

Analysis of Voglibose by Post-column Fluorometric Detection Method

α -Glucosidase hydrolyzes the α -D-glucoside bonds of disaccharides and helps digestion and absorption of sugars. Voglibose, α -glucosidase inhibitor, obstructs this enzyme's function. It is used as a drug for diabetes.

Since voglibose exhibits no UV absorption, special detection methods are necessary for HPLC analysis. The commonly used method is post-column

fluorescence derivatization method with taurine/periodic acid reagent.

This Application News introduces an example of analyzing voglibose using the Shimadzu LC-VP Reducing Sugar Analysis System with the post-column fluorescence derivatization method with taurine /periodic acid reagent.

■ Analytical Method

Fig.1 shows the structure of voglibose. Since voglibose only absorbs UV in the low-wavelength region, it cannot be directly detected with high sensitivity. Therefore, the post-column fluorescence derivatization method with taurine/periodic acid reagent¹⁾, which is effective for detecting sugar alcohols and non-reducing sugars at high sensitive, is commonly used.

In this method, voglibose is separated using an amino column, the taurine/periodic acid reagent is continuously added to the column eluate, and the fluorescent material generated by reaction at 100°C is detected.

By using this method with the Shimadzu LC-VP Reducing Sugar Analysis System, voglibose can be analyzed with high sensitivity. Fig.2 shows the flow diagram and Table 1 shows the typical analytical conditions.

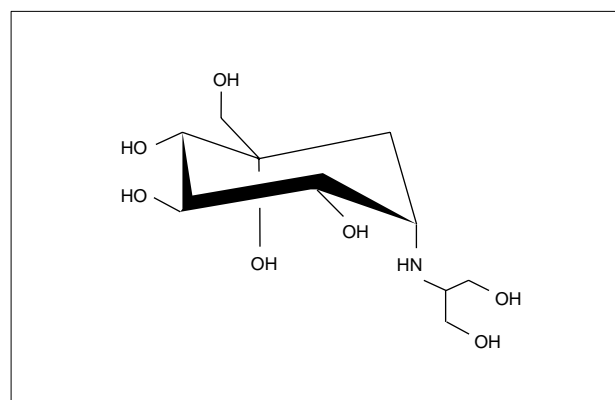


Fig.1 Structure of Voglibose

Table 1 Analytical Conditions

Column	:Asahipak NH2P-50 4E (250mmL.×4.6mmI.D.)
Guard Column	:Asahipak NH2P-50G 4A (10mmL.×4.6mmI.D.)
Mobile Phase	:A / B = 1 / 2 (v/v) A:20mM (Sodium) phosphate buffer (pH 6.50) B:Acetonitrile
Flow Rate	:0.6 mL/min
Column Temp.	:25°C
Reaction Reagent	:Taurine / Sodium periodate solution
Reagent Flow Rate	:0.6 mL/min
Reaction Temp.	:100°C
Detector	:RF-10AXL Super Ex at 350nm, Em at 430nm

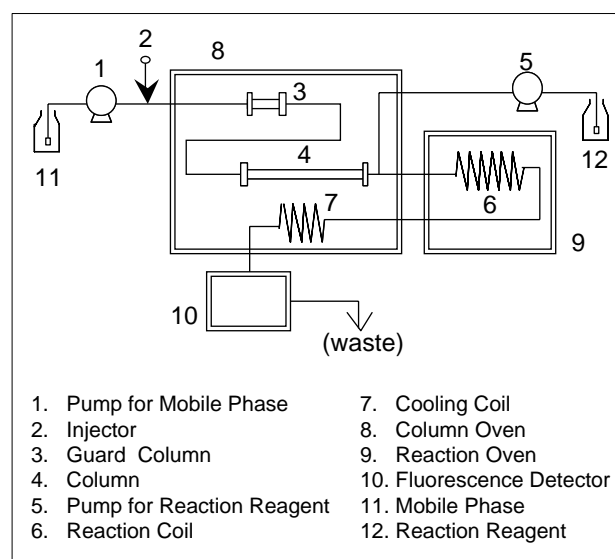


Fig.2 Flow Diagram

■ Analysis of Standard Voglibose

Fig. 3 shows the results of injecting 50 μ L of a standard voglibose solution (200 μ g/L).

Fig. 4 shows the calibration curve obtained by injecting

50 μ L of standard solutions at concentrations of 20, 40, 100 and 200 μ g/L. This result indicates that a linearity can be given at this concentration range.

