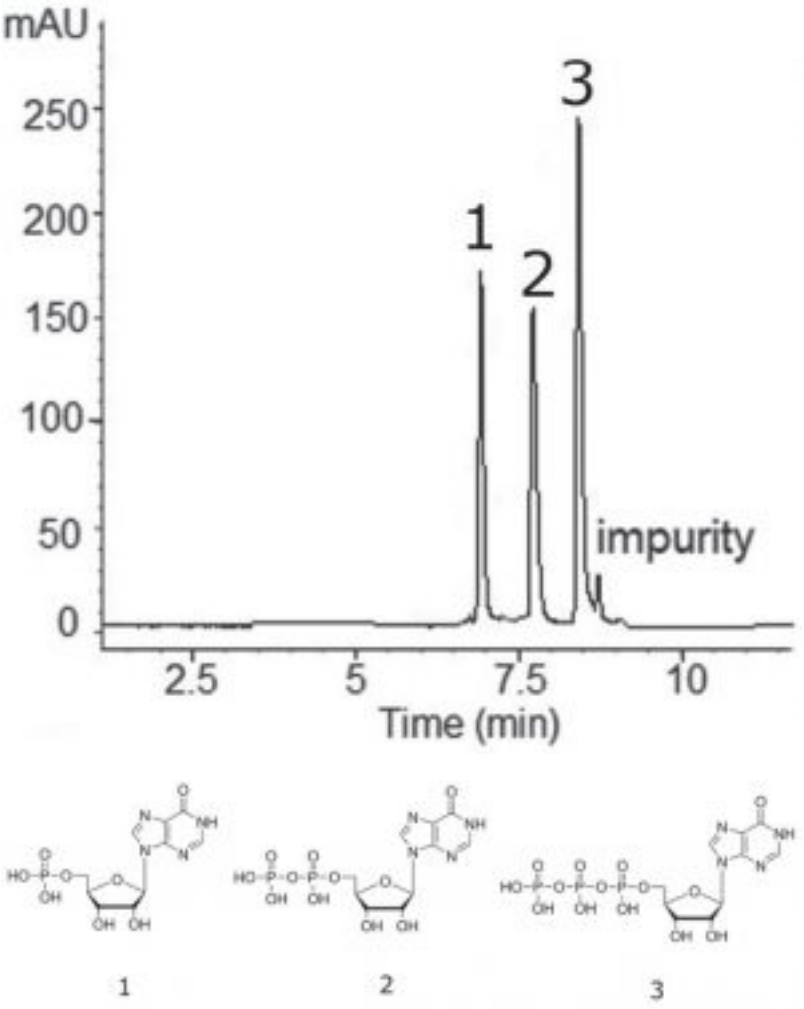
Separation of Inosine Nucleotides – AppNote

IMP, IDP, and ITP Analyzed by HPLC

The figure shows the optimized separation of ITP (Inosine 5’-monophosphate), IDP (Inosine 5’-diphosphate) and IMP (Inosine 5’-triphosphate) 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.



Peaks:

1. IMP – Inosine 5’-monophosphate
2. IDP – Inosine 5’-diphosphate
3. ITP – Inosine 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 Formate

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

Gradient:

Time *(minutes)*	%B
0	100
1.5	100
13	30
20	30
20.1	100

Temperature: 25˚C

Post Time: 3 minutes

Injection vol.: 1 µL

Flow rate: 0.4mL / minute

Detection: UV @ 254nm

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

Attachment

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