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A Method for the Rapid and Simultaneous Analysis of Sweeteners in Various Food Products Using the ACQUITY Arc System and ACQUITY QDa Mass Detector

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APPLICATION BENEFITS

- The ACQUITY® QDa® Mass Detector facilitates simultaneous analysis of sweeteners in a single run.
- Quantification of natural and artificial non-nutritive sweeteners in less than 10 minutes.
- Mass detection provides information-rich orthogonal detection for co-eluting compounds.
- The ACQUITY QDa Mass Detector provides sensitive and selective determination of UV transparent sweeteners.

WATERS SOLUTIONS

ACQUITY Arc™ System

ACQUITY QDa Mass Detector

Empower® 3 Chromatography Data System

KEYWORDS

Non-nutritive sweetener, mass detection, ACQUITY Arc System

INTRODUCTION

Sugars are renowned for their sweet taste and are often added to manufactured foods to enhance human perception of flavor. Due to the negative health effects of excessive consumption of sugar, alternative non-nutritive sweeteners are commonly used in food and beverage products. Examples include soft drinks, table-top sweeteners, chocolates, dairy products, and many other so-called "diet" foods. In many cases it is a combination of various sweeteners that are used to impart overall sweetness to these products.

Aspartame, saccharin, acesulfame-K, neotame, and sucralose are approved artificial sweeteners by the U.S. Food and Drug Administration (FDA).[1] Compounds such as rebaudioside A and stevioside, which originated from the South American Stevia plant, are also becoming more popular in the United States. In 2010, the European Food Safety Authority (EFSA), approved the use of stevioside as a sweetener.

The European Union (EU) Directive 94/35/EC, along with four amendments: 96/83/EC, 2003/115/EC, 2006/52/EC and 2009/163/EU, restrict the level of sweeteners in specific types of food. The EU Commission Regulation 1129/2011 lists the maximum level of the sweeteners permissible in various food products. Hence the determination of the amount of these sweeteners in foods is also important in order to ensure consistency in product quality.

The most common method for the detection of sweeteners is HPLC coupled to a UV detector. This configuration enables the detection of some sweeteners such as acesulfame-K, aspartame, saccharin, and neotame. However cyclamate and sucralose cannot be analyzed by UV because they lack chromophores. The ability to analyze all of these sweeteners using a single method with mass detection would be ideal. Waters has developed the ACQUITY QDa Mass Detector to allow food and beverage scientists to incorporate mass detection into their existing chromatographic workflows.

The ACQUITY QDa not only allows for the detection of all sweeteners in a single run, but also brings improved discrimination to the analysis, thereby eliminating the requirement of baseline separation of all compounds. The combination of Waters® ACQUITY Arc System with the ACQUITY QDa Mass Detector is extremely beneficial for food and beverage manufacturers for the identification and quantification of sweeteners in their products using a single analysis method.

In this application note, a fast, reliable and sensitive method was developed to analyze sweeteners in food and beverage samples.

