Determination of the Base Contents of Liver DNA of Rats by Reversed-Phase High Performance Liquid Chromatography

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Abstract The base contents of liver deoxyribonucleic acid DNA of rats living at an altitude of 2.3 km were determined by reversed-phase high performance liquid chromatography. At first 0.05 mol/L KH₂PO₄ pH 4.0 was used to dissolve the DNA acid hydrolysis products with 8-bromoguanosine BrG as an internal standard. Then the DNA hydrolysis products with BrG were chromatographed on a Supelcosil LC-18 column with UV detection at 254 nm and eluted by the mobile phase of MeOH-0.05 mol/L KH₂PO₄ pH 4.0 at 20:80 V/V at the flow rate of 0.8 mL/min. Under these conditions several bases were separated effectively. From the results the relatively constant proportions of the bases in DNA were found. The contents were 17.4% of cytosine C, 28.8% of adenine A, 23.3% of guanine G and 25.3% of thymine T. RSDs of the determination of these bases were 1.7%, 1.5%, 1.3% and 2.1% respectively. At the same time the methylation level of liver DNA of the rats determined by the internal standard method was 6.2%.

Key words reversed-phase high performance liquid chromatography deoxyribonucleic acid DNA base methylation level liver rat
1.2

Supercosil LC-18 250 mm × 4.6 mm i.d., 5 μm, pH 4.0, 0.05 mol/L KH2PO4

ABCD

1.3 DNA

DNA 2.5 mg/mL

ABCDEF

1.4 DNA

m1 = f j × m2 × A s × C m5C

f1 = m2 × A s × C

DNA m5C m5C C C

Table 1 Determmation results of the sample

<table>
<thead>
<tr>
<th>Base</th>
<th>Content (%)</th>
<th>SD</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytosine C</td>
<td>17.4</td>
<td>0.29</td>
<td>1.7</td>
</tr>
<tr>
<td>Guanine G</td>
<td>23.3</td>
<td>0.34</td>
<td>1.5</td>
</tr>
<tr>
<td>Adenine A</td>
<td>28.8</td>
<td>0.37</td>
<td>1.3</td>
</tr>
<tr>
<td>Thymine T</td>
<td>25.3</td>
<td>0.54</td>
<td>2.1</td>
</tr>
<tr>
<td>5-Methyl cytosine m5C</td>
<td>5.2</td>
<td>0.05</td>
<td>0.9</td>
</tr>
</tbody>
</table>

The methylation level of DNA 6.2%