

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

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ABSTRACT

Many of the recently commercialized sweeteners tend to have increased potency, and so the amount of the active ingredient being added to beverages and other food products is reduced. This may result in cost-savings for the manufacturer. But this 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. Such product characterization is required for quality and safety issues. Traditional HPLC-UV approaches are inappropriate as these compounds typically do not possess any chromophore. The work in this study describes a number of HPLC-CAD methods that can be used to study 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 methods provide sensitivity at low (ng) levels with good reproducibility and accuracy, and correlation to the component concentrations. Stevia products were analyzed by CAD[®] and UV; the CAD 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 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, RI, LC-MS, ELSD, and HPLC-pulsed amperometric approaches. The HPLC-CAD platform can be used throughout the manufacturing process, to ensure finished-quality, and to ensure batch to batch uniformity.

INTRODUCTION

The Corona[®] is a mass-sensitive universal detector for non-volatile and some semi-volatile analytes. Unlike absorbance, fluorescence, refractive index (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 CAD 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
Column Temperature:	30 °C
Injection Volume:	50 μL
Flow Rate:	1.0 mL/min
Mobile Phase:	A) Deionized water B) Acetonitrile + 0.1% trifluoroacetic acid
Gradient:	2 to 40% B over 25 minutes, 40 to 60% 25–30 minutes
Samples:	1.2 to 20 μg on column

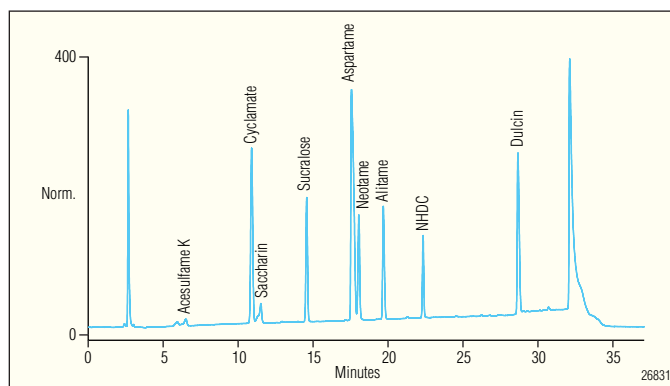


Figure 1. Chromatogram of artificial sweeteners.

Universal Gradient Method for Artificial Sweeteners

Several different artificial sweeteners are used commercially. Their sweetness 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 levels of 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

HPLC Parameters

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

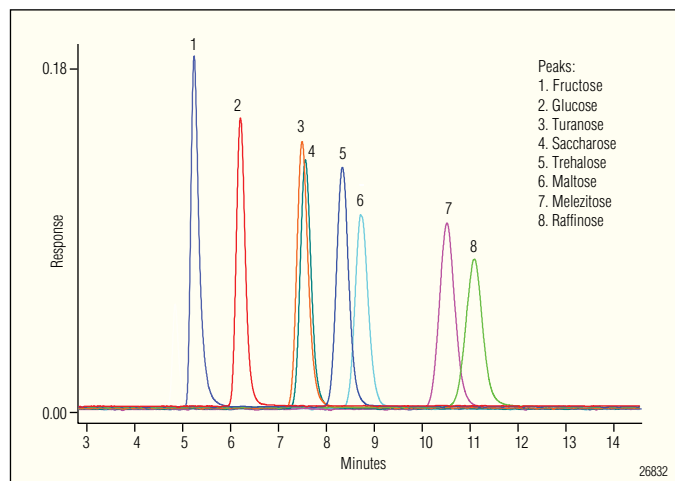


Figure 2. Chromatogram of honey sugars.

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

- Charged Aerosol 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®

HPLC Parameters

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

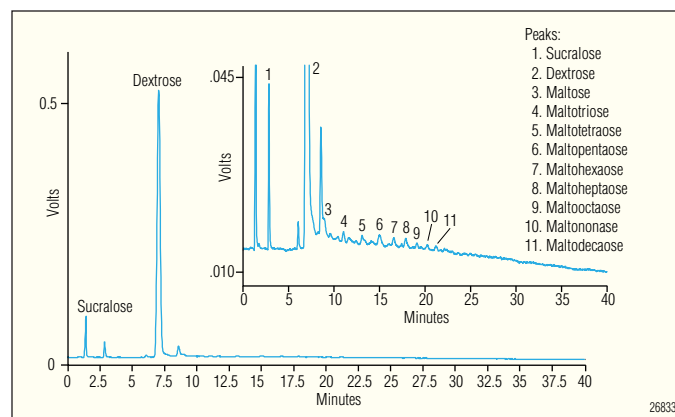


Figure 3. Chromatogram of Splenda sweeteners.

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 Corona CAD with its wide dynamic range and sensitivity is ideal for the routine measurement of product content and quality.

