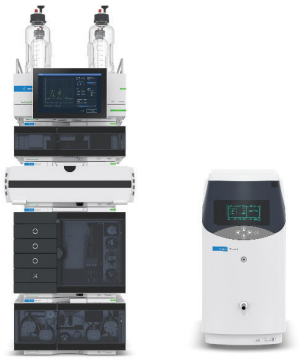
Application Note

Food and Beverage Testing

Liquid Chromatographic Separation of Sugars

Using the Agilent 1290 Infinity III LC System coupled with ELSD



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Abstract

This application note successfully demonstrates the separation of eight commonly tested sugars in food and beverages, including the challenging glucose/galactose epimer pair, on the Agilent 1290 Infinity III LC System coupled with an Agilent 1290 Infinity III Evaporative Light Scattering Detector (ELSD). The method has been proven to be robust, with a low detection limit of quantification (LOQ), excellent calibration linearity of greater than 0.99, and confirmed injection repeatability.

Introduction

The most abundant components in food are carbohydrates, with monosaccharides and disaccharides (in short sugars) being two of the major classes.[1] The identification and quantification of sugars are important procedures for quality control, nutritional labelling, and authenticity study purposes.

Many analysis techniques have been reported, and high-performance liquid chromatography (HPLC) is one of many popular techniques.[1] A refractive index detector (RID), a universal detector, is often used with HPLC in sugar analysis as sugars lack chromophores, meaning that they do not absorb ultraviolet light to generate a sufficient absorbance response. The drawback of RID is that it is not compatible with gradient elution, which leads to long elution times of analytes and a resultant long analysis time. As a result, ELSD has been introduced as an alternative detector which is gradient-elution compatible and provides better sensitivity.

In this study, the chromatographic separation of eight common sugars was demonstrated on the Agilent 1290 Infinity III LC System coupled with the Agilent 1290 Infinity III ELSD, together with an Agilent ZORBAX Original 70Å Carbohydrate Analysis column. This column selection is crucial for the successful separation of sugars, especially the glucose/galactose epimer pair. Glucose and galactose have the same atomic mass, and differ only in the location of the hydroxyl (OH) group at the carbon-4 position (Figure 1). The analytical method described here was validated based on the limit of quantification (LOQ), calibration linearity, and injection repeatability to demonstrate method robustness.

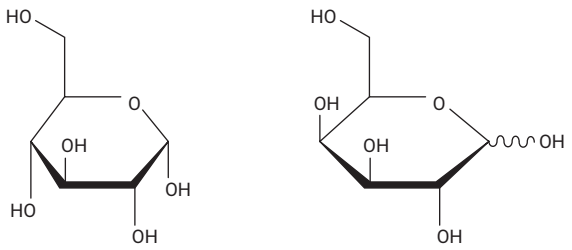

Figure 1. Structures of glucose and galactose.

Experimental

Instrumentation

The Agilent 1290 Infinity III LC System comprises the following modules, as shown in Table 1.

Table 1. Agilent 1290 Infinity III LC System instrumentation.

Part Number	Instrument
G7120A	1290 Infinity III High-Speed Pump
G7167B	1290 Infinity III Multisampler
G7116B	1290 Infinity III Multicolumn Thermostat
G7102A	1290 Infinity III ELSD

Chromatographic conditions

Table 2 describes the chromatographic conditions for HPLC and ELSD for the separation of sugars.

Table 2. Chromatographic conditions for HPLC and ELSD.

