LC-MS/MS quantitation of artificial sweeteners in beverages

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This technical note describes a direct injection method for the analysis of 9 artificial sweeteners in beverage samples (advantame, saccharin, sucralose, aspartame, acesulfame K, stevioside, rebaudioside, neotame, and sorbitol). Using the SCIEX QTRAP 4500 system, the in-sample limits of quantitation (LOQ) ranged from 0.125 µg/mL for acesulfame K to 10 µg/mL for sucralose. Matrix spikes in a cola beverage (n=5) showed mean accuracies between 72% and 107% for the 1x LOQ spike, and between 91% and 114% for the 5x LOQ spike. The mean precision (%CV) was <13% for all analytes at both spiking levels. The sample preparation method consisted of a simple 500x dilution with diluent. The Phenomenex Synergi™ 2.5 µm Polar RP column provided good retention of the most polar analytes while achieving separation from the matrix interferences. Further, the method was applied to the analysis of 7 artificial sweetener-containing soft drinks (example shown in Figure 1). Six different artificial sweeteners were detected in the beverages with varying compositions and concentrations.

Key benefits for the analysis of sweeteners in beverages on the SCIEX QTRAP 4500 system

- **Method accuracy and precision in matrix spike samples.** Matrix spikes showed good accuracy (71.5–114%) and precision (%CV ≤13%) for all the compounds at 1x and 5x LOQ concentrations
- **Rapid sample preparation method.** A simple 500x dilution reduced sample preparation time, while maintaining sensitivity to analyze artificial sweeteners in commercial beverages
- **Method sensitivity.** Using the SCIEX QTRAP 4500 system, in-sample LOQs ranged from 0.125 µg/mL for acesulfame K to 10 µg/mL for sucralose
- **Good analyte peak shape and retention.** The Phenomenex Synergi Polar-RP column showed good peak shape, chromatographic retention, and retention reproducibility for the artificial sweeteners

Direct injection

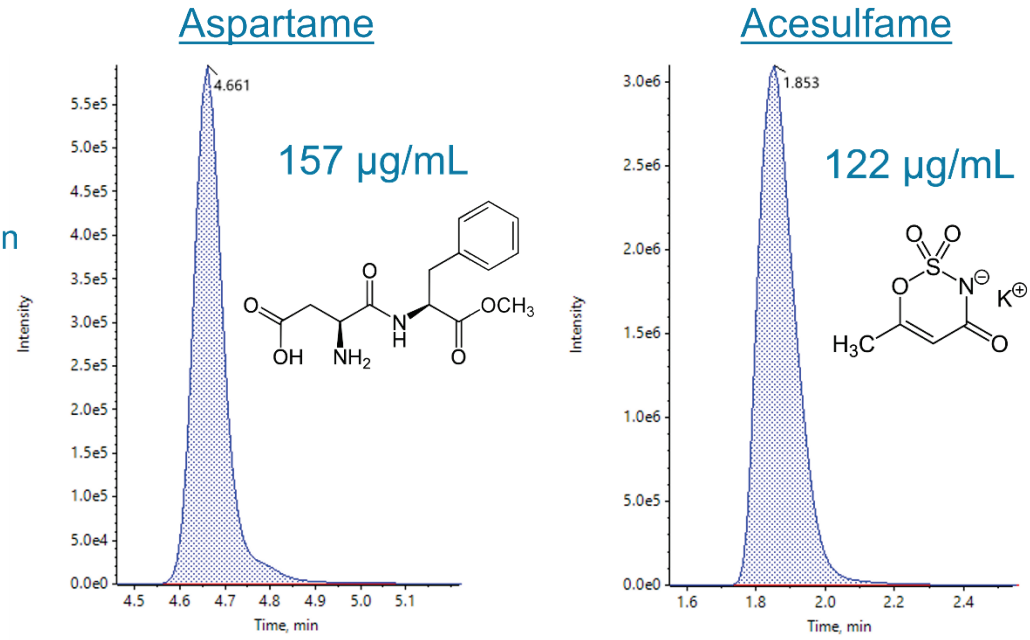

Figure 1. Artificial sweeteners were detected in beverage sample #2 using the SCIEX QTRAP 4500 system. Representative extracted ion chromatograms (XICs) are shown for the quantifier transition of aspartame and acesulfame K with their corresponding in-sample concentrations.

