Rapid Determination of Lactose and Lactulose in Dairy Products Using High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection

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Overview

Purpose: Demonstrate fast separations of lactose and lactulose in dairy samples by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD) using a Thermo Scientific™ Dionex™ ICS-5000+ High-Pressure™ Ion Chromatography (HPIC™) system.

Methods: Dairy samples were incubated with Carrez solution, diluted, and the precipitate pelleted by centrifugation. Following neutralization and filtration by a Thermo Scientific™ Dionex™ OnGuard™ IIA cartridge, the carbohydrates were separated on a Thermo Scientific™ Dionex™ CarboPac™ SA10-4μm column using an HPIC system and quantified by comparing the signal from pulsed ampeometric detection to that of standards.

Results: Lactose and lactulose eluted within 8 min with good baseline resolution. The method demonstrated accurate (100–113% recovery) and reproducible (<4 RSDs) from sub-mg/L to double digit mg/L quantifications.

Introduction

Lactose and lactulose are important components in milk-based products. Lactose is the major milk disaccharide which is metabolized with the aid of lactase to the monosaccharides, glucose and galactose. Lactase-deficient and lactose-intolerant individuals have difficulties in digesting milk products resulting in uncomfortable intestinal symptoms such as diarrhea and bloating. To meet the demands of this population, lactose-free products are commercially produced by enzymatic hydrolysis with lactase.[1] However, the enzymatic hydrolysis process is not 100% efficient. Therefore commercial suppliers need accurate and robust methods to determine lactose concentrations in milk products and residual lactose in lactose-free products. Currently there are no defined lactose concentration limits or regulations governing lactose-free products, however lactose determinations are needed to meet the ingredient labeling requirements.

Pasteurization heat treatment is recommended for sterilization of milk products, but as a result, some lactose is isomerized to lactulose.[2] Lactulose is not found in nature, and therefore not absorbed by the human digestive system. For the same reason, lactulose is used as a sugar substitute in calorie-reduced foods. However, lactulose can be hydrolyzed to galactose and fructose by microbial activity in the intestinal tract, providing digestive relief as a by-product. The concentration of lactulose is of interest as an indication of milk product degradation and when being used as a sugar substitute or pharmaceutical ingredient. Lactose has been analyzed by many methods including photometric, polarimetry, and fluorometry, but these methods are time consuming and not specific for lactose and lactulose.[3] High-Performance Anion-Exchange (HPAE) chromatography with Pulsed Amperometric Detection (PAD) is a well established sensitive method that selectively and directly determines carbohydrates.[1] With the introduction of 4 µm resin particle Dionex CarboPac SA10-4µm fast carbohydrate columns, these analytes can be separated with both increased signal-to-noise and shorter analysis times than have been previously possible.

Methods

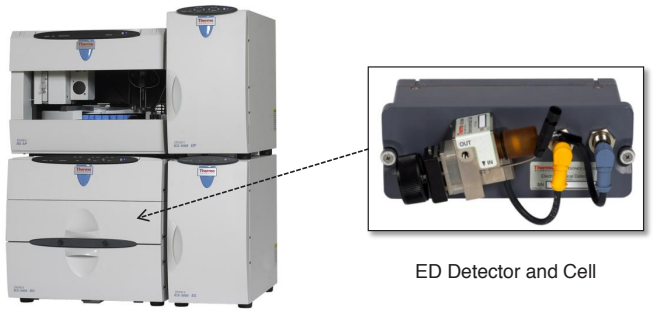
Sample Preparation

Pasteurized Grade A 2% milk, raw milk (unpasteurized), and lactose-free yogurt were treated with Carrez I and Carrez II solutions to precipitate proteins and other high molecular weight molecules while keeping carbohydrates in solution. Following dilution, the mixture was centrifuged and the supernatant filtered and neutralized using a Dionex OnGuard IIA sample treatment cartridge. For a complete method description, see Thermo Scientific Technical Note (TN) 146: *Fast Determinations of Lactose and Lactulose in Milk Products Using HPAE-PAD*.[4]

Equipment and Data Analysis

Dionex ICS-5000+ HPIC system with an electrochemical detection (ED) module
Thermo Scientific Dionex AS-AP Autosampler
Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS) software

FIGURE 1. A Dionex ICS-5000⁺ HPIC system with the module containing the electrochemical detector (ED) and cell indicated.



