

# HIGH PERFORMANCE LIQUID CHROMATOGRAPH

# No. L226

## Fluorometric Detection of Non-reducing Saccharides and Oligosaccharides with Post-Column Derivatization

Fluorometric detection method introduced here uses taurocyamine [ $\text{H}_2\text{N}(\text{NH})\text{CNHCH}_2\text{CH}_2\text{SO}_3\text{H}$ , mw/167.18] as the reagent for post-column derivatization. This method was developed by the research group of Professor Kinoshita, Pharmacology Department, Kitazato University. [J. of Liquid Chromatography, 14 (10) 1929-1938 (1991)]

This method is particularly suited for the fluorometric detection of non-reducing saccharides and oligosaccharides. Of course, simultaneous detection of oligosaccharides is possible and it permits selective and highly sensitive detection of saccharides.

### ■ Detection of Sugar Alcohols

In the presence of sodium periodate, taurocyamine derivatives sugar alcohols, which are non-reducing, to detectable species. Fig.1 shows the chromatogram of a standard solution of sorbitol. Under these conditions, reducing sugars, which in this analysis are glucose and fructose, were detected. A mixture of 500pmol each of the three components was injected. Fig.2 shows the flow diagram, and Table 1 shows the analytical conditions.

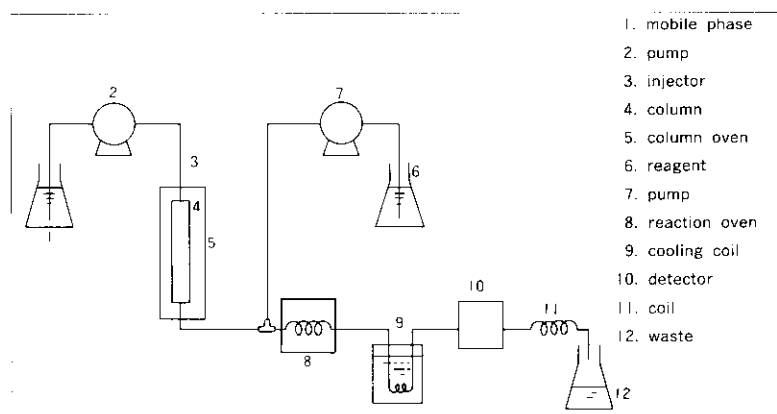


Fig. 2 Flow Diagram

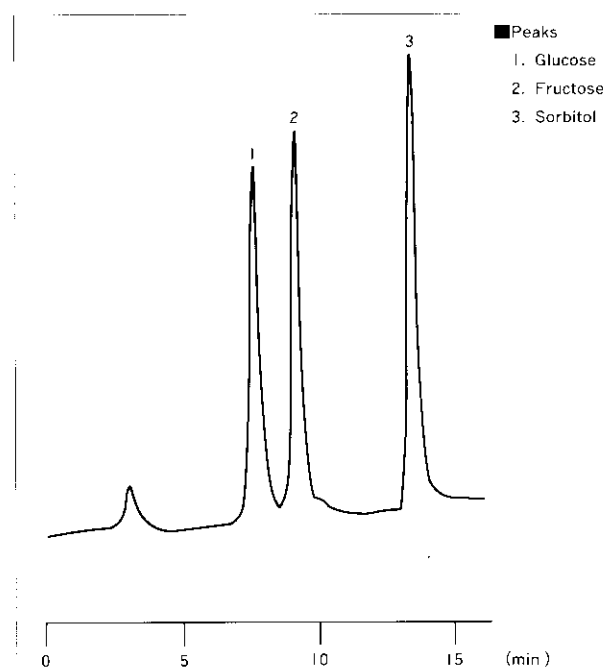


Fig. 1 Simultaneous Detection of Non-Reducing Saccharide and Reducing Saccharides

Table 1 Analytical Conditions

Sample	: Sorbitol, Glucose, Fructose Standard Solution 500pmol each
Column	: Shim-pack SCR-101C (7.9mm I.D. x 30cm L.)
Column Temp.	: 80°C
Mobile Phase	: Water
Flow Rate	: 1.0 mL/min.
Detection	
Reagent	: 0.1M Potassium Tetraborate containing 20mM Taurocyamine and 1mM Sodium Periodate (pH=10.5 adjust with 10N KOH)
Flow Rate	: 1.0 mL/min.
Reaction Temp.	: 150°C
Reaction Coil	: 0.5 mm I.D. x 5 mL.
Cooling Coil	: 0.3 mm I.D. x 2 mL.
Detector	: RF-550 Ex=320 nm, Em=450 nm IV INT Connector Sense=High ATTEN=3

## ■ Detection of Non-Reducing Oligosaccharides

Fig.3 shows a chromatogram of a standard solution of the mixture of sucrose, raffinose and stachyose. The

