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SHIMADZU

Application News

High Performance Liquid Chromatography No.L406

Applications of the Prominence RF-20Axs Fluorescence Detector (Part 5) Analysis of Voglibose with Postcolumn Derivatization System

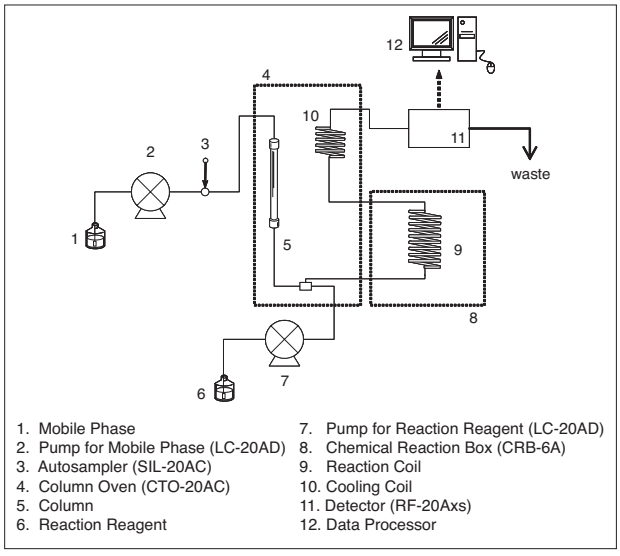
Voglibose is a diabetes drug which inhibits the activity of α-glucosidase. HPLC post-column derivatization is specified as the test method for voglibose in the Japanese Pharmacopeia, Fifteenth Edition (voglibose purity test, quantitation of voglibose in pharmaceutical tablets). Here we introduce an example of voglibose tablet testing as specified in the Japanese Pharmacopeia, Fifteenth Edition, using the Prominence post-column derivatization system with the Prominence RF-20Axs fluorescence detector.

■ Analysis of Standard Solution

Fig. 1 shows the flow diagram of this post-column derivatization system. The Japanese Pharmacopeia specifies that after mixing with the reaction solution (taurine / sodium periodate solution) at about 100 °C, the solution is to be cooled at a constant temperature of about 15 °C. However, since the RF-20Axs is equipped with cell temperature control, we adjusted the cell temperature to 15 °C. Not only does this eliminate the requirement for a temperature-controlled cooling bath, it can also provide improved accuracy and reduce fluorescence quenching at elevated temperatures.

Fig. 2*3 shows the chromatogram obtained following injection of 50 µL of the voglibose standard solution*1 (250 µg/L*2, prepared using mobile phase), and Table 1*4 shows the analytical conditions.

*1: The voglibose standard was provided by Sawai Pharmaceutical Co., Ltd.
*2: The Japanese Pharmacopeia specifies a standard solution concentration of 40 mg/L in the quantitative method.
*3: The peak in the vicinity of 5 minutes in Fig. 2 and Fig. 4 originates from the sample solvent.
*4: The Japanese Pharmacopeia indicates use of a column with an inner diameter of 4 mm, but here analysis was conducted using a column having a 4.6 mm inner diameter.



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

Fig. 1 Flow Diagram

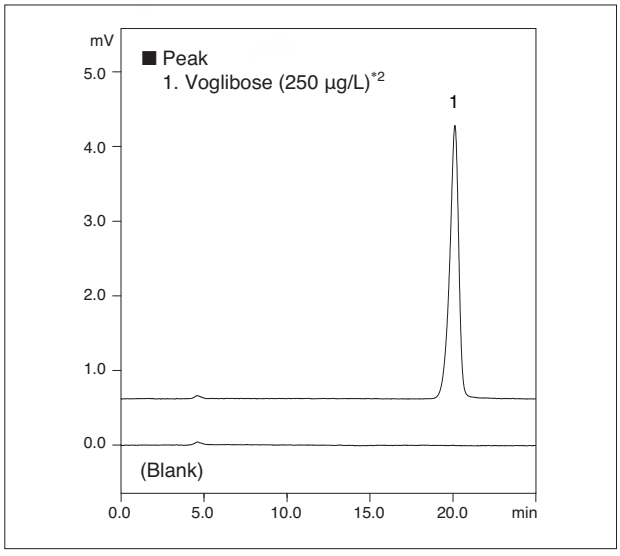


Fig. 2 Chromatogram of Voglibose Standard (250 µg/L, 50 µL injected)

Table 1 Analytical Conditions

<Separation>		<Detection>	
Column	: Shim-pack CLC-NH_2 (M) (150 mm L. × 4.6 mm I.D., 5 µm)	Reaction Reagent	: Taurine / Sodium periodate aq. solution
Mobile Phase	: (Sodium) phosphate buffer (pH 6.5) / Acetonitrile = 300 / 600 (v/v)	Flow Rate	: 0.8 mL/min
Flow Rate	: 0.8 mL/min	Reaction Coil	: PTFE, 20 m L. × 0.5 mm I.D.
Column Temp.	: 25 °C	Reaction Temp.	: 100 °C
Injection Volume	: 50 µL	Cooling Coil	: PTFE, 2 m L. × 0.3 mm I.D.
		Cooling Temp.	: 25 °C (CTO-20AC) → 15 °C (RF-20Axs)
		Detection	: RF-20Axs Ex. at 350 nm, Em. at 430 nm
		Cell Temp.	: 15 °C

■ Linearity

Fig. 3 shows the calibration curve obtained from analysis of voglibose standard solutions with concentrations of 2-250 µg/L (50 µL injected). Excellent linearity was obtained, with an R^2 value greater than 0.9999.

