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Application Note

Analysis of Sucralose in Beverages Using an ACQUITY™ UPLC™ H-Class PLUS with the ACQUITY UPLC Refractive Index Detector

Kim Van Tran, Euan Ross, Peter Hancock

Waters Corporation

Abstract

Sucralose is a non-nutritive sweetener that can be found in a variety of foods and soft drinks. Sucralose is derived from sucrose through the selective replacement of three hydroxyl groups which are substituted with three chlorine atoms. A common detection method for sweeteners is the use of high-performance liquid chromatography (HPLC) coupled with ultraviolet/visible light (UV/Vis) detection, however, sucralose requires alternative detection due to a lack of chromophore. Alternative detectors for the analysis of sucralose include refractive index (RI), evaporative light scattering (ELS), or mass spectrometry (MS). This application note highlights a method for the analysis of sucralose using an ACQUITY UPLC H-Class PLUS coupled with an ACQUITY Refractive Index Detector. Combined with a CORTECS™ T3 Analytical Column and Empower™ 3 Chromatography Data System, this set-up provides a simple isocratic method for the determination of sucralose in beverages, such as soft drinks and energy drinks.

Benefits

- Simple isocratic LC method with ease of method setup for routine product quality control

- The ACQUITY RI Detector has a low internal volume which delivers low dispersion and a stable baseline performance for reliable quantitative results
- The thermally isolated optics bench of the ACQUITY RI Detector and highly efficient temperature equilibration of the incoming eluent, minimizes baseline drift
- Excellent method reproducibility, accuracy, and precision

Introduction

Sucralose is a high-intensity sweetener used as a sugar substitute in a variety of food and beverage products. Sucralose is derived from sucrose through the selective replacement of three hydroxyl groups which are substituted with three chlorine atoms. When ingested, sucralose does not contribute to calories, and it is widely used in sugar-free products. HPLC coupled to UV/Vis detection is a common approach to the analysis of sweeteners such as aspartame and acesulfame potassium (Ace-K).[1] Sucralose has no chromophore, and as a result, alternative detectors such as RI, ELS, or MS are required for its determination in food and beverage products.

This application note highlights an example analytical method for the quantification of sucralose in various energy drinks. Whilst the use of refractive index detection limits the method to an isocratic mobile phase, this also results in a simple method with no re-equilibration steps in-between injections. A CORTECS T3 Column was used for the retention of sucralose and acceptable separation between sucralose and other ingredients was observed in the products tested.

Experimental

Materials and Reagents

The sucralose standard was obtained from Sigma Aldrich, purity ≥98.0% (HPLC).

Reagents

Methanol (HPLC grade) was obtained from Honeywell Research Chemicals.

Water from PureLab flex ELGA system (LabWater United States of America).

Products containing sucralose were obtained from local sources (Massachusetts).

Preparation

Standards Preparation

A sucralose standard was prepared at a concentration of 500 µg/mL in 800 µL water and 200 µL of methanol and then stored at 4 °C. A calibration curve with a range of 7.8 µg/mL to 500 µg/mL was then prepared by serial dilutions in 80:20 water:methanol.

Sample Products

Samples G1 and P2: 800 µL of the samples were taken and 200 µL methanol was added (1.25X dilution).
Sample 5H: a 10 µL sample was taken and 790 µL water and 200 µL methanol (100X dilution).
After dilution, samples were vortexed and filtered with PTFE 0.2 µm (p/n: WAT200556 < https://www.waters.com/nextgen/global/shop/sample-preparation--filtration/wat200556-acrodisc-minispike-syringe-filter-ptfe-13-mm-02--m-non-polar-100.html>), and 10 µL of G1, P2, or 5 µL of 5H samples were injected. Samples were prepared in six replicates.

