APPLICATION NOTE 73341

Determination of sugars in dairy products using HPAE-PAD

Authors: Jingli Hu and Jeffrey Rohrer
Thermo Fisher Scientific, Sunnyvale, CA

Keywords: Dionex CarboPac PA1-2mm column, Dionex ICS-5000+ HPIC system, milk, cheese, infant formula, whey powder, carbohydrate, electrochemical detection, Dionex ICS-6000 HPIC system, ISO

Goal

To demonstrate that ISO/DIS 22184 determination of sugars (galactose, glucose, fructose, sucrose, lactose, and maltose) in dairy products can be executed with a Thermo Scientific™ Dionex™ CarboPac™ PA1-2mm column using a Thermo Scientific™ Dionex™ ICS-5000+ HPIC system

Introduction

Carbohydrates are present in almost all dairy products, including milk, infant formula, yogurt, and cheese. The most common carbohydrates in dairy products are mono- and disaccharides. Lactose is the primary sugar in natural milk, but other sugars such as galactose, glucose, fructose, sucrose, and maltose are also found in dairy products. Sugar content needs to be specified on nutrition facts labels of dairy products; and therefore, dairy products must be analyzed for carbohydrates.

High-performance anion-exchange chromatography (HPAE) with pulsed amperometric detection (PAD) is a widely used technique for carbohydrate analysis. This technique offers the advantage of high-resolution separation, direct analysis (no sample derivatization), and high sensitivity. This technique has been applied in an ISO standard method for the sugar analysis of instant coffee[1] and a CEN/TS standard method for the sugar analysis of animal feed[2]. Application of HPAE-PAD for the fast and sensitive determination of lactose in lactose-free dairy products has been discussed in previous application notes.[3,4,5]

An International Organization for Standardization (ISO) method was recently developed for the determination of the six sugars in dairy products with a detailed description of the experimental method provided.[6,7] In this method, arabinose is added to each sample as the internal standard. Sample sugars are extracted with an aqueous ethanol buffer solution. The sugar extract is then clarified with Carrez reagent to remove protein. After removing the precipitate, the solution is diluted and analyzed by HPAE-PAD.

In this application note, ISO/DIS 22184 was evaluated with a Dionex CarboPac PA1 (2 × 250 mm) column using an ICS-5000+ HPIC system. The experimental design and description are the same as described in the ISO method and the publication that preceded it.[6,7] Key performance parameters were evaluated including separation, linearity, and precision. Fourteen dairy samples provided for the collaborative study of ISO/DIS 22184 were analyzed and the main six sugars (lactose, galactose, glucose, fructose, sucrose, maltose) were determined.

Experimental

Equipment

- Dionex ICS-5000+ HPIC system including*:
  - Dionex ICS-5000+ DP Pump module
  - Dionex ICS-5000+ DC Detector/Chromatography module with ED Electrochemical Detector
  - Thermo Scientific™ Dionex™ AS-AP Autosampler with sample tray cooling, 250 µL sample syringe (P/N 074306),1200 µL buffer line (P/N 074989) and 1.5 mL vial trays (P/N 074936).
- Dionex ICS-5000+ ED Electrochemical Detector Cell (P/N 072044)
- Gold on PTFE Disposable working electrodes with 2 mil gaskets (P/N 066480)
- Reference electrode pH, Ag/AgCl (P/N 061879)
- Knitted reaction coil, 375 µL, unpotted (P/N 043700)
- Three-way manifold (P/N 048227)
- Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) Software, version 7.2.5

*This method can be run on a single Dionex ICS-5000+ or Dionex ICS-6000 system using a Thermo Scientific™ Dionex™ AXP pump to add the post-column reagent.

Consumables

- Thermo Scientific™ Nalgene™ Syringe Filter, PES membrane, 0.2 µm (Fisher Scientific P/N 725-2520)
- Polypropylene autosampler vials, 1.5 mL with caps and split septa (P/N 079812)
- Dionex AS-AP Autosampler Vials 10 mL (P/N 074228)
- Thermo Scientific™ Nalgene™ Rapid-Flow 0.2 µm filter units, 1000 mL, nylon membrane, 90 mm diameter (Thermo Scientific P/N 164-0020)
- Disposable centrifuge tube, polypropylene, 50 mL (Fisher Scientific P/N 05-539-6)
- Helium, ultrahigh purity grade from Airgas

Reagents and standards

- Deionized (DI) water, Type I reagent grade, 18 MΩ·cm resistivity or better
- Sodium acetate salt, electrochemical grade (Thermo Scientific Dionex P/N 059326)
- Sodium hydroxide, 50% (w/w) (Fisher Scientific P/N SS254-500)
- Sodium hydroxide pellets (Fisher Scientific P/N S318-100)
- Arabinose (Sigma P/N A3256-25G)
- Galactose (Sigma P/N G0750-25G)
- Glucose (Sigma P/N G8270-100G)
- Fructose (Sigma P/N F2543-100G)
- Sucrose (Sigma P/N S7903-250G)
- Lactose monohydrate (Sigma P/N 61339-25G)
- Maltose monohydrate (Sigma P/N M5885-100G)
- Hydrochloric acid, 37% (Fisher Scientific P/N A142-212)
- Acetonitrile, HPLC grade (Fisher Scientific P/N A998-1)
- Ethanol (Fisher Scientific P/N A4094)
- Methanol (Fisher Scientific P/N A412-500)
- Potassium hexacyanoferrate trihydrate, $K_4Fe(CN)_6 \cdot 3\ H_2O$ (Fisher Scientific P/N P236-500)
- Zinc acetate dihydrate, $Zn(CH_3COO)_2 \cdot 2H_2O$ (Fisher Scientific P/N Z20-500)
- Glacial acetic acid (Fisher Scientific P/N A38S-500)
- PIPES, Piperazine-N, N'-bis(2-ethanesulfonic acid) (Fisher Scientific P/N BP304-100)

Samples

Fourteen dairy samples were received from the ISO international collaborative study organizer.