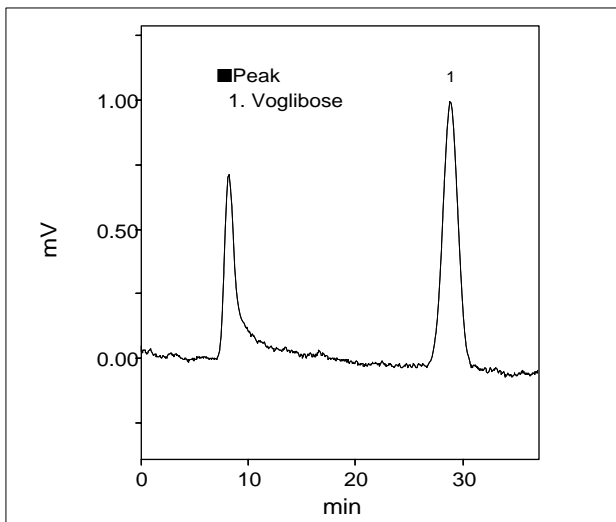


Fig.3 Standard Voglibose Sample (200 μ g/L, 50 μ L inj.)

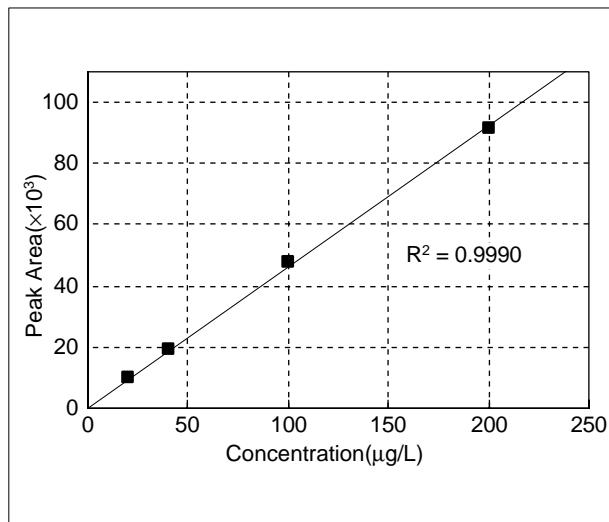


Fig.4 Calibration Curve

■ Repeatability

In order to evaluate repeatability near the lower detection limit, 50 μ L of a standard solution at 20 μ g/L was analyzed five times. Table 2 shows the results. A good result with 2.32% RSD was obtained.

Table 2 Repeatability

	Peak Area
1st	9579
2nd	9340
3rd	9212
4th	9158
5th	9647
Average	9387.2
RSD(%)	2.32

■ Effect of Flow Cell Temperature

Generally speaking, in fluorometric detection method, the sensitivity varies depending on the temperature. Fig. 5 shows the results of analyzing the voglibose peak area values (50 μ L injected at 40 μ g/L) at different cell temperatures using the RF-10AxL Super with a cell temperature control. It is clearly shown that the area value increases as the cell temperature decreases.

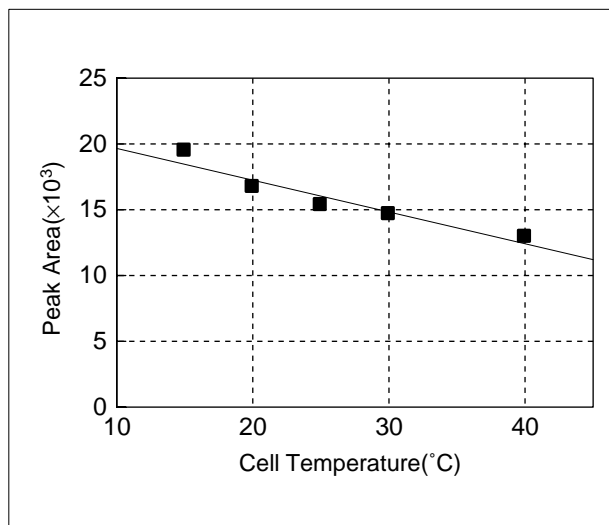


Fig.5 Relationship Between Flow Cell Temperature and Peak Area

Reference:

1) Takehiko Kato, Toshio Kinoshita; Bunseki Kagaku, 35, 869-874 (1986)



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