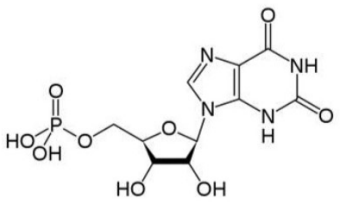
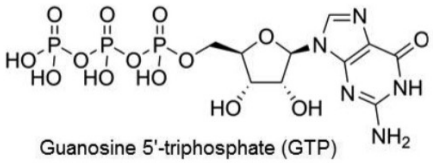
Purine Nucleotides

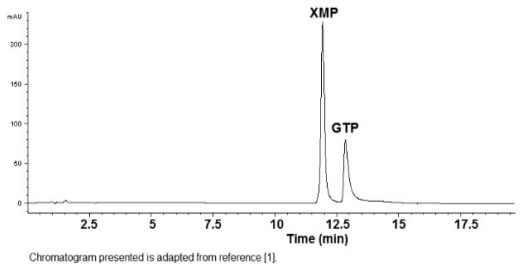
Fast Separation of XMP and GTP



Xanthosine 5'-monophosphate (XMP)



Guanosine 5'-triphosphate (GTP)



Chromatogram presented is adapted from reference [1].

Notes: Among the most important purine nucleotides, XMP and GTP are often analyzed from the lymphocytes of healthy people and HIV-1 seropositive patients at different stages of the disease (ARC-AIDS). Several differences in metabolism of purine nucleotides in the lymphocytes of the AIDS patients, were observed [2]. XMP does not normally appear in free nucleotide cell extracts, however it is the product of the important cell differentiation enzyme IMP dehydrogenase in ribavirin therapies.

[1]. "Aqueous normal phase retention of nucleotides on silica hydride- based columns: Method development strategies for analytes relevant in clinical analysis", M.T. Matyska, J.J. Pesek, J. Duley, M. Zamzami, S.M. Fisher, J. Sep. Sci. (2010/, 33, 930-938.

[2]. "Analysis of purine nucleotides in lymphocytes from healthy subjects and AIDS patients", A Tabucchi, F Carlucci, E Ramazzotti, MC Re, F Marinello, M Rubino, R Pagani , Biomedecine & Pharmacotherapy, Volume 46, Issue 1, 1992, Pages 25-29.

Method Conditions

Column: **Cogent Diamond Hydride™**, 4µm, 100Å

Catalog No.: 70000-15P-2

Dimensions: 2.1 x 150 mm

Solvents: A: DI H_2O + 0.1% ammonium formate
B: 90% Acetonitrile/ 10% DI H_2O/ 0.1% ammonium formate

Gradient:

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

Temperature: 25°C

Post Time: 5 min

Injection vol.: 1µL

Flow rate: 0.4 mL/min

Detection: UV diode array

Sample: 1. XMP: Xanthosine -5'-monophopate
2. GTP: Guanosine -5'-triphosphate

Discussion

This fast, simple and easy to use method achieves the separation of nucleotides XMP from GTP. The analysis of these polar compounds is achieved at high concentration of an organic solvent as part of the mobile phase (usually acetonitrile, but acetone may be used as well when MS detection is used) which provides increased sensitivity. Retention times were very reproducible with %RSD approximately 0.4, even when red blood cells extracts were injected in between the standard samples. Using a mass sprectometer this method can be specific and sensitive.

MANUFACTURED BY:
MICROSOLV TECHNOLOGY CORPORATION

9158 Industrial Blvd NE
Leland, NC 28451

p: 1.732.380.8900
f: 1.910.769.9435

customers@mtc-usa.com
www.Cogent-HPLC.com