Chromatographic conditions

Parameter	Value
Columns	Dionex CarboPac PA1 Guard, 2 × 50 mm (P/N 057179) Dionex CarboPac PA1 Separation, 2 × 250 mm (P/N 057178)
Eluent	Gradient (Table 1)
Flow rate	0.25 mL/min
Column temperature	20 °C
Injection volume	5 µL (Full loop)
Autosampler temperature	5 °C
Reference electrode	Ag/AgCl
Working electrode	Gold on PTFE disposable electrode with a 2 mil gasket (1 mil = 25.4 µm)
Post-column reagent	0.3 M NaOH
Post-column reagent flow rate	0.13 mL/min delivered by pump 2
Detection	Pulsed amperometric detection (electrochemical detection)
Detection compartment temperature	20 °C
Detection waveform	Gold, carbohydrates, 4-potential (Table 2)
System backpressure	~1500 psi (100 psi = 689.5 kPa)
Run time	52 min

Table 1. Eluent gradient

Time (min)	A (%) 1 M NaOAc	B (%) 200 mM NaOH	C (%) H_2O	D (%) 25 mM NaOAc
Initial	0	5	88	7
0	0	5	88	7
10	0	5	88	7
15	0	17	76	7
25	0	93	0	7
28.1	20	73	0	7
32	20	73	0	7
32.1	0	5	88	7
52	0	5	88	7

Table 2. Carbohydrate, 4-potential waveform

Time (s)	Potential (V)	Integration
0	0.1	Off
0.2	0.1	On
0.4	0.1	Off
0.41	-2.0	Off
0.42	-2.0	Off
0.43	0.6	Off
0.44	-0.1	Off
0.5	-0.1	Off

Preparation of solutions and reagents

Eluent preparation

Eluent A (1 M NaOAc)*

Degas 2 L of DI water by sparging helium gas in a plastic eluent bottle for at least 15 min. Dissolve 82 g of sodium acetate in 800 mL of degassed DI water. Vacuum filter this solution through a 0.2 µm Nalgene, 1 L nylon filter to remove particles from the sodium acetate that can damage parts of the pump. Transfer the solution to a 1 L volumetric flask and bring to volume. Mix well and transfer eluent into a 2 L eluent bottle. Immediately blanket eluent with helium gas at 5–8 psi.

Eluent B (0.2 M NaOH)

Degas 2 L of DI water as indicated for eluent A. Add 800 mL of degassed water into a 1 L volumetric flask, add 10.4 mL or 16 g of 50% (w/w) sodium hydroxide solution (the Fisher Scientific product – not the 50% solution prepared from pellets and used to prepare the PIPES buffer) and bring to volume. Mix well and transfer eluent into a 2 L eluent bottle. Immediately blanket eluent with helium gas at 5–8 psi.

Eluent C (Water)

Degas 2 L of DI water as indicated for eluent A. Immediately blanket eluent with helium gas at 5–8 psi.

Eluent D (25 mM NaOAc)*

Degas 2 L of DI water as indicated for eluent A. Dissolve 2.05 g of sodium acetate in 800 mL of degassed DI water. Vacuum filter this solution through a 0.2 µm Nalgene, 1 L nylon filter to remove particles from the sodium acetate that can damage parts of the pump. Transfer the solution to a 1 L volumetric flask and bring to volume. Mix well and transfer eluent into a 2 L eluent bottle. Immediately blanket eluent with helium gas at 5–8 psi.

Post-column reagent (0.3 M NaOH)

Degas 2 L of DI water as indicated for eluent A. Add 800 mL of degassed water into a 1 L volumetric flask, add 15.6 mL, or 24 g of 50% (w/w) sodium hydroxide solution (Fisher Scientific product) and bring to volume. Mix well and transfer eluent into a 2 L eluent bottle. Immediately blanket eluent with helium gas at 5–8 psi.

For additional details on eluent preparation refer to Thermo Scientific Technical Note 71.[8]

*These eluents were prepared as directed by the ISO Method. Thermo Fisher Scientific does not recommend preparing acetate eluents without added hydroxide.[7] When using eluents without sodium hydroxide, the analyst should be careful to avoid system contamination and be aware of the signs of system contamination.

Standards preparation

Sugar standard stock solutions

Weigh 260 mg of galactose, glucose, fructose, 421 mg of lactose monohydrate and maltose monohydrate, and 400 mg of sucrose into a 500 mL volumetric flask (Table 3). Add 200 mL of DI water and dissolve the sugars. Add 25 mL of acetonitrile, fill up to the mark with DI water, and mix the solution.

Table 3. Sugar stock solution concentration (500 mL)

Analyte	Analyte amount (mg)	Compound	Compound amount (mg)	Conc. (mg/L)
Fructose	260	Fructose	260	520
Galactose	260	Galactose	260	520
Glucose	260	Glucose	260	520
Lactose	400	Lactose monohydrate	421	800
Maltose	400	Maltose monohydrate	421	800

Arabinose internal standard stock solution

Weigh 7 g of arabinose into a 50 mL volumetric flask. Add about 30 mL of water and dissolve the arabinose. Add 2.5 mL of acetonitrile, fill up to the mark with water, and mix.

Working standard solutions

Prepare the different levels of sugar calibration standards according to Table 4. Add the specified volumes of the sugar standard stock solution and internal standard stock solution in a 200 mL volumetric flask, add 50 mL of DI water. Add 10 mL of acetonitrile and fill up to the mark with water, and mix. The sugar concentrations of the calibration standards are shown in Table 5.

Table 4. Calibration solution preparation (200 mL)

Sugar calibration standard solution	Sugar standard stock solution (mL)	Arabinose internal standard stock solution (µL)
L1	0.2	50
L2	1	50
L3	6	50
L4	10	50
L5	20	50
L6	40	50
L7	80	50
L8	100	50

Table 5. Calibration standard concentration (mg/L)

Analyte	L1	L2	L3	L4	L5	L6	L7	L8
Fructose	0.52	2.6	15.6	26	52	104	208	260
Galactose	0.52	2.6	15.6	26	52	104	208	260
Glucose	0.52	2.6	15.6	26	52	104	208	260
Lactose	0.8	4	24	40	80	160	320	400
Maltose	0.8	4	24	40	80	160	320	400
Sucrose	0.8	4	24	40	80	160	320	400

Carrez reagent preparation

Carrez reagent I

Add 106 g of potassium hexacyanoferrate trihydrate in a 1 L volumetric flask, dissolve in 800 mL of DI water, fill up to the mark with DI water, and mix the solution. Store in the refrigerator.

