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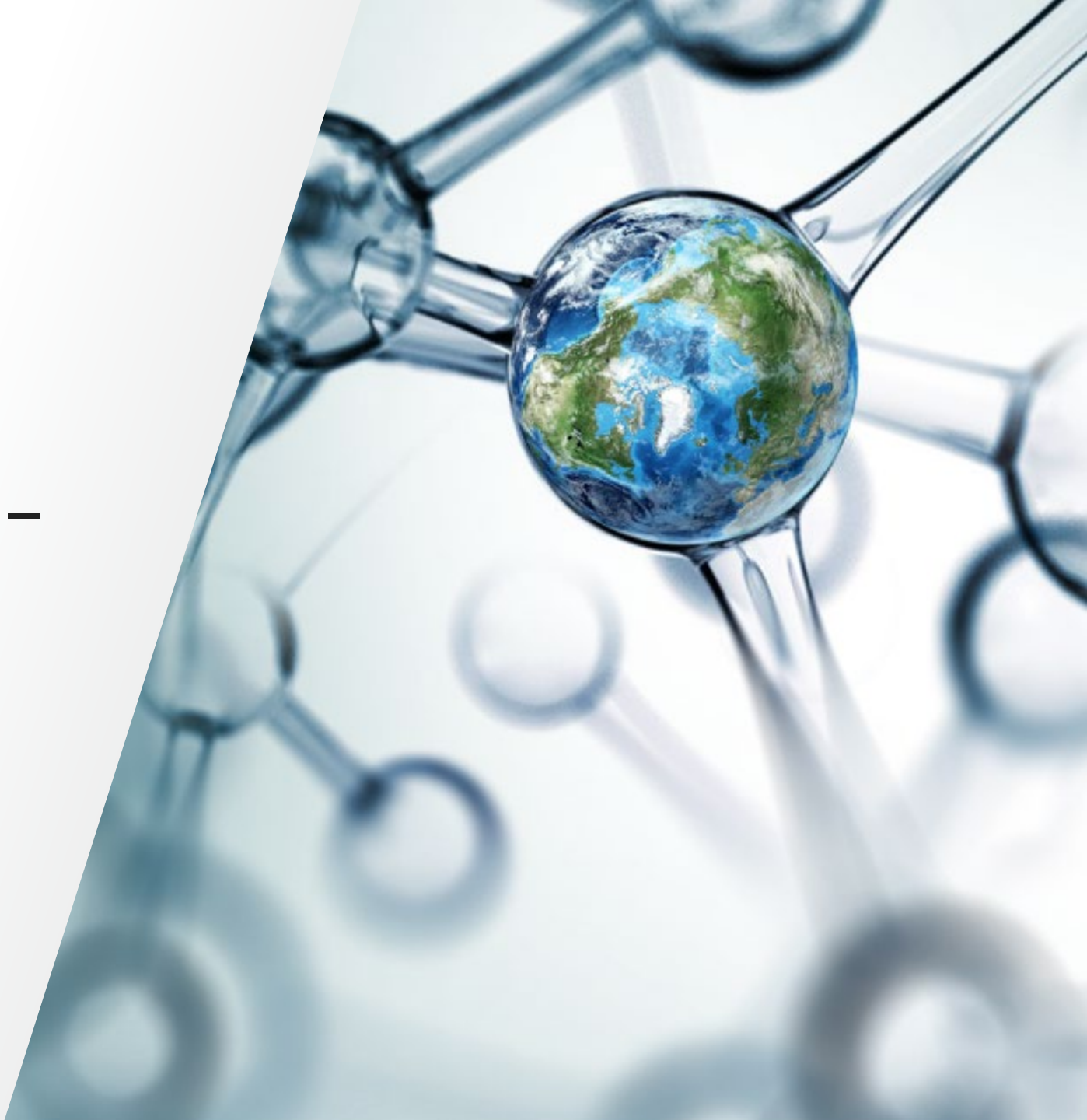
# Column troubleshooting guide – HILIC



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# Column troubleshooting guide – HILIC

## Select option

General approach

General information on how to start the column troubleshooting process

Key topics

Choose one of the topics

This guide is designed to aid the troubleshooting of columns in HILIC mode. While the content may apply in other applications, this is not the intent of the guide.

