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Application News

No. AD-0126

Water Analysis / LCMS-8060

Quantitative Analysis of Residual Artificial Sweeteners in Surface Water by Highly Sensitive LC/MS/MS Method

❑ Introduction

Artificial sweeteners, being low caloric sugar substitutes, are widely used in food and beverages for decades. Researches on environmental occurrence and eco-toxicological effects of artificial sweeteners have increased in recent years [1]. Artificial sweeteners are found in surface waters and wastewaters at levels from ng/L to µg/L [1-3], which are classified as new emerging environmental contaminants. The artificial sweeteners which have been studied most in this field are acesulfame (ACE), cyclamate (CYC), saccharin (SAC) and sucralose (SUC) [1-4]. LC/MS/MS techniques have been used in these studies. However, pre-concentration of water sample is often required because of the needs for detection of trace levels of the compounds. A LC/MS/MS method was developed previously on LCMS-8040 for identification and quantification of ten artificial sweeteners in beverages [5]. In this Application News, a new LC/MS/MS method on LCMS-8060 is described, aiming at achieving ultra high sensitivity for direct quantitation of above-mentioned five artificial sweeteners in surface waters and drinking waters.

❑ Experimental

Analytical conditions

A high sensitivity triple quadrupole system LCMS-8060, which is coupled with a Nexera UHPLC system, was employed in this study. A biphenyl column obtained from Phenomenex was used, which is described with features of enhanced selectivity and aqueous stability. A gradient elution program was developed for the five artificial sweeteners. The details of UHPLC and MS/MS conditions are compiled into Table 1.

Preparation of standards and samples

Stock solutions of the five artificial sweetener standards were prepared from powder chemicals in pure methanol. A mixed standard was prepared from the stocks and was diluted in series using Milli-Q water to 1, 5, 20, 50, 100, 250, 500 and 1000 ng/L as calibrants. The testing samples were obtained from a third-party laboratory, including treated water and local reservoir sampling water samples and a few spiked samples as controls. All the waters samples were injected to LC/MS/MS without any pre-treatment or enrichment.

Table 1: Analytical conditions of artificial sweeteners on LCMS-8060

Column	Kinetex 2.6µm Biphenyl 100Å (100 mm L. x 2.10mm I.D.)
Mobile Phase	A: Water B: Methanol
Elution Program	Gradient elution, 1%B (0.0-0.5 min), 30%B (1.5-2.0 min), 80%B (3.5-4.5 min), 1%B (4.6-6.0 min)
Flow Rate	0.3 mL/min
Oven Temp.	40 ºC
Injection	10 µL
Interface	ESI Heated by heating gas
MS Mode	MRM, Positive and Negative mode
Block Temp.	500 ºC
DL Temp.	300 ºC
Interface Temp.	400 ºC
Nebulizing gas	N_2, 3 L/min
Drying gas	N_2, 0 L/min
Heating Gas	Zero air, 20 L/min

❑ Results and Discussion

A. High sensitivity MRM method for artificial sweeteners

The MRM transitions of the five artificial sweeteners optimized on LCMS-8060 are shown in Table 2. Acesulfame (ACE), cyclamate (CYC) and saccharin (SAC) are ionized in negative ESI/MRM mode, which is in accordance with that was reported in literatures [2-3]. For sucralose (SUC) and aspartame (ASP), positive ESI/MRM mode was used, because it gave better sensitivity and fragmentation spectrum. It was also reported by Noora Perkola et al. [4] that the positive MRM of sucralose is more sensitive than that of negative ESI/MRM mode. The parent ion of sucralose (SUC) in positive mode is sodium adduct ion m/z419.1, which produces two main product ions of m/z239 and m/z221.

With a fast gradient program, the five artificial sweeteners are eluted as sharp peaks as illustrated in Figure 1. Linear calibration curves were established based on quantifying MRMs of the five artificial sweeteners. The calibration curves and performance information of the quantitation method are shown in Figure 1 and Table 2.

