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APPLICATION NOTE 72780

Determination of lactose in lactose-free dairy products using HPAE coupled with PAD and MS dual detection

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Keywords

Dionex ICS-5000+, Dionex CarboPac PA210-4µm column, ISQ EC single quadrupole mass spectrometer, dairy products, pulsed amperometric detection, Dionex ICS-6000 system

Goal

To develop a method for determination of lactose and its derivatives in lactose-free dairy products using HPAE coupled with PAD and MS dual detection

Introduction

Lactose is the major disaccharide found in milk and is metabolized to glucose and galactose by the enzyme lactase. Lactase-deficient individuals have difficulty digesting the lactose in milk products, resulting in diarrhea and bloating, and may benefit from lactose-free dairy products. These 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.

In addition, milk products undergo heat treatment procedures[1,2] to eliminate microbes that can cause food spoilage. 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. Allolactose, lactulose, and epilactose are some of the common derivatives that are formed during these processes.[3,4] Lactulose and epilactose are prebiotic isomers of the milk sugar lactose.[5,6]

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Currently, there are no legally defined lactose concentration limits or regulations governing lactose-free products in either U.S. or EU legislation except for infant formula, in which lactose should be no more than 610 mg/100 kcal (Commission Directive, 2006).[7] 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 0.01/100 g to 0.1 g/100 g of final product (EFSA, 2010).[8] A concentration of < 0.01% is generally accepted as “lactose-free”. The increased market demand for lactose-free products has created a need for a fast, reliable, and sensitive method to analyze them.

For the determination of carbohydrates, high-performance anion-exchange (HPAE) coupled with pulsed amperometric detection (PAD) is the analytical method of choice, as previously demonstrated.[9-12] Carbohydrates at µg/L concentrations in complex matrices can be demanding. Therefore, a low-mass confirmatory detection method, such as a mass spectrometry (MS) optimized for low mass, is needed to confirm the analyte’s presence. In MS detection, ionic species in the gaseous phase are separated by mass/charge (*m/z*), thereby providing molecular specificity, selectivity, and confirmatory information. Combining HPAE with PAD and MS takes advantage of the strengths of both techniques. HPAE separation with eluent generation provides chromatographic selectivity and analytes in the ionic form. Electrospray ionization (ESI) introduces the liquid HPAE stream after it passes through a desalter as a fine spray into the MS source. The heated electrospray ionization (HESI-II) probe improves the ESI interface by allowing the use of high temperatures and voltage to deliver better desolvation and enhanced sensitivity; as a result, make-up solvent is not needed.

This application is an extension of our work in AN72632: Fast and Sensitive Determination of Lactose in Lactose-Free Products using HPAE-PAD.[13] Here we used MS detection along with PAD detection to detect residual lactose in lactose-free dairy products. HPAE separation employs high-pH eluents, which cannot be directly pumped into the mass spectrometer. Thus, we used a post-column device to covert the sodium (or potassium) hydroxide eluent to water. A Thermo Scientific™ Dionex™ CarboPac™ PA210-4µm column separated lactose from structurally similar milk sugars using KOH eluent produced online by an eluent generator. A “tee” was placed immediately after the column to split the flow, thus enabling both PAD and MS detection of the same separation. Approximately 45% of the total flow goes to the PAD detector and 55% to the mass spectrometer through an electrolytically regenerated desalter. Coupling MS to the HPAE separation provides higher detection selectivity and sensitivity for this application.

Experimental

Equipment

- Thermo Scientific™ Dionex™ ICS-5000⁺ HPIC™ system, including:
  - DP Dual Pump
  - EG Eluent Generator
  - DC Detector/Chromatography Compartment
  - CD Conductivity Detector Cell (Optional) (P/N 061830)
  - 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™ ISQ™ EC Single Quadrupole Mass Spectrometer (P/N ISQEC000IC)
- Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) was used for all data acquisition and processing.

