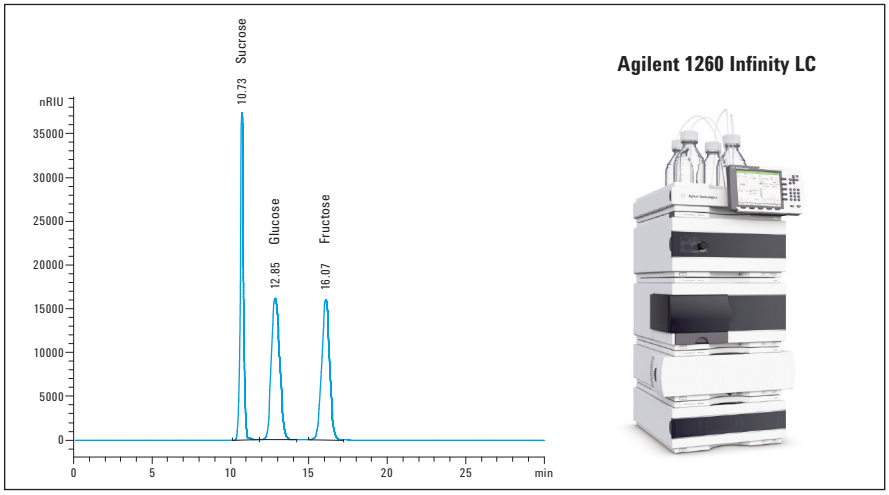
Agilent Application Solution

Determining total sugar content in maple syrup to meet FDA nutrition labeling requirements

Application Note

Food Safety

Suitable for Agilent
1260 Infinity III LC



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Abstract

Nutrition labels on food items provide valuable information to consumers. To ensure authenticity, the Food and Drug Administration (FDA) checks the reported values on the nutrition labels. This Application Note shows a total sugar analysis method for commercially available maple syrup using the Agilent 1260 Infinity Binary LC system coupled with the Agilent 1260 Infinity Refractive Index Detector. The method involved the use of Agilent HiPlex Ca column with 100% water as mobile phase. The method was validated for area and retention time (RT) precision, linearity, and accuracy. Robustness of the method was tested by varying pump flow, column temperature, injection amount, and refractive index optical unit temperature. The total sugar value reported on the maple syrup's nutrition label was verified. This method can be used by food manufacturers to report total sugar content to meet nutrition labeling requirements.

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Introduction

The nutrition Labeling and Education Act (NLEA) requires vendors to report ≥ 1 g per serving size of total sugar. The FDA monitors food items for compliance with the labeling requirement[1]. Total sugars or sugar alcohols are reported separately from carbohydrates. Total carbohydrates are measured[1] by subtracting the sum of crude protein, total fat, moisture and ash from the total weight of the food. The total sugars are measured as the sum of disaccharides (sucrose, lactose, mannose) and monosaccharides (glucose, galactose and fructose). The choice of method in sugar analysis is based on the prior knowledge of composition of sugars in food. For example, sucrose, a disaccharide, is the major constituent present in maple syrup which also may contain trace amount of glucose and fructose. An acceptable method to analyze total sugars in maple syrup would require analysis of sucrose.

UV absorbance at ~200 nm shows poor response for sugars due to low extinction coefficients. The AOAC method, 920.189[2], describes the analysis of sucrose in maple products using polarimeter before, and after inversion using Invertase enzyme. Due to advancements in chromatography and universal analysis of sugars by refractive index detectors (RI)[3], RI is the preferred method for sugar analysis. The AOAC methods, 977.20 and 980.13, for analysis of total sugars in honey, and chocolates[4,5] involve carbohydrate column and refractive index detector. Here we report a total sugar analysis method for commercially available maple syrup using Agilent HiPlex Ca Column, a cation exchange column and Agilent 1260 Infinity Refractive Index Detector.