Parameter	Value
HPLC	
Mobile Phase	A) Water B) Acetonitrile
Gradient	Time (min) %B 0 81 5.5 81 13.0 60
Stop Time	13.0 min
Post Run	3.0 min
Flow Rate	1.5 mL/min
Injection Volume	10 µL
Needle Wash	1:1 Water:acetonitrile; 3 sec
Column	Agilent ZORBAX Original 70Å Carbohydrate Analysis column, 4.6 × 150 mm, 5 µm (p/n 843300-908)
Column Temperature	25 °C Agilent InfinityLab Quick Connect Heat Exchanger, standard flow (p/n G7116-60015)
ELSD	
Evaporator Temperature	60 °C
Nebulizer Temperature	90 °C
Gas Flow Rate	1.50 SLM
Data Rate	10 Hz
Smoothing	30

Software

Agilent OpenLab CDS, version 2.8 was used for data acquisition and interpretation.

Solvents and standards

The solvents that were used in the analysis are as follows:

- Acetonitrile (HPLC grade, purchased from Aik Moh, Singapore)
- De-ionized water (Milli-Q, purchased from Millipore Sigma, Burlington, MA)

The following sugar standards were purchased from Sigma-Aldrich, St. Louis, MO: arabinose, fructose, glucose, galactose, maltose, lactose, sucrose, and xylose.

Standard preparation

A sugar stock standard solution of 10 mg/mL was prepared by dissolving 100 mg of each sugar (arabinose, fructose, glucose, galactose, maltose, lactose, sucrose, and xylose) in 10 mL of water. It was then further diluted with water to prepare the calibration solution (0.1, 0.2, 0.5, 1, 2, 5, and 10 mg/mL).

Results and discussion

Peak separation

Figure 1 illustrates the separation of eight sugars on a ZORBAX Original 70Å Carbohydrate Analysis column, using gradient elution with common HPLC mobile phase solvents (water and acetonitrile). ELSD enables the use of gradient elution to elute sugars in a shorter time. Unlike a refractive index detector, where only isocratic elution is allowed, the peaks tend to be broader as the analytes are eluted over a longer period. On the other hand, it can be challenging to resolve glucose and galactose if the column is not appropriately chosen, as both sugars are structurally similar. Using a ZORBAX Original 70Å Carbohydrate Analysis column, good glucose and galactose separation can be achieved.