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

Consumables

- Thermo Scientific™ Dionex™ EGC 500 KOH Eluent Generator Cartridge (P/N 075778)
- Thermo Scientific™ Dionex™ CR-ATC 500 column (P/N 075550)
- Thermo Scientific™ Dionex™ 6-port high-pressure valve (P/N 22153-60014)
- Thermo Scientific™ Dionex™ ERD 500 Electrolytically Regenerated Desalter, 2 mm (P/N 60-095089)
- Thermo Scientific™ Dionex™ Micro Tee assembly PEEK-1/16 inch (P/N 302852)
- 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)
- Lactose Isotope Standard, Cambridge Isotope Laboratories (P/N CLM-44230)
- 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

HPAE separation			
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 Generator Cartridge		
Eluent:	0–14 min: 19 mM KOH 14–19 min: 100 mM KOH 19–30 min: 19 mM KOH		
Flow Rate:	0.8 mL/min		
Injection Volume:	10 µL		
Inject Mode:	Push full		
Loop Overfill Factor:	5		
System Backpressure:	~3850 psi		
Run Time:	30 min		
Detection 1	**Pulsed Amperometry**		
Working Electrode:	Gold on PTFE Disposable Electrode with 0.002 inch gasket		
Reference Electrode:	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	
Background:	25–30 nC		
Noise:	~20 pC/min peak-to-peak		

* Note: Here we used a Dionex EGC 500 KOH Eluent Generator Cartridge to generate eluent, but this application can be performed using manually prepared eluent. Please refer to Technical Note 71 for instructions on proper manual eluent preparation for HPAE-PAD.[14]

Detection 2	Mass Spectrometry
MS Detector:	ISQ EC single quadrupole MS
Ionization Interface:	Electrospray Ionization (ESI), negative mode
Sheathe Gas Pressure:	50 psi
Aux Gas Pressure:	5 psi
Sweep Gas Pressure:	0 psi
Source Voltage:	-2500 V
Vaporizer Temp:.	250 °C
Ion Transfer Tube Temp.:	200 °C
Chrom. Filter Peak Width:	32 s
Advanced Scan Mode:	See table below

Scan Name	Mass List (amu)	Dwell or Scan Time (s)	SIM Width (amu)	Ion Polarity	Source CID Voltage
Glucose	179.2	0.2	0.3	Negative	0
Lactose	341.3	0.2	0.3	Negative	0
Lactose ISTD	347.3	0.2	0.3	Negative	0

Instrument setup and installation

System set up

The Dionex ICS-5000⁺ HPIC system is configured for electrochemical detection, operating under high pressure conditions up to 5000 psi. To install this application, connect the Dionex AS-AP Autosampler, Dionex ICS-5000⁺ IC system modules, and ISQ EC MS as shown in Figure 1. In addition, a tee, Dionex ERD 500 Electrolytically Regenerated Desalter, and a six-port diverter valve are plumbed in the system as shown in Figure 1. The tee is placed after the column to split the flow, thus enabling both PAD and MS detection of the same separation. The Dionex ERD 500 desalter is placed between the tee and the CD cell to convert KOH to water, thus preventing KOH from going into the MS. A diverter valve is placed between the CD and mass spectrometer to prevent high concentrations of eluent during the wash step / sample matrix from going into the ESI source of mass spectrometer. It is optional to have a CD cell in the HPAE-PAD/MS setup. If a CD cell is not used, then the Dionex ERD 500 desalter is connected directly to the diverter valve.