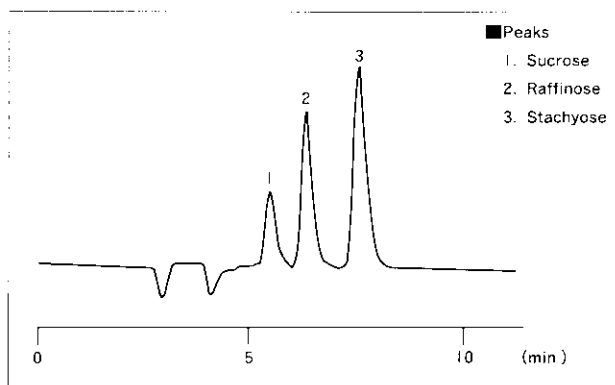


Fig. 3 Detection of Non-Reducing Oligosaccharides

## ■ Detection of Malto-Oligosaccharides

Fig.4 shows a chromatogram of standard solution of malto-oligosaccharides. The injected amount of each component was 10ng (Ca. 10pmol equivalents with

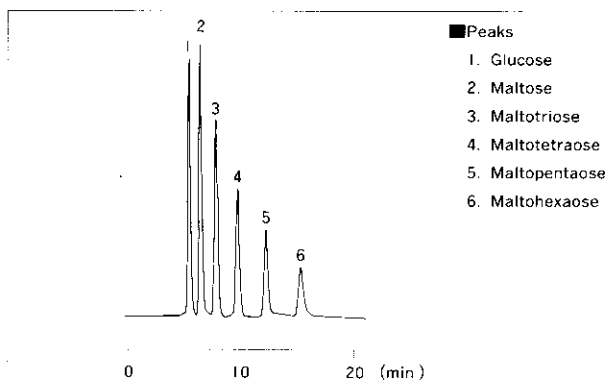


Fig. 4 Detection of Malto-Oligosaccharides

## ■ Detection of Reducing Monosaccharides

Fig.5 shows an example of detection of reducing monosaccharides using the reagent. The amount injected

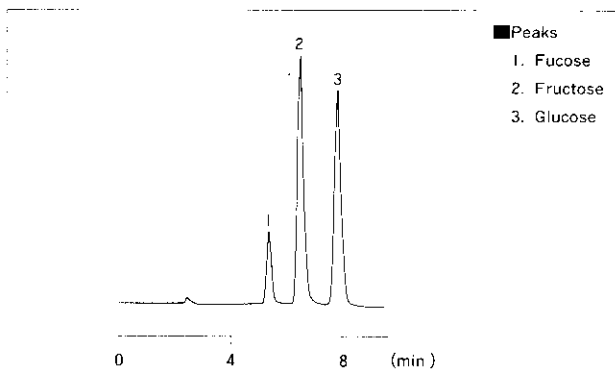


Fig. 5 Detection of Reducing Monosaccharides

injected sample contained 500pmol of each component. The analytical conditions are shown in Table 2.

Table 2 Analytical Conditions

Sample	: Sucrose, Raffinose, Stachyose, Standard Solution, 500pmol each	Flow Rate : 1.0m <sup>3</sup> /min. Reaction Temp. : 150°C Reaction Coil ; 0.5mmI.D.×5mL. Cooling Coil ; 0.3mI.D.×2mL.
Column	: Asahipak NH:P-50 (4.6mmI.D.×25cmL.)	Detector ; RF-550 Ex=320nm. Em=450nm.
Column Temp.	: 40°C	1V INT Connector
Mobile Phase	: Water/Acetonitrile (35/65)	Sense=High ATTEN=3
Flow Rate	: 1.0m <sup>3</sup> /min.	
Detection	Reagent : 0.1 M Potassium Tetraborate Containing 20mM Taurocyamine and 1mM Sodium Periodate (pH=10.5 adjusted with 10N KOH)	

respect to maltohexaose). Table 3 shows the analytical conditions.

Table 3 Analytical Conditions

Sample	: Malto-Oligo. saccharides Standard Solution 10ng each	Flow Rate : 1.0m <sup>3</sup> /min. Reaction Temp. : 150°C Reaction Coil ; 0.5mmI.D.×5mL.
Column	: Asahipack NH:P-50 (4.6mmI.D.×25cmL.)	Cooling Coil ; 0.3mI.D.×2mL. Detector ; RF-550
Column Temp.	: 40°C	Ex=320 nm.
Mobile Phase	: Water/Acetonitrile (35/65)	Em=450 nm 1V INT Connector
Flow Rate	: 1.0m <sup>3</sup>	Sense=High ATTEN=3
Detection	Reagent : 0.1M Potassium Tetra. borate Containing 20mM Taurocyamine (pH=12.5 adjusted with 10N KOH)	

was ca. 40pmol of each component. The analytical conditions are shown in Table 4.

Table 4 Analytical Conditions

Sample	: Fucose, Fructose, Glucose, Standard Solution 40pmol each	Flow Rate : 1.0m <sup>3</sup> /min. Reaction Temp. : 150°C Reaction Coil ; 0.5mmI.D.×5mL.
Column	: Asahipak NH:P-50 (4.6mmI.D.×25cmL.)	Cooling Coil ; 0.3mI.D.×2mL. Detector : RF-550.
Column Temp.	: 40°C	Ex=320nm. Em=450nm.
Mobile Phase	: Water/Acetonitrile (25/75)	1V INT Connector.
Flow Rate	: 1.0m <sup>3</sup> /min.	Sense=High ATTEN=3
Detection	Reagent : 0.1M Potassium Tetra borate Containing 20mM Taurocyamine (pH=10.5 adjusted with 10N KOH)	



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