

Sensitive Analysis of Commonly Used Artificial and Natural Sweeteners Including Stevia and Their Impurities and Degradation Products

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Abstract

Many recently commercialized sweeteners tend to have increased potency, reducing the amount of active ingredient added to beverages and other food products and often providing cost savings to the manufacturer. Using increased potency has also contributed to a need for sensitive analytical methods to quantify the active product and to detect low levels of breakdown products and impurities, which are required for quality and safety issues. Because compounds typically do not possess any chromophore, traditional HPLC-UV approaches are inappropriate. The work in this study describes several methods using HPLC with the Thermo Scientific Dionex Corona™ Charged Aerosol Detector (CAD™) for the study of common natural sugars (fructose, glucose, turanose, saccharose, trehalose, maltose, melezitose, and raffinose); artificial sweeteners (sucralose, aspartame, saccharin, and acesulfame K); and newly introduced products containing stevia extracts (rebaudioside A and stevioside). These HPLC-CAD methods provide sensitivity at low levels (ng) with good reproducibility and accuracy, and correlation to the component concentrations. Stevia products were analyzed by both charged aerosol detection and UV. Charged aerosol detection showed a greater than fivefold improvement in sensitivity over UV for all major components. Finally, the UHPLC methods developed showed a decreased run time and an increased sensitivity for glucose, lactose, and sucrose. Typical limits of detection (LOD) were found to be <500 pg (on column) for glucose and other mono- and disaccharides. HPLC-CAD is a very flexible approach to measuring sweeteners and overcomes many of the limitations of UV, refractive index (RI), LC-MS, evaporative light scattering detection (ELSD), and HPLC-pulsed amperometric approaches. The HPLC-CAD platform can be used throughout the manufacturing process to ensure finished quality and batch-to-batch uniformity.

Introduction

The Dionex Corona CAD is a mass-sensitive universal detector for nonvolatile and some semivolatile analytes. Unlike absorbance, fluorescence, RI, or electrochemical detection, analyte response is independent of chemical

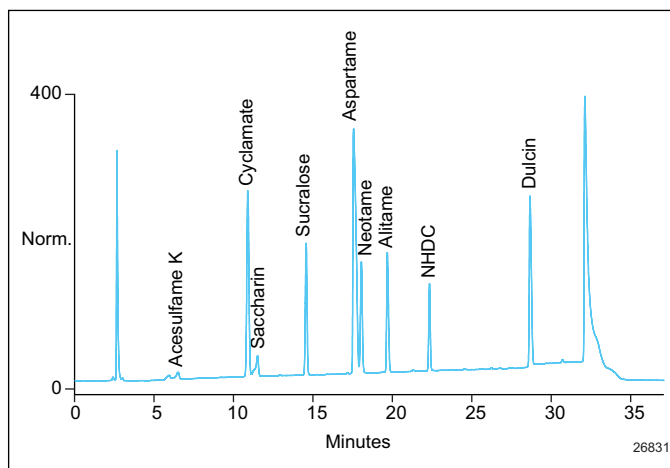
structure. Carbohydrates lack chromophores and the typical approaches used for their analysis may lack sensitivity, require derivatization, or cannot be used with gradient. The work in this study examines different HPLC-CAD methods for the analysis of honey sugars, artificial sweeteners, and other natural products including stevia extracts. Finally, a new UHPLC method for fast analysis of simple carbohydrates is presented. The Corona CAD system is easy to use and offers a simple, sensitive, reproducible, and direct approach for the routine analysis of natural and artificial sweeteners.

Artificial Sweeteners Global Method

HPLC Parameters

Column: ACE®, C18 4.6 × 250 mm, 5 μm
Mobile Phase: A) Deionized water
B) Acetonitrile + 0.1% trifluoroacetic acid
Gradient: 2 to 40% B over 25 min,
40 to 60% 25–30 min
Flow Rate: 1.0 mL/min
Inj. Volume: 50 μL
Column Temp.: 30 °C
Samples: 1.2 to 20 μg on column

FIGURE 1. Chromatogram of artificial sweeteners.



