thermo scientific

APPLICATION NOTE 72632

Fast and sensitive determination of lactose in lactose-free products using HPAE-PAD

Authors

Manali Aggrawal and Jeffrey Rohrer
Thermo Fisher Scientific
Sunnyvale, CA

Keywords

Dionex ICS-5000+, Dionex CarboPac PA210 4µm column, dairy products, milk, baked goods, cookies, chocolate, ICS-6000

Goal

To develop an HPAE-PAD method for the determination of allolactose, lactose, and lactulose in lactose-free dairy products as well as lactose-free baked goods

Introduction

Lactose is the major disaccharide found in milk and is metabolized to glucose and galactose by the enzyme lactase. Lactose-intolerant individuals have a lactase deficiency; therefore, lactose is not completely metabolized. These individuals have difficulty digesting milk products, resulting in uncomfortable intestinal symptoms such as diarrhea and bloating. The demand for low-lactose dairy products is increasing and more lactose-free products are now available. These lactose-free products are produced by enzymatically breaking down lactose into glucose and galactose. However, the enzymatic hydrolysis process is not 100% efficient and the resulting products contain varying amounts of residual lactose. Currently there are no legally defined lactose concentration limits or regulations governing lactose-free products either in USA or in EU legislation, except for infant formula in which lactose should be 610 mg/100 kcal (Commission Directive, 2006).[1] However, lactose determinations are needed to meet ingredient labeling requirements. Some EU states have set threshold levels for the use of the terms ‘‘lactose-free”, ‘‘very low lactose”, and ‘‘low lactose” for various foodstuffs. These threshold levels vary from 0.01 to 0.1 g/100 g of final product (EFSA, 2010).[2]

ThermoFisher SCIENTIFIC

Milk products undergo heat treatment procedures to eliminate microbes that can cause food spoilage. The United States Public Health Service/Food and Drug Administration (USPHS/FDA) recommends a pasteurization heat treatment process to sterilize milk products to meet public health and safety guidelines.[3,4] During this process, lactose and other saccharides present are converted thermally, enzymatically (β-galactosidase), or by bacterial fermentation (lactic acid bacteria) to many different derivatives.[5] Allolactose, lactulose, and epilactose are some of the common derivatives that are formed during these processes. Allolactose is a lactose isomer formed by β-galactosidase during the transgalactosylation reaction.[6] It is a disaccharide, consisting of the monosaccharides D-galactose and D-glucose linked through a β1-6 glycosidic linkage instead of the β1-4 linkage of lactose. Lactulose is another milk disaccharide that is not found in raw milk but is produced during the heat treatment of milk by isomerization of lactose. Lactulose and epilactose are potentially prebiotic isomers of the milk sugar lactose.[7, 8] They can be produced enzymatically by cellobiose 2-epimerases. Due to their chemical similarity, these sugars are very hard to separate and thus quantify.

The increased market demand for lactose-free products has created a need for a fast, reliable, and sensitive method to analyze these products. High-performance anion-exchange chromatography coupled with pulsed amperometric detection (HPAE-PAD) is one of the most powerful techniques for carbohydrate determinations. HPAE-PAD is a direct-detection technique and therefore eliminates errors associated with analyte derivatization. It is a well-established technique for lactose and lactulose determinations. The common anion-exchange chromatography columns used for the analysis of lactose as described in the literature are Thermo Scientific™ Dionex™ CarboPac™ PA1, PA10, PA20, and PA100 columns.[7, 9-12] In this work, we developed an HPAE-PAD method using the recently introduced Thermo Scientific™ Dionex™ CarboPac™ PA210-4µm column to separate lactose from other structurally similar milk sugars. This column was developed to provide fast, high-resolution separations for most mono- through tetrasaccharides in a variety of food and beverage samples. These columns are packed with a hydrophobic, polymeric, microporous anion exchange resin that is stable over the entire range of pH 0–14. In this work, 11 commercial lactose-free products including both dairy products and baked goods were tested and analyzed for their lactose content using HPAE-PAD. In addition, structurally similar sugars, allolactose and lactulose, were determined and quantitated in these samples.

Experimental

Equipment

- Thermo Scientific™ Dionex™ ICS-5000⁺ HPIC™ system, including:
  - SP Single Pump or DP Dual Pump
  - DC Detector/Chromatography Compartment
  - ED Electrochemical Detector (No cell, P/N 072042)
  - ED Cell (no reference or working electrode; P/N 072044)
  - ED Cell Polypropylene support block for use with disposable electrodes* (P/N 062158)
  - Gold on PTFE Disposable Electrodes (Pack of 6) P/N 066480)
  - pH-Ag/AgCl Reference Electrode (P/N 061879)
  - EG Vacuum Degas Conversion Kit (P/N 063353)
- Thermo Scientific™ Dionex™ AS-AP Autosampler with tray temperature control option (P/N 074926)
- Thermo Scientific™ Dionex™ EGC 500 KOH Eluent Generator Cartridge (P/N 075778)
- Thermo Scientific™ Dionex™ CR-ATC 500 Column (P/N 075550)
- Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software was used for all data acquisition and processing.

*This method can also be executed with a conventional gold working electrode, although all data presented in this application note were collected with disposable gold working electrodes.

