

HPLC Carryover—Decreased Sample Carryover Using the Surveyor Autosampler

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Overview

Chromatographers have always been concerned with sample carryover. For the purpose of this application note, sample carryover is defined as sample from a previous injection detected during the analysis of the subsequent injections.

Sample carryover is observed when the needle, injection port, transfer tube, or valve is inadequately flushed. The results show that using an adequate amount of flush volume in the Thermo Scientific Surveyor Autosampler decreases the sample carryover in classical HPLC to a negligible amount.

Introduction

Sample carryover can cause serious problems with peak quantification and identification. The Surveyor Autosampler flush feature allows the user to flush the needle, injection port, transfer tube, and the valve. Once the flush liquid exits the valve, it goes to waste. This feature is programmable, allowing for a maximum sample pathway flush of 6 mL. The flush occurs automatically after each sample injection and can be manually activated using the direct commands feature.

In typical HPLC applications, liquid flow in tubing is laminar, which causes the liquid to travel faster in the center of the passage than along the walls. Therefore, a large amount of flush solvent is needed to properly flush the walls of the sample passage area.

Methods

These experiments were designed to measure the amount of sample carryover in the Surveyor Autosampler.

The first experiment, with data shown in Figure 2, is a series of 10 μL partial loop injections of 2000 ppm acetophenone. The first injection is followed by the default 400 μL flush, the second by a 1000 μL flush, the third by a 3000 μL flush, and the fourth by a 6000 μL flush of 100% methanol.

The second experiment, with data shown in Figure 4, is a 10 μL partial loop injection of a 2000 ppm solution of acetophenone followed by a 1000 μL flush of 100% methanol. In order to measure the amount of sample carryover, the next 25 injections are 10 μL partial loop injections of mobile phase. Each mobile phase injection is followed by a 1000 μL flush of 100% methanol. Since there are 25 mobile phase injections following one injection of 2000 ppm acetophenone, the sample trapped between the rotor and stator, retained inside the polymeric rotor material, is washed away as the valve rotates.

System Parameters:

Flow Rate: 1 $\mu\text{L}/\text{min}$
Mobile Phase: 55/45 MeOH/H₂O
System Pressure: 1600 psi
Injection Sample: 2000 ppm acetophenone
Injection Volume: 10 μL partial loop
Column Oven Temperature: 30°C
Syringe Flush Solvent: 100% methanol
Valve: Rheodyne®, 7739-039 for the Surveyor Autosampler
Column: Thermo Scientific Hypersil BDS column, 5 μm , 100 \times 4.6 mm

Calculations

Since UV detectors do not have the linear dynamic range to accurately quantify over five orders of magnitude, a 4 ppm peak was injected and the area extrapolated to 2000 ppm using the following calculation:

2000 ppm acetophenone peak area:

4 ppm Peak Area = 251,050 mAu

2000 ppm Peak Area = 251,050 mAu

* 50 = 125,525,000 mAu

% Carryover = (Carryover Peak Area / 2000 ppm Acetophenone Peak Area) * 100%

Key Words

- Surveyor™ Autosampler
- High-throughput Analysis
- Low Sample Carryover
- Hypersil™ BSD Column

The schematic in Figure 1 illustrates the sample pathway from the needle to the column. Once the sample is aspirated into the needle it is injected into the injection

port and travels through the sample transfer tube and into the valve. The valve is then rotated so that the sample will be transferred to the column.

