Characterization of carbohydrates in commercial fruit juices using the Agilent 1200 Series evaporative light scattering detector

Application Note

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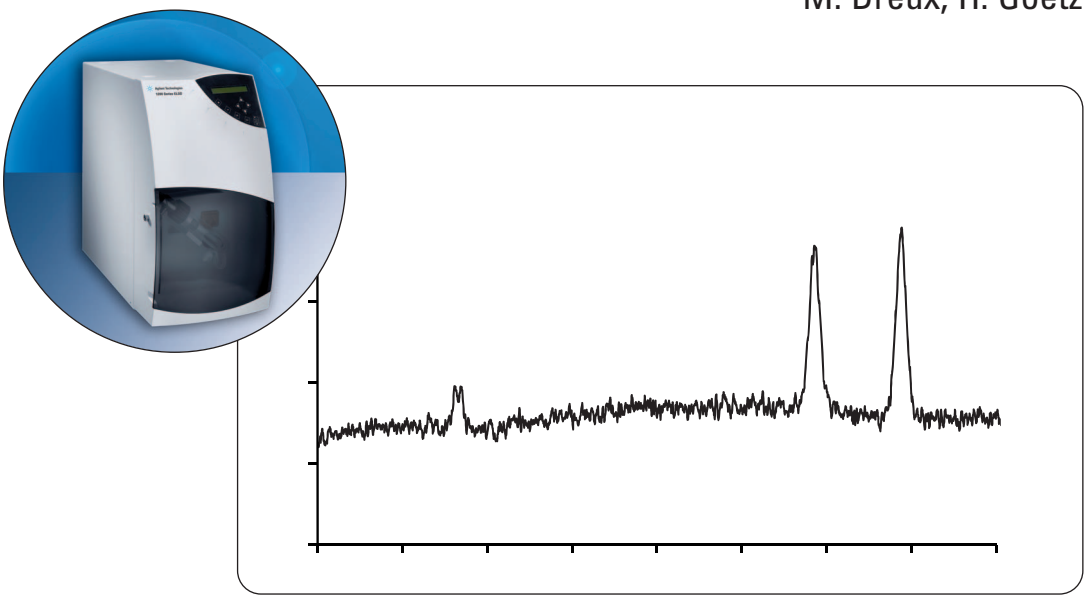

Abstract

This Application Note describes the quantitative analysis of carbohydrates in fruit juices. The analysis technique deployed the Agilent 1200 Series evaporative light scattering detector, which proved to be a viable alternative to refractive index detection because of lower susceptibility to temperature fluctuations and the possibility to perform gradient analyses. Correlation coefficients greater than 0.99 were achieved for the carbohydrates glucose, fructose, sucrose and sorbitol.

Agilent Equipment

- 1200 Series LC system
- 1200 Series ELSD

Application Area

- Foods and beverages

Agilent Technologies

Introduction

Fruit juices are one of the most popular drinks in the world. Directly extracted from fruits or concentrated before dilution, these tasty aqueous solutions contain a wide range of polyphenols, organic acids and carbohydrates. For many purposes, the carbohydrate composition may be of interest, giving a fingerprint of the quality of fruits and juices. From the literature, many systems have been proposed, but most of these use low sensitivity refractive index detection (RID). Evaporative light scattering detection (ELSD) is a good alternative for detecting these carbohydrates because the solutes, which do not possess chromophores, are non-volatile. Further, in contrast to RID, ELSD allows the use of gradient elution and is not sensitive to changes in room or mobile phase temperatures.

Experimental

Equipment

- Agilent 1200 Series LC system comprising binary pump, vacuum degasser and thermostatted column compartment
- Agilent 1200 Series evaporative light scattering detector

Sample preparation

The objective of this study was to provide a straightforward analysis of carbohydrates found in several fruits. The carbohydrates analyzed were sucrose, glucose and fructose. In addition, sorbitol was also considered because this solute is present in some juices and can be analyzed using the same system.

To decrease the time for pre-treatment, solutions (juices) were simply filtered (0.2 µm) and then diluted to avoid detector saturation (1/100 and 1/1000 dilutions).

Chromatographic conditions

Mobile phase:	Water/acetonitrile
Gradient:	20/80 to 35/65 in 30 min
Flow rate:	1 mL/min
Column:	ALLTECH Prevail Carbohydrate ES column, 5 µm, 150 x 4.6 mm
Detection:	Gain 9, temperature 50 °C, pressure 3.5 bar
Elution order:	Fructose, sorbitol, glucose, sucrose

Results and discussion

Using the new Agilent 1200 Series evaporative light scattering detector the sensitivity for carbohydrates was found to be in the range of 1 to 10 nanograms on-column under comparable conditions (figure 1). In addition, the design of the Agilent detector makes it ideal for analysis of coarse mixtures. From nebulization to detection, parameters are optimized for a contamination-free process. Working with coarse mixtures or complex matrixes is now possible, without causing damage to the detector.

