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Application News

No. AD-0052

LCMS-2020

Quantitative Analysis of Sweeteners in Beverages and Mouth Rinse Using Ligand Exchange Chromatography-MS with APCI Interface and Post-column Reagent Addition

As sugar replacers and food additives, sugar alcohols and artificial sweeteners have been used widely and also thoroughly assessed for health and safety concerns. Analytical methods of high sensitivity and selectivity are in demand for quantitative analysis of sweeteners in foods and beverages in research laboratory and industry. Here, we introduce a novel LC/MS method using ligand exchange chromatography and single quadrupole mass spectrometer with APCI interface for separation and detection of a total of nine sugar alcohols and artificial sweeteners with pure water as the mobile phase. It is known that sugar molecules are not easily ionized on LCMS interface and the pure water mobile phase is not a favourite condition for ionization. Therefore, post-column addition of organic solvents (e.g. methanol) and ionization reagents (e.g. chloroform) are normally used for enhancement of ionization efficiency of carbohydrates [1, 2]. However, chloroform may cause strong ion suppression and contamination to the interface and ion optics of LC/MS system. A reduced amount of chloroform in the post-column addition line was used and evaluated in this study.

❑ Experimental

A single quadrupole LCMS-2020 (Shimadzu Corporation) was employed in this work. A ligand exchange chromatography column (Shim-pack SCR-101 P, 7.9 x 300 mm) was used for separation of sugars and sweeteners with pure water as the mobile phase. The LC and MS conditions are shown in Table 1. Nine commonly used sweetener compounds (see Table 2) were obtained in powders from Sigma Aldrich, Fluka, and SUPELCO. A mixed stock solution of the nine sweeteners was prepared in pure water. A calibrant series ranging from 0.04 mg/L to 500 mg/L was prepared from the mixed standard stock solution with water as diluent.

Table 1: LC/MS analytical conditions of Sweeteners

LC Conditions

Column	Shim-pack SCR-101 P (7.9 x 300 mm)
Flow Rate	0.60 mL/min
Elution Mode	Isocratic elution
Mobile Phase	Water
Post Column solvent	Methanol:Chloroform, 95:5 (0.1 mL/min)
Oven Temp.	80 ºC
Injection Volume	10 µL

MS Conditions (Shimadzu LCMS-2020)

Interface	APCI
MS Mode	Negative Mode (SIM)
Interface Temp.	450 ºC
Block Temp.	200 ºC
DL Temperature	250 ºC
Nebulizing Gas Flow	Nitrogen, 2.5 L/min
Drying Gas Flow	Nitrogen, 5.0 L/min

❑ Results and Discussion

Method Development

Figure 1 shows the SIM chromatograms of the nine sweeteners on Ligand Exchange Chromatography-MS. The ligand exchange chromatography separation of the compounds was carried out using pure water as the mobile phase. The chloroform reagent of 5% in MeOH was pumped at 0.1 mL/min before the APCI interface through a post-column addition flow line. The content of choloroform in mobile phase entering into APCI was 0.7%, which is much lower than the reported literatures. Most of the sweeteners and sugar alcohols studied formed chlorine adduct ions $[M+Cl]^-$ in negative mode. Acesulfame potassium and saccharin were ionized directly in negative mode to form $[M-K]^-$ and $[M-H]^-$, respectively.

As shown in Figure1, three pairs of compounds co-eluted with very closed retention times, acesulfame potassium and saccharin (6.91/7.09 min), sucralose and mannitol (24.36/25.03 min), meso Erythritol and Lactitol (19.79/20.68 min). Acesulfame potassium and saccharin were detected separately in different m/z values (161.9 $[M-K]^-$ and 181.9 $[M-H]^-$) in SIM mode. The rest two pairs were also separated due to different m/z values of the adduct ions $[M+Cl]^-$.

Calibration curves of the nine sweeteners were established using mixed standard samples of concentrations from 0.04, 0.1 or 0.5 mg/L to 500 mg/L. Linear calibration curves were obtained for all compounds (r2 > 0.999) as shown in Figure 2.

The limits of detection (LODs) of these compounds in neat solutions were at 0.01~1 mg/L depending on compounds. The repeatability of the method was evaluated and the RSD (%, n=6) of peak area obtained for 50 mg/L concentration (except saccharin of 20 mg/L) were found below 7.0% except for maltitol (9.44%) and lactitol (8.59%) (See Table 2).