Introduction

Artificial sweeteners, also known as low- and no-calorie sweeteners (LNCS), are food additives widely used in food and beverages due to their high sweetness and low caloric content. Awareness of diseases linked to natural sugar consumption, such as type-2 diabetes, has increased the use of artificial sweeteners in our food. While many artificial sweeteners are approved for human consumption, the World Health Organization's International Agency for Research on Cancer (IARC) recently designated 1 artificial sweetener, aspartame, as "possibly carcinogenic to humans".[1] Accurate and robust analytical methods for the measurement of artificial sweeteners in beverages are necessary for the protection of human health. Methods must be applicable to the diversity of chemical structures in artificial sweeteners including small, polar compounds such as saccharin, and larger glycosides such as stevioside and rebaudioside.

Methods

Samples and reagents: Neat standards of the 9 artificial sweeteners were purchased from Sigma Aldrich. Analyte stock solutions of sucralose, aspartame, acesulfame K, stevioside, rebaudioside, and neotame were prepared using LC-MS grade water, while saccharin and advantame stock solutions were prepared using 50:50 (v/v) water/methanol. The calibration standards were prepared using the diluent, 95:5 (v/v) water/acetonitrile with 0.05% acetic acid.

Procedural recoveries in matrix spikes: Matrix spikes were performed using a cola-based beverage that did not list artificial sweeteners on the ingredient list. Method recovery was determined by performing matrix spike experiments at 1x and 5x the in-sample LOQ (n=5). In the case of sorbitol, background levels were detected in a broad survey of cola beverages, for example, approximately 12.5 µg/mL in the sample used for the matrix spikes. Therefore, sorbitol was spiked at 45 µg/mL which represented 3–5x the background level. Sample preparation was performed by transferring 1 mL of the beverage sample to a 3 mL centrifuge tube, spiking with the stock solutions, and vortexing for 2 min. Then, the sample was diluted 500x using the diluent (Table 2) and vortexed for an additional 2 minutes. The diluted samples were filtered through a Phenomenex CLARIFY-PVDF 0.22 µm syringe filter (P/N AF8-7706-12) prior to analysis. Analyte recovery was quantified against an external solvent calibration curve. The in-sample spiking levels for the 9 artificial sweetener analytes are shown in Table 1.

Table 1: Matrix spike concentrations for the nine artificial sweeteners in beverages

Analyte name	In-sample spike concentration (µg/mL)	
	1x LOQ	*5x LOQ*
Advantame	0.50	2.5
Saccharin	1	5.0
Sucralose	10	50
Aspartame	0.25	1.25
Acesulfame K	0.125	0.625
Stevioside	5	25
Rebaudioside	5	25
Neotame	0.25	1.25
	Sorbitol, 3-5x background levels	
Sorbitol[1]	45	

[1] Sorbitol had high background levels in the unspiked samples and therefore only one spiking level was performed at 45 µg/mL.

Analysis of commercial beverages. The method was applied to 7 commercially available beverages that listed at least one artificial sweetener on the ingredient list. Sample preparation followed the method above without the additional of the spiking stocks.

Chromatography: An ExionLC AD system was used with the Phenomenex Synergi™ 2.5 µm Polar RP column for chromatographic separation (100 x 3.0 mm, P/N 00D-4371-Y0). The mobile phases were 10mM ammonium acetate in water and acetonitrile. The flow rate was 0.400 mL/min and the gradient conditions are shown in Table 2. The injection volume was 10 µL and the column oven temperature was 40°C. The autosampler temperature was set to 15°C, and 0.5 mL of rinsing solution was used for the needle washing.

Mass spectrometry: Samples were analyzed on the SCIEX QTRAP 4500 system operated with electrospray ionization mode. Data were acquired using multiple reaction monitoring (MRM) with polarity switching. Optimized source and compound-specific parameters are presented in Tables 3 and 4, respectively. Two selective MRM transitions were monitored (Table 4). Confirmation of the targeted analytes was based on the ion ratio.

Table 2: Chromatographic gradient for the analysis of sweeteners.

Time (Min)	Flow rate (ml/min)	Mobile phase A (%)	Mobile phase B (%)
0.0	0.400	98	2
1.25	0.400	98	2
8.0	0.400	2	98
10.0	0.400	2	98
10.1	0.600	90	10
14.0	0.600	90	10
14.1	0.400	98	2
18.0	0.400	98	2

Rinsing solution: 50:50 (v/v), water/acetonitrile
Diluent: 95:5 (v/v) water acetonitrile with 0.05% acetic acid

Table 3: Source and gas parameters for the analysis of artificial sweeteners using the SCIEX QTRAP 4500 system.