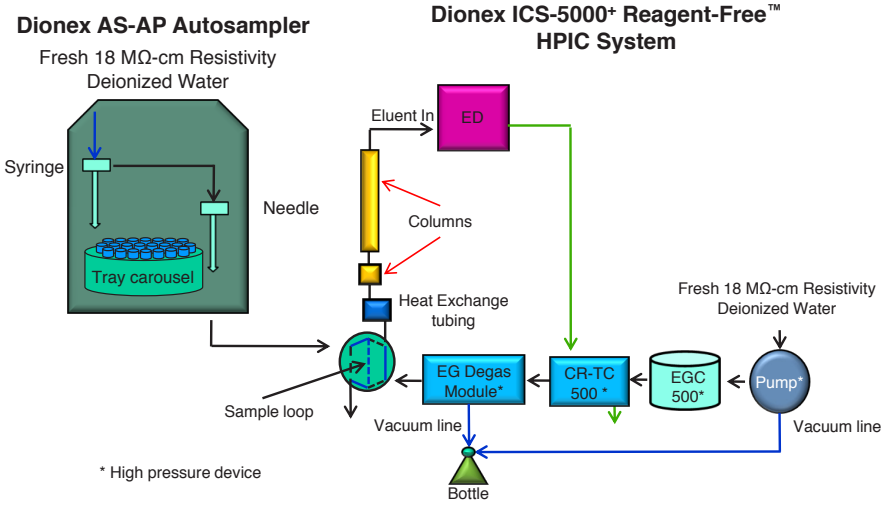
ED Detector and Cell

Conditions

Columns: Dionex CarboPac SA10 guard and Dionex CarboPac SA-10-4µm separation columns, 4 mm i.d
Eluent Source: Thermo Scientific Dionex EGC 500 KOH cartridge
Detection: PAD, Four-potential carbohydrate waveform

The HPIC setup and flow path is shown in Figure 2.

FIGURE 2. Flow diagram for the Dionex ICS-5000⁺ HPIC system with an electrochemical detector (ED).



Results

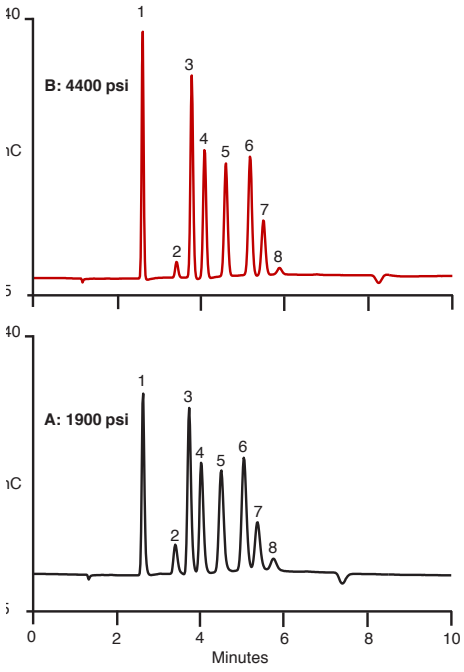
The Dionex CarboPac SA10-4µm anion-exchange carbohydrate column was selected for this application for its characteristic fast isocratic separations of monosaccharides and disaccharides without the need for manually prepared acetate eluents. The new 4 µm resin particle format results in highly efficient separations and higher signal-to-noise ratios than larger particle formats, resulting in higher reporting reliability. With smaller particle size columns, a high-pressure capable IC system, such as the Dionex ICS-5000⁺ HPIC system, is needed to facilitate analysis.

