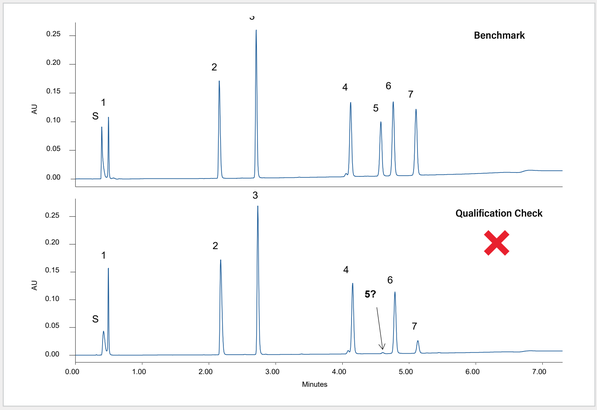
HPLC Troubleshooting: A System Monitoring Approach



Step 1

Benchmark Performance
Use a Quality Control Reference Material (QCRM) to **establish a benchmark** on your LC system when the system is in a **known good state**, such as after installation or performance maintenance and testing by a service engineer.

Step 2

Qualification Check
Perform periodic qualification checks to confirm **continued good system performance.** A failed qualification check should trigger troubleshooting to identify the cause.

For best troubleshooting results, use a qualification standard that contains a variety of analyte types, such as Waters Reversed Phase QCRM, P/N 186006363.

Step 3

List Failure Indicators
Identify which failure indicators are present in your qualification check chromatogram from the menu below. In the example to the left we have two failure indicators:

1. Missing Peak (Peak 5)
2. Decreased Peak Area (Peak 7)

Step 4

List Analyte Properties and Pass/Fail Category
Identify the properties of your peak analytes and list whether they pass or fail the qualification check:

1. Uracil – polar, neutral, PASS
2. Propranolol – basic, PASS
3. Amitriptyline – basic, PASS
4. Butylparaben – neutral, PASS
5. Naphthalene – neutral, volatile, FAIL
6. Dipropyl phthalate – neutral, PASS
7. Acenaphthene – neutral, volatile, FAIL

Step 5

Eliminate Causes that are Not Consistent
If there is more than one failure indicator, eliminate the causes that are not present in all lists, because they do not explain all the failure indicators. **Only causes that are consistent across applicable lists should remain.**

Decrease in Peak Area
The new peak area is stable but does not match the benchmark data.

- Evaporative loss of analytes
- Degradation of analytes
- ~~Leak in system (between sample injector and detector)~~
- ~~Change in split factor (for systems with a splitter only)~~
- ~~Sample preparation error~~
- ~~Sample volume low in the vial~~
- ~~Weak needle wash empty or low~~
- ~~Leak in sample fluidics~~
- ~~Bubble in sample fluidics line~~
- ~~Weak needle wash not compatible with sample~~
- ~~Loss of detector sensitivity~~
- Injection Needle damaged
- ~~Injection needle not drawing enough liquid~~

Step 6

Eliminate Causes that Do Not Explain Other Known Information
Eliminate additional causes that don't make sense. In this example, we can eliminate "Loss of detector sensitivity" because it would not explain why only two of the analytes are affected. We can also eliminate "sample preparation error" because we are using a reference standard sample.

Missing Peak(s)
An analyte does not elute inside the gradient or is not detected within the run time of the sample.

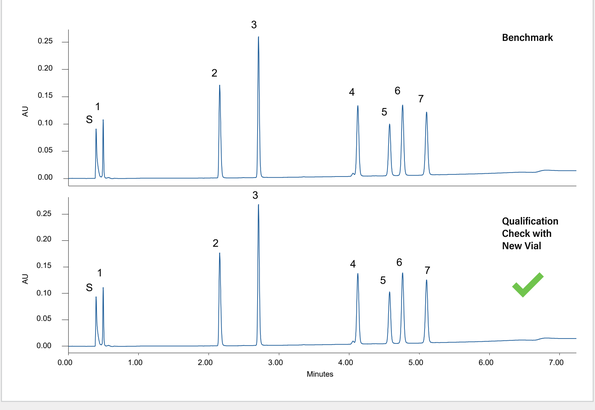
- Evaporative loss of analyte
- Degradation of analyte
- ~~Sample preparation error~~
- ~~Loss of detector sensitivity~~
- ~~Co-elution with another analyte~~
- Wrong sample injected

Step 7

Test Possible Failure Causes
Once you have narrowed down the list of possible failure causes, test the **most likely** or **easiest to test** causes first. If the problem is unresolved, move on to the next most likely cause, and continue until your problem is resolved. In this example, it is very easy to test the two remaining possible failure causes at the same time by using a **new vial** of our reference standard.

Step 8

Re-Run Qualification Check
Confirm that the problem has been resolved. In this example, we can conclude that the failure cause was "Evaporative loss of analytes" because we can see that both of our failed peaks are volatile compounds, while the non-volatile compounds remained at a similar concentration to benchmark, and the problem was resolved when we injected from a new vial.



Failure Indicators Menu

Failure Indicators represent how the chromatogram failed and appear in your chromatograms as changes from benchmark values in six different categories. Each failure indicator has a list of possible Failure Causes, which represent why the chromatogram failed. The failure cause is the source of the failure indicator, and is typically instrument, column, method, or analyst related.

Change in Peak Retention Time

Increase in Retention Time
The new retention time is stable but does not match the benchmark data.