Table 2: Summary of MRM transitions, retentions, calibration and sensitivity for five artificial sweeteners on LCMS-8060 with 10 µL injection

Compound (Abbr.)	Chemical Formula	MRM Transitions & Parameter				RT, Calibration and Sensitivity					
		Precursor (m/z)	Product (m/z)	CE (V)	Relative Intensity	RT (min)	Range (ng/L)	Linearity R^2	LOD (ng/L)	LOQ (ng/L)	%RSD (n=3)
Acesulfame (ACE)	$C_4H_5NO_4S$	162.1	82.1	16	100	0.86	5 – 1000	0.9994	0.27	0.82	2.6
			78.0	32	29						
Cyclamate (CYC)	$C_6H_{13}NO_3S$	178.1	79.9	26	100	1.03	5 – 1000	0.9968	1.1	3.4	4.7
			95.6	20	8						
Saccharin (SAC)	$C_7H_5NO_3S$	182.0	105.8	18	100	1.66	5 – 1000	0.9978	2.5	7.5	5.1
			41.9	24	112						
Sucralose (SUC)	$C_{12}H_{19}Cl_3O_8$	419.1	239.0	-21	100	3.56	20 – 1000	0.9986	30.6	92.7	9.2
			220.9	-21	90						
Aspartame (ASP)	$C_{14}H_{18}N_2O_2$	295.1	120.0	-27	100	4.12	1 – 1000	0.9995	0.99	3.0	1.7
			180.0	-15	41						

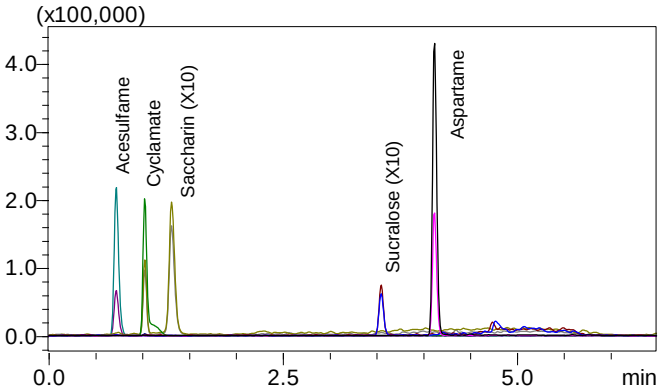

Figure 1: MRM chromatogram of five artificial sweeteners of a mixed standard of 500 ng/L on LCMS-8060 with 10 µL injection.

B. Analyses of treated water and reservoir water

The MRM method established was applied for screening and quantitation of the targeted artificial sweeteners in treated water (S1) and reservoir water (S2) samples. The samples were analyzed with an injection of 10 µL and without any pre-treatment or sample enrichment. In addition, two spiked diluents (Milli-Q water) at low and high concentrations as controls (C/L and C/H) prepared by a third-party laboratory were also analyzed with the method. The analysis results are shown in Table 3.

Table 3: Results of five artificial sweeteners in water samples* by direct MRM method on LCMS-8060.

Compd	RT	C (L)	C (H)	S1	S2
ACE	0.77	20	130	2.1	12
CYC	1.03	21	74	65	52
SAC	1.16	69	149	32	89
SUC	3.06	36	153	N.D.	34
ASP	4.12	3	92	N.D.	N.D.

** S1: Treated water, S2: Reservoir water, N.D.: Not Detected*

The LODs of the method for the target artificial sweeteners are better than 2.5 ng/L, except for sucralose (SUC). The individual MRM peaks of mixed standards and reservoir sample (S1) are displayed in Figure 3. It can be seen that the peak intensity of SUC is relatively low in comparison with the other four compounds. The analysis results were based on an injection volume of 10 µL. With an increased injection volume of 30 µL, the sensitivity of the method is expected to increase to be able to achieve a LOD of 10 ng/L for SUC.

