

SIL-HTc COMPETITIVE CARRYOVER PERFORMANCE ANALYZED BY LC-MS/MS: a Collaborative Study between Shimadzu Marketing Center and Covance Laboratories*

(*Madison, WI, USA)

Introduction

Advances in the field of drug discovery have resulted in a tremendous production of potentially therapeutic compounds and, thus, another “bottleneck” downstream from synthesis to drug development. Candidate compounds need to be rapidly analyzed for structure elucidation and evaluated for efficacy and toxicity. Bioanalysis is now perhaps the most prominent activity in drug development and the preferred tools for these types of analyses are High Performance Liquid Chromatography (HPLC) coupled with triple quadrupole mass spectrometry (LC-MS/MS). Successful development of accurate and dependable bioanalytical methods is therefore extremely critical. Method development to determine levels of drugs and their metabolites in biological fluids can present an array of analytical problems that can interfere with the assay. The causes of assay interference are often attributed to *sample carryover*, particularly with “sticky” compounds having an affinity for the flow-path composition and/or uncleared micro dead volumes in the HPLC system and, in particular, the autosampler. In addition, the increased sensitivity of triple quadrupole mass spectrometry tends to magnify the detection of carryover. Carryover has been conventionally reported using UV detection. Mass spectrometry was used in this collaborative study with Covance Laboratories (Madison, WI, USA) as a more sensitive determination of sample carryover using the new high-speed SIL-HTc autosampler. Carryover performance of the SIL-HTc was compared to that of other commercially available HPLC autosamplers on the market.

Experimental

Equipment

LC-MS/MS Components:

- (1) SIL-HTc High Throughput Autosampler (Model C has Cooling Capability) with built-in system controller (below).
- (2) Commercially available HPLC Autosamplers denoted as: A, B, C and D.

SIL-HTc High Throughput Autosampler



All autosamplers were configured with the same two Shimadzu LC-10ADvp analytical pumps (high pressure mixing) and MS/MS (Sciex API 365) for direct comparison of carryover performance.

LC-MS/MS Conditions

The Shimadzu LC system was configured with the SIL-HTc and the commercially available autosamplers (A, B, C or D) and were coupled with a Sciex API 365 triple quadrupole mass spectrometer. A typical C18 (50 x 4.6mm, 3.5 μ m particle size) column was employed; flow rate: 0.400mL/min.; column temperature: 40 °C; injection volume: 20 μ L.

The mobile phase consisted of: (a) 5 mM ammonium formate in 0.1% formic acid: 0.1% formic acid in

acetonitrile (55:45, v:v). The mass spectrometer was operated in positive ionspray mode. MRM ions for Compound X: 386.3/167.1; dwell time: 300 msec; capillary voltage: 2.5 kV; source temperature: 400 °C; collision gas: N₂; collision energy: 37V.

Sample Preparation

Sample Compound X (**Covance Laboratories**, Madison, WI, USA) was used as the model compound for testing the carryover of various HPLC autosamplers. Sample solutions were prepared by diluting the stock standard solution of Compound X (1.00 mg/mL in methanol) with methanol/water (1:1,v/v). The tested samples included high standard (1.00 µg/mL), low standard (0.500 ng/mL) and blank solvent (5 mM ammonium formate: acetonitrile: formic acid, 60:40:0.1 v: v: v).

Test Procedure. For each test, 20 µL of a blank sample was injected into the LC-MS/MS immediately

after a high calibration standard. The percent carryover was calculated as the relative peak area of the blank against that of the high calibration standard.

Note 1: The sample for the study was proprietary and therefore structural information was not available; however, the sample was described to be basic and very hydrophobic

Note 2: The rinse protocols for the other autosamplers may have included extensive rinsing with one or more solvents to eliminate carryover. The SIL-HTc was used in default, 20-second cycle time without extensive rinsing.