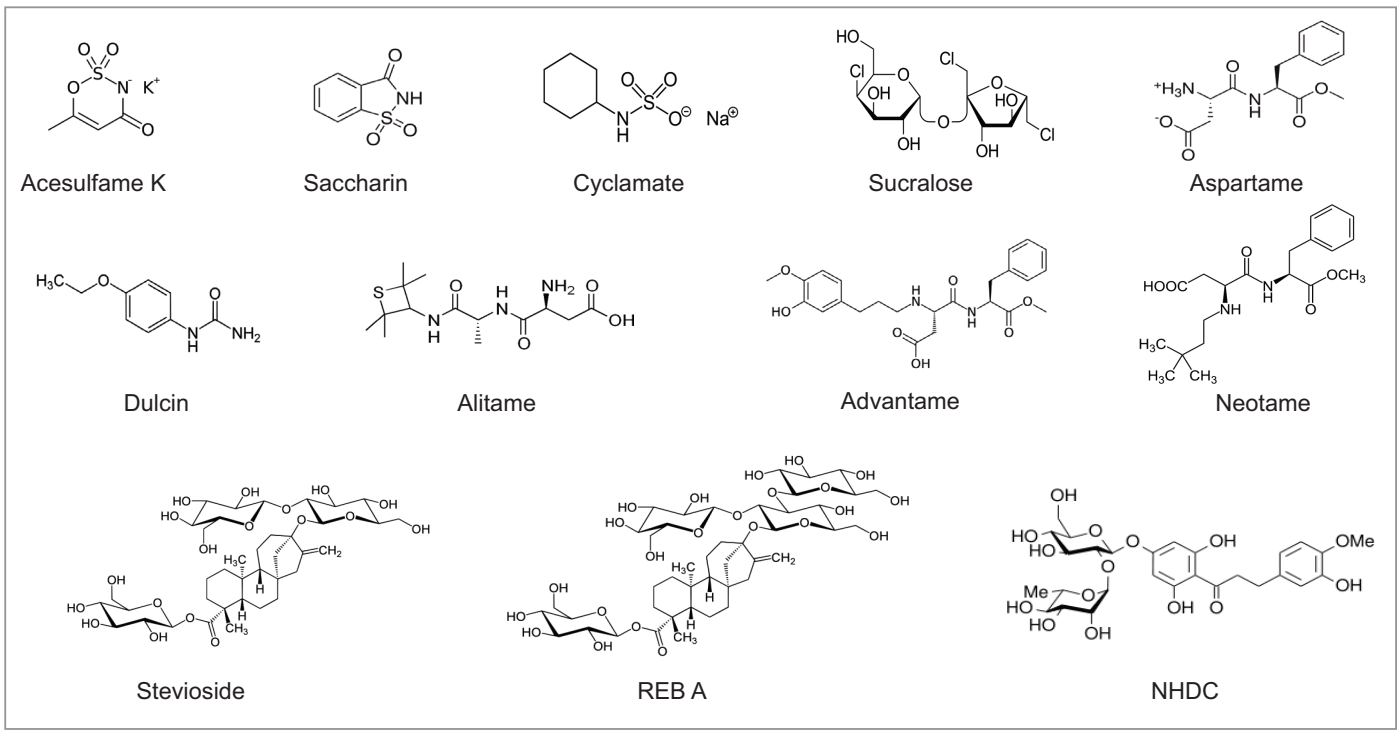

Figure 1. Chemical structures of the sweeteners analyzed.

EXPERIMENTAL

UHPLC conditions

UHPLC system:	ACQUITY Arc System
Column:	Waters CORTECS® T3, 3 x 100 mm, 2.7 µm
Column temp.:	40 °C
Injection volume:	2 µL
Flow rate:	0.55 mL/min
Mobile phase A:	Water with 0.1% formic acid
Mobile phase B:	Acetonitrile with 0.1% formic acid
Runtime:	8.5 min

	Time (min)	Flow rate (mL/min)	%A	%B
1.	Initial	0.55	90	10
2.	2.0	0.55	90	10
3.	8.0	0.55	40	60
4.	8.5	0.55	0	100
5.	9.5	0.55	0	100
6.	9.6	0.55	90	10

Table 1. Elution gradient.

MS conditions

MS detector:	ACQUITY QDa (Performance)
Ionization mode:	ESI-, ESI+
Capillary voltage:	0.8 kV
Probe temp:	600 °C
Cone voltage:	15 V
Sampling rate:	2 points/second
Acquisition:	100–1000 *m/z* centroid
SIR channels:	See Table 2

UV conditions

Detector:	Waters 2998 PDA Detector
Wavelength:	210–360 nm
Sampling rate:	10 points/second
Analog channel:	214 nm

Standard preparation

Stock solutions (1000 ppm) of each of the sweeteners were prepared in water. From these, a mixed stock of 25 ppm for 10 sweeteners and 50 ppm for REB A and sucralose was prepared in 2:3 (v/v) acetonitrile-water. From this, seven additional dilutions were made in 2:3 acetonitrile-water to produce eight levels of concentrations from 0.25 to 25 mg/L (ppm) for 10 sweeteners, and 0.5 to 50 mg/L (ppm) for REB A and sucralose.

Sample preparation

A total of seven different samples were analyzed for this work. The samples included three table top sweeteners, a diet candy, a diet chocolate pudding, a diet soft drink and beverage standard.

The table top sweeteners (~1 g) were dissolved in 100 mL water and further diluted at two additional levels 1:20 and 1:10. All three levels were injected to cover the different levels of sweeteners in these samples.

The candy (5.29 g) and pudding (2.93 g) were dissolved separately in 100 mL water and diluted 1:10. The solutions were filtered through a 0.2 µ PVDF filter and injected. The diet soft drink was degassed through sonnication, filtered as above diluted 1:20 and injected. The beverage standard was diluted 1:20.

