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Quantitative Analysis of Carbohydrates and Artificial Sweeteners in Food Samples Using GFC- MS with APCI Interface and Post-column Reagent Addition

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1. Introduction

Sensitive and simple analytical method for quantification of a variety of carbohydrates is increasingly in demand in foods, nutrition and biochemical research fields. As sugar replacers and food additives, sugar alcohols and artificial sweeteners were used widely and thoroughly assessed for health and safety concerns. The conventional analytical methods based on GC/MS or HPLC with ELSD, UV or fluorescence detection were used normally for few sugars or carbohydrates. It is desired to use a single method to detect and quantify more and different carbohydrates and sweeteners. Here we introduce a novel LC/MS method using gel filtration chromatography (GFC) and single quadruple mass spectrometer with APCI interface for separation and detection of a total of sixteen carbohydrates (sugars and sugar alcohols) and three artificial sweeteners with pure water as the mobile phase, the results of the method were applied to food samples and a mouth rinse product. It is known that sugar molecules are not easily to be 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.

2. Experimental

A single quadrupole LCMS-2020 (Shimadzu Corporation) was employed in this work. A GFC column (Shim-pack SCR-101 P, 7.9 × 300 mm) was used for separation of carbohydrates and sweetneners with pure water as the mobile phase. The LC and MS conditions are shown in Table 1. Nineteen commonly used carbohydrates and sweetener compounds (see Table 2a and Table 2b) were obtained in powders from Sigma Aldrich, AnalaR Normapur, Wako Chemicals, Fluka, Merck, TCI and SUPELCO. Two groups of mixed stock solutions of the standard compounds were prepared in pure water. The mixed standard solutions were diluted into two calibrant series ranging from 0.1 mg/L to 400 mg/L and 0.04 mg/L to 500 mg/L.

Table 1 LC and MS Conditions for Nineteen Carbohydrates and Sweeteners Analysis

LC Conditions

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

MS Conditions (Shimadzu LCMS-2020)

Interface	APCI
MS Mode	Negative (SIM)
Interface Temp.	450 °C
Block Temp.	200 °C
DL Temp.	250 °C
Nebuliz. Gas Flow	N2, 2.5 L/min
Drying Gas Flow	N2, 5.0 L/min

3. Results and Discussion

Method development:

The SIM chromatograms of 12 carbohydrates in group one and 9 sweeteners in group 2 on GFC-MS were showed in Fig. 1 (a) and (b) . The GFC 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 into APCI was 0.7%, which is much lower than that reported literatures. Most of the carbohydrates and sweeteners 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.

One of the advantages of LC/MS method is the capability of separation of co-eluting compounds with different molecular masses. In this analysis, there were two pairs of co-eluting compounds: galactose and rhamnose (RT at 14.78 min and 14.81 min); myo Inisitol and glycerol (RT at 19.42 min and 19.47 min), and three pairs of compounds coeluted 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 pairs were also separated due to different *m/z* values of the adduct ions $[M+Cl]^-$.

The calibration curves were set up using mixed standard samples for group one of the 12 carbohydrates with concentrations from 0.1 or 0.5 mg/L to 400 mg/L and group two of the nine sweeteners with 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) (selected curves were shown in Fig. 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 each compounds at different concentration levels were reported at Table 2a and Table 2b.

