

Determination of Lactose in Lactose-Free Products by High-Performance Anion-Exchange Chromatography with Electrochemical Detection

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INTRODUCTION

Lactose is a disaccharide found in milk products and is catabolized into glucose and galactose by the enzyme lactase. Lactose-intolerant individuals have a lactase deficiency; therefore, lactose is not completely catabolized. Commercially available lactose-free products are produced by breaking down lactose into glucose and galactose by enzymatic hydrolysis. However, the resulting milk products contain varying amounts of residual lactose. This has created the need for simple, reliable, and accurate analytical methods to quantify lactose.

Milk changes structurally and chemically when heat-treated, but the extent of the change depends on the temperature and duration of the heating. Lactulose is a disaccharide containing galactose and fructose that is not naturally found in raw milk but is formed during the heat treatment of milk by the isomerization of lactose. Lactulose levels in milk can be used to determine the method used to sterilize the milk. The average lactulose content when using in-container sterilization is 744 mg/L, but only 3.5 mg/L in milk treated by low-temperature pasteurization methods.¹

The AOAC Method 984.15 uses enzymatic hydrolysis of lactose to glucose and galactose at pH 6.6 by β -galactosidase. This method is time-consuming by requiring extensive reagent preparations. The reported detection limits of this assay may not allow for the analysis of lactose in lactose-free samples.² The work shown here describes a sensitive and accurate method to determine lactose and lactulose in dairy products, including lactose-free products, using high-performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD) in six different commercial products. Lactose-free Gouda and Havarti cheeses had no detectable lactose, while cottage cheese and lactose-free milk had 2.9 mg/mL and 0.58 mg/mL of lactose, respectively. Spike recoveries for lactose and lactulose ranged from $85 \pm 6\%$ to $101 \pm 4\%$, suggesting method accuracy.

EXPERIMENTAL

Instrument

Dionex ICS-3000 system consisting of:

SP Single pump (P/N 061707), or dual pump (P/N 061712)
with degas option

DC Detector compartment (P/N 061767) single or dual temperature zone
Electrochemical detector (P/N 061719)

Disposable Au on PTFE Working Electrode, Pack of 6 (with 2 mil
gaskets included) (P/N 066480)

pH-Ag/AgCl Reference electrode (P/N 061879)

AS Autosampler (P/N 061289) with cooling tray option (recommended)

Chromeleon[®] Chromatography Data System (CDS) software

Method Conditions

Columns: CarboPac[®] PA20 Analytical column, 3 × 150 mm
(Dionex P/N 060142)
CarboPac PA20 Guard column, 3 × 30 mm
(Dionex P/N060144)

Flow Rate: 0.4 mL/min

Injection Volume: 10 μ L

Tray Temp: 4 °C

Detection: Integrated pulsed amperometry, Au on PTFE
disposable or conventional Au working electrodes

Waveform: Carbohydrate (standard quad)

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Time (s)	Potential (V)	Gain Region*	Ramp*	Integration
0.00	+0.1	Off	On	Off
0.20	+0.1	On	On	On
0.40	+0.1	Off	On	On
0.41	-2.0	Off	On	Off
0.42	-2.0	Off	On	Off
0.43	+0.6	Off	On	Off
0.44	-0.1	Off	On	Off
0.50	-0.1	Off	On	Off

**Settings required when using the ICS-3000 or ICS-5000 systems, but not used for older Dionex systems.*

Eluent: A: Water
 B: 200 mM sodium hydroxide
 C: 100 mM sodium acetate, 200 mM sodium hydroxide
 D: 1 M sodium acetate, 200 mM sodium hydroxide

Time (min)	A%	B%	C%	D%
0–10	94	6	0	0
20–31	90	7.5	2.5	0
33–43	0	25	0	75
43.1	0	100	0	0
49	0	100	0	0
49.1	94	6	0	0
65	94	6	0	0

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SAMPLES AND STANDARDS

Standards were prepared using α -lactose, monohydrate, β -D-glucose, D-galactose, lactulose, and sucrose (Sigma P/N L-3625, G-5250, G-0625, L-7877, and S-9378, respectively). Samples were prepared using the Carrez method, followed by applying the sample to the OnGuard® IIA cartridges, to remove any anionic contaminants and neutralize the samples. For more extensive details of the method, refer to reference 3.