RESULTS AND DISCUSSION

The chemical structures for the twelve sweeteners analyzed are shown in Figure 1. These sweeteners were separated on a reversed-phase column and detected using the ACQUITY QDa Mass Detector. The retention times, along with the Single Ion Recording (SIR) mass-to-charge ratio (*m/z*), and detection polarity of these sweeteners are shown in Table 2.

Analyte	SIR and Polarity	RT (min)
Acesulfame K	162.0 (-)	1.19
Saccharin	182.0 (-)	1.62
Cyclamate	178.2 (-)	1.94
Sucralose	397.1 (-)	4.57
Aspartame	295.1 (+)	4.88
Dulcin	181.0 (+)	5.80
Alitame	332.1 (+)	5.93
Advantame	459.2 (+)	7.50
Neotame	379.2 (+)	8.08
Stevioside	641.5 (-)	7.87
REB A	803.5 (-)	7.83
NHDC	611.3 (-)	7.26

Table 2. The SIR channels' m/z values, the ESI polarity, and the retention times for the 12 sweeteners studied.

The mass spectrum of each analyte was acquired using a Total Ion Current (TIC) scan (positive and negative). Results are shown in Figure 2. Please note the formate adduct for sucralose was observed. This adduct has been reported elsewhere.[1] The mass spectra of the sweeteners REB A and stevioside showed fragment ions as the main species for these compounds (Figure 3).

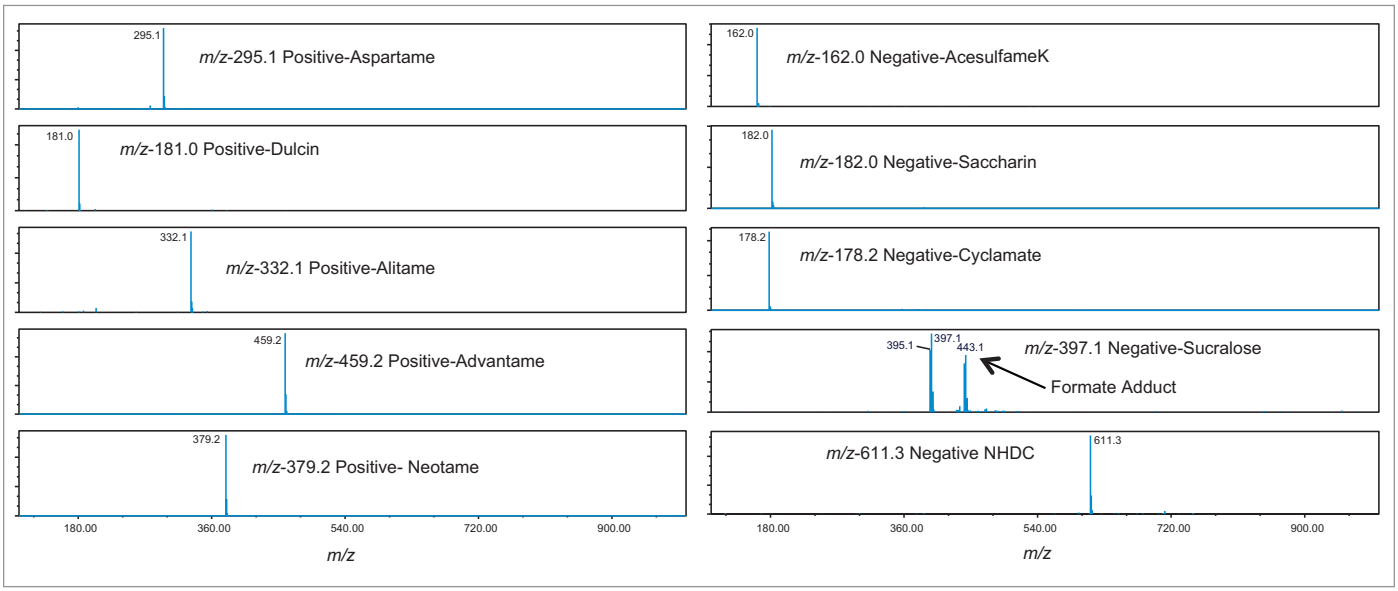

Figure 2. ESI positive and negative mass spectra of 10 sweeteners. The m/z, polarity, and the analyte name are shown in each mass spectrum. The molecular ions are the main ions in these mass spectra, except in sucralose MS, a large formate adduct ion is observed. Chlorine isotope pattern is also observed for sucralose.