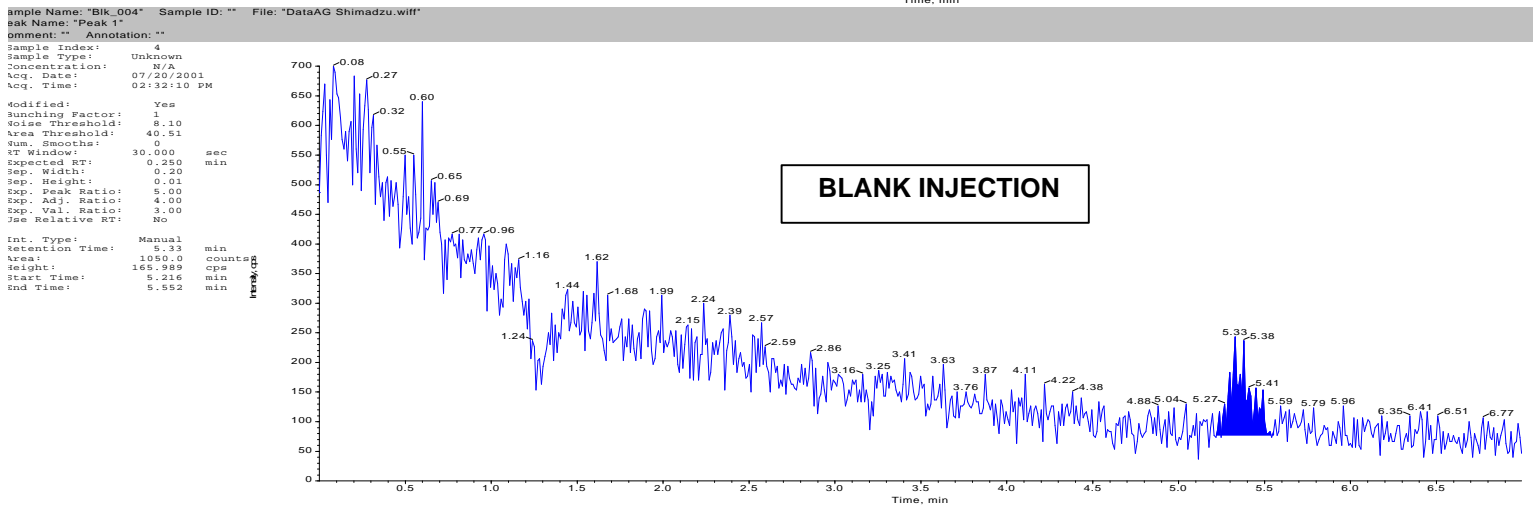
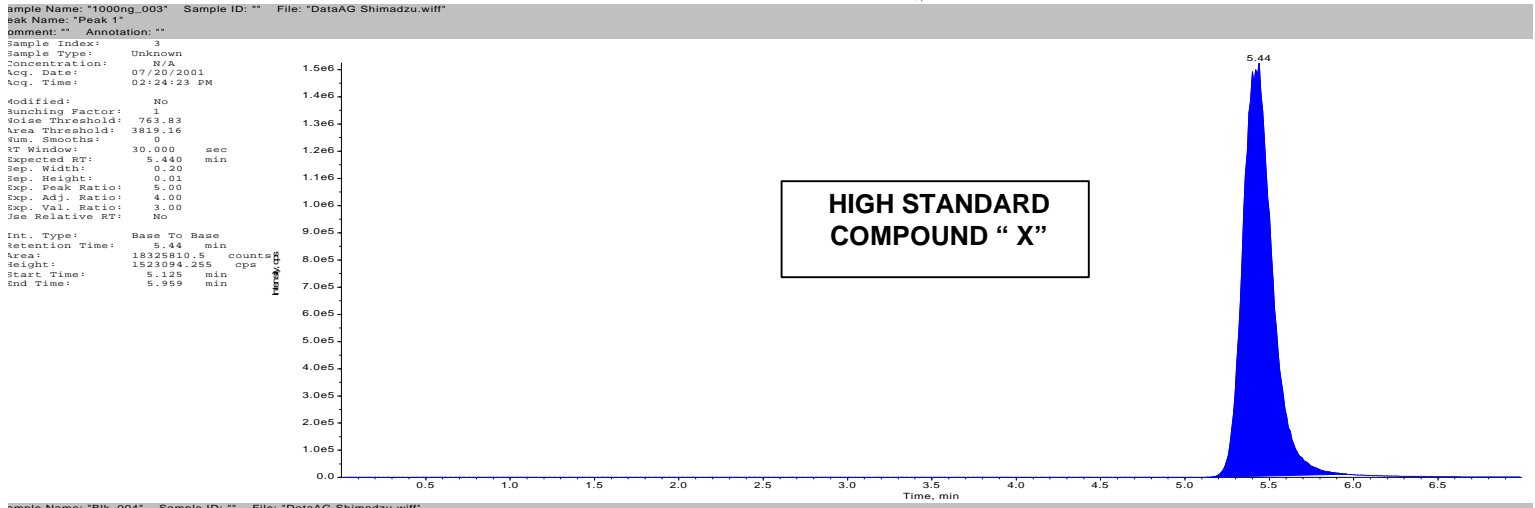
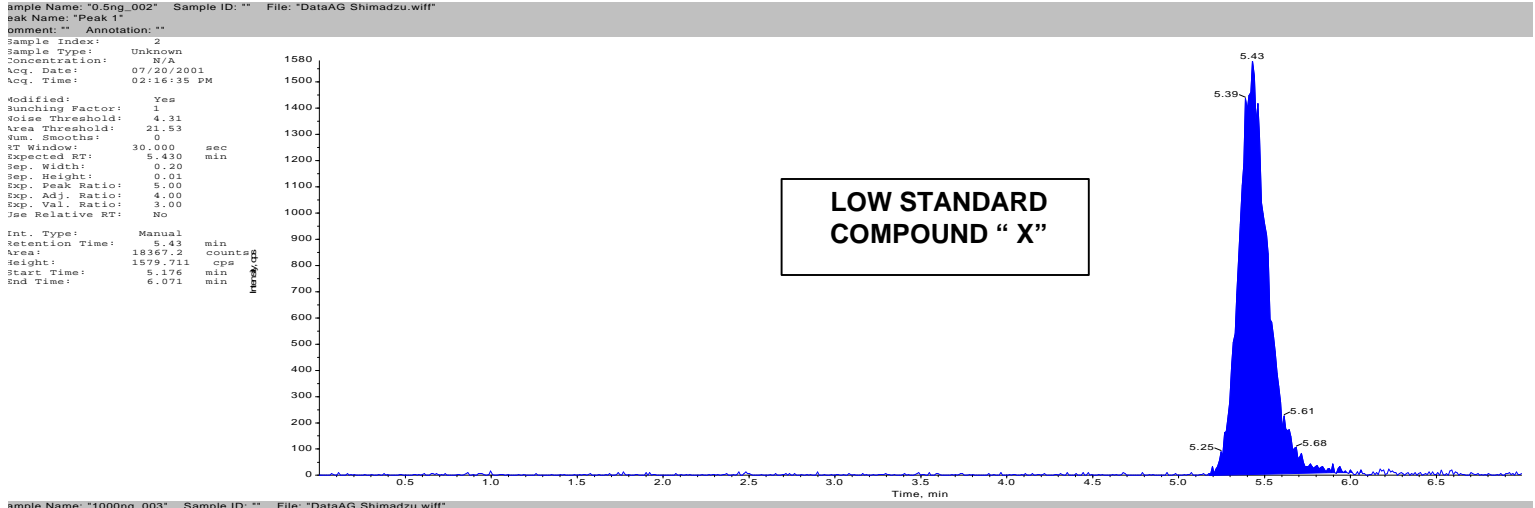
RESULTS