Universal Gradient Method for Artificial Sweeteners

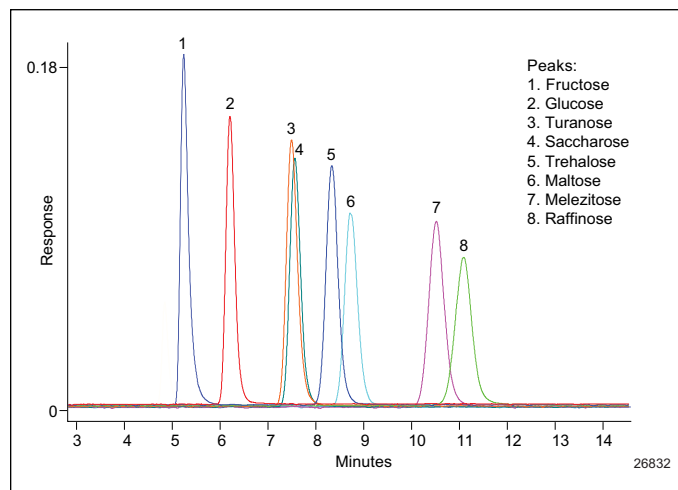
The sweetness of the several different artificial sweeteners commercially available ranges in potency from 30 to 13,000 times that of sucrose. Their chemical structure varies significantly as do their UV responses. The gradient method presented here is sensitive (easily measuring the low-level degradants and impurities). Unlike UV detection, all compounds give a similar response independent of chemical structure, thereby simplifying method development. This global method is a good starting point for the simultaneous analysis of artificial sweeteners with application to product development and quality control.

Honey Sugars Method

HPLC Parameters

Column: Shodex Asahipak, NH2P-50 4E,
4.6 × 250 mm, 5 μm
Mobile Phase: 70/30 (v/v) Acetonitrile, deionized water
Flow Rate: 1.0 mL/min
Inj. Volume: 10 μL
Column Temp.: 35 °C

FIGURE 2. Chromatogram of honey sugars.



The traditional approaches used for the analysis of carbohydrates include RI, UV following derivatization, and pulsed amperometric detection (PAD).

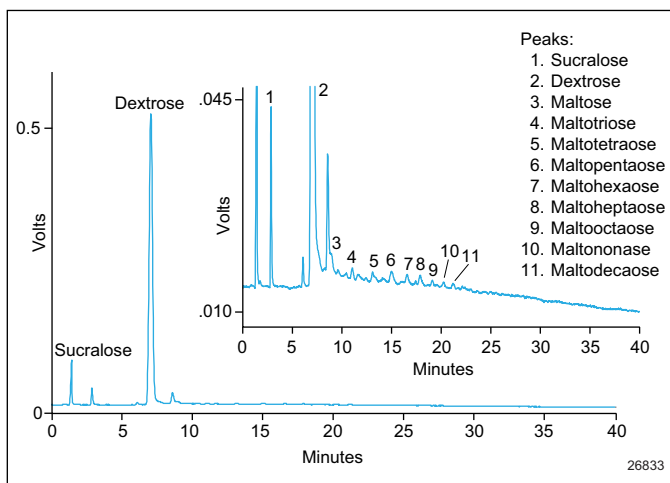
- Charged aerosol detection is more sensitive than RI and can be used with gradient chromatography. It measures compounds directly without the added complication of derivatization. Separation of carbohydrates using HILIC-based approaches extends the range of columns beyond the ion-exchange columns typically used with PAD.
- Eight carbohydrate standards commonly found in honey were analyzed (shown above at 1 μg on column each). The method was used to compare differences in forest, fir, and acacia honey samples.
- The LOD for simple sugar analysis is in the low ng levels on column.

Splenda Method

HPLC Parameters

Column: Shodex Asahipak, NH2P-50 4E,
4.6 × 250 mm, 5 μm
Mobile Phase A: Acetonitrile
Mobile Phase B: Deionized water
Gradient: 30% to 70% B in 40 min
Flow Rate: 1.0 mL/min
Inj. Volume: 10 μL
Column Temp.: 30 °C

FIGURE 3. Chromatogram of Splenda® sweeteners.



Gradient Analysis of Splenda Sweetener

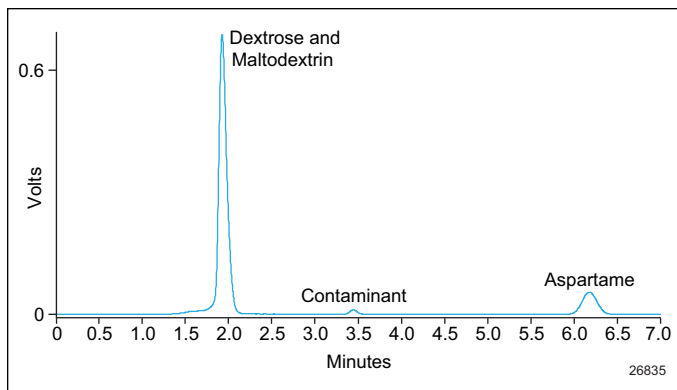
A packet of Splenda was dissolved in mobile phase A and diluted to obtain 10 µg of the sweetener on column. The gradient method enabled the separation of the active ingredient sucralose, the filler dextrose, and low levels of maltodextrins. All of these compounds are reported on the product packaging and must remain below government specified levels to be sold as a zero-calorie sweetener. As sucralose is so sweet, its relative abundance compared to the other ingredients in the product is low. The Dionex Corona CAD detector, with its wide dynamic range and sensitivity, is ideal for the routine measurement of product content and quality.

