Application Note

Food & Beverage Testing

Determine Sugars and Artificial Sweeteners in a Single Run

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Abstract

The determination of sugars in food and beverages is an established procedure in many analytical laboratories. With an industry and consumer trend towards products with reduced sugar content and added low-calorie sweeteners, analytical laboratories need to run a second method to analyze the sweeteners. This application note presents a method enabling simultaneous determination of sugars and sweeteners in a single run, reducing the effort and time spent on the analysis.

Introduction

The excessive consumption of sugars as part of a widespread poor diet has been identified by the World Health Organization (WHO) as a major risk factor for premature deaths.[1] The WHO therefore strongly recommends reducing the intake of free sugars. To limit the addition of sugars to food and beverages, many countries have installed excise duties on added sugar in food and beverages. For this reason, the food and beverage industry seeks to reduce added sugar in products. To maintain sensory identity and quality of a product, however, omitted sugar is frequently replaced with low-calorie sweeteners. This trend is reflected in decreasing sales or consumption of added sugars and increase of sweeteners (e.g., in Canada[2], the United States[3,4], and the European Union[5]). The increasing number of products containing both sugars and sweeteners drives the need for analytical methods enabling simultaneous analysis of both analyte classes. Whereas most artificial sweeteners exhibit a chromophore, making them amenable to UV detection, sugars lack a chromophore and thus cannot be reliably and sensitively detected by a UV detector. A refractive index detector (RID), on the other hand, can detect any compound distinct from the mobile phase, which makes it an ideal detector for sugars.

This application note presents a method for simultaneous separation and quantitation of four commonly used sweeteners and five mono- and disaccharides found in natural ingredients as well as food additives.

Experimental

Instrumentation

The Agilent 1260 Infinity II LC consisted of the following modules:

- Agilent 1260 Infinity II Isocratic Pump (G7110B)
- Agilent 1260 Infinity II Vialsampler (G7129A)
- Agilent 1260 Infinity II Multicolumn Thermostat (G7116A)
- Agilent 1260 Infinity II Refractive Index Detector (G7162A)

Column

Agilent ZORBAX Carbohydrate Analysis, 4.6 × 250 mm, 5 µm (part number 840300-908)

Software

Agilent OpenLab CDS, version 2.6, or later versions

Solvents

HPLC gradient-grade acetonitrile (ACN) was purchased from VWR (Darmstadt, Germany). Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22 µm membrane point-of-use cartridge (Millipak).

Chemicals and samples

Acesulfame, ammonium acetate, aspartame, cyclamate, fructose, glucose, lactose monohydrate, maltose monohydrate, saccharine, and sucrose were obtained from Merck (Darmstadt, Germany).

A calibration stock solution of all standards was made up in 20 mM aqueous ammonium acetate/acetonitrile, 1:1 (by volume). Pure stock solution was used for the highest calibration point; other calibration points were created by diluting the stock solution with mobile phase. Calibration curves were constructed in a range from 31 to 1,000 µg/mL for sweeteners, and from 156 to 5,000 µg/mL for sugars. Each point was measured in triplicate.

Soda samples were sourced from a local grocery store. Before injection, the sample was centrifuged for 5 minutes at 14,100 × g, filtered using an Agilent Captiva premium syringe filter (0.45 µm, regenerated cellulose, part number 5190-5107), and diluted with pure ACN or mobile phase.

Method settings

Table 1. Chromatographic conditions.

Parameter	Value
Mobile Phase	20 mM ammonium acetate in acetonitrile/water 75:25 (v:v)
Flow Rate	1.5 mL/min
Injection Volume	5 µL
Sample Temperature	Ambient
Column Temperature	35 °C
RI Detector	35 °C Peak width >0.025 min (0.5 s response time, 18.5 Hz) Signal polarity: positive (+)

Results and discussion

Figure 1 shows the chromatogram of the separation of the highest calibration point. Four artificial sweeteners and five sugars were successfully separated. Resolution between all analytes was typically larger than 2; the only exception was a resolution ranging from 1.6 to 1.7 between aspartame and fructose, which is just acceptable for quantitation. Between fructose and glucose (peaks 5 and 6), each calibration point exhibited a negative peak. The negative signal was largest in the highest calibration sample and decreased with increasing dilution of the calibrant; a blank injection of pure mobile phase did not show any negative peaks. It is therefore hypothesized that the different buffer concentration between calibration solvent and mobile phase was detected by the RID. The lower the buffer concentration in the less diluted calibration samples, the larger the negative peak area. To integrate peak 6 in a reproducible way, the intercept of the baseline after peak 5 and 6 was treated as the start of peak 6.