Consumables

- 10 µL PEEK™ Sample Loop (P/N 042949)
- Thermo Scientific™ Nalgene™ Syringe Filters, PES, 0.2 µm (Fisher Scientific, P/N 09-740-61A)
- AirTite™ All-Plastic Norm-Ject® Syringes, 5 mL, sterile (Fisher Scientific, P/N 14-817-28)
- Vial Kit, 10 mL Polypropylene with Caps and Septa (P/N 055058)
- Thermo Scientific™ Nalgene™ Rapid-Flow™ Sterile Disposable Filter Units with Nylon Membrane (1000 mL, 0.2 µm pore size, Fisher Scientific P/N 09-740-46)

Reagents and standards

- Deionized (DI) water, Type I reagent grade, 18 MΩ·cm resistivity or better
- Lactose monohydrate, Sigma-Aldrich® (P/N L3625)
- Lactulose, Sigma-Aldrich (P/N L7877)
- Allolactose, Carbosynthe (P/N OG09259)
- Epilactose, Sigma-Aldrich (P/N G0886)
- Potassium hexacyanoferrate(III) ACS reagent, ≥99.0% Sigma-Aldrich (P/N 244023)
- Zinc sulfate monohydrate ≥99.9% trace metals basis; Sigma-Aldrich (P/N 307491)

Experimental conditions

System:	Dionex ICS-5000+ HPIC system		
Columns:	Dionex CarboPac PA210 Guard, 4 × 30 mm (P/N 088955) Dionex CarboPac PA210 Analytical, 4 × 150 mm (P/N 088953)		
Eluent source:	Dionex EGC 500 KOH*		
Eluent:	0–10 min: 23 mM KOH 10–15 min: 100 mM KOH 15–30 min: 23 mM KOH		
Flow rate:	0.8 mL/min		
Injection volume:	10 µL		
Inject mode:	Push full		
Loop overfill factor:	5		
Detection:	Pulsed amperometry, Carbohydrate Certified Disposable Gold Working Electrode with 0.002 inch gasket, Ag/AgCl reference		
Waveform (TN21):	*Time (s)*	*Potential (V)*	*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	
System back pressure:	~3700 psi		
Background:	34–37 nC		
Noise:	~30 pC/min peak-to-peak		
Run time:	30 min		

* Note: Here we used a Dionex EGC 500 KOH to generate eluent, but this application can be performed using manually prepared eluent. Please refer to technical note TN71 for instructions on proper manual eluent preparation for HPAE-PAD.[13]

Preparation of solutions and reagents

Carrez I solution

Dissolve 15.0 g potassium hexacyanoferrate(III) in 75 mL DI water and filter through a 0.20 µm filter. Transfer to a 100 mL volumetric flask and bring to volume.

Carrez II solution

Dissolve 30.0 g zinc sulfate monohydrate in 75 mL DI water and filter through a 0.20 µm filter. Transfer to a 100 mL volumetric flask and bring to volume.

Standards

All standard concentrates (Stock Standards) can be stored for up to 6 months at -40 °C. Diluted intermediate standards are stable for 3 months at -40 °C, and working and mixed standards are stable for two weeks at 2–4 °C. Standards and samples will degrade within days if not stored properly.

1000 mg/L standard concentrates

Prepare individual stock standards of 1000 mg/L of each carbohydrate including allolactose, lactose, and lactulose. Working standards in mg/L concentrations are prepared by diluting the stock standards.

Working standards and standards for method linearity

To prepare working standards, use a calibrated pipette to deliver the appropriate volume of 1000 mg/L stock standard into a volumetric flask and dilute to volume with DI water. For method linearity studies, the following standards of lactose and lactulose were used: 20, 10, 5, 2.5, 1, and 0.25 mg/L.

Sample preparation (milk, yogurt samples)

Step 1:
Weigh 1 g of sample in a 100 mL volumetric flask and add 10 mL DI water to the sample.

Step 2:
Add 200 µL Carrez I solution and 200 µL Carrez II solution to the mixture, shaking after each addition. Bring the volume to 100 mL with DI water. The samples are treated with Carrez I and Carrez II solutions to remove fats, proteins, and other redox compounds that can interfere with analysis.

Step 3:
Centrifuge a portion of this sample at 3000 RPM. Aspirate the supernatant and filter through a 0.20 µm syringe filter.

Step 4:
Prepare a Thermo Scientific™ Dionex™ OnGuard™ IIA, 2.5 cc cartridge by flushing it with 15 mL DI water at a flow rate of less than 2 mL/min, then discard the effluent. Load 8 mL of sample, discard the first 6 mL into a waste container, and collect the next 2 mL for analysis.

The Dionex OnGuard IIA cartridge treatment removes the anionic contaminants and neutralizes the sample thereby minimizing baseline disturbances from a highly acidic sample.

Sample preparation (cheese, butter, cookie, and chocolate samples)

Step 1:
Weigh 1 g of sample (crushed and powdered) in a 100 mL volumetric flask and add 10 mL DI water to it. Place the volumetric flask with a stirring bar in it on a hot plate. Heat it at 65 °C with constant stirring for ~15 min.