Figure 3 compares the separations of a carbohydrate standard mixture using the same conditions on both the 6 µm and 4 µm resin particle versions of the Dionex CarboPac SA10 column. As can be seen, the peak efficiencies increased by > 60% when the performance of the 4 µm particle size column is compared to that of the 6 µm particle size column.

Method Optimization

To achieve baseline resolution, while preserving fast run times, the following conditions were selected for this method: 4 mM KOH at 1.45 mL/min and 35 °C, which resulted in all analyte peaks eluting before 8 min (Figures 4–6).

FIGURE 3. Comparison of separations on 6 µm (A) and 4 µm (B) resin particle columns.



Column:	A: Dionex CarboPac SA10, 4 mm i.d. B: Dionex CarboPac SA10-4µm, 4 mm i.d.
Eluent Source:	Dionex EGC 500 KOH cartridge
Eluent:	1 mM KOH
Flow Rate:	1.5 mL/min
Inj. Volume:	10 µL
Column Temp.:	45 °C
Detection:	PAD, Au on PTFE disposable, Four-potential carbohydrate waveform
Gasket:	0.002" thick PTFE
Ref. Electrode:	pH-Ag/AgCl
Standard:	5 mg/L each

Peaks:

	Peak Efficiencies (EP)* A	B	
1. Fucose	8400	14000	plates
2. Sucrose	6000	10600	
3. Arabinose	9400	15800	
4. Galactose	8200	14000	
5. Glucose	7700	18000	
6. Xylose	9000	15000	
7. Mannose	8700	15000	
8. Fructose	7500	12000	

* Approximate values based on individual columns

To determine the accuracy of the method, the recoveries of lactose and lactulose added to the samples was measured (Table 1). The diluted milk samples were separately spiked with 0.5 mg/L of lactulose or 5 mg/L lactose, whereas the diluted yogurt sample was spiked with 0.5 mg/L lactose and lactulose. The recoveries ranged from 99 to 113% for lactose and 89 to 94% for lactulose.

TABLE 1. Results of lactose and lactulose recovery experiments.

Sample	Lactose			Lactulose		
	Added (mg/L)	Recovered* (mg/L)	%	Added (mg/L)	Recovered* (mg/L)	%
Lactose-free yogurt	0.5	0.48 ± 0.02	99.8	0.5	0.448 ± 0.020	89.0
Raw milk	5.0	10.0 ± 0.04	113	0.5	0.478 ± 0.014	95.6
2% Pasteurized milk	5.0	9.62 ± 0.06	108	0.5	0.470 ± 0.008	94.0

*n = 3

The method exhibited good stability based on retention time (data not shown) and peak area reproducibilities (RSDs < 4; Table 2).

TABLE 2. Lactose and lactulose peak area reproducibility.

100-fold Diluted Sample*	Lactose		Lactulose	
	Peak Area (nC-cm)	RSD	Peak Area (nC-cm)	RSD
Lactose-free yogurt	--	--	--	--
Lactose-free yogurt + 0.5 mg/L standard	0.21 ± 0.02	0.69	0.104 ± 0.01	1.92
Raw milk	1.80 ± 0.02	0.22	--	--
Raw milk + standard**	3.77 ± 0.02	0.44	0.103 ± 0.00	2.51
2% Pasteurized milk	1.99 ± 0.01	0.62	--	--
2% Pasteurized milk + standard**	4.00 ± 0.11	2.8	0.104 ± 0.01	1.92
0.5 mg/L Standard	0.22 ± 0.02	0.7	0.106 ± 0.01	3.28
5.0 mg/L Standard	2.21 ± 0.02	0.6	1.04 ± 0.01	1.21