EQUAL® AND AN UNKNOWN IMPURITY

HPLC Parameters

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

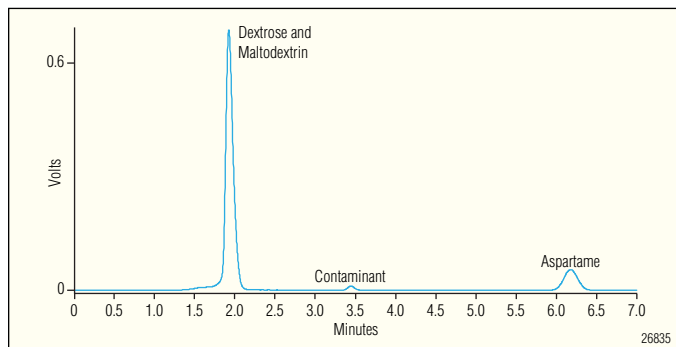


Figure 4. Chromatogram of Equal sweeteners.

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 Corona CAD.
- 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 C18 AQ, 4.6 x 250 mm, 5 µm (ESA #88-92044)
 Column Temperature: 50 °C
 Injection Volume: 10 µL
 Flow Rate: 1.0 mL/min
 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 minutes

The commercial use of the herb Stevia contains extracts from the Stevia rebaudina, Bertoni plant. The two major glycosides are Stevioside and 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 CAD, but not by UV at 210 nm.

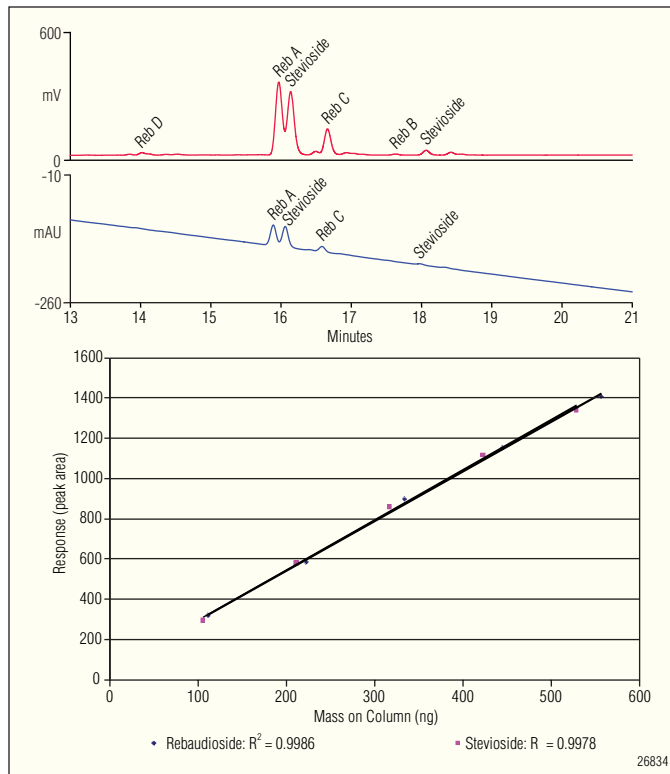


Figure 5. Selected portion of chromatogram of SweetLeaf® Stevia Extract at ~860 ng on column run with UV at 210 nm and CAD in series (top). Overlay of curves for Rebaudioside A (Reb A) and Stevioside from ~500 to 100 ng on column each (bottom). Average of 3 injections, each fit to a linear correlation.

STEVIA SWEETENERS

Table 1. Samples of Sweeteners Used Here

Product	Distributor	Classification	Serving Size 1 Packet (g)	Injection Concentration (mg/mL)
Truvia™	Cargill, Inc.	Table sugar	3.5	5.9
PureVia™	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

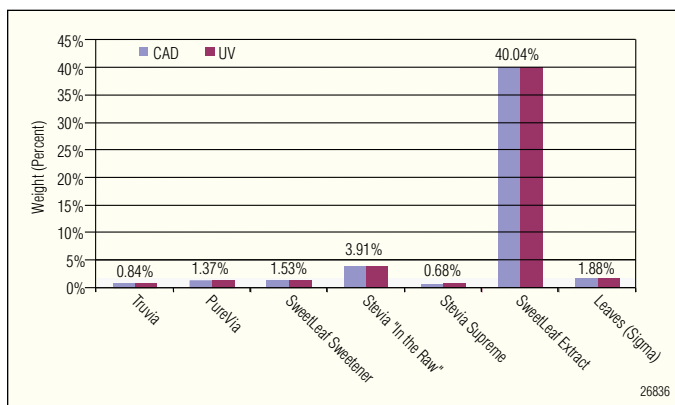


Figure 6. Percentage of Reb A in Stevia containing products.

