

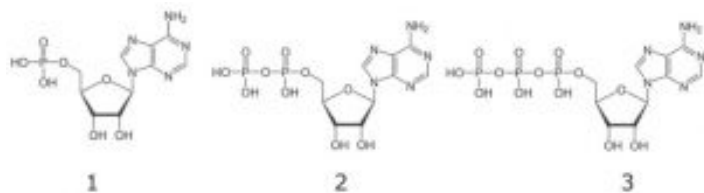
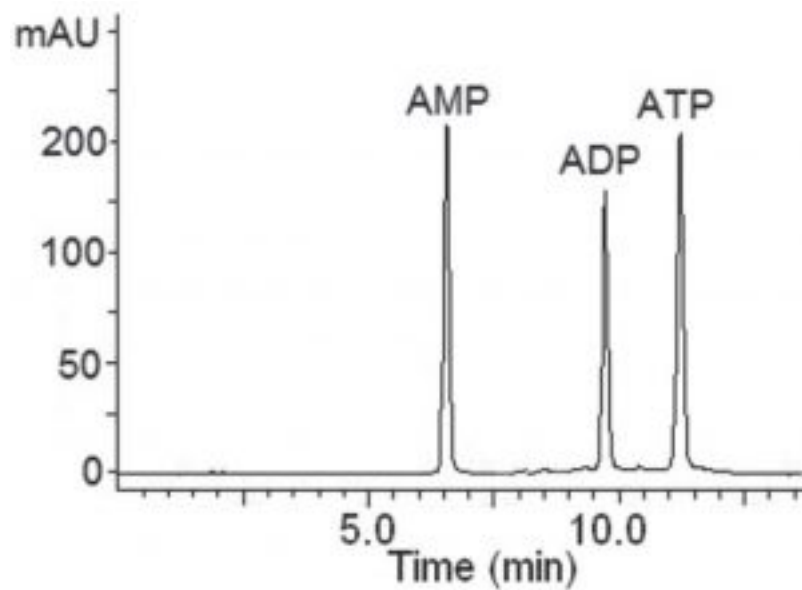


Separation of Adenine Nucleotides - AppNote

Separation of AMP, ADP, ATP by HPLC

The figure shows a separation of three energy nucleotides. All three are baseline separated in order of increasing polarity as is expected when ANP chromatography is used.

It is worth noting that the retention of nucleotides increased as the buffer concentration was increased (data not shown). 16.0mM concentration of the buffer in Solvents A and B was the maximum concentration still compatible with MS detection.



Peaks:

1. AMP - Adenosine 5'-monophosphate



2. ADP - Adenosine 5'-diphosphate
3. ATP - Adenosine 5'-triphosphate

Method Conditions

Column: Cogent UDA™, 4µm, 100Å

Catalog No.: 40031-05P-2

Dimensions: 2.1 x 50mm

Mobile Phase:

A: DI Water / 16.0mM Ammonium Formate

B: 90% Acetonitrile / 10% DI Water / 16.0mM Ammonium Formate

Gradient:

Time (minutes)	%B
0	95
0.5	95
10	70
15	30
20	30
20.1	95



Temperature: 25°C

Post Time: 3 minutes

Injection vol.: 1 µL

Flow rate: 0.4mL / minute

Detection: UV @ 254nm

Sample Preparation: Stock Solution: 1mg / mL solutions in DI Water. Samples were diluted 1:10 into 50% Acetonitrile / 50% DI Water mixture. Before injection, samples were filtered through a 0.45µm Nylon Syringe Filter (MicroSolv Tech Corp.).

t₀: 0.7 minutes

Note: The ratio of the Adenine Nucleotides (Adenosine ATP/ADP/AMP) is measured to indicate cell energy status or cell apoptosis/death, or ischemia in a tissue.



Attachment

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