr>
<td>Range × 1, Sensitivity High</td>
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<tr>
<td>Acetonitrile/aqueous KH₂PO₄ (10 mmol/L)/glacial acetic acid, 500/500/10 by volume</td>
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<tr>
<td>1.5 mL/min</td>
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<td><strong>Chart speed</strong></td>
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<td>5 mm/min</td>
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<td>25 µL</td>
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Department of Community Health Science, Saga Medical School, Nabeshima, Saga 840-01, Japan.

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other fluorescent substances. The chromatogram of a normal urine reflects a CP concentration of 64 μg/L. In the chromatogram of a lead worker's urine, the peaks correspond to a CP concentration of 325 μg/L. The retention time for urinary CP in this method was between 4 and 6 min, much shorter than the 16 to 20 min reported by Sakai et al. (6). The analysis time for urinary CP appears to be shortened by both omission of a guard column and use of a shorter reversed-phase column (150 mm). We analyzed more than 500 samples for urinary CP without using a guard column, without deleterious effects on the analytical column.

In the method of Sakai et al. (6) excitation/emission wavelengths of the detector were set at 400/600 nm; in our method, they were set at 400/620 nm. The analytical sensitivity for urinary CP at the latter setting was about double that at the 400/600 nm setting.

Effect of time after sample preparation on concentrations of urinary CP. After dilution with glacial acetic acid, the concentration of urinary CP gradually increased for 60 min, after which it became almost constant. These findings suggest that metal-binding CP (perhaps to zinc) other than free CP exists in the urine, which gradually is dissociated from the metal by the addition of acetic acid. We therefore allow the acid-treated urine samples to stand for at least 1 h at room temperature before injecting them onto the column.

Analytical recovery. HPLC of known amounts of CP I standard added to the normal urine at final concentrations of 50, 100, 250, and 500 μg/liter of urine gave analytical recoveries of 100 to 105% (mean 102%).

Comparison with conventional method. We determined on the same day the concentration of urinary CP in 41 lead workers by the present HPLC method (y) and the conventional method (x). The results were well correlated: y = 1.12x - 23.67 (r = 0.94; range of concentrations, about 0–300 μg/L).

Effect of frozen storage on urinary CP. Each of five lead workers' urines, with urinary CP ranging from 45 to 325 μg/L, was divided into two groups, then frozen at -20 °C for 2 and 16 weeks. The frozen samples were thawed in a water bath at 37 °C and the supernatants analyzed by HPLC analysis. The results are shown in Figure 2.

No loss of urinary CP was observed in the urine sample frozen at -20 °C for two weeks; however, the same sample frozen at -20 °C for 16 weeks lost about 50% of its CP content. Therefore, assays of urinary CP should be performed as soon as possible, even for urine samples stored at -20 °C. At present, we have not studied whether storage of urine samples at -20 °C after acidification with acetic acid keeps urinary CP stable for longer periods.

In conclusion, the present HPLC method for measuring urinary CP is simpler and more rapid than the spectrophotometric method and is useful in mass screening for lead exposure.

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References