

# Application News

## No. L431

### High Performance Liquid Chromatography

## High Speed, High Resolution Analysis (Part 41) Carryover Evaluation of Glibenclamide in Human Plasma by Nexera HPLC

LC/MS/MS high-speed, high-resolution analysis methods with columns packed with particles of 2  $\mu\text{m}$  or less are often used for analysis of drugs in human blood plasma. The small particle columns produce dispersion that is even less than that obtained with conventional columns (5  $\mu\text{m}$  particle size), so peaks are taller, thereby enabling quantitation at even higher sensitivity. While this high-sensitivity analysis has become possible, the effect of autosampler carryover on high-sensitivity LC/MS/MS analysis has become a problem.

In Application News No. L425, we evaluated the

performance of the Nexera SIL-30AC autosampler in the analysis of reserpine in human plasma, and reported its low carryover performance. In addition to the high potential of the SIL-30AC, we believe that rinsing with its powerful and sophisticated multi-solvent configuration was very effective.

Here, we report on the effectiveness of the rinse method reported in Application News No. L425, and demonstrate its effectiveness as a rinse method applicable to measurement of a wide range of drugs in plasma.

### ■ Preparation of Glibenclamide in Human Plasma

We selected glibenclamide as the target substance for the evaluation of carryover in the SIL-30AC. Glibenclamide is a second-generation oral hypoglycemic medication that was developed in 1966 by Boehringer Mannheim GmbH and Farbwerke Hoechst AG. It acts to lower the sugar level in the blood by stimulating the  $\beta$  cells in the pancreas, causing the discharge of insulin. It is typically used to treat patients with non-insulin dependent diabetes, also referred to as type 2 diabetes.

The structural formula of glibenclamide is shown in Fig. 1. It has an S-phenyl sulfonyleurea structure consisting of a para-phenyl group, a sulfonyl group, and a urea bond. Because of the hydrophobic rings, it is a compound that would be expected to be particularly prone to carryover. For the evaluation, LC/MS/MS measurement of glibenclamide in human plasma was conducted using a high-sensitivity MS/MS instrument.

Fig. 2 shows the pretreatment process used for spiking the human plasma with glibenclamide. Commercially available human plasma was used for the experiment. Three  $\mu\text{L}$  of the prepared glibenclamide-spiked sample was injected into the Nexera-MS/MS system using the SIL-30AC.

MRM (Multiple Reaction Monitoring) quantitation was conducted using the glibenclamide proton-adduct molecular ion ( $[\text{M}+\text{H}]^+$ :  $m/z=494$ ) as the precursor ion, and  $m/z=369$  as the product ion.

Quantitation of the glibenclamide in human plasma used the analytical conditions shown in Table 1. With this method, high-speed analysis was conducted with an analysis time of just 4 minutes, and even at a low concentration of 0.005 ng/mL in the plasma, high-sensitivity analysis was achieved with an S/N ratio of 2.

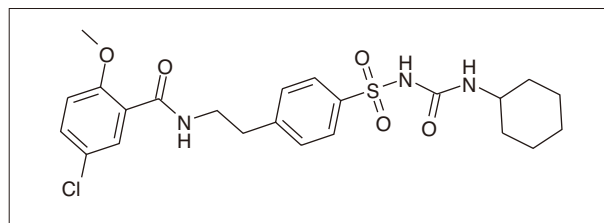


Fig. 1 Structure of Glibenclamide

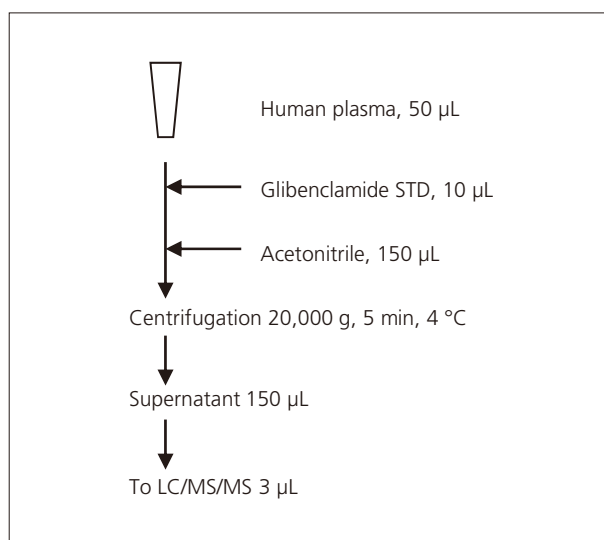


Fig. 2 Pretreatment Protocol

## ■ Carryover Evaluation of Glibenclamide in Human Plasma

Table 1 shows the analytical conditions, including the autosampler rinse liquids and rinsing sequence. The SIL-30AC can use 4 different wash solutions to wash the inner and outer needle surfaces.