Parameter	Value
Polarity	Positive/ negative
Ion spray voltage	5000/ -4500 V
Curtain gas	35 psi
CAD gas	8
Source temperature	550°C
Ion source gas 1	50 psi
Ion source gas 2	55 psi

Table 4: Compound-specific MRM parameters for the analysis of artificial sweeteners using the SCIEX QTRAP 4500 system.

ID	Q1 (Da)	Q3 (Da)	DP (V)	CE (V)	CXP (V)
Advantame_01	459.2	84.0	96	64	13
Advantame_02	459.2	102.0	96	46	10
Saccharin_01	181.9	106.0	-40	-25	-12
Saccharin_02	181.9	42.0	-40	-47	-10
Sucralose_01	395.0	359.0	-64	-13	-13
Aspartame_01	293.0	200.0	-69	-20	-13
Aspartame_02	293.0	145.9	-69	-23	-12
Acesulfame K_01	162.0	82.0	-31	-19	-13
Acesulfame K_02	162.0	78.0	-31	-48	-7
Stevioside_01	803.3	641.2	-139	-61	-9
Rebaudioside_01	965.4	803.3	-141	-40	-12
Rebaudioside_02	965.4	641.3	-141	-84	-17
Neotame_01	377.2	200.0	-79	-25	-13
Neotame_02	377.2	345.2	-79	-19	-15
Sorbitol_01	181.0	89.0	-64	-20	-5
Sorbitol_02	181.0	84.9	-64	-22	-5

Compound ID_01 = quantifier transition
Compound ID_02 = qualifier transition

Data processing: All data were processed using the SCIEX OS software (version 2.1.6). Peaks were automatically integrated using the MQ4 algorithm and a weighting of $1/x^2$ was used for quantitation.

Chromatographic separation of the 9 sweeteners using an 18-minute gradient

The chromatographic conditions were extensively optimized to achieve good retention and void volume separation for the 9 artificial sweetener analytes (Figure 2). A linear 18-min gradient (Table 2) was developed to separate the diverse analytes and avoid co-elution with matrix interferences. During the method development, various mobile phases and columns were tested. Good retention and peak shape were achieved using the Phenomenex Polar-RP column and mobile phases comprised of water modified with ammonium acetate and acetonitrile. The most polar analytes (sorbitol and acesulfame K) eluted after the void volume as shown through the retention factor (k') of 0.05 for sorbitol and 0.53 for acesulfame K. Adequate separation from the void volume reduced the potential impact from unretained interferences.

Sensitivity, precision, and linear dynamic range of the solvent-based calibration standards

The solvent-based calibration curve was plotted using triplicate injections for each standard level, and a good linear dynamic range was shown with r^2 values >0.99 (Table 5). The calibration curves of neotame and aspartame are shown in Figure 3. The mean accuracies were between 84.1% and 108% when considering all analytes across the entire calibration range (Table 5). Good sensitivity was achieved on the SCIEX QTRAP 4500 system and the in-vial LOQs ranged from 0.25 ng/mL to 20 ng/mL in the solvent-based standards. The selection criteria for LOQ were based on the following passing for both the quantifier and qualifier transitions: S/N ratio >10, accuracy ±30% and precision < 15%, and ion ratio tolerance ± 30%. At the LOQ concentration, the mean accuracies ranged from 94.7% to 102%, and the mean %CV ranged from 1.7% to 10% (Table 5).