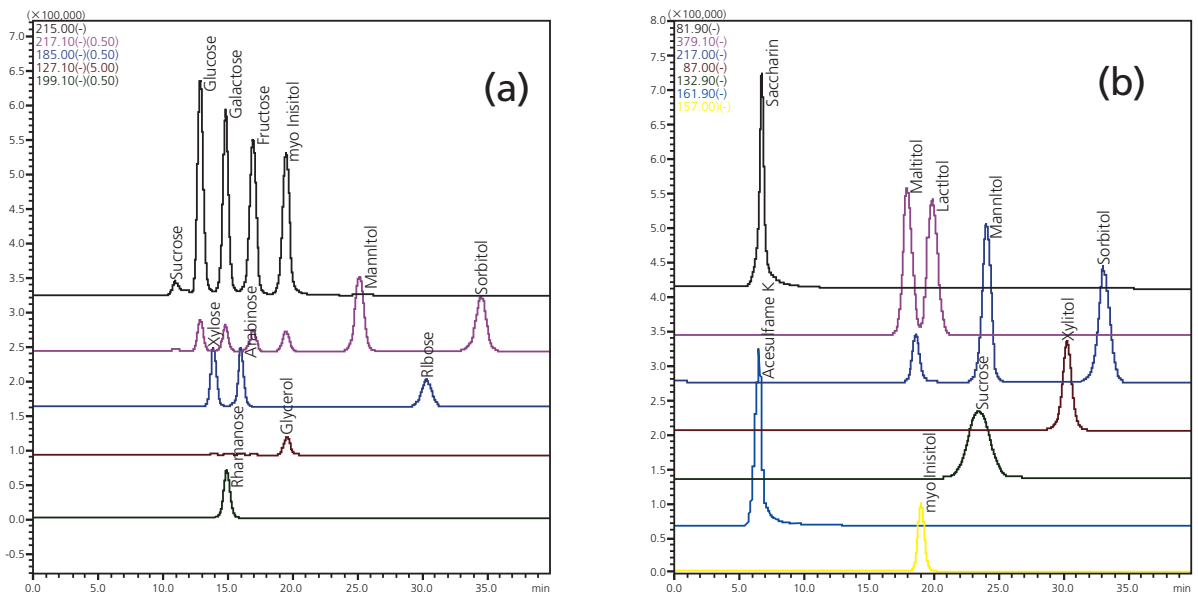

Fig. 1 SIM Chromatograms of 12 carbohydrate (a) and 9 sweeteners (b) by LC/MS.
Concentration: 100 mg/L each, except for saccharin of 40 mg/L

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Table 2a Calibration Curve and Repeatability of the Method for group one - 12 Carbohydrates

Carbohydrate	MW	$[M+Cl]^-$	RT (min)	Calibration Conc. range / mg/L	r2 value	%RSD Peak Area (10 mg/L)
Sucrose	342.3	$[M-C_6H_{10}O_5+Cl]^-$ 215.1	10.74	0.5 - 100	0.9998	4.7
Glucose	180.16	215.1	12.82	0.1 - 400	0.9999	1.6
Xylose	150.13	185.1	13.84	0.1 - 400	0.9998	3.2
Galactose	180.16	215.1	14.78	0.1 - 400	0.9998	1.5
Rhamnose	164.17	199.1	14.81	0.1 - 400	0.9993	4.2
Arabinose	150.13	185.1	15.94	0.1 - 400	0.9997	4.1
Fructose	180.16	215.1	16.86	0.1 - 400	0.9996	4.1
myo Inisitol	180.16	215.1	19.42	0.1 - 400	0.9994	2.5
Glycerol	92.09	127.1	19.47	0.1 - 400	0.9995	3.1
Mannitol	182.17	217.1	25.07	0.1 - 400	0.9997	3.2
Ribose	150.13	185.2	30.19	0.1 - 400	0.9994	6.9
Sorbitol	182.17	217.1	34.40	0.1 - 400	0.9995	2.8

Table 2b Calibration Curve and Repeatability of the Method for group two - 9 sweeteners

Sweetener	MW	Detect Ion (*m/z*)			RT (min)	Concentration range (mg/L)	r2 value	%RSD Peak Area (50 mg/L)
		$[M+Cl]^-$	$[M-K]^-$	$[M-H]^-$				
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

