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Profiling Mono and Disaccharides in Milk and Infant Formula Using the ACQUITY Arc System and ACQUITY QDa Detector

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APPLICATION BENEFITS

- The ACQUITY Arc™ System provides a choice of two flow paths to emulate HPLC or UHPLC separations.
- Chromatographic separation of the difficult isomeric pair, glucose and galactose.
- The ACQUITY® QDa® Detector provides complimentary detection to Refractive Index (RI) or Evaporative Light Scattering (ELS) detectors that are commonly employed for carbohydrate analysis.

WATERS SOLUTIONS

ACQUITY® Arc™ System

ACQUITY QDa®

XBridge® BEH Amide XP Column

KEYWORDS

Mass detection, infant formula, milk, carbohydrate, mono and disaccharide

INTRODUCTION

Sugars and sugar alcohols are classes of carbohydrates that are natural constituents of foods and provide important nutritional benefits. Some sugars are added to processed foods in order to enhance flavor or to mimic fresh food products. With the increasing incidence of obesity and diabetes across the developed world, the need to monitor sugar intake has grown in recent years. Consequently, there are now requirements to provide accurate information about sugar content on food product labels in order to comply with increasingly stringent regulatory demands.

The analysis of these compounds is challenging because they lack chromophores within their compound structures, and because of the close similarity among the various molecules, many of which are simple isomers of one another. Structures and formulae are shown in Figure 1. Due to its separation power, accuracy and speed of analysis, HPLC has become the method of choice for the analysis of sugars.[1] HPLC techniques typically employ RI or ELS detection. RI detection requires careful control of the mobile phase to avoid any changes during the analysis and therefore requires isocratic elution. With RI detection it is also difficult to change the mobile phase composition from one analysis to the next because the RI detector may require several hours to equilibrate when a different mobile phase composition is introduced. ELS detection is more robust for mobile phase composition changes, but ELS often does not meet the sensitivity demands for the detection of sugars in complex matrices.

An alternative gaining traction is the use of mass detection with electrospray ionization (ESI). Waters® ACQUITY QDa Detector offers the opportunity to decrease detection limits as well as the ability to obtain mass spectral information on components in the sample. The combination of chromatographic retention time and mass information can provide improved selectivity for the profiling of sugars and sugar alcohols. The ACQUITY QDa Detector is the only mass detector that has been holistically designed to be incorporated with an LC system. It fits in the LC stack, occupying the same amount of space as a PDA detector. Extensive training is not required, so users already familiar with HPLC can quickly take advantage of the improved selectivity and sensitivity that mass detection affords. In this application note we describe the use of the ACQUITY QDa Detector coupled to the ACQUITY Arc System for the profiling of sugars in milk and infant formula.

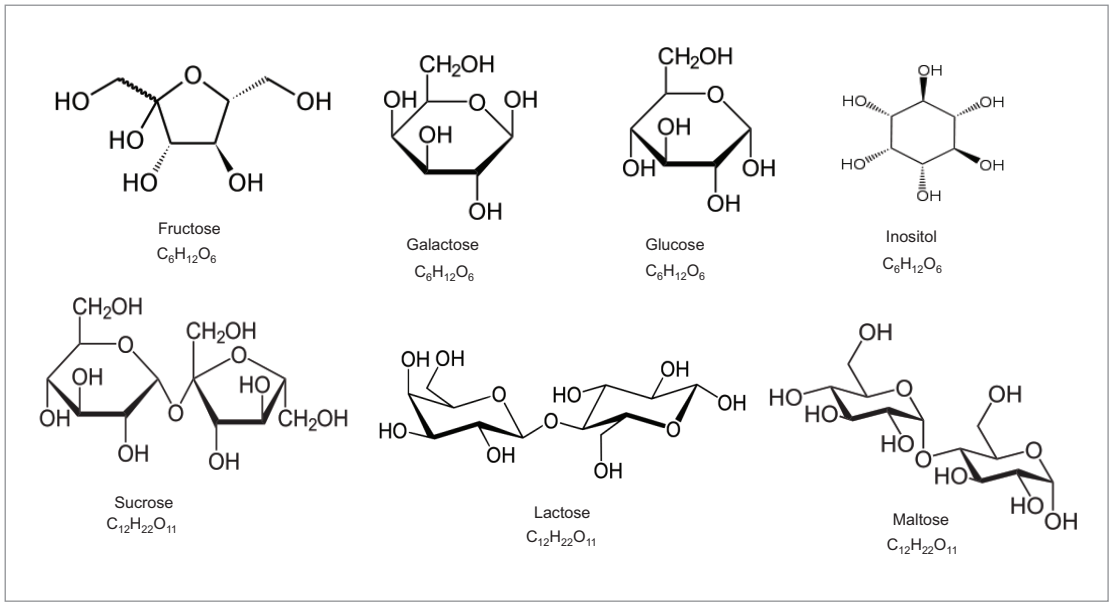

Figure 1. Structures and formulas for the sugar compounds analyzed.

