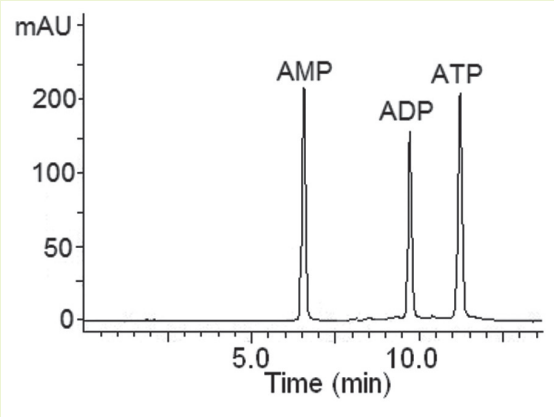
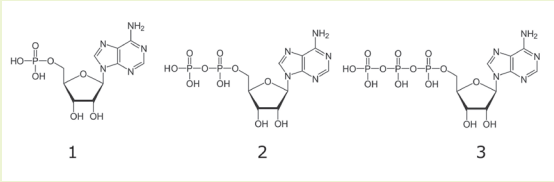
# Separation of Adenine Nucleotides

## AMP, ADP, ATP on UDA column




**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.

## Method Conditions

**Column:** **Cogent UDA™**, 4µm, 100Å

**Catalog No.:** 40031-05P-2

**Dimensions:** 2.1 x 50 mm

**Solvent:** A: DI $H_2O$ / 16.0 mM ammonium formate
B: 90% acetonitrile / 10% DI $H_2O$ / 16.0 mM ammonium acetate

**Gradient:**

| time (min.) | %B |
|---|---|
| 0 | 95 |
| 0.5 | 95 |
| 10 | 70 |
| 15 | 30 |
| 20 | 30 |
| 20.1 | 95 |

**Temperature:** 25˚C

**Post Time:** 3 min

**Injection vol.:** 1 microL

**Flow rate:** 0.4 mL/min

**Detection:** UV 254 nm

**Samples: Stock Solution:** 1 mg/mL solutions in DI $H_2O$. Samples were diluted 1:10 into 50% acetonitrile / 50% DI $H_2O$ mixture. Before injection, samples were filtered through a 0.45µm nylon syringe filter (MicroSolv Tech Corp.).

**Peaks:** 1. AMP – adenosine 5'-monophosphate
2. ADP – adenosine 5'-diphosphate
3. ATP – adenosine 5'-triphosphate

**$t_0$:** 0.7 min

## Discussion

The figure shows a separation of three energy nucleotides using the Cogent UDA HPLC column and a simple gradient. All three nucleotides 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). 16.0 mM concentration of the buffer in solvents A and B was the maximum concentration still compatible with MS detection.



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