RAPID ANALYSIS OF EMERGING SWEETENERS IN BEVERAGES USING UPLC WITH THE ACQUITY UPLC H-CLASS SYSTEM

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INTRODUCTION

The past few years have seen the emergence of several artificial sweeteners such as potassium acesulfame, neohesperidine dihydrochalcone (NHDC), neotame and aspartame. They are popular additives in non-caloric soft drinks and other foods for those who must restrict calories or avoid natural sugar and its derivatives.

Of more recent interest is Stevia, which is derived from the species Asteraceae, native to Central and South America. Stevia's compounds responsible for providing the sweet taste are mainly stevioside and rebaudioside A (Reb A), although much smaller fractions of Reb B, Reb C, Reb D and Reb E can be found in the plant extract. Reb A exhibits the least bitter taste, making it the key compound of interest. Due to its pleasant taste and possible health benefits,[1] Reb A has garnered attention as a sugar substitute. It is sold under several trade names and has achieved GRAS (generally regarded as safe) status in the US[2]. Last year France's food safety agency (the AFFSA) approved Reb A[3] but an EU decision is still pending.

As food and beverage manufacturers bring new products to market based on Stevia, they have a need to develop methods for incoming raw material testing, alternative supplier approval, testing new formulations and quality control of their finished products. In doing so, manufacturers have a need for fast chromatographic methods to identify and separate Reb A from its impurities. The work presented here highlights the benefits of using the ACQUITY UPLC® H-Class System, which delivers UPLC® performance with HPLC simplicity.

Figure 1. Waters ACQUITY UPLC H-Class System.

METHODS

Reb A in three packet products and four beverages:

System: Waters ACQUITY UPLC H-Class / eλ PDA detection
Column: ACQUITY UPLC HSS T3, 2.1x100 mm, 1.8 µm @ 40 °C
Injection Volume: 5 µL
Sampling Rate: 20 pts/sec
Filter Response: 0.1
Detection: PDA (190-340 nm) Extracted @ 205 nm
Software: Empower™ 2
Mobile Phase A: Water (0.1% formic acid)
Mobile Phase B: Methanol (0.1% formic acid)
Flow Rate: 0.5 mL/min
Gradient Profile:

Time	%A	%B	Curve
Initial	60.0	40.0	-
3.0	5.0	95.0	6
4.0	5.0	95.0	6
4.1	60.0	40.0	6

Mixture of Reb A with other alternative sweeteners:

Conditions as above except gradient was 10-82 % methanol in 6.0 minutes.

Mixture of Reb A with other Steviol Glycosides:

Conditions as above with the following exclusions
Column: 2.1 x 100 mm UPLC BEH Amide @ 35 °C
Mobile Phase A: Water (0.1% NH_4OH)
Mobile Phase B: Acetonitrile (0.1% NH_4OH)
Flow Rate: 0.3 mL/min, 22% A, 78% B (Isocratic)

Standard Preparation:

A 2800 ppm stock solution of Reb A (Chromadex) was made in 10:90 water/methanol. Dilutions of this stock in water were used to create a five point calibration curve. Each standard level was injected in duplicate.
Individual 1 mg/mL stocks of acesulfame K, sodium saccharin, aspartame, NHDC, and neotame were prepared in water. 100 µL of each along with 50 µL of the Reb A standard described above were added to 450 ul of water and 2µL was injected.
Individual stocks of Reb C, Reb D and stevioside were made as for the Reb A stock described above.
50 µl of each of the stock solutions were added to 800 µl of a 22:78 water/acetonitrile (0.1% NH_4OH) mix and 3 µL was injected.

Sample Preparation:

Three different commercial Reb A packet preparations were procured from grocery stores. The contents of each sample packet were weighed and dissolved in 50 ml water. The solution was then filtered through a 0.45 micron membrane and injected in duplicate.

In addition, four different Reb A fortified water beverages were also purchased. Portions of these beverages were filtered and injected as above. In addition, Beverage C was injected seven times for a reproducibility study.

RESULTS

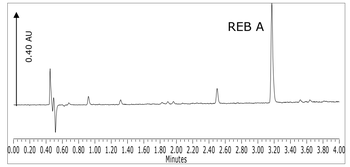

Figure 2. Chromatogram of a 280 ppm Reb A standard.

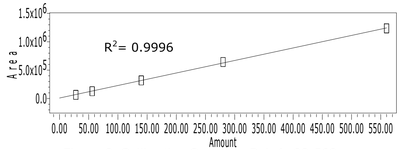

Figure 3. Calibration Curve for Reb A, 28-560 ppm.