RESULTS AND DISCUSSION

To optimize the separation of lactose and lactulose in the presence of expected sample carbohydrates, a mixed carbohydrate standard was prepared.

SEPARATION

Figure 1 shows a chromatogram of a mixed carbohydrate standard with an optimized gradient for the separation of lactose and lactulose. The retention times of galactose, glucose, sucrose, lactose, and lactulose were 9.63, 10.65, 13.79, 22.98, and 24.36 in respectively. All the carbohydrates were well separated from each other, including lactose and lactulose.

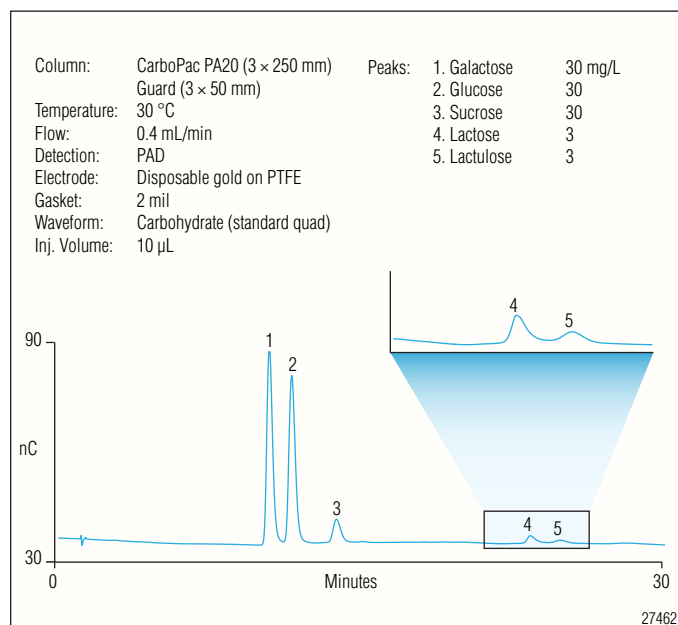


Figure 1. Separation of a mixed-carbohydrate standard, including lactose and lactulose.

COLUMN-TO-COLUMN REPRODUCIBILITY

Due to the close elution of lactose and lactulose, the authors confirmed the method ruggedness by evaluating the separation on three columns from three different lots. Figure 2 shows that for each column, lactose and lactulose are well resolved.

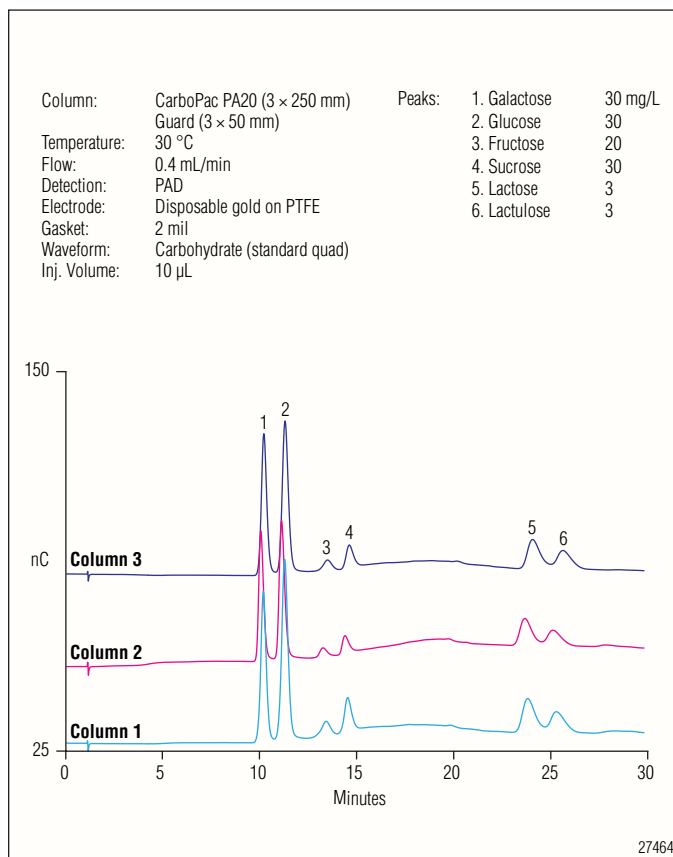


Figure 2. Overlay of the lactose and lactulose separation on columns from three different lots.

