Analysis of Commercially Available Products Containing Stevia

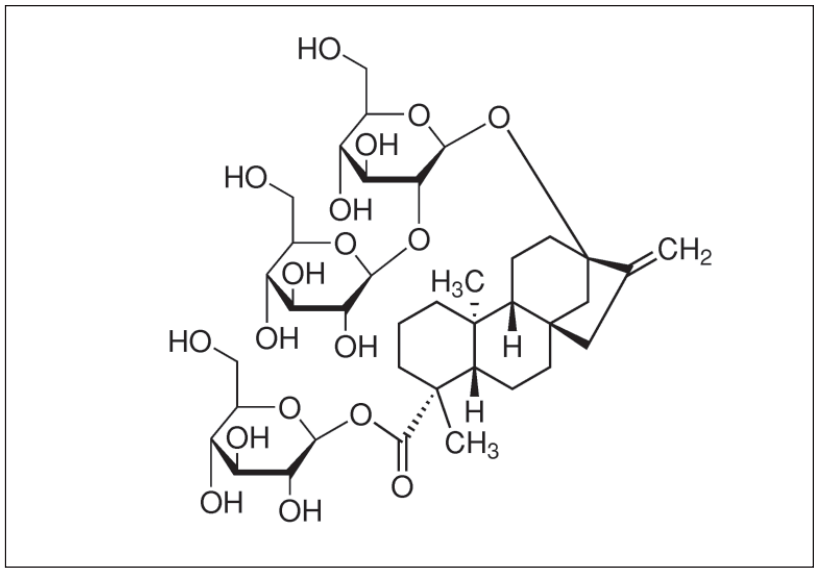

Figure 1. Stevioside.

The commercial use of the herb stevia refers to the extracts from the *Stevia rebaudina*, Bertoni plant. Although originally native to Paraguay and Brazil, it is now cultivated around the world. The plant contains at least eight diterpene glycosides ranging in both relative sweetness and abundance. For example, the two major glycosides stevioside (Figure 1) and rebaudioside A (Reb A) occur at ~3–10% and ~1–2% w/w, respectively, in the dried plant. They are reported to be up to 250 times sweeter than sucrose.

In a few countries (e.g., Japan), extracts from the stevia plant have been used as a sweetener for centuries. However, up until recently, regulations in the United States prohibited the use of Stevia extracts in food or beverages, even though they could be purchased as nutritional supplements. This recently changed. In December 2008, the FDA issued generally recognized as safe (GRAS) affirmations for two commercial products containing Reb A: Truvia® (The Coca-Cola Company) and Pure Via™ (PepsiCo). Both companies have also announced that beverages containing this sweetener will be available in 2009. The FDA GRAS affirmation does not provide approval for the use of all Stevia components in food products, just Reb A. Therefore, Stevia products must be highly purified and characterized prior to use.

This application note describes a routine gradient HPLC-Thermo Scientific Dionex Corona Charged Aerosol Detector method that can be used to characterize Stevia components in raw and commercially available products. Universal detection has merits over the use of absorbance-based detectors for this analysis.

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Method Parameters

Column:	C18 AQ, 4.6 × 250 mm, 5 µm
Temperature:	50 °C
Detector:	Corona™ Charged Aerosol Detector Plus
Nebulizer Heater:	On
Filter:	None
Mobile Phase A:	Deionized water (DI), acetonitrile, trifluoroacetic acid (TFA) (95:5:0.1)
Mobile Phase B:	Acetonitrile, DI (95:5)
Gradient Profile:	Table 1
Flow Rate:	1.0 mL/min
Injection Volume:	10 µL at 10 °C
UV Wavelength:	210 nm

Sample Preparation and Analysis

Commercially available products containing Stevia were purchased at a local supermarket. The products ranged in their classifications and recommended serving sizes (Table 2). The samples and standards were prepared in an aqueous methanol (20%) solution; sample injection concentrations are listed in Table 2.

The Stevia rebaudiana Bertoni leaves were purchased from Sigma-Aldrich (St. Louis, USA). The dry leaf extract was prepared by placing 83 mg of dry leaf in 10 mL of hot deionized water. The solution was then filtered through a 0.2 µm nylon filter and the filter was washed with an additional 15 mL of the aqueous methanol solvent. The solution was then diluted three-fold in order to fall within the standard curve concentrations.

