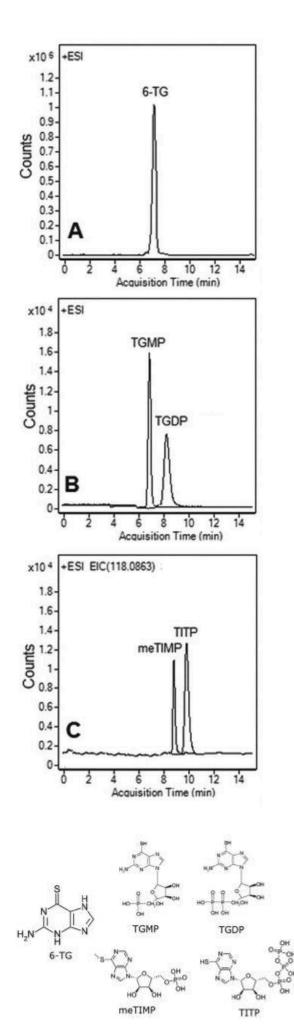


Analysis of Thiopurines - AppNote

Separation using LCMS Gradient Method

The Cogent Diamond Hydride Column with gradient elution was used for analysis of Thiopurines. *Figure A* shows the Peak of 6-TG, which also can be analyzed using Isocratic conditions. Good retention and symmetrical peak shape were obtained under the analysis conditions.

Figure B represents two separated Thiopurines (Mono and Di- Phosphate forms). Figure C shows two Inosine compounds, one with an additional Methyl group, being separated.



Peaks:

A: Thioguanine (6-TG) at m/z = 168.0338 [M+H]+

B: 6-Thioguanosine -5´-Phosphate (TGMP) at m/z = 380.3, 6-Thioguanosine -5´-Diphosphate (TGDP) at m/z = 460.3 C: 6-Methyl-Thioinosine-5´-Monophosphate (meTIMP) at m/z = 379.3 and 6-Thioinosine-5´-Triphosphate (TITP) at m/z = 525

Method Conditions

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

Catalog No.: <u>70000-15P-2</u> **Dimensions:** 2.1 x 150mm

Mobile Phase:

A: DI Water / 50% Methanol / 0.1% Formic Acid (v/v)

B: Acetonitrile / 0.1% Formic Acid (v/v)

Gradient:

Time (minutes)	%B
0	100
12	30
14	30
15	0
19	0
20	100

Post Time: 2 minutes Flow rate: 0.4 mL/minute

Detection: ESI – POS - Agilent 6210 MSD TOF Mass Spectrometer

Injection vol.: 1µL

Sample Preparation: 0.4 mg/mL solutions in DI Water. For MS analysis, samples were diluted 1:100 into 50% Acetonitrile / 50% DI Water mixture. Before injection, samples were filtered through a

0.45µm Nylon Syringe Filter (MICROSOLV Tech Corp.).



Attachment

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