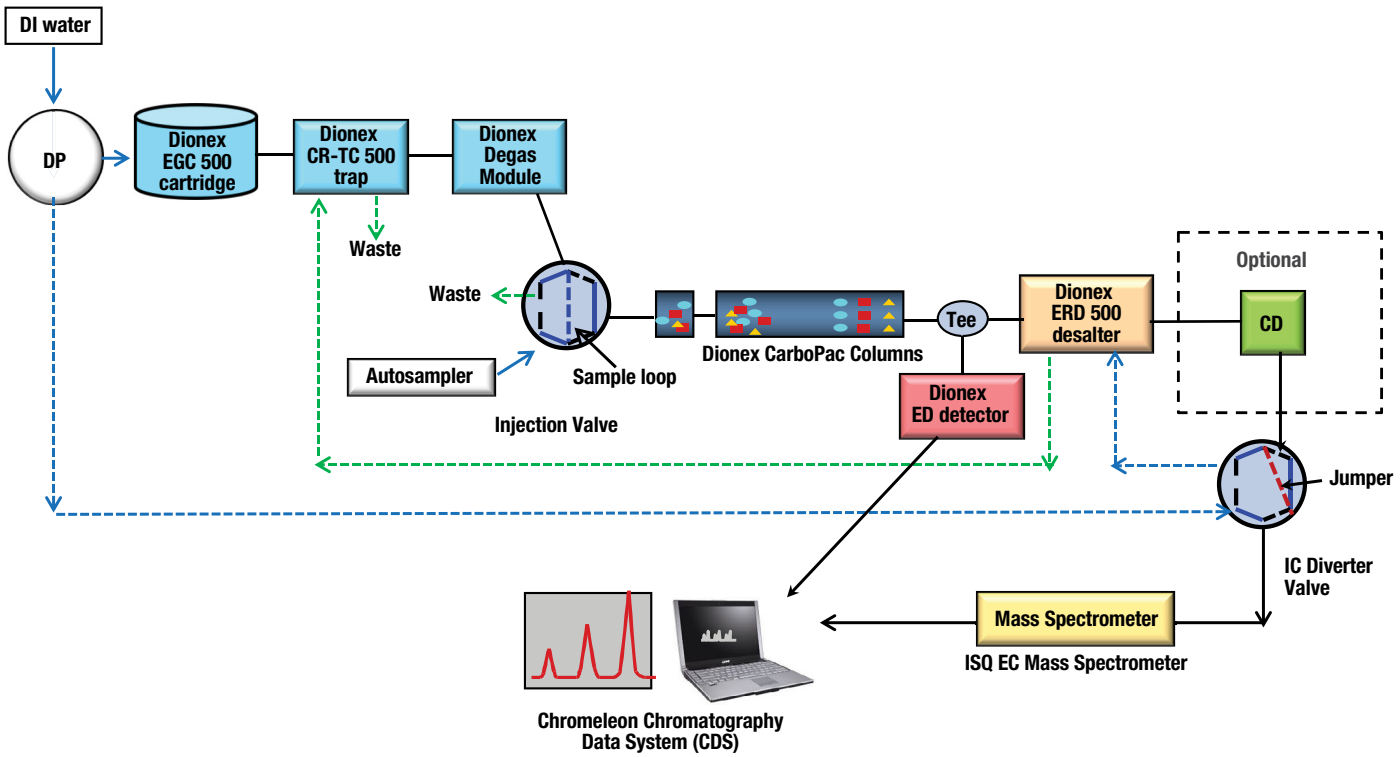

Figure 1. Flow diagram for HPAE-PAD/MS set up

Diverter valve position

As shown in Figure 2, the diverter valve can be configured (in the script editor in the instrument method) in two positions, Position A (Inject) and Position B (Load). Position A directs the eluent flow from CD to MS and external DI water to the desalter Regen In. Position B directs the eluent flow from CD to desalter Regen In and external DI water to the MS.

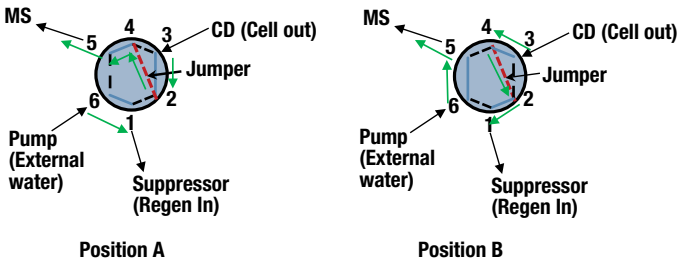

Figure 2. Diverter valve position

In a HPAE-PAD/MS set up, it is critical to use tubing of appropriate length to minimize the volume delay from column to mass spectrometer as well as to maintain back pressure ≤ 100 psi on the Dionex ERD 500 desalter. We recommend the following tubing lengths for the setup:

- Micro Tee to ED detector cell (P/N 302846)*
  0.005 × 0.062 × 7” PK, RED**
- Micro Tee to ERD 500 desalter (P/N 302847)
  0.005 ×0.062 × 27.5” PK, RED*
- Dionex ERD 500 desalter to CD cell in
  0.005 × 0.062 × 5.9” PK, RED
- CD Cell Out to Diverter valve
  0.005 × 0.062 × 13.8” PK, RED
- Diverter valve to MS
  0.005 × 0.062 × 21.7” PK, RED
- Diverter valve to Regen In
  0.03 × 0.062 × 23.6” PK, GRN
- Jumper tubing
  0.005 × 0.062 × 0.59” PK, RED
- Backpressure flow adjusters (3 options)
  0.005 × 0.062 × 66.9” PK, RED
  0.005 × 0.062 × 70.9” PK, RED
  0.005 × 0.062 × 74.8” PK, RED

* Pre-cut tubing available to purchase
** Internal Diameter (i.d) in inches × Outer Diameter (o.d) in inches x length in inches

Use the backpressure flow adjusters to determine the flow to the mass spectrometer

1. Set the pump flow to the initial eluent conditions at 0.8 mL/min, and measure the flow through the suppressor by collecting the suppressor eluent channel elution over a 1 min period into a tared vial.

2. Subtract the tare weight (mg) from the collected weight (mg) to obtain the flow in µL/min (each mg is a µL).

3. If the flow is less than 50% of the total flow (i.e., < 400 µL/min), stop the pump and exchange the 180 mm tubing (0.005 × 0.062 × 70.9’’ PK, RED) for the 190 mm (0.005 × 0.062 × 74.8’’ PK, RED) tubing at the ED cell outlet. If the flow is more than 60% of the total flow (>500 µL/min), stop the pump and exchange the 180 mm tubing for the 170 mm (0.005 × 0.062 × 66.9’’ PK, RED) tubing at the ED cell outlet.

4. Repeat steps 1 and 2 to verify the eluent flow through the suppressor is between 450 and 500 µL/min.

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 were prepared by diluting the stock standards.

Working standards and standards for method calibration

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: 5, 2.5, 1, and 0.25, 0.1, and 0.05 mg/L.

Sample preparation

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 matrix, thereby minimizing baseline disturbances from a highly acidic sample.

Precautions

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

2. 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 residual lactose concentration in 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 determination of lactose as described in the literature are the Thermo Scientific™ Dionex™ CarboPac™ PA1, PA10, PA20, and PA100 columns.[9-12] In this application note, we used 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 tetra-saccharides in a variety of food and beverage samples. This column was previously applied to the determination of mono- to tetra saccharides in honey.[15]

Sugar separations were performed on a 4 × 150 mm Dionex CarboPac PA210 column at 0.8 mL/min flow rate. The column flow was split immediately post column and approximately ~55–60% of the total flow was diverted to the MS and ~40–45% to the ED detector. A desalter device is placed before MS to convert KOH to water and thus prevent KOH from entering the MS. Desalting is accomplished with a Dionex ERD 500 Electrolytically Regenerated desalter using 1 mL/min regenerant water flow. The sugars were detected in parallel by PAD and MS. Electrospray ionization by the HESI II microprobe was used to introduce the ion flow into the ISQ EC single quadrupole MS where the ions were analyzed in selected ion monitoring (SIM) mode. Allolactose, lactose, lactulose, and epilactose have the same molecular weight of 342.297 g/mol, thus signal was detected at a SIM of 341.3 *m/z* (deprotonated form) in negative ionization mode; the negative ionization mode was chosen for the best selectivity and reduced noise.

As discussed in AN72632[13] two different eluent concentrations were tested and 23 mM KOH was chosen as it showed the best separation of lactose and its derivatives in standards as well as samples. Although the chromatography was good with 23 mM KOH and PAD detection, the resolution with MS detection was not good due to the additional tubing to the mass spectrometer. For this reason, we tried a slightly lower eluent concentration of 19 mM KOH.

Figure 3 displays the chromatographic profiles (PAD and MS profiles) of a 0.5 mg/L standard mix (allolactose, lactose, lactulose, and epilactose) run at 19 mM KOH. The three lactose derivatives are well separated under these conditions, including epilactose and lactulose, which are chemically similar and theoretically difficult to separate. In comparison to the PAD detection, the peak resolution is poor with MS detection.