Carrez reagent II

Add 220 g of zinc acetate dihydrate in a 1 L volumetric flask, dissolve in 800 mL of DI water, add 30 mL of glacial acetic acid, fill up to the mark with DI water, and mix the solution. Store in the refrigerator.

IMPORTANT: Do not prepare Carrez reagent II with zinc sulfate

Buffer solution preparation

NaOH solution (1 M)

Add 40 g of sodium hydroxide pellets in a 1 L plastic volumetric flask, dissolve in about 500 mL of DI water, after cooling down, fill up to the mark with DI water and mix the solution.

NaOH solution 33% (w/w)
Add 66 mL of NaOH (50% w/w) (Fisher Scientific product) in a 100 mL volumetric flask, fill up to mark with DI water, and mix the solution.

PIPES buffer 1.5 M, pH 6.9
Add 20 mL of 1 M NaOH in a 100 mL Erlenmeyer flask, add 22.5 g of PIPES and dissolve. Adjust the pH to 6.9 with 33% NaOH (w/w) in water. Transfer the PIPES buffer solution to a 50 mL polypropylene tube and fill up to mark with DI water. The pH of the obtained buffer solution must be within the range of 6.8–7.0.

Mixture of ethanol (95%) and methanol (5%)
Mix 95 mL of ethanol with 5 mL of methanol.

Sample preparation

1. Weigh 1 g ± 0.1 g of sample in a 50 mL centrifuge tube.

2. Add 4 mL of ethanol and methanol mixture, 125 µL of arabinose internal standard stock solution, and 500 µL of PIPES buffer solution. Note: the aqueous ethanol buffer solution was used to inhibit probiotic activities of the sugars in samples.

3. Mix well by vortexing and let the sample suspension stand for 10 min at ambient temperature.

4. Fill up the centrifuge tube to the 25 mL mark with 40 °C DI water, and vortex for 1 min.

5. Add 500 µL of Carrez I reagent, mix and then add 500 µL of Carrez II reagent solution and 2.5 mL acetonitrile, and fill up to the 50 mL mark with DI water and mix well.

6. Centrifuge the solution at 5000 × g for 10 min and collect the supernatant.

7. Filter the supernatant through a Nalgene 0.2 µm PES syringe filter.

8. Prepare two different dilutions directly in the sample vials.
   - 10-fold dilution: Mix 100 µL of filtered supernatant with 900 µL of DI water.
   - 50-fold dilution: Mix 20 µL of filtered supernatant with 980 µL of DI water.

System preparation and setup

The Dionex ICS-5000+ HPIC system diagram configured for ED detection is shown in Figure 1. A Dionex ICS-5000+ dual system has two pumps. Use the first pump to deliver the eluent and the second pump to deliver the post-column reagent. Connect approximately five inches of tan (0.001¨ i.d.) PEEK tubing to the second pump outlet to achieve ~1500 psi pressure for lowering baseline noise.

The post-column addition of the NaOH-solution will require the installation of a knitted reaction coil after the column but before the detector. Install a PEEK mixing tee (P/N 048227) after the column and use the second pump of the DP to deliver the post-column solution to the tee. Direct the third port on the tee to the reaction coil that is followed by the electrochemical detector cell.

Assemble the cell following the Dionex ICS-5000+ operator's manual[9] and the Dionex ED User's Compendium for Electrochemical Detection[10]. While running the ED cell, bubbles may be trapped in the cell. Air bubbles in the cell can cause spikes in the baseline. To prevent air from becoming trapped in the cell, increase the backpressure on the cell by connecting backpressure tubing to the cell outlet. The backpressure limit for the ED cell is 690 kPa (100 psi). Do not exceed this limit. Six feet of black (0.01" i.d.) PEEK tubing at the cell outlet can generate 30–40 psi backpressure, which can prevent bubble formation.

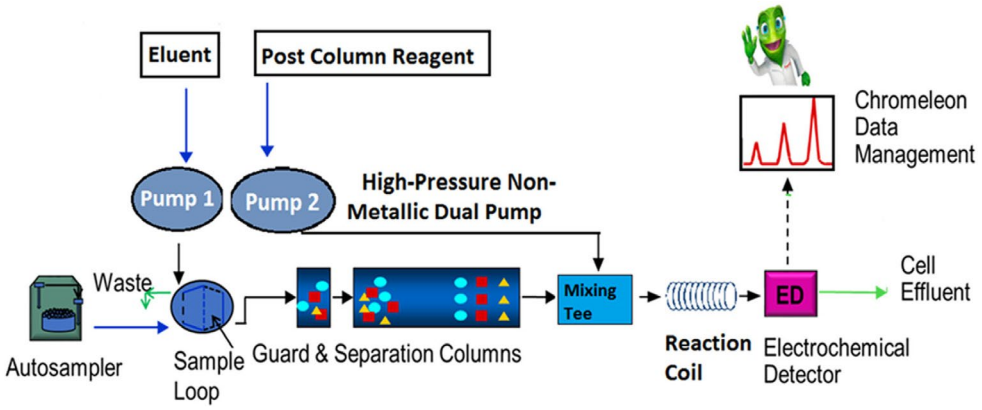


Figure 1. Dionex ICS-5000+ HPIC system configured for ED detection

Results and discussion

Separation

The Dionex CarboPac PA1 column is a general-purpose column for the separation of mono-, di-, and oligosaccharides by high-performance anion-exchange chromatography, coupled with pulsed amperometric detection (PAD). It is one of the recommended columns for analyzing common sugars in food samples. Figure 2 shows a separation of monosaccharides (galactose, glucose, and fructose), disaccharides (sucrose, lactose, and maltose), and the internal standard (arabinose). The seven sugars were well resolved with resolution values for all components >2.0.

Method calibration ranges

Calibration curves with eight concentration levels (L1–L8) were constructed for the sugar standards using the internal standard calibration method (Figure 3). The calibration curves deviate from linearity in the selected calibration range. Therefore, the peak area versus concentration data was fit using a quadratic regression function. Table 6 summarizes the calibration data. The coefficient of determination (r^2) was greater than 0.999 for each component except sucrose and maltose.

