

Determination of Adenine and Guanine Nucleotides in Tissue Extracts

Polymeric Reversed Phase HPLC Column 2.1 x 150 mm PRP-1

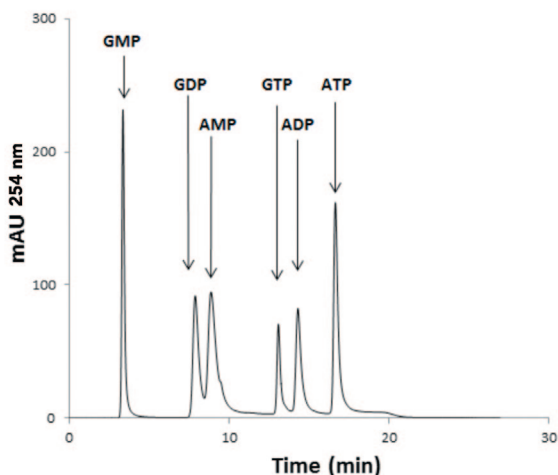
Adenosine triphosphate (ATP) is the universal energy currency in biological systems. ATP is synthesized from adenosine mono and di-phosphate substrates. The relative ratio of ATP, ADP and AMP is an important indicator of metabolic standing: the energy charge ($EC = \frac{[ATP] + 0.5[ADP]}{[ATP] + [ADP] + [AMP]}$) is considered a quantitative measure of energy status, and serves as a barometer of sorts, as aberrant change in EC is associated with onset of numerous pathological states. Measurement of adenosine nucleotides, therefore, represents an important and insightful diagnostic.

In the present study, an ion pair reversed phase HPLC method was developed to separate ATP, ADP and AMP from like-phosphorylated guanosine metabolic products (e.g., GTP, GDP, GMP) enabling quantification of these nucleotides from tissue extracts.

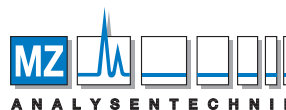
Column:	PRP-1, 5 µm, 2.1 x 150 mm														
Part Number:	79366														
Mobile Phase A:	100 mM Monopotassium phosphate (adjust pH to 7 with potassium hydroxide), 1 mM tetrabutylammonium phosphate, 2.5% methanol														
Mobile Phase B:	Eluent A + 20% methanol														
Flow Rate:	300 µL/min														
Gradient:	<table><thead><tr><th>Time (min)</th><th>%B</th></tr></thead><tbody><tr><td>0</td><td>1</td></tr><tr><td>3</td><td>1</td></tr><tr><td>10</td><td>15</td></tr><tr><td>15</td><td>55</td></tr><tr><td>16</td><td>95</td></tr><tr><td>19</td><td>95</td></tr></tbody></table>	Time (min)	%B	0	1	3	1	10	15	15	55	16	95	19	95
Time (min)	%B														
0	1														
3	1														
10	15														
15	55														
16	95														
19	95														
Injection Volume:	5 µL														
Sample Concentration:	0.02 mM														
Detection:	UV at 254 nm														
Temperature:	50 °C														

Compounds

1. Guanosine monophosphate
2. Guanosine diphosphate
3. Adenosine monophosphate
4. Guanosine triphosphate
5. Adenosine diphosphate
6. Adenosine triphosphate



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