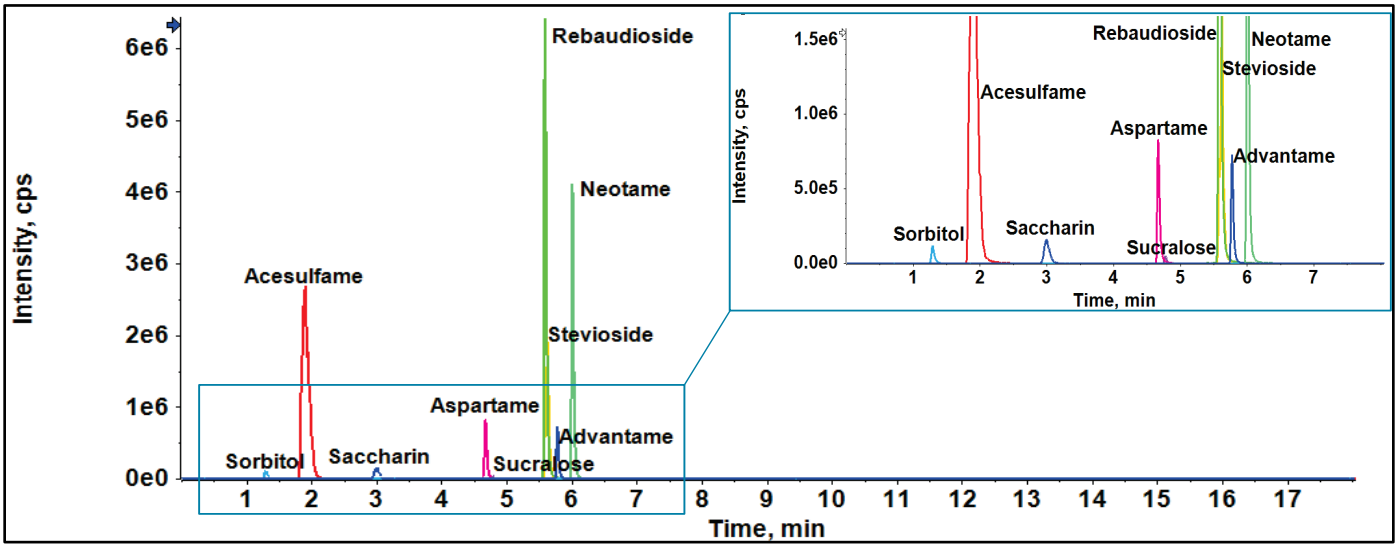

Figure 2: XICs of 9 artificial sweeteners at 250 ng/mL for 7 compounds and 2500 ng/mL for stevioside and rebaudioside in the solvent-based standards. Good chromatographic separation and separation from void volume retention were achieved using the Phenomenex Polar-RP column.

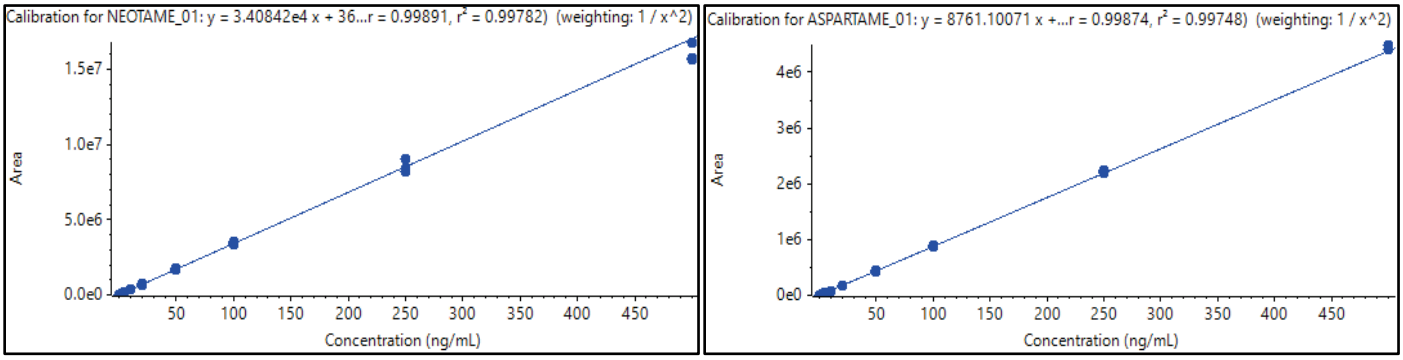

Figure 3. Representative calibration curves for neotame (377.2 / 200.0) and aspartame (293.0 / 200.0) from the quantifier transition. A linear curve across the range of 0.5–500 ng/mL with an r^2-value of > 0.99 was achieved using a weighing factor of $1/x^2$.

Table 5. Sensitivity, correlation coefficient (r^2), accuracy and precision of the solvent-based calibration standards (n=3). Accuracy values are shown for the full calibration range and the LOQ level, whereas precision (%CV) is shown for LOQ only. Accuracy and precision values are given for the quantifier transition.