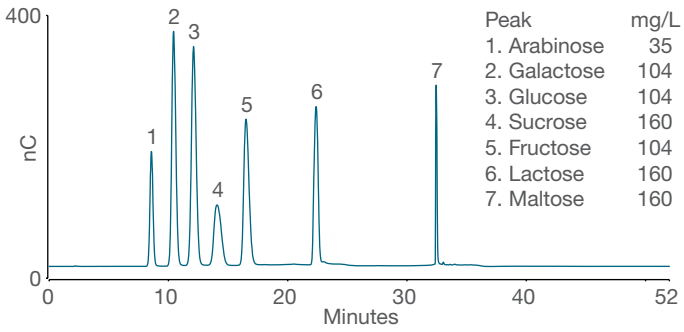


Figure 2. Separation of a seven sugar standard using a Dionex CarboPac PA1-2mm column

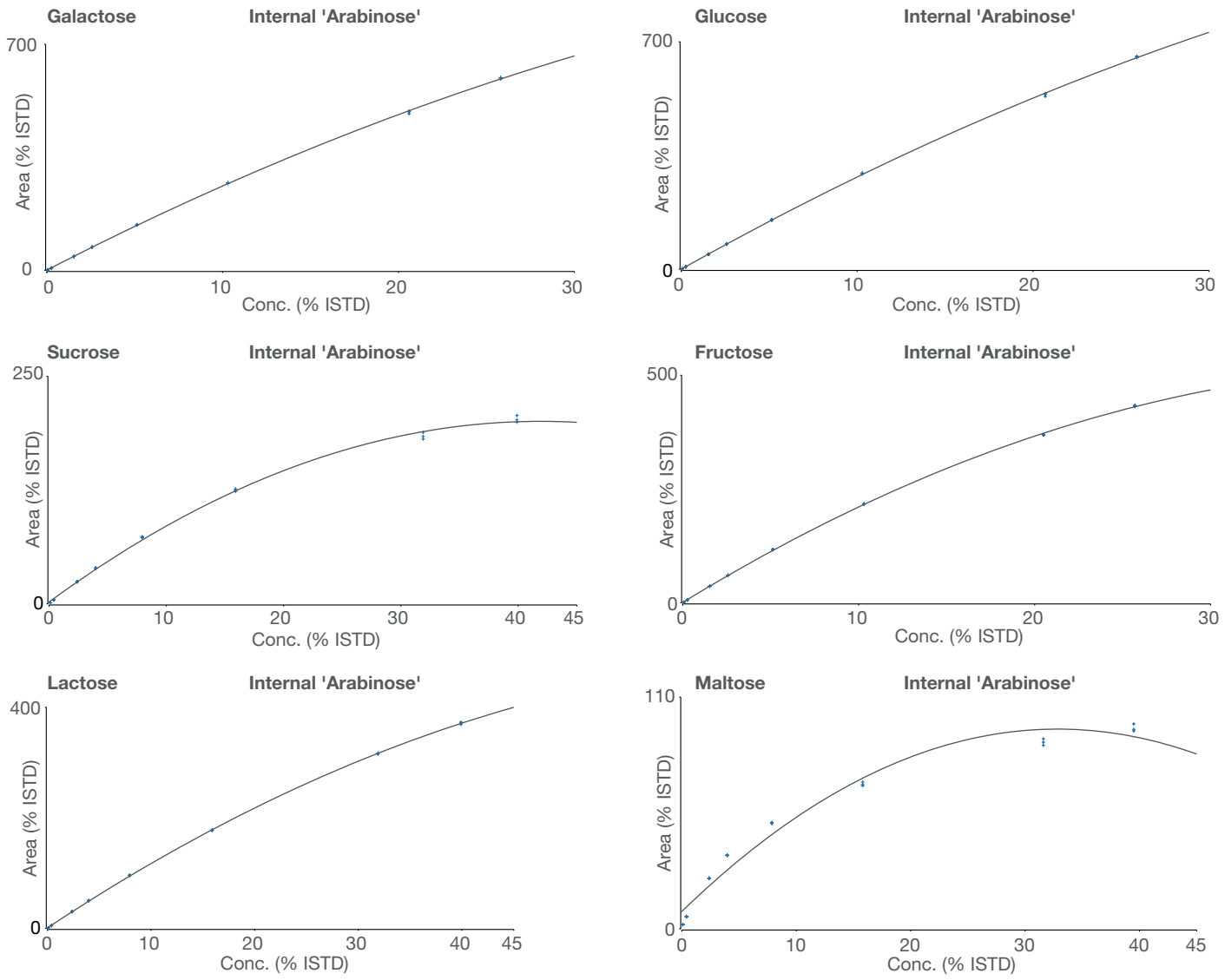


Figure 3. Six carbohydrate calibration curves with quadratic fitting, L1-L8

Table 6. Calibration (L1-L8)

Standard	Range (mg/L)	Calibration	Coefficient of determination (r^2)
Galactose	0.52–260	Quadratic	0.9999
Glucose	0.52–260	Quadratic	0.9999
Sucrose	0.8–400	Quadratic	0.9985
Fructose	0.52–260	Quadratic	0.9999
Lactose	0.8–400	Quadratic	0.9999
Maltose	0.8–400	Quadratic	0.9785

Table 7. Calibration table for sample analysis

Standard	Calibration sandard	Range (mg/L)	Calibration type	Coefficient of determination (r^2)
Galactose	L1–L8	0.52–260	Quadratic	0.9999
Glucose	L1–L8	0.52–260	Quadratic	0.9999
Sucrose	L1–L7	0.8–320	Quadratic	0.9995
Fructose	L1–L8	0.52–260	Quadratic	0.9999
Lactose	L1–L8	0.8–400	Quadratic	0.9998
Maltose	L1–L5	0.8–80	Quadratic	0.9999

Figure 4 shows the chromatogram of calibration standard L8 where sucrose overloads the column and/or detector. While we observed an overload for sucrose in L8, we were informed that not all participants in the collaborative study observed this overload. The coefficient of determination (r^2) of sucrose was 0.9995 if L1–L7 is used for calibration. (Figure 5). The sucrose concentration in all 14 samples is below L7. Therefore, for sucrose L1–L7 was used to prepare the calibration curve for sample analysis. (Table 7).

