

Fast Determinations of Lactose and Lactulose in Milk Products Using HPAE-PAD

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Key Words

Food, Dionex CarboPac SA10-4 μ m, lactose, lactulose, Dionex ICS-5000⁺

Goal

Demonstrate fast separations of lactose and lactulose in food samples by HPAE-PAD using a high-pressure Thermo Scientific™ Dionex™ ICS-5000⁺ HPIC™ system.

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.

The United States Public Health Service/Food and Drug Administration (USPHS/FDA) and the Centers for Disease Control and Prevention (CDC) recommend a pasteurization heat treatment process to sterilize milk products primarily from *Salmonella*, *Escherichia coli* O157, and *Campylobacter* bacteria to meet public health and safety.²⁻⁴ As a result of the heat treatment process, some



lactose is isomerized to lactulose.⁵ 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 microbiological activity in the intestinal tract. The resulting effect may provide digestive comfort by increasing bowel motion. As such, lactulose is an approved EU food additive for digestive comfort or prescribed at higher doses to treat constipation.^{6,7} The concentrations of lactulose are of interest to both the consumer and commercial milk producers as an indication of milk product degradation. For example, lactulose concentrations average around 744 mg/L in milk when using in-container sterilization but ~200-fold lower (3.5 mg/L) by low temperature pasteurization methods.⁵ Assays are also needed when using lactulose 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.⁸⁻¹¹ The standard method by the Association of Official Analytical Chemists (AOAC) is Method 984.15 which uses enzymatic hydrolysis of lactose at pH 6.6 by β -galactosidase. This method is also time consuming, requires extensive reagent preparations, and is not sufficiently sensitive for the determination of lactose in lactose-free samples.¹² High performance anion-exchange (HPAE) chromatography with pulsed amperometric detection (PAD) is a well established sensitive method that selectively and directly determines carbohydrates, such as lactose and lactulose.^{1,13,14} With the introduction of 4 μ m resin particle Thermo Scientific™ Dionex™ CarboPac™ SA10-4 μ m fast carbohydrate columns, these analytes can be separated with both increased signal-to-noise and shorter analysis times than have been previously possible. Here we demonstrate fast separations of lactose and lactulose in milk products by HPAE-PAD on the 4 mm Dionex CarboPac SA10-4 μ m column, which was facilitated by the high-pressure capable Dionex ICS-5000+ HPIC system.

Equipment

High-Pressure Dionex ICS-5000+ HPIC system including:

- Thermo Scientific Dionex ICS-5000+ SP/DP Pump module
- Thermo Scientific Dionex ICS-5000+ EG Eluent Generator module with high-pressure degas module
- Thermo Scientific Dionex ICS-5000+ DC Detector/Chromatography module with ED detector module
- Thermo Scientific Dionex AS-AP Autosampler with temperature control
- EG/DP/SP Vacuum Degas Conversion Kit, P/N 063353

Thermo Scientific™ Dionex™ Chromeleon™ 7.2 Chromatography Data system (CDS) software

Reagents and Standards

18 M Ω -cm resistivity degassed deionized water

50% (w/w) Sodium hydroxide (Fisher Scientific, P/N SS254-500)

Fisher Scientific reagents, ACS grade

- Monosaccharide and disaccharide reagents
- Potassium hexacyanoferrate(III)
- Zinc sulfate heptahydrate
- pH Buffer solutions, pH = 7, pH = 10

Samples

Pasteurized Grade A 2% milk, raw milk (unpasteurized), and lactose-free yogurt.

Conditions

Columns:	Dionex CarboPac SA 10 guard and Dionex CarboPac SA10-4 μ m separation columns, 4 \times 250 mm
Eluent Source:	Thermo Scientific Dionex EGC 500 KOH cartridge
Eluent:	4 mM KOH from -3 to 8 min*
Flow Rate:	1.45 mL/min
Column Temp.:	35 °C
Inj. Volume:	10 μ L
Detection:	PAD, Four-Potential Carbohydrate waveform (Table 1) ¹⁵
Working Electrode:	Gold on PTFE Disposable Electrode
Reference Electrode:	pH-Ag/AgCl
Detection Temp.:	20 °C
Autosampler Temp.:	10 °C
Background:	20–40 nC
Noise:	< 20 pC
System backpressure:	4800 psi

* Add a column wash every 10 sample injections at 100 mM KOH with a 5 min equilibration at 4 mM KOH to maintain column stability.

The four-potential waveform is optimized to provide a stable gold oxide layer which results in reproducible carbohydrate determinations (Table 1).