Figure 2. ACQUITY Arc System shown with the PDA and ACQUITY QDa detectors.

EXPERIMENTAL

LC conditions

LC system:	ACQUITY Arc Path 1
Run time:	30.0 min
Column:	XBridge BEH Amide XP 2.5 µm, 3.0 × 150 mm
Column temp.:	85 °C
Mobile phase:	90:5:5 Acetonitrile-water-2-propanol with 0.05 % diethylamine and 500 ppb guanidine hydrochloride
Flow rate:	0.8 mL/min
Injection volume:	1 µL

MS conditions

MS system:	ACQUITY QDa (Performance)
Ionization mode:	ESI-
Capillary voltage:	0.8 V
Cone voltage:	5.0 V
Probe temp.:	600 °C
Acquisition rate:	2 Hz
Full scan:	100 to 500 *m/z*
Curve fit:	Quadratic, 1/x weighting
Smoothing:	Mean, Level 7
SIR $\{M+Cl^-\}^-$:	215.0 fructose, glucose, galactose, inositol 377.0 sucrose, lactose, maltose

Standard preparation

A 50 mg/L stock of the seven saccharides listed above was prepared in 1:1 acetonitrile-water. This stock was further diluted to produce nine individual levels (0.5, 1, 2, 2.5, 5, 10, 20, 25, and 50 mg/L).

Sample preparation

Samples of a non-fat dry milk powder, a dairy-based infant formula, a soy based infant formula, and a low fat milk were purchased. These were prepared based on the procedure described by Chavez-Servin et al[2] as follows:

- Add approximately 0.6 g sample to a 25 mL volumetric flask.
- Add 10 mL 1:1 ethanol-water.
- Sonicate in a water bath at 60 °C for 25 min.
- Cool, add 250 µL Carrez 1* reagent, stir for 1 min.
- add 250 µL Carrez 2** reagent, stir for 1 min.
- Add 5 mL acetonitrile, mix.
- Make to 25 mL with 1:1 ethanol-water.
- Mix well and transfer to a 50 mL centrifuge tube, allow to settle.
- Centrifuge at an rcf of 2465 g for 30 min.
- Filter supernatant through a 0.2 µm PVDF filter.
- *Carrez 1 reagent: dissolve 0.36 g $K_4[Fe(CN_6).3H_20$ in 10 mL water.
- **Carrez 2 reagent: dissolve 0.72 g $ZnSO_4.7H_2O$ in 10 mL water.

The supernatant was diluted with 1:1 water:acetonitrile, 1:500 for the analysis of sucrose and lactose, and 1:20 for the analysis of fructose, glucose, galactose and inositol. The initial dilution of 25 was included for the final quantification calculations.

RESULTS AND DISCUSSION

Figure 2 shows the ACQUITY Arc System with the ACQUITY QDa and PDA detectors. Although shown with PDA, UV detection was not used for this analysis. Figure 3 shows the SIR chromatograms of the seven saccharide standards at a 5 ppm level (standard 5) used in the study. The annotated *m/z* (215 and 377) represent the $[m+ Cl]^-$ adducts. Figure 4 shows the mass spectra for the analytes. Addition of guanidine hydrochloride to the mobile phase shifts the equilibrium to the chloride adducts, *m/z* 215 for the monosaccharides and inositol, and 377 *m/z* for the disaccharides. Note the baseline separation of galactose and glucose using this chemistry, and also that lactose eluted before maltose.

