CARRYOVER MITIGATION USING NEEDLE WASH SOLVENT CHEMISTRY AND AUTOSAMPLER FEATURES OF A UPLC-MS SYSTEM

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INTRODUCTION

The greater sensitivity provided by mass detection is driving a need for improved carryover mitigation in LC applications. Carryover has many sources, but the most common source of carryover is related to the autosampler and occurs when sample components adhere to or absorb onto the outside of the needle. A key attribute of modern autosamplers is the ability to effectivity remove sample from all active surfaces of the autosampler. The design of the sample flow path in the autosampler, features controlling needle washing, and the chemistry of the wash solvent all play a role in eliminating detectable carryover.

In this study, an ACQUITY UPLC H-Class PLUS system configured with an ACQUITY QDa Mass Detector is utilized to examine the impact of wash solvent chemistry and autosampler features for controlling carryover. Granisetron HCl, an antinauseant and antiemetic agent commonly used in cancer therapy[1], was selected as the model compound for this study. The study is designed to quantify carryover to 0.0002% or 2pg on column, to this end the ACQUITY Diverter Valve, installed on the ACQUITY QDa, was programmed to divert the flow to waste when the highly concentrated Granisetron challenge sample was injected. This precaution was taken to avoid saturation of the QDa mass detector which would interfere with accurate quantitation.

METHODS

UPLC System:	ACQUITY UPLC H-Class PLUS System (176810005), ACQUITY QDa detector and a single column heater with the Active Pre-heating (CH-A).
Column:	UPLC HSS T3, 1.8 µL 3.0 x 5 0 mm (186004679)
Column temp.:	35 °C
Sample temp.:	15 °C
Injection volume:	1 µL
Flow rate:	0.9 mL/min
Needle wash solvent:	Water:acetonitrile, 90:10 and 50:50 Water:methanol, 90:10 and 50:50 Acetonitrile, 100% Methanol, 100%
Needle wash mode :	Default (6 seconds post-injection) 6 seconds pre & post-injection 12 seconds pre- and post- injection
Mobile Phase A:	0.1% Formic acid in water
Mobile Phase B:	0.1% Formic acid in acetonitrile
Gradient:	Isocratic 80:20 (mobile phase A : mobile phase B)
Run time:	3 minutes,
SIR:	313.1 m/z
Data Management:	Chromatography Data Software Empower 3 FR3 Hotfix 1

SAMPLES

Granisetron HCl (Catalog#: PHR1616) was purchased from MilliporSigma.

All solutions were prepared in 85:15, water:acetonitrile (diluent). The Granisetron challenge solution was prepared at 1 mg/mL. This solution was then serially diluted to 0.0002, 0.0005, and 0.0010% of the challenge solution (2, 5 and 10 ng/mL respectively) which were used to generate the calibration curve for quantitation. These calibrators represent 2, 5 and 10 pg on column. The concentration of the calibrators was selected based on the mass detection linear dynamic range. Sample diluent was used for pre-standard and post-challenge blank sample injections.

RESULTS & DISCUSSION

STUDY DESIGN AND QUANTIFICATION OF CARRYOVER

Granisetron HCl was analyzed on an ACQUITY UPLC H-Class PLUS System configured with an ACQUITY QDa w/ ACQUITY Diverter Valve. Three replicate injections were performed for each wash solvent and needle wash mode tested.

The method selected to evaluate carryover employed a highly concentrated challenge solution. The ACQUITY Diverter Valve[2], installed on the ACQUITY QDa, was programmed to divert the flow to waste when the highly concentrated Granisetron challenge sample was injected. This precaution was taken to avoid saturation of the QDa mass detector which would interfere with accurate quantitation.

A three-point standard calibration curve was generated to quantify carryover of the highly concentrated challenge sample. The calibration curve represents 0.0002% to 0.001% of the challenge sample. The concentration of the calibrators was selected based on the mass detection linear dynamic range. The sequence for this study was as follows: pre-standard blank, standard solutions (levels 1, 2 & 3), challenge solution, followed by 5 post-challenge blanks (Figures 1 & 2).

