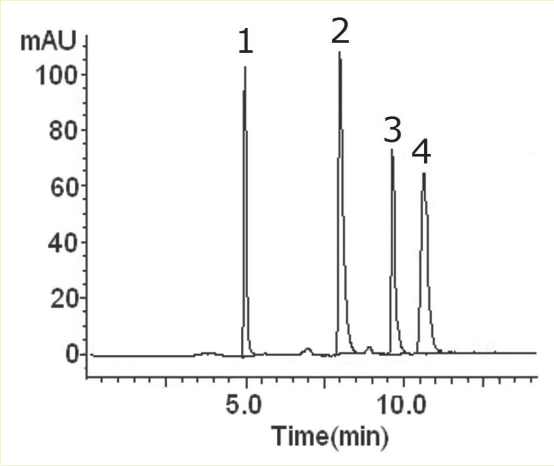
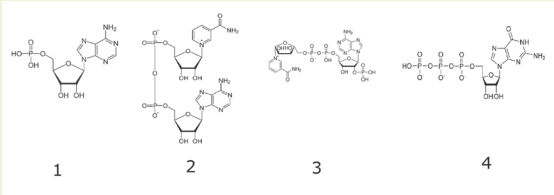
Separation of Nucleotides

AMP, NAD, NADP, and GTP on UDA column





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.

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 acetate
B: 90% acetonitrile / 10% DI H_2O / 16.0 mM ammonium acetate

Gradient:

time (min.)	%B
0	95
0.5	95
10	75
15	30
20	30
20.1	95

Temperature: 25˚C

Post Time: 3 min

Injection vol.: 1µL

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. NAD - beta-nicotinamide adenine dinucleotide
3. NADP – NAD - phosphate
4. GTP - guanosine 5' - triphosphate

t_0: 0.7 min

Discussion

The Cogent UDA HPLC column was used for analysis of nucleotides which are not well retained under reversed phase conditions due to their highly polar nature (the presence of one or more phosphate groups). In the case of the Cogent UDA column, weak cation-exchange interactions can provide additional retention/selectivity along with the ANP retention of the hydride surface.

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