The guide is best navigated by using the buttons; links to the previous page or to the start can be found in the bottom right of the page.

# General approach to troubleshooting

What led us to question the column performance:

- Instrument reported an error (red lights, instrument alarm, Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) popup on screen, etc.)
- Sample queue failed to run to completion
- Physical observation (wet floor, rusting, liquid leak, etc.)
- Analysis of samples does not produce the expected result (calibration curve is not linear, SST results fail, etc.)
- Chromatography shows poor quality (peak shape not symmetrical, resolution issue, baseline issues, etc.)

Steps to solve the issue:

- Separate instrument issues / chromatography issues / application issues
- Determine if instrument is functioning properly?
- Determine if sample is valid (model with a standard)?
- Does the column need to be replaced?
- Contact technical support when more ideas are needed

**Select option**

Checking product specifications

QAR/COA retesting

Instrument checks

Understanding HILIC

How to contact us

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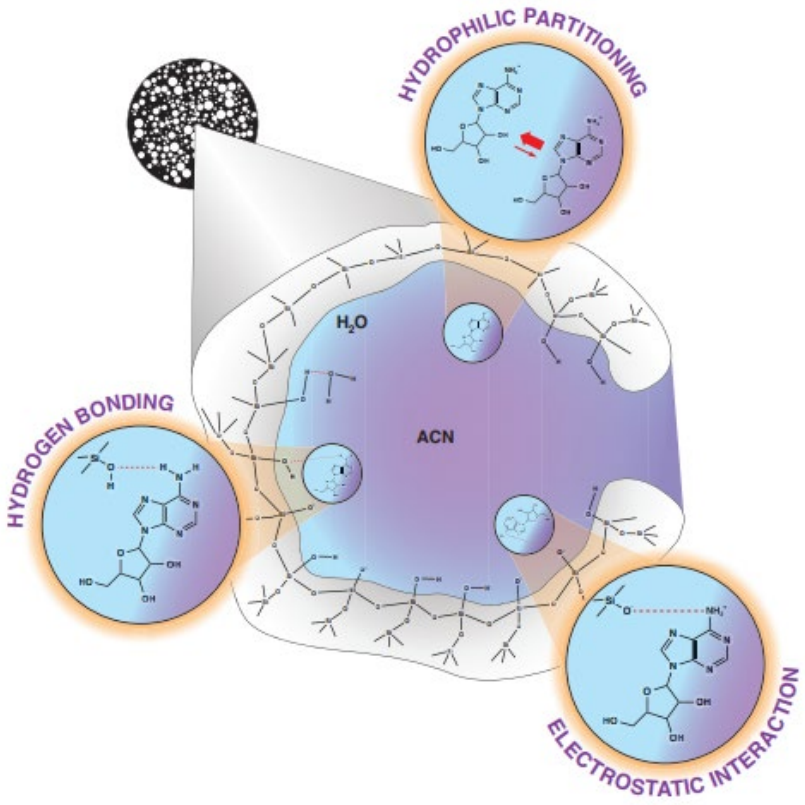
# General approach – Understanding HILIC




- HILIC is a variation of Reversed Phase Liquid Chromatography (RPLC) in which a polar stationary phase and high organic/low aqueous concentration (typically acetonitrile) mobile phase is utilised
- The water in the mobile phase forms an aqueous rich layer adsorbed to the polar surface of the stationary phase
- The retention mechanism is complex, but it is based on the preference of polar analytes to partition into the aqueous rich layer on the columns surface
- This causes an elution order which is roughly opposite of what is observed in reversed phase separation

- Further information can be found in the HILIC technical guide

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# General approach – Instrument reported an error

Is the error obvious?

- Physical leak
- Sample in the wrong location
- Mobile phase bottles ran empty

Can the audit trail identify the problem?

- Overpressure
- Module was not connected or disconnected unexpectedly
- Detector was not turned on properly
- Issues with the instrument method (lower maximum pressure, flow rate changes etc.)

Can the chromatography data system (CDS) or modules be restarted?

Can the queue be restarted?

Can the problem be identified and fixed?

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