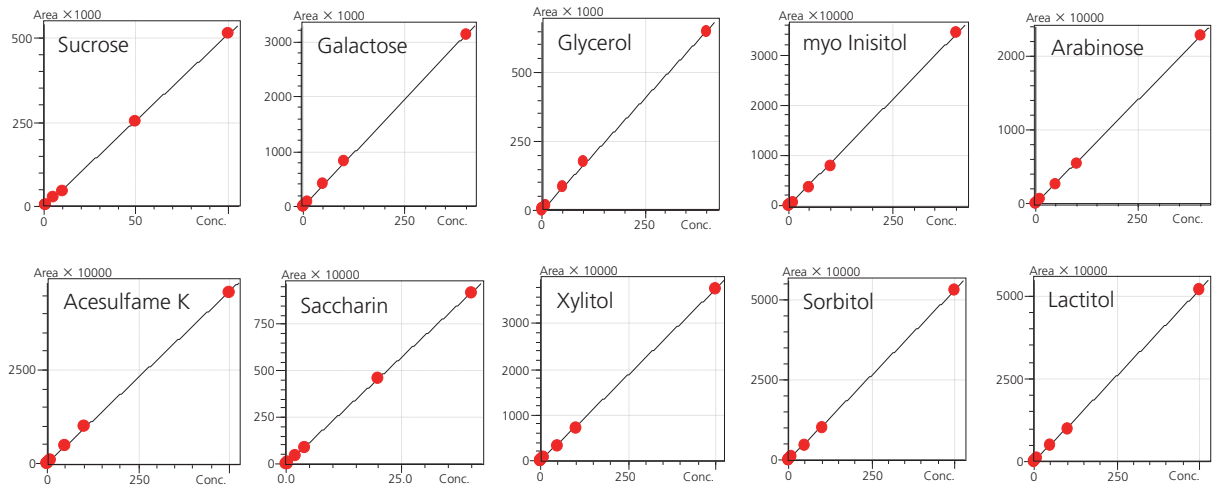

Fig. 2 Selected Calibration Curves of carbohydrate and sweeteners , peak area ~ concentration (mg/L)

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Analysis of Food Samples and Mouthrinse

The LC/MS method established was applied to a variety of liquid samples including beverage, food (Japanese "Sake", soya sauces and soft drink/diet drink/probiotic drink) and mouthrinse. The liquid samples investigated were diluted 1000 times in water and were filtered with 2 µm filters before injection on to GFC-MS. The SIM chromatograms of the samples tested are shown in Fig. 3 The results of identification about the types of sugars, sugar alcohols and artificial sweeteners as well as their quantification were in accordance with the contents available on the product labels (Table 3).

Table 3 Results of carbohydrate and sweetener contents in Samples Tested (g/L).

Carbohydrate	Sample group A		
	Sake	Soya sauce	Soft drink
Sucrose	-	27.09	-
Glucose	26.49	3.52	40.99
Galactose	-	2.25	-
Fructose	-	1.51	59.2
myo Inisitol	-	0.87	-
Glycerol	-	19.76	-
Mannitol	-	0.34	-

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

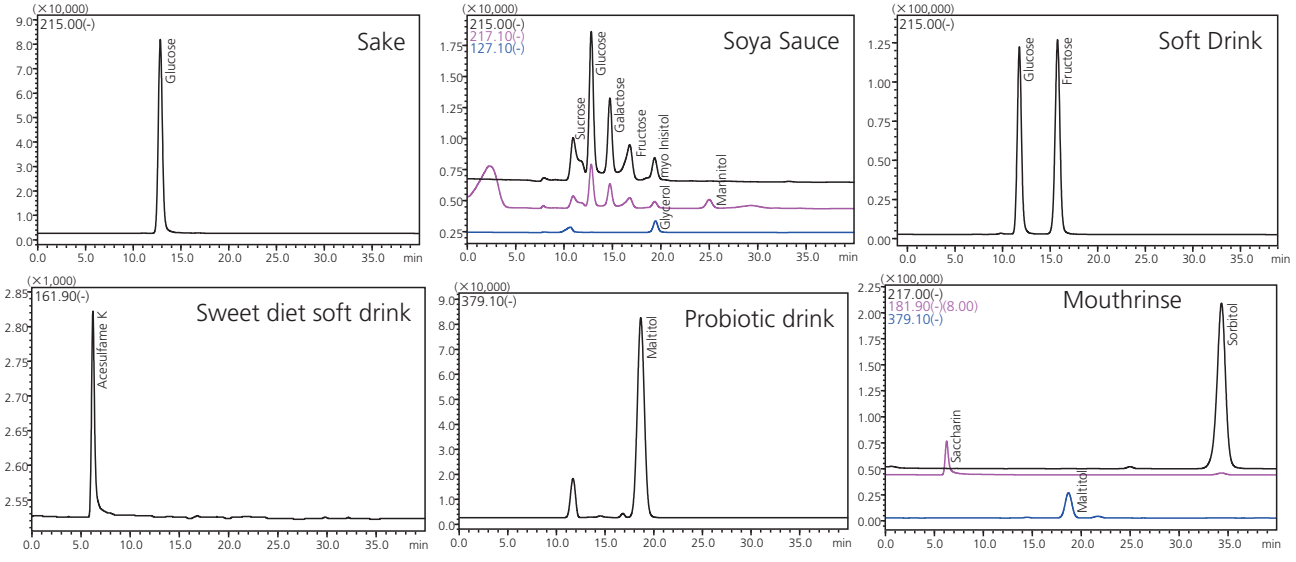

Fig. 3 SIM Chromatograms of Food samples and mouthrinse at 1000x dilution

4. Conclusions

A new APCI-GFC/MS method was developed for quantitative analysis of total nineteen carbohydrates and sweeteners. The results showed that as low as 0.7% of chloroform as post-column addition reagent was sufficient for effective ionization of the nineteen carbohydrates and 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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