% Carryover was calculated using the following equation:
% Carryover = (calculated concentration in post-challenge blank / concentration of the challenge sample) * 100

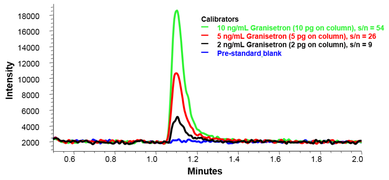

Figure 1: Mass Detected Calibrators. *The mass detected chromatographic results for the pre-standard blank and three calibrators. A flat baseline was established before the calibrators and challenge solution. The s/n for the calibrators was ≥ 9 .*

	Level	X Value (ng/mL)	Response (Area)	Calc. Value (ng/mL)	% Deviation
1	Level 1	2	15905	2.1	5.1
2	Level 1	2	14462	1.9	-4.0
3	Level 1	2	14106	1.9	-6.3
4	Level 2	5	36503	4.7	-5.8
5	Level 2	5	39507	5.1	1.8
6	Level 2	5	41758	5.4	7.4
7	Level 3	10	74837	9.6	-4.4
8	Level 3	10	78575	10.0	0.3
9	Level 3	10	81111	10.4	3.5

Figure 2: Calibration Results. *Calibration curve results used to quantitate carryover. The % Deviations of the calibrators demonstrate reliable low-level quantitation.*

IMPACT OF NEEDLE WASH SOLVENT CHEMISTRY ON CARRYOVER

The function of needle wash solvent is to solubilize the compound(s) of interest that remain on the sample needle after injection. Therefore, the composition of an optimal wash solvent is application and compound specific.[3,4] Important considerations when formulating wash solvent include the choice and ratio of organic to aqueous solvent as well as the addition of volatile pH modifiers to the wash solvent.[5,6] Optimization of the needle wash solvent chemistry is ideally performed during the development stages of an LC method. Granisetron HCl is readily soluble in water[7] therefore varying concentrations of water, acetonitrile and methanol in the needle wash solvent were investigated to demonstrate the impact of wash solvent composition. To prevent bacterial growth in the wash solvent, organic solvent was present in all wash solvents tested.

There was a noticeable increase in carryover when 100% organic solvent was used as the needle wash solvent. The data suggests that granisetron HCl may be more soluble in 100% methanol than 100% acetonitrile. Granisetron's ability to readily solubilize in water was evident in the wash solutions tested. The best results were found with a 50/50 mixture of water and acetonitrile. (Figures 3 & 4).

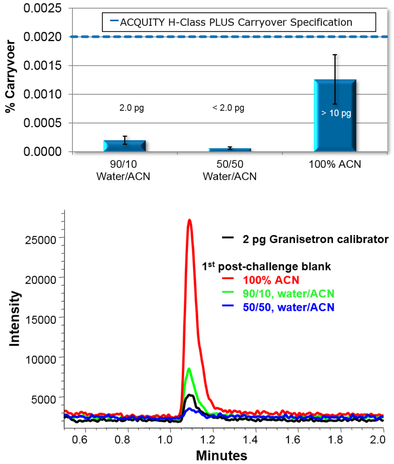

Figure 3: Carryover, Acetonitrile-Based Solvents. *Carryover results of triplicate injections for Granisetron using acetonitrile-based solvents as the needle rinse solvent. Needle wash program: Default, 6 sec. post-injection.*

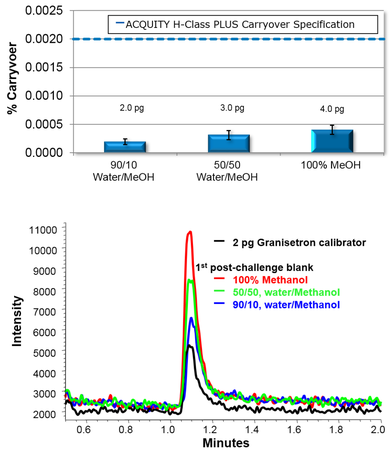

Figure 4: Carryover, Methanol-Based Solvents. *Carryover results of triplicate injections for Granisetron using methanol-based solvents as the needle rinse solvent. Needle wash program: Default, 6 sec. post-injection.*

IMPACT OF NEEDLE WASH MODE AND AUTOSAMPLER DESIGN ON CARRYOVER

The ACQUITY UPLC H-Class PLUS Sample Manager FTN employs a flow through needle design where the needle is part of the flow path (Figure 5) and the interior of the needle is flushed by the chromatographic mobile phase.

