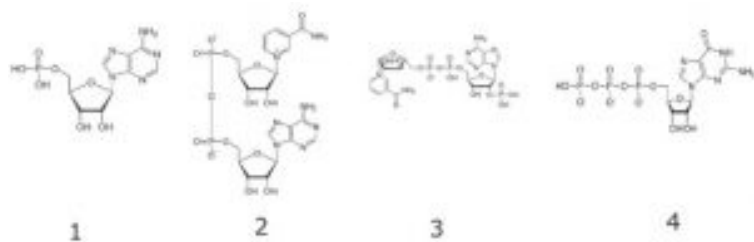
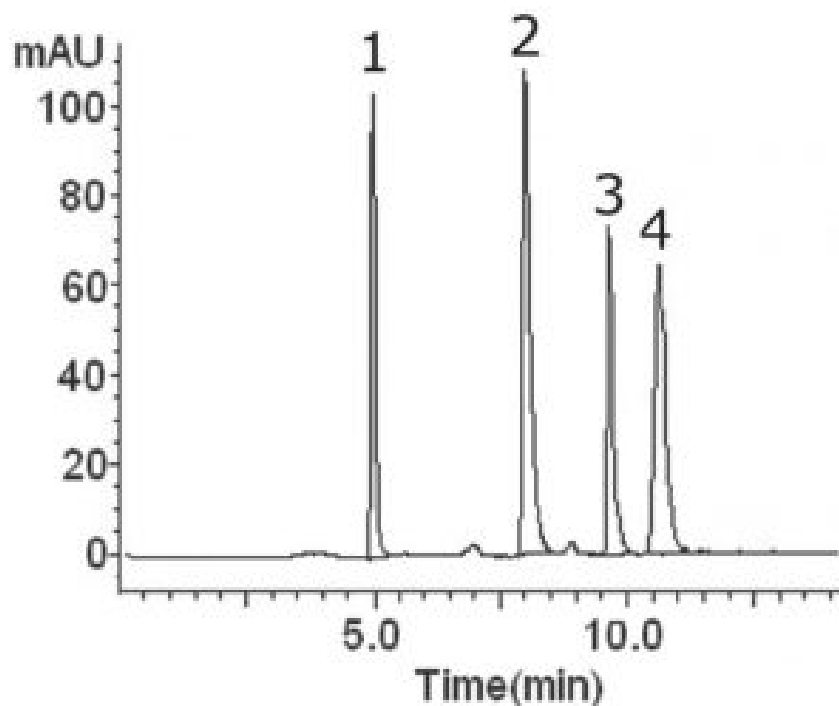


## Separation of Nucleotides - AppNote

### AMP, NAD, NADP, and GTP on UDA Column

Nucleotides are not well retained under Reversed Phase conditions due to their highly polar nature (the presence of one or more Phosphate groups). In this method, weak cation-exchange interactions can provide additional retention/selectivity along with the ANP retention of the Hydride surface.



#### Peaks:

1. AMP - Adenosine 5'-Monophosphate
2. NAD - Beta-nicotinamide Adenine Dinucleotide
3. NADP - NAD - Phosphate
4. GTP - Guanosine 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 Acetate

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

**Gradient:**

Time (minutes)	%B
0	95
0.5	95
10	75
15	30
20	30
20.1	95

**Temperature:** 25°C

**Post Time:** 3 minutes

**Injection vol.:** 1µL

**Flow rate:** 0.4mL / minute

**Detection:** UV @ 254nm

**Sample Preparation:** Stock Solution: 1 mg / 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.).

**t<sub>0</sub>:** 0.7 minutes

**Note:** The ratio of NAD to NADP has biological relevance when studying redox profiling and redox potential in the study of new generation NAD depleting Cytotoxic drugs. For metabolic screening, the Erythrocytes of Lesch-Nyhan Disease patients have grossly raised levels of NAD relative to NADP, while GTP is very low. Sensitive assay of GTP levels is also relevant to binding studies of G-proteins.



**Attachment**

No 260 Separation of Nucleotides pdf 0.2 Mb [Download File](#)