n = 3

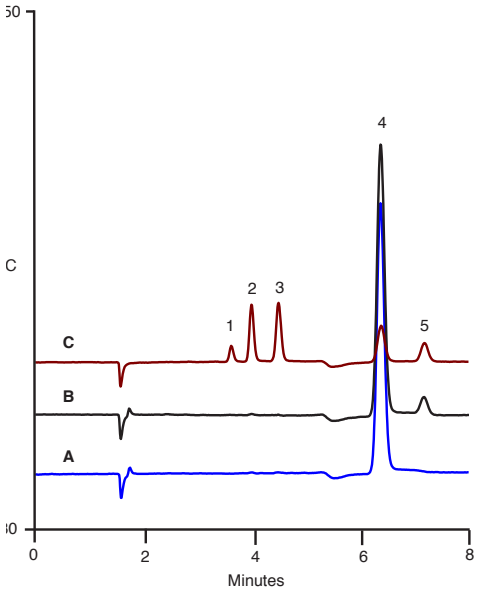
* Diluted after sample preparation

** 5 mg/L of lactose and 0.5 mg/L of lactulose

Sample Analysis

Figures 4–5 compare the chromatograms of spiked samples to those of the samples prior to any addition of lactose and/or lactulose. In Figure 4, lactulose is well resolved from lactose and shows approximately equivalent quantification when the spiked sample is compared to the carbohydrate standard.

FIGURE 4. Lactose and lactulose in raw unpasteurized milk.

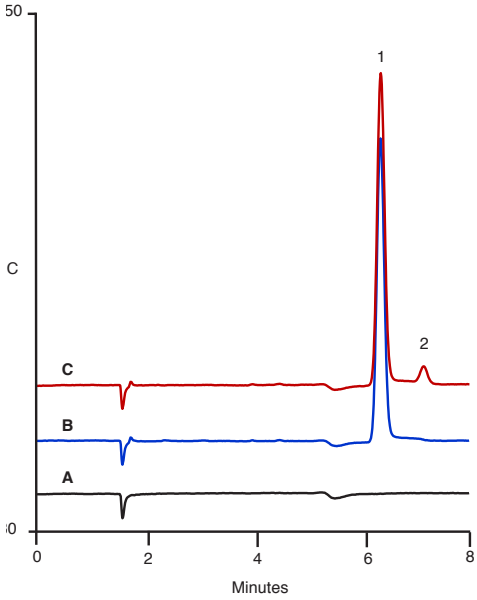


Column:	Dionex CarboPac SA10-4μm and guard, 4 mm i.d.
Eluent Source:	Dionex EGC 500 KOH cartridge
Eluent:	4 mM KOH
Flow Rate:	1.45 mL/min
Inj. Volume:	10 μL
Column Temp.:	35 °C
Detection:	PAD, Au on PTFE disposable, Four-potential carbohydrate waveform
Gasket:	0.002" thick PTFE
Ref. Electrode:	pH-Ag/AgCl
Sample Prep.:	Carrez digestion, centrifuge, filter, Dionex OnGuard IIA cartridge
Sample:	A: 100-fold diluted raw, unpasteurized milk B: Sample A + 0.5 mg/L lactulose C: 0.5 mg/L carbohydrate standard

Peaks:	A	B
1. Sucrose	--	-- mg/L
2. Galactose	--	--
3. Glucose	--	--
4. Lactose	3.75	3.77
5. Lactulose	--	0.48

Comparable recovery of lactulose was obtained for 2% fat Grade A pasteurized milk following addition of this carbohydrate as shown in Figure 5.

FIGURE 5. Lactose and lactulose in 2% fat Grade A pasteurized milk.