Standards of Reb A, stevioside and isosteviol were purchased from ChromaDex (Irvine, USA) and were certified to have >97% purity. The stock solutions for stevioside, Reb A, and isosteviol were prepared at 167, 159, and 91 ppm, respectively. The five-point calibration curve solutions, ranging from 11 to 56 ppm, as well as limit of detection (LOD) solutions were prepared by appropriate dilutions of the stock solutions. Two injections of each sample and three injections of the individual standards were analyzed. Injections of the standards and samples were interlaced over a three-day period.

Table 1. Gradient profile

Time	% Mobile Phase B
0.0	5
3.0	5
30.0	90
35.0	90
40.0	5
45.0	5

Table 2. Product information

Product	Distributor	Classification	Serving Size 1 packet (g)	Injection Concentration (mg/ml)
Truvia	Cargill, Incorporated	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	Dietiary Supplement	1	1.0
Stevia Supreme™	Stevita Company, Inc.	Dietary Supplement	1	1.1
SweetLeaf® Stevia Extract	Wisdom Natural Brands	Dietary Supplement	0.025	0.086

Results and Discussion

Linearity, Reproducibility, Sensitivity, and Accuracy

Both charged aerosol detector and UV data were evaluated for linearity, sensitivity, and accuracy. Three repetitive injections were made of the five different calibration solutions and the data obtained were analyzed for linearity and reproducibility. For the charged aerosol detector, linear regression analysis was used (Figure 2); charged aerosol detector and UV results are listed in Table 3.

The reproducibility of the analysis was evaluated by calculating the %RSD for repetitive injections of each point on the five point curves. The average relative standard deviation (RSD) calculations for the charged aerosol detector and UV detection are listed in Table 4.

The sensitivity of the charged aerosol detector and UV was evaluated by determining the LOD for the stevioside and Reb A (Table 5). The charged aerosol detector was far more sensitive than UV for all components of Stevia samples (see Figures 3 and 4).

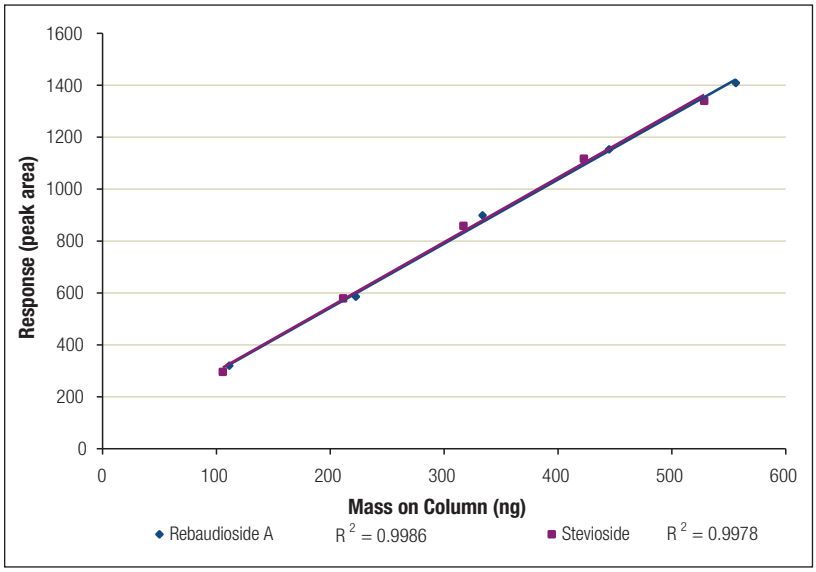

Figure 2. Analyte linearity.

Table 3. Linearity Data

Detector	Linear Correlation Coefficients	
	Rebaudioside A	Stevioside
Charged Aerosol Detector	0.9986	0.9978
UV at 210	0.9992	0.9994

Table 4. RSD data

Detector	Average %RSD	
	Rebaudioside A	Stevioside
Charged Aerosol Detector	0.77%	0.52%
UV at 210	2.44%	1.75%

Table 5. Limit of detection

Detector	Limit of Dection (mass on column)		
	Rebaudioside A	Stevioside	Isosteviol
Charged Aerosol Detector	4 ng	4 ng	60 ng
UV at 210	65 ng	65 ng	>900 ng

