

Alliance Carryover Performance Part 1: Carryover Improvement Achieved Through Instrument Design Changes for the Alliance HPLC System

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APPLICATION BENEFITS

- Improved carryover performance for the 2018 Alliance™ HPLC System with the newly designed injector assembly

WATERS SOLUTIONS

[Alliance HPLC Systems](#)

[XBridge™ C₁₈ Columns](#)

[CORTECS™ C₁₈ Columns](#)

[HPLC with UV Standards Kit](#)

[Empower™ Chromatography Data Software](#)

KEYWORDS

Carryover, seal pack, quetiapine fumarate, coumarin, chlorhexidine, caffeine, Alliance HPLC System

INTRODUCTION

Sample carryover is a common problem for analytical laboratories and may adversely affect chromatographic methods. Sample carryover occurs when material from an injection is present in subsequent injections. The impact of carryover may result in failing batches, out of specification results, and poor reproducibility, to name a few. There are several factors that can influence carryover including the chemistry of the analyte, the analytical column, and the design of the HPLC system injector assembly. For example, an HPLC system based upon a flow-through needle design can provide reduced carryover because the interior of the sample injection needle is continuously washed by the mobile phase. In addition, most LC systems include some type of needle washing mechanism to rinse the exterior of the needle with an appropriate wash solvent in order to further reduce sample carryover.

In this study, we will examine the improved carryover obtained after design optimization of the seal pack components within the Alliance HPLC System injector assembly. Four compounds, each with different chemical properties, were selected to demonstrate the injector design improvements.

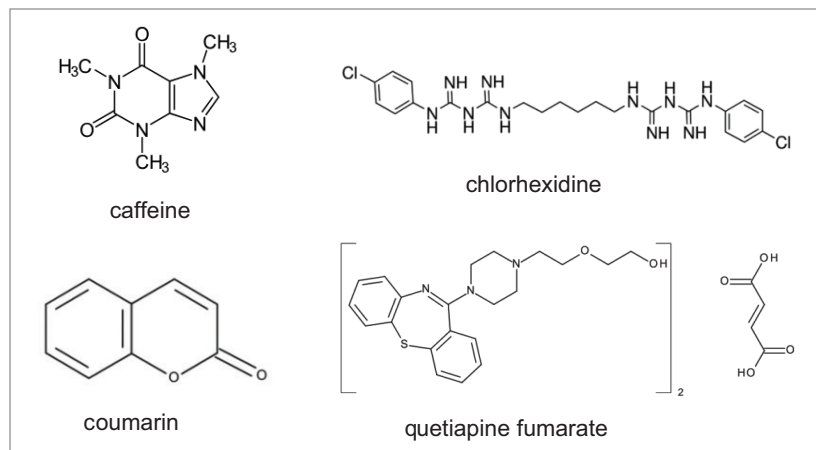


Figure 1. Structures of caffeine, chlorhexidine, coumarin, and quetiapine fumarate.

EXPERIMENTAL

LC conditions

LC systems

Alliance: Alliance e2695 Separations Module with 100 µL syringe, 2998 PDA Detector, and CH-30 equipped with passive column preheater. Firmware 3.03

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

Caffeine: Challenge solution: 4.0 mg/mL Caffeine (Solution 7), Standard at 0.01%: 0.4 µg/mL Caffeine (Solution 8), and Blank (Solution 9) from Waters HPLC with UV Standards Kit (p/n: [700003741](#))

Column: XBridge BEH C₁₈, 3.5 µm, 4.6 × 50 mm (p/n: [186003031](#))

Column temp.: 35 °C

Sample temp.: 4 °C

Injection volume: 10 µL

Flow rate: 1.0 mL/min

Needle wash: Methanol

Needle wash time: Normal

Mobile phase A: Water

Mobile phase B: Methanol

Gradient:

Time (minutes)	Mobile phase A (%)	Mobile phase B (%)
0.0	70	30
2.5	70	30
3.0	10	90
9.0	10	90
9.5	70	30

Run time: 23 minutes

PDA wavelength: 273 nm at 1.2 nm resolution

Sample 2

Chlorhexidine: Standard Solution: 1.0 mg/mL chlorhexidine in 0.1% TFA in water. Blank: 90:10 water:acetonitrile

Column: CORTECS C₁₈, 2.7 µm, 3 mm × 100 mm (p/n: [186007372](#))

Column temp.: 50 °C

Sample temp.: Room temp.

