Nucleotides Analyzed with LCMS – AppNote

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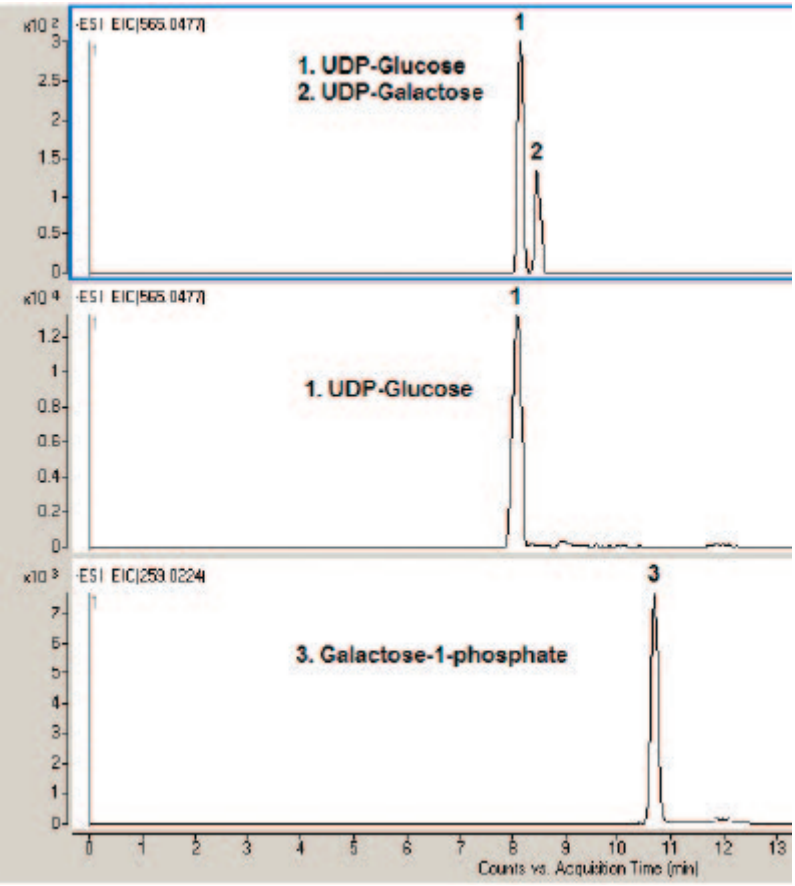
UDP-Glucose, UDP-Galactose and Galactose-1-phosphate from Blood Cells

For most physiologically relevant Nucleotides, use of Mass Spectrometry is the most practical approach for the analysis with biological samples. With Mass Spec, it is essential to Separate UDP-Glucose and UDP-Galactose chromatographically because they are Isomers or Isobaric Compounds, *(same molecular weight)* and cannot be easily differentiated.

This Method was developed using MS and the samples were blood cell extracts. The Method resulted in excellent Precision.



Peaks:

1. UDP-Glucose: Uridine 5′-Diphosphateglucose, 565.0477 m/z (M-H)-
2. UDP-Galactose: Uridine 5′-Diphosphategalactose, 565.0477 m/z (M-H)-
3. Galactose-1-phosphate, 259.0224 m/z (M-H)-

Method Conditions

Column: Cogent Diamond Hydride™ 4µm, 100Å.

Catalog No.: 70000-15P-2

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Dimensions: 2.1mm x 150mm

Mobile Phase:

A: DI Water / 0.1% Formic Acid

B: 90% Acetonitrile /10% DI Water / 16.5mM Ammonium Acetate

Gradient:

Time *(minutes)*	%B
0	95
1	95
3	85
6	85
7	75
9	75
10	50
12	50
13	30
15	30
15.01	95

Post Time: 5 minutes

Flow Rate: 0.4mL / minute

Samples Preparation:

Stock Standard Solutions for analysis were prepared in DI Water (1mg / mL of each sample) and were stored at -20°C. For LCMS Analysis samples were diluted 1:100 with 50% Acetonitrile / 50% DI Water Solution.

Detection: ESI – NEG: – Agilent 6210 MSD TOF Mass Spectrometer.

Notes: *UDP-Glucose, UDP-Galactose and Galactose 1-phosphate determination can be used for diagnosis of Galactosemia in newborn babies [1-3].*

Literature: *[1]. Ji-Seon Jeong, Ha-Jeong Kwon, Hye-Ran Yoon, Yong-Moon Lee, Tae-Young Choi, Seon-Pyo Hong, A pulsed amperometric detection method of galactose 1-phosphate for Galactosemia diagnosis, Analytical Biochemistry 376 (2008) 200-2005.*

[2]. Ji-Seon Jeong, Hye-Ran Yoon, Seon-Pyo Hong, Development of a new diagnostic method for Galactosemia by high-performance anion exchange chromatography with pulsed amperometric detection, J. Chromatography A, 1140 (2007) 157-162.

[3]. Christopher J. Easley, Lian Ji Jin, Katja B. Presto Elgstoen, Egil Jellum, James P. Landers, Jerome P. Ferrance, Capillary Electrophoresis with Laser-induced Fluorescence Detection for laboratory diagnosis of Galactosemia, J. Chromatography A, 1004 (2003) 29-37.

Attachment

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