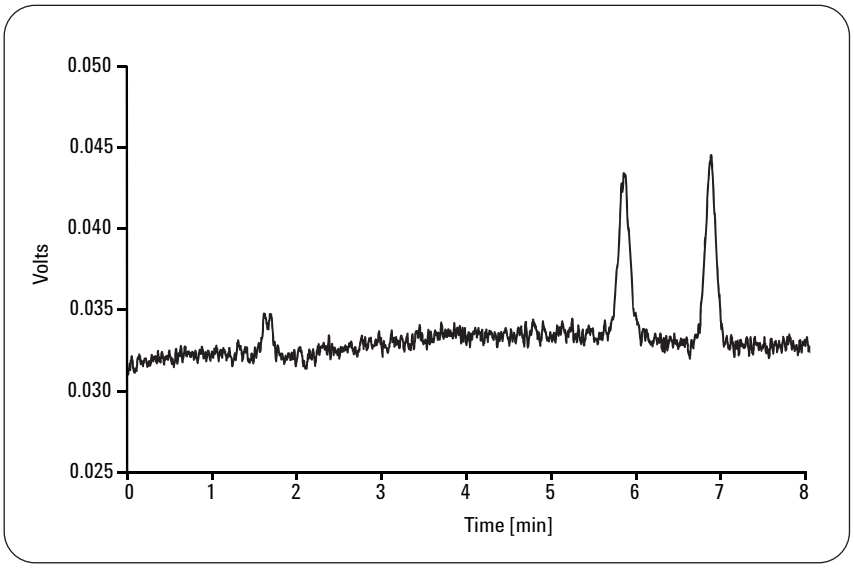

Figure 1
Analysis of 50 ng each of glucose and sucrose using the Agilent 1200 Series ELSD at 40 °C and 1 mL/min flow.

Separation

Gradient elution was found to perform the analysis in the shortest duration. To protect the carbohydrate separation column and to avoid any contamination, a short C8 pre-column was used. A water/acetonitrile gradient was used to separate the carbohydrates. The four selected components were separated in less than 12 minutes with complete resolution using a linear gradient pattern (figure 2). For such an analysis, sensitivity is not an issue because the carbohydrates were present at high levels in juices. The injected masses on ciolumn were in the µg range.

Linearity and quantification

Linearity tests were done using the Agilent 1200 Series ELSD. The standard flow nebulizer was chosen accordingly to the flow rate (1 mL/min). Five nebulizers are available for the Agilent 1200 Series ELSD and each nebulizer is optimized to provide the best sensitivity and the best repeatability for a selected flow rate range. For quantification, the best choice was to plot the logarithm of area against the logarithm of injected mass. This provided a linear relationship with a good correlation coefficient, typically close to r2 > 0.99 (figure 3). As a demonstration, this process was successfully applied to the separation of glucose, fructose, sorbitol and sucrose.

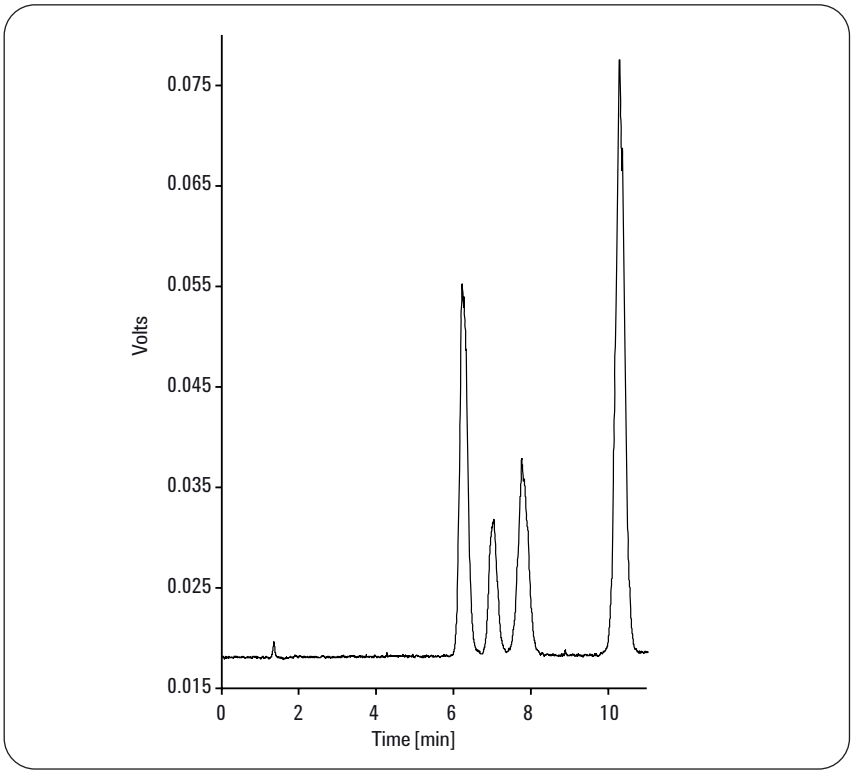

Figure 2
Analysis of carbohydrate standards.

