

Experimental conditions

HPAEC-PAD	High-performance anion-exchange chromatography with pulsed amperometric detection
Device	Thermo Scientific Dionex ICS-5000 ⁺
Mobile phases	A water (pure), B NaOH 100 mM
Stationary phase	Column: CarboPac™ PA1, 2 x 250 mm Pre-column: CarboPac™ PA1, 2 x 50mm
Electrode	Gold working electrode for carbohydrates Dionex™ ED disposable electrode for electrochemical detectors (Dionex Gold Disposable Electrode)
Reference electrode	Ag/AgCl
Temperature	25 °C column temperature
Injection volume	5 µL
Software	Chromeleon 7.2

Gradient elution

[min]	Flow [mL/min]	% A	%B	curve
0	0.20	80	20	5
16	0.20	80	20	5
21	0.25	0	100	5
26	0.25	0	100	5
29	0.20	80	20	5
33	0.20	80	20	5

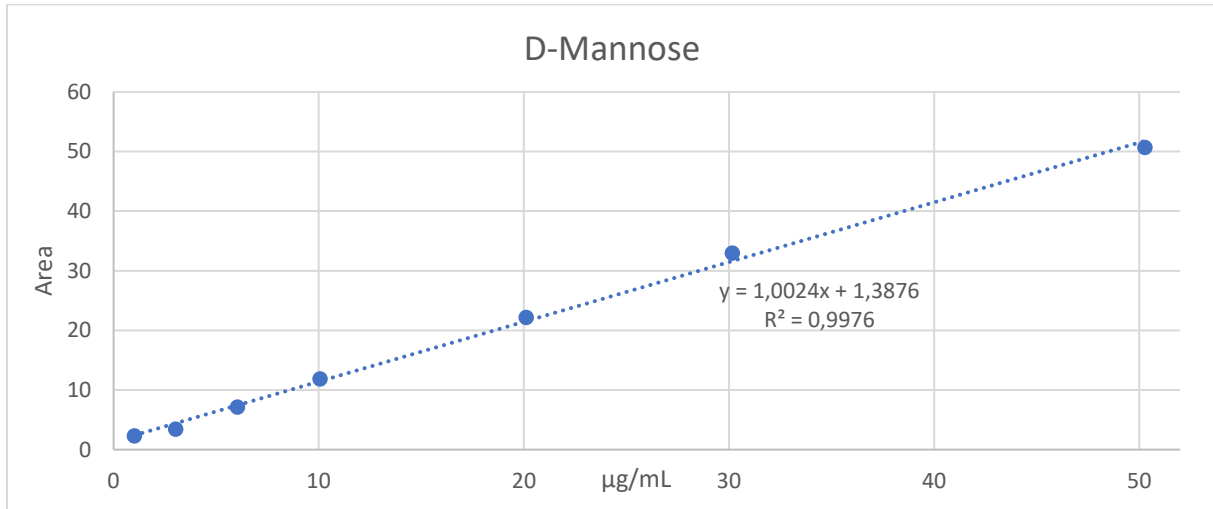
Waveform (for carbohydrates)

[sec]	Potential (V) vs. Ag/AgCl	Integration
0.00	+0.1	
0.20	+0.1	Begin
0.40	+0.1	End
0.41	-2.0	
0.42	-2.0	
0.43	+0.6	
0.44	-0.1	
0.50	-0.1	

Calibration with D-Mannose

Linearity

All solutions in water (pure); 7 concentration levels: c [$\mu\text{g}/\text{mL}$] = 50.28 / 30.17 / 20.11 / 10.06 / 6.03 / 3.02 / 1.01



Sequence-Calibration

Three concentration levels (6 / 20 / 32 [$\mu\text{g}/\text{mL}$]) before and after each sample series.

Table of weights

All solutions in water (pure)

Declared mannose content [g/unit]	Weighing-in	1 st volumetric flask: volume [mL]	2 nd volumetric flask: dilution [mL/mL]	Expected concentration in the test sample [$\mu\text{g}/\text{mL}$]
0.5	1 unit	250	5 / 500	20
1.0	1 unit	500	5 / 500	20
2.0	1 unit	1000	5 / 500	20

Sample preparation

Weigh out the mannose preparations exactly according to the table, transfer with water into the 1st volumetric flask (250 – 1000 mL), place in the ultrasonic bath for extraction (solution) for 5 min, fill up to the mark with water.

