

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.

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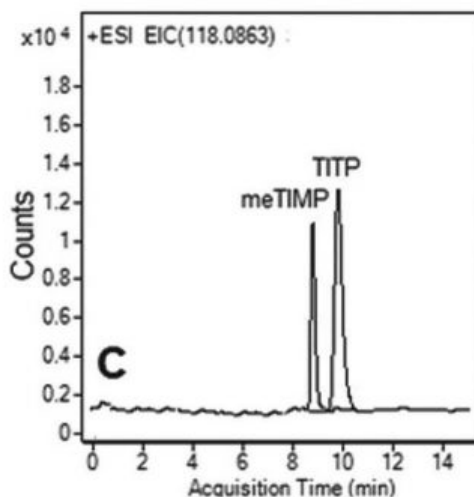
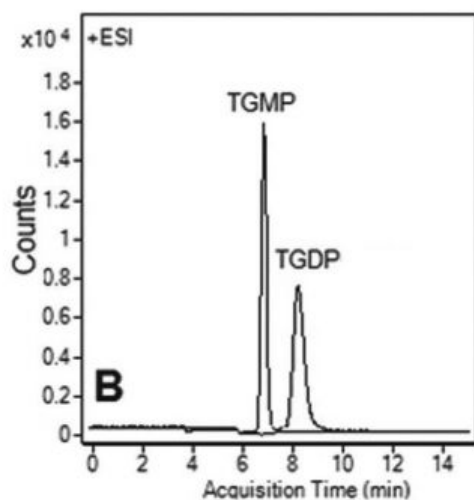
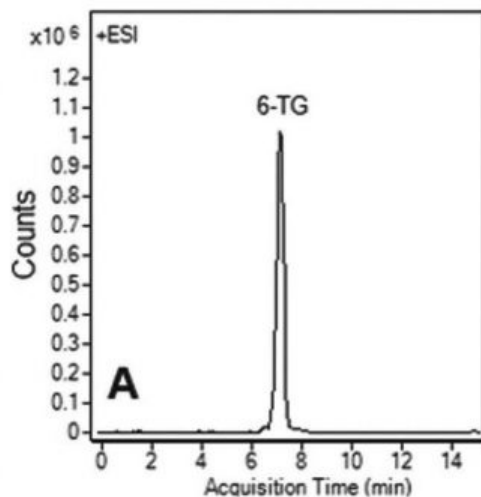
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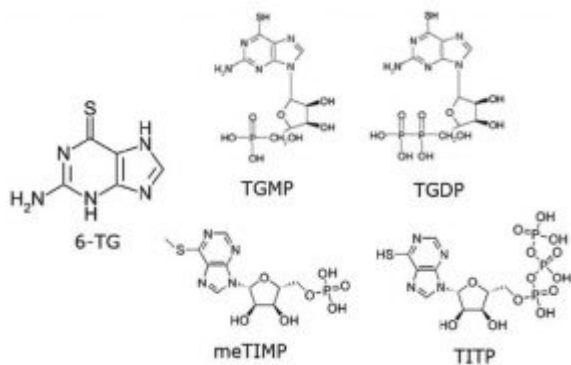
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Peaks:

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™, 4 μ m, 100Å

Catalog No.: 70000-15P-2

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.: 1 μ L

Sample Preparation: 0.4 mg/mL solutions in DI Water. For MS analysis, samples were diluted 1:100 into 90% Acetonitrile / 50% DI Water mixture. Before injection, samples were filtered through a 0.45 μ m Nylon Syringe Filter (MicroSolv Tech Corp.).

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Attachment

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