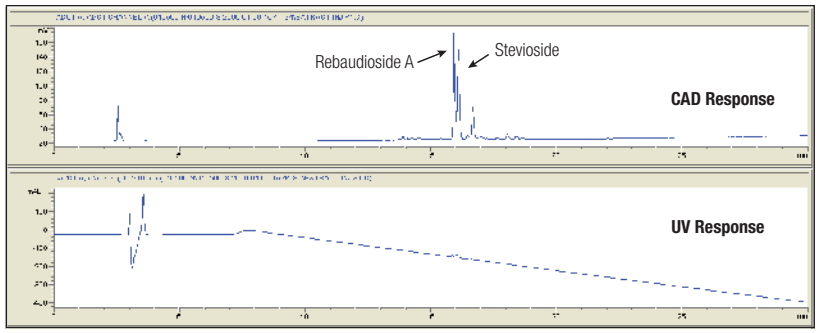

Figure 3. Difference in response between detectors.

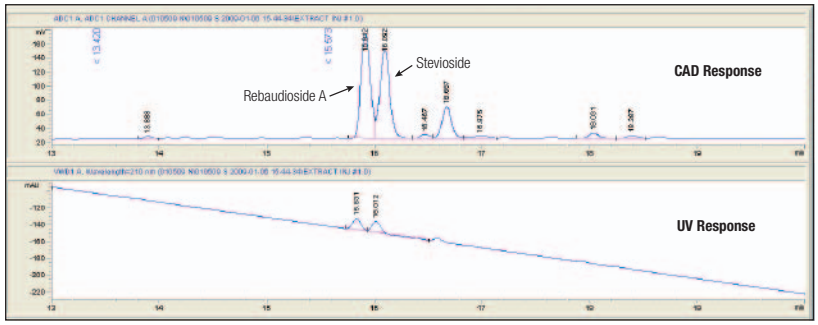

Figure 4. Difference in response between detectors, presented at higher sensitivity.

The accuracy of the analysis was evaluated two ways:

- By spiking a known amount of the standards into a sample and calculating recovery. The recovery for Reb A was 98.4% for the charged aerosol detector compared to 92.2% recovery for the UV detector. Recovery of stevioside was 103.4% for the charged aerosol detector and 101.2% for the UV detector.
- From the manufacturer's reported value. The Reb A reported value was 40% of Reb A in SweetLeaf Stevia Extract compared to a charged aerosol detector value of 40.04% (representing 100.1% recovery).

Rebaudioside A and Stevioside Concentrations in Commercial Products

Linear regression analysis of the five-point calibration was used to calculate a w/w% of the two major glycosides in each of the products indicated in Table 2. All the products contained Reb A and values were calculated and are illustrated in Figure 5 for both the UV and charged aerosol detector. The Truvia, Pure Via, and Stevia Extract In the Raw are purified Reb A sweeteners and no stevioside was detected.

The weight % of the stevioside was calculated for each of the other products and is presented in Figure 6. The charged aerosol detector shows good correlation to the UV values obtained for all products (containing either all Reb A or similar concentrations of both Reb A and stevioside) – between 99 and 105% charged aerosol detector/UV. For the Stevia Supreme and the Stevia leaves, the Reb A correlation was 76% and 95%, respectively, while those of stevioside were 108% and 94%, respectively. Unfortunately, the actual content of each component in these products is not available from the manufacturer, so comparison to calculated results is not possible.

Stevia in Beverages

The FDA GRAS declaration now permits the commercial production of Stevia-based zero-calorie beverages. Zevia (Culver City, USA) began producing 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 diluted by diluting 1 mL of each drink with 5 mL of aqueous methanol solvent. The content of Reb A was calculated to be 0.016% in both drinks; the content of stevioside was calculated to be 0.015% w/w in the Natural Twist flavor and 0.016% w/w in the Natural Cola flavor.