METHOD PERFORMANCE

Table 1 shows intraday reproducibility measured by making 30 consecutive injections of a mixed-carbohydrate standard containing 30 mg/L each of galactose, glucose, and sucrose; 3 mg/L each of lactose and lactulose; and 20 mg/L fructose. The method exhibited good short-term reproducibility; the intraday retention time RSDs ranged from 0.12 for lactulose to 0.16 for sucrose, and the peak area RSDs ranged from 1.03 for galactose to 5.06 for lactulose.

The MDLs for lactose and lactulose were determined by making seven injections of a low level solution fortified with lactose and lactulose at 3 to 5 times the estimated MDL. The calculated MDLs in DI water obtained by this method were 0.12 mg/L for lactose and 0.23 mg/L for lactulose. Table 2 summarizes the data for this determination.

Table 1. Intraday Reproducibility				
Analyte	Conc. (mg/L)	RSD		
		Ret. Time	Peak Area	Peak Height
Galactose	30	0.15	1.03	1.01
Glucose	30	0.14	1.30	1.08
Sucrose	30	0.16	1.51	1.37
Lactose	3	0.13	3.70	5.74
Lactulose	0	0.12	5.06	5.42

Table 2. Linearity and MDLs for Lactose and Lactulose				
Analyte	Range (mg/L)	r ²	Calculated MDL (mg/L)	MDL Standards (mg/L)
Lactose	0.25–100	0.9966	0.12	0.5
Lactulose	0.5–100	0.9942	0.23	1.0

SAMPLE ANALYSIS

The optimized separation was applied to two samples matrices; lactose-free Gouda cheese and lactose-free Havarti cheese. Figure 3A shows overlaid chromatograms of fortified and unfortified Gouda cheese. Trace 1 shows a separation of unfortified cheese with no detectable lactose. Trace 2 shows the separation of a Gouda cheese sample fortified with 10 mg/L each of lactose and lactulose. This chromatogram shows that lactose and lactulose are well separated from each other and matrix-related interferants. Figure 3B shows overlaid chromatograms of fortified and unfortified Havarti cheese. Trace 1 shows a separation of unfortified Havarti cheese with no lactose detected. Trace 2 shows the separation of a Havarti cheese sample fortified with 10 mg/L each of lactose and lactulose.

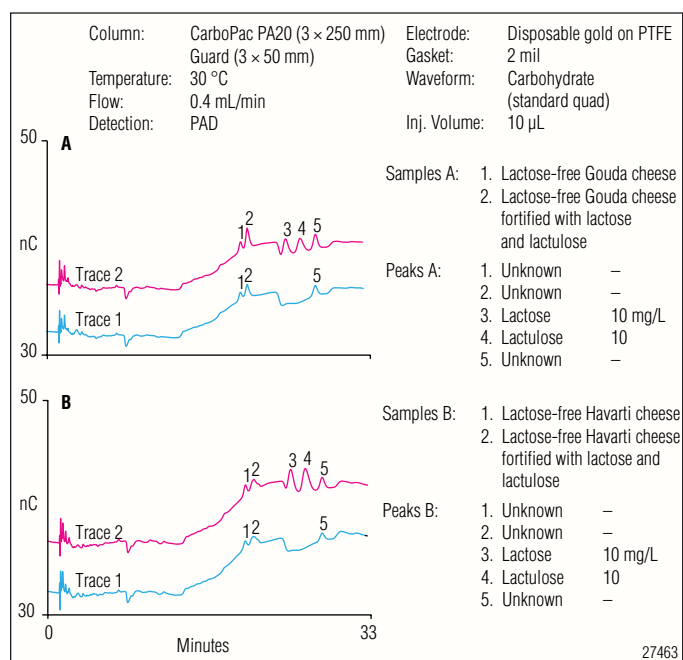


Figure 3. A) Separation of carbohydrates in fortified and unfortified lactose-free Gouda cheese samples. B) Separation of carbohydrates in fortified and unfortified lactose-free Havarti cheese