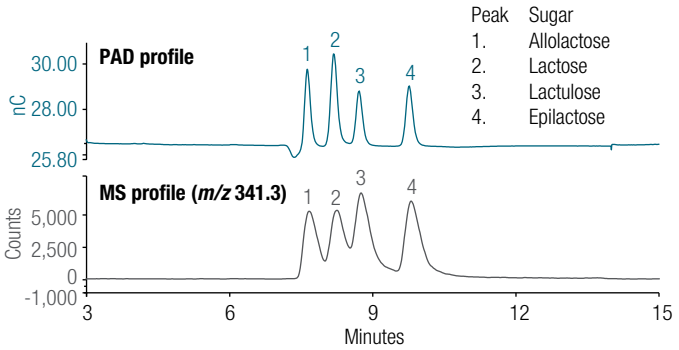

Figure 3. PAD profile (top) and MS profile (bottom) of 0.5 ppm standard mix

The retention time for lactose (PAD profile) is ~8.15 min. A delay time of 0.25 min is applied to MS profile. The delay time is the time required for the analyte to travel from one detector to another if they are in series. Here, the analyte goes through the desalter and the CD cell before going into MS, thus a delay time is applied to MS to match the PAD profile.

Sample analysis

Lactose concentration was determined in five commercial lactose-free dairy products. Table 1 lists the products analyzed for their lactose content. All products were indicated as "100% lactose-free" or "99% lactose-free". Figure 4 displays the chromatographic profiles (PAD and MS) of lactose-free, fat-free milk. Besides allolactose and lactose, additional peaks (Peak 1, 4, and 5) are detected at *m/z* 341.3. Peak 1 is sucrose (molecular weight of 342.3 g/mol, same as allolactose/lactose) and Peaks 4 and 5 are unknown sugars/compounds of the same molecular weight present in the sample. All the peaks are well separated. Similarly, other lactose-free products were analyzed for lactose concentration by dual detection. All lactose-free dairy products have similar chromatographic profiles (Figures 5 and 6). Lactulose and epilactose were not detected in any of the tested samples.

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

Sample	Lactose-free Product	Label
1	Fat-free milk	100% lactose free
2	Half & half milk	100% lactose free
3	Kefir (cultured low-fat milk smoothie)	99% lactose free
4	Yogurt	Lactose free
5	Sour cream	Lactose free

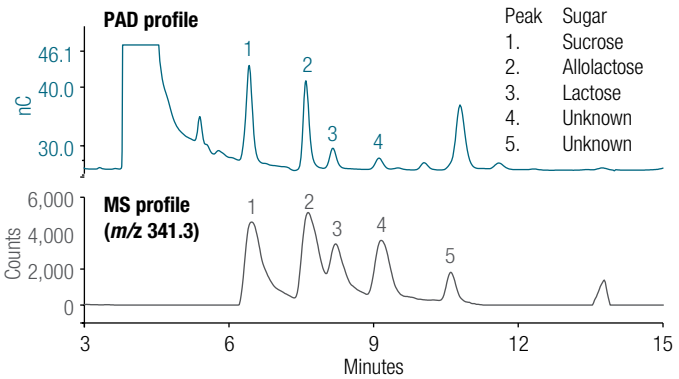

Figure 4. PAD profile (top) and MS profile (bottom) of lactose-free, fat-free milk (Sample 1)

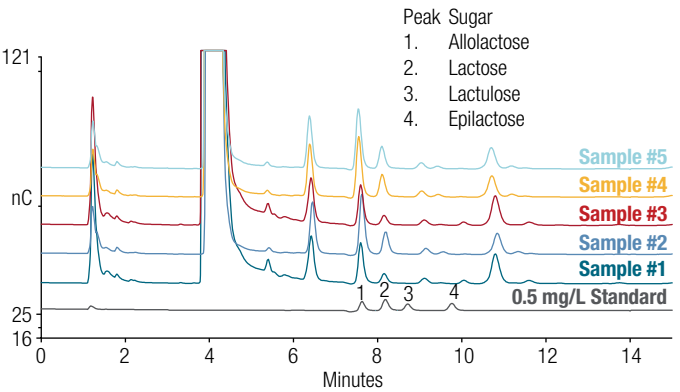

Figure 5. PAD profile of lactose-free samples along with 0.5 mg/L standard sugar mix