Table 1. Waveform A, four-potential carbohydrate waveform.¹⁵

Time (sec)	Potential (V)	Gain*	Ramp*	Integration
0.00	+ 0.100	Off	On	Off
0.20	+ 0.100	On	On	On
0.40	+ 0.100	Off	On	Off
0.41	- 2.000	Off	On	Off
0.42	- 2.000	Off	On	Off
0.43	+ 0.600	Off	On	Off
0.44	- 0.100	Off	On	Off
0.50	- 0.100	Off	On	Off

* Settings for Thermo Scientific Dionex ICS-5000, Dionex ICS-5000+ HPIC and Thermo Scientific Dionex ICS-3000 IC systems.

Table 2. Consumables list.

Product Name	Type	Part Number
Dionex EGC 500 KOH*	High-pressure Anion Eluent Generator cartridge	075778
Thermo Scientific Dionex CR-ATC 500* Continuously Regenerated Anion Trap Column	High-pressure electrolytic anion trap column	075550
Columns	Dionex CarboPac SA10-4µm Carbohydrate guard column, 4 × 50 mm	088234
	Dionex CarboPac SA10-4µm Carbohydrate separation column, 4 × 250 mm	088233
EG/DP/SP Vacuum Degas Conversion Kit	Conversion kit to allow vacuum pump to degas eluent for carbohydrate determinations	063353
Dionex HP EG Degasser*	High-pressure analytical EG Degasser module	075522
Dionex HP fittings (blue)*	High-pressure Bolts / Ferrules	074449 / 074373
Electrochemical Detector	ED Detector module for capillary or analytical flow rates.	072042
Electrochemical Cell	ED Cell assembly includes cell body, one reference electrode, and spacer block, and yoke-knob assembly. (Does not include working electrodes.)	AAA-061756
Reference Electrode	pH-Ag/AgCl	061879
For 100-fold Diluted Samples Using 0.002" thick gaskets		
Disposable Working Electrodes	Gold on PTFE working electrodes, package of six with two (0.002" thick) gaskets	066480
	Extra Teflon gaskets (0.002" thick) for disposable gold working electrodes	060141
	Extra spacer blocks for use with 0.001", 0.002", 0.015" thick gaskets	062158
Filtering flask, 1 L	Filtering flask for vacuum filtration using nylon 0.2 µm membrane	0974046**
Thermo Scientific™ Dionex™ OnGuard™ IIA cartridge	Sample preparation trap columns, 2.5 mL, package of 48	057092
Dionex AS-AP autosampler vial kit, polypropylene	1.5 mL vials and caps, package of 100	079812
	0.3 mL vials, package of 100	055428
PEEK Tubing	Yellow (0.003 in, 0.075 mm i.d.)	052301
	Black (0.010 in, 0.25 mm i.d.)	052306

* High-pressure device

** Fisher Scientific P/N

Standard and Sample Preparation

Tip: It is important to use 18 M Ω -cm resistivity deionized water for standards, eluent, and autosampler flush solution. It is recommended to degas the deionized water intended for eluent used for anion determinations. (An appropriate degassing method is vacuum filtration with ultrasonic agitation.) Using deionized water with resistivity less than 18 M Ω -cm resistivity can reduce sensitivity, introduce contamination, and affect calibration, thereby resulting in inaccurate quantification. Results can vary and contamination introduced from samples can affect the chromatography.

Sodium Hydroxide Cleaning Solution

Tip: For carbohydrate determinations, it is important to decontaminate the IC system of biological contamination using a >200 mM sodium hydroxide solution prior to installing the application. To prepare a 200 mM sodium hydroxide solution, degas 1 L of deionized water using vacuum filtration. Add 16 g (10.5 mL) of 50% (w/w) sodium hydroxide to 984 g of degassed deionized water in a 2 L eluent bottle. Cap bottle and swirl to mix. Connect the 200 mM NaOH cleaning solution to the pump using the Eluent A line and prime the pump.

Standard Preparation

Prepare individual stock standards of 1000 mg/L solution of each carbohydrate including lactose and lactulose. Working standards in mg/L concentrations were prepared by diluting the stock standards. Stock standards are stable for six months when stored at -40 °C and working standards when stored at 2–4 °C are stable for 2 weeks. Standards and samples will degrade within days if not stored properly.