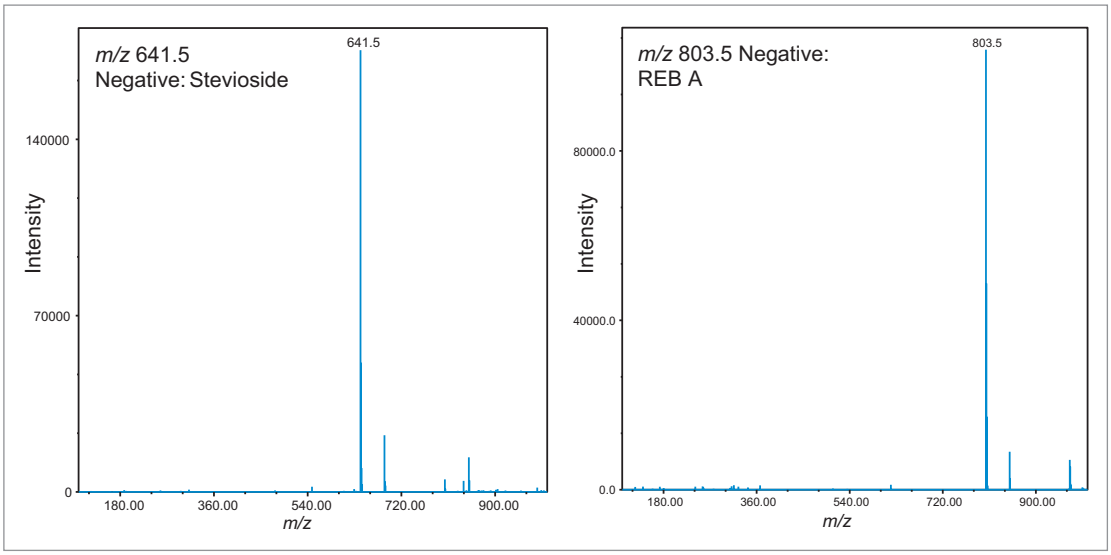

Figure 3. Mass spectra for REB A and stevioside. The base peaks in these mass spectra are fragment ions.

The *m/z* of 641 for stevioside has also been reported by others.[2] The 12 SIR chromatograms of a 12 sweetener standard mix in a solvent solution are shown in Figures 4. Calibration curves and regression coefficients are shown in Figure 5. A quadratic fit with 1/x weighting was appropriate for these analytes. The correlation coefficient (R^2) is greater than 0.996 for all compounds. Figure 6 is an overlay of the UV trace at 214 nm and the SIR channels at *m/z* 295.1 and 397.2 of aspartame and sucralose respectively. Sucralose being UV transparent does not appear in the UV trace but has a strong signal at *m/z* 397.2 showing the strength of mass detection augmenting UV.