Compound	Linear range (ng/mL)	LOQ [1] ng/mL	Correlation coefficient (r^2)	Accuracy (%) across full calibration range	Mean LOQ % accuracy (n=3)	Mean LOQ %CV (n=3)
Advantame	1.0 - 500	1.0	0.997	95.2–107	101	5.9
Saccharin	2.0 – 500	2.0	0.995	89.1–107	95.9	4.4
Sucralose	20 - 2500	20	0.990	88.8–107	96.6	4.8
Aspartame	0.5 - 500	0.5	0.997	96.4–103	103	10
Acesulfame K	0.25 - 250	0.25	0.992	85.4–108	94.7	9.8
Stevioside	10 - 5000	10	0.997	94.1–105	100	5.7
Rebaudioside	10 - 5000	10	0.995	84.1–106	97.7	1.7
Neotame	0.5 - 500	0.5	0.998	94.3–102	100	6.5
Sorbitol	20 - 5000	20	0.998	96.1–106	97.3	2.1

[1] LOQ values were selected based on 2 selective MRM transitions, S/N ratio >10 for quantifier and qualifier of calibration standard, accuracy within ± 30%, % CV <15%, and ion ratio tolerance within ± 30%.

Quantitative performance in matrix spiked soft drink samples

Commercial soft drinks were purchased to evaluate potential matrix candidates for the method development experiments. Although the labels did not indicate the presence of artificial sweeteners, the samples were initially screened to determine potential background levels. All beverages were found to be free of artificial sweeteners except for sorbitol. Ultimately, one cola-based beverage was selected for the matrix spikes. Matrix spikes were performed at 1x and 5x the LOQ for all sweeteners except for sorbitol. Sorbitol was detected in all beverage samples at approximately 12.5 µg/mL. Therefore, sorbitol was spiked at 45 µg/mL which represented 3–5x of the background level.

The sample preparation consisted of a simple 500x dilution. The relatively large dilution factor minimized the matrix effects, while still maintaining adequate sensitivity to quantify artificial sweeteners levels in commercial beverages.

The in-sample matrix spiking concentrations are shown in Table 1. The matrix spike samples (n=5) were quantified against the external solvent calibration curve. Mean accuracies ranged from 71.5% to 107% for the 1x LOQ spike, and from 90.6% to 114% for the 5x LOQ spike (Table 6). The mean precision (%CV) was <13% for all analytes at both spiking levels. The XICs for the 1x LOQ matrix spikes are shown in Figure 4.

Table 6. Mean accuracy and precision of the 1x and 5x LOQ matrix spike samples (n=5). Values are shown for the quantifier ion transition.

Compound	1x LOQ matrix spikes			5x LOQ matrix spikes		
	1x LOQ (µg/mL)	Mean accuracy (%)	Mean precision (%CV)	5x LOQ (µg/mL)	Mean accuracy (%)	Mean precision (%CV)
Advantame	0.5	105	13	2.5	109	8.9
Saccharin	1.0	107	7.0	5.0	114	2.6
Sucralose	10	105	11	50	107	3.2
Aspartame	0.25	88.5	13	1.25	95.9	6.6
Acesulfame K	0.125	93.0	7.2	0.625	108	4.0
Stevioside	5	103	7.7	25	99.7	6.0
Rebaudioside	5	106	3.1	25	107	6.8
Neotame	0.25	71.5	9.1	1.25	90.6	7.3
Sorbitol matrix spikes, 3-5x background levels						
Sorbitol[1]	45	71.1	3.1	-	-	-

[1] *The sorbitol spiking level was 3-5x greater than the background concentration (45 µg/mL).*