Equal and an Unknown Impurity Method

HPLC Parameters

Column: Shiseido C18 SG300,
4.6 × 150 mm, 5 µm
Mobile Phase: Acetonitrile, deionized water,
trifluoroacetic acid (85:15:0.05) Column
Flow Rate: 1.0 mL/min
Inj. Volume: 10 µL
Column Temp.: 30 °C

FIGURE 4. Chromatogram of Equal® sweeteners.



Detection of Unknown Peak in Equal

- Equal contains multiple components including the active ingredient, aspartame, along with the fillers dextrose and maltodextrin.
- All components were separated using reversed-phase chromatography and detected by the Dionex Corona CAD detector.
- During method development a trace impurity/contaminant was found. Although several potential degradants (e.g., phenylalanine, aspartic acid) were analyzed, none corresponded to the impurity.

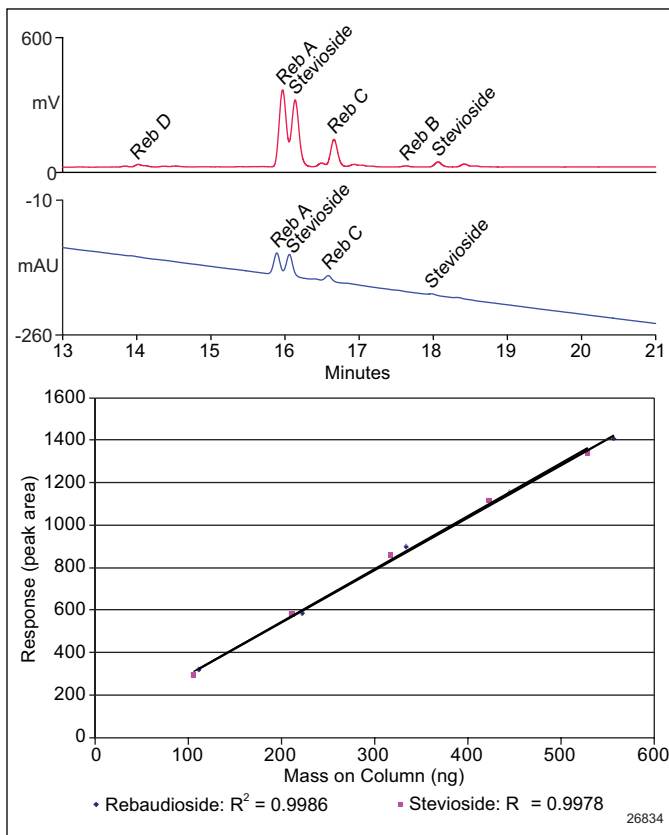
Stevia Method

HPLC Parameters

Column: Shiseido Capcell PAK C18AQ®,
4.6 × 250 mm, 5 µm
Mobile Phase A: Deionized water, acetonitrile,
trifluoroacetic acid (95:5:0.1)
Mobile Phase B: Acetonitrile, deionized water (95:5)
Gradient: 5 to 90% B over 30 min
Flow Rate: 1.0 mL/min
Inj. Volume: 10 µL
Column Temp.: 50 °C

The commercial use of the herb stevia contains extracts from the *Stevia rebaudina*, Bertoni plant. The two major glycosides—stevioside and rebaudioside A (Reb A)—and other minor glycosides (Reb B, C, and D) are resolved and detected by this method. Interestingly, several low level impurities are detected by the Dionex CAD detector, but not by UV at 210 nm.

FIGURE 5. Selected portion of chromatogram of SweetLeaf Stevia extract at ~860 ng on column run with UV at 210 nm and CAD in series (top). Overlay of curves for Reb A and stevioside from ~500 to 100 ng on column each (bottom). Average of three injections, each fit to a linear correlation.