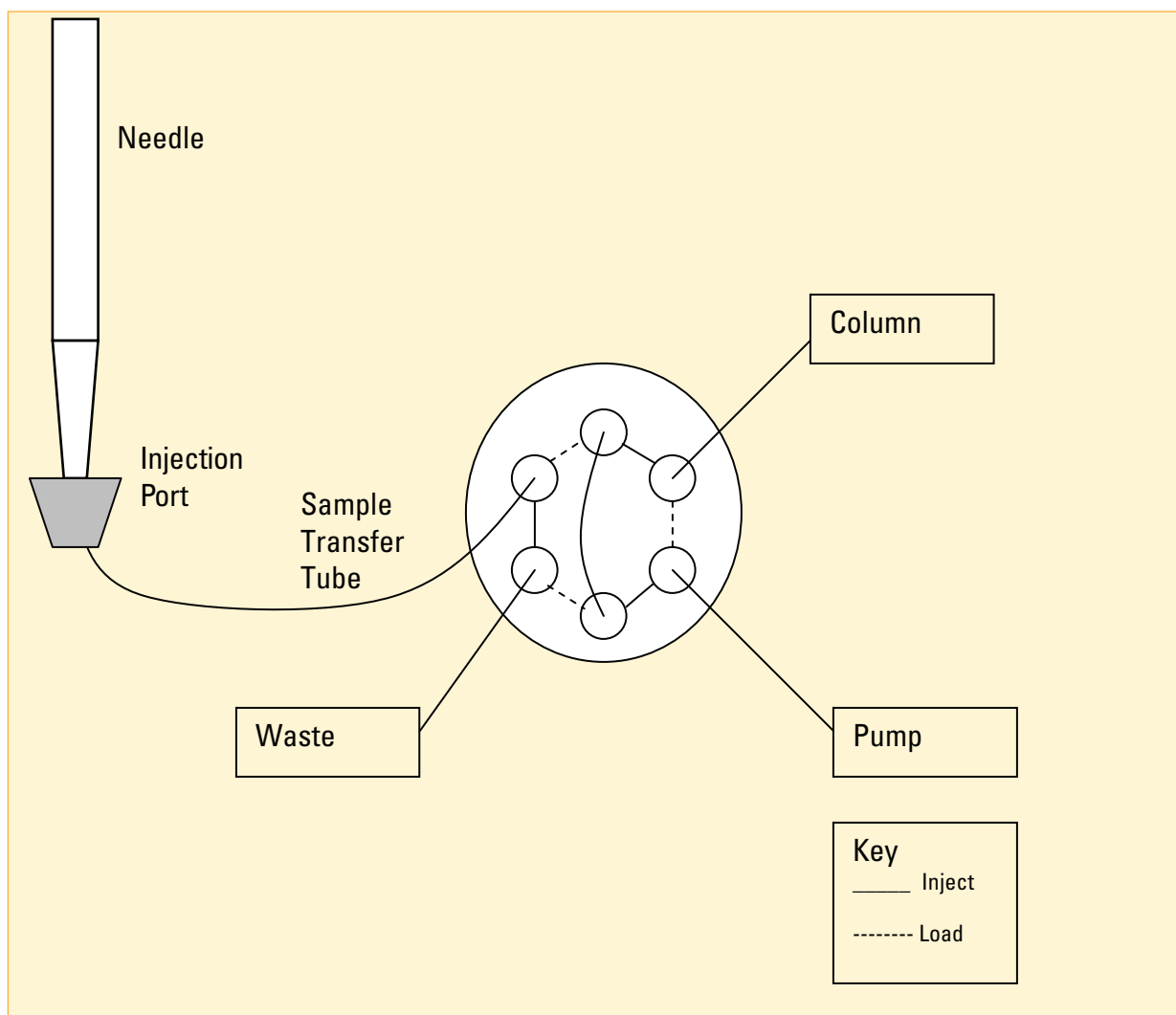


Figure 1. Schematic of sample pathway and valve

The results from experiment 1, displayed in Figure 2, show the percentage of carryover in the valve and solvent pathway does not vary much by increasing the flush amount. Whether the chromatographer uses the default

400 μL or the maximum 6000 μL flush, the percentage of sample carryover ranges from 0.0089% down to 0.0076%. The actual carryover is less than one tenth the carryover specification of 0.1%.

Results

	Peak Area	Carryover After Flush			
		1.0 $\mu\text{L}/\text{min}$	1.0 $\mu\text{L}/\text{min}$	1.0 $\mu\text{L}/\text{min}$	1.0 $\mu\text{L}/\text{min}$
2000 ppm acetophenone sample	125525000	11201	11196.2	10016	9538
Percent Carryover		0.0089%	0.0089%	0.0080%	0.0076%
Total Flush Solvent Volume (μL)		400	1000	3000	6000

Figure 2. Results from experiment 1: amount of carryover using different syringe flush volumes.

The chromatogram in Figure 3 shows an example of sample carryover from one injection to another. In this particular injection the sample carryover is a separate peak. A less desirable situation would be if the sample carryover was part of the desired peak because the area of the desired peak would be abnormally high and the user would not know why.

