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Application News

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LCMS-2020

Quantitative Analysis of Carbohydrates in Food Samples Using APCI-LC/MS with Post-column Reagent Addition and Ligand Exchange Chromatography

Sensitive LC/MS methods for quantification of carbohydrates are in demand increasingly in foods, nutrition and biochemistry fields. The conventional analytical methods based on GC/MS or HPLC with ELSD, UV or fluorescence detection were used normally for few sugars or carbohydrates and derivatization of the compounds prior to analysis was needed mostly. It is desired to use LC/MS method without derivatization for quantitative determination of more carbohydrates. It is well known that ionization of carbohydrates by atmospheric pressure ionization (API) is difficult. Therefore, post-column addition of reagent such as chloroform is required [1, 2]. However, the high content of chloroform in the mobile phase may cause strong ion suppression and contamination to the interface and ion optics of LC/MS. Here, we report a new LC/MS method using a reduced content of chloroform by post-column addition in mobile phase and ligand exchange chromatography for analysis of twelve carbohydrates.

❑ Experimental

A single quadrupole LCMS-2020 (Shimadzu Corporation) was employed in this work. The LC and MS conditions are shown in Table 1. Twelve carbohydrates (see table 2) used as standards were obtained in powders from Sigma Aldrich, AnalaR Normapur, Wako Chemicals, Fluka, Merck and TCI. A mixed stock solution of the 12 carbohydrates was prepared with pure water as the solvent. The mixed standard solution was diluted into a calibrant series ranging from 0.1 mg/L to 400 mg/L.

Table 1: LC and MS Conditions for Carbohydrates Analysis

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 12 carbohydrates using LCMS-2020. The LC separation of the compounds was carried out by ligand exchange chromatography using a Shim-pack SCR-101P column (7.9mmID x 300mmL) with pure water as the mobile phase (0.60 mL/min, isocratic mode). Chloroform reagent of 5% in MeOH was pumped at 0.1 mL/min into APCI interface through a post-column addition flow line to promote ionization of the carbohydrates to form $[M+Cl]^-$ ions in negative mode.

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). Having different m/z of $[M+Cl]^-$, for example, galactose with m/z of 215.1 and rhamnose with m/z of 199.1, they could be detected separately in SIM mode.

The calibration curves of the 12 carbohydrates were set up using mixed standard samples with concentrations from 0.1 or 0.5 mg/L to 400 mg/L. Linear calibration curves were obtained for all compounds (r2 > 0.999) (shown in Figure 2). The limits of detection (LODs) of these compounds in neat solutions were at 0.05~1 mg/L depending on compounds.

The repeatability of the method was evaluated and the RSD (%, n=6) of peak area obtained for 10 mg/L concentration were found below 5.0% except for ribose (6.9%) (See Table 2).

