A Simple Thin-layer-chromatographic Method for Detection of Urinary Homocystine

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For the detection of homocystine in urine specimens, a simple thin-layer chromatographic method was devised. Thirty μl of urine specimens without any prior treatment were applied onto a silica gel-G plate, and developed by ascending chromatography using a phenol water mixture (3:1) containing 0.05 % potassium cyanide. After developing and drying the plate, a slightly modified potassium iodoplatinate reagent was sprayed. Homocystine, cystine, methionine, cystathionine and histidine were clearly separated with different Rf values of 23-27, 12-13, 59-64, 14-16 and 40-42, respectively.

The present report described a simple rapid method for detection of homocystine in urine specimens by using a thin-layer chromatography.

METHODS

According to the procedure of Stahl, silica gel-G, 500 μm in thickness, was applied to a 20×20 cm glass plate. After keeping the plate at room temperature for about 5 minutes, the silica plate was activated by heating at 110°C for one hour. Along a base line about 3 cm from the bottom of the silica plate, spotting was done by using 30 μl each of urine specimens and of standard solution containing authentic preparation of homocystine, cystine, methionine, cystathionine or histidine. Each spot was placed 2 cm apart from each other.

The ascending chromatography, at 30°C for 3 hours, was carried out by the use of a developing solvent of a phenol/water mixture (3:1) containing 0.05% (w/v) potassium cyanide. After completion of the development, the plate was dried at 80°C then sprayed with a slightly modified potassium iodoplatinate reagent consisting of 20 ml of 5% H₂PtCl₆·6H₂O in 1N HCl, 100 ml of 10% potassium iodide and 400 ml of distilled water. This reagent was stable for at least one month when kept at 4°C.

After being sprayed with the potassium iodoplatinate reagent, the plate was stained pink in color, and homocystine, cystine, methionine, cystathionine and histidine were found as bleaching spots with different Rf as shown in Table 1 and Figs. 1 and 2.

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TABLE 1. *Rf* values of homocystine, cystine, methionine, cystathionine and histidine on a thin-layer chromatogram by author’s method

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>Rf</th>
<th>Color of spot</th>
<th>Minimum amount to be detected (γ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homocystine</td>
<td>23−27</td>
<td>White</td>
<td>0.7−1.0</td>
</tr>
<tr>
<td>Cystine</td>
<td>12−13</td>
<td>Yellow</td>
<td>2.0−3.0</td>
</tr>
<tr>
<td>Methionine</td>
<td>59−64</td>
<td>White</td>
<td>2.0−3.0</td>
</tr>
<tr>
<td>Cystathionine</td>
<td>14−16</td>
<td>White</td>
<td>2.0−3.0</td>
</tr>
<tr>
<td>Histidine</td>
<td>40−42</td>
<td>Yellowish-white</td>
<td>2.0−3.0</td>
</tr>
</tbody>
</table>

**Fig. 1.** Thin-layer chromatogram of methionine (M), homocystine (H), cystine (C), cystathionine (Cy) and histidine (Hi) by author’s method. Each authentic preparation was dissolved in distilled water at a concentration of 20 mg/100 ml. Thirty μl of each were spotted.

**Fig. 2.** Thin-layer chromatogram of homosystine (H), cystine (C), methionine (M), cystathionine (Cy) and histidine (Hi) by author’s method. Each authentic preparation was dissolved in urine from a healthy adult at a concentration of 20 mg/100 ml. Thirty μl of each were spotted.

On the examination of urine specimens from a patient with homocystinuria, the presence of homocystine was clearly demonstrated as shown in Fig. 3.

**DISCUSSION**

Homocystinuria was reported firstly by Field and his associates in 1962. These authors succeeded in the identification of homocystine in urinary specimens from patients with homocystinuria by using column chromatographic analysis.

Fig. 1. Thin-layer chromatogram of methionine (M), homocystine (H), cystine (C), cystathionine (Cy) and histidine (Hi) by author’s method. Each authentic preparation was dissolved in distilled water at a concentration of 20 mg/100 ml. Thirty μl of each were spotted.
Field et al.\textsuperscript{3} also reported a paper chromatographic method for detecting urinary homocystine where homocystine was converted into homocysteic acid by treatment with hydrogen peroxide prior to the two dimensional paper chromatography using phenol and ‘collidine’ as developing solvents.

Subsequently in 1965, Carson and his associates\textsuperscript{5} reported further ten cases of homocystinuria, and they also used paper chromatographic method for identification of urinary homocystine where homocystine was oxidized into homocysteic acid by treatment with hydrogen peroxide and molybdate prior to paper chromatography with use of phenol and ‘collidine’ as solvents. They\textsuperscript{5} stated that homocystine did not appear on phenol-lutidine paper chromatograms owing to its decomposition and that this was probably why such a relatively common amino-aciduria had been missed on the intensive survey.

In 1965 Schimke et al.\textsuperscript{6} reported 38 cases of homocystinuria and they adopted a high voltage paper electrophoresis with successful separation of homocystine from cystine in the urine.

In 1964 Gerritsen and Waisman\textsuperscript{7} reported 2 cases of homocystinuria where urinary homocystine was identified by column chromatography as well as by paper chromatography using a mixture of n-butanol, acetic acid and water (60: 20: 20) as a solvent after oxidation of homocystine with a few drops of 30\% $\text{H}_2\text{O}_2$ added directly onto the paper.

Fig. 3. Thin-layer chromatogram of urine specimens from a normal subject and a patient with homocystinuria.
1: Normal. 2: Homocystinuria. 3: Standard solution containing homocystine (H), cystine (C) and methionine (M).
Recently Werder et al.\textsuperscript{8} reported first 2 cases of homocystinuria from Europe by using automatic amino acid analyzer for detecting urinary homocystine.

It may be said that as compared with various methods reported so far for detection of homocystine in urinary specimens, the author’s thin-layer chromatography is simple and easy in the procedure, and more convenient for screening test for homocystinuria.

References