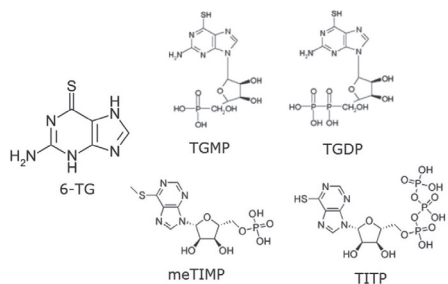
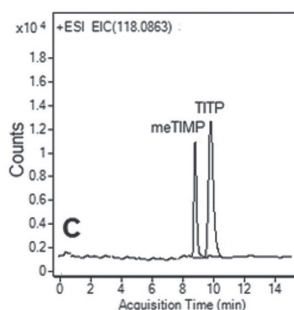
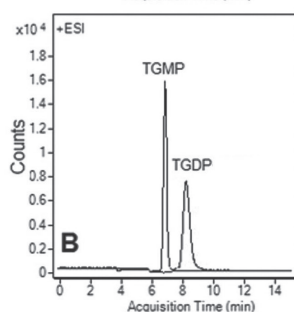
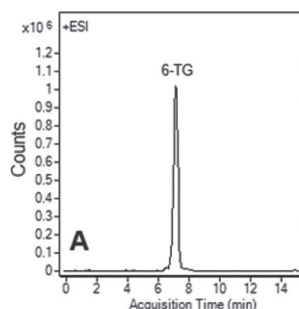


Analysis of Thiopurines

Separation using LC-MS gradient method



Figures:

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.: 70000-15P-2

Dimensions: 2.1 x 150 mm

Mobile Phase: A: DI H₂O / 50% methanol / 0.1% formic acid (v/v)
 B: Acetonitrile / 0.1% formic acid (v/v)

Gradient:	time (min.)	%B
	0	100
	12	30
	14	30
	15	0
	19	0
	20	100

Post Time: 2 min

Injection vol.: 1 microL

Flow rate: 0.4 mL/min

Detection: ESI - POS - Agilent 6210 MSD TOF mass spectrometer

Sample: Stock Solution: 0.4 mg/mL solutions in DI H₂O. For MS analysis, samples were diluted 1:100 into 50% acetonitrile / 50% DI H₂O mixture. Before injection, samples were filtered through a 0.45 µm nylon syringe filter (MicroSolv Tech Corp.).

Discussion

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. The peak shapes are all symmetrical.