Injection volume: 5 µL

Flow rate: 1.0 mL/min

Needle wash: 50:50 water:acetonitrile

Needle wash time: Normal

Mobile phase A: 0.1% TFA in water

Mobile phase B: 0.1% TFA in acetonitrile

Gradient: Isocratic (67:33 mobile phase A: mobile phase B)

Run time: 10 minutes

PDA wavelength: 257 nm at 4.8 nm resolution

Sample 3

Coumarin: Stock solution: 8 mg/mL coumarin in methanol. Challenge solution: 2 mg/mL coumarin in water. Standard at 0.01%: 0.2 µg/mL coumarin in water. Blank: water

Column: CORTECS C₁₈, 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

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 4Quetiapine Fumarate Assay USP 40 NF35 S1¹

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 C₈, 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

Needle wash time: Normal

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 optimized injector design on sample carryover, multiple compounds with varying chemical properties were analyzed on the Alliance HPLC System, and the 2018 Alliance HPLC System equipped with the e2695 Enhancement Kit. The compounds include caffeine, chlorhexidine, coumarin, and quetiapine fumarate. Each compound was prepared individually and injected in replicates of six.

In this study, two methods were used to assess carryover. One method uses a highly concentrated challenge solution, which saturates the detector. Because an accurate peak area cannot be defined due to detector saturation, a standard solution prepared at 0.01% of the challenge solution concentration is also injected. The injection sequence is as follows: a 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 caffeine and coumarin.

In the second methodology, the challenge solution falls within the linear range of the detector, allowing for the 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 chlorhexidine and quetiapine fumarate.

INJECTOR DESIGN CONSIDERATIONS AND IMPACT ON CARRYOVER

The injector of the Alliance HPLC System uses a flow-through needle, which provides washing of the interior of the needle during the programmed method. The exterior surface of the needle is washed with an additional wash solvent for a specified time while the needle is seated in the seal pack of the injector assembly.² The 2018 Alliance HPLC System includes a re-designed seal pack that improves and optimizes the needle wash flow over the exterior surface of the injector needle. For existing Alliance systems, an e2695 Enhancement Kit is available to convert the injector components to the new seal pack design.