Step 2:
Take the flask off the hot plate and add 200 µL Carrez I solution and 200 µL Carrez II solution to the mixture, shaking after each addition. Bring the volume to 100 mL with DI water. Follow Steps 3 and 4 as described above for dairy samples.

Samples can be stored at -4 ºC for up to 2 weeks.

Precautions

1. Each chromatographic run must have a 5 min wash step and a 15 min equilibration step to ensure retention time reproducibility.

2. When running cookie samples, an additional longer wash step of 10 min is needed. A thorough cleanup with 1 M MSA is required (please refer to the column manual for details) after about 40–50 injections (before starting the wash, remove the ED cell from the flow path and reverse the column order).

3. The working electrode shows some loss of peak area response (~10–12%) over 3–4 weeks of continuous sample injections, and thus calibration standards should be run daily for the best results.

Results and discussion

Separation

For the determination of low lactose concentrations in all kinds of lactose-free products, a good separation is required to avoid overestimation of the content as a result of analyte co-elution. The common anion exchange chromatography columns used for the analysis of lactose as described in the literature are the Dionex CarboPac PA1, PA10, PA20, and PA100 columns. In this application note, we developed an HPAE-PAD method using the recently introduced Dionex CarboPac PA210-4µm column to separate lactose from other structurally similar milk sugars. This column was developed to provide fast, high-resolution separations for most mono- through tetra-saccharides in a variety of food and beverage samples. This column was previously applied to the determination of mono- to tetrasaccharides in honey.[14]

To obtain a fast separation with baseline resolution of lactose from other structurally similar sugars, the method was evaluated at different isocratic eluent conditions ranging from 12 to 25 mM KOH. Initially, 14 mM KOH was chosen for the analysis as the peaks were well separated. Later during the analysis of samples, unknown peaks were observed eluting at the retention time of lactulose, and thus lactulose could not be distinguished from other unknown saccharides in the samples evaluated. Using 23 mM KOH, the peaks are sharper and lactulose was well separated from interfering peaks. It was later confirmed that there was no lactulose in these samples. (Please see below in the Sample Recovery section for details). Figure 1 shows the chromatographic profile (run at 23 mM KOH) of a 5 ppm sugar standard mix of fucose, arabinose, galactose, glucose, fructose, sucrose, allolactose, lactose, lactulose, epilactose, and raffinose. The retention time for lactose is approximately 7.25 min. Epilactose and lactulose are nicely separated with 23 mM KOH despite being chemically similar and theoretically hard to separate. Not all 11 carbohydrates are completely resolved, but the goal of this separation was to resolve allolactose, lactose, lactulose, and epilactose.

Sample analysis

Lactose concentration was determined in a broad range of commercial lactose-free products including dairy and baked products. Lactose-free dairy products were purchased from a US supermarket and were from US producers, and lactose-free cookies and chocolates were acquired in Germany and were from German manufacturers. Table 1 lists the commercial lactose-free products analyzed for their lactose content. All products were indicated as “lactose-free” or “zero lactose”. In the product information, lactose amounts of 0.0 g/100 g, <0.1 g/100 g, or <0.01 g/100 g were reported.

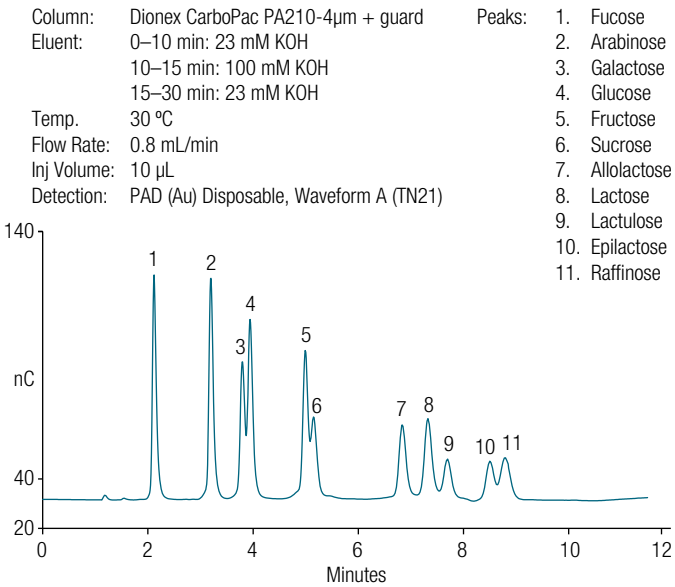

Figure 1. Chromatogram of a 5 ppm sugar standard mix

Table 1. List of low-lactose/lactose-free products used in this study

Sample #	Product	Label
1	Half & half milk	100% Lactose free
2	Fat free milk	100% Lactose free
3	Kefir (cultured low fat milk smoothie)	99% Lactose free
4	Yogurt	Lactose free
5	Aged Cheese	None
6	Butter	Lactose free
7	Sandwich cookie	Lactose free; <0.1g/100 g
8	Butter cookie	Lactose free; <0.1g/100 g
9	Shortbread cookie	Lactose free and sugar free
10	Milk chocolate	Lactose free; <0.1g/100 g
11	Caramel cookie	Lactose free and gluten free