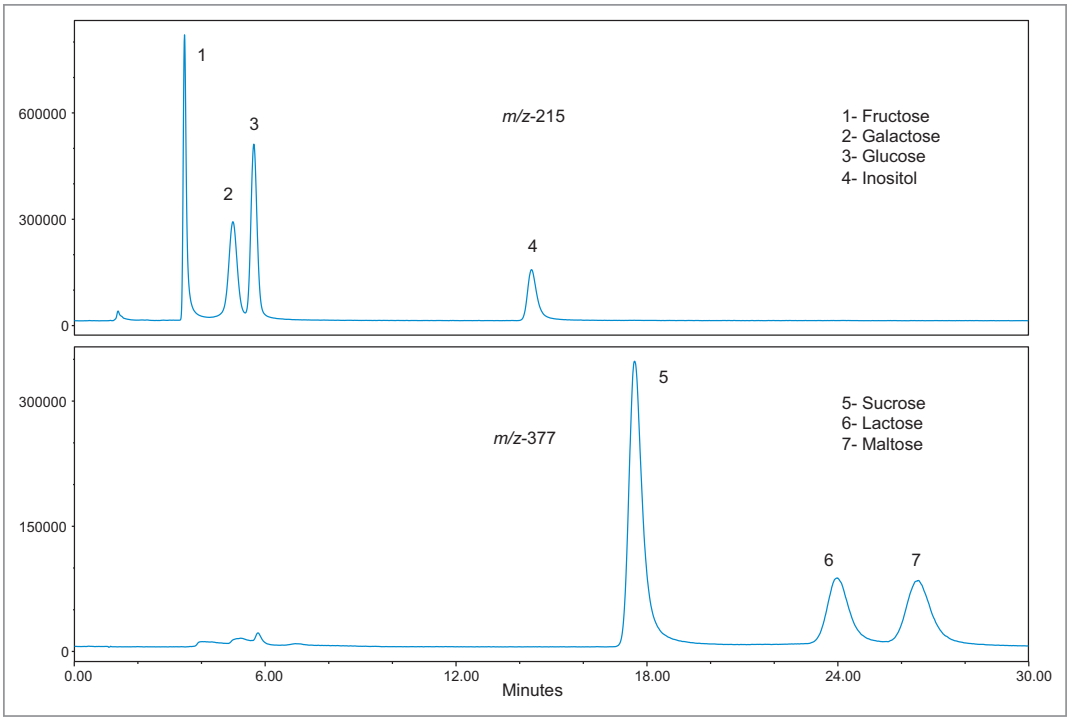

Figure 3. SIR chromatograms of the seven saccharide standards used in the study, the annotated m/z represents the $[m+ Cl]^-$ adducts.

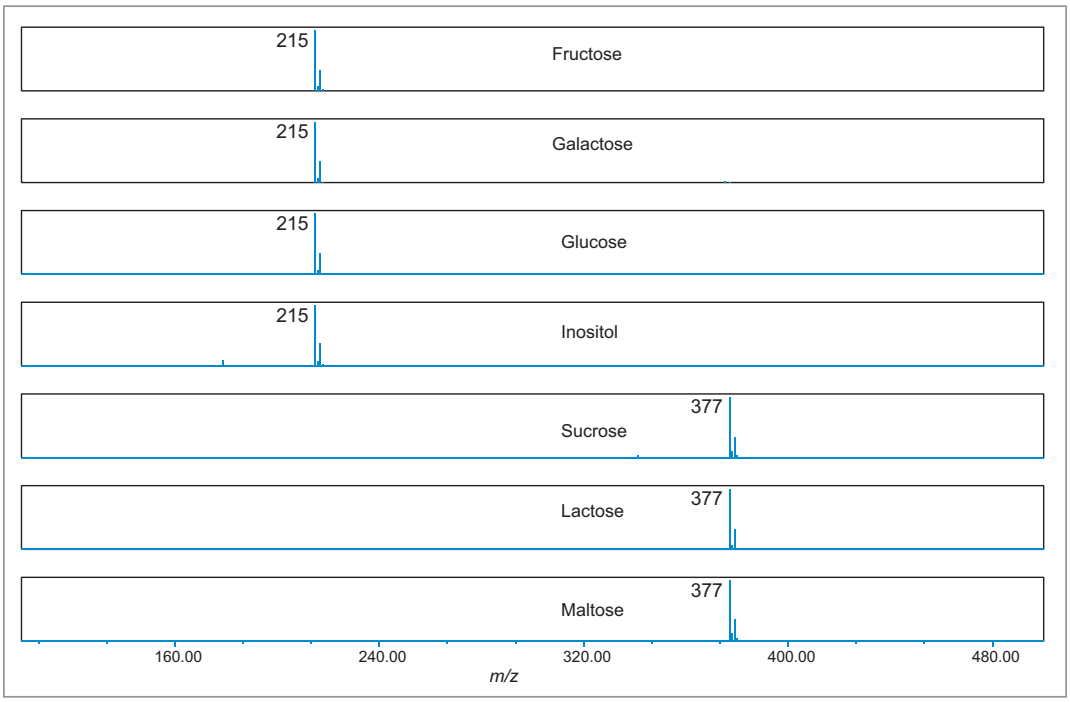

Figure 4. Mass spectral information extracted from SIRs of the seven saccharide standards. The annotated m/z represents the $[m+ Cl]^-$ adducts.

