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Highly Sensitive and Robust LC/MS/MS Method for Quantitative Analysis of Artificial Sweeteners in Beverages

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Introduction

Artificial sweeteners described as intense, low-calorie and non-nutritive are widely used as sugar substitutes in beverages and foods to satisfy consumers' desire to sweet taste while concerning about obesity and diabetes. As synthetic additives in food, the use of artificial sweeteners must be approved by authority for health and safety concerns. For example, Aspartame, Acesulfame-K, Saccharin, Sucralose and Neotame are the FDA approved artificial sweeteners on the US market. However, there are also many other artificial sweeteners allowed to use in EU and many other countries (Table 2), but not in the US. In this regard, analysis of artificial sweeteners in beverages and foods has become essential due to the relevant regulations in protection of consumers' benefits and safety concerns in many countries [1, 2]. Recently, artificial sweeteners are found as emerging environmental contaminants in surface water and waste water [3]. Initially, HPLC analysis method with ELSD detection was adopted, because many artificial sweeteners are non-UV absorption compounds [2]. Recently, LC/MS/MS methods have been developed and used for identification and quantitation of artificial sweeteners in food and beverages as well as water for its high sensitivity and selectivity [3, 4]. Here we report a high sensitivity LC/MS/MS method for identification and quantitation of ten artificial sweeteners (Table 2) in beverage samples. An ultra-small injection volume was adopted in this study to develop a very robust LC/MS/MS method suitable for direct injection of beverage samples without any sample pre-treatment except dilution with solvent.

Experimental

Ten artificial sweeteners of high purity as listed in Table 2 were obtained from chemicals suppliers. Stock standard solutions and a set of calibrants were prepared from the chemicals with methanol/water (50/50) solvent as the diluent. Three brand soft-drinks and a mouthwash bought from local supermarket were used as testing samples in this study. The samples were not pretreated by any means except dilution with the diluent prior to injection into LCMS-8040 (Shimadzu Corporation, Japan), a triple quadrupole LC/MS/MS system. The front-end LC system connected to the LCMS-8040 is a high pressure binary gradient Nexera UHPLC. The details of analytical conditions of LC/MS/MS method are shown in Table 1.

Table 1: LC/MC/MS analytical conditions of artificial sweeteners on LCMS-8040

Column	Synergi, Polar-RP C18 (100 x 2 mm, 2.5µm)
Flow Rate	0.25 mL/min
Mobile Phase	A: water with 0.1% Formic acid - 0.03% TA B: MeOH with 0.1% FA - 0.03% Trimethylamine
Gradient program	B: 10% (0.01 to 0.5 min) → 95% (8 to 9 min) → 10% (9.01 to 11min)
MS mode	ESI, MRM, positive-negative switching
ESI condition	Nebulizing gas: 3L/min, Drying gas: 15L/min, Heating block: 400°C, DL: 250°C
Inj. Vol.	0.1uL, 0.5uL, 1uL, 5uL and 10uL

Results and Discussion

Method development

First, precursor selection and MRM optimization of the ten sweeteners studied was carried out using an automated MRM optimization program of the LabSolutions. Six compounds were ionized in negative mode and four in positive mode as shown in Table2. For each compound, two optimized MRM transitions were selected and used, with the first one for quantitation and the second one for confirmation.

The ten compounds were well-separated as sharp peaks between 2 min and 8.2 min as shown in Figure 1. Linear calibration curves of wide concentration ranges were established with mixed standards in diluent as summarized in Table 2. We also investigated the performance of the LC/MS/MS method established by employing very small injection volumes (0.1, 0.5, 1 and 5 uL). This is because actual beverages usually contain very high contents of sweeteners (>>1ppm) to MS detection. Analysts normally dilute the samples before injection into LC/MS/MS. An alternative way is to inject a very small volume of samples even without dilution. Figs 2 & 3 show a chromatogram and calibration curves established with 0.1uL injection, which demonstrates the feasibility of an ultra-small injection volume combined with high sensitivity LC/MS/MS.