Figure 2 shows the separation of carbohydrates in lactose-free dairy samples 1, 3, and 5 along with a 1 ppm sugar standard mix. The chromatogram of sample #1 (lactose-free half and half milk sample) shows that it contains large amounts of glucose and galactose and small amounts of allolactose and lactose. Sample #3 is a 99% lactose-free low-fat milk smoothie; it was diluted 40 times to bring the lactose concentration into the calibration range. Sample #3 contains almost the same amounts of galactose, glucose, sucrose, and lactose. Sample #5 is an aged cheese sample. Its chromatogram shows a very small amount of lactose. In ripened hard cheese, the sugars (e.g., lactose, galactose, and glucose) are naturally metabolized by microflora. As a result, aged cheese contains a very low concentration of lactose.[10]

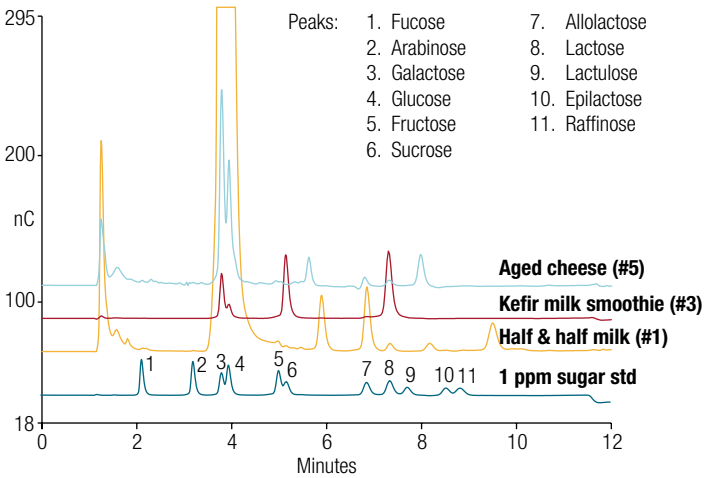

Figure 2. Chromatograms of dairy samples (half & half milk, kefir milk smoothie, and aged cheese) along with an 11 sugars standard mix

Figure 3 shows the separation of carbohydrates in lactose-free cookie samples #7 and #9 along with a 1 ppm sugar standard mix. The chromatogram of Sample #7 shows a very broad allolactose peak, which could indicate that there is another sugar eluting very close to allolactose. Lactose peak shape is good and well separated from other peaks. Sample #9 is a lactose-free and sugar-free cookie sample, thus only very small peaks were detected for glucose, fructose, and sucrose and almost no peaks for allolactose, lactose, and lactulose. However, epilactose and raffinose peaks were detected. Also, large early eluting peaks were detected in this sugar-free cookie; these could be peaks of sugar substitutes used in baking sugar-free cookies. All cookie samples we analyzed have raffinose. In comparison to cookie samples, a chocolate sample (Figure 4) had high amounts of allolactose and lactose. None of the samples had lactulose. In all products tested, the lactose concentration determined was <0.01 g/100 g (Table 2) except Samples #2 and #10, each with less than 0.1 g/100 g. The other exception is Sample #3, which has about 3 g/100 g of lactose in it. While the label says it is up to 99% lactose free, it has more than 1% lactose in it. Among all the lactose-free samples tested, lactose-free butter has the lowest amount of allolactose and lactose. Sample #9 (lactose-free and sugar-free cookie) does not have any detected lactose or allolactose.

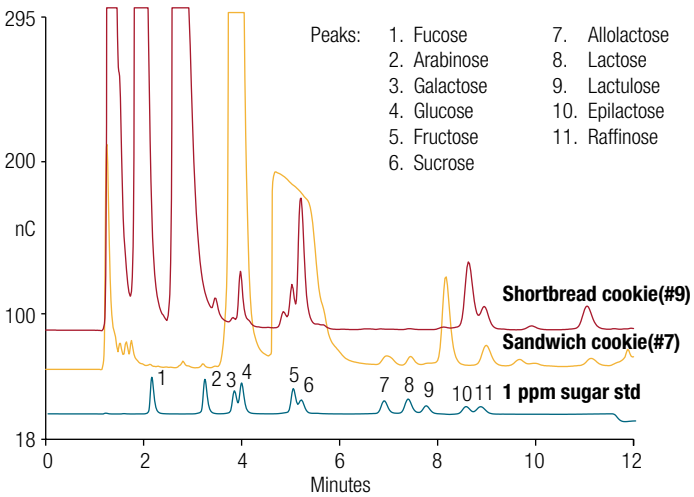

Figure 3. Chromatograms of lactose-free cookie samples (sandwich cookie and shortbread cookie) and an 11 sugars standard mix

Table 2. Concentrations of allolactose and lactose in lactose-free samples

	Sample	Allolactose		Lactose	
		Amount g/100 g	RSD	Amount g/100 g	RSD
1	Lactose-free half & half milk	0.058	0.88	0.009	0.86
2	Lactose-free fat free milk	0.114	0.13	0.029	0.04
3	Lactose-free low-fat milk smoothie	0.06	0.47	3.16	0.32
4	Lactose-free fat-free yogurt	0.006	0.48	0.002	0.00
5	Aged cheese	0.008	0.39	0.006	0.84
6	Lactose-free butter	0.003	0.30	0.001	0.17
7	Lactose-free sandwich cookie	-	-	0.009	0.41
8	Lactose-free butter cookie	0.018	0.05	0.007	0.03
9	Lactose-free shortbread cookie	-	-	-	-
10	Lactose-free chocolate	0.083	0.65	0.046	0.72
11	Lactose-free caramel cookie	-	-	0.003	0.12