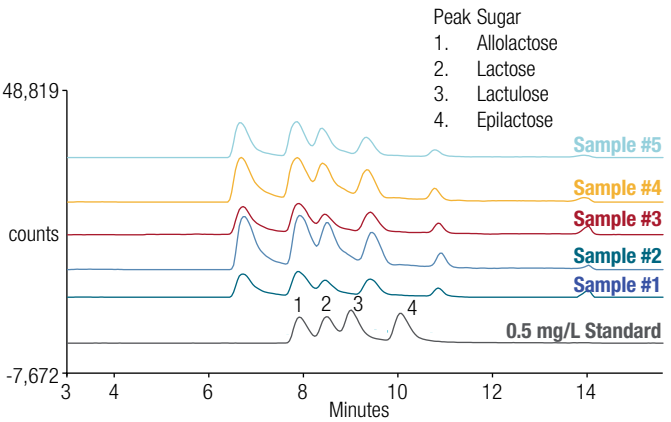

Figure 6. MS (@*m/z* 341.3) profile of lactose-free samples along with 0.5 mg/L sugar standard mix

Calibration and quantification

Calibration standards for all four sugars (allolactose, lactose, lactulose, and epilactose) were prepared in DI water. We used six calibration standards ranging from 0.05 mg/L to 5 mg/L. For PAD detection, the external calibration method was used.

For MS, we used the internal standard calibration method. Lactose: H_2O (Glucose-$^{13}C_6$, 98%+) standard was used as the internal standard. The internal standard method provides a means to account for losses in ionization efficiencies due to components of the matrix that may compete for ion formation in the source. The use of isotopically labeled internal standards ensures that both compound identification and compound quantification are of the highest degree of precision and accuracy possible. A calibration curve is created using a series of standard solutions for the analyte that are spiked with the labeled internal standard and the response factor (% ISTD; Instrument Response for analyte/instrument response for internal standard) is determined. This is done automatically in the Chromeleon CDS software when internal standard calibration is selected. For all four sugars, the signal response was found slightly nonlinear at high concentrations, thus calibration curves (Figure 7) were each fit using a quadratic equation. It was observed with PAD detection as well.[13,16] The coefficient of determination (r^2) is greater than 0.999 for all sugars. Table 2 summarizes the calibration data for calibration curves obtained by injecting calibration standards between 0.05 and 5 mg/L. Over the calibration range, the relative standard deviations of the retention times of all four peaks (in six calibration standards) ranged from 0.08 to 0.15%.

