Analysis of human plasma and urine purines using high performance liquid chromatography (HPLC)

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The recent development of microparticulate, chemically bonded packing materials for high performance liquid chromatography (HPLC) has allowed the development of sensitive methods for the detection and quantification of purine bases and nucleosides in biological fluids. Using reverse phase HPLC methodology developed by Hartwick and others we undertook quantitative analyses of purine nucleosides and bases in normal human plasma and adapted these methods to obtain a qualitative profile of urine purine excretion products.

Instrumentation consisted of a Waters P/N 80060 modular system I chromatograph comprising twin pumps, solvent flow programmer, injection, 254 nm fixed wave absorbent detector, and M730 data module. The column used is a pre-packed Bondapak (C18) column consisting of a porous silica support with an octadecyl (C18) chemically bonded stationary phase (particle size 10 μm, column dimensions 3.9 × 300 mm). A dry-packed precolumn of the same material (37–50 μm particle size) protects the main column. The solvent system employed is a linear gradient; 100% 0-02 mol/l phosphate buffer pH 5.6 to 40% methanol/water (60% v/v) in 35 minutes at ambient temperature and a flow rate of 1.5 ml/min.

40 μl plasma or 10 μl of a 1 in 10 dilution of urine are injected after ultra filtration in Amicon cones (CF 50A).

The identification of plasma purine and nucleoside peaks has been verified by cochromatography with pure compounds and enzyme shift methods. The table shows normal values for plasma constituents that may be accurately quantified. The lower limit for detection of plasma purines and nucleosides is approximately 0.2 μmol/l.

Urinary purine base and nucleoside concentrations are much more variable and dependent on diet and hydration. The definitive identification of all urine components detected has not been completed and many are partly excreted as methylated metabolites.

The urine chromatogram may, however, be used to provide a qualitative 'profile' of purine excretion in addition to quantitative measurements of uric acid and creatinine.

Table 1 Normal values (μmol/l) plasma constituents. Figures are means (SD)

<table>
<thead>
<tr>
<th></th>
<th>Women (n = 16)</th>
<th>Men (n = 9)</th>
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</thead>
<tbody>
<tr>
<td>Creatinine</td>
<td>92.6 (17.14)</td>
<td>97.8 (12.4)</td>
</tr>
<tr>
<td>Uric acid</td>
<td>182.4 (32.9)</td>
<td>297.5 (64.1)</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>44.7 (21.9)</td>
<td>40.1 (35.8)</td>
</tr>
<tr>
<td>Hypoxanthine</td>
<td>2.21 (2.67)</td>
<td>1.96 (1.47)</td>
</tr>
<tr>
<td>Uridine/Xanthine</td>
<td>2.63 (1.05)</td>
<td>2.94 (0.98)</td>
</tr>
<tr>
<td>Inosine</td>
<td>1.38 (0.91)</td>
<td>1.08 (0.94)</td>
</tr>
<tr>
<td>Guanosine</td>
<td>Generally below limit of estimation</td>
<td></td>
</tr>
<tr>
<td>Adenosine</td>
<td>1.55 (1.39)</td>
<td>1.05 (0.78)</td>
</tr>
</tbody>
</table>

Reference

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