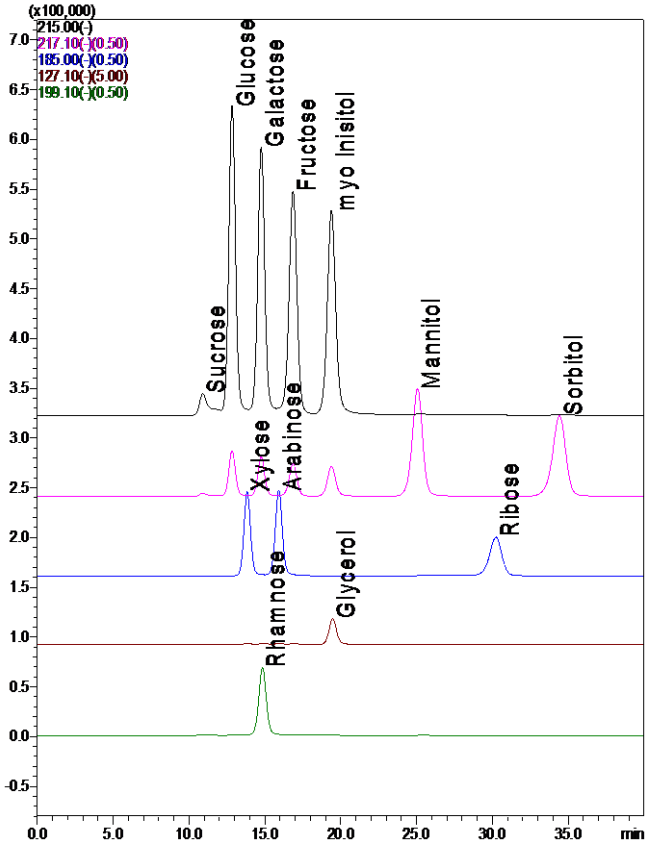


Fig 1: SIM chromatograms of 12 carbohydrates by LC/MS. Concentration: 100 mg/L.

Analysis of Food and Beverage Samples

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

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

Carbohydrate	Sample		
	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	-

Table 2: Calibration curve and repeatability of the method for quantitative analysis of 12 carbohydrates

Carbohydrate	MW	$[M+Cl]^-$	RT (min)	Calibration Conc. range / mg/L	r^2 value	%RSD Peak Area
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

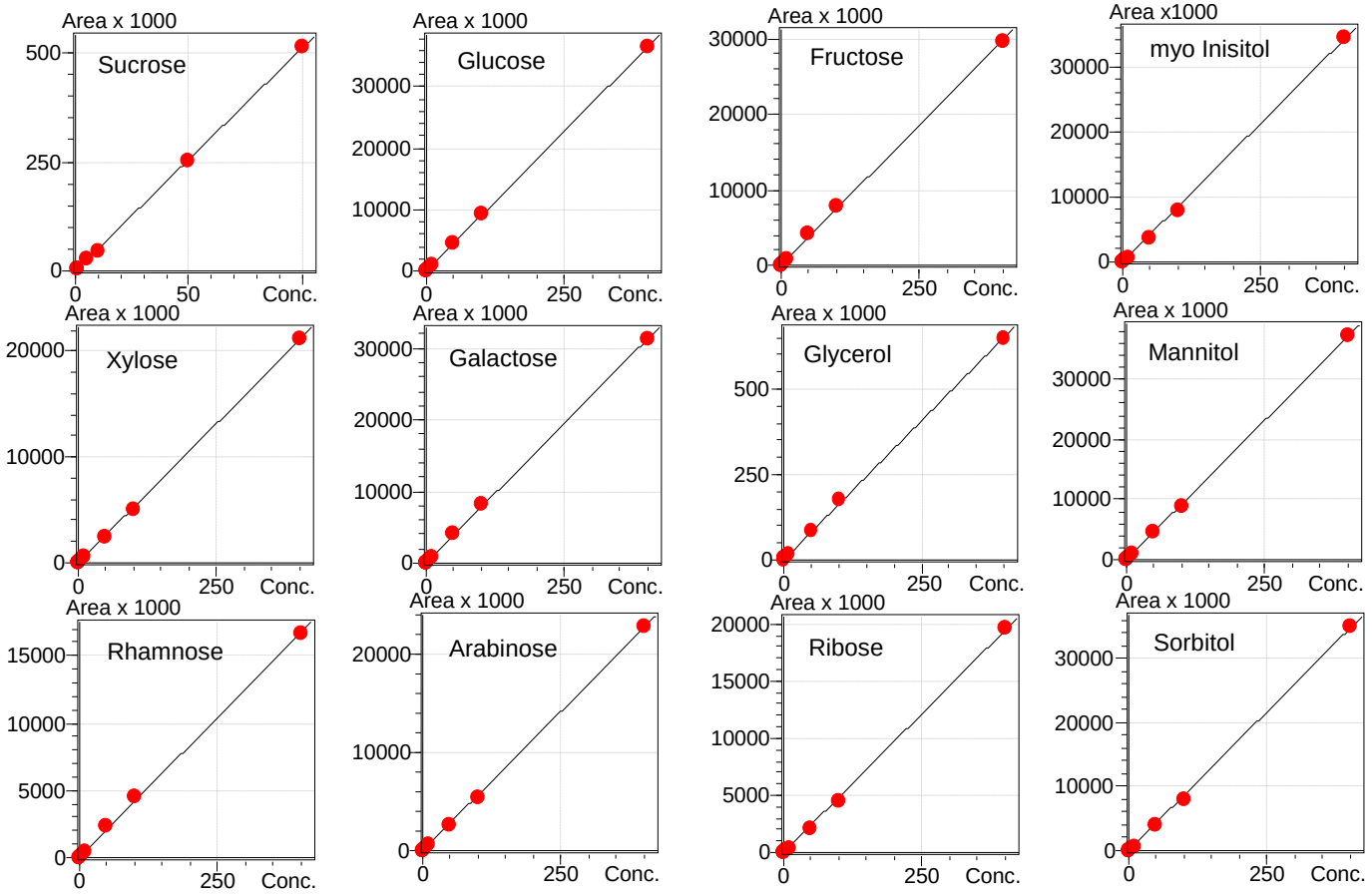
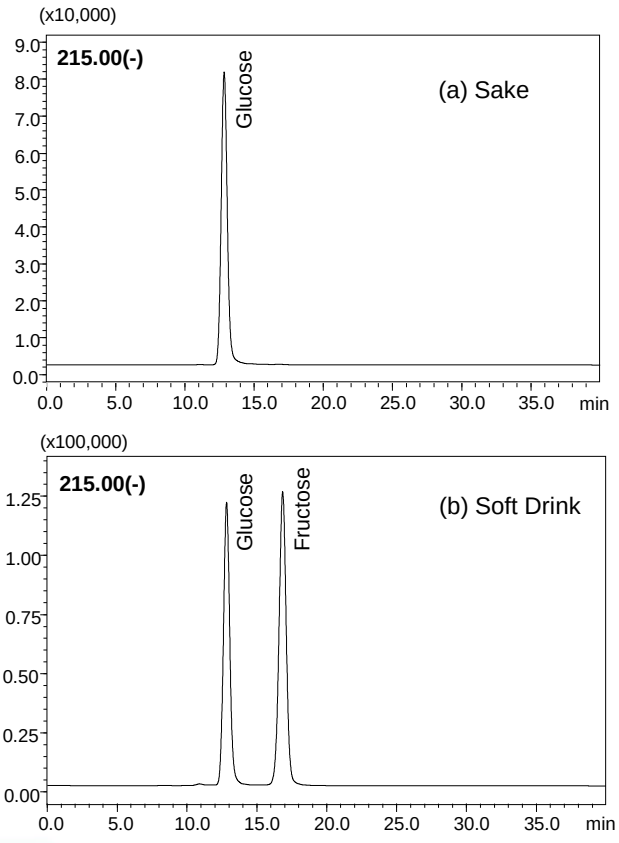