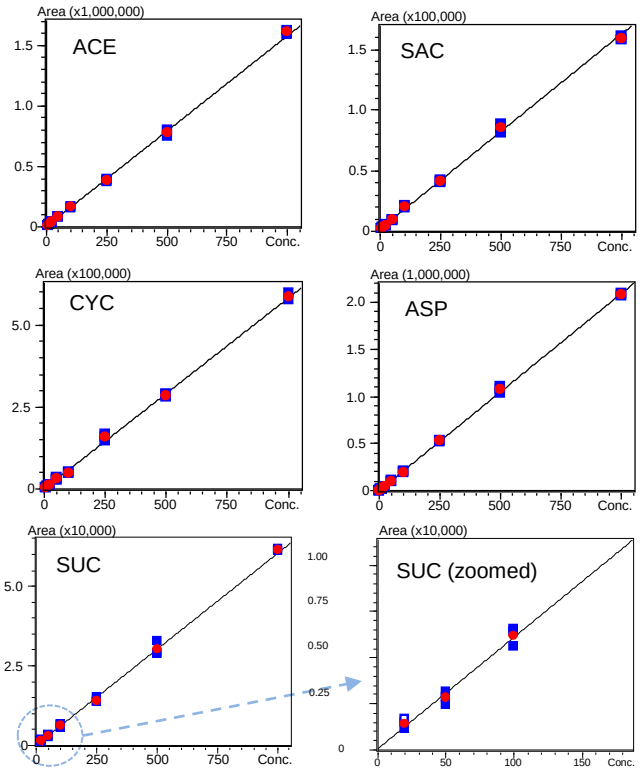

Figure 2: Calibration curves of five artificial sweetener standards in MRM mode on LCMS-8060 with 10 µL injection.

❑ Conclusions

A LC/MS/MS method with fast gradient elution of 6 minutes was established and applied in detection and quantitation of five artificial sweeteners, acesulfame (ACE), cyclamate (CYC), saccharin (SAC), sucralose (SUC) and aspartame (ASP), in surface water samples. Without any sample pre-treatment or enrichment, the LODs of the method have achieved the level of low ng/L. This results indicate the possibility to apply the method for direct analysis of the target artificial sweeteners in surface waters and drinking waters without the need of sample enrichment.

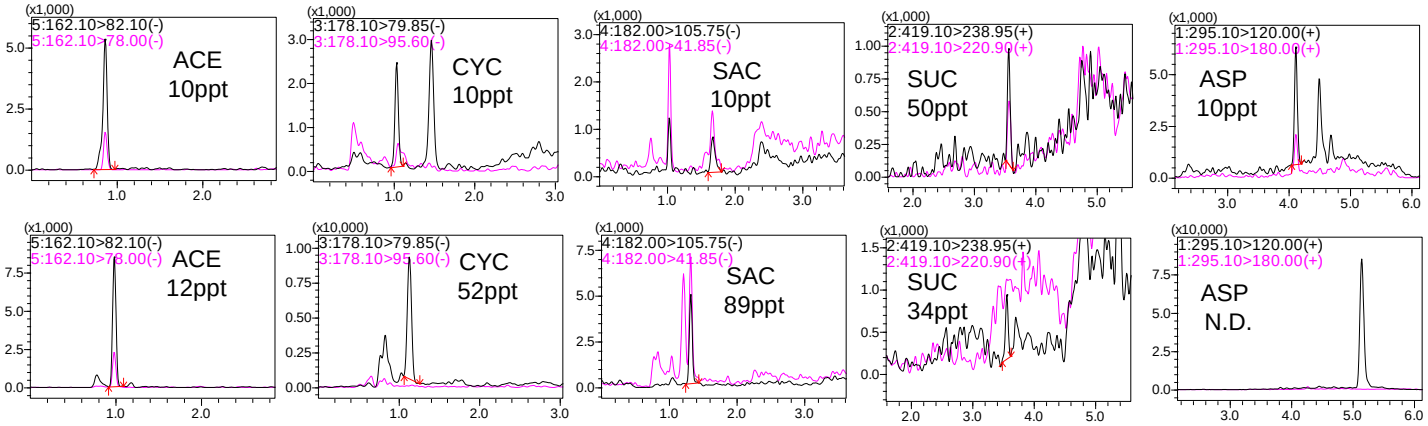

Figure 3: Individual MRM chromatograms of five artificial sweeteners on LCMS-8060 with Injection volume of 10 µL. Top: standards in Milli-Q water; Bottom: reservoir water sample.

❑ References

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