Sample Preparation

Prepare Carrez I and Carrez II solutions as described in Thermo Scientific Application Note (AN) 248 *Determination of Lactose in Lactose-Free Milk Products by High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection*.¹

The samples (1 g in 10 mL deionized water) are treated with Carrez I and Carrez II solutions, mixed, diluted to 100 mL, centrifuged, and the supernatant filtered and treated with a Dionex OnGuard IIA sample preparation cartridge. The Dionex OnGuard IIA cartridge treatment removes the anionic contaminants and neutralizes the sample matrix, thereby minimizing baseline disturbances from a highly acidic sample. This sample preparation method is described in detail in AN 248.¹

Instrument Setup

Tip: To achieve the best chromatography with high-pressure IC, it is important to use high-pressure connectors and ferrules (see Table 1) for all connections prior to the suppressor. The high-pressure Dionex ICS-5000+ HPIC Reagent-Free™ IC system is designed to operate from 2000 to 5000 psi.

To set up this application:

1. Set up the high-pressure Dionex ICS-5000+ instrument modules (Figure 1), install the ED detector, power-up the system, and configure the system electronically with Chromeleon CDS version 7.
2. Decontaminate and rinse the system.
3. Install the degassed deionized water for eluent generation.
4. Install and condition the Dionex EGC 500 KOH cartridge, Dionex CR-ATC 500 trap column, and columns.
5. Install the EG/DP/SP Vacuum Degas Conversion Kit.
6. Condition and calibrate the pH-Ag/AgCl reference electrode.
7. Clean the electrochemical cell body.
8. Install the Teflon gasket, Disposable Gold on PTFE (carbohydrate) working electrode, spacer block, and yoke-knob assembly.
9. Install the pH-Ag/AgCl reference electrode.
10. Install the cell into the ED detector.
11. Finish plumbing (Figure 1)

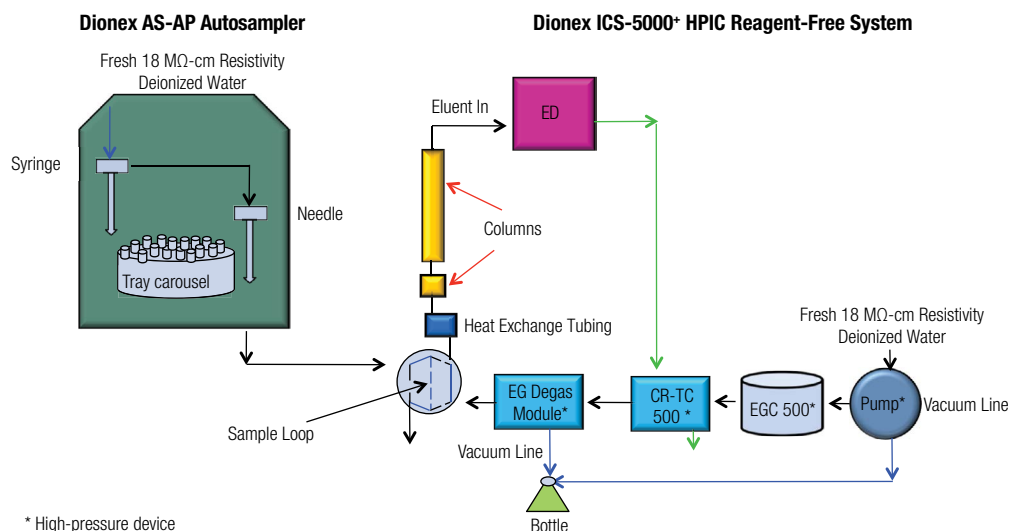


Figure 1. Flow diagram for the high-pressure Dionex ICS-5000+ HPIC reagent-free system.

Decontaminate the System

Tip: Carbohydrates are desirable food sources for many microorganisms. For successful carbohydrate applications, it is recommended to decontaminate the IC system with strong base, such as >200 mM NaOH. It is also important that this eluent be flushed from the system with deionized water to minimize future pump maintenance.

Install 1 to 2 ft of yellow PEEK tubing (0.003 in, 0.075 mm i.d.), 3 ft of black PEEK tubing (0.010 in, 0.25 mm i.d.) and the injection loop tubing from the DP pump to a waste line. Install the 1 L of 200 mM NaOH cleaning solution on Channel A. Prime the pump, and pump the cleaning solution at 0.5 mL/min for at least 1 h. Remove the cleaning solution bottle and install a 2-L bottle of degassed deionized water on the same channel, prime the pump and flush the system with deionized water overnight. Disconnect the tubing assembly but use this tubing to plumb the application.