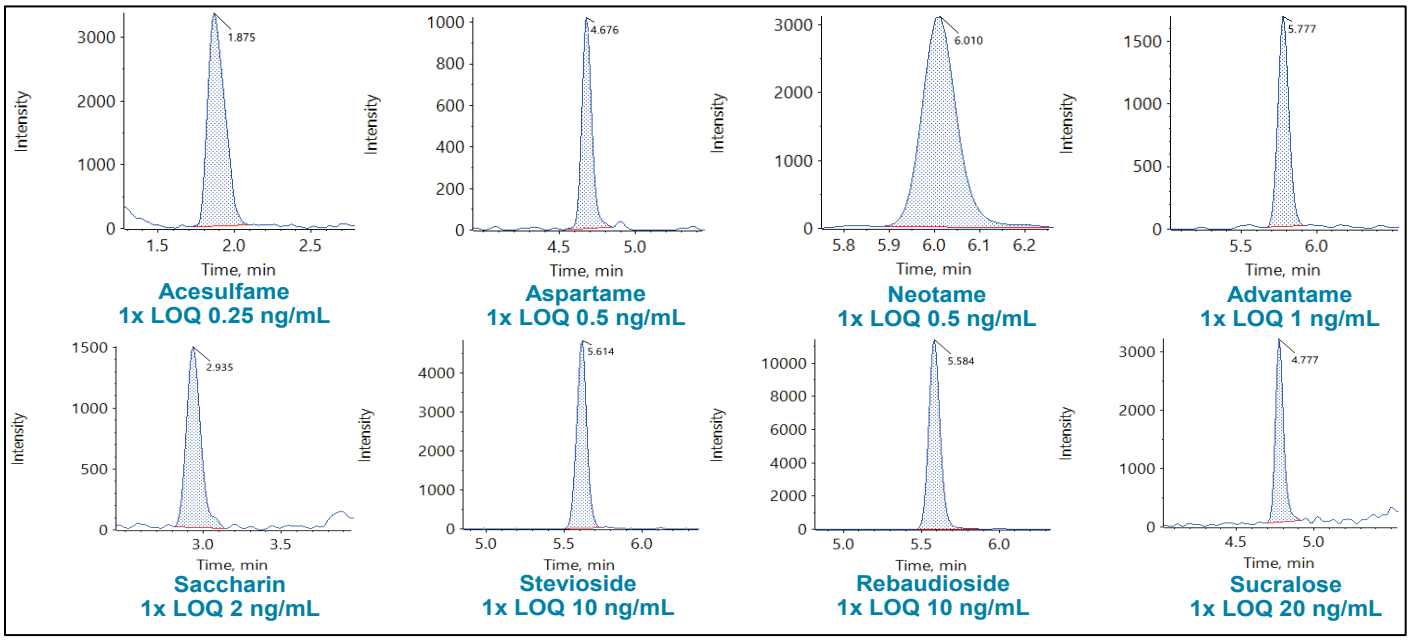

Figure 4. Representative XICs of 8 sweeteners at 1x LOQ spike in a soft drink sample. Chromatograms are shown from the quantifier transition for all XICs.

Quantitative performance in beverage samples

Seven artificial sweetener-containing beverages were purchased and analyzed to demonstrate the method's applicability. Samples were extracted using the developed method and injected in triplicate. The mean concentrations for the artificial sweeteners detected are presented in Table 7, XICs for sample #2 are shown in Figure 1. This brief survey of cola-based drinks shows the diversity of artificial sweeteners used in commercial beverages.

Further, it shows the broad range of artificial sweetener concentrations, ranging from 1.6 µg/mL for stevioside in sample #2 to 180 µg/mL for sucralose in sample #3. Overall, these results demonstrate the method's capability for analyzing artificial sweeteners in beverage samples using the SCIEX QTRAP 4500 system.

Table 7. Mean concentrations (µg/mL) of artificial sweeteners detected in different soft drinks samples (n=3).

Compound	LOQ (µg/mL)	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6	Sample #7
Sucralose	10	nd	167	180	151	nd	nd	nd
Aspartame	0.25	157	nd	nd	nd	nd	nd	nd
Acesulfame K	0.125	122	40	89	nd	nd	nd	nd
Stevioside	5	nd	1.6	nd	20	9.3	13	10
Rebaudioside	5	nd	1.4	nd	75	32	48	37
Sorbitol	10	nd	<LOQ	nd	nd	16	<LOQ	15

Conclusions

In this technical note, a method was developed to analyze 9 artificial sweeteners in beverages using the SCIEX QTRAP 4500 system. This technical note demonstrated:

- Optimal chromatographic peak shape and separation from the void volume for the diverse analyte panel using the Phenomenex Synergi™ Polar RP column
- The 4500 QTRAP system sensitivity allowed for a simplified sample preparation, consisting of a 500x dilution only, to reduce matrix effects and matrix interferences
- Matrix spike accuracies ranged from 72% to 114% with %CV<13% for all compounds at the 1x and 5x LOQ levels
- Method applicability through the analysis of artificial sweeteners in 7 beverage products

References

1. Aspartame hazard and risk assessment results released. World Health Organization, 14 July 2023.

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