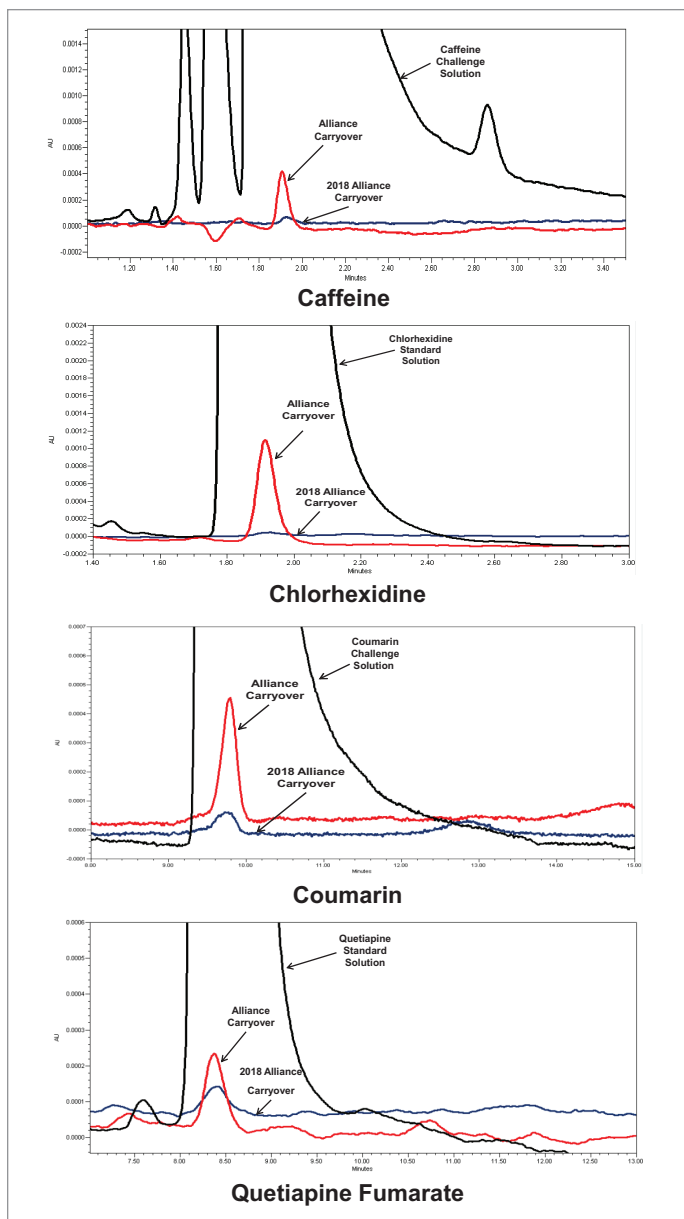


Figure 2. The chromatographic results for the Alliance HPLC System (red) and the 2018 Alliance HPLC System (blue) for the post-challenge blank are represented in the figure above for each of the compounds, caffeine, chlorhexidine, coumarin, and quetiapine fumarate. The black chromatogram represents the challenge solution for caffeine and coumarin while the black chromatogram represents the standard solution for chlorhexidine and quetiapine fumarate.

To illustrate the impact of these changes, carryover of four analytes was evaluated on the Alliance HPLC System, and the 2018 Alliance HPLC System (Table 1 and Figure 2). Two common compounds that are regularly used to assess potential carryover on an HPLC system are caffeine and chlorhexidine, while coumarin and quetiapine fumarate are compounds known to carryover on HPLC systems.^{3,4}

Both Alliance HPLC systems showed low levels of carryover for all compounds evaluated under the specified conditions. The 2018 Alliance HPLC System, however, was able to significantly reduce carryover further due to the improvements in the seal pack design. Specifically, caffeine carryover was reduced by a factor of seven, chlorhexidine carryover was reduced by a factor of twenty, coumarin carryover was reduced by a factor of three, and quetiapine carryover was reduced by a factor of one point five.

Compound	Alliance HPLC System	2018 Alliance HPLC System	Carryover improvement
Caffeine	0.0011%	0.00016%	6.9x
Chlorhexidine	0.010%	0.0005%	20x
Coumarin	0.0002%	0.00006%	3.3x
Quetiapine fumarate	0.028%	0.019%	1.5x
System specification*	≤0.01%	≤0.0025%	NA

Table 1. Carryover results for caffeine, chlorhexidine, coumarin and quetiapine fumarate on the Alliance HPLC System, and the 2018 Alliance HPLC System using the default 'Normal' wash mode setting. *The carryover instrument specification for the Alliance HPLC System⁵ and the 2018 Alliance HPLC System⁶ is based upon the compound caffeine under specified method conditions.

CONCLUSIONS

The 2018 Alliance HPLC System incorporates a newly designed seal pack which improves needle wash flow over the exterior of the injector needle. This newly designed seal pack results in a significant reduction in carryover for a wide range of chemical compounds. This data suggests that the 2018 Alliance HPLC System can reduce the amount of carryover present regardless of the specific compounds being analyzed.

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