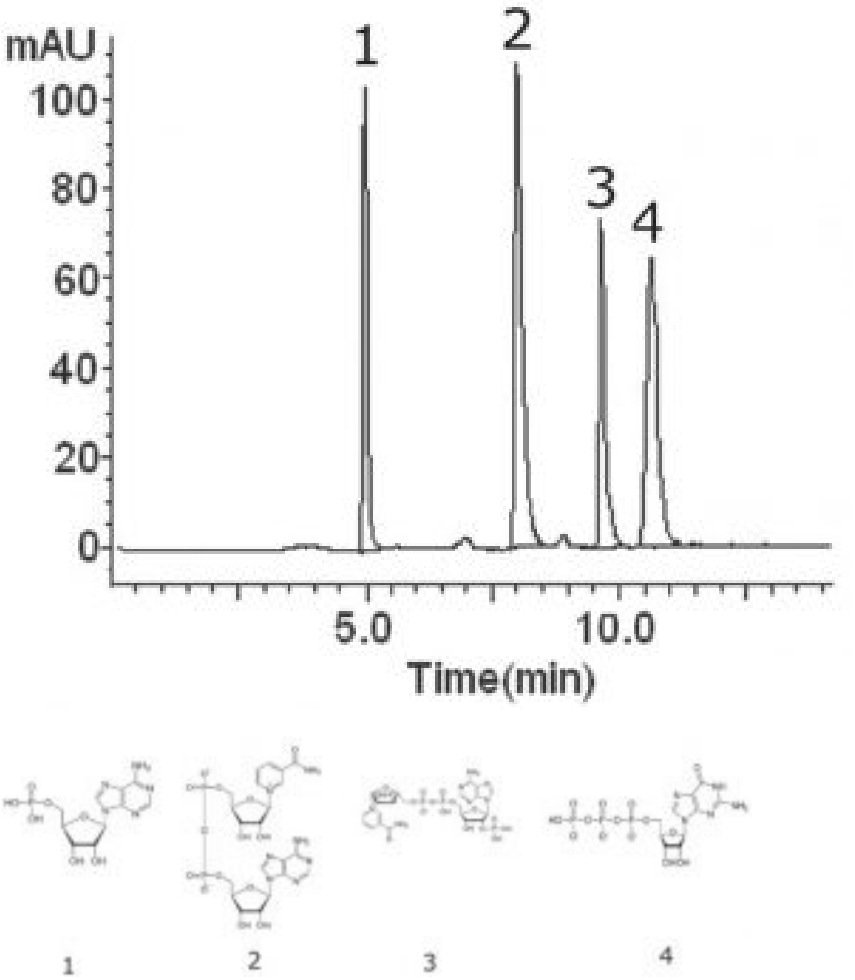
Separation of Nucleotides – AppNote

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_0: 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

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