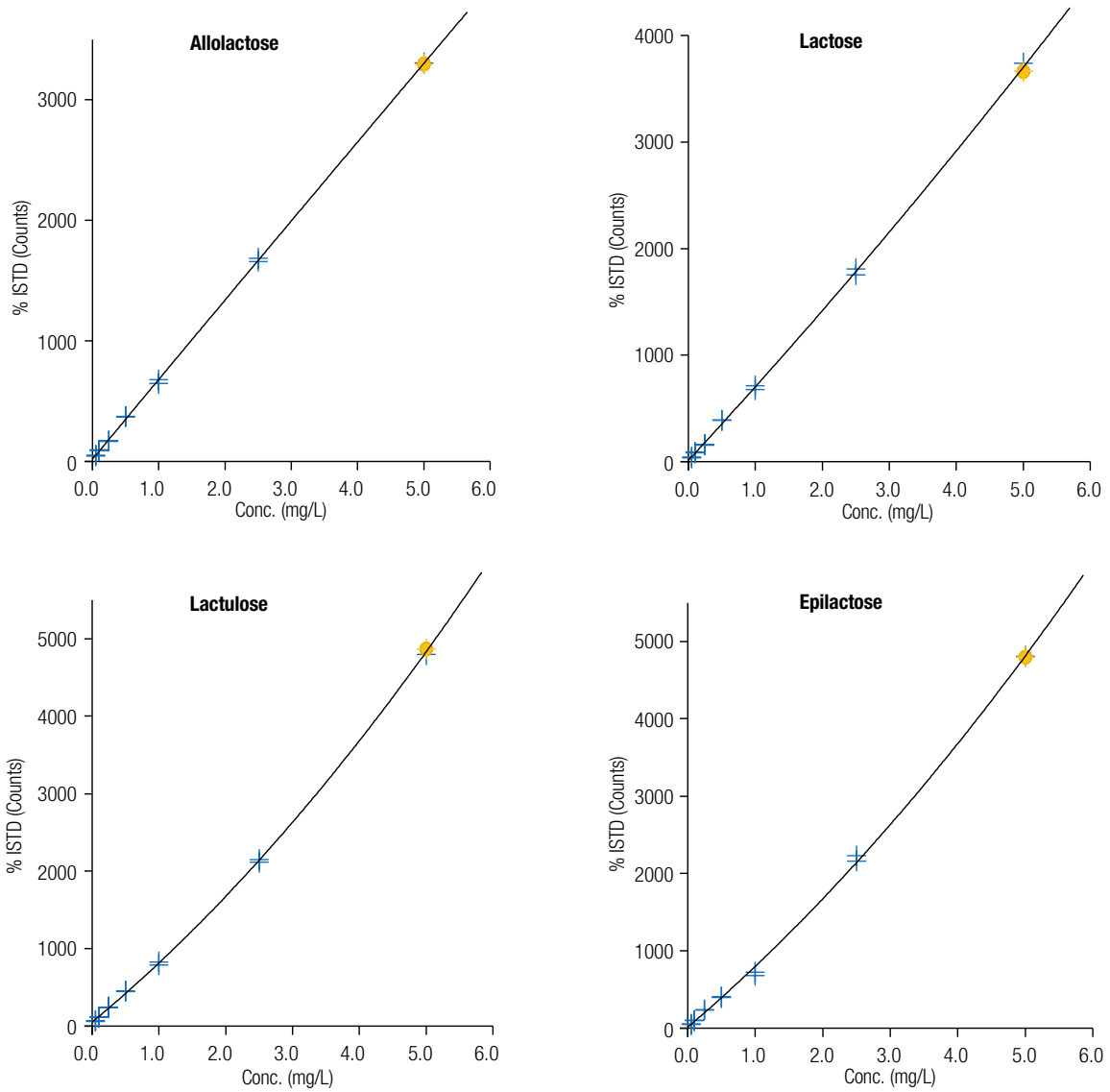

Figure 7. Calibration curves of allolactose, lactose, lactulose, and epilactose using Lactose: H_2O (Glucose-$^{13}C_6$, 98%+) as an internal standard.

Table 2. Results for calibration, LOD, and LOQ for allolactose, lactose, lactulose, and epilactose

Carbohydrate	Range (mg/L)	PAD detection				MS detection			
		Calibration type	Coefficient of determination (r^2)	LOD (mg/L)	LOQ (mg/L)	Calibration type	Coefficient of determination (r2)	LOD (mg/L)	LOQ (mg/L)
Allolactose	0.05–5	Quad, WithOffset	0.9999	0.008	0.025	Quad, WithOffset	0.9998	0.003	0.010
Lactose	0.05–5	Quad, WithOffset	0.9999	0.007	0.022	Quad, WithOffset	0.9995	0.004	0.016
Lactulose	0.05–5	Quad, WithOffset	0.9998	0.011	0.037	Quad, WithOffset	0.9998	0.003	0.009
Epilactose	0.05–5	Quad, WithOffset	0.9999	0.009	0.030	Quad, WithOffset	0.9991	0.003	0.010

To determine the LOD and LOQ, the baseline noise was first determined by measuring the peak-to-peak noise in a representative 1 min 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 0.02 mg/L standard. The LOD and LOQ were determined by 3× and 10× S/N, respectively, of a 0.02 mg/L standard (n = 7). The estimated LODs and LOQs for all four sugars are summarized in Table 2. In comparison to PAD detection, LODs and LOQs are ~2–3 fold lower with MS detection for all four sugars.