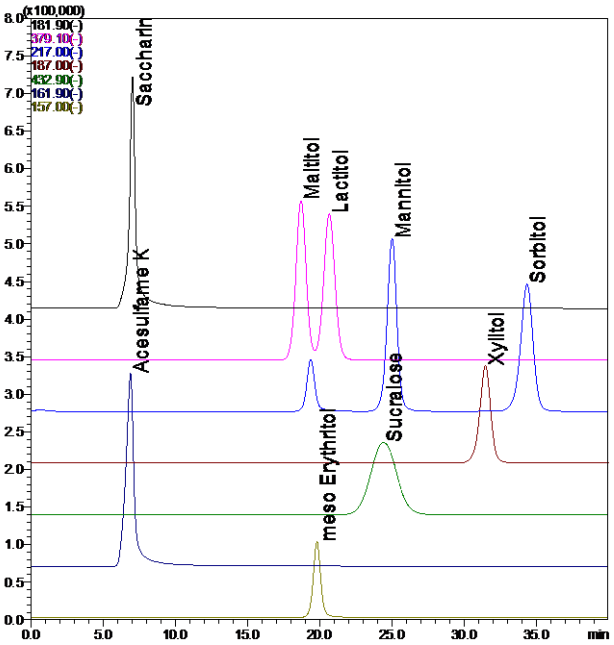


Figure 1: SIM chromatograms of nine sugar alcohols and sweeteners by LC/MS. Conc.: 100 mg/L except for saccharin of 40 mg/L

Analysis of Beverages and Daily Product

The LC/MS method established was applied to a variety of liquid samples including beverage and daily product (sweet soft diet drink, probiotic drink and mouthrinse). The liquid samples investigated were diluted 1000 times in water and sonicated to remove gas bubbles. All samples were filtered with 2 µm filters before injection on to Ligand Exchange Chromatography-MS. The SIM chromatograms of the sample tested are shown in Figure 3. The identification results of the types of artificial sweeteners and sugar alcohols as well as their quantification were in accordance with the contents available on the product labels (Table 3).

Table 3: Results of sweeteners in the samples tested (g/L)

	Sweet soft diet drink	Mouthrinse	Probiotic drink
Acesulfame K	0.07	-	-
Saccharin	-	0.47	-
Maltitol	-	11.45	39.05
Sorbitol	-	88.82	-

Table 2: Calibration curve and repeatability of the method for quantitative analysis of 9 sweeteners

Sweetener	MW	Detect Ion (m/z)			RT (min)	Concentration range (mg/L)	r^2 value	%RSD Peak Area
		$[M+Cl]^-$	$[M-K]^-$	$[M-H]^-$				
Acesulfame K	201.24	-	161.9	-	6.91	0.1 - 500	0.9996	0.92
Saccharin	183.18	-	-	181.9	7.087	0.04 – 40	0.9999	0.57
Maltitol	344.31	379.1	-	-	18.692	0.5 – 500	0.9999	9.44
meso Erythritol	122.12	157	-	-	19.799	0.5 – 500	0.9996	3.66
Lactitol	344.31	379.1	-	-	20.685	0.5 – 500	0.9998	8.59
Sucralose	397.64	432.9	-	-	24.361	0.1 – 500	0.9999	2.02
Mannitol	182.17	217	-	-	25.026	0.1 – 500	0.9998	7.06
Xylitol	152.15	187	-	-	31.492	0.1 – 500	0.9997	2.58
Sorbitol	182.17	217	-	-	34.371	0.1 - 500	0.9997	5.94