Table 2: Artificial Sweeteners, MRM transitions and calibration curves on LCMS-8040

Cat[1]	Compd. & Abbr. Name	MRM parameter					RT & Calibration Curve[4]		
		Trans. (m/z)	Pola. (+/-)	Q1 (V)	CE (V)	Q3 (V)	RT (min)	Conc. R. (ug/L)	R^2
A2	Acesulfame K (Ace-K)	161.9 >82.1	-	11	14	29	1.99	1 - 20000	0.9999
		161.9 >78.0	-	11	32	28			
A5	Cyclamate (CYC)[3]	178.3 >80.1	-	19	24	30	2.87	5 - 20000	0.9996
		178.3 >79.0	-	12	27	10			
A3	Saccharin (SAC)	181.9 >106.1	-	13	20	15	3.28	1 - 20000	0.9984
		181.9 >42.1	-	13	36	13			
A4	Sucralose[2] (SUC)	441.0 >395.1	-	20	11	25	4.61	5 - 20000	0.9983
		441.0 >359.1	-	20	15	23			
A1	Aspartame (ASP)	295.1 >120.1	+	-19	-25	-25	5.15	0.1 - 2000	0.9999
		295.1 >180.1	+	-19	-14	-20			
A6	Neotame (NEO)	379.3 >172.2	+	-18	-23	-20	7.51	0.05 - 1000	0.9998
		379.3 >319.3	+	-18	-18	-24			
B1	Alitame (ALI)	332.2 >129.1	+	-23	-19	-26	5.44	0.1 - 2000	0.9995
		332.2 >187.1	+	-23	-16	-21			
B3	Dulcin (DUL)	181.1 >108.1	+	-22	-25	-21	5.58	5 - 10000	0.999
		181.1 >136.1	+	-21	-18	-26			
B2	Neohespiridin Dihydrochalcone (NHDC)	611.3 >303.1	-	30	38	30	6.71	0.5 - 2000	0.9988
		611.3 >125.3	-	30	47	20			
C1	Glycyrrhi-Zinate (GLY)	821.5 >351.2	-	22	46	20	8.19	5 - 1000	0.9996
		821.5 >193.2	-	22	52	19			

1. *A1~A6: US FDA, EU and others approval; B1~B3: only EU and other countries approval. C1: natural sweetener, info not available.*
2. *Sucralose precursor ion m/z 441.0 is formic acid adduct ion.*
3. *Sodium cyclamate known as "magic sugar" was initially banned in the US in 2000. FDA lifted the ban in 2013.*
4. *Injection volume: 10 uL*

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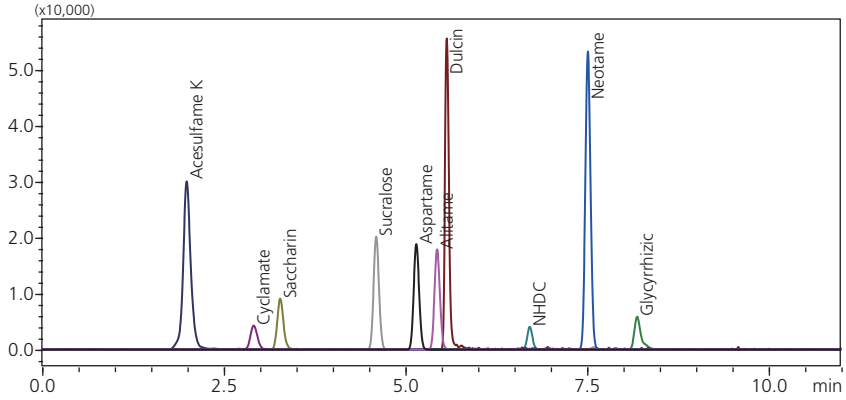

Figure 1: MRM Chromatogram of ten sweeteners by LC/MS/MS with 10uL injection:
Asp & Ali 1ppb, Neo 0.5ppb, Dul, Gly, Ace-K, Sac, Suc and Cyc 10ppb, NHDC 1ppb.