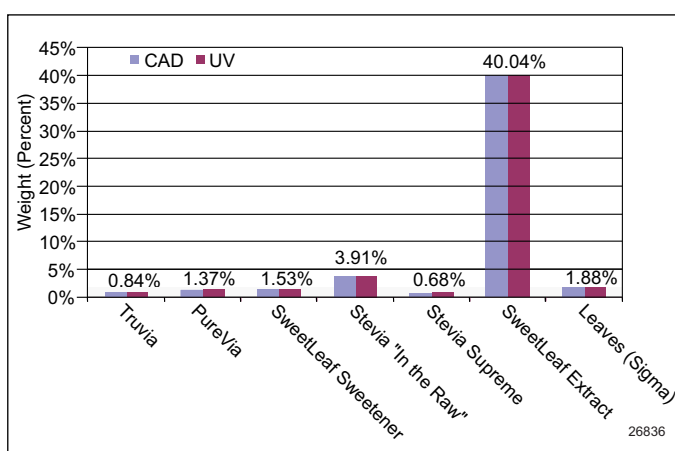


Stevia Sweeteners Method

Table 1. Samples of Sweeteners Used

Product	Distributor	Classification	Serving Size 1 Packet (g)	Injection Conc. (mg/mL)
Truvia®	Cargill, Inc.	Table Sugar	3.5	5.9
Pure Via™	Whole Earth Sweetener Company	Table Sugar	2	3.6
SweetLeaf Sweetener	SweetLeaf	Dietary Supplement	1	2.6
Stevia Extract (in the raw)	Cumberland Packing Corp	Dietary Supplement	1	1.0
Stevia Supreme	Stevia Company, Inc.	Dietary Supplement	1	1.1
SweetLeaf® Stevia Extract	Wisdom Natural Brands	Dietary Supplement	0.025	0.086

FIGURE 6. Percentage of Reb A in stevia-containing products.



Analysis of Reb A in Stevia-Containing Products

Although several stevia-containing products were sold as dietary supplements, it was not until late 2008 that the FDA issued Generally Recognized As Safe (GRAS) affirmations for two commercial products to be sold as all natural, zero-calorie sweeteners: Truvia (Coca Cola) and Pure Via (Pepsi). The GRAS affirmation was for purified Reb A sweeteners only and not for products that contain the other glycosides found in the stevia leaf.

The products listed in the table above were analyzed using the gradient charged aerosol detection method and the percent by weight of Reb A was determined. The data generated by UV at 210 nm and the Dionex CAD detector were comparable. However, the Dionex CAD has the advantage that all compounds are determined independent of chemical structure, whether a chromophore is present or not. The Dionex CAD detector is ideal for the measurement of trace contaminants and impurities.

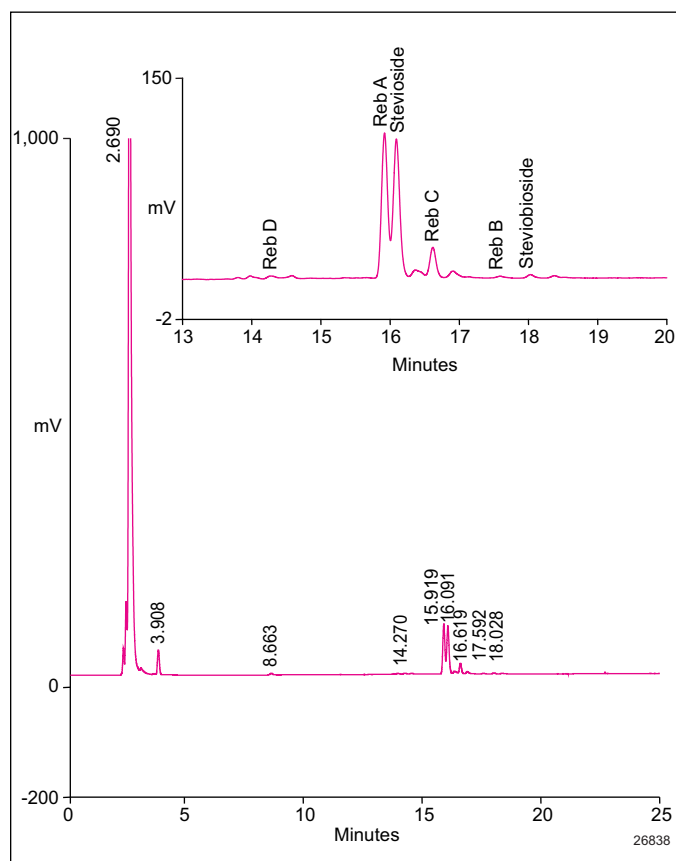
Sensitive Measurement of Stevia in Beverages