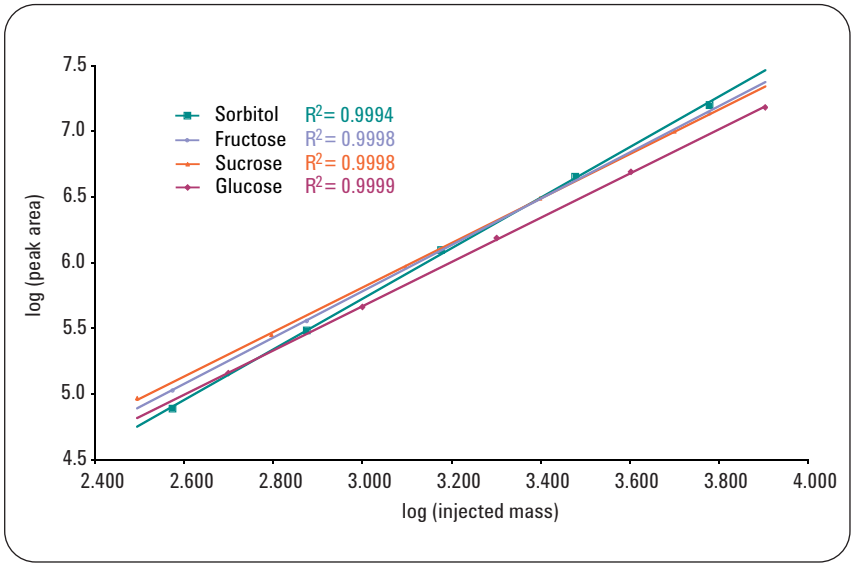

Figure 3
Quantification of carbohydrates.

An injected mass range from 1 µg to 30 µg was chosen, which corresponded to the levels found in the 1/100 or 1/1000 dilutions. Four juices were purchased in a local supermarket (orange, grape, pineapple and apple). These were either pure juices, or juices diluted from concentrates. All juices were filtered using a 0.2 µm filter and diluted to 1/100 or 1/1000, depending on the concentration level of carbohydrates. Quantification was performed successfully based on the quantitative data established using the carbohydrate standards. The results expressed in milligram per liter in each juice are given in table 1 and figure 4.

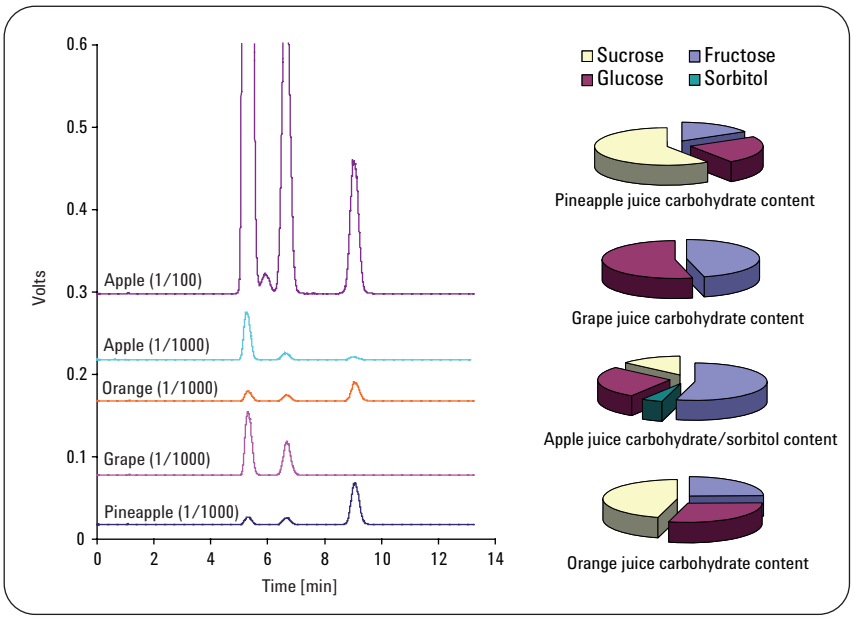

Figure 4
Quantitative data from analysis of fruit juices.

Conclusion

An original separation method has been developed for the separation of carbohydrates in coarse fruit juice mixtures. It was demonstrated that the use of a rapid filtering process before dilution of the sample and the use of a short reverse phase C8 pre-column were sufficient to protect the column and avoid matrix effects. The Agilent 1200 Series ELSD was used to analyse the carbohydrate mixtures and generate quantitative data. The correlation coefficients of the quantitative plots were excellent, confirming the quantitative capability of evaporative light scattering detection. Combining an optimized separation with the Agilent 1200 Series ELSD provides for acquisition of quantitative data from coarse fruit juice mixtures, without the need for sample and time-consuming pretreatments.

		Pineapple	Orange	Grape	Apple
Carbohydrate [g/L]	Fructose	23.7	27.3	76.2	64.8
	Glucose	26.7	24.2	66.2	26.7
	Sucrose	64.8	40.3	–	–
Polyol [g/L]	Sorbitol	–	–	–	4.7

Table 1
Quantitative data from analysis of fruit juices.

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Published April 1, 2008
Publication Number 5989-7889EN

Agilent Technologies