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Alliance Carryover Performance Part 2: Carryover Improvement Achieved Through Needle Wash Optimization for the 2018 Alliance HPLC System

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APPLICATION BENEFITS

- Optimization of the needle wash mode and the needle wash solvent composition to reduce sample carryover on the 2018 Alliance™ HPLC System

WATERS SOLUTIONS

Alliance HPLC System

XBridge™ C_{18} Column

CORTECS™ C_{18} Column

Empower™ Chromatography Data Software

KEYWORDS

Carryover, seal pack, needle wash solvent, quetiapine fumarate, coumarin, Alliance HPLC System

INTRODUCTION

Sample carryover, a common problem for analytical laboratories, occurs when material from an injection is present in subsequent injections. There are several factors that can influence carryover including the chemistry of the analyte, the composition of the needle wash solvent, as well as the selected needle wash mode of the liquid chromatography (LC) system. The 2018 Alliance HPLC System employs a flow-through needle design and includes multiple wash modes to clean the exterior of the injector needle[1] with an appropriate wash solvent. Optimization of the needle wash composition and wash mode can significantly reduce sample carryover.

In this study, the 2018 Alliance HPLC System will be used to examine the impact of changes to needle wash composition and wash modes on resulting carryover. Two compounds, coumarin and quetiapine fumarate, will be used for the evaluation.

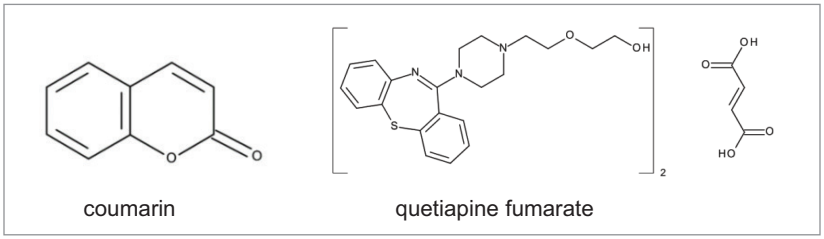


Figure 1. Structures of coumarin and quetiapine fumarate.

EXPERIMENTAL

LC conditions

LC system: 2018 Alliance: Alliance e2695 Separations Module with 100 µL syringe, 2998 PDA Detector, CH-30 equipped with passive column preheater, and the e2695 Enhancement Kit. Firmware 3.04.

Sample 1

Coumarin:	Challenge solution: 2 mg/mL coumarin in water. Standard solution: 0.2 µg/mL coumarin in water. Blank: water
Column:	CORTECS C_{18}, 2.7 µm, 3 mm × 100 mm (p/n: 186007372)
Column temp.:	30 °C
Sample temp.:	4 °C
Injection volume:	4 µL
Flow rate:	0.8 mL/min
Needle wash:	90:10 water:acetonitrile
Needle wash time:	Normal, double, extended
Mobile phase A:	Water
Mobile phase B:	Acetonitrile
Gradient:	Isocratic (90:10 mobile phase A:mobile phase B)
Run time:	15 minutes
PDA wavelength:	275 nm at 4.8 nm resolution

Sample 2

Quetiapine fumarate assay USP 40 NF35 S1

Quetiapine:	Standard solution: 0.16 mg/mL of quetiapine fumarate in mobile phase (standard stock solution for USP monograph assay). Blank: water
Column:	XBridge BEH C_8, 5 µm, 4.6 mm × 250 mm (p/n: 186003018)
Column temp.:	25 °C
Sample temp.:	4 °C
Injection volume:	50 µL
Flow rate:	1.3 mL/min
Needle wash:	90:10 water:acetonitrile 70:30 methanol:water
Needle wash time:	Normal, double, extended
Mobile phase:	54:7:39 methanol:acetonitrile:buffer premixed and filtered with 0.45 µm filter
Buffer:	2.6 g/L of dibasic ammonium phosphate adjusted to pH 6.5 with phosphoric acid
Gradient:	Isocratic
Run time:	15 minutes
PDA wavelength:	230 nm at 4.8 nm resolution

Data management

Empower 3 Chromatography Data Software, FR 3, Hot Fix 1

RESULTS AND DISCUSSION

STUDY DESIGN AND QUANTIFICATION OF CARRYOVER

In order to examine the impact of the needle wash settings and composition on sample carryover, coumarin and quetiapine fumarate were analyzed on the 2018 Alliance HPLC System equipped with the e2695 Enhancement Kit. Each compound was prepared individually and injected in replicates of six.

