Method Conditions

Column: Cogent Diamond Hydride™ 4µm, 100Å.
Catalog No.: 70000-15P-2
Dimensions: 2.1x150mm
Solvents:
A: DI water + 0.1% ammonium formate
B: 90% acetonitrile/10% DI water/0.1% ammonium formate
Gradient:

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>%B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>95.0</td>
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<tr>
<td>0.5</td>
<td>95.0</td>
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<tr>
<td>10.0</td>
<td>75.0</td>
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<tr>
<td>15.0</td>
<td>30.0</td>
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<tr>
<td>20.0</td>
<td>30.0</td>
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<tr>
<td>20.1</td>
<td>95.0</td>
</tr>
</tbody>
</table>

Post Tme: 5 min
Temperature: 25°C
Flow Rate: 0.4 mL/min
Injection: 1 µL
Samples:
1. XMP: Xanthosine -5'-monophosphate
2. GTP: Guanosine -5' triphosphate
Detection: UV diode array using chromatograms at 254 nm and 280 nm, extracted from the UV spectra scanned from 230 to 310 nm for all runs.

Discussion

This fast, simple and easy to use method achieves the separation of nucleotides XMP from GTP. The analysis of these polar compounds is achieved at high concentration of an organic solvent as part of the mobile phase (usually acetonitrile, but acetone may be used as well when MS detection is used) which provides increased sensitivity. Retention times were very reproducible with %RSD approximately 0.4, even when red blood cells extracts were injected in between the standard samples. Using a mass spectrometer this method can be specific and sensitive.

For more information visit www.MTC-USA.com

Notes:
Among the most important purine nucleotides, XMP and GTP are often analyzed from the lymphocytes of healthy people and HIV-1 seropositive patients at different stages of the disease (ARC-AIDS). Several differences in metabolism of purine nucleotides in the lymphocytes of the AIDS patients, were observed [2]. XMP does not normally appear in free nucleotide cell extracts, however it is the product of the important cell differentiation enzyme IMP dehydrogenase in ribavirin therapies.