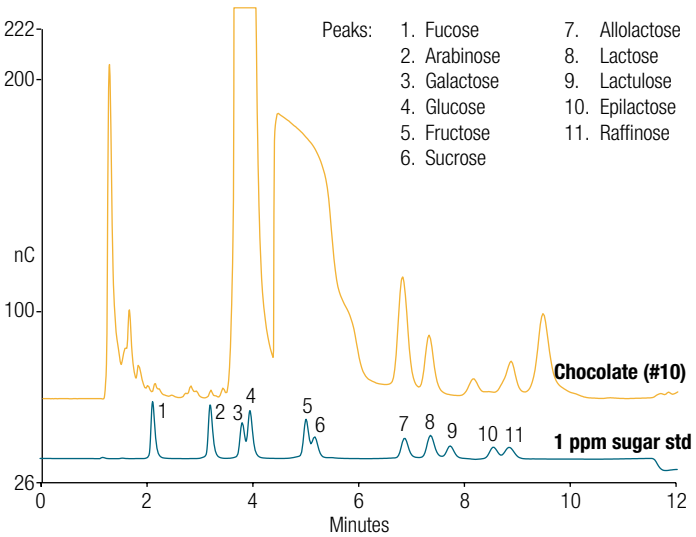

Figure 4. Chromatograms of lactose-free chocolate sample and an 11 sugar standard mix

Calibration and quantification

Calibration standards for all three sugars (allolactose, lactose, and lactulose) were prepared in DI water. Table 3 summarizes the calibration data for calibration curves obtained by injecting calibration standards between 0.25 and 20 mg/L. The calibration curves for these three sugars are shown in Figure 5. The coefficient of determination (r^2) is greater than 0.999 for all sugars. Over the calibration range (0.25–120 mg/L), the relative standard deviations of the retention times of all three peaks (in six calibration standards) ranged from 0.13% to 0.15%.

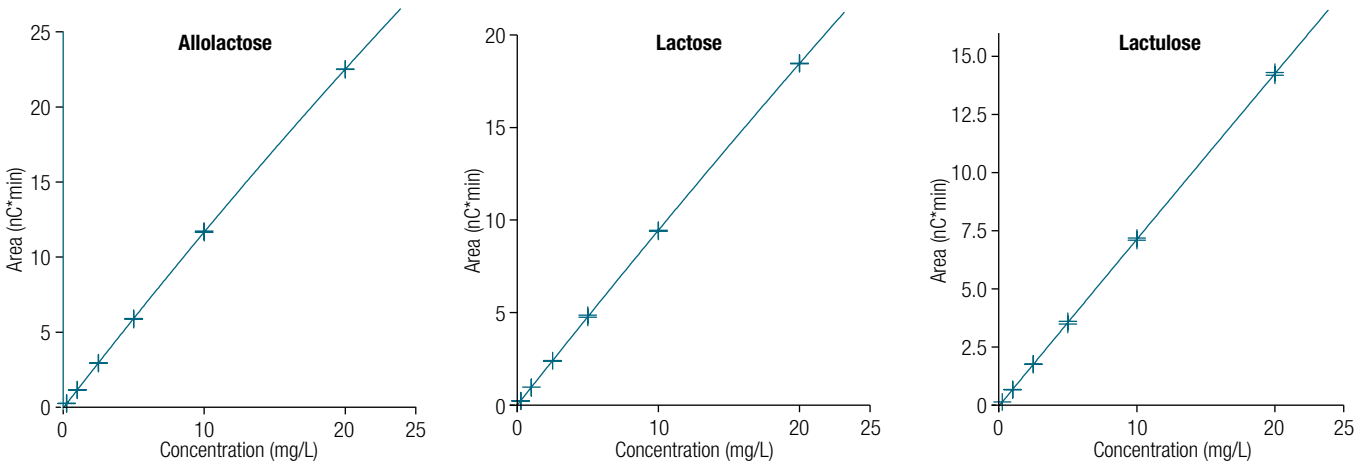

Figure 5. Calibration curves of allolactose, lactose, and lactulose

To determine the LOD and LOQ, the baseline noise was first determined by measuring the peak-to-peak noise in a representative one-minute segment of the baseline where no peaks elute, but close to the peak of interest. The signal was determined from the average peak height of three injections of 0.25 mg/L standard. The LOD and LOQ were determined by 3× and 10× S/N, respectively, of a 0.25 mg/L standard (n = 7). The estimated LODs and LOQs for all three sugars are summarized in Table 3.

Sample recovery

Method accuracy was evaluated by measuring recoveries of sugar standards containing allolactose, lactose, and lactulose spiked into lactose-free samples. A duplicate of each of the samples was fortified with known amounts of allolactose, lactose, and lactulose prior to sample preparation.