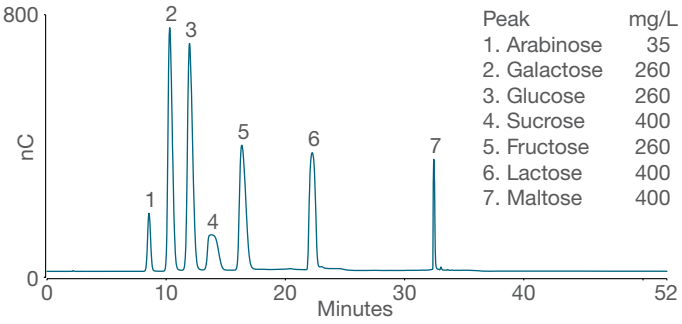


Figure 4. Chromatogram of the L8 calibration standard

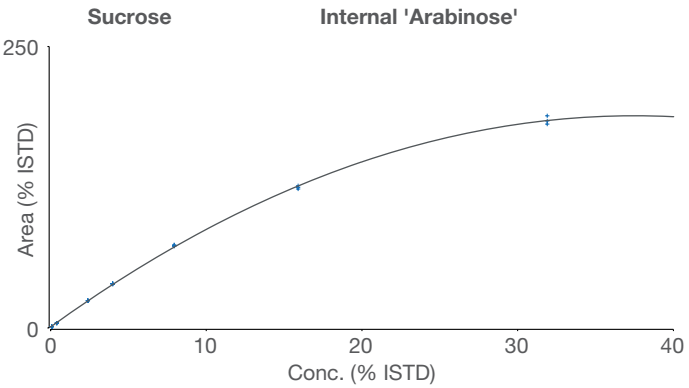


Figure 5. Calibration of sucrose using L1–L7

The maltose calibration curve deviates from the quadratic fit in the selected calibration range (L1–L8), with a coefficient of determination (r^2) of only 0.9785. This is probably because maltose co-elutes with the baseline disturbance that is caused by the eluent gradient (Figure 6). The gradient could be modified to move maltose away from the baseline spike. In this study, we did not modify the gradient because we followed the conditions that were provided for the ISO collaborative study. The coefficient of determination (r^2) increases to 0.9999 if L1–L5 is used. (Figure 7). The maltose concentration of all 14 samples is below L5. Therefore, for maltose L1–L5 was used to prepare the calibration curve for sample analysis (Table 7).

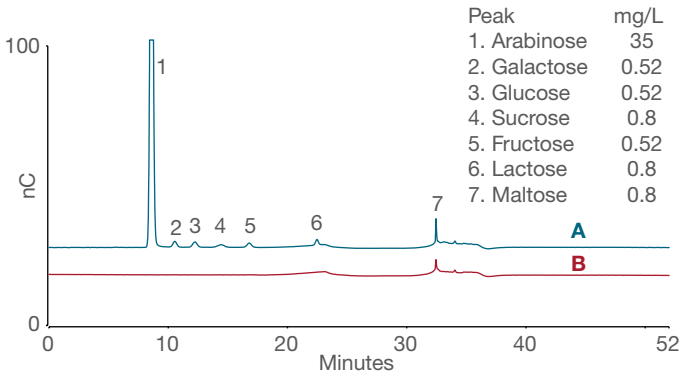


Figure 6. Overlay sugar calibration standard L1(A) with DI water (B)

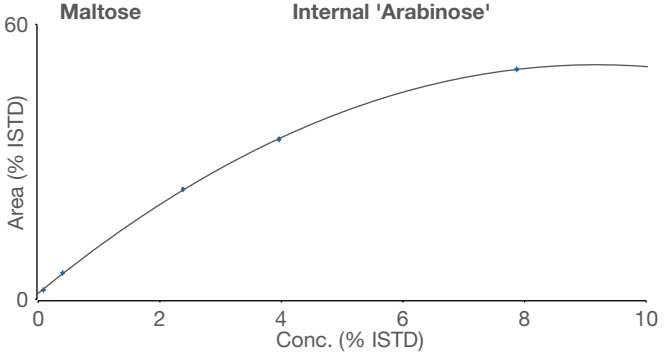


Figure 7. Calibration of maltose using level L1-L5

Method precision

The precision of the method was determined by triplicate injections of the level 3 calibration standard on three separate days over a week. As shown in Table 8, the calculated peak area precision varied from 0.46 to 1.98%, with retention time precision <0.22% for all target carbohydrates.

Sample analysis

Fourteen dairy samples were received from the ISO collaborative study organizer. Seven types of dairy samples were selected to confirm the method is applicable for different dairy products. These samples were milk, milk powder, sweetened condensed milk, cheese, whey powder, and infant formula (Table 9). Duplicate samples were analyzed for each sample type to evaluate method reproducibility. Figures 8–14 show chromatograms of each sample type. Lactose in samples 3 and 4, and sucrose in samples 7 and 9 overload the detector and/or column, thus 50-fold diluted sample chromatograms are used for the quantification of those sugars. Table 10 summarizes the results of sugar analysis. The primary sugar found in whey powder and infant formula is lactose with an average concentration of 72% and 23%, respectively. The main sugar found in sweetened condensed milk and low lactose infant formula is sucrose with an average concentration of 44% and 29%, respectively. Maltose is found in all four infant formula samples with a concentration range of 1.7–2.9%. Galactose and glucose are found in low lactose UHT milk with concentrations of 2.2% and 2.3%, respectively. The duplicate samples had similar results, which indicates that this method is reproducible.