- Leak in system
- Organic solvent not being pumped properly (pump plungers, seals or check valves degraded)
- Column not properly equilibrated (either to starting mobile phase conditions or column temperature)
- Change in mobile phase pH
- Wrong solvent/method being used
- Bubble in fluidic lines
- Column degradation

Decrease in Retention Time
The new retention time is stable but does not match the benchmark data.

- Organic solvent not being pumped properly (pump plungers, seals or check valves degraded)
- Column not properly equilibrated (either to starting mobile phase conditions or column temperature)
- Change in mobile phase pH
- Sample diluent effect
- Wrong solvent/method being used
- Column overload
- Column degradation

Erratic Retention Time
Unstable retention time across multiple injections, with no trend.

- Solvent delivery failure
- Gradient mixing failure
- Column temperature variability
- Mobile phase additive variations
- Mobile phase line partially blocked
- Column degradation

Drifting Retention Time
A trend of increasing or decreasing retention time across multiple injections.

- Column not properly equilibrated (either to starting mobile phase conditions or column temperature)
- Solvent delivery failure
- Gradient mixing failure
- Degradation of mobile phase additives
- Mobile phase composition change
- Mobile phase not prepared properly
- Column degradation

Change in Peak Area

Drifting Peak Area
A trend of increasing or decreasing peak area across multiple injections.

- Drifting decrease
- Evaporative loss of analyte
- Degradation of analyte
- Sample temperature not equilibrated
- Drifting increase
- Evaporative loss of sample diluent
- Sample temperature not equilibrated
- Non-specific adsorption

Erratic Peak Area
The new retention time is stable but does not match the benchmark data.

- Leak in sample fluidics
- Sample syringe plunger degraded
- Syringe damaged
- Bubble in sample fluidics
- Fluidic volumes not characterized/calibrated
- Leak in system
- Loss of detector sensitivity
- Column degradation/failure

Decrease in Peak Area
The new peak area is stable but does not match the benchmark data.

- Evaporative loss of analytes
- Degradation of analytes
- Leak in system (between sample injector and detector)
- Change in split factor (for systems with a splitter only)
- Sample preparation error
- Sample volume low in the vial
- Weak needle wash empty or low
- Leak in sample fluidics
- Bubble in sample fluidics line
- Weak needle wash not compatible with sample
- Loss of detector sensitivity
- Injection needle damaged
- Injection needle not drawing enough liquid
- Non-specific adsorption

Increase in Peak Area
The new peak area is stable but does not match the benchmark data.

- Concentration of analytes
- Evaporative loss of sample diluent
- Wrong method/injection volume used
- Change in split factor (for systems with a splitter only
- Sample preparation error
- Co-elution of peaks

Change in Peak Shape

Peak Tailing
Peak symmetry value is increased compared to the benchmark data.

- Secondary interaction with stationary phase
- Change in mobile phase pH
- System tubing not seated properly
- Slight overload of analyte
- Sample diluent effects
- Column degradation
- Non-specific adsorption

Peak Fronting
Peak symmetry value is decreased compared to the benchmark data.

- Secondary interaction with stationary phase
- Change in mobile phase pH
- System tubing not seated properly
- Column overload
- Sample diluent effects
- Column degradation
- Change in detector settings

Peak Splitting
A single analyte yields two or more peaks that share a base.

- Sample diluent effects
- Particulates accumulating on inlet frit
- System tubing not seated properly
- Sample volume too large
- Change in mobile phase pH (mild splitting)
- Column degradation

Peak Shouldering
A single analyte yields two partially separated peaks that are not as well separated as a split peak.

- Sample diluent effects
- Particulates accumulating on inlet frit
- Column overload
- Weak needle wash not compatible with separation
- System tubing not seated properly
- Change in mobile phase pH
- Column degradation
- Non-specific adsorption

Change in System Pressure

Increase in System Pressure
The new pressure is stable but does not match the benchmark data.

- Particulates accumulating in column frits
- Sample precipitation onto column
- Clog in system tubing
- Wrong method being used
- Change in column temperature
- Column degradation

Drifting System Pressure
A steady increase or decrease in system pressure across replicate injections with a consistent trend.

- Particulates accumulating in column inlet frit
- Sample precipitation onto column
- Column degradation

Decrease in System Pressure
The new pressure is stable but does not match the benchmark data.

- Leak in system
- Mobile phase lines obstructed
- Check valve degradation
- Air in solvent lines
- Change in column temperature
- Column degradation

Erratic System Pressure
The system does not maintain steady pressure across replicate injections/within a single injection.

- Plunger/seal degradation
- Check valve problem
- Solvent inlet filter clogged
- Solvent mixer failure
- Column degradation
- Air in solvent lines

Change in Peak Width

Peak Narrowing
Peaks are symmetrical, but narrower compared to the benchmark.

- Change in system tubing
- Decrease in system bandspreading
- Change in mobile phase pH
- Change in detector settings

Peak Widening
Peaks are symmetrical, but wider compared to the benchmark.

- Change in system tubing
- Increase in system bandspreading
- System tubing not seated properly
- Change in mobile phase pH
- Sample diluent effects
- Volume overload
- Change in detector settings
- Column degradation

Change in Number of Peaks

Extra Peak
There is an extra peak in the separation.

- Carryover from previous injection
- Sample preparation failure
- Analyte separating as two peaks (see Peak Splitting)
- Contaminated mobile phase
- Contaminated system

Missing Peak(s)
An analyte does not elute inside the gradient or is not detected within the run time of the sample.

- Evaporative loss of analyte
- Degradation of analyte
- Sample preparation failure
- Loss of detector sensitivity
- Co-elution with another analyte
- Wrong sample injected
- Non-Specific adsorption

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