Figure 6 shows the comparison of the chromatogram of unspiked and spiked Sample #1 run under two eluent concentrations—14 mM and 23 mM KOH. As shown here, lactose is very well separated from similar disaccharides using both eluent conditions. However, the lactulose peak appears misshapen with much of the peak area eluting later than the standard using 14 mM KOH and symmetrical using 23 mM KOH. Similarly, other samples exhibited the same behavior using both 14 and 23 mM KOH and spiking experiments confirmed that none of the samples tested here contained lactulose.

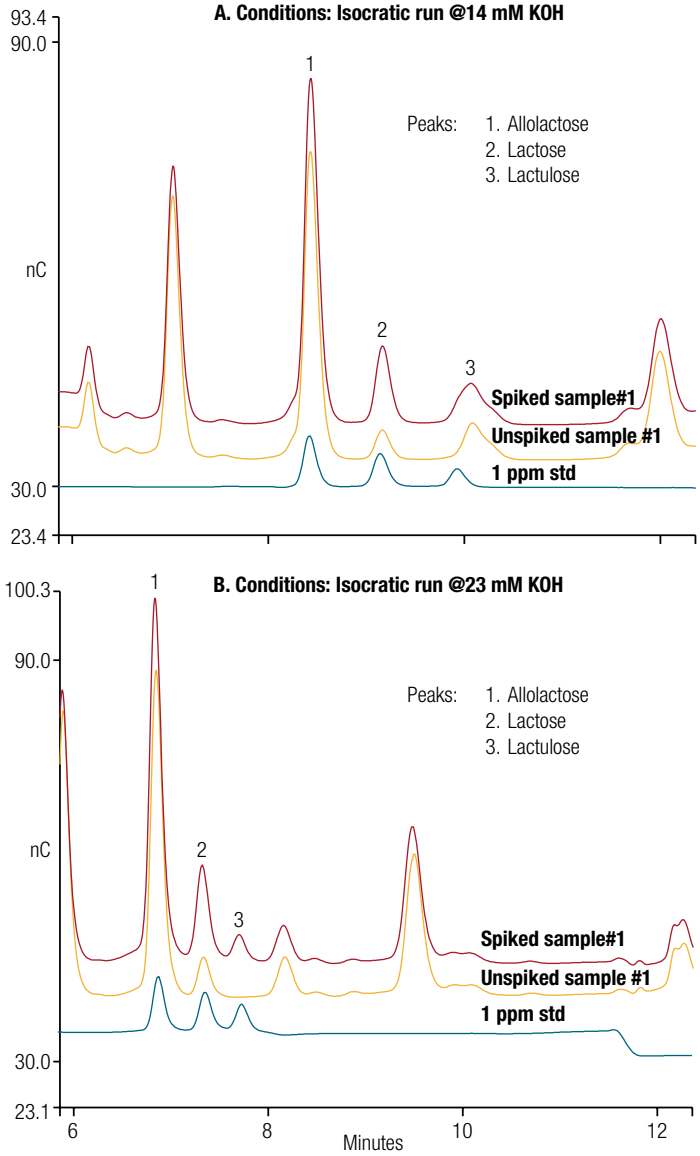

Figure 6. Comparison of chromatograms of lactose-free sample #1 with (A) 14 and (B) 23 mM KOH eluent

Table 3. Results for calibration, LOD, and LOQ for allolactose, lactose, and lactulose

Carbohydrate	Range (mg/L)	Calibration Type	Coefficient of Determination (r^2)	LOD (mg/L)	LOQ (mg/L)
Allolactose	0.25–20	Quad, WithOffset	0.99999	0.009	0.029
Lactose	0.25–20	Linear, WithOffset	0.99981	0.010	0.034
Lactulose	0.25–20	Quad, WithOffset	0.99993	0.017	0.056

Figures 7–11 show the chromatograms of unspiked and spiked lactose-free samples #4, #5, #6, #7, and #10, respectively. The recovery percentages were calculated using the formula shown below:

$$Recovery\ \% = \frac{C\ spiked\ sample - C\ unspiked\ sample}{C\ analyte\ added} * 100$$

