SHIMADZU

Application News

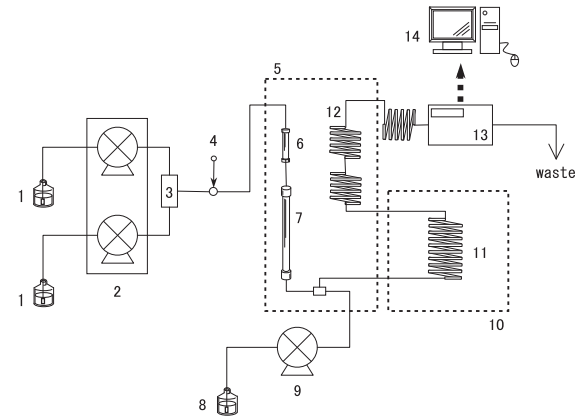
High Performance Liquid Chromatography No.L394A

Applications of Prominence RF-20Axs Fluorescence Detector (Part 3) Analysis of Saccharides Using Post-Column Derivatization System

The Shimadzu Prominence reducing sugar analysis system is a post-column derivatization system; after separation of saccharides by the column, an arginine/boric acid reagent solution is continuously added to the column eluent to convert the saccharides to fluorescent derivatives for detection[1]. This system allows detection of saccharides at high sensitivity and with excellent selectivity, furthermore with the new Prominence RF-20Axs, even higher sensitivity is achieved. Additionally, the temperature controlled flowcell in the RF-20Axs allows highly reliable analysis that is unaffected by ambient temperature fluctuations. Here we present some examples of analyzing saccharides using the reducing sugar analysis system (anion exchange mode) with the Prominence RF-20Axs.

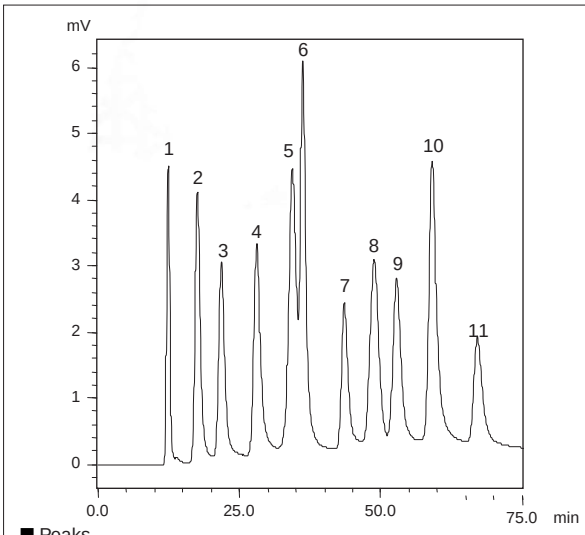
■ Analysis of Standard Solution

Fig. 1 shows a flow diagram of the Prominence reducing sugar analysis system, and Fig. 2 shows the chromatogram of a standard solution of 11 saccharides. Table 1 shows the analytical conditions.



1. Mobile Phase
2. Pump for Mobile Phase (LC-20AB)
3. Mixer
4. Autosampler (SIL-20AC)
5. Column Oven (CTO-20AC)
6. Guard Column (Guard Column ISA)
7. Column (ISA-07/S2504)
8. Reaction Reagent
9. Pump for Reaction Reagent (LC-20AD)
10. Chemical Reaction Box (CRB-6A)
11. Reaction Coil
12. Cooling Coil
13. Detector (RF-20Axs)
14. Data Processor

Fig. 1 Flow Diagram of the System



■ Peaks
1. Sucrose, 2. Cellobiose, 3. Maltose, 4. Lactose, 5. Rhamnose, 6. Ribose
7. Mannose, 8. Arabinose, 9. Galactose, 10. Xylose, 11. Glucose

Fig. 2 Chromatogram of a Standard Mixture of 11 Saccharides (200 μmol/L each, except sucrose at 2 mmol/L, 10 μL injected)

Table 1 Analytical Conditions

<Separation>		<Detection>	
Column	: Shim-pack ISA-07/S2504 (250 mm L. × 4.0 mm I.D.)	Reaction Reagent	: 10 g/L Arginin, 30 g/L Boric acid
Guard Column	: Shim-pack Guard Column ISA (50 mm L. × 4.0 mm I.D.)	Flow Rate	: 0.5 mL/min
Mobile Phase	: A ; 0.1 mol/L Potassium borate buffer (pH8)	Reaction Coil	: SUS, 10 m L. × 0.8 mm I.D.
	B ; 0.4 mol/L Potassium borate buffer (pH9)	Reaction Temp.	: 150 ˚C
	A → B Linear gradient elution	Cooling Coil	: SUS, 6 m L. × 0.3 mm I.D.
Flow Rate	: 0.6 mL/min	Detection	: RF-20Axs Ex. at 320 nm, Em. at 430 nm
Injection Volume	: 10 μL	Cell Temp.	: 25 ˚C
Column Temp.	: 65 ˚C		

■ High-Sensitivity Analysis

The RF-20Axs offers unprecedented levels of sensitivity; a water Raman S/N ratio is at least 2000. This allows the reducing sugar analysis system to detect saccharides with much higher sensitivity.
Fig. 3 shows a chromatogram obtained from analysis of a standard solution of saccharides (2 μmol/L each, except sucrose at 20 μmol/L, 10 μL injected). In this case, glucose is clearly detected even when injected at the amount of 20 pmol (3.6 ng).

