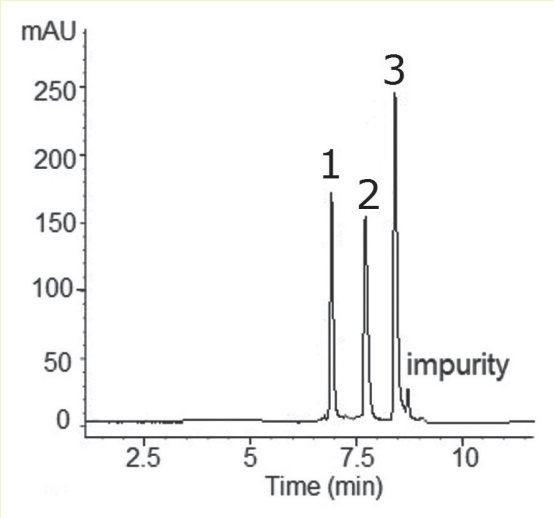
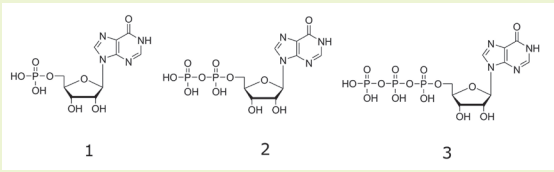
Separation of Inosine Nucleotides

IMP, IDP, and ITP on UDA column





Note: Deficiency of the enzyme ITP pyrophosphohydrolase is a common genetic defect in human populations and has aroused recent interest for its putative pharmacogenetic relevance to thiopurine therapy. The enzyme is part of a nucleotide ''futile cycle'', which converts IMP to IDP and ITP then back to IMP.

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	100
0.5	100
13	30
20	30
20.1	100

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 0.45 µm nylon syringe filter (MicroSolv Tech Corp).

Peak: 1. IMP – inosine 5'-monophosphate
2. IDP – inosine 5'-diphosphate
3. ITP – inosine 5'-triphosphate

t_0: 0.7 min

Discussion

The figure shows the optimized separation of ITP, IDP and IMP using the Cogent UDA column. The three targeted analytes were separated in the order of increasing phosphate content similar to anion exchange. The presence of at least one impurity near ITP and possibly a second near IMP precluded accurate determination of peak symmetry.

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