Figure 4 shows a decrease in the percentage of sample carryover relative to the number of flushes. A flush is automatically conducted after each mobile phase injection, meaning it is washing both the valve rotor and stator. After two 1000 μL flushes, the percentage of sample carryover seen in mobile phase run 2 is a mere 0.0023%. As the number of mobile phase injections increased, so did the amount of 100% methanol that was flushed through the sample pathway and valve. The sample carryover is 0.0008% after 6000 μL s have been flushed through the polymeric rotor material.

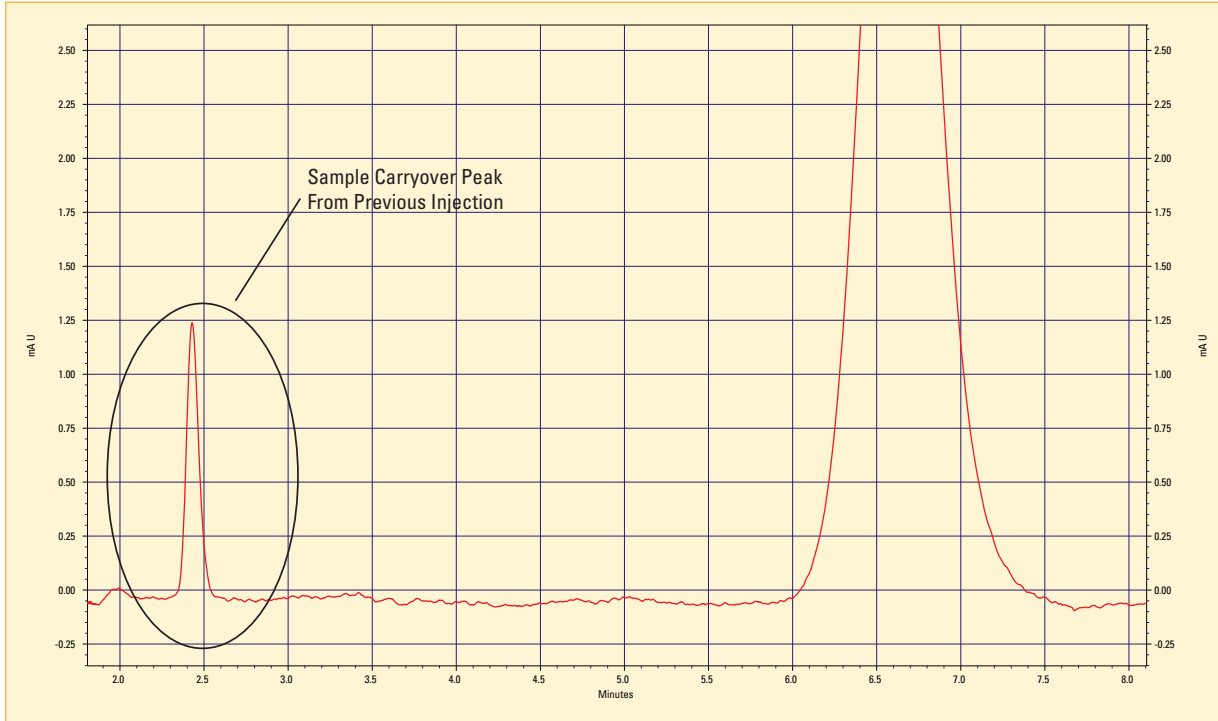


Figure 3. A chromatogram of the sample carryover peak.