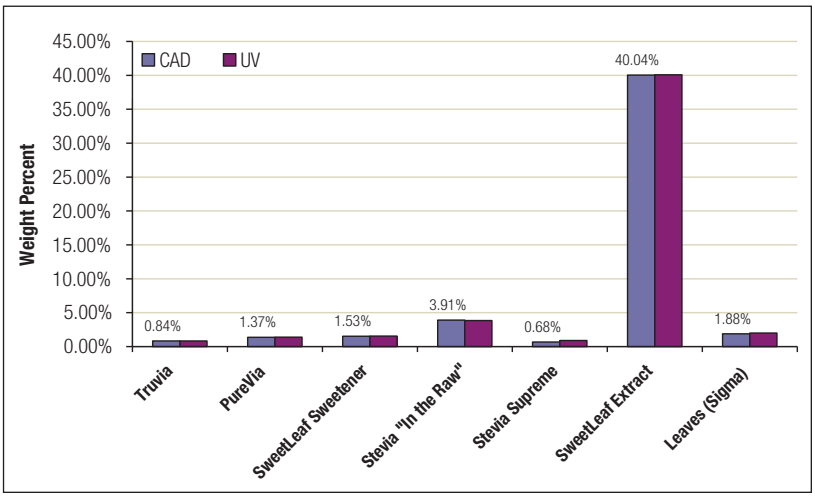

Figure 5. The weight percent of Rebaudioside A in six commercially available Stevia sweeteners and the dried Stevia leaves calculated by using Corona Charged Aerosol Detector and UV detection at 210 nm. % data for charged aerosol detector.

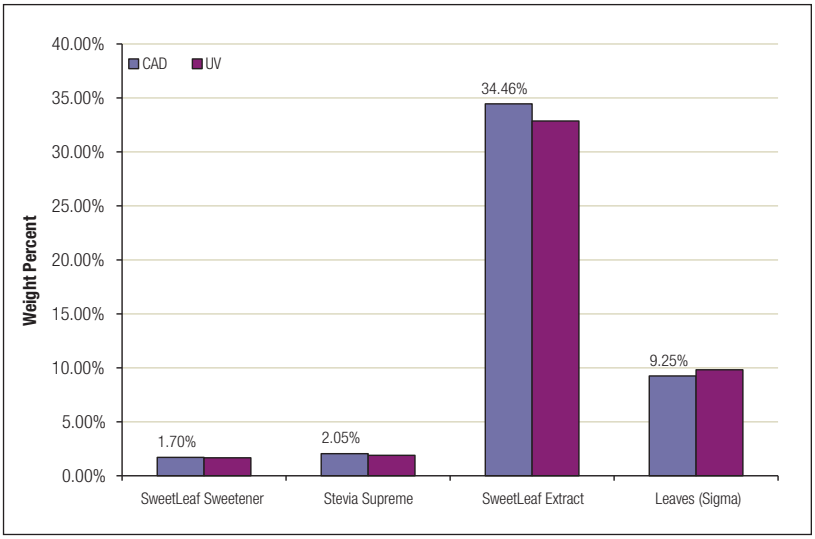

Figure 6. The weight percent of stevioside in three commercially available Stevia sweeteners and the dried Stevia leaves calculated by using Corona Charged Aerosol Detector and UV detection at 210 nm. % data for charged aerosol detector.

Isosteviol and Other Components

Isosteviol is a derivative of stevioside and has been evaluated to determine whether there are any negative health implications when using Stevia sweeteners. This material possesses a weak chromophore and no signal was observed for the 90 ppm standard by UV detection at 210 nm. On the other hand, the signal on the charged aerosol detector was strong—the limit of detection was determined to be ~60 ng on column. The compound was not detected in any of the commercial products tested.

As shown in Figures 5 and 6, the diterpene glycosides from Stevia constitute a small percentage of the table sugars' total mass. Other major components in Stevia products include sugar alcohols (erythritol), dextrose, and inulin soluble fiber. None of these compounds have strong UV chromophores, but all can be detected by charged aerosol detection. In this method, these compounds are not retained—other charged aerosol detector methods exist for their measurement.

Conclusion

A gradient HPLC-UV-charged aerosol detector method was developed for the routine analysis of sweeteners obtained from the Stevia rebaudina, Bertoni plant. The method is accurate, sensitive, and reproducible.

Since the sweetness and potential aftertaste of the different diterpene gyclosides is very important, many companies must ensure that their products are produced consistently by using a viable analytical method. The Corona charged aerosol detector has great applicability throughout the manufacturing process to ensure that product quality and consistency remains uniform. The Corona Charged Aerosol Detector's universality and enhanced sensitivity over UV detection allows for additional components from Stevia to be detected in foods and beverages.

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