Condition Consumables

Install and hydrate the Dionex EGC 500 KOH cartridge, and Dionex CR-ATC 500 trap column, according to the product manual instructions and the flow diagram shown in Figure 1. Complete the installation according to the flow diagram. Use the previously cleaned black PEEK tubing for all plumbing connections. Use high-pressure connectors and ferrules (blue) for all of the fittings from the pump to the electrochemical cell. Standard pressure fittings can be used for the cell connections. Detailed instructions are described in Thermo Scientific Technical Note (TN) 129 *Configuring High-Pressure IC Systems for Analytical Flow Rates*, the product manuals, and the instrument installation and operator's manuals.¹⁶⁻²¹

Install EG/DP/SP Vacuum Degas Conversion Kit

Tip: To achieve the best chromatography with HPAE-PAD, it is important to install the vacuum pump bypass kit.

Install the EG/DP/SP Vacuum Degas Conversion Kit according to the instructions included with the kit. First install the two barbed fittings and the plug fitting on the 250 mL vacuum bottle using an o-ring on each fitting. Then screw-on the cap to achieve an air tight seal. Turn off and disconnect the power cord to the DP pump.

Open the DP pump side door and install the fitting in the back of the DP pump. Locate the vacuum pump that is associated with the application pump (System 1 or System 2). Cut the vacuum tubing, insert the T-connector, attach a piece of vacuum tubing of sufficient length from the end of the T-connector to fit onto the inside connection of the previously installed back wall fitting. Connect a length of vacuum tubing to connect the outside surface of the DP pump back wall fitting to one of the barbed fittings on the 250 mL vacuum bottle. Connect vacuum tubing from the vent port of the HP EG Degasser module (in the EG module) to the second barbed fitting on the 250 mL vacuum bottle. Close the DP pump door, connect the power cord, and power-up the DP pump. The vacuum pump will turn on immediately and should stop running in <2 min as it achieves a vacuum. If the vacuum pump continues to run longer than 2 min, check for leaks by re-tightening all fittings.

Electrochemical Cell

To set up the electrochemical cell, first condition and calibrate the pH-Ag/AgCl reference electrode using pH = 7 and pH = 10 buffers according to the instructions on the ED Panel in the Chromeleon CDS software. Rinse the electrochemical cell body, Teflon gasket, and spacer block with deionized water and dry with a lab wipe. Rinse the disposable Gold on PTFE working electrode and shake-off the excess deionized water. Do not touch the Gold on PTFE electrode with any paper products. This electrode was selected for its robustness at higher eluent concentrations that are used in the column wash. The typical Gold on PTFE working electrode has a working life of three to four weeks under these conditions. Assemble the cell according to the ED User's Compendium by installing the gasket, working electrode, spacer block, and yoke-knob assembly.²² Tighten the yoke-knob assembly to "three clicks". Install the pH-Ag/AgCl reference electrode and then install the cell into the ED detector. Finish plumbing the cell by connecting the tubing from the column to the cell inlet. Turn on the pump, set the eluent concentration. Install 4 in. of black PEEK tubing after eluent is observed flowing from the outlet. When the pH is >10, turn the cell on and download the four-potential carbohydrate waveform. Allow to stabilize for at least 1 h prior to experiments.

Results and Discussion

The Dionex CarboPac SA10-4 μ m 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 μ m 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 2 compares the separations of a carbohydrate standard mixture using the same conditions on both the 6 μ m and 4 μ m resin particle versions of the Dionex CarboPac SA10 column. Similar run times can be achieved relative to earlier versions (Dionex CarboPac SA10 column, P/N 074641) with a >60% increase in peak efficiencies resulting in higher signal to noise and higher resolution. The smaller particle size also results in a higher column pressure (~4400 psi) requiring a high-pressure capable IC system, such as the Dionex ICS-5000⁺ HPIC system.

Method

The method presented here significantly reduces the run time from the 30–40 min described in AN 248¹ (the most recent application note detailing the separation of milk sugars) to under 8 min. To obtain a fast separation with baseline resolution of lactose from lactulose and any adjoining peaks, the method was evaluated at 1.0 to 1.5 mL/min, 30 to 45 °C, and 1 to 4 mM KOH. At higher temperatures, there was an unacceptable baseline disturbance between lactose and lactulose which would result in poor quantification, whereas, lower temperatures, eluent strength, and flow rates resulted in longer run times. The temperature effects on disaccharide separations are also discussed in the column manual.¹⁹ To achieve baseline resolution, while preserving fast run times, compromise 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 (Figure 3). Additionally to eliminate an unknown peak (that elutes at ~ 11 minutes) from chromatographic analysis, a three minute equilibration time was added to the method, which resulted in this peak eluting prior to sample injection. This method has increased resolution of lactose from lactulose, as discussed later with Figures 4 to 6.

