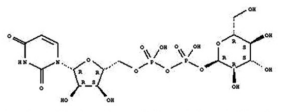
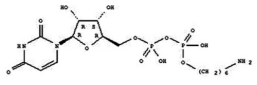
Activated Nucleotide Sugar

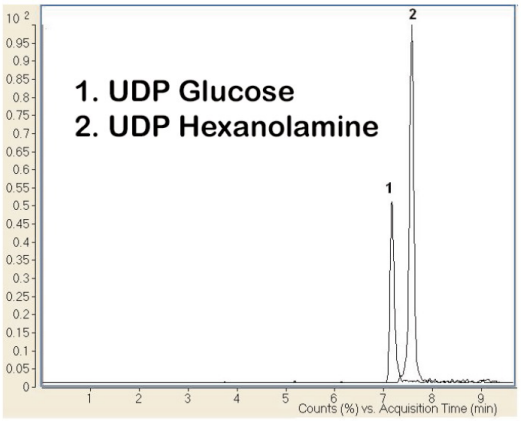
Analyzing UDP Glucose



1. Uridine 5′-diphosphate (UDP)–glucose



2. UDP - hexanolamine



Notes: Activated nucleotide sugars, such as UDP-glucose [UDP-Glc] are donor substrates of glycosyltransferases involved in protein glycosylation processes. Therefore, their availability can influence the glycosylation of proteins.
The HPLC method developed can also be used to quantify UDP-Glc, an important metabolic intermediate and the substrate of enzymes catalyzing glycosylation reactions. Thus several enzymes producing UDP-Glc can be directly assayed using the HPLC method described in this application note.

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	100
3	95
6	70
7	70
8	50
9	50

Post Time: 5 min

Flow rate: 300µL/min

Detection: ESI – neg – Agilent 6410 Triple Quadrupole LC/MS

Compounds: 1. UDP glucose - the monitored MRM transitions were m/z 565 to m/z 323
2. UDP hexanolamine (internal standard) - the monitored MRM transitions were m/z 502 to m/z 258
(MRM - multiple reaction monitoring in LC/MS/MS)

Discussion

The Aqueous Normal Phase (ANP) inverse gradient method shown above was used to analyze UDP glucose. UDP hexanolamine was used as an internal standard. The method is rapid and simple and it can be used in measuring the metabolite. The advantages of using this method to assay nucleotide sugars are the short separation time, excellent long term stability and rapid equilibration time when a gradient is used.

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