Figure 5 shows the SIR chromatograms of the saccharide standards at 5 ppm (standard 5) along with the dairy and soy based infant formulas at *m/z* 215. Note the absence of galactose in the soy based formula as would be expected. However, inositol is present in both formulations as it is an important nutrient in infant formula.[3] Inositol is highlighted in Figure 6.

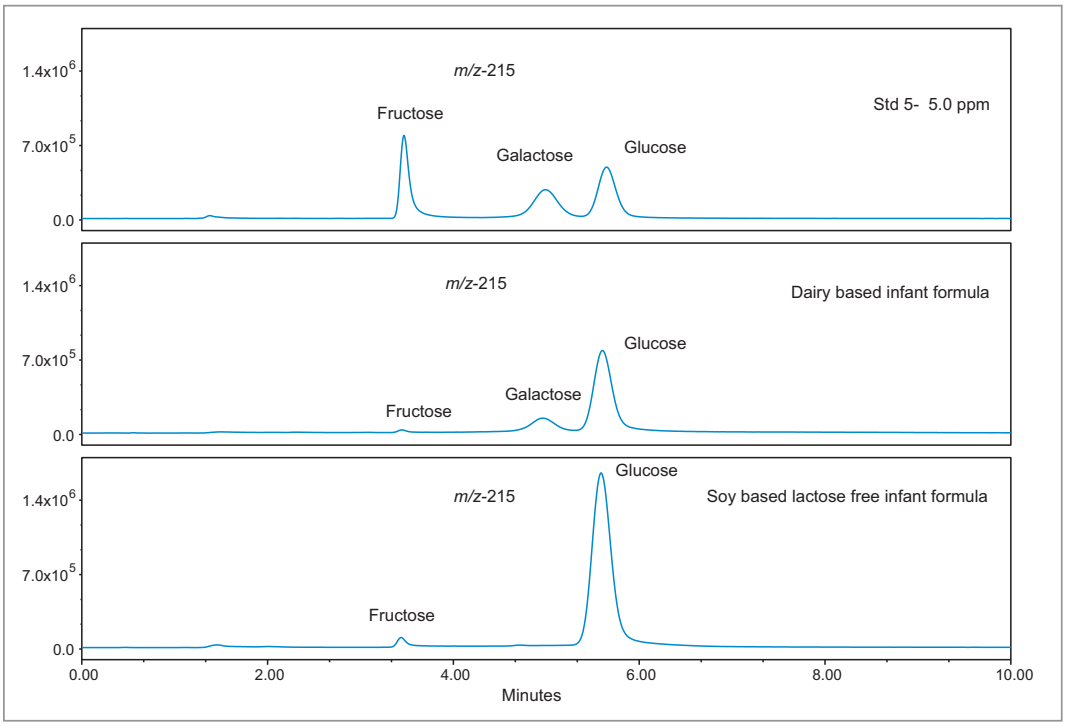

Figure 5. SIR chromatograms of Standard 5 with a dairy and soy based infant formula at m/z 215 for the saccharides fructose, galactose and glucose.