To determine the linearity, the peak responses of single injections of five standards from 0.5 to 25 mg/L were evaluated. The results showed linearity with $r^2 > 0.996$ for lactose and lactulose. The LOD and LOQ were determined by 3x and 10x S/N, respectively, of a 0.5 mg/L standard ($n = 7$). The estimated LODs and LOQs were 0.036 and 0.12 mg/L for lactose and 0.087 and 0.289 mg/L for lactulose, respectively.

Columns:	A: Dionex CarboPac SA10 column, 4 mm	Peak Efficiencies (EP)*	Peaks: A B	
	B: Dionex CarboPac SA10-4 μ m column, 4 mm		1. Fructose	8400 14000 plates
			2. Sucrose	6000 10600
Eluent Source:	Dionex EGC 500 KOH cartridge		3. Arabinose	9400 15800
Eluent:	1 mM KOH		4. Galactose	8200 14000
Flow Rate:	1.5 mL/min		5. Glucose	7700 18000
Inj. Volume:	10 μ L		6. Xylose	9000 15000
Column Temp.:	45 °C		7. Mannose	8700 15000
Detection:	PAD, Au on PTFE disposable, Four-Potential Carbohydrate waveform		8. Fructose	7500 12000
Gasket:	0.002" thick PTFE		*Approximate values based on individual columns	
Ref. Electrode:	pH-Ag/AgCl			
Standard:	5 mg/L each			

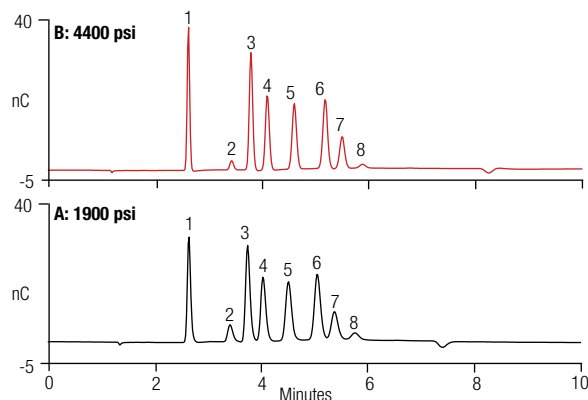


Figure 2. Comparison of separations on a 6 μ m and 4 μ m resin particle columns.

Column:	Dionex CarboPac SA10-4 μ m and guard columns, 4 mm	Peaks:*	A	B	C
Eluent Source:	Dionex EGC 500 KOH cartridge	1. Sucrose	—	—	2.8 mg/L
Eluent:	4 mM KOH	2. Galactose	—	—	2.3
Flow Rate:	1.45 mL/min	3. Glucose	—	—	2.1
Inj. Volume:	10 μ L	4. Unknown	—	—	—
Column Temp.:	35 °C	5. Lactose	3.75	4.43	—
Detection:	PAD, Au on PTFE disposable, Four-Potential Carbohydrate waveform	6. Unknown	—	—	—
Gasket:	0.002" thick PTFE	* Lactulose not detected.			
Ref. Electrode:	pH-Ag/AgCl				
Sample Prep.:	Carrez digestion, centrifuge, filter, Dionex OnGuard IIA cartridge, 100-fold dilution				
Sample:	A: Raw unpasteurized milk				
	B: U.S. Grade A Pasteurized 2% fat milk				
	C: Lactose-free yogurt				

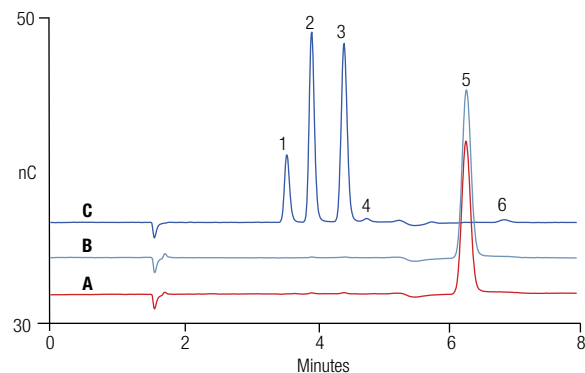


Figure 3. Lactose and lactulose in milk products.