In this study, two methods to evaluate carryover were used. The first method uses a challenge solution, at a concentration in which the detector is saturated, and a standard solution at 0.01% of the challenge solution. The injection sequence for this study is as follows: pre-blank, standard solution, challenge solution, post-challenge blank. Carryover is then calculated by:

$$\% \text{ carryover} = (\text{post-challenge blank peak area}) / (\text{standard peak area}) * 0.01$$

This methodology was used to assess carryover for coumarin.

In the second methodology, the challenge solution falls within the linear range of the detector, allowing for the direct quantification of carryover based on the challenge sample solution. For this method, carryover is calculated by:

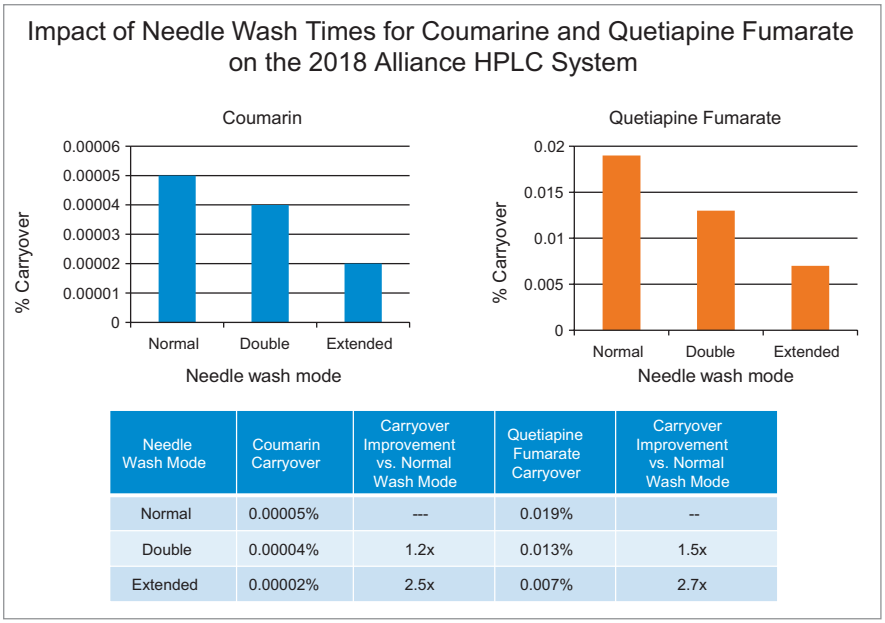
$$\% \text{ carryover} = (\text{post-challenge blank peak area}) / (\text{standard peak area}) * 100$$

This methodology was used for the quetiapine fumarate compound.

IMPACT OF THE NEEDLE WASH SETTING ON CARRYOVER

The needle wash settings that determine the exterior rinse of the injector needle can have a considerable impact on sample carryover. There are three needle wash mode settings available: normal (default), double, and extended. 'Double' increases the duration of the needle wash, which is performed prior to injection, while 'extended' enables washing of the needle before and after sample injection. Note: Firmware 3.04 is required for these changes in wash time and cycle.

For this study, the three needle wash settings were evaluated for the analysis of coumarin and quetiapine fumarate (Figure 2). As expected, carryover decreased when the wash mode was changed from 'normal' to 'double', and was further reduced using the 'extended' wash mode. The 'double' needle wash setting displayed a 1.2× and a 1.5× reduction in carryover for coumarin and quetiapine fumarate, respectively, over the default setting of 'normal'. The ability to wash the needle after an injection using the 'extended' wash setting is significant given that compounds that are prone to carryover might not be completely removed during the needle wash prior to the injection. Since coumarin and quetiapine fumarate are prone to carryover, using the 'extended' needle wash mode reduced carryover 2.5× and 2.7×, respectively, compared to the 'normal' wash setting. The carryover improvement was greater for quetiapine fumarate; however, using the advanced needle wash settings significantly reduced carryover for both compounds.