Table 2. Means of Detection for Stevioside Components

Detector	Limit of Detection (Mass On Column)		
	Rebaudioside	Stevioside	Isodeviol
CAD	4 ng	4 ng	60 ng
UV at 210 nm	65 ng	65 ng	>900 ng

- The Dionex Corona CAD detector demonstrated greater sensitivity for all of the compounds of interest in the stevia evaluation.
- Limit of quantification (LOQ) was defined by a signal-to-noise ratio ≥ 10 .
- LOD was defined by a signal-to-noise ratio ≥ 3 .

FIGURE 7. Determination of caffeine (retention time 3.9 min) and five glycosides in a commercially available product.



Analysis of Stevia in Soft Drinks

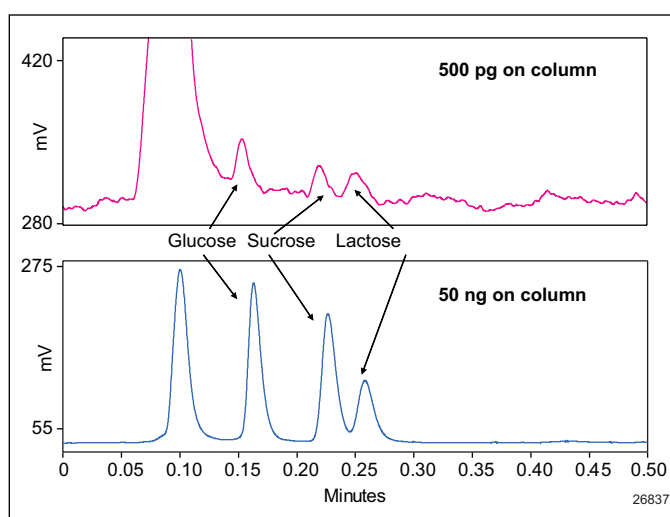
The FDA GRAS declaration now permits the commercial production of stevia-based zero-calorie beverages. However, the Zevia company (Seattle, WA) began selling a line of alternative soft drinks with stevia early in 2008 marketed as “carbonated stevia supplements.” The Zevia™ Natural Cola and the Zevia Natural Twist flavors were purchased and prepared by diluting 1 mL of each beverage with 5 mL methanol solvent prior to analysis. The content of Reb A was calculated to be 0.016% in both beverages with similar concentrations of stevioside. As shown in the chromatogram above (Zevia Natural Cola), caffeine is determined along with many unknown components.

UHPLC Carbohydrate Analysis

HPLC Parameters

Column: Waters BEH HILIC, 2.1 × 50 mm, 1.7 μm
 Mobile Phase: Acetonitrile/5 mM ammonium formate
 pH = 3 (91:9)
 Flow Rate: 1.8 mL/min
 Inj. Volume: 2 μL
 Column Temp.: 40 °C
 Dionex Corona
ultra Filter: Medium

FIGURE 8. Comparison of picogram vs nanogram levels of carbohydrates.



High Sensitivity and Rapid Analysis

Figure 8 shows the separation of three common carbohydrates run under UHPLC-HILIC conditions. The method, using the Dionex Corona *ultra*™ detector, was rapid (<30 s) and could reproducibly detect <1 ng on column of the sugars. The LOD for glucose was estimated to be ~250 pg on column. Traditional HPLC-based platforms cannot maintain excellent analyte resolution with the rapid analysis of a nonchromophoric material.

Conclusion

- Both the Dionex Corona CAD and Dionex Corona *ultra* are extremely versatile detectors. The Dionex Corona *ultra* detector is fully compatible with UHPLC.
- As the Dionex Corona is a mass sensitive detector, with response being independent of chemical structure, a wide variety of compounds can be detected making it ideal for analysis of the many different compounds used in the food and beverage industry.
- Unlike RI detection, the Dionex Corona CAD can be used with gradient chromatography to improve speed and resolution.
- The sensitivity of the Dionex CAD detector (low ng on column) is greater than that of RI, approaches the sensitivity of PAD, and is about 10× more sensitive than UV at 210 nm. Subnanogram sensitivity can be achieved when UHPLC techniques are employed.
- The analysis of the major glycosides in stevia yielded similar data to UV, but due to its sensitivity and consistent inter-analyte response, it was capable of measuring trace impurities that were missed using UV detection.

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