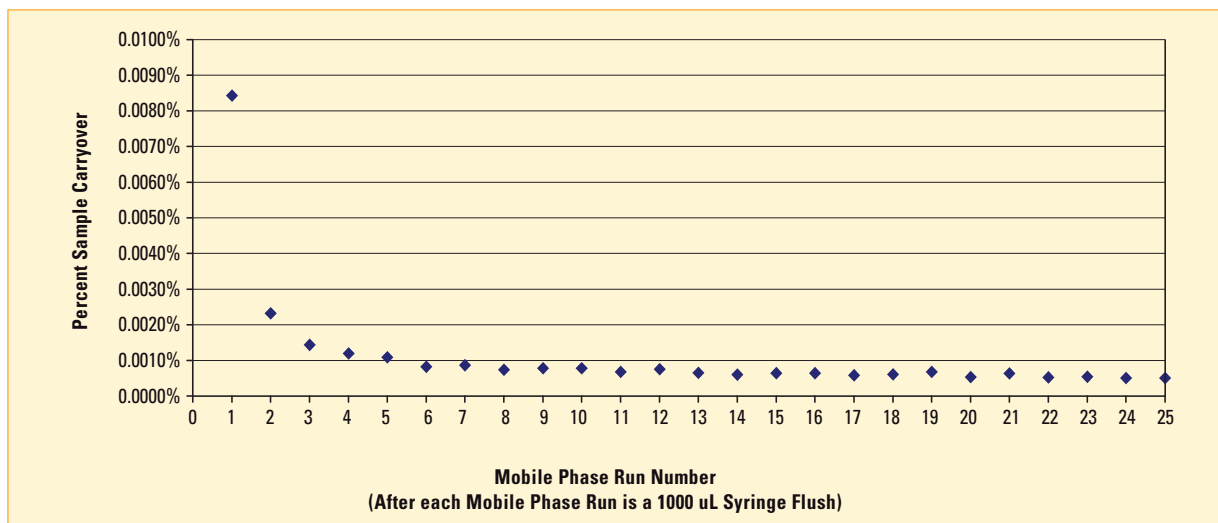


Figure 4. Percent sample carryover vs. mobile phase run number

Figure 5 was plotted on a logarithmic scale and shows two distinct slopes, indicating there are two distinct mechanisms taking place. The first is the flushing of the needle, injection port, transfer tube, and valve ports, and the second is the flushing of the sample that is retained inside the polymeric rotor material.

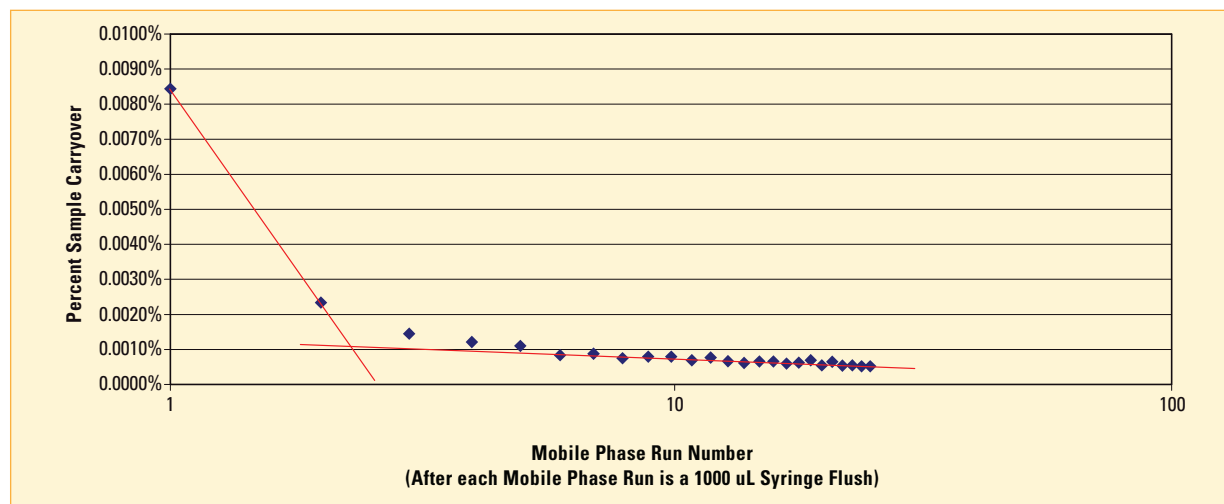


Figure 5. Percent sample carryover vs. mobile phase run number, on a logarithmic scale.

Conclusions

When a user conducts a normal flush, the needle, injection port, transfer tube, and valve ports are flushed. However, sample residue stays on the polymeric rotor material of the valve until it is rotated during the next injection. If the next injection is different than the previous sample, carryover will be noticed. The best method for completely flushing the system of carryover is to conduct a syringe flush while rotating the valve, ensuring the polymeric rotor material is flushed.

It has been demonstrated that with classical standalone HPLC, the Finnigan Surveyor Autosampler has an extremely low sample carryover of 0.0089% using the default 400 μ L flush and it can be as low as 0.0008%, if 6000 μ L are flushed through the solvent pathway and the polymeric rotor material with valve rotation.

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