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Several milk-based products were evaluated for their lactose and lactulose content. Figure 4A shows the separation of carbohydrates in whole milk. The prepared milk samples were diluted 1:10 to prevent overloading with lactose. The diluted milk sample showed some galactose and glucose and large amounts of lactose. Figure 4B shows the separation of carbohydrates in lactose-free milk. The chromatogram shows that lactose-free milk contains high concentrations of galactose and glucose and trace amounts of lactose (0.6 mg/L, 0.00006%). This product showed the lowest detected lactose concentration among the products evaluated.

A duplicate of each of the samples was fortified with known amounts of lactose and lactulose prior to sample preparation. Recoveries were calculated following analysis of both native and spiked samples. Recoveries of lactose and lactulose for all matrices were 85 to 100% (Table 3).

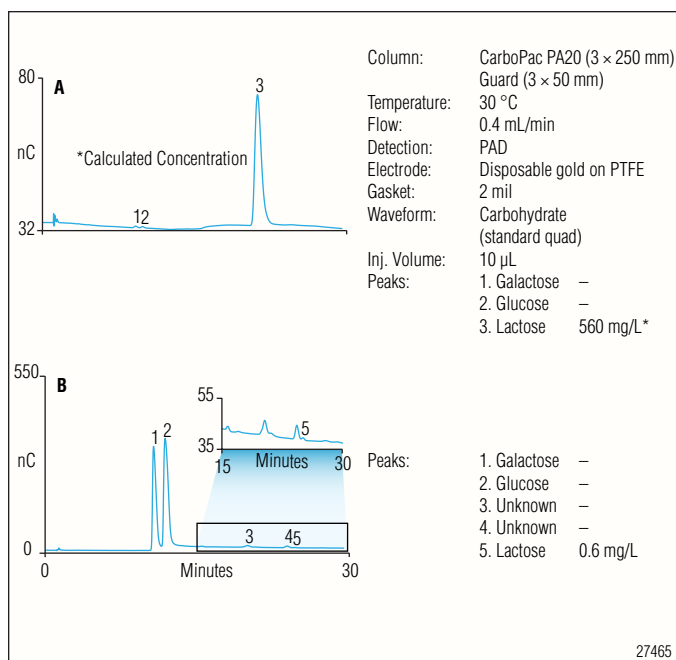


Figure 4. A bi-panel showing the following chromatograms: A) separation of carbohydrates in whole milk, and B) separation of carbohydrates in lactose-free low-fat milk.

Table 3. Recovery of Lactose and Lactulose from Various Matrices

Matrix	Amount Added (mg/L)	Lactose Recovery (%) n = 3	Lactulose Recovery (%) n = 3
Whole milk (1:10 diluted)	10	85.3	98.1
Lactose-free low fat milk	10	97.6	94.5
Lactose-free gouda cheese	10	90.1	100.8
Lactose-free havarti cheese	10	99.7	93.2

CONCLUSION

- A sensitive and accurate method to extract, separate, and quantify lactose and lactulose from other carbohydrates commonly found in milk-based products is described.
- The method accurately determines lactose at low concentrations in lactose-free products without the error and labor associated with analyte derivatization
- Recoveries of lactose and lactulose for all matrices were 86–100%.

REFERENCE

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2. Lynch, J. M.; Barbano, D. M. Determination of Lactose Content of Fluid Milk by Spectroscopic Enzymatic Analysis Using Weight Additions and Path Length Adjustment: Collaborative Study. *J. AOAC Int*. **2007**, *90*, 196–216.
3. Dionex Corporation. *Determination of Lactose in Lactose-Free Milk Products by High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection*. Application Note 248, LPN 2503, 2009, Sunnyvale, CA.

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