Calibration curves for all analytes were constructed measuring six points in triplicate. Concentration levels were 31.25, 62.5, 125, 250, 500, and 1,000 µg/mL for the sweeteners, and 156.25, 312.5, 625, 1,250, 2,500, and 5,000 µg/mL for the sugars. Excellent correlation (R^2) and standard deviations of the procedure (s_{x0}) were found for all analytes, as shown in Table 2. Limits of detection and quantitation were calculated for each analyte based on the signal-to-noise ratio, with the noise determined according to the ASTM method E 685-93.

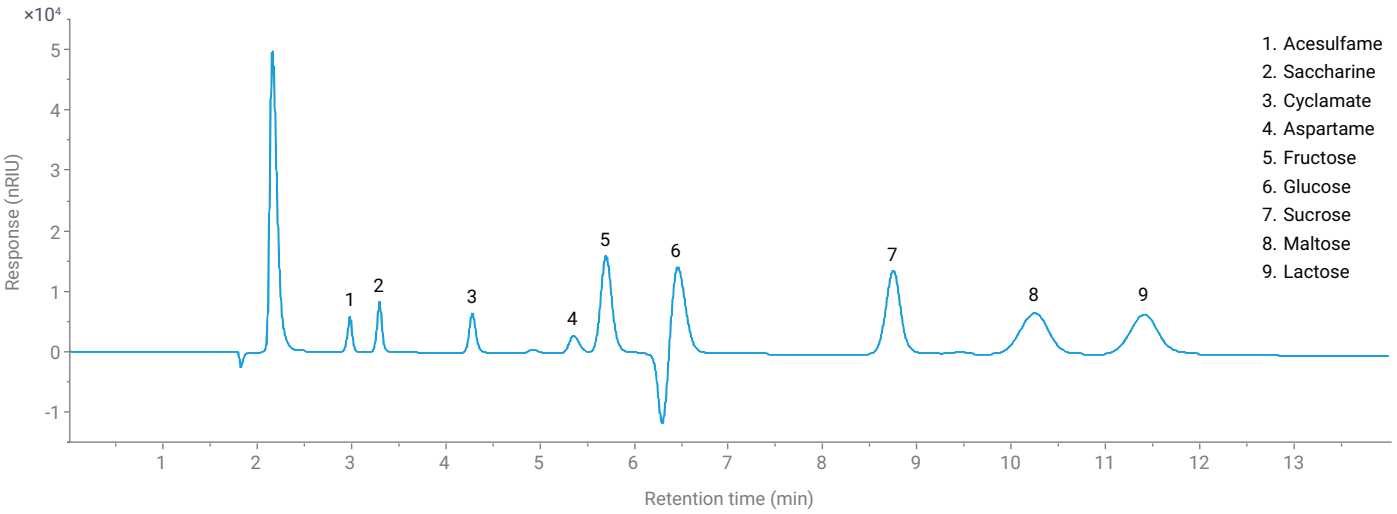


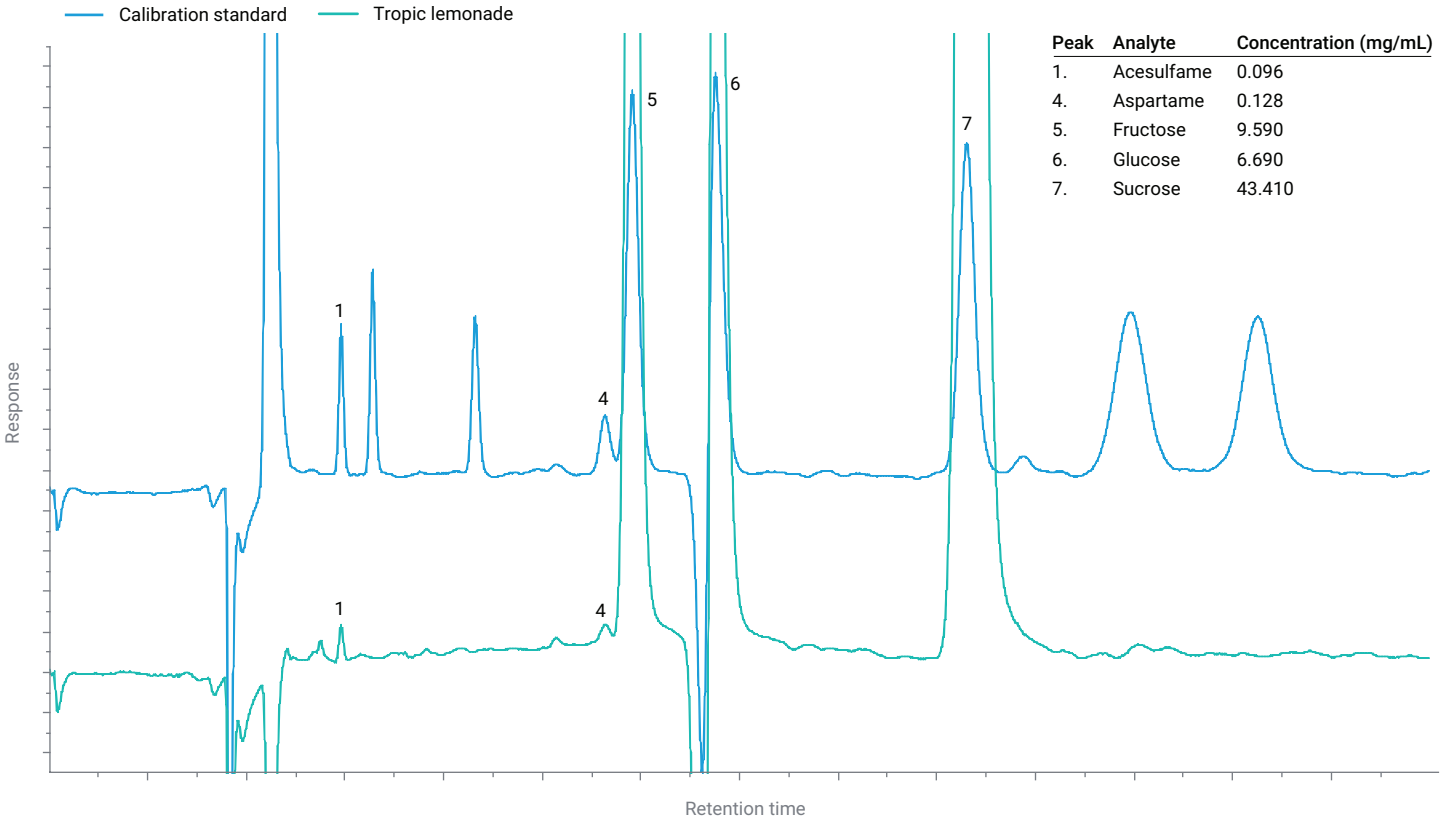
Figure 1. Separation of the calibration sample.

Table 2. Statistics of the calibration.

