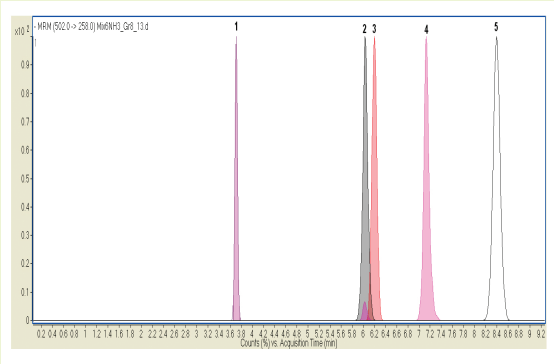
Phosphorylated Sugars by LCMS

UDP, ADP, TDP and CDP



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 pH 7.2
B: 90% acetonitrile / 10% DI H_2O / 0.1% ammonium formate pH 6

Gradient:

time (min.)	%B
0.0	95
10.0	75
12.0	75
12.1	95
15.0	95

Flow rate: 0.3 mL/min

Peaks: 1. ADP – glucose, RT = 3.68 min, monitored MRM transitions were m/z 588 to m/z 346
2. Proprietary sugar nucleotide, RT = 6.03 min, monitored MRM transitions were m/z 563 to m/z 321
3. Proprietary sugar nucleotide, RT = 6.20 min, monitored MRM transitions were m/z 606 to m/z 385
4. CDP – glucose, RT = 7.13 min, monitored MRM transitions were m/z 564 to m/z 322
5. UDP hexanolamine (internal standard), RT = 8.40 min, monitored MRM transitions were m/z 502 to m/z 258

MRM – multiple reaction monitoring in LC/MS/MS

Samples: 400mL DI H_2O / 400mL acetonitrile / 20mL of stock solution of each compound / 5mL of 12% ammonia

Detection: ESI – NEG - Agilent 6210 MSD TOF mass spectrometer

Note: Please note the addition of small amount of ammonia to the sample matrix. The alkaline environment of the sample matrix assured efficient and symmetrical peaks for all analytes.

Discussion

UDP hexanolamine was used as the internal standard in the analysis of nucleotide sugars in this rapid analysis method. The mobile phase was designed to maximize the detector response in LC-MS for maximum efficiency. The simple “inverse gradient” which produces ANP (aqueous normal phase) HPLC method was required for the results shown, therefore a Cogent Diamond Hydride column was chosen. This method can be used in measuring metabolite concentration.

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