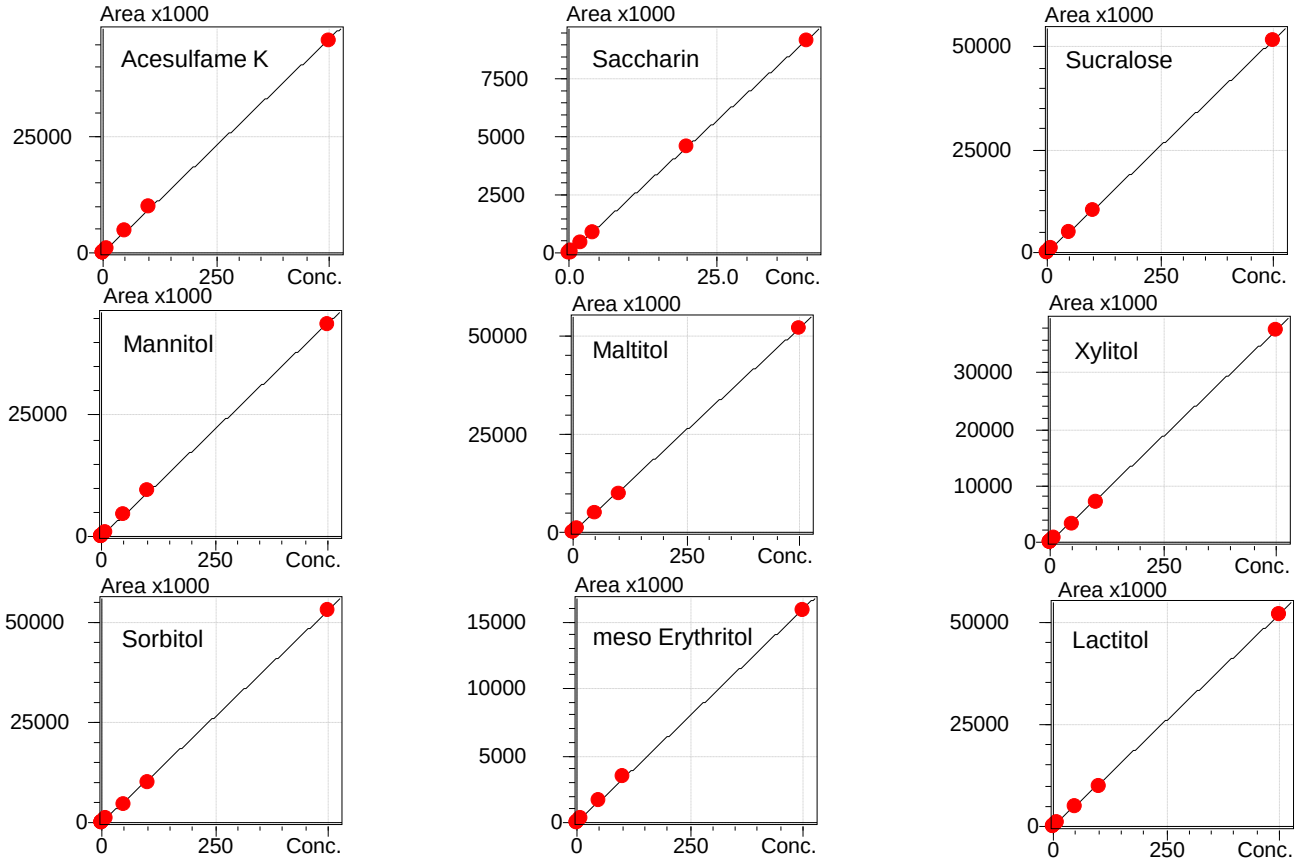
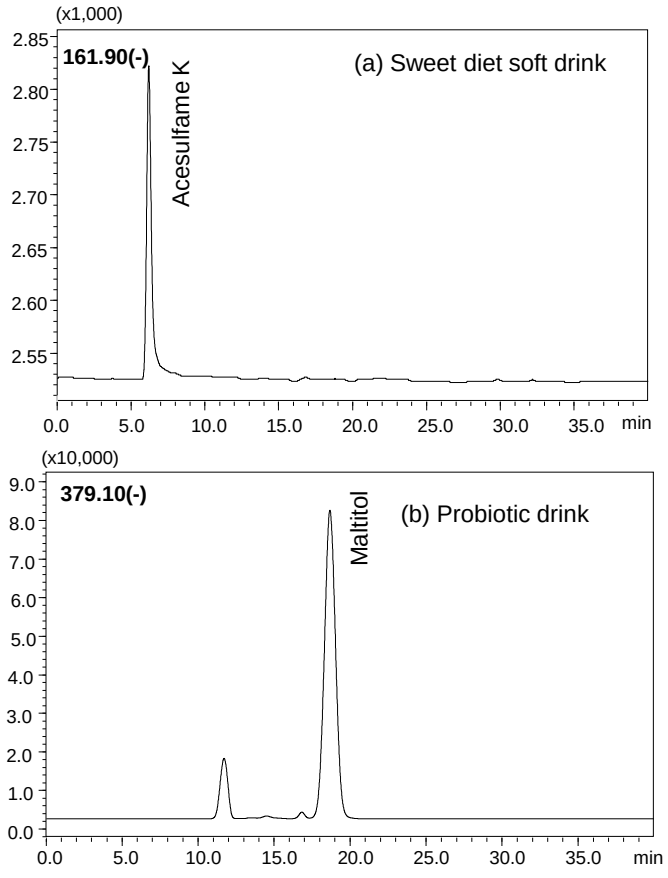


Figure 2: Calibration curves of 9 sweetener standards, peak area ~ concentration (mg/L)



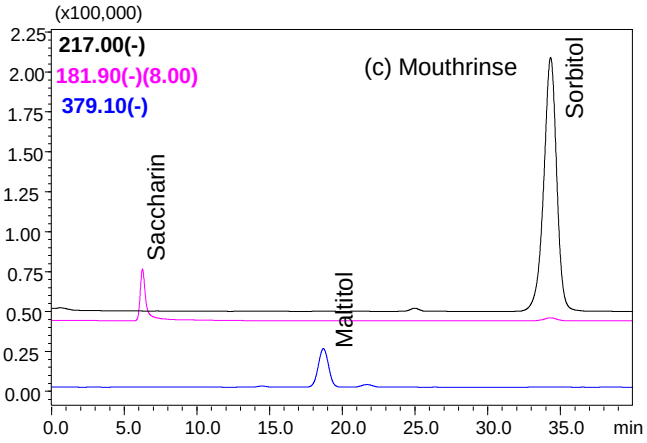


Figure 3: Beverage and daily product sample at 1000x dilution (a) Sweet diet soft drink (b) Probiotic drink and (c) Mouthrinse

❑ Conclusions

A new APCI-Ligand Exchange Chromatography/MS method was developed for quantitative analysis of nine sweetener compounds. The results showed that as low as 0.7% of chloroform as post-column addition reagent was sufficient for effective ionization of the nine sweeteners studied to achieve desired sensitivity of 0.01~1 mg/L.

❑ References

[1] Application News No. C74, Shimadzu, http://www.shimadzu.com/appli/index.html
[2] Kato, Y. Numajiri, Y., J. Chromatography 562, 81-97 (1991).

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