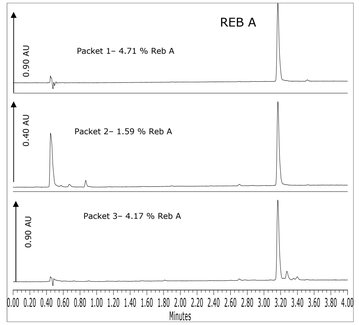

Figure 4. Chromatograms from three different commercial sweetener packets containing Reb A. The calculated percentage Reb A by weight for each sample is shown.

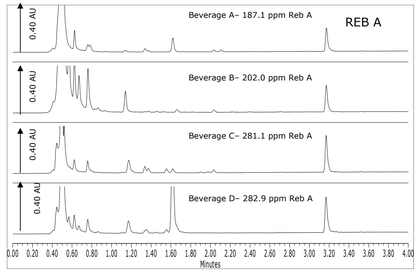

Figure 5. Chromatograms of four different beverages containing Reb A. The calculated concentration of Reb A for each beverage is shown.

Component	% RSD RT	% RSD Concentration
Reb A	0.044	0.670

Table 1. Reproducibility data for Retention Time and Concentration for 7 injections of Beverage C.

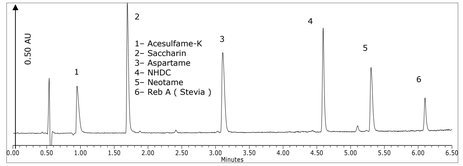

Figure 6. Chromatogram showing the separation of Reb A with other artificial sweeteners.

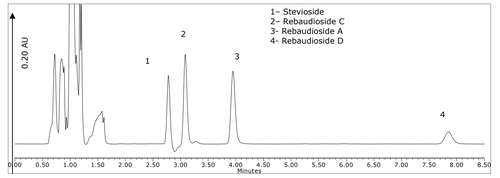

Figure 7. Chromatogram showing the separation of Reb A and other Steviol Glycosides.

DISCUSSION

Figure 2 shows a chromatogram of a 280 ppm Reb A standard. The calibration curve for five standard levels of Reb A is displayed in Figure 3. As seen in the figure, excellent linearity was obtained with an r^2 value of 0.9996.

The chromatograms for each of the three sweetener packets are shown in Figure 4. Using the sample weights, dilution factor and calibration curve, Empower Software calculated the % Reb A by weight in each of the packets. The results indicate great variations between Packet 2 and the other two products, illustrating significant discrepancies among commercial products.

Figure 5 shows the chromatograms for the four beverages tested. Differences in the chromatographic profiles for each beverage are evident. The concentration of Reb A (ppm) in each of the drinks was calculated and is also reported on Figure 5. In addition, Beverage C was injected seven times. Excellent reproducibility was achieved for the concentration and retention time, as shown in Table 1.

The separation of Reb A with several other artificial sweeteners is displayed in Figure 6. A different gradient was used to separate the weakly retaining Acesulfame– K and saccharin. The retention time of Reb A was approximately 6.2 minutes under these conditions. This separation is useful for screening and quantifying artificial sweeteners from different products in one single method.

Figure 7 shows the separation of Reb A along with several other steviol glycosides. Here a different column chemistry (BEH Amide) is required to separate the key analytes. This separation would be useful for monitoring the purification process of the raw plant extract or testing the purity of raw materials prior to their incorporation into commercial products.

CONCLUSION

- **Reb A can be analyzed from packet materials and beverages with a run time of less than 3.5 minutes using the ACQUITY UPLC H-Class System.**
- **Steviol glycosides can be effectively separated in raw extracts for process and quality control purposes using a BEH Amide column.**
- **Reb A along with several other artificial sweeteners can be separated in under 6.5 minutes.**
- **This work highlights the utility of ACQUITY UPLC H-Class based technology for the analysis of alternative sweeteners for the food and beverage industry.**

References

1. Geuns Jan, " Steviol Glycosides as Food Additives " European Stevia Association, 2007, pp 17
2. http://www.fda.gov/Food/default.htm GRAS Notice 000287; GRAS Notice 000282
3. http://www.afssa.fr/
4. Afssa – Saisine n° 2009-SA-0269, Saisines liées n° 2006-SA-0231, n° 2008-SA-0108, n° 2008-SA-0321, n° 2009-SA-0012, n° 2009-SA-0119

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