Analyte	Calibrated Range (µg/mL)	Resolution	R^2	LOQ (mg/mL)	LOD (mg/mL)	s_{x0} (mg/mL)
Acesulfame	31.25–1,000	–	0.99998	0.006	0.002	0.002
Saccharine	31.25–1,000	3.4–3.6	0.99994	0.003	0.001	0.003
Cyclamate	31.25–1,000	8.8–9.0	0.99999	0.004	0.001	0.001
Aspartame	31.25–1,000	6.6–6.8	0.99947	0.011	0.003	0.010
Fructose	156.25–5,000	1.6–1.7	0.99998	0.010	0.003	0.010
Glucose	156.25–5,000	3.3–3.4	0.99999	0.010	0.003	0.005
Sucrose	156.25–5,000	8.5–8.9	0.99999	0.010	0.003	0.006
Maltose	156.25–5,000	3.5–3.6	0.99999	0.019	0.006	0.005
Lactose	156.25–5,000	2.0–2.1	0.99999	0.020	0.006	0.008

The different calibration ranges for sweeteners and sugars were chosen to account for the expected concentrations of these two analyte classes in real samples. The range between sweeteners and sugars in the selected samples was in fact so high that it required dilution to accurately quantify the sugar amount. To avoid sample breakthrough, aliquots of the samples were diluted with the threefold volume of pure ACN, mimicking the composition of the mobile phase. If the sugar concentration was still out of the calibration range, another aliquot of the sample was diluted tenfold with pure mobile phase. Two lemonades were analyzed: one with reduced sugar content and added sweeteners ("tropic lemonade"), and one diet lemonade based on whey, with added sweeteners but without added sugar ("whey lemonade").

Figure 2 shows the analysis of the tropic lemonade, diluted 1:4 with ACN. Two sweeteners and three sugars were detected. To quantify the amount of sugar, a tenfold dilution of the same sample needed to be analyzed (not shown). Acesulfame and aspartame were quantified at 96 and 128 µg/mL, respectively. Fructose, glucose, and sucrose were found in larger amounts, namely 9.59, 6.69, and 43.41 mg/mL. The sum of these three sugars, 59.69 mg/mL, matches the amount given on the nutrition label (60 mg/mL). The amount of added sweeteners was not provided on the label, but the measured concentration is in good agreement with the amounts that can be found in the literature: 126 ±72 µg/mL for acesulfame, and 162 ±120 µg/mL for aspartame, based on a survey of 57 different drinks.[6]



Peak	Analyte	Concentration (mg/mL)
1.	Acesulfame	0.096
4.	Aspartame	0.128
5.	Fructose	9.590
6.	Glucose	6.690
7.	Sucrose	43.410

Figure 2. Chromatogram overlay of the tropic lemonade sample (diluted 1:4 with ACN) with a calibration standard.

The analysis of the whey lemonade is shown in Figure 3. Again, two sweeteners could be detected, this time acesulfame at 168 µg/mL and cyclamate at 244 µg/mL. Since this lemonade is based on whey, a significant amount of lactose was expected, and lactose was quantified at 14.05 mg/mL. The label of the lemonade only declared the total sugars, which were given at 15 mg/mL. This number, however, includes the amount of caramelized sugar that is added for color. In response to a direct inquiry, the manufacturer reported a lactose concentration of 14 mg/mL. Neither of the sweeteners was given with a quantity on the label, but again, the numbers found are in accordance with the literature[6]: 126 ±72 µg/mL for acesulfame, 207 ±47 µg/mL for cyclamate.

The sum of sweeteners found in the whey lemonade (412 µg/mL) is higher than in the tropic lemonade (224 µg/mL), which can be explained in two ways: First, the whey lemonade only contains a quarter of the amount of sugar found in the tropic lemonade. To meet customer expectations of lemonade sweetness, more added sweetener might be required. Second, cyclamate, found in the whey lemonade but not in the tropic lemonade, has a sweetening power six times lower than aspartame and acesulfame[7], which were added to the tropic lemonade. With this factor calculated out of the cyclamate concentration, the sum of sweeteners is about the same in both lemonades (224 compared to 209 µg/mL).