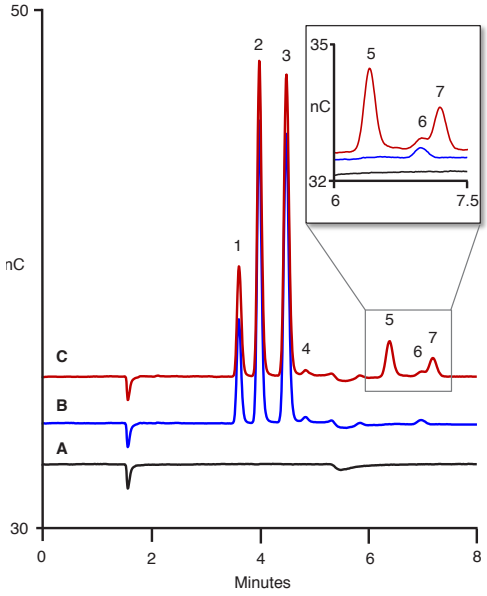


Column:	Dionex CarboPac SA10-4μm and guard, 4 mm i.d.
Eluent Source:	Dionex EGC 500 KOH cartridge
Eluent:	4 mM KOH
Flow Rate:	1.45 mL/min
Inj. Volume:	10 μL
Column Temp.:	35 °C
Detection:	PAD, Au on PTFE disposable, Four-potential carbohydrate waveform
Gasket:	0.002" thick PTFE
Ref. Electrode:	pH-Ag/AgCl
Sample Prep.:	Carrez digestion, centrifuge, filter, Dionex OnGuard IIA cartridge
Sample:	A: Water blank B: 100-fold diluted 2% fat, U.S. Grade A Pasteurized milk C: Sample B + 0.5 mg/L lactulose

Peaks:	B	C
1. Lactose	4.43	4.40 mg/L
2. Lactulose	--	0.47

The chromatogram of the lactose-free yogurt (Figure 6) shows baseline resolution (Rs (EP) = 2.1) of lactose from the next eluting peak (Peak 6). However, this unknown peak (Peak 6), which may result from a different anomeric carbohydrate form, is barely resolved from lactulose (Rs (EP) = 1.0). To obtain more accurate determinations in the presence of this peak, it may be necessary to modify the processing conditions so that the integration is dropped vertically to the baseline.

FIGURE 6. Lactose and lactulose in lactose-free yogurt.



Column:	Dionex CarboPac SA10-4μm and guard, 4 mm i.d.
Eluent Source:	Dionex EGC 500 KOH cartridge
Eluent:	4 mM KOH
Flow Rate:	1.45 mL/min
Inj. Volume:	10 μL
Column Temp.:	35 °C
Detection:	PAD, Au on PTFE disposable, Four-potential carbohydrate waveform
Gasket:	0.002" thick PTFE
Ref. Electrode:	pH-Ag/AgCl
Sample Prep.:	Carrez digestion, centrifuge, filter, Dionex OnGuard IIA cartridge
Sample:	A: Water blank B: 100-fold diluted lactose-free yogurt C: Sample B + 0.5 mg/L lactose and 0.5 mg/L lactulose

Peaks:	B	C
1. Sucrose	2.8	2.8 mg/L
2. Galactose	2.3	2.3
3. Glucose	2.1	2.1
4. Unknown	--	--
5. Lactose	--	0.48
6. Unknown	--	--
7. Lactulose	--	0.47

Conclusion

This poster demonstrates a fast, accurate, and reproducible method for lactose and lactulose determinations in diluted milk samples and lactose-free products. The method measures lactose and lactulose from sub-mg/L to double digit mg/L concentrations that is accurate (100 to 113% recoveries) and reproducible (<4 RSDs). Lactose and lactulose elutes within 8 min with good baseline resolution, Rs (EP) = 7, in the milk samples. In the yogurt sample, the unknown peak eluting near lactulose reduces the lactose and lactulose resolution to Rs (EP) = 3 and Rs (EP) = 1.1, respectively.

References

1. Thermo Fisher Scientific Application Note AN 248: Determination of Lactose in Lactose-Free Milk Products by High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection. Sunnyvale, CA, 2012. [Online] http://www.thermoscientific.com/content/dam/tfs/ATG/CMD/CMD%20Documents/Application%20&%20Technical%20Notes/Chromatography/Ion%20Chromatography/AN-248-Lactose-Milk-Products-HPAE-PAD-AN70236-EN.pdf (accessed Aug. 5, 2014).
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