Allolactose and lactose determination

Table 3 lists the concentration of allolactose calculated by dual detection (PAD and MS). The amount of allolactose calculated by PAD is ~2–3 fold higher than by MS detection. It could be that there is a coelution that MS does not pick up. The other possibility is suppression of the allolactose signal. Due to the unavailability of an allolactose internal standard we were not able to confirm ionization suppression.

The lactose concentrations (Table 4) calculated by two methods of detection are similar. Lactose concentrations were in the range of 0.01 g/100 g to 0.1 g/100 g in all lactose-free products tested except Sample 3, which had more than 0.1 g/100 g. The label of Sample 3 says it is up to 99% lactose free. The fat-free milk sample has the lowest amount of allolactose and lactose.

Table 3. Concentrations of allolactose in lactose-free samples

#	Lactose-free product	PAD detection		MS detection	
		Amount (g/100 g)	RSD	Amount (g/100 g)	RSD
1	Fat-free milk	0.024	0.29	0.008	0.48
2	Half & half milk	0.140	0.13	0.058	0.21
3	Low-fat milk smoothie	0.969	0.07	0.384	0.18
4	Fat-free yogurt	0.144	0.03	0.053	1.38
5	Sour cream	0.142	0.89	0.057	0.17

Table 4. Concentrations of lactose in lactose-free samples

#	Lactose-free product	PAD detection		MS detection	
		Amount (g/100 g)	RSD	Amount (g/100 g)	RSD
1	Fat-free milk	0.004	0.81	0.004	0.46
2	Half & half milk	0.042	0.92	0.044	0.43
3	Low-fat milk smoothie	0.161	0.78	0.163	0.43
4	Fat-free yogurt	0.045	0.69	0.043	1.20
5	Sour cream	0.045	0.59	0.044	0.49

Sample recovery

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

Figure 8 shows the chromatographic profiles (PAD and MS) of unspiked and spiked lactose-free Sample 3. The recovery percentages were calculated using the formula shown below:

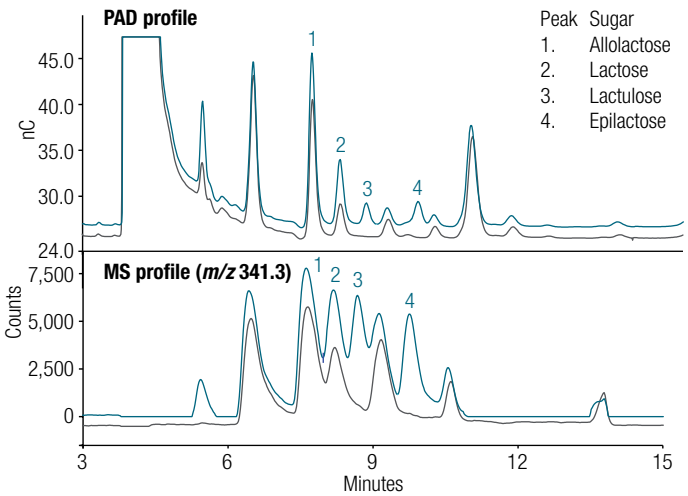

Figure 8. PAD profile (top) and MS profile (bottom) of Sample 3 and spiked Sample 3

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

The recovery percentages for lactose in all the sample matrices (Table 5) are in the range of 80–110% as calculated by dual detection.

Conclusion

Five commercial lactose-free dairy products (milk, half and half milk, cultured milk smoothie, yogurt, and sour cream) were analyzed for their lactose content. With dual detection, lactose was detected down to 0.004% with good precision and accuracy with recovery range of 85–110%. The combination of PAD and MS detections after HPAE separation allows both the quantification of lactose in low-lactose/lactose-free dairy products and peak confirmation.

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Table 5. Recovery of lactose calculated by PAD and MS detection

Sample	PAD detection			MS detection		
	Found (mg/L)	Added (mg/L)	Recovery (%)	Found (mg/L)	Added (mg/L)	Recovery (%)
1	0.402	0.50	101	0.403	0.50	99.3
2	1.14	0.50	88.7	1.09	0.50	93.9
3	0.402	0.50	102	0.406	0.50	111
4	1.13	0.50	89.1	1.08	0.50	85.3
5	1.12	0.50	90.7	1.10	0.50	107

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