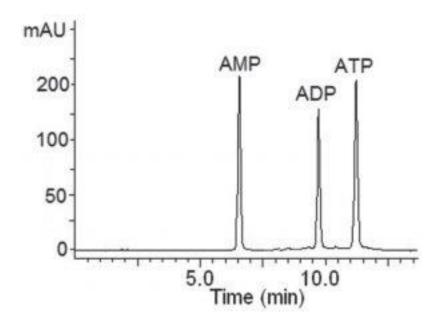


# Separation of Adenine Nucleotides - AppNote

# 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.:** <u>40031-05P-2</u> **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:**

%B
95
95
70
30
30
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  $0.45\mu m$ 

Nylon Syringe Filter (MicroSolv Tech Corp.).

to: 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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