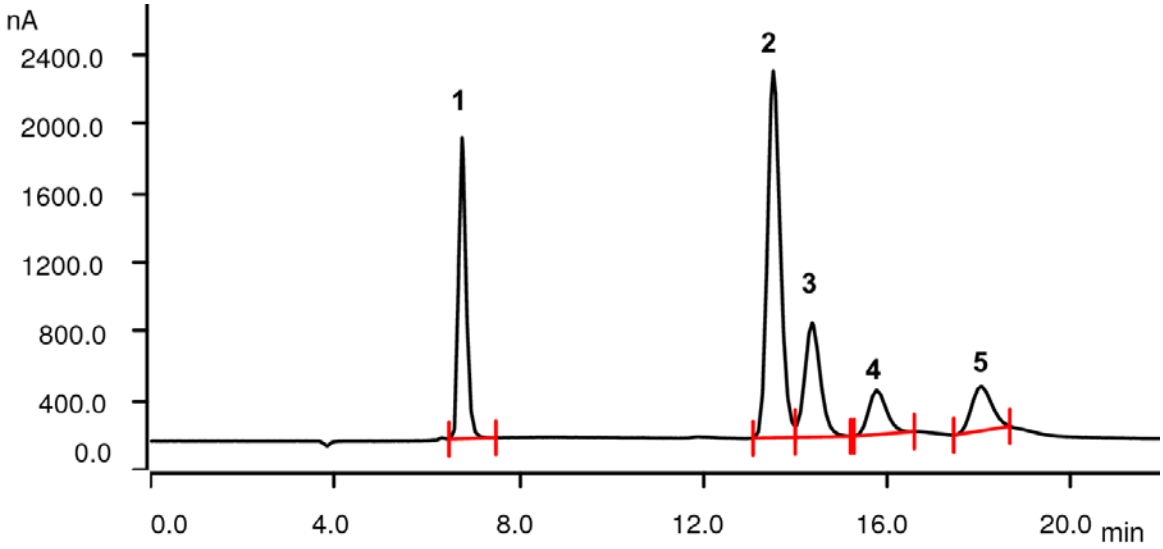
IC Application Note P–77

Proof of concept for the determination of lactose and its derivatives as well as sialic acid* in fermentation broths



The separation of lactose, lactobionic acid, sialic acid*, 6'-sialyllactose, and 3'-sialyllactose is shown as a proof of concept for the control of these components in fermentation process for a pharmaceutical product. The acceptance criterion of a minimum resolution of the peaks (< 1.3) is reached. The separation is achieved on a Metrosep Carb 2 - 250/4.0 column with subsequent Pulsed Amperometric Detection.

Results

Component	Concentration [mg/L]	Resolution
Lactose	5.0	16.8
Lactobionic acid	5.0	1.5
Sialic acid*	5.0	2.1
6'-sialyllactose	5.0	3.0
3'-sialyllactose	5.0	-

* Sialic acid = N-acetlyneuraminic acid

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Sample

Standards

Sample preparation

None

Columns

Metrosep Carb 2 - 250/4.0	6.1090.430
Metrosep Carb 2 Guard/4.0	6.1090.500

Solutions

Eluent	300 mmol/L sodium hydroxide 100 mmo/L sodium acetate

Parameters

Flow rate	0.5 mL/min
Injection volume	20 µL
Pmax	20 MPa
Recording time	22 min
Column temperature	40 °C

PAD Parameters

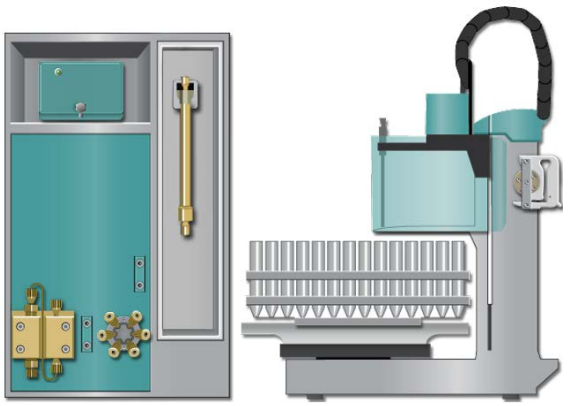
Cell	Wall-Jet cell
Working electrode	Gold
Reference electrode	Palladium
Spacer	50 µm
Measuring potential	0.05 V
Measuring duration	100 ms
Cycle duration	550 ms
Measuring range	200 µA
Temperature	35 °C
Mode	PAD

Analysis

Pulsed amperometric detection

Instrumentation

930 Compact IC Flex Oven/Deg	2.930.2160
IC Amperometric Detector	2.850.9110
858 Professional Sample Processor	2.858.0020
IC equipment Wall-Jet cell: Carb (Au, Pd)	6.5337.010



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