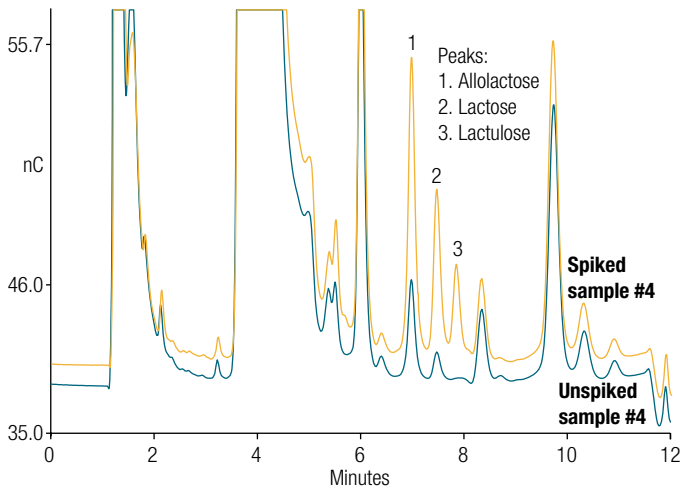

Figure 7. Chromatogram of unspiked and spiked lactose-free yogurt

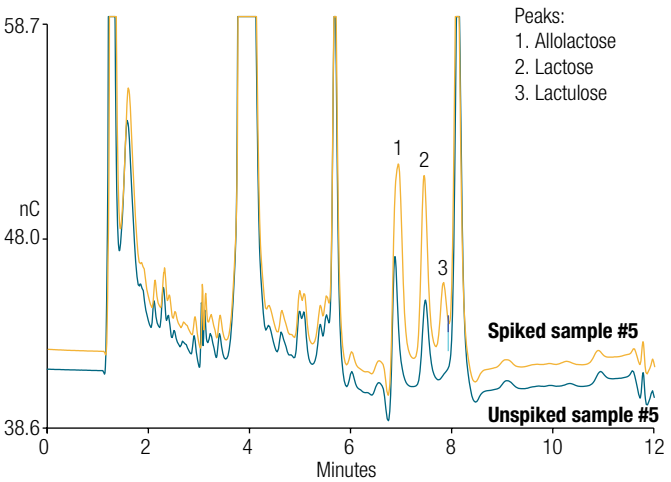

Figure 8. Chromatogram of unspiked and spiked aged cheese

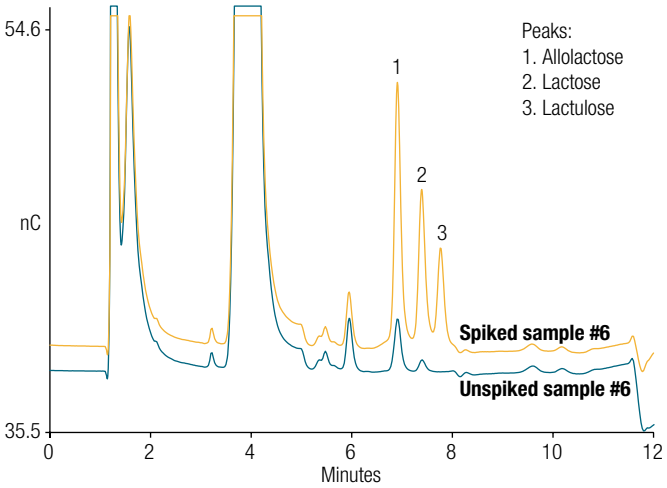

Figure 9. Chromatogram of unspiked and spiked lactose-free butter

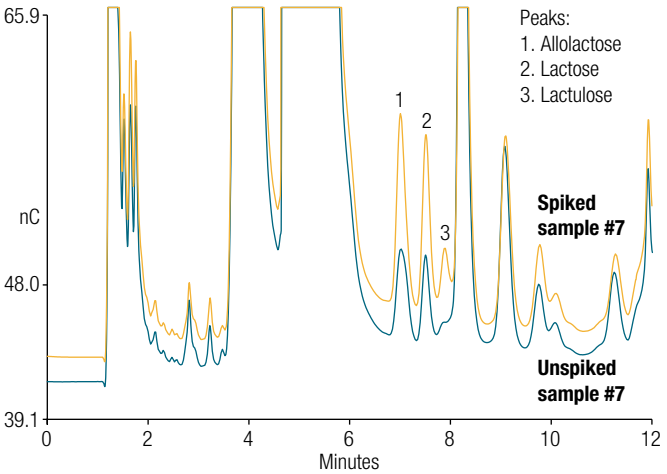

Figure 10. Chromatogram of unspiked and spiked lactose-free sandwich cookie

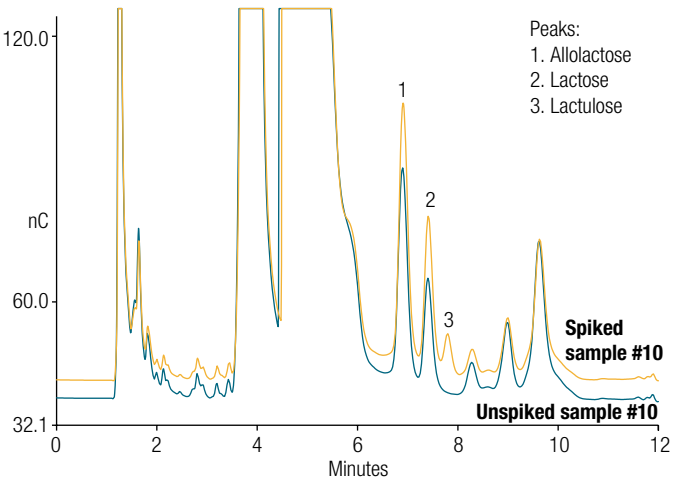

Figure 11. Chromatogram of unspiked and spiked lactose-free milk chocolate

Recoveries of all three sugars for all samples were 80% to 110% (Table 4), with the exception of allolactose in aged cheese and sandwich cookie. This is thought to be due to the poor peak shape for allolactose in these two samples (Figures 8 and 10). The poor peak shape is due to the co-elution of an unknown saccharide or other electrochemically active compound, which interferes with the spike recovery experiment, and thus recovery was not calculated for allolactose in these two samples. The recovery percentages for lactose in all the samples are in the range of 87% to 107%.