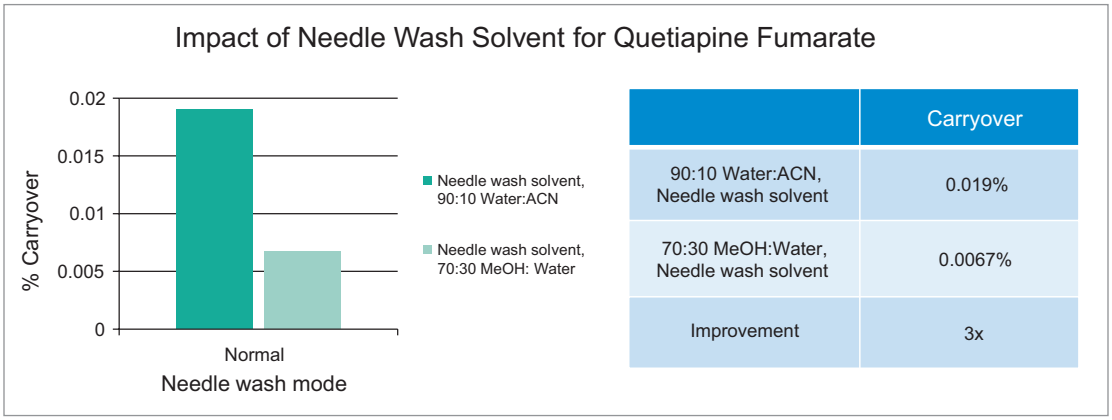


Needle Wash Mode	Coumarin Carryover	Carryover Improvement vs. Normal Wash Mode	Quetiapine Fumarate Carryover	Carryover Improvement vs. Normal Wash Mode
Normal	0.00005%	---	0.019%	--
Double	0.00004%	1.2x	0.013%	1.5x
Extended	0.00002%	2.5x	0.007%	2.7x

Figure 2. The observed impact of the advanced features for the needle wash modes for coumarin and quetiapine fumarate on the 2018 Alliance HPLC System.

IMPACT OF NEEDLE WASH SOLVENT ON CARRYOVER

In addition to wash settings, the composition of the needle wash solvent itself is important for reducing carryover. The needle wash solvent should readily solubilize the compound of interest; therefore it is application specific. The USP monograph assay method for quetiapine fumarate[2] does not provide any guidance on an appropriate needle wash solvent, therefore a generic solvent of 90:10 water: acetonitrile was originally used (Figure 3). The measured carryover is considerable and can be minimized by changing the composition of the needle wash solvent. To optimize the needle wash composition, select conditions (solvents and/or pH) that will effectively solubilize the sample compound. A good starting place is the initial elution conditions in the chromatographic method. The quetiapine fumarate assay uses a mobile phase consisting of 61% organic; therefore formulating the needle wash composition with a higher organic percentage should improve the solubility of the quetiapine fumarate onto the injector needle. In this example, using a slightly higher concentration of organic (70% methanol) in the needle wash solvent[3] allowed for the amount of carryover of quetiapine fumarate to be reduced by a factor of three (Figure 3).



	Carryover
90:10 Water:ACN, Needle wash solvent	0.019%
70:30 MeOH:Water, Needle wash solvent	0.0067%
Improvement	3x

Figure 3. Carryover results for the two different needle wash compositions for the quetiapine fumarate USP assay method on the 2018 Alliance HPLC System.

CONCLUSIONS

Choosing the optimal needle wash setting and needle wash solvent composition is essential to decreasing sample carryover of an HPLC method. When combined, the needle wash mode and the needle wash composition significantly reduce carryover on the 2018 Alliance HPLC System.

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