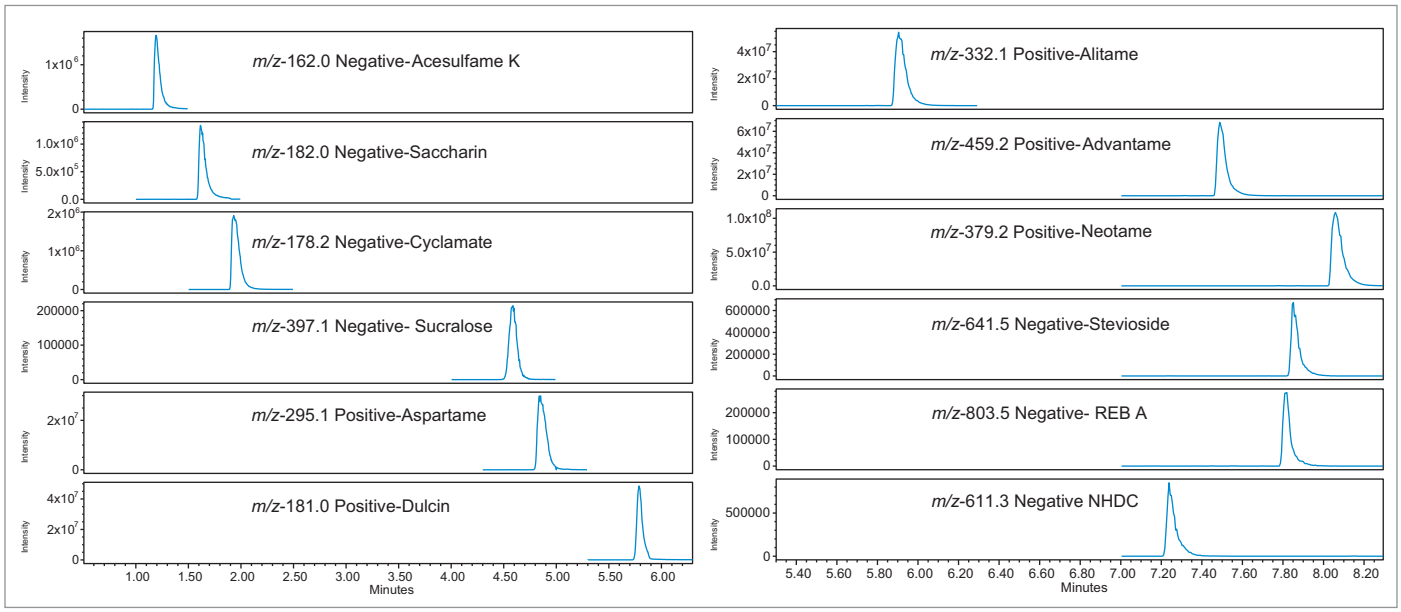

Figure 4. SIR chromatograms for sweeteners in a solvent standard mix.

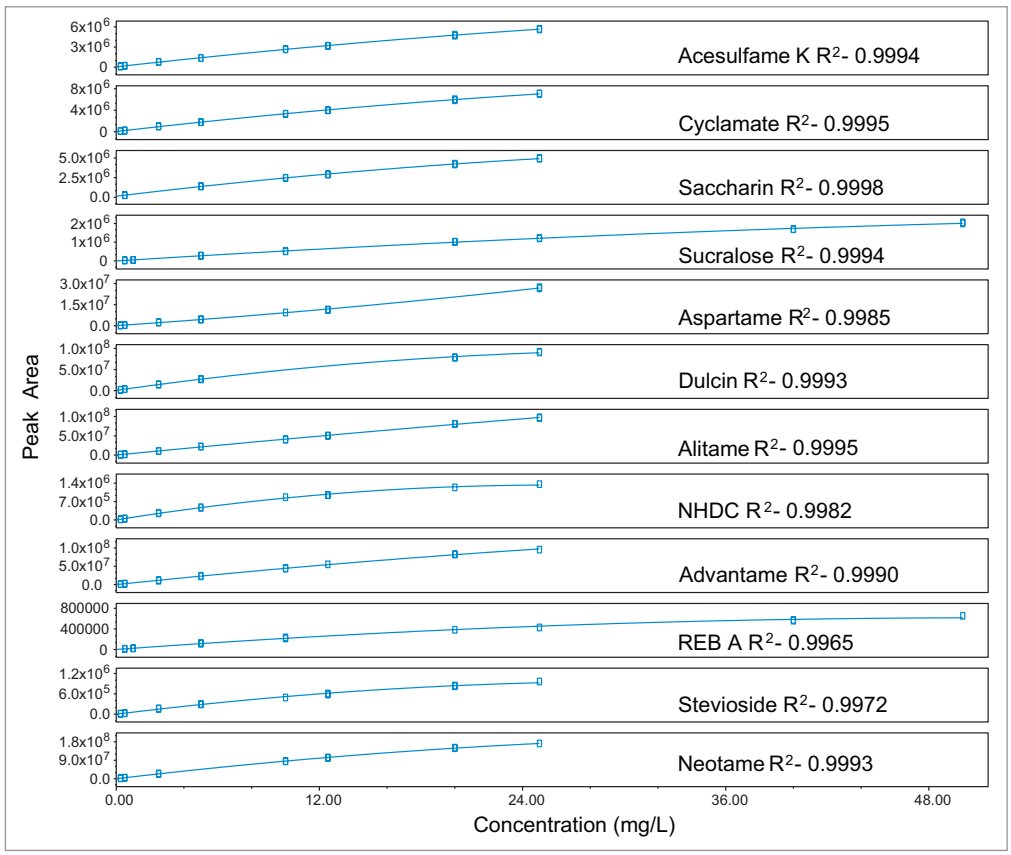

Figure 5. Calibration plots for 12 sweeteners. A quadratic fit with 1/x weighting was used for all compounds. The analyte and the correlation coefficient (R^2) are shown in each individual plot. R^2 is greater than 0.996 for all compounds.