Table 9. Samples

Sample ID	Sample
S1	UHT milk, low lactose
S2	UHT milk, low lactose
S3	Infant formula
S4	Whey powder
S5	Infant formula
S6	Whey powder
S7	Sweetened condensed milk
S8	Sweetened condensed milk
S9	low lactose Infant formula
S10	low lactose Infant formula
S11	Processed cheese
S12	Processed cheese
S13	Cream/fresh cheese
S14	Cream/fresh cheese

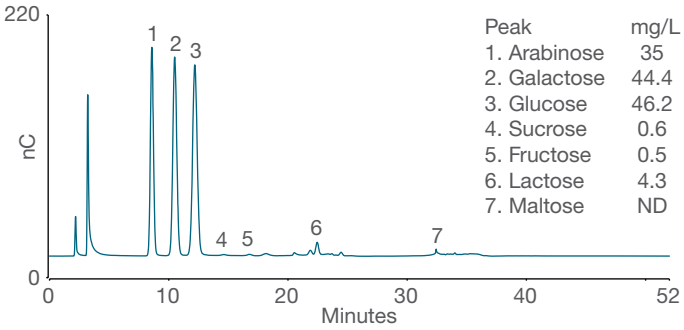


Figure 8. Sugars in 10-fold diluted sample #1 (low lactose UHT milk)

Table 8. Retention time and peak area precisions for triplicate injections

Analyte	Galactose	Glucose	Sucrose	Fructose	Lactose	Maltose
Retention time RSD	0.06	0.12	0.10	0.22	0.06	0.06
Peak area RSD	0.73	0.48	1.98	1.00	0.46	1.50

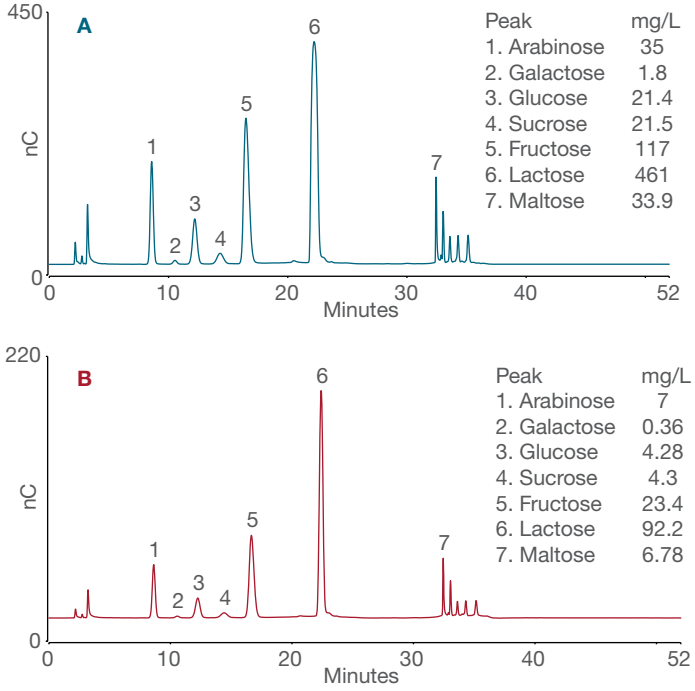


Figure 9. Sugars in sample #3 (infant formula), (A) 10-fold diluted and (B) 50-fold diluted

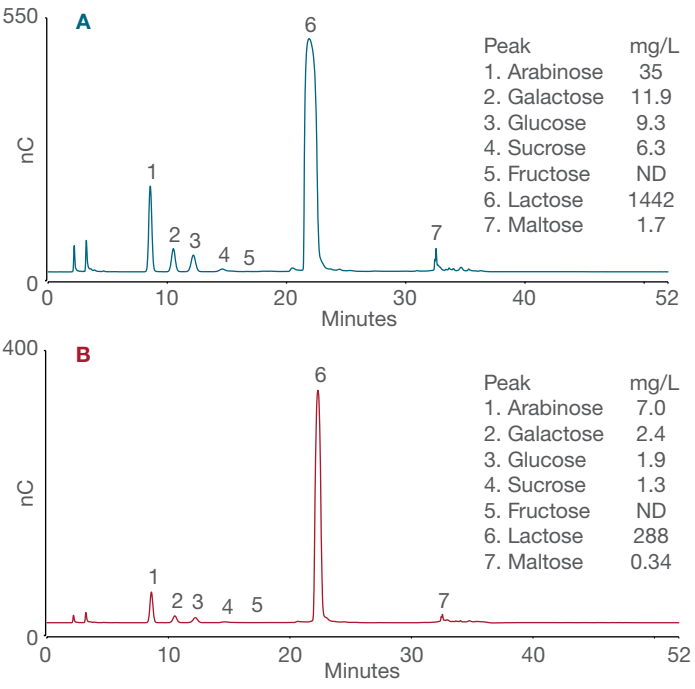


Figure 10. Sugars in sample #4 (whey powder), (A) 10-fold diluted and (B) 50-fold diluted

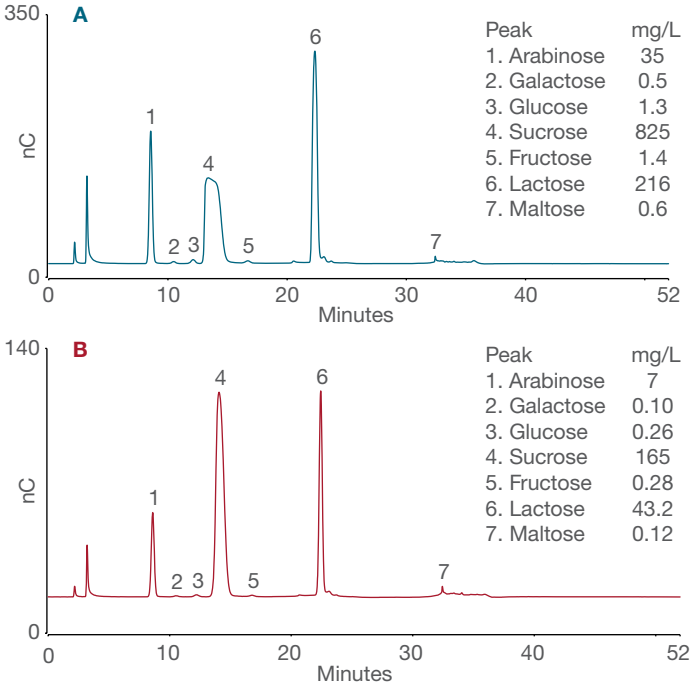


Figure 11. Sugars in sample #7 (sweetened condensed milk), (A) 10-fold diluted and (B) 50-fold diluted

