

Separation of ATP, UDP-Glucose UDP-Galactose In Red Blood Cell Extracts

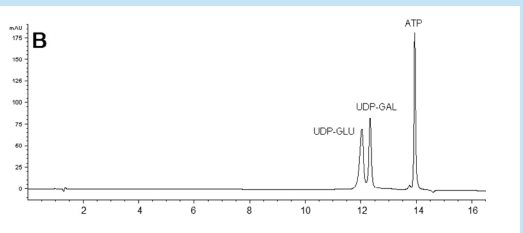
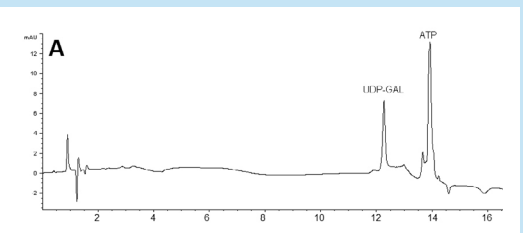
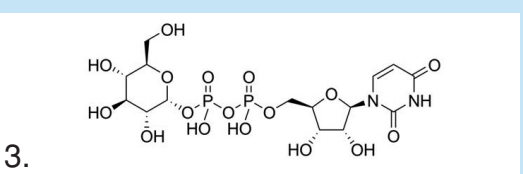
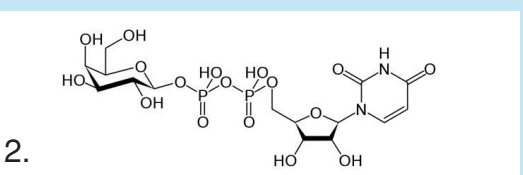
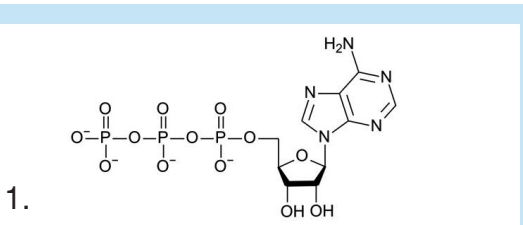


Figure adapted from reference 2

Method Conditions

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

Catalog No.: 70000-7.5P

Dimensions: 4.6x75mm

Solvents: A: DI water/0.1% ammonium acetate
B: 90% acetonitrile/10% DI water/0.1% ammonium acetate

Gradient:	Time (min)	%B	Time (min)	%B
	0.0	100.0	10.0	80.0
	1.0	95.0	10.5	50.0
	8.0	95.0	14.0	50.0
	9.0	80.0	14.0	100.0

Post Time: 5 min.

Flow Rate: 0.4 mL/min

Injection: 1 mL

Samples: 1. UDP-GLU:

UDP-glucose: uridine 5'-diphosphateglucose, 1 mg/mL

2. UDP-GAL:

UDP-galactose: uridine 5'-diphosphategalactose, 1 mg/mL

3. ATP: *Adenosine 5'-triphosphate, 1 mM*

Detection: UV:254 nm

Discussion

Figure A shows a sample (spiked with UDP-Galactose) of a red blood cell extract from a patient for monitoring. Figure B shows separation of standards using the same method. Retention times were very reproducible with %RSD around 0.4. The desired separation between UDP-Glu and UDP-Gal was achieved, all other nucleotides eluted later and did not interfere with the analysis. ATP represents the most abundant nucleotide in the red blood cell extracts and in this example it eluted after both analytes.

Notes: Even with UV detection it is possible to detect individual nucleotides in the presence of a biological matrix. The retention time can be compared to the standard (Figure B) showing that the values of the real sample and the standard are very close. UDP-glucose, UDP-galactose and galactose 1-phosphate determination can be used for diagnosis of galactosemia in newborn babies [1-2].

1. 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.
2. M.T. Matyska, J.J. Pesek, J. Duley, M. Zamzami, S.M. Fischer, Aqueous normal phase retention of nucleotides on silica hydride-based column: Method development strategies for analytes relevant in clinical analysis, *J. Sep. Sci.* 33 (2010) 930-938

Cat. No.	Description
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70000-7.5P	Cogent Diamond Hydride™ HPLC Column, 100Å, 4µm, 4.6x75mm
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