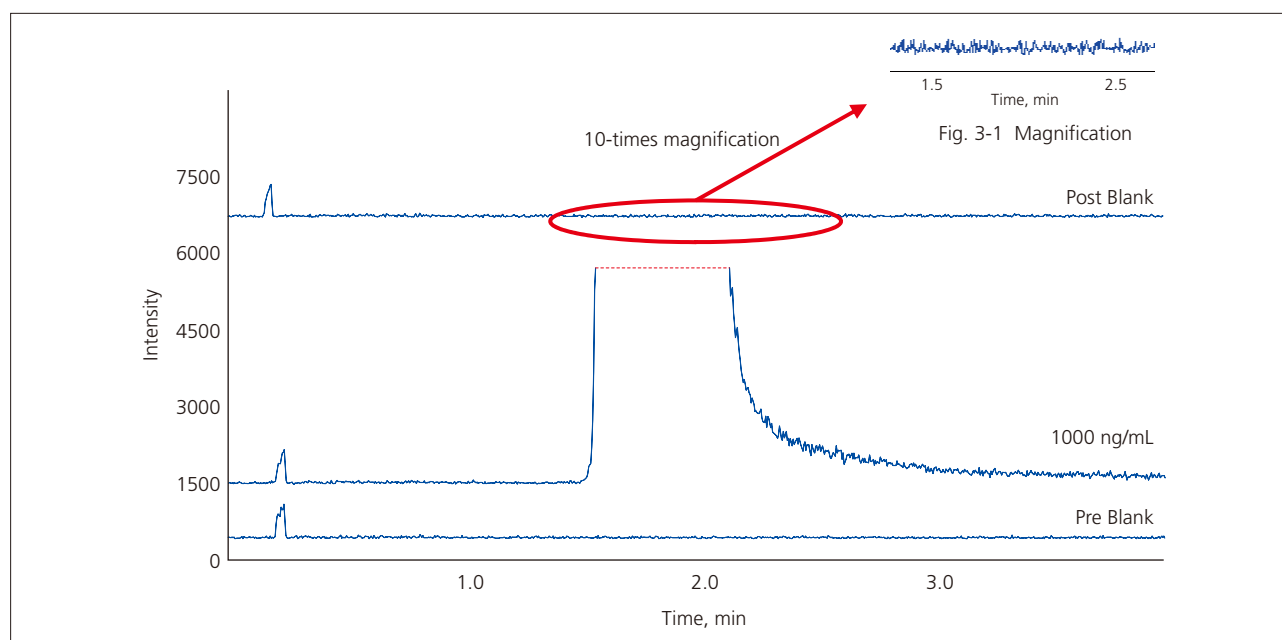
One possible source of carryover of a drug substance in plasma is the adhesion of phospholipids in the plasma to the sampling device, and the secondary adhesion of the drug substance on top of that. Therefore, considering that phospholipids are easily dissolved in 2-propanol, we programmed internal needle washes with high-concentration 2-propanol as "R1" and a combination of 2-propanol with other solvents as "R2", conducting 2 cycles of alternating washes with R1 and R2, using 300 µL of wash solution for each wash. Additionally, the external surface of the needle was washed for 1 second with "R3" (same solution as "R1"), using the active rinse pump.

Fig. 3 shows the results of the carryover evaluation for glibenclamide in human plasma.

First, we injected a human plasma blank (sample prepared according to Fig. 2, but using methanol instead of glibenclamide). That MRM chromatogram is denoted as "Pre Blank". Next, after injecting 3 µL of sample containing 1000 ng/mL of glibenclamide in human plasma (denoted as "1000 ng/mL" in Fig. 3), the plasma blank was run again (denoted as "Post Blank"), and then carryover was evaluated based on the glibenclamide elution position (in the vicinity of 1.7 minutes). A 10-times magnification of that area of the chromatogram is shown in Fig. 3-1, and it is clear that glibenclamide is not detected. These results indicate that carryover was less than 0.005 ng/mL (less than 0.0005 %). Thus, in LC/MS/MS drug analysis using human plasma samples, the Nexera SIL-30AC autosampler can deliver excellent low-carryover performance using the rinse/wash methods described here.

**Table 1 Analytical Conditions**

<HPLC Conditions>		[Auto Sampler Option]	
Column	: ODS column (50 mm L. × 2.0 mm I.D., 2.3 µm)	Wash Solution	
Mobile Phase	: A: 0.1 % Formic acid in water B: 0.1 % Formic acid in acetonitrile Isocratic elution, A/B = 7/3	R0 : Mobile phase A	
Flow Rate	: 0.5 mL/min	R1 : 50 % 2-Propanol	
Column Temperature	: 40 °C	R2 : Acetonitrile / 2-Propanol / Water / Formic acid (700/200/100/2)	
Injection Volume	: 3 µL	R3 : 50 % 2-Propanol Internal Rinse	
<MS Conditions>		R1 → R2 → R1 → R2 → R0	
Ionization Mode	: ESI positive	Wash volume 300 µL each	
	Glibenclamide ( 494 > 369)	External Rinse	
		R3 1 s	
		Rinse Mode	: Before and after aspiration
		Rinse Method	: Rinse pump then port



**Fig. 3 Carryover Evaluation of Glibenclamide in Human Plasma**

Note) The published data was not acquired using an instrument registered by Japanese pharmaceutical affairs law.

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> Nexera series  
Ultra High Performance Liquid Chromatograph

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