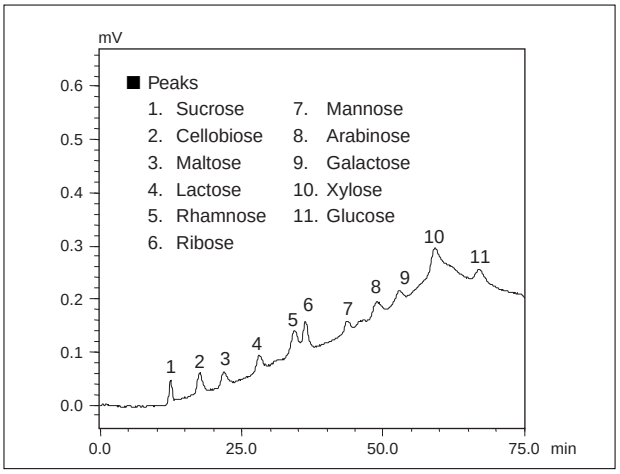
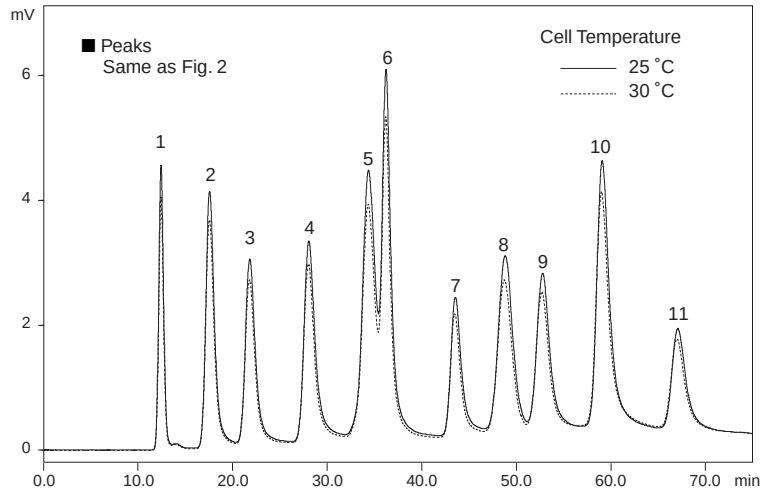


Fig. 3 Chromatogram of a Standard Mixture of 11 Saccharides (2 μmol/L each, except sucrose at 20 μmol/L, 10 μL injected)

■ Effect of Cell Temperature Control

It is generally known that the fluorescence intensity drops as the temperature rises; because the molecular collisions increase in frequency with increase in the temperature, therefore molecules lose their potential energy.[2]. In other words, a fluctuation of the ambient (detection) temperature changes the fluorescence intensity of some compounds, and this negatively influences the accuracy of analysis.
The RF-20Axs has a temperature-controlled cell as a standard feature, ensuring the high reliability of analysis that is not affected by a temperature fluctuation.
Fig. 4 shows a comparison of the peak intensities at cell temperatures of 25 ˚C and 30 ˚C (using the same saccharide solution and analytical conditions as in Fig. 2). The comparison of two chromatogram at cell temperatures of 25 ˚C and 30 ˚C reveals a decrease in peak intensity over 10 % for every compound at the higher cell temperature. By maintaining a constant cell temperature, peak intensity and detection sensitivity will not be compromised if the room temperature changes during the sequence run.



	Peak Height		
	25 ˚C	30 ˚C	30 ˚C / 25 ˚C (%)
Sucrose	4546	4043	88.9
Cellobiose	4082	3639	89.1
Maltose	2966	2645	89.2
Lactose	3175	2830	89.1
Rhamnose	4232	3717	87.8
Ribose	5852	5118	87.5
Mannose	2201	1961	89.1
Arabinose	2820	2445	86.7
Galactose	2489	2184	87.7
Xylose	4263	3759	88.2
Glucose	1623	1454	89.6

Fig. 4 Effect of Cell Temperature on Peak Intensity

[References]
1) H. Mikami and Y. Ishida: Bunseki Kagaku, 32, E207 (1983)
2) H. Nakamura, Supervisor: Liquid Chromatography Knacks (Detection Chapter), Maruzen (2006), P40

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