Sample	100-fold Diluted Sample		Calculated Total		100-fold Diluted Sample
	Lactose (mg/L)	RSD	(mg per g)	(wt %)	Lactulose (mg/L)
Raw milk	3.75 ± 0.01	0.37	37.5	3.75	—
2% Pasteurized milk	4.43 ± 0.04	0.96	44.3	4.43	—
Lactose-free yogurt	—	—	—	—	—

n = 3

Samples

Sample Preparation

The method was applied to three samples: raw milk, 2% pasteurized milk, and lactose-free yogurt. To evaluate the reproducibility of the sample preparation process, each sample was prepared in triplicate. The results show good reproducibility for 100-fold diluted samples, with RSDs of 1.21, 1.73, and 5.69 for the raw unpasteurized milk, U.S. Grade A pasteurized milk with 2% fat, and yogurt samples, respectively.

Sample Analysis

The results and total concentrations are summarized in Table 3. Total concentrations were calculated by correcting for the dilution and the sample preparation. Both the raw unpasteurized milk sample and U.S. Grade A pasteurized 2% fat milk sample had similar results: 3.75 ± 0.06 and 4.43 ± 0.04 mg/L lactose (n = 3), respectively. In contrast, the lactose-free yogurt sample had 2.1 to 2.8 mg/L of sucrose, galactose, and glucose with no lactose peak detected. Lactulose was not detected in any of the samples. The chromatograms of the 100-fold diluted samples are compared in Figures 3 to 6. The chromatography shows symmetrical peaks and good baseline separation of all peaks. The separation of lactose from lactulose was well resolved in the milk samples (Figures 4 to 6) with resolutions (R_s , ((EP; European Pharmacopeia)) of ~ 7.0 , well above the typical minimum value of 2.0 for accurate quantification (Gaussian, 8σ -separation).²³

The method exhibited good stability based on retention time (data not shown) and peak area reproducibilities (Table 4). While the lactose peak was evaluated in the diluted samples, lactulose (not natively present) was measured in samples that had been spiked with 0.5 mg/L of this sugar. The retention time reproducibilities were good with lactose at 6.38 to 6.41 min (RSDs <0.08) and lactulose at 7.16 to 7.18 min (RSDs <0.07). The peak areas were reproducible with RSDs <4.

Column:	Dionex CarboPac SA10-4 μ m and guard columns, 4 mm	Sample:	A: 100-fold diluted raw, unpasteurized milk
Eluent Source:	Dionex EGC 500 KOH cartridge		B: Sample A + 0.5 mg/L lactulose
Eluent:	4 mM KOH		C: 0.5 mg/L carbohydrate standard
Flow Rate:	1.45 mL/min		
Inj. Volume:	10 μ L		
Column Temp.:	35 °C		
Detection:	PAD, Au on PTFE disposable, Four-Potential Carbohydrate waveform	Peaks:	A B
Gasket:	0.002" thick PTFE	1. Sucrose	— — mg/L
Ref. Electrode:	pH-Ag/AgCl	2. Galactose	— —
Sample Prep.:	Carrez digestion, centrifuge, filter, Dionex OnGuard IIA cartridge	3. Glucose	— —
		4. Lactose	3.75 3.77
		5. Lactulose	— 0.48

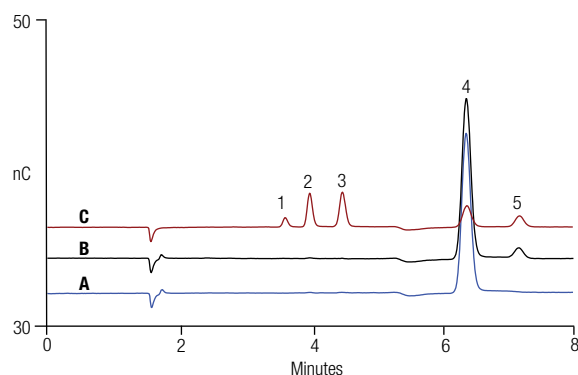


Figure 4. Lactose and lactulose in raw unpasteurized milk.

Table 4. Lactose and lactulose peak area reproducibility.

100-fold Diluted Sample*	Lactose		Lactulose	
	Peak Area (μ S-cm)	RSD	Peak Area (μ S-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 were added separately

Table 5. 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

To determine the accuracy of the method, we measured the recoveries of added lactose and lactulose to the samples (Table 5). 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.

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.