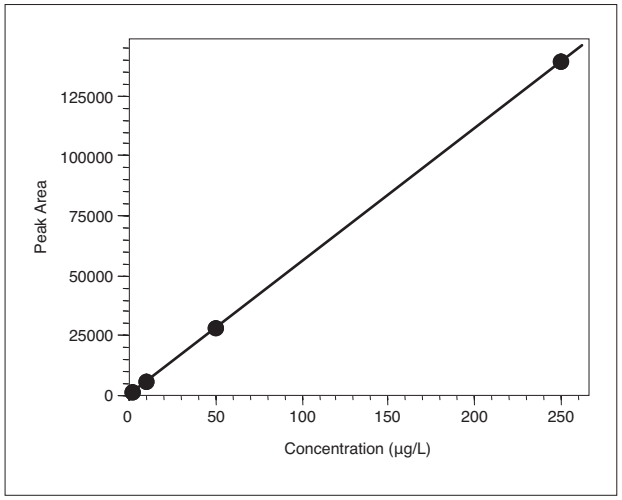


Fig. 3 Calibration Curve (2-250 µg/L, 50 µL injected)

■ Analysis of Voglibose at High Sensitivity

Fig. 4 shows the chromatogram obtained following injection of 50 µL of a 2 µg/L voglibose standard solution. The voglibose peak area repeatability (n = 5) for this analysis was 1.4 % RSD.
Use of the RF-20Axs permits micro-level analysis of voglibose at high sensitivity and with high accuracy.

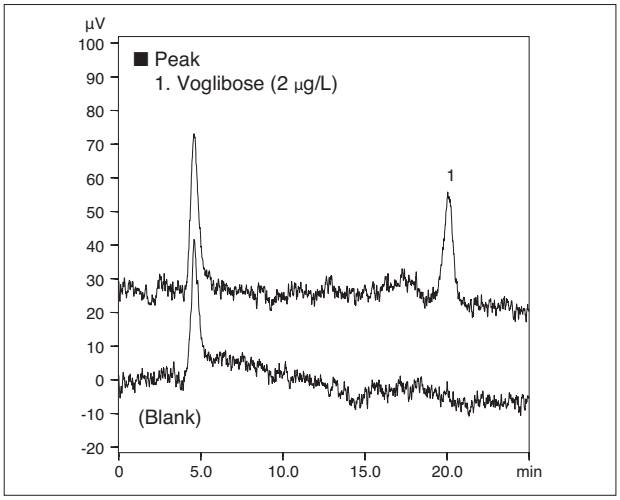


Fig. 4 Chromatogram of Voglibose Standard at High Sensitivity (2 µg/L, 50 µL injected)

■ System Suitability Test

We conducted system suitability testing for voglibose tablets as specified in the Japanese Pharmacopeia, Fifteenth Edition. The left-hand chromatogram in Fig. 5 was obtained using a test solution containing lactose and voglibose. The 8.2 peak resolution clearly satisfies the official criterion value (4 or greater). The overlaid chromatograms at the right in Fig. 5 were obtained using 50 µL injections of 40 mg/L voglibose standard solution. The peak area repeatability for these voglibose analyses was 0.16 % RSD (n = 6), satisfying the criterion value of 2.0 % or less.

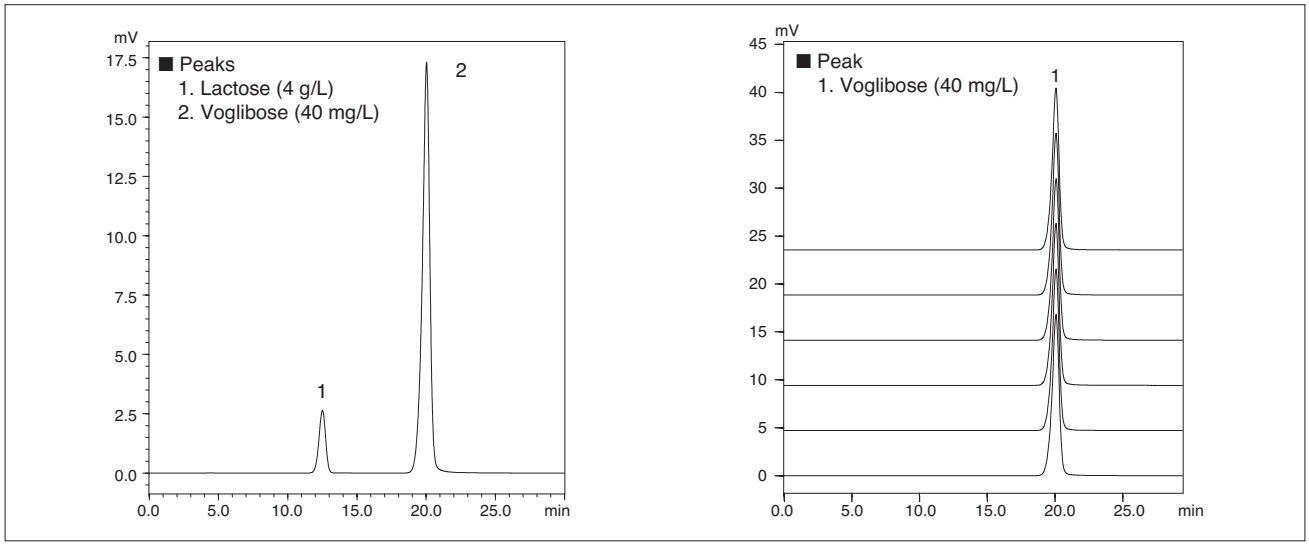


Fig. 5 System Suitability Test / System Performance (Left), System Repeatability (Right)

[References]
The Japanese Pharmacopeia, Fifteenth Edition (Edited by Pharmaceutical and Medical Device Regulatory Science Society of Japan)

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