ANALYSIS OF SAMPLES

The seven samples were prepared as previously described and analyzed in duplicate. The quantitation results are listed in Table 3. The first table-top sweetener contained sucralose (14.27 mg/g). The second table-top sweetener was found to contain aspartame (16.05 mg/g) and saccharin (4.17 mg/g). The third table-top sweetener listed Reb A as the main sweetener. However, besides the Reb A (27.72 mg/g), we note a small amount of stevioside (0.03 mg/g) was present although it is not listed as an ingredient. With the discrimination and sensitivity of mass detection, it was easily apparent that both of these co-eluting sweeteners were present in the sample, even though the stevoiside was present at a much lower level and was not labeled to be present. This is another example of the power of mass detection to detect low levels of analytes. The beverage standard was certified as 150 mg/L acesulfame K and 100 mg/L saccharin. Our values of 137.95 mg/L for acesulfame K and 97.66 mg/L for saccharin agree well here.

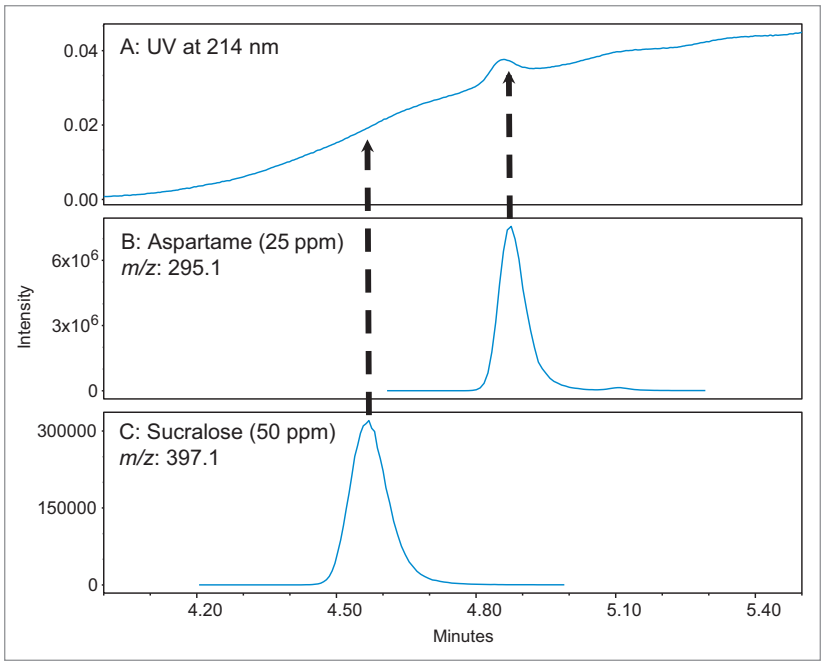

Figure 6. Overlay of UV chromatogram at 214 nm and the SIR chromatograms of aspartame (m/z 295) and sucralose (m/z 397.1).

Sample	Acesulfame K	Aspartame	REB A	Saccharin	Stevioside	Sucralose	Units
Table-top Sweetener 1	–	–	–	–	–	14.27	mg/g
Table-top Sweetener 2	–	16.05	–	4.17	–	–	mg/g
Table-top Sweetener 3	–	–	27.72	–	0.03	–	mg/g
Diet candy	–	–	–	–	–	0.21	mg/g
Diet Pudding	0.17	–	–	–	–	0.10	mg/g
Diet Soft Drink	80.14	294.51	–	–	–	87.33	mg/L
Beverage Standard	137.95	–	–	97.66	–	–	mg/L

Table 3. Quantification results of the seven samples tested in this study.

CONCLUSIONS

This application note has described a method for the separation, detection and quantification of twelve natural and artificial non nutritive sweeteners in less than 14 minutes (including equilibration time) using the ACQUITY Arc System coupled with the ACQUITY QDa Mass Detector.

The ACQUITY QDa Mass Detector enables a single method to analyze both UV transparent and non-transparent sweeteners, and this method can be incorporated into existing workflows – with or without UV detection. Mass detection provides a much higher level of analyte discrimination and it does not require baseline separation of the analytes with different masses. This helps minimize method development times and removes the need for separate injections of individual standards to verify compound identifications.

References

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2. D. Yang and B. Chen. "Simultaneous Determination of Nonnutritive Sweeteners in Foods by HPLC/ESI-MS" *J. Agric. Food Chem.* 2009, 57, pp. 3025.

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