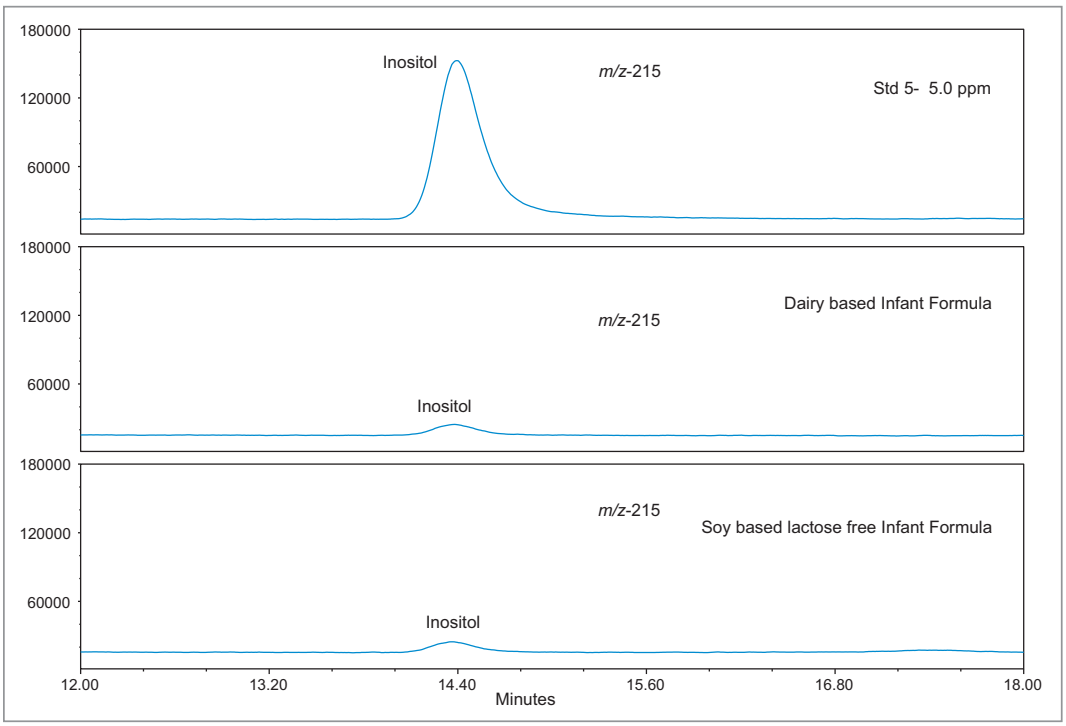

Figure 6. Annotated SIR profile of inositol in standard 5, along with a dairy and soy based infant formula.

Figure 7 shows the SIR chromatograms of the saccharide standards at 5 ppm (standard 5), along with the dairy and soy-based infant formulas at *m/z* 377. Here we see the absence of lactose in the soy formulation. However sucrose and maltose are present, which are absent in the dairy formulation. This is also to be expected as these two sugars are derived from plant based sources and should not be found in a dairy matrix unless added artificially. Figure 8 shows the calibration curves for the analytes. The regression coefficient (R^2) was >0.998 for all analytes.

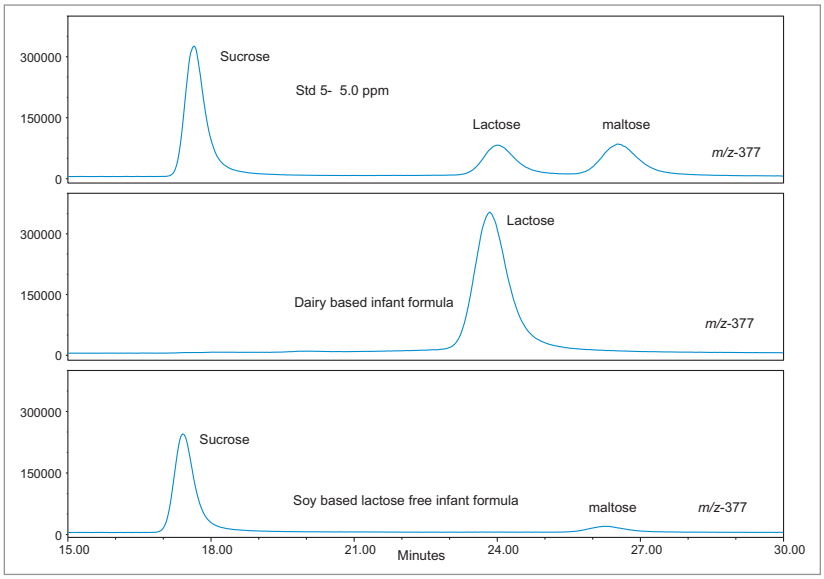

Figure 7. Annotated SIR profile of sucrose, lactose, and maltose in standard 5, along with a dairy and soy based infant formula.