Dilution: Take aliquot according to table and pipette into a 2nd volumetric flask (500 mL), fill up to the mark with water.

Sample chromatograms

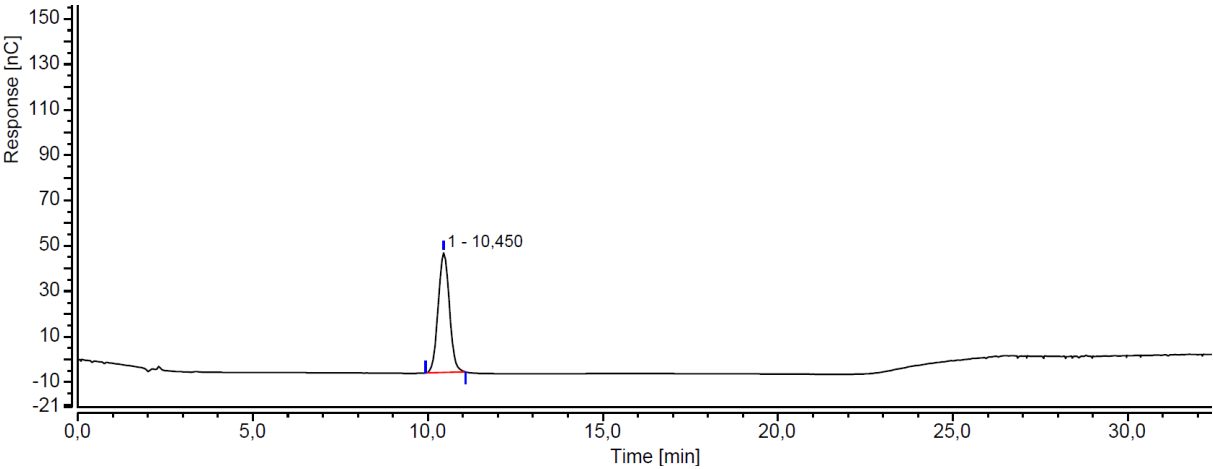


Fig. 1 Sample #6 (2013,7 mg / 1000 mL; dilution 1:100 prior to injection)

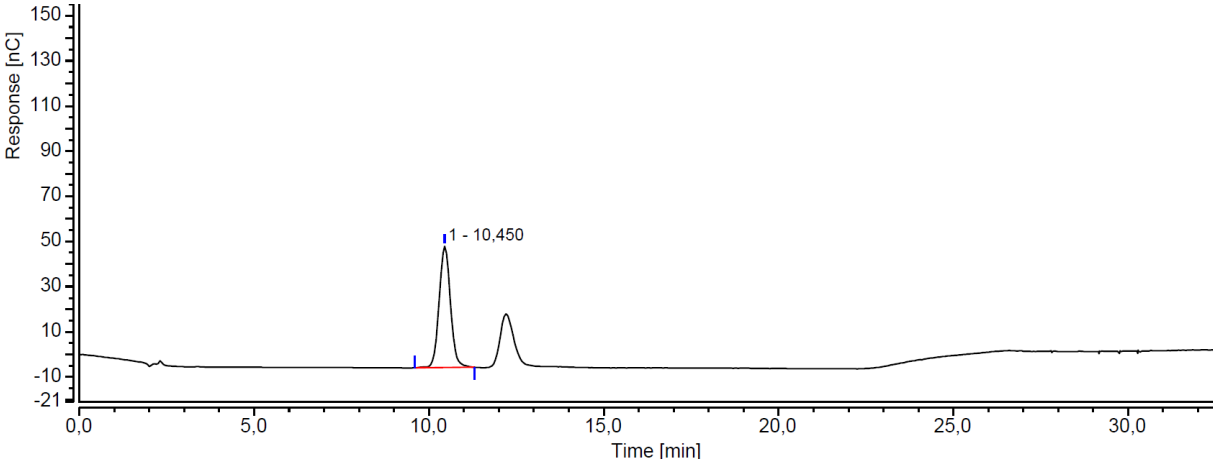


Fig. 2 Sample #4 (1 sachet / 1000 mL; dilution 1:100 prior to injection). 2nd peak (12.2 min) = Fructose