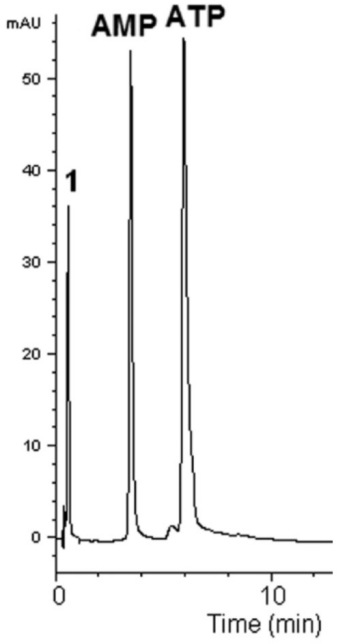
# Analysis of Nucleotides by ANP

## ATP & AMP




### Method Conditions

**Column:** **Cogent Diamond Hydride™**, 4µm, 100Å

**Catalog No.:** 70000-10P-2

**Dimensions:** 2.1 x 100 mm

**Solvents:** A: DI $H_2O$/ 0.1% ammonium formate
B: 90% acetonitrile/ 10% DI $H_2O$/ 0.1% ammonium formate

**Gradient:**

| time (min.) | %B |
|---|---|
| 0 | 95 |
| 10 | 70 |

**Post Time:** 5 min

**Injection vol.:** 2µL

**Flow rate:** 0.3 mL/min

**Detection:** UV 254 nm

**Samples:** 0.3 mg of each in 50% acetonitrile/ DI $H_2O$ + ammonia

**Peaks:** 1. adenosine-3',5'-cyclic monophosphate
2. adenosine 5'-monophosphate (AMP)
3. adenosine 5'-triphosphate (ATP)

### Discussion

The chromatogram shows an example of the separation possible with this method for three adenosine analytes. The Aqueous Normal Phase (ANP) gradient starts at a high percentage of acetonitrile in the mobile phase and decreases to 70% over a 10 minute period. The last nucleotide, adenosine 5'-triphosphate (ATP) displays noticeable tailing in comparison to the first two compounds when a sample is injected without ammonia. An improvement in peak shape can be achieved by adding this to the sample. The amount used in this note (5µL of 12% ammonia/mL) does not appreciably affect the retention time.

**Notes:** Nucleotides are important phosphate containing compounds that are found in living cells and are associated with a broad array of metabolic and biological processes. They have significant roles in the synthesis of DNA and RNA, are involved in signal transduction pathways, function as coenzymes in biosynthetic pathways and serve as energy reservoirs in biological systems.



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