Conclusions

Using the Dionex CarboPac PA210-4µm column, an HPAE-PAD method was successfully developed and validated for lactose determinations in 11 commercial lactose-free products including dairy as well as baked products. This column allows the separation of lactose from structurally similar saccharides allolactose, lactulose, and epilactose in less than 9 min with overall cycle time of 30 min. PAD is sensitive, thus allowing the determination of low concentration of lactose in lactose-free products, while at the same time detecting the high concentrations of the major components, galactose, glucose, sucrose, and fructose. The method showed good precision and accuracy with a recovery range of 80% to 110% in the samples tested.

Table 4. Recovery of allolactose, lactose, and lactulose in all samples

Sample #	Allolactose			Lactose			Lactulose		
	Found (mg/L)	Added (mg/L)	Recovery %	Found (mg/L)	Added (mg/L)	Recovery %	Found (mg/L)	Added (mg/L)	Recovery %
1	5.78	0.99	85.4	0.88	1.10	97.5	0.00	0.98	80.7
2	11.41	0.98	96.7	2.89	1.02	106	0.00	1.00	87.4
3	0.15	1.00	98.6	7.89	1.00	100	0.00	1.01	86.0
4	0.56	1.02	95.5	0.18	0.99	87.0	0.00	0.97	80.3
5	Not calculated			0.65	1.00	91.3	0.00	1.00	81.3
6	0.32	0.99	97.9	0.08	0.99	94.5	0.00	0.98	93.0
7	Not calculated			0.93	1.10	88.1	0.00	0.98	94.7
8	1.78	1.12	92.5	0.70	0.98	91.6	0.00	1.00	80.8
9	0.00	1.00	85.7	0.00	1.11	107	0.00	1.01	88.5
10	0.00	1.11	110	0.29	0.98	97.0	0.00	1.02	81.6
11	8.34	2.01	90.1	4.57	1.99	101	0.00	2.10	91.4

References

1. Commission Directive 2006/141/EC of 22 December 2006, (2006). Official Journal of the European Union, 1–31. 20/12/2006. L 401.
2. EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies) Scientific opinion on lactose thresholds in lactose intolerance and galactosaemia. EFSA Journal. 2010, 8(9), 29. Online: http://dx.doi.org/10.2903/j.efsa.2010.1777 (accessed on December 2017)
3. 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.
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. *Emerg Infect Diseases.* **2012,** *18*(3), 385–391.
5. Seki, N.; Saito, H. Lactose as a source for lactulose and other functional lactose derivatives, *Int. Dairy J.* **2012,** *22,* 110–115.
6. Fransen, C.T.M.; van Laere, K.M.J.; van Wijk, A.A.C.; Brüll, L.P.; Dignum, M.; Thomas-Oates, J.E.; Haverkamp, J.; Schols, H.A.; Voragen, A.G.J.; Kamerling, J.P.; Vliegenthart, J.F.G.; α-D-Glcp-(1↔1)-β-D-Galp-containing oligosaccharides, novel products from lactose by the action of β-galactosidase, *Carbohydr. Res.* **1998,** *314,* 101–114.
7. Adachi, S. Formation of lactulose and tagatose from lactose in strongly heated milk, *Nature.* **1958,** *181,* 840–841.
8. Kuschel, B.; Seitl, I.; Stressler, T. et al. Analyse von Präbiotika in Milch: Laktose, Lactulose und Epilactose, *Biospektrum.* **2017,** *23,* 833. [Online] https://doi.org/10.1007/s12268-017-0877-2
9. van Riel, J.; Olieman, C. Selectivity control in the anion-exchange chromatographic determination of saccharides in dairy products using pulsed amperometric detection, *Carbohydrate Research,* **1991,** *215*(1), 39–46.
10. Monti, L.; Negri, S.; Meucci, A.; Stroppa, A.; Galli, A. Contarini, G. Lactose, galactose and glucose determination in naturally lactose free hard cheese: HPAEC-PAD method validation, *Food Chem.* **2017,** *220,* 18–24.
11. Thermo Fisher Scientific. Application Note 248, Determination of Lactose in Lactose-Free Milk Products by High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection, [Online: https://appslab.thermofisher.com/App/1555/an248-determination-lactose-lactosefree-milk-products-by-highperformance-anionexchange-chromatography-with-pulsed-amperometric-detection Accessed on November 2017.
12. Van Scheppingen, W.B.; van Hilten, P.H.; Vijverberg, M.P.; Duchateau, A.L. Selective and sensitive determination of lactose in low-lactose dairy products with HPAEC-PAD, *J. Chromatogr. B.* **2017,** *1060,* 395–399.
13. Thermo Fisher Scientific. Technical Note 71: Eluent Preparation for High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection. [Online: http://tools.thermofisher.com/content/sfs/brochures/TN-71-Eluent-Preparation-for-High-Performance-Anion-Exchange-Chromatogarphy-with%20APD-TN-70669.pdf] Accessed on December 2017.
14. Thermo Fisher Scientific. Application Note 1158: HPAE-PAD Determination of Carbohydrates in Honey to Evaluate Samples for Quality and Adulteration. [Online: https://appslab.thermofisher.com/App/3610/an1158-hpaepad-determination-carbohydrates-honey-evaluate-samples-for-quality-adulteration] Accessed on December 2017.

Find out more at **thermofisher.com/IC**

ThermoFisher SCIENTIFIC