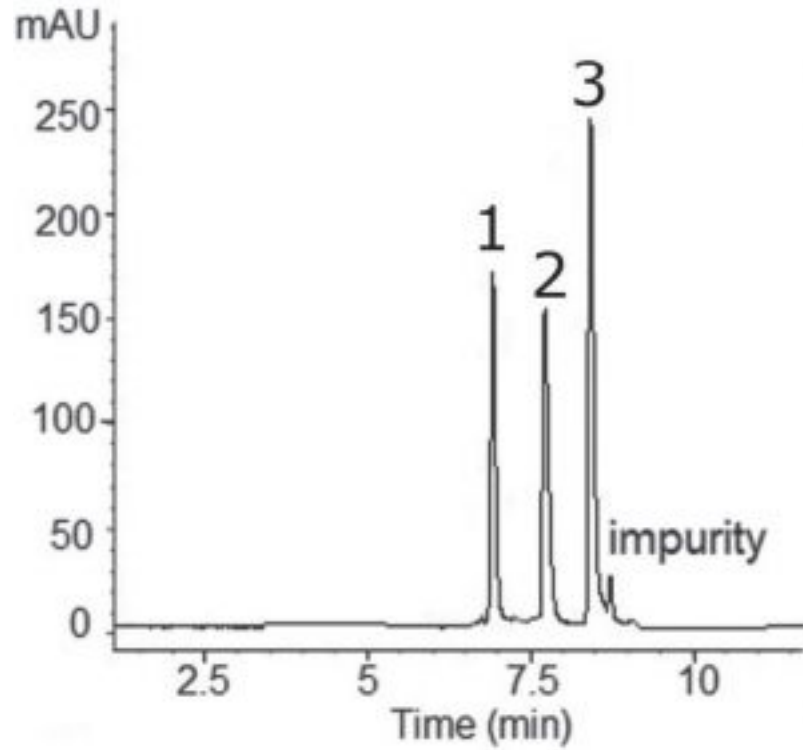


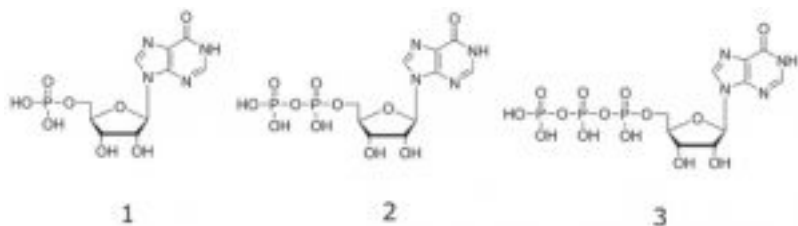


## Separation of Inosine Nucleotides - AppNote

### **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<sub>0</sub>:** 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.

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## Attachment

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