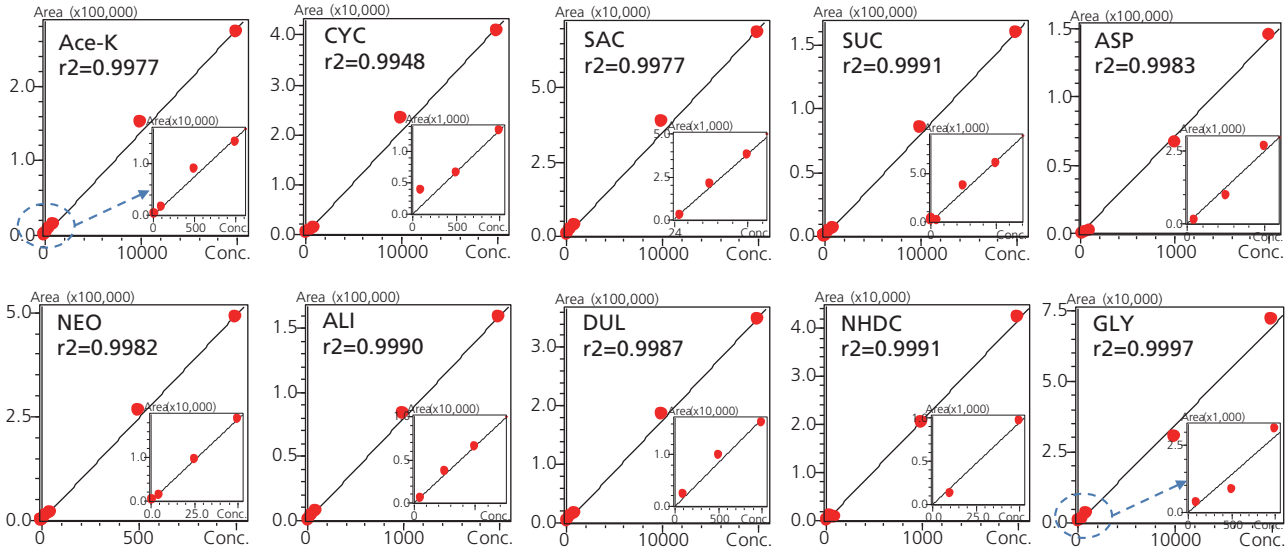

Figure 3: Calibration curves of artificial sweeteners on LCMS-8040 with
an ultra-small injection volume (0.1 uL) of same set of calibrants as shown in Table 2.

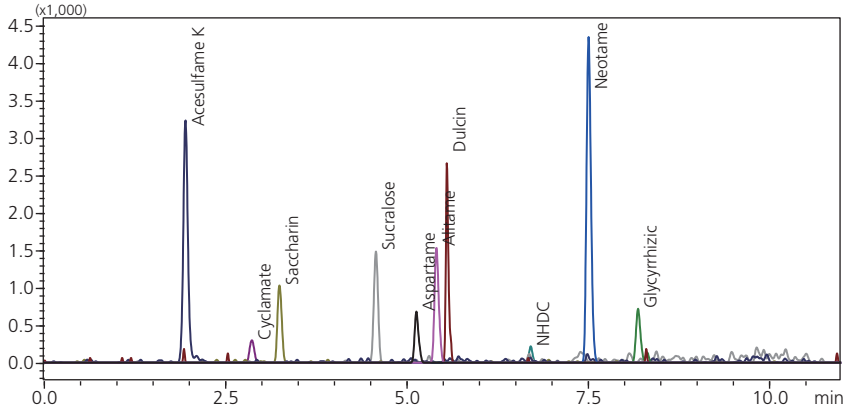

Figure 2: MRM Chromatogram of ten sweeteners by LC/MS/MS with 0.1uL injection:
Asp & Ali 0.1ppm, Neo 0.05ppm, Dul, Gly, Ace-K, Sac, Suc and Cyc 1ppm, NHDC 0.1ppm.

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Method performance

Table 3 summarizes the results of repeatability and sensitivity of the method with mixed standards. The method was not evaluated with beverage spiked samples. However, because beverage samples are normally diluted many times, matrix effect and interferences can be ignored for high sensitivity LC/MS/MS analysis. The results indicate that the method with ultra-small injection volume exhibits good linearity, repeatability and sensitivity.

Table 3: Repeatability and Sensitivity of LC/MS/MS method of artificial sweeteners

Name	Repeatability (peak area), 10uL				Sensitivity (ug/L)					
	Conc. (ug/L)	RSD%	Conc. (ug/L)	RSD%	LOQ/LOD (0.1 µL inj)		LOQ/LOD (0.5 µL inj)		LOQ/LOD 10 (µL inj)	
Ace-K	20	5.1	100	5.2	200	50	40	10	4.0	1.33
CYC	20	11.7	100	8.1	800	500	200	90	14	4.5
SAC	20	8.0	100	5.8	250	100	50	20	4.5	1.5
SUC	20	7.5	100	2.7	200	100	50	15	2.4	0.8
ASP	2	7.8	10	3.0	80	20	20	4	0.5	0.17
NEO	1	5.3	5	1.0	5	3	2	1	0.03	N.A.
ALI	2	8.6	10	1.7	40	25	10	5	0.2	N.A.
DUL	20	7.5	100	3.1	160	50	30	10	1.4	0.5
NHDC	2	9.2	10	4.6	100	25	40	6	0.5	0.18
GLY	20	8.2	100	5.4	400	150	15	5	5.0	1.8