Table 1: Percent carryover comparison of the commercial autosamplers and the SIL-HTc

AUTOSAMPLER	% CARRYOVER
A	0.0129
B	0.00924
C	0.0170
D	0.0144
Shimadzu SIL-HTc	0.00572

According to the results in **Table 1**, the SiL-HTc autosampler outperformed or gave the least amount of measurable carryover (~ 2 to 3 times less) compared to the other autosamplers tested (see FIGURE 1 below). As mentioned in **Note 2** above, extensive rinsing, multiple rinse solvents (when applicable) and/or both were used for the other autosamplers. Extensive rinsing correlates with increased cycle time. The *SIL-HTc* was tested using the default injection routine consisting of a 20-sec injection cycle time without employing extensive rinsing.

FIGURE 1: TYPICAL CHROMATOGRAM of SIL-HTc CARRYOVER



CONCLUSION

Sample carryover has progressively become more of a problem in drug development, especially in the area of bioanalysis, for a number of reasons. Given that carryover is dependent upon compound structure and thus the resulting chemical properties of that structure in a multitude of different environments, there are other obvious variables to consider. The sample matrix or vehicle is critical when developing an accurate and quantitative assay. The compound and its metabolites are most likely in biological fluids such as blood, urine plasma and or serum to name a few, which further complicates assay development because of the very nature of these fluids (proteins, salts and other interfering components). The compound/metabolites then usually need to be extracted before reconstituting into an appropriate solvent or buffer for HPLC-MS, MS-MS analyses. Providing that most of these problems have been addressed, the analytical equipment is then suspect and the carryover seems to be magnified by employing the increased sensitivity of a mass spectrometer as the detector. The autosampler would then be the most logical component of the HPLC system contributing to sample carryover.

Shimadzu has approached the carryover problem with a newly designed high throughput autosampler, the SIL-HTc. The rapid cycle time does not sacrifice throughput and the extremely low carryover makes it the perfect autosampler for these types of HPLC-MS-MS applications and others. It was clear that the SIL-HTc had the least amount of carryover in this study. Shimadzu incorporated several new design features in the SIL-HTc to accomplish this. The newly engineered SIL-HTc development features include:

- (a) special metallic coating of the sample needle
- (b) the use of a PEEK™ rotor seal
- (c) modification of the flow paths

As a result, the new SIL-HTc had the least amount of sample carryover, minimal rinsing with a rapid cycle time of 20 seconds.

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