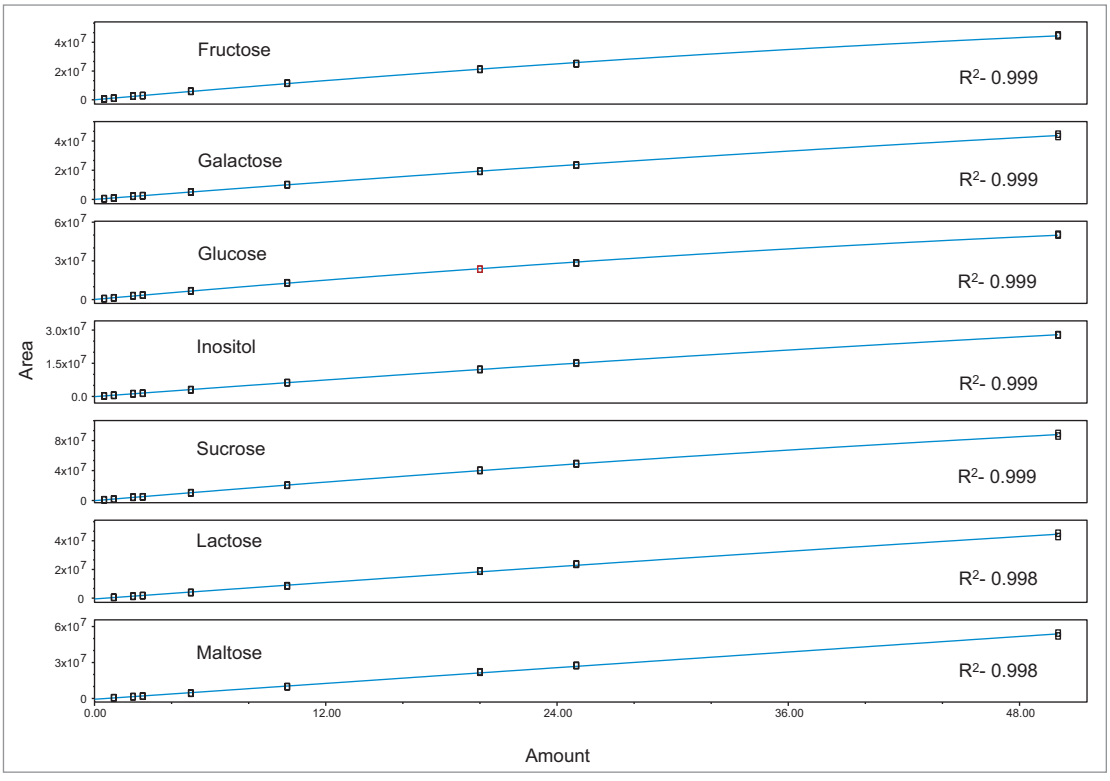

Figure 8. Calibration curves for the seven saccharide standards showing R^2 values, a quadratic fit was used.

Table 1 lists the quantitated values for the saccharides studied. The values for inositol in the dairy and soy based infant formula generally agree with the values reported by Indyk.[4] Likewise, the value for lactose in the dairy infant formula generally agrees with that reported by Ferreira.[5] Table 2 lists reproducibility data for 7 injections of the soy based infant formula. RSD's were <0.25% for retention time and 8.20% for amount.

Sample	Fructose	Galactose	Glucose	Inositol	Sucrose	Lactose	Maltose
Non Fat Dry Milk	0.04	0.837	1.295	0.389	ND	381.647	ND
Dairy Infant Formula	0.113	2.377	7.681	0.339	ND	394.996	ND
Soy Infant Formula	0.481	ND	19.579	0.359	83.705	ND	28.594
Low Fat Milk	ND	0.130	0.125	0.078	ND	42.775	ND

Table 1. Calculated concentrations from the quantification studies of the various dairy and infant formulas profiled in this study (g/kg).

Analyte	RT	% RSD	Amount	%RSD
Fructose	3.44	0.10	0.48	8.17
Glucose	5.59	0.10	19.58	3.71
Inositol	14.34	0.10	0.36	3.14
Sucrose	17.41	0.14	83.71	1.42
Maltose	26.27	0.22	28.59	2.02

Table 2. Reproducibility data for retention time (Min) and amount (g/kg) for seven injections of a soy based infant formula.

CONCLUSIONS

The analysis of carbohydrates in dairy products can be challenging because of the mix of closely related UV transparent compounds. The combination of the ACQUITY Arc System, ACQUITY QDa Detector, and the XBridge BEH Amide Column offers scientists the advanced performance expected of ACQUITY separations, high resolution, sensitivity, and improved throughput, along with a complimentary mass detector to RI and ELS that provides the additional advantages of:

- Improved analytical selectivity by combining both retention time and mass analysis for compound identification.
- Detection of UV transparent molecules using a sensitive and selective detector.
- Chromatographic separation of the difficult isobaric pair, galactose and glucose.

References

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3. HE Indyk and DC Woolard. Determination of Free Myo-Inositol in Milk and Infant Formula by High Performance Liquid Chromatography. *Analyst*. 119: 397-402, 1994.
4. Ibid p 401.
5. Ferreira, Op. Cit. p 227.

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