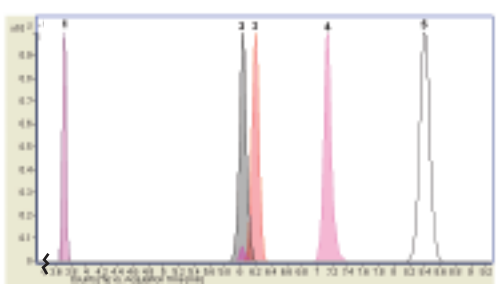
APPLICATION NOTES

Cogent™ Diamond-Hydride

HPLC
ANP

Phosphorylated Sugars by LCMS

UDP, ADP, TDP and CDP



Notes:
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.

Method Conditions

Column: Cogent Diamond Hydride™ 4µm, 100Å.
Catalog No.: 70000-15P-2
Dimensions: 2.1 x150 mm
Solvents: A: DI water + 0.1% ammonium formate pH 7.2
B: 90%acetonitrile + 10% DI water + 0.1% ammonium formate pH 6

Mobile phase:

Gradient:	*Time*	*%B*	*Time*	*%B*
	0.0	95	12.1	95
	10.0	75	15.0	95
	12.0	75		

Flow rate: 0.3 µL/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
Sample Preparation: 400 mL DI water+400 mL acetonitrile+20 mL of stock solution of each compound + 5 mL of 12% ammonia.
Detection: ESI – neg - Agilent 6210 MSD TOF mass spectrometer.

For more information visit www.MTC-USA.com

Cat. No.	Description
70000-15P-2	Cogent Diamond Hydride™ HPLC Column, 100Å, 4µm, 2.1 x 150mm

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