Experimental

Instrument/Experimental parameters	Details
Column:	Agilent Hi-Plex Ca Column, 7.7 mm × 300 mm, 8.0 µm (p/n PI1170-6810), operated at 85 °C
Mobile phase:	100% water (Milli-Q), isocratic run
Flow:	0.5 mL/min
Injection volume:	3 µL, samples maintained at room temperature
Run time:	30 min
Agilent 1260 Infinity Binary Pump	G1312C
Agilent 1260 Infinity High Performance Autosampler	G1367E
Agilent 1290 Infinity Thermostatted Column Compartment	G1316C
Agilent 1260 Infinity Refractive Index Detector	G1362A
Agilent 1260 Infinity Standard Degasser	G1322A
Agilent ChemStation for Liquid Chromatography	B.04.02[SP1]
Agilent 1260 Infinity Refractive Index Detector parameters	
Optical Unit Temperature:	30 °C
Attenuation:	500000 nRIU

Material and instrumentation

Maple syrup samples were obtained from local stores in the Unites States of America. Sucrose, glucose and fructose standards were obtained from Sigma-Aldrich and dried for 12 hrs at 50 °C under vacuum.

Extraction procedure

0.75 mL of maple syrup was added into pre-weighed 50 mL centrifuge tube. The difference in weight was recorded. Twenty-five mL of water was added and vortexed. The centrifuge tubes were placed in sonicator for 25 min and the solution was filtered using glass microfiber and cellulose nitrate 2 in 1 syringe filter 0.45 µm (p/n 5042-1391). The filtered solution was used for analysis.

Recovery studies

The recovery experiments were performed by spiking commercially available maple syrup with sucrose, at two different sucrose concentrations. At a high concentration spike, sucrose was spiked to 40% higher sucrose than reported on the nutrition label. At a low concentration spike, where sucrose was spiked at 20% higher sucrose than reported on the nutrition label. The recovery is measured as a difference between high concentration spike and low concentration spike where the area obtained was back calculated to concentration using the linearity curve. The density of maple syrup used in the calculation was empirically determined as 1.3 g/mL. Due to the viscous nature of maple syrup, the weight was used to obtain accurate volume and also to normalize samples (mg of sucrose per gram of maple syrup). The variation from the added value was reported in percentage as recovery.

Method validation

The sucrose method for maple syrup was validated using aqueous standards of sucrose, glucose and fructose. Precision, linearity, accuracy and carry-over were obtained for sucrose. The validation for glucose and fructose was performed separately and the limit of detection (LOD) and the limit of quantitation (LOQ) were determined. The analyte concentration that provides a signal to noise ratio (S/N) of >3 was considered as LOD and analyte concentration with S/N > 10 was considered as LOQ. A linearity curve for each compound was constructed from the LOQ level to a maximum concentration. Each linearity solution was injected six times. Accuracy was determined as back calculated concentration from the linearity equation. To determine the sensitive parameters in the method, method robustness was performed by varying pump flow (± 2%), column temperature (± 5%), Injection volume (± 5%), and RI optical unit temperature (± 5%)[6]. Each experimental variation run was performed for six replicates. Robustness testing was done using a mixture of 27 mg/mL of sucrose, 0.5 mg/mL each of glucose and fructose.