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

Column:	Dionex CarboPac SA10-4µm and guard columns, 4 mm	Sample:	A: Water blank
Eluent Source:	Dionex EGC 500 KOH cartridge	B:	100-fold diluted 2% fat, U.S. Grade A Pasteurized milk
Eluent:	4 mM KOH	C:	Sample B + 0.5 mg/L lactulose
Flow Rate:	1.45 mL/min	Peaks:	B C
Inj. Volume:	10 µL	1. Lactose	4.43 4.40 mg/L
Column Temp.:	35 °C	2. Lactulose	— 0.47
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		

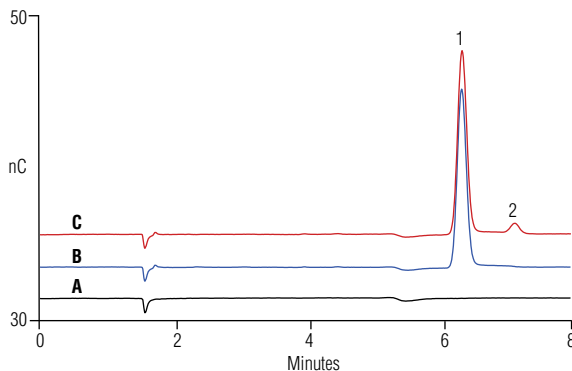


Figure 5. Lactose and lactulose in 2% fat Grade A pasteurized milk.

The chromatogram of the lactose-free yogurt (Figure 6) shows baseline resolution (R_s (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 (R_s (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.

Column:	Dionex CarboPac SA10-4µm and guard columns, 4 mm	Sample:	A: Water blank
Eluent Source:	Dionex EGC 500 KOH cartridge	B:	100-fold diluted lactose-free yogurt
Eluent:	4 mM KOH	C:	Sample B + 0.5 mg/L lactulose and 0.5 mg/L lactulose
Flow Rate:	1.45 mL/min	Peaks:	B C
Inj. Volume:	10 µL	1. Sucrose	2.8 2.8 mg/L
Column Temp.:	35 °C	2. Galactose	2.3 2.3
Detection:	PAD, Au on PTFE disposable, Four-Potential Carbohydrate waveform	3. Glucose	2.1 2.1
Gasket:	0.002" thick PTFE	4. Unknown	— —
Ref. Electrode:	pH-Ag/AgCl	5. Lactose	— 0.48
Sample Prep.:	Carrez digestion, centrifuge, filter, Dionex OnGuard IIA cartridge	6. Unknown	— —
		7. Lactulose	— 0.47

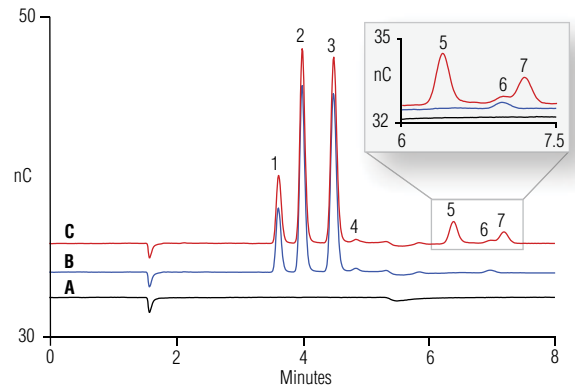


Figure 6. Lactose and lactulose in lactose-free yogurt.

Conclusion

This application demonstrates the advantages of high pressure IC using a Dionex ICS-5000+ HPIC system to provide fast, high-resolution carbohydrate separations on standard bore columns. The high backpressure capability of the Dionex ICS-5000+ HPIC system permits fast separations across standard bore, microbore, and capillary scale formats.

