

Troubleshooting Carryover

This document describes how to prevent and troubleshoot carryover in Waters UPLC, HPLC, LC/MS, LC/MS/MS, and Purification applications. It includes:

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NOTE: To be certain you have the [latest version of this procedure](#), please visit the *Waters Portal*.

Definition of carryover

Carryover is the appearance of material from a prior injection in subsequent injections, in the form of unexpected or extra (ghost) peaks. It is usually expressed as a percentage of the compounds in one sample injection found in the consecutive injection of a blank solution. It can compromise the quality of data obtained from even the most robust chromatographic methods.

Carryover occurs when a small amount of sample gets trapped somewhere in the wetted flow path. Although it can never be completely eliminated, it can be minimized to an acceptable level.

Dilution carryover

Often carryover appears in injections immediately after the original injection. Sometimes called dilution carryover, this type of carryover can be washed from the system with a couple of blank injections or by improving the washing procedures. An example is the overloading of columns with sample (i.e., concentration effects).

Adsorptive carryover

Carryover can also appear more slowly and randomly (i.e., several injections after the original one). This happens when a small amount of sample gets adsorbed to or stuck on a surface somewhere in the sample path (e.g., on a column or tubing) and elutes from the system more slowly. This type is sometimes called adsorptive carryover. In such cases more systematic investigation is necessary to find and fix the problem.

Distinguishing carryover from contamination

Extra peaks can arise from contamination as well as carryover. Although the terms carryover and contamination are often used interchangeably, carryover is actually a specific type of contamination. It refers exclusively to sample left over from a previous injection.

By contrast, contamination is the presence of *any* unwanted substance in an LC/MS system. Typical contaminants are impurities in organic solvents, residual mass spectrometer calibration solution, hand cream, detergents, metal ions, siloxanes, and the phthalates (plasticizers) that abound in laboratory environments.

More random in appearance and more difficult to troubleshoot, contamination degrades the quality of a chromatogram or mass spectrum by increasing the background, reducing the sensitivity, and introducing interfering ions.

Both carryover and contamination must be minimized during method development, assessed during method validation, and routinely monitored during study samples analysis.

To determine whether your extra peaks are the result of carryover or contamination, follow the process illustrated in [Figure 1](#).

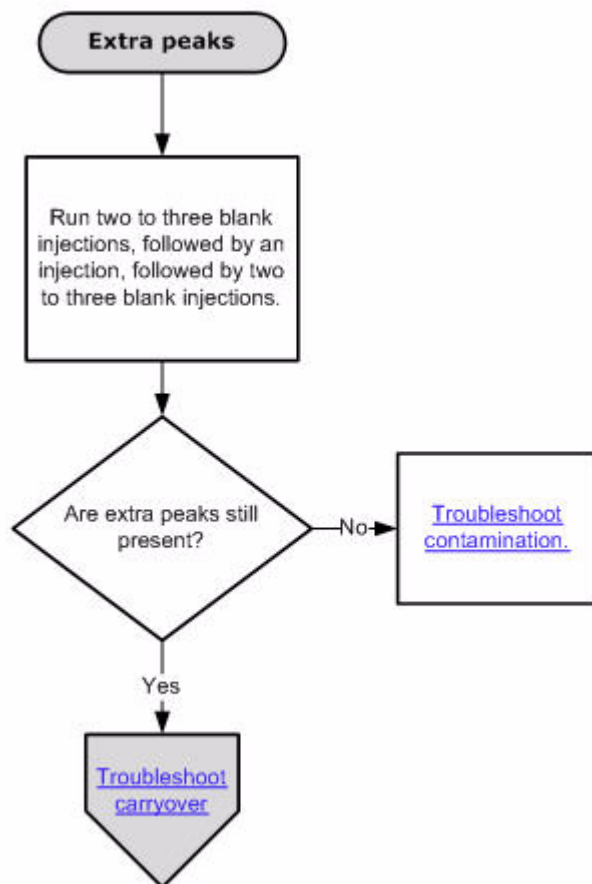


Figure 1 - Determining the cause of extra peaks

Troubleshooting carryover

Once you've determined that your extra peaks are the result of carryover, you must use a systematic process to find the root cause of the carryover.

[Table 1](#) lists possible causes of carryover and their corrective actions. After investigating each cause, retest for carryover (see [Testing for carryover, page 4](#)).

NOTE: Unless otherwise noted, the recommendations apply to all systems.

Table 1: Causes of carryover^a

Possible cause of carryover	Corrective action
Wash solvent problem	1. Use appropriate wash solvents (and replace or clean reservoir bottles). Strong wash must be at least as strong as the highest percent organic in the gradient. 2. Flush the sample manager several times (see Flushing the sample manager to remove carry-over, page 5). 3. If problem persists, increase the wash volume or number of wash cycles. 4. Use more organic solvent or even 100% of strong solvent in wash. WARNING: THE MATRIX ALSO MUST BE SOLUBLE IN THE WASH SOLVENTS. PROTEINS ARE NOT SOLUBLE IN >40% ORGANIC. 5. Adjust wash pH. See your system's operator's manual for solvent recommendations.
Wash probe vent not selected (purification only)	1. Ensure wash probe vent box is selected in the Wash tab of the sample manager. 2. Prime the 2767 (from the Inlet method page, select Waters 2767 and then Prime system).
Sample inject volume to the 2767 has changed (purification only)	1. Verify appropriate injector volumes, depending on 2767 configuration. 2. Ensure that syringe and holding loop match.
System includes multiple sample types or large (20-30) sample batches	Run a blank injection between different sample types and batches.
Gradient not strong enough to flush the loop	Modify the end of the gradient to include high organic for a longer time. See Testing for carryover, page 4.
Poor sample solubility	Ensure sample is soluble in both the weak and strong wash solvents.