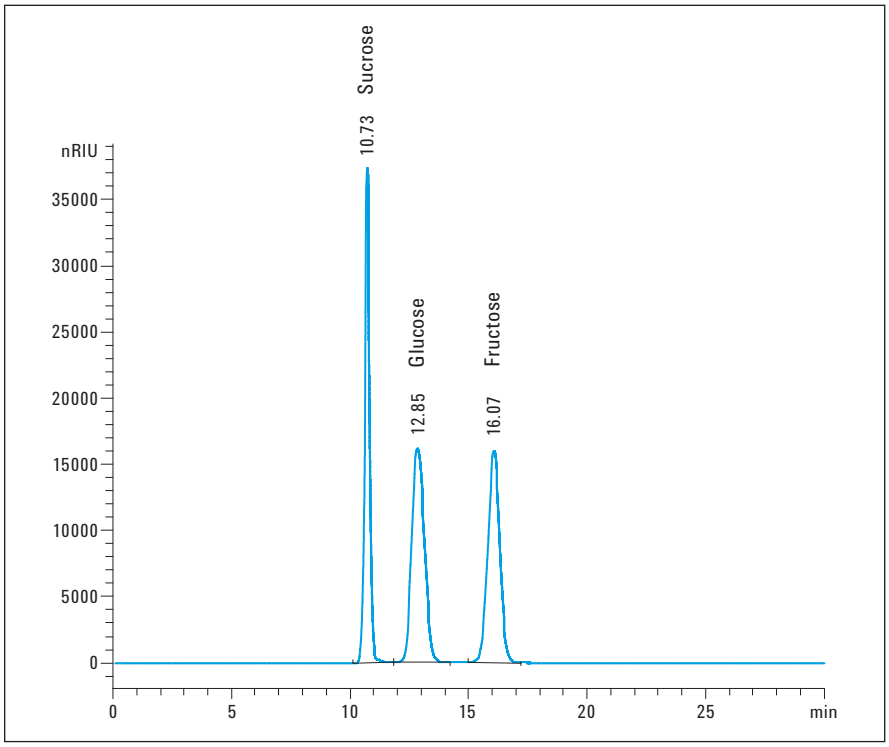

Figure 1
Chromatogram showing the separation of 10 mg/mL each of sucrose, glucose and fructose using an Agilent HiPlex Ca column.

Results and discussion

Method development

Individual standards of monosaccharide and disaccharides were injected on a Hi-Plex Ca column showing good resolution for sucrose, glucose and fructose as shown in Figure 1. Hi-Plex Ca is a ligand exchange column which requires low flow rate and high column temperature for analysis. Water was used as mobile phase which displayed a flat baseline with the refractive index detector.

Filter test

Filters were tested using a standard preparation by measuring the peak area with and without the filter. The results show that nylon, PTFE and regenerated cellulose filters showed an area recovery of 60% while glass microfiber showed area recovery of 100%. Glass microfiber and cellulose nitrate 2 in 1 syringe filters showed an area recovery of 104%. This filter was selected for the analysis because of their pore size of 0.45 u, as small pore size helps to remove particulates from samples. For sample analysis, the area recovery from syringe filters are incorporated in calculations of total sucrose.

Extraction procedure

To obtain an extraction method optimized for maximum recovery, maple syrup was tested by dissolving it in either water or 50% acetonitrile-water followed by either heating at 85 °C or sonication for 25 minutes. In all cases, the extracted solvent was filtered with cellulose nitrate and glass microfiber 2 in 1 syringe filter. Dissolving in water followed by sonication yielded better chromatography. Comparable extraction was achieved using 50% acetonitrile-water solution followed by heating. Water as dissolution solvent followed by sonication was used in recovery and sample analysis.

Recovery studies

The recovery experiments were performed by spiking maple syrup with sucrose. Since sucrose free maple syrup is not available, the recovery is measured as a difference between high concentration spike and low concentration spike. In food analysis, if a blank matrix is available, standards are spiked at two or three different concentrations and compared with the blank[7]. For this study, since a blank matrix is not available, sucrose is spiked at two