LC Conditions

LC system:	ACQUITY UPLC H-Class PLUS System (Quaternary Solvent Manager)
Detection:	ACQUITY UPLC RI Detector: Sampling rate 20 points/sec
Vials:	LCGC Certified Clear Glass, Max Recovery, with Cap and Preslit PTFE/Silicone Septum, 1.5 mL (p/n: 186000327C)

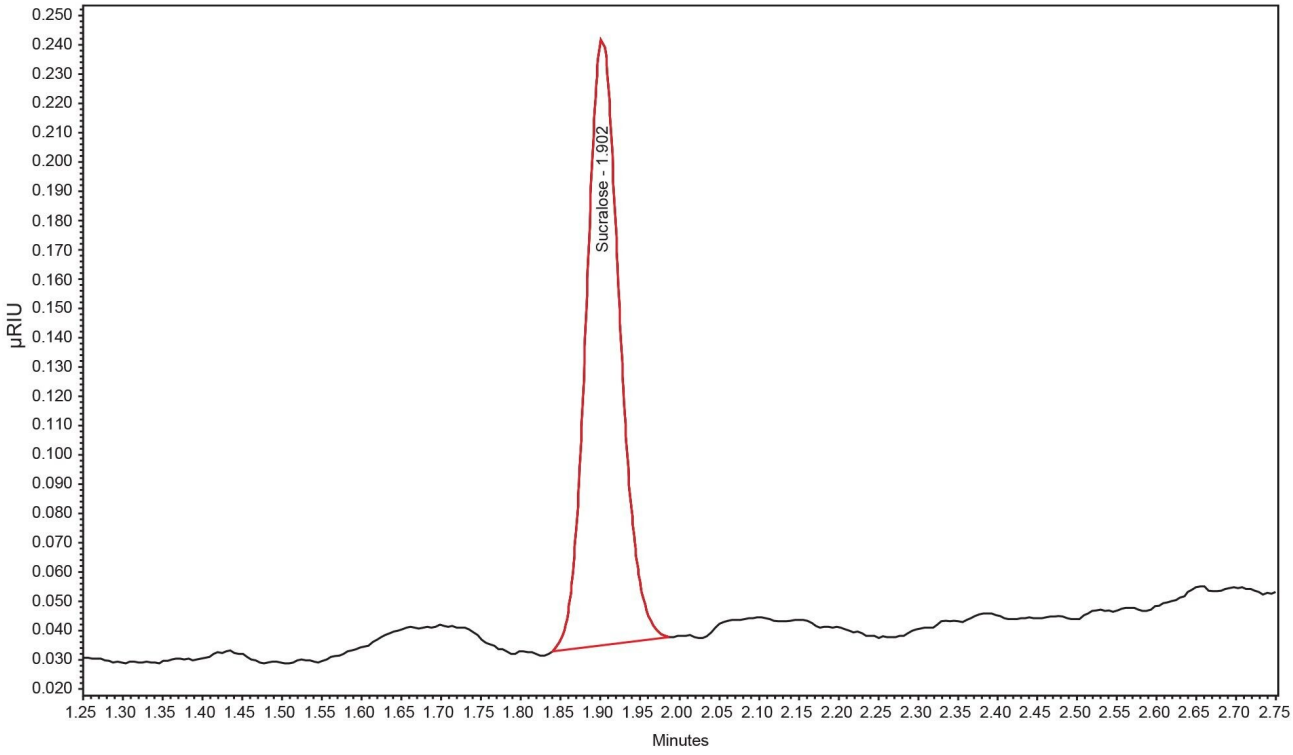
Filter:	Syringe Filter 0.2 µm PTFE (p/n: WAT200556)
Column(s):	CORTECS T3 Column, 120 Å, 2.7 µm, 3 mm x 100 mm, (p/n: 186008489)
Column temp.:	50 °C
Sample temp.:	25 °C
Detector temp.:	50 °C
Injection volume:	5 or 10 µL
Flow rate:	1 mL/min
Mobile phase:	80:20 Water:methanol
Sample diluent:	80:20 Water:methanol
Seal wash:	80:20 Water:methanol
Needle wash:	80:20 Water:methanol
Purge solvent:	80:20 Water:methanol

Data Management

Chromatography software:	Empower 3 Chromatography Data Software (CDS)

Results and Discussion

The analysis of sucralose was performed using an ACQUITY UPLC H-Class PLUS coupled to an ACQUITY UPLC RI Detector. A CORTECS T3 Column was used with an isocratic mobile phase with a composition of 80:20 water and methanol. These mobile phase conditions provided acceptable retention, peak shape, and separation from other ingredients in the energy drinks. A temperature of 50 °C gave the best response from the RI detector. To reduce any baseline fluctuations the column temperature and RI detector temperature were both set to 50 °C. Signal to noise for the lowest calibration standard (7.8 µg/mL) was determined using the Empower CDS Software. Figure 1 shows the chromatogram of the lowest calibration standard and the signal to noise and the peak tailing factor calculated in Empower.