Analysis of beverage samples

The LC/MS/MS method established was applied for screening and quantitation of the targeted sweeteners in three brand beverages: S1, S2 and S3, and a mouthwash S4. The results are shown in Figure 4 and Table 4. It is interested to note that glycyrrizinate was found in the mouthwash.

Table 4: Screening and quantitation results for ten artificial sweeteners in beverages and mouthwash (mg/L)

Artificial Sweetener	S1	S2	S3	S4
ASP	116.9	127.9	ND	ND
Ace-K	143.9	165.9	97.2	ND
Saccharin	ND	ND	ND	208.7
SUC	55.1	ND	183.4	ND
GLY	ND	ND	ND	449.3
Others	ND	ND	ND	ND

1. *S2 was diluted 100 times, the rests were diluted 10 times. 1 uL injection.*
2. *ND = not detected.*

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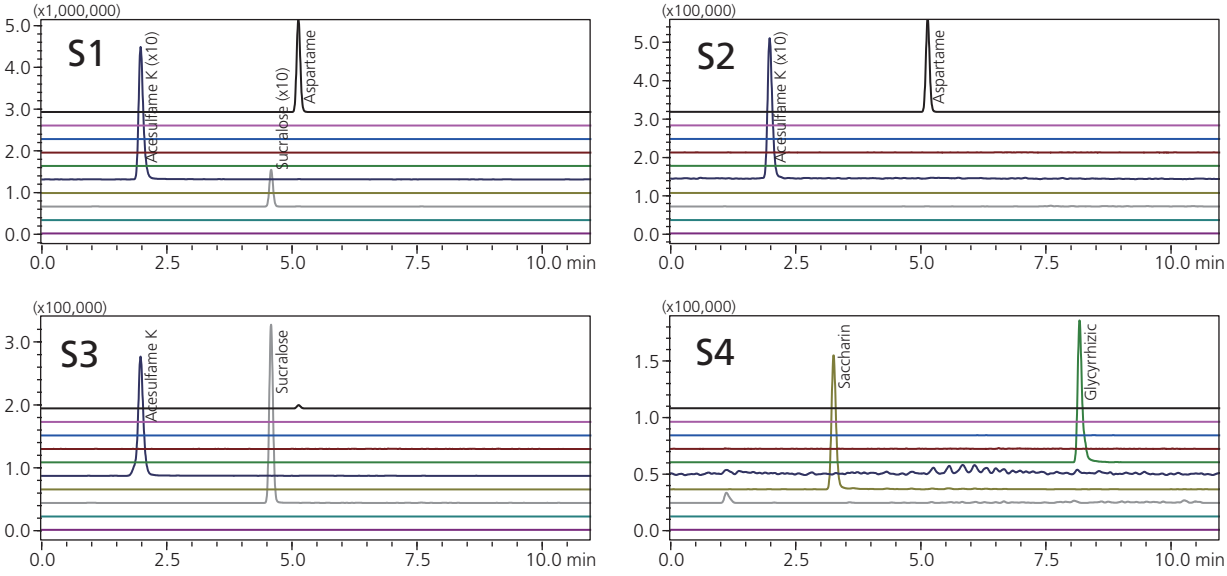

Figure 4: Screening and quantitation for 10 targeted artificial sweeteners in beverage and mouthwash samples by LC/MS/MS with 1uL injection.

Conclusions

A MRM-based LC/MS/MS method was developed and evaluated for screening and quantitation of ten artificial sweeteners in beverages. This high sensitivity LC/MS/MS method combined with small or ultra-small injection volume (0.1~1.0 uL) was proven to be feasible and reliable in actual samples analysis of the targeted sweeteners in beverages, achieving high throughput and free of sample pre-treatment (except dilution). The method is expected to be applicable to surface water and drinking water samples. For wastewater and various foods, sample pre-treatment is usually required. However, the advantages of the method in high sensitivity and ultra-small injection volume are expected to enable it tolerates relatively simple sample pre-treatment procedures.

References

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