Fig 2: Calibration curves of 12 carbohydrate standards, peak area ~ concentration (mg/L)



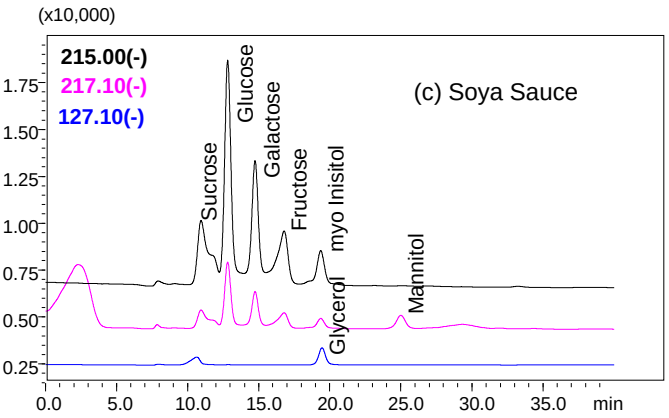


Fig 3: Food and beverage sample at 1000x dilution,
(a) Sake, (b) Soft drink and (c) Soya sauce

❑ Conclusions

A new APCI-Ligand Exchange Chromatography/MS method was developed for quantitative analysis of twelve carbohydrates. The results showed that as low as 0.7% of chloroform as post-column addition reagent was sufficient for effective ionization of the twelve carbohydrates studied to achieve desired sensitivity of 0.05~1 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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