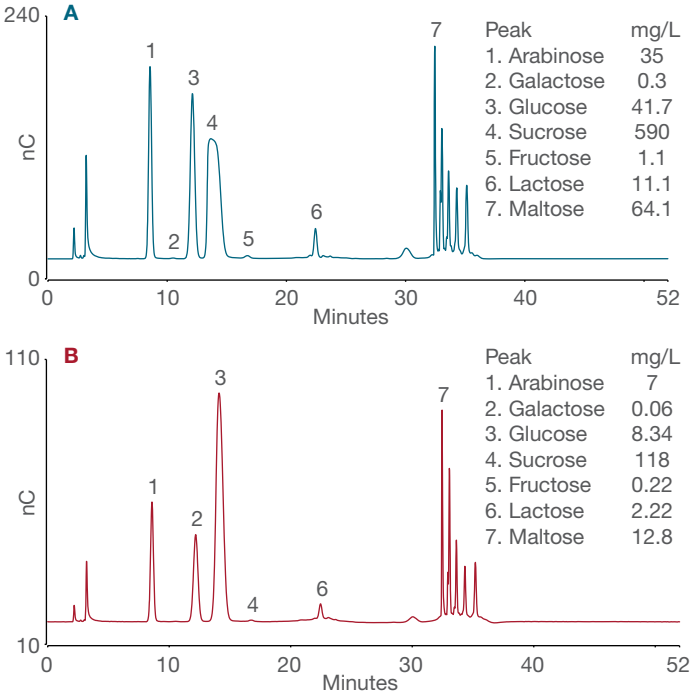


Figure 12. Sugars in sample #9 (infant formula), (A)10-fold diluted and (B) 50-fold diluted

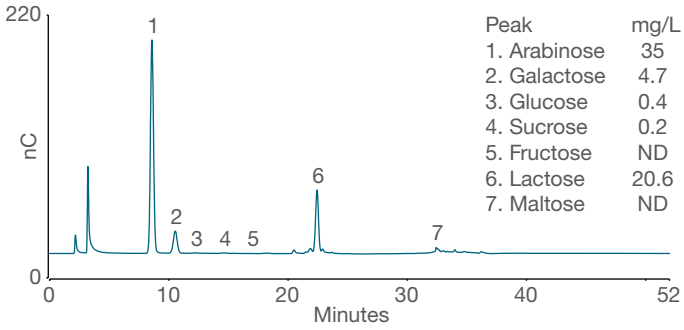


Figure 13. Sugars in sample #11 (processed cheese)

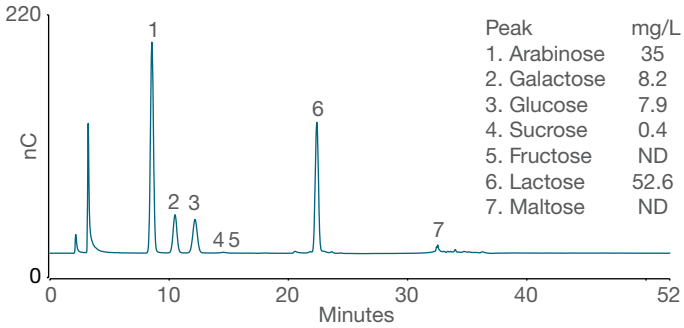


Figure 14. Sugars in sample #13 (cream/fresh cheese)

For each sample, a 10-fold dilution and a 50-fold dilution were prepared. The internal standard in the 50-fold dilution sample is 5-fold more diluted than the internal standard in the 10-fold dilution sample and calibration standards. Therefore, the Chromeleon CDS variable internal standard function is used. Instructions on how to use the variable internal standard are shown in Figure 15.

However, the variable internal standard function does not work for all situations. For example, 50-fold dilution sample #3 is used for quantification of lactose. The internal standard in the sample is 5-fold diluted, the peak area ratio (lactose/internal standard) is much higher than the calibration range, and thus Chromeleon CDS cannot produce a result. In this case, an external calibration curve should be used for the quantification of lactose in sample #3.

Table 10. Sugar contents of the samples (g/100g)

Sample	Galactose	Glucose	Sucrose	Fructose	Lactose	Maltose
1	2.16	2.24	0.0215	0.0324	0.193	ND
2	2.22	2.31	0.0218	0.0431	0.201	ND
3	0.0860	1.03	5.81	1.04	22.9	1.68
4	0.580	0.461	0.0067	0.307	70.3	0.154
5	0.0909	1.07	5.44	1.09	23.2	1.88
6	0.602	0.482	0.0075	0.317	73.3	0.138
7	0.0396	0.0785	0.0643	43.9	11.5	0.0324
8	0.0364	0.0768	0.0626	43.5	11.0	0.0311
9	0.0123	2.00	0.0450	28.3	0.517	2.61
10	0.0134	1.98	0.0472	28.6	0.509	2.93
11	0.239	0.0194	ND	0.002	1.10	ND
12	0.240	0.0192	ND	0.001	1.12	ND
13	0.417	0.396	ND	0.0146	2.76	ND
14	0.404	0.384	ND	0.0137	2.65	ND

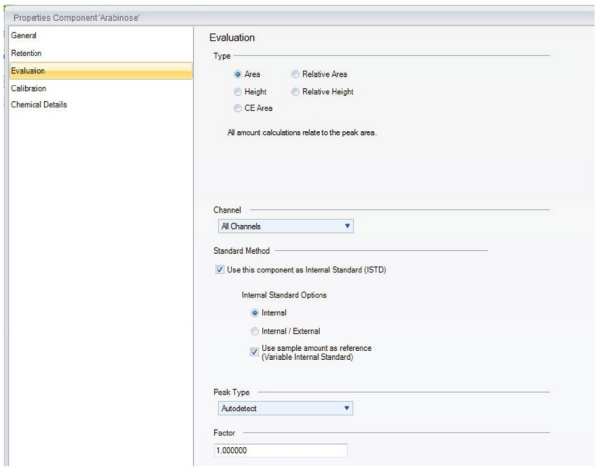
Detection | Component Table | Calibration | Peak Group Table | Chromatogram Subtraction | Advanced Settings | SST/IRC

Component Table

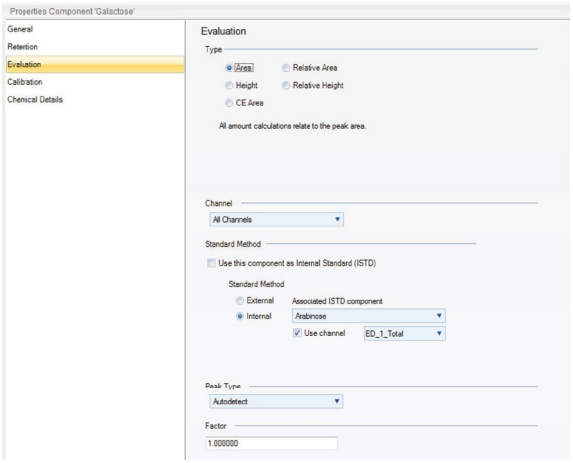
Group Area Drag a column header here to group by that column. Run Component Table Wizard... Show Properties...