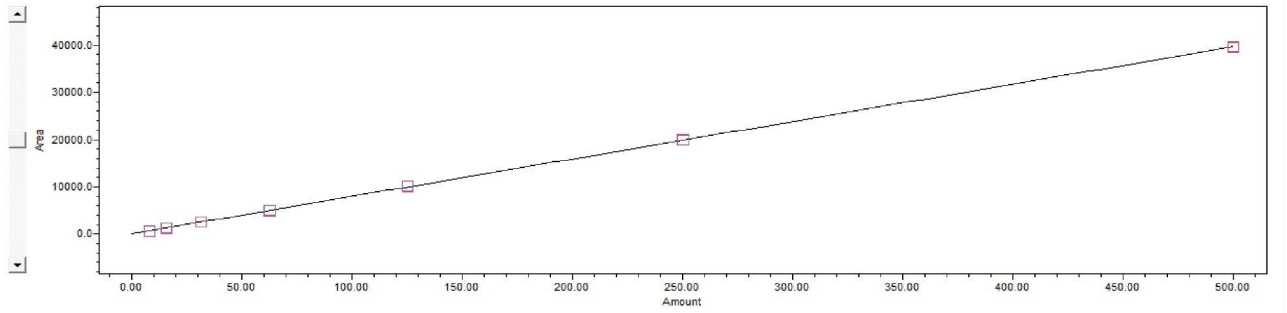


Standard	Retention time	USP s/n	USP tailing	Width at baseline
Sucralose	1.90	37.30	1.10	0.15

Figure 1. Chromatogram of sucralose standard (7.8 µg/mL) using a CORTECS T3 Column.

A multi-point calibration curve for sucralose was prepared in six replicates via serial dilution in 80:20 water and methanol. The calibrated range was from 7.8 µg/mL to 500 µg/mL and showed good linearity (R^2>0.999) and is

shown in Figure 2.



	Project Name	Name	Processing Method	Order	R^2	Equation	Calibration Type	Fit Type	Points	X-axis	Y-axis	Units
1	SucraloseRI	Sucralose	Sucralose_RI_30point_2	1	0.999962	Y = 7.93e+001 X + 5.68e+001	LC	Linear (1st Order)	42	Amount	Area	ppm

Figure 2. Calibration curve for sucralose from 7.8 to 500 µg/mL using the ACQUITY RI Detector.

Method Robustness

The column performance and pressure trace

The pressure trace of the column was monitored over 100 injections of standards and samples. There were no significant changes across the pressure trace (a*bout 23 psi difference).* The reproducibility of the retention time from the column was monitored for 100 injections from the first injection of 500 µg/mL standards overlaid with the last injection of a 500 µg/mL standard, interspersed with matrix samples. (Figure 3a and 3b).