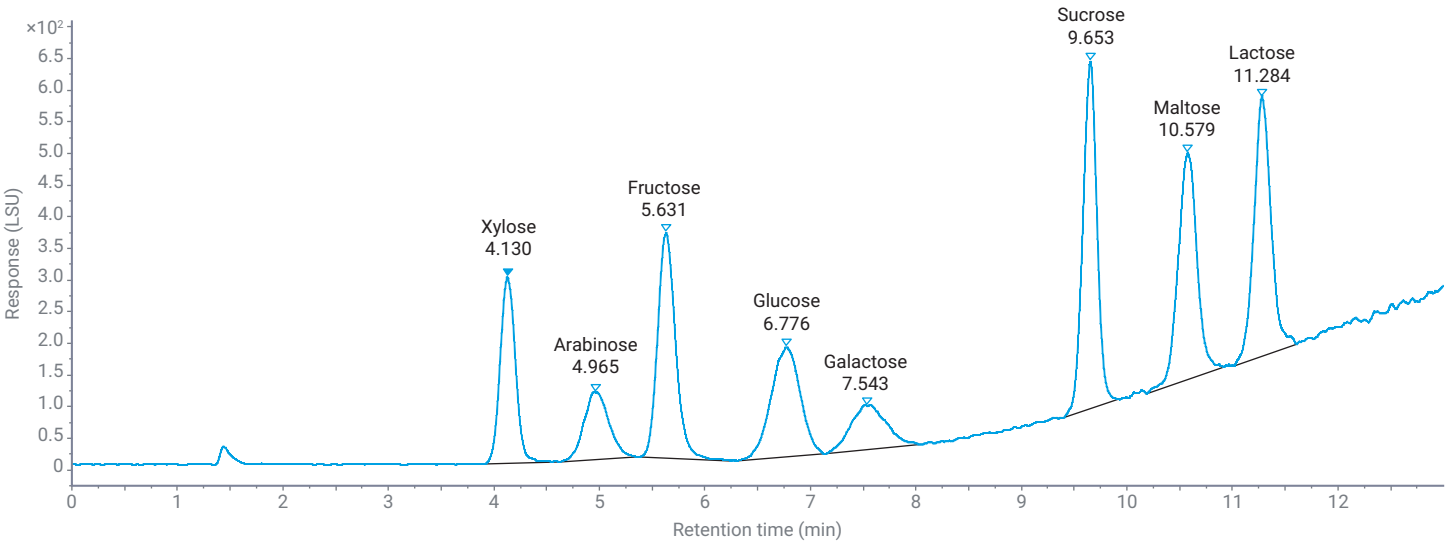

Figure 2. Representative chromatogram of 0.5 mg/mL sugar standard solution.

Method validation

This analytical method was evaluated in terms of limit of quantification, calibration linearity, and injection repeatability.

Limit of Quantification (LOQ)

The LOQ of an analyte is commonly defined as the concentration at which the signal-to-noise (S/N) ratio of the analyte is observed to be 10. The LOQs for this study, estimated from the lower concentration of the sugar standard solution, is listed in Table 3.

Table 3. LOQ and calibration parameters of each sugar.

Compound	LOQ (mg/mL)	Calibration Level (mg/mL)	Curve Model	R^2
Xylose	0.03	0.1 to 10	Log/log	1.000
Arabinose	0.07	0.1 to 10	Log/log	0.999
Fructose	0.04	0.1 to 10	Log/log	1.000
Glucose	0.04	0.1 to 10	Log/log	0.999
Galactose	0.11	0.2 to 10	Log/log	0.998
Sucrose	0.03	0.1 to 10	Log/log	0.998
Maltose	0.07	0.1 to 10	Log/log	0.998
Lactose	0.07	0.1 to 10	Log/log	0.999

Calibration linearity

A series of standard solutions with varying concentrations of sugars were injected to construct the calibration curves. These curves demonstrated excellent linearity, with correlation coefficients (R^2) close to 1. Table 3 details the calibration parameters for each sugar. As the ELSD response is nonlinear, the log/log curve model was used.

Injection repeatability

Six consecutive injections of 0.5 mg/mL sugar standard solution were conducted to assess repeatability, evaluated based on the relative standard deviation (RSD) of retention time (RT) and peak area. Overall, the repeatability was excellent, with an RT RSD of less than 0.2% and a peak area RSD of less than 2%. The data are provided in Table 4.

Table 4. Repeatability data (N = 6) of each sugar.

Compound	Average RT (min)	RT RSD (%)	Average Area	Area RSD (%)
Xylose	4.125	0.11	3000.57	1.44
Arabinose	4.967	0.15	1808.72	1.32
Fructose	5.621	0.13	4227.23	1.18
Glucose	6.755	0.19	3291.09	1.49
Galactose	7.538	0.05	1627.31	1.88
Sucrose	9.642	0.07	5168.84	1.63
Maltose	10.568	0.07	4434.48	1.72
Lactose	11.276	0.06	4475.81	1.96

Conclusion

Eight sugar analytes were successfully separated and detected using the Agilent 1290 Infinity III LC System coupled with ELSD on an Agilent ZORBAX Original 70Å Carbohydrate Analysis column. It is worth noting that good separation between the glucose and galactose epimer pair was achieved. The analytical method has been validated, and has proven to demonstrate a low detection limit, excellent calibration linearity, and verified injection repeatability.

Reference

1. Debebe, A.; Temesgen, S.; Redi-Abshiro, M.; Chandravanshi, B. S.; Ele, E. Improvement in Analytical Methods for Determination of Sugars in Fermented Alcoholic Beverages. *Journal of Analytical Methods in Chemistry 2018*, **2018**, 1–10. DOI:10.1155/2018/4010298.

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DE-008929

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Printed in the USA, August 21, 2025
5994-8594EN

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