#	Name	Ret.Time	Eval.Type	Stand.Meth.	Cal.Type	Level "1"	Li... e...	Level "8"	Conc.Unit
1	Arabinose	8.580	Area	ISTD Var.Internal	Lin, WithOffset	1.000000	1	1.000000	mg/mL
2	Galactose	10.510	Area	Var.Internal Arabinose (ED_1_Total)	Quad, WithOffset	0.000540	0	0.258000	mg/mL
3	Glucose	12.190	Area	Var.Internal Arabinose (ED_1_Total)	Quad, WithOffset	0.000540	0	0.259000	mg/mL
4	Sucrose	14.240	Area	Var.Internal Arabinose (ED_1_Total)	Quad, WithOffset	0.000840	0	0.399000	mg/mL
5	Fructose	16.630	Area	Var.Internal Arabinose (ED_1_Total)	Quad, WithOffset	0.000540	0	0.257000	mg/mL
6	Lactose	22.430	Area	Var.Internal Arabinose (ED_1_Total)	Quad, WithOffset	0.000840	0	0.399000	mg/mL
7	Maltose	32.500	Area	Var.Internal Arabinose (ED_1_Total)	Quad, WithOffset	0.000830	0	0.395000	mg/mL

Step1. Processing method - Component Table



Step 2. Arabinose check “Variable internal standard”



Step 3. For each sugar component, choose internal standard method

#	Name	IntStd
151	Sample 10 F diluted	1.0000
152	Sample 50 F diluted	0.2000
153	Std 2	1.0000
154	Std 4	1.0000
155	Std 6	1.0000
156	Std 8	1.0000

Step 4. Sequence file, input InStd 0.2 for 50-fold diluted sample

Figure 15. Chromeleon CDS variable internal standard, steps 1–4

thermo scientific

Conclusions

This application note demonstrated that ISO/DIS 22184 for dairy product sugar analysis[6] could be successfully executed with a Dionex CarboPac PA1-2mm column using a Dionex ICS-5000+ HPIC system. The separation, linearity, reproducibility, and sensitivity were excellent. This method is reliable and can be used for the determination of the major sugars (lactose, galactose, glucose, fructose, sucrose, maltose) in dairy products.

References

1. ISO 11292: (1995) "Instant Coffee-Determination of free and total carbohydrate contents-Method using high performance anion-exchange chromatography," International Organization for Standardization, Geneva, Switzerland, https://www.iso.org/standard/19270.html
2. CEN/TS 15754: 2008(2008) "Animal feeding stuffs-Determination of sugar content-High-performance anion-exchange chromatographic method (HPAEC-PAD)," European Committee for Standardization Technical Specification. https://www.evs.ee/products/cen-ts-15754-2008
3. Thermo Scientific Application Note 248: Determination of lactose in lactose-free milk products by high-performance anion-exchange chromatography with pulsed amperometric detection https://assets.thermofisher.com/TFS-Assets/CMD/Application-Notes/AN-248-IC-Lactose-Milk-AN70236-EN.pdf
4. Thermo Scientific Application Note 72632: Fast and sensitive determination of lactose in lactose-free products using HPAE-PAD https://assets.thermofisher.com/TFS-Assets/CMD/Application-Notes/an-72632-ic-hpae-pad-lactose-free-products-an72632-en.pdf
5. Thermo Scientific Application Note AN 72780: Determination of lactose in lactose-free dairy products using HPAE coupled with PAD and MS dual detection https://assets.thermofisher.com/TFS-Assets/CMD/Application-Notes/an-72780-ic-ms-lactose-free-dairy-an72780-en.pdf
6. ISO/WD 22184 & IDF/WD 244, Milk and milk products-Determination of the sugar contents – High performance anion exchange chromatographic method (HPAEC-PAD), May 1st, 2017
7. Sanders, P.; Ernste-Nota, V.; Visser, K.; van Soest, J.; Brunt, K. The Determination of Sugars in Dairy Products: Development of a New Standard Method for the International Dairy Federation and the Internal Organization for Standardization. *J AOAC Int.* **2017** Sep 1, *100*(5), 1577-1581. doi: 10.5740/jaoacint.17-0068. Epub 2017 May 11.
8. Thermo Scientific Technical Note 71: Eluent Preparation for High-Performance Anions-Exchange Chromatography with Pulsed Amperometric Detection http://tools.thermofisher.com/content/sfs/brochures/TN-71-Eluent-Preparation-for-High-Performance-Anion-Exchange-Chromatogarphy-with%20APD-TN-70669.pdf
9. Thermo Scientific Electrochemical Detection User's Compendium, P/N 065340-02, April 2013 https://assets.thermofisher.com/TFS-Assets/CMD/manuals/Man-065340-Electrochemical-Detection-Man065340-EN.pdf
10. Thermo Scientific Dionex ICS-5000+ Ion Chromatography System Operator's Manual P/N 065446.December 2014 https://assets.thermofisher.com/TFS-Assets/CMD/manuals/Man-065446-IC-ICS-5000-Operators-Manual.pdf
11. Thermo Scientific Dionex CarboPac PA1 Columns Product Manual P/N 065547-01, November 2013 https://assets.thermofisher.com/TFS-Assets/CMD/manuals/Man-065547-CarboPac-PA1-Column-Man065547-EN.pdf

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

ThermoFisher SCIENTIFIC