This application demonstrates a fast, accurate, and reproducible method for lactose and lactulose determinations in diluted milk samples and lactose-free products. For well-resolved peaks the Dionex CarboPac SA10-4 μ m carbohydrate column only requires low eluent strength, which is accurately, precisely, and reproducibly delivered inline using eluent generation to minimize time, labor and the variability normally seen in manually prepared eluents. 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, R_s (EP) = 7, in the milk samples. In the yogurt sample, the unknown peak eluting near lactulose reduces the lactose and lactulose resolution to R_s (EP) = 3 and R_s (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, AN70236_E 10/12S, Sunnyvale, CA, 2012.
2. U.S. Department of Health and Human Services Public Health Service Food and Drug Administration. Grade "A" Pasteurized Milk Ordinance (Includes provisions from the Grade "A" Condensed and Dry Milk Products and Condensed and Dry Whey-Supplement I to the Grade "A" PMO) 2009 Revision. <http://www.fda.gov/downloads/Food/GuidanceRegulation/UCM209789.pdf> (Accessed October 2013.)
3. Center of Disease Control. Food Safety and Raw Milk. <http://www.cdc.gov/foodsafety/rawmilk/raw-milk-index.html>. (Accessed October 2013.)
4. Langer, A.J.; Ayers, T.; Grass, J.; Lynch, M.; Frederick J. Angulo, F.J.; Mahon, B.E. Nonpasteurized Dairy Products, Disease Outbreaks, and State Laws—United States, 1993–2006. *Emerging Infectious Diseases*, **March 2012**, *18*(3), 385–391. www.cdc.gov/eid
5. Marconi, E.; Messia, C. M.; Amine, A.; Mascone, D.; Vernazza, F.; Stocchi, F.; and Palleschi, G. Heat-Treated Milk Differentiation by a Sensitive Lactulose Assay. **2004**, 447–450.
6. Minnesota Gastroenterology, P.A., Premier Gastro enterology Care. Hepatic Encephalopathy and Lactulose. <http://www.mngastro.com/gi-system-amp-disorders/gi-system-amp-disorders/hepatic-encephalopathy-and-lactulos>. (Accessed October 2013.)
7. European Commission. Health and Consumers, Food, Lactulose. http://ec.europa.eu/food/food/biotechnology/novelfood/nfnetweb/mod_search/index.cfm?action=mod_search.details&seqfce=304. (Accessed October 2013.)
8. Luzzana, M.; Agnellini, D.; Cremonesi, P.; Caramenti, G.; DeVita S. *Milk Lactose and Lactulose Determination by the Differential pH Techniques*. **2003**, 409–416.
9. Guan, R.; Liu, D.; Ye, X.; Yang, K. *Use of Fluorometry for Determination of Skim Milk Powder Adulteration in Fresh Milk*. **2005**, (11), 1101–1106.

10. Monica, R. G.; Mar, V.; Rosina, L. F. *Effect of Homogenization on Protein Distribution and Proteolysis during Storage of Indirectly Heated UHT Milk*. 2002, 589–599.
11. Moscone, D.; Bernado, R. A.; Marconi, E.; Amine, A.; Palleschi, G. *Rapid Determination of Lactulose in Milk by Microdialysis and Biosensors*. 1999, 325–329.
12. 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*. 2007, 196–216.
13. Hurum, D.; Rohrer, J. Application Note AN 1006, Determination of Carbohydrates in Urine by HPAE-PAD. LPN AN70088_E_08/12S. Thermo Fisher Scientific, Sunnyvale, CA, USA, 2012.
14. Christison, T.; Zhang, A.; Doyle, M., Lopez, L. Technical Note, TN 137 Determination of Carbohydrates in Urine by Capillary HPAE-PAD, TN70696_E 06/13S, Thermo Fisher Scientific, Sunnyvale, CA, USA, 2013.
15. Thermo Fisher Scientific. Technical Note TN 21, Optimal Settings for Pulsed Amperometric Detection of Carbohydrates Using the Dionex ED40 Electrochemical Detector, LPN 034889-03 3.5M 10/98, Sunnyvale, CA, USA, 1998.
16. Thermo Fisher Scientific. Dionex Technical Note TN 129, Configuring High-Pressure IC Systems for Analytical Flow Rates, TN70317_E 11/12S, Sunnyvale, CA, USA, 2012.
17. Thermo Fisher Scientific. Dionex AS-AP Operator's Manual. Dionex Doc No. 065361, Sunnyvale, CA, USA, 2012.
18. Thermo Fisher Scientific. *Product Manual for the Continuously Regenerated Trap Column (CR-TC)*. Doc No. 031910, Sunnyvale, CA, USA, 2010.
19. Thermo Fisher Scientific. *Product Manual for the Dionex CarboPac SA10-4µm Columns*, Doc No. 065579, Sunnyvale, CA, USA, 2013.
20. Thermo Fisher Scientific. Eluent Generator Cartridge Product Manual, P/N 065018-2, Sunnyvale, CA, USA, 2012.
21. Thermo Fisher Scientific. ICS-5000+ Installation manual. Dionex Doc No. 065446, Sunnyvale, CA, USA, 2012.
22. Thermo Fisher Scientific. *ED User's Compendium for Electrochemical Detection*. Doc. No. 065340, Sunnyvale, CA, USA, 2010.
23. Weiss, J. *Theory of Chromatography*. In Handbook of Ion Chromatography, 3rd Eds.; Wiley-VCH, Verlag GmbH & Co. KGaA: Weinheim, Federal Republic of Germany; 2004. Vol. 1, pp 13–26.

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