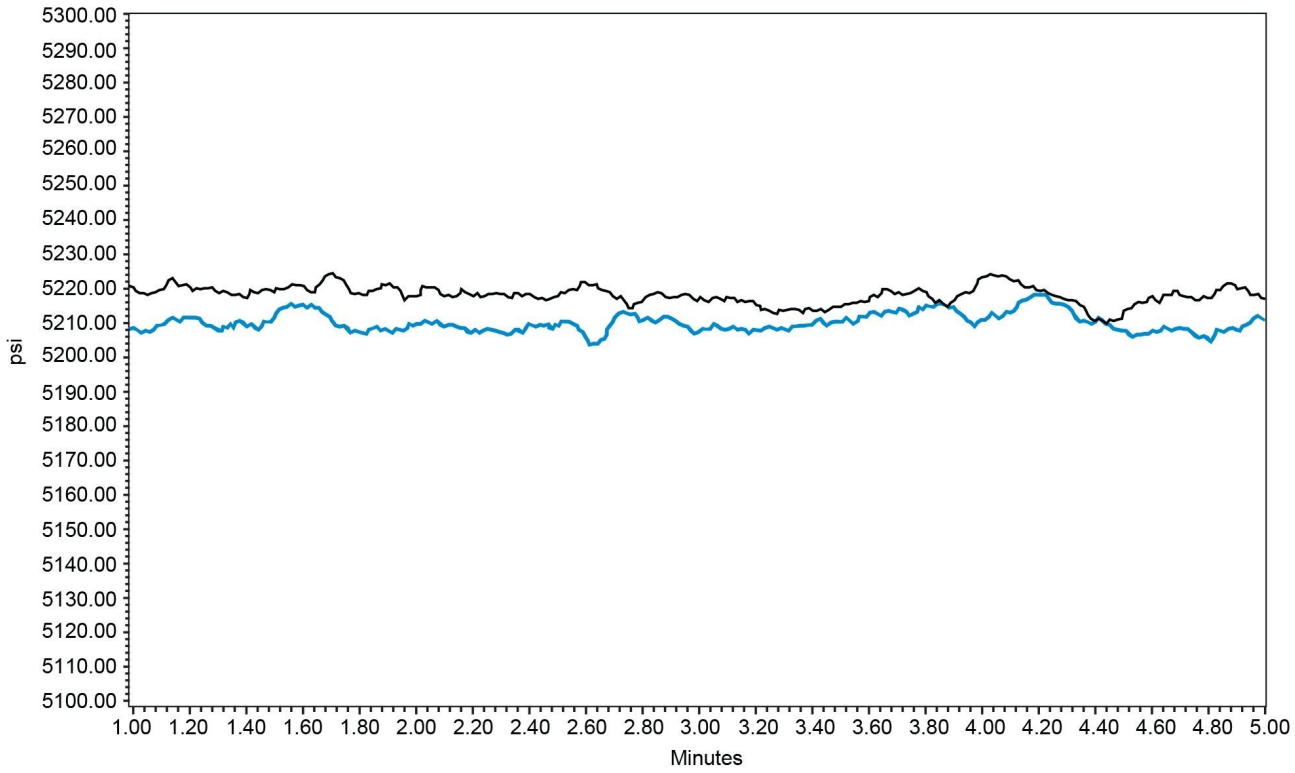


Figure 3a. Pressure trace over 100 injections.