The exterior of the needle is actively washed with wash solvent which enters at the bottom of the injection/wash port and exits to waste at the top of the injection/wash port. This design ensures that the portion of the needle exterior that contacts the sample is washed with clean, fresh wash solvent. The exterior needle wash can be performed pre-injection and/or post injection for a duration from 0 up to 99 seconds. During the pre-injection exterior needle wash, the needle is positioned above the needle seal and the wash solvent begins to flow before the needle is lowered into the needle seal. Performed by default, the post-injection exterior needle wash occurs after the needle is in the seal position and the injection has begun.

IMPACT OF NEEDLE WASH MODE AND AUTOSAMPLER DESIGN ON CARRYOVER (Continued)

The volume of the combined injection/wash port of the ACQUITY UPLC H-Class PLUS FTN-H is approximately 40 µL. In the default wash mode (6s post-injection) the injection/wash port is flushed with approximately 25x injection/wash port volumes. This large flush to port volume ratio minimizes the possibly of residual contamination remaining in the injection/wash port.

The dedicated wash solvent delivery pump & sample syringe prevent the wash solvent from entering the sample stream containing mobile phase as these are separate flow paths.[5] This provides greater freedom in formulating the composition of the wash solvent.

Overall the wash solvent composition must be strong enough and the duration of the wash long enough to dissolve any sample remaining on the exterior of the needle and fully flush the injection/wash port.

The wash solvent containing 100% acetonitrile was selected as the wash solvent to demonstrate the impact of needle wash mode on carryover for its visual impact. Carryover was reduced 3-fold when the needle wash mode was changed from the default wash (6 second post-inject) to a 12 second pre- and post-injection wash (Figure 6).

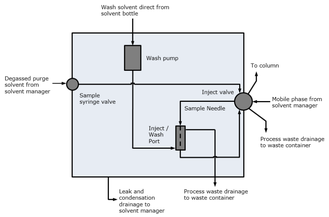

Figure 5: Autosampler Design. *The diagram shows how the SM-FTN PLUS functions as part of the ACQUITY UPLC PLUS system. One can choose two external needle wash modes, pre-injection or post-injection. Neither wash sequence allows wash solvent to enter the sample stream.*

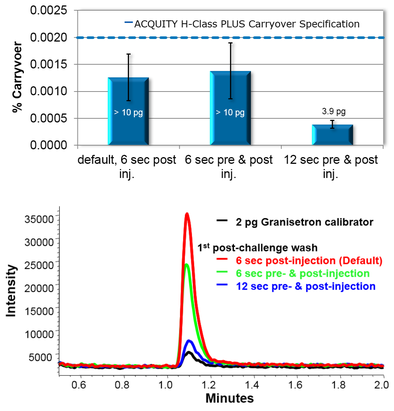

Figure 6: Needle wash Mode Impact on Carryover. *Chromatographic results of observed carryover with varying needle wash modes using 100% ACN as the needle wash solvent. The extra needle rinse time improved carryover 3-fold.*

CONCLUSION

Optimization of the needle wash solvent and wash settings are important aspects of LC method development. The needle wash solvent should be able to solubilize the components of the sample. A common starting place for many reverse phase applications is a mixture of water and the strong solvent used in the gradient. Investigation of the wash settings available on the autosampler should be included when optimizing needle wash during LC method development.

The efficient design of the ACQUITY UPLC H-Class PLUS FTN easily managed carryover from Granisetron HCl providing greater flexibility to manage carryover and increased reliability of quantitation for LC applications with minimal impact to cycle time.

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