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 PureVia (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 CAD method (left) and the percent by weight of Reb A was determined. The data generated by UV at 210 nm and the CAD were comparable. However, the CAD has the advantage that all compounds are determined independent of chemical structure, and whether a chromophore is present or not. The CAD 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 of Column)		
	Rebaudioside	Stevioside	Isodeviol
CAD	4 ng	4 ng	60 ng
UV @ 210	65 ng	65 ng	>900 ng

- The CAD demonstrated greater sensitivity for all of the compounds of interest in the Stevia evaluation.
- Limit of Quantification was defined by a signal to noise ≥ 10
- Limit of Detection was defined by a signal to noise ≥ 3

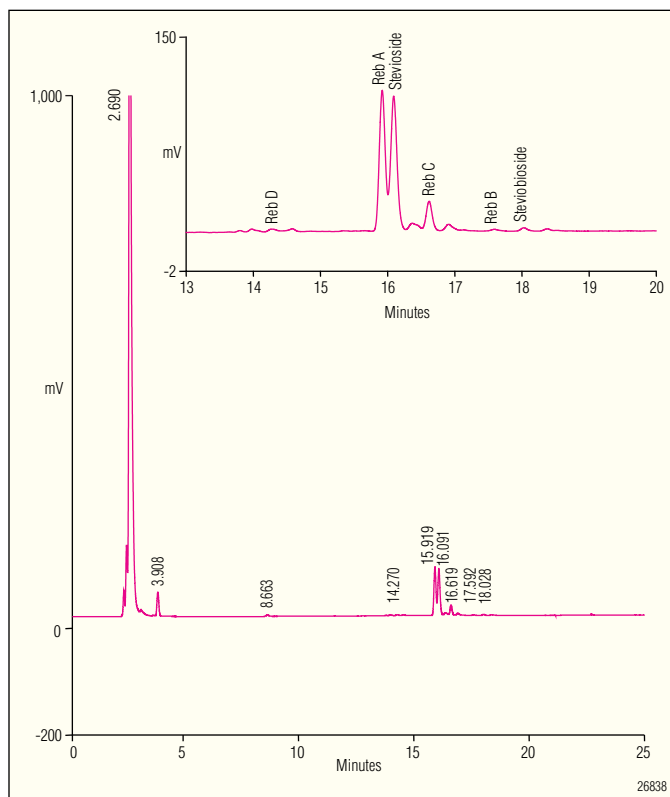


Figure 7. Determination of caffeine (retention time 3.9 min) and five glycosides in a commercially available product.

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 drink with 5 mL methanol solvent prior to analysis. The content of Reb A was calculated to be 0.016% in both drinks with similar concentrations of Stevioside. As shown in the chromatogram above (Zevia Natural Cola), caffeine is determined along with many unknown components.

UHPLC ANALYSIS OF CARBOHYDRATES

HPLC Parameters

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

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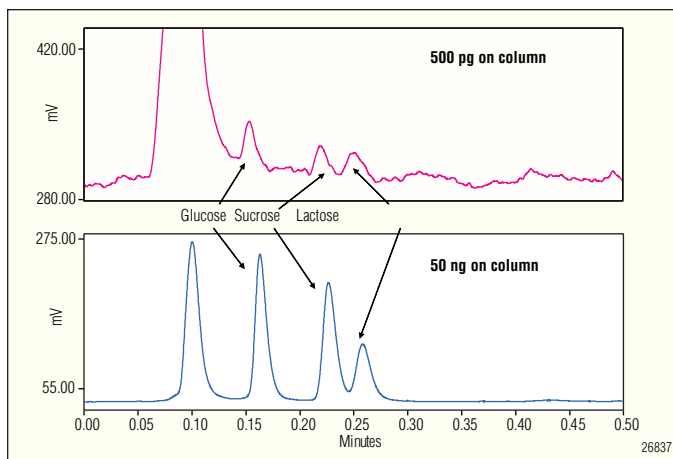


Figure 8. Comparison of picogram vs. nanogram levels of carbohydrates.

High Sensitivity and Rapid Analysis

The above chromatogram shows the separation of three common carbohydrates run under UHPLC-HILIC conditions. The method using a Corona *ultra*[™] detector was rapid (< 30 sec) and could reproducibly detect < 1 ng on column of the sugars. The LOD for glucose was estimated to be ~250 pg on column. The rapid analysis of a nonchromophoric material while maintaining excellent analyte resolution is not possible with traditional HPLC-based platforms.

CONCLUSION

- Both the Corona CAD and Corona *ultra* are extremely versatile detectors. The Corona *ultra* is fully UHPLC.
- Since the 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 Corona CAD can be used with gradient chromatography to improve speed and resolution.
- The sensitivity of the CAD (low nanograms on column) is greater than that of RI, approaches the sensitivity of PAD, and is about 10x more sensitive than UV at 210 nm. Sub-nanogram 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.

ACKNOWLEDGEMENTS

ESA is grateful to Dr. Mark Nightingdale (Durham County Council, UK) for giving his permission to use his data on the global analysis of artificial sweeteners.

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LPN 2403-02 3/10
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