Table 1: Causes of carryover^a

Possible cause of carryover	Corrective action
Wrong injection mode	Use appropriate injection mode. NOTE: For UPLC only: Disable loop offline and load-ahead techniques when troubleshooting. In general, partial loop mode exhibits the least carryover; because sample is diluted by the weak wash in the loop, less of it is introduced. In addition, this mode does not introduce sample to the VDD. See Injection mode parameters, page 6 .
Tubing is contaminated or defective	Replace the tubing.
Kalrez O-ring in the wash station is contaminated or defective (UPLC only)	Replace the O-ring. See Replacing the O-ring .
Wash station is contaminated or defective	Clean or replace the wash station. See Replacing the Needle Wash Housing .
Sample loop is contaminated or defective	Flush with mobile phase between injections.
	Check/tighten fittings. If necessary, replace sample loop (see your system's operator's guide).
Column contaminated	Remove the column and perform a direct injection
Sample needle/probe is damaged or defective	Replace the sample needle/probe (see your system's operator's guide).
Sample needle is incorrect material for the application	Try a different needle material. NOTE: PEEK needles are more likely to cause carryover than stainless steel needles. PEEKsil needles are preferable to PEEK needles but are not as good as stainless steel.
Syringe or injection port/wash station is dirty	Flush the port or wash station with suitable solvent and use a clean syringe if necessary.
Piercing needle or probe clogged or touching sample	Replace piercing needle or probe.
Piercing needle touching sample	Adjust needle position or sample volume.
Sample contaminated	Replace with clean sample (use clean vial).
Use of non-recommended cap mats or septa	Use Waters-recommended cap mats and septa.
Injector cartridge (POD) defective (UPLC only)	Replace injector cartridge.
Volume detection device (VDD) is contaminated or defective (UPLC only)	Replace the VDD. See Calibrating the Volume Detection Device .
Blow or suck (BOS) tube clogged (UPLC only)	Replace BOS tube.
Column contaminated	Replace the column.

a. Unless otherwise noted, the recommendations are universal to all integrated systems.

Testing for carryover

To test for carryover, use a high sample concentration followed by two blanks.

Flushing the sample manager to remove carryover

NOTE: Some of the recommendations regarding solvent washes may need adjustment depending on your application.

To flush the sample manager, follow the steps below. After each step, check to see if the problem is resolved. If the problem persists, go to the next step.

1. Use another injection mode.
2. Increase wash solvent volumes. If problem persists:
3. Change the solvent composition. Adjust the pH of the strong and weak wash solvents.
4. Adjust strong-to-weak wash ratio to 1:3. Ensure concentration of strong solvent is no stronger than needed.

NOTE: Strong wash must be at least as strong as the highest percent organic in the gradient.

5. Ensure weak wash is the same as the initial mobile phase. Try flushing with the sample loop offline. This may flush out sample residues in the Rheodyne dead volume.

Sample loop offline parameters:

- Delay time = $N \times \text{loop volume} / \text{flow rate}$ ($N \geq 5$)
- Increase strong wash volume.
- Use a washing time that outlasts the loop cycle.

Recommendation (UPLC only): Never use sample loop offline if carryover is a problem. It is the most likely cause.

6. For UPLC only: If problem persists: manually flush the system (from the Console).

Considerations when transferring HPLC methods to UPLC

When transferring a method from HPLC to UPLC, it is particularly important to optimize the method. HPLC has larger dispersion, dwell volume, and column volume than UPLC. Thus a peak introduced into an HPLC system is three times more diluted than that in an ACQUITY UPLC system.

The following practices are recommended:

1. Measure dispersion by looking at bandspread, or how much peak broadening occurs. Typical dispersion is 10 to 12 μL for ACQUITY UPLC, and 35-50 μL for HPLC.

NOTE: For more information on measuring dispersion, see "Measuring extra-column bandspreading of the system" in [Troubleshooting the ACQUITY UPLC System](#).

2. Add a blank injection after every 20 to 30 samples injected.
3. Ensure that final conditions are at organic strength and at sufficient time to allow the system fluidics to be fully exposed to the highest strength.
4. Randomize the order of injection for samples within the sample list.

5. Understand the use of loop offline and load-ahead techniques (see [Table 2](#)).

Table 2: Injection mode parameters

Injection mode	Injection volume on column	Sample used and in contact with parts of SM	Carryover considerations
Partial loop (pressure assist)	1 µL	1 µL	Best choice to prevent carryover, because: • Weak wash dilutes sample in the loop, so less of the sample is introduced. • This mode does not expose the VDD to sample. NOTE: Use partial loop mode for proteomic applications.
Partial loop needle overfill (PLUNO)	1 µL	16 µL	Risk: exposes the VDD to sample. To avoid carryover, use the maximum overfill factor with loop offline programmed.
Full loop	10 µL	40 µL	To avoid carryover, use the maximum overfill factor. NOTE: For proteomic applications, use full loop only for quantification studies, where reproducibility is important.
Loop offline and load-ahead techniques			Disable this mode when troubleshooting. Avoid loop offline whenever you suspect carryover.