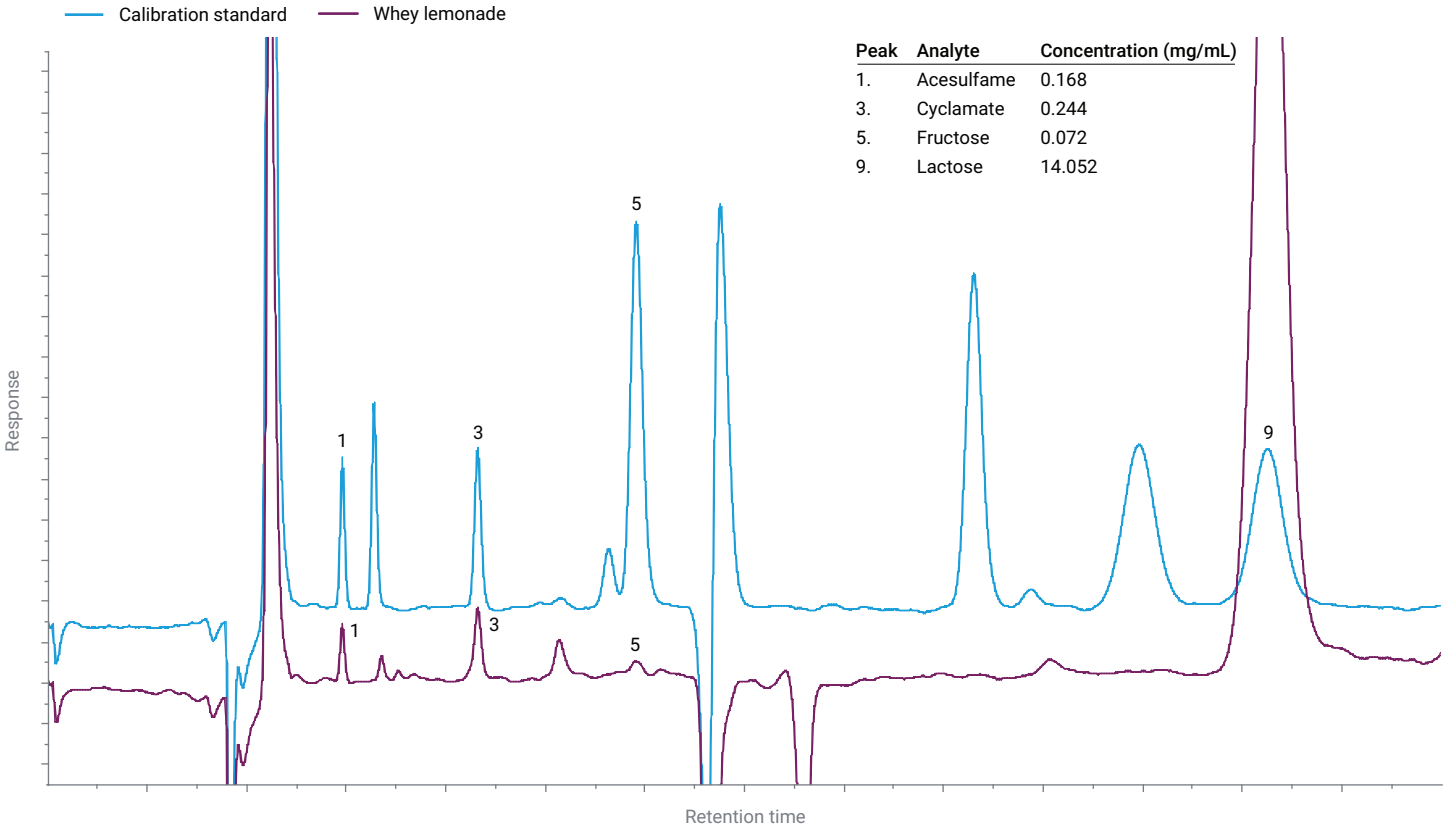


Figure 3. Chromatogram overlay of the whey lemonade sample (diluted 1:4 with ACN) with a calibration standard.

Conclusion

This application note presents an isocratic method capable of simultaneously analyzing four sweeteners and five mono- and disaccharides found in food and beverages. Within 14 minutes, the nine analytes were separated and quantified. For all compounds except maltose and lactose, LOQs were at 11 µg/mL or lower, equaling 55 ng on column. The simultaneous determination of sweeteners and sugars in a single run can significantly reduce the analysis time of samples containing both analyte classes, which makes the analysis faster and more cost-efficient.

References

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