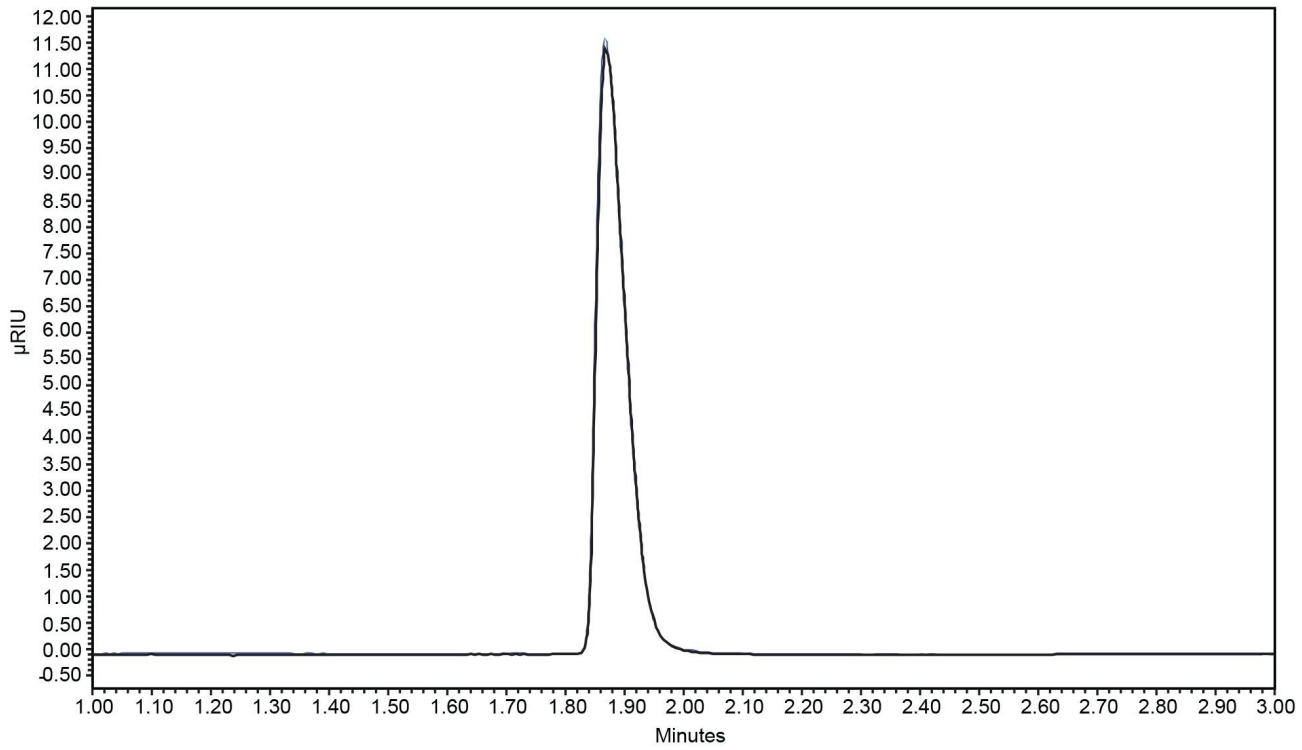


Figure 3b. Reproducibility of 500 µg/mL standard over 100 injections.

Analysis of Sucralose in Beverages

The quantitative results from the analysis of different sucralose beverages with six replicates are shown in Figure 4. Samples were diluted to fit into the calibrated range. The results have shown that sucralose was well separated from the other ingredients and the results were reproducible.

Samples	Retention time	Amount (ppm)
	n=6 (%RSD)	n=6 (%RSD)
Sample G1	1.90 (0.1)	57.3 (1.1)
Sample P2	1.90 (0.1)	73.0 (0.6)
Sample 5H	1.89 (0.1)	1423 (1.6)

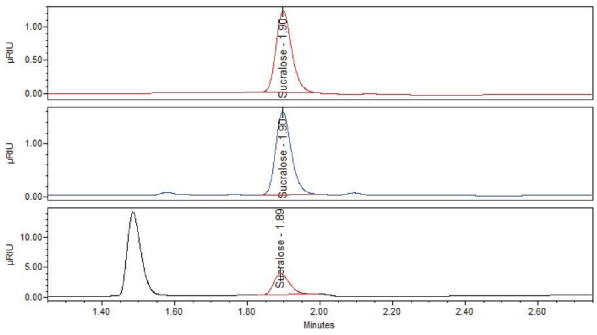


Figure 4. Results of quantitative analysis and representative chromatograms of sucralose beverages.

Conclusion

- When combined with the ACQUITY UPLC H-Class PLUS and ACQUITY RI Detector, the CORTECS T3 Column enabled efficient quantitation of sucralose using a simple isocratic method.
- The ACQUITY RI Detector has low dispersion that helps maintain a high degree of temperature stability, low baseline noise, and a wide linear dynamic range.
- The CORTECS T3 Column showed excellent reproducibility of the retention time and the backpressure.
- This method demonstrated excellent linearity, precision, and accuracy.
- This analytical method may be suitable for supporting manufacturers in standardizing the analyses for sucralose in beverages.

References

1. Jinchuan Yang, Paul D. Rainville. Analysis of Soft Drink Additives with No Interference from Aspartame Degradants Using Arc HPLC System with PDA Detection. Waters Application Note, 720007219, 2021.

Featured Products

ACQUITY UPLC H-Class PLUS System <https://www.waters.com/10138533>

ACQUITY UPLC Refractive Index Detector <https://www.waters.com/134726507>

Empower Chromatography Data System <https://www.waters.com/10190669>

720007622, May 2022