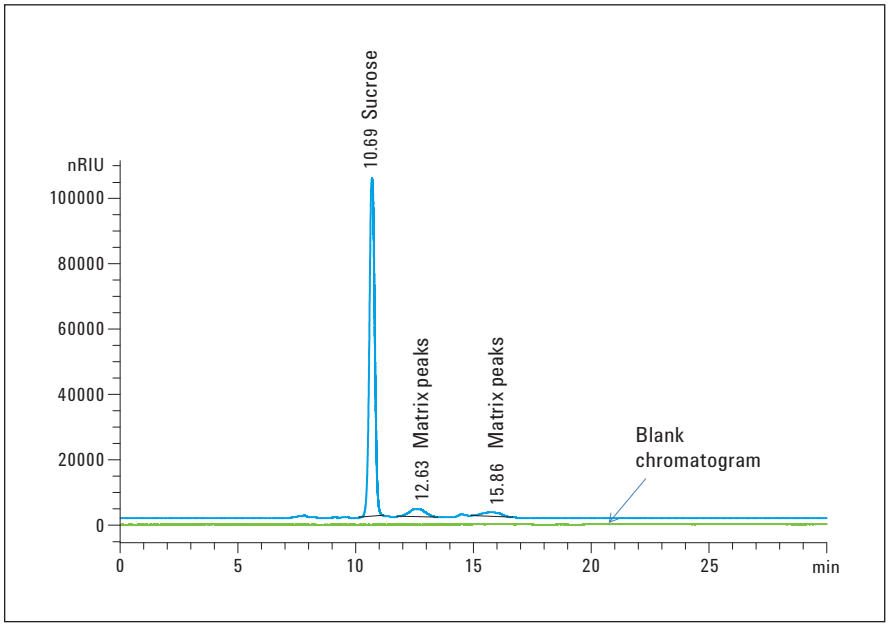

Figure 2
Agilent HiPlex Ca column separation of maple syrup extract spiked with high concentration of sucrose, glucose and fructose peaks. Glucose and fructose peaks contribute 2% of the sucrose peak area. Matrix peaks occurs at 12.63 min and 15.86 min which overlap with the glucose and fructose peaks (see Figure 1). The blank chromatogram showed flat baseline and low background interference to analyte peak.

level concentrations to account for compounds already present in the matrix or account for matrix interfering peaks. The high concentration spike chromatogram over a 100% water injection (blank) is displayed in Figure 2. The results show recovery of 90 ± 5% with no sample carry over.

The nutrition label of maple syrup reports 53 g of total sugars in a serving size of 60 mL. A sampling of 0.75 mL of maple syrup dissolved in 25 mL of water would yield a sucrose concentration of 26.5 mg/mL. At this concentration of sucrose, two matrix peaks of sucrose (10% of peak area) are seen that overlap with retention time for glucose and fructose peaks. An Agilent ZORBAX carbohydrate column was used to separate these matrix peaks from the spiked glucose and fructose peaks. The results show that these peaks have trace or no contribution to total sugar content in maple syrup. For chromatographic methods both, Agilent ZORBAX carbohydrate columns as well as HiPlex Ca columns provides separation of sucrose, glucose and fructose and both can be used for analysis of sucrose in maple syrup.

Method validation

The method was validated using a standard aqueous preparation of sucrose and standard aqueous samples of glucose and fructose separately. The results of method validation are shown in Tables 1 and 2. The validation table lists the passing criteria wherein the not more than (NMT) and not less than (NLT) limit was set. The linearity range for sucrose (9 mg/mL to 50 mg/mL) was chosen for the extraction concentration to be in the middle of the calibration curve. Similarly, the linearity

Validation protocol	Experimental runs	Results
	Sucrose	
Precision: Area and RT:	11 concentrations, n = 6 (9 mg/mL to 50 mg/mL)	Maximum RSD of area: 0.31% Maximum RSD of RT: 0.032% Limit set for RSD area: NMT 0.5% Limit set for RSD RT: NMT 0.2%
Linearity:	11 concentrations, n = 6 (9 mg/mL to 50 mg/mL)	Ave Response factor (amt/area) NMT: 0.0000196 Std. deviation NMT: 0.000000046. R^2 NLT: 0.999
Accuracy:	11 concentrations, n = 6 (9 mg/mL to 50 mg/mL)	Minimum value obtained: 99% Limit set for accuracy: NLT 97%
Carryover:	6 injections of stock solution followed by blank injections	–8 nRIU units baseline. No detectable sucrose peak.
	Glucose and fructose	
Precision: Area and RT:	7 concentrations, n = 6 (0.1 mg/mL to 5 mg/mL)	Maximum RSD of RT for glucose: 0.076% Limit RSD of RT for glucose: 0.1% Maximum RSD of RT for fructose: 0.046% Limit RSD of RT for fructose: 0.1% Maximum RSD of area for glucose: 3.7% Limit RSD of area for glucose: 6% Maximum RSD of area for fructose: 4.2% Limit RSD of area for fructose: 6%
Linearity:	7 concentrations, n = 6 (0.1 mg/mL to 5 mg/mL)	Ave response factor (amt/area) NMT: 0.0000176 Std. deviation NMT: 0.00000061 Ave response factor (amt/area) NMT: 0.0000179 Std. deviation NMT: 0.00000071 R^2 NLT: 0.999
Accuracy:	7 concentrations, n = 6 (0.1 mg/mL to 5 mg/mL)	Glucose: L2 to L8 accuracy > 98%–102%, L1 lowest value was 88% Fructose from L1 to L8 accuracy > 93%–105%
LOD and LOQ:		Glucose and fructose: LOD: 0.05 mg/mL (S/N = 6) LOQ: 0.1 mg/mL (S/N = 13)

Table 1
The validation results performed using an aqueous sucrose standard and aqueous mixture of glucose and fructose. NMT: not more than, NLT: not less than.

curve for glucose and fructose (0.1 mg/mL to 5 mg/mL) were chosen to include the lower limit of reporting concentration of 1g in 60 mL serving size. The results show excellent linearity of greater than 0.999 for both sucrose and glucose/fructose samples. A robustness test performed on the mixture of sucrose, glucose and fructose showed that RT and area are susceptible to changes in flow rate and column compartment temperature (see Table 2). A 2% change in RT is seen for a low flow rate and as high as a 32% deviation in area is observed for low column temperature. The method requires that the column temperature (TCC) and pump flow to be stable during sample analysis. The TCC used in this analysis was G1316C as it would allow column temperature to be as high as 100 °C.

Parameters	Changes	Sucrose		Resolution of fractose	Glucose		Fractose	
		% area	% RT	% resolution	% area	% RT	% area	% RT
Flow: 0.5 mL/min ± 2%	High: 0.51 mL/min	2	2	1	4	2	5	2
	Low: 0.49 mL/min	1	2	1	1	2	3	2
TCC: 85 °C ± 5%	High: 89.3 °C	7	0.1	1	32	0	29	1
	Low: 80 °C	4	0.2	1	27	0.4	25	1
Injector: 3 µL ± 5%	High: 3.15 µL	4	0.1	1	5	0.1	3	0.1
	Low: 2.85 µL	6	0.0	1	1	0.1	2	0.2
RI optical unit	High: 31.5 °C	1	0.1	1	5	0.2	4	0.2
temperature: 30 °C ± 5%	Low: 28.5 °C	1	0	1	4	0	3	0.2

Table 2
Robustness studies performed on the sample containing the mixture of 27 mg/mL sucrose, 0.5 mg/mL of glucose and 0.5 mg/mL of fructose. The % area, % RT and % resolution represent the % deviation in area, retention time and resolution (fructose/glucose) from the experimental conditions respectively.

Analysis of samples

Three types of maple syrup samples were analyzed to verify the nutrition label information. Here the sucrose content was estimated using the chromatographic method and compared with the concentration claimed on the nutrition label. The grade A medium amber maple syrup matrix was used in earlier recovery experiments. Each sample was prepared in three separate extractions and three trials were run on each separation. The results of sample analysis are shown in Table 3. The values were obtained with and without

Maple syrup type	Nutrition label per serving size of 60 mL	Calculated value per serving size of 60 mL	Calculated value with recovery per serving size of 60 mL
Grade A – Medium amber*	53 g	51 g	56 g
Grade A – Dark amber	53 g	49 g	55 g
Grade B – Dark and delicious	53 g	48 g	53 g

Table 3
The total sugar analysis from maple syrup samples shows that upon incorporation of recovery values, the values on the nutrition labels underrepresented total sugar content by 5% for one sample. The sample* was used in recovery experiments and was applied to other maple syrup brands.

recovery and syringe filter correction. For corrections, the area was corrected before determining its concentration. Results are reported to the nearest gram as per NLEA requirements. The results show that for one of the maple syrup samples, the total sugar content was underrepresented by 5%. Glucose and fructose were present in trace levels in the maple syrups and so sucrose content alone accurately represented the total sugar content.

Conclusions

A method for the determination of total sugar in food sample was developed using Agilent 1260 Infinity LC System coupled with an Agilent Refractive Index detector. Hi-Plex Ca ion exchange column separate sucrose peaks from the matrix giving a recovery of 90%. A robust and partial validated method was developed that requires control of column compartment temperature and flow rate for optimal performance. A maple syrup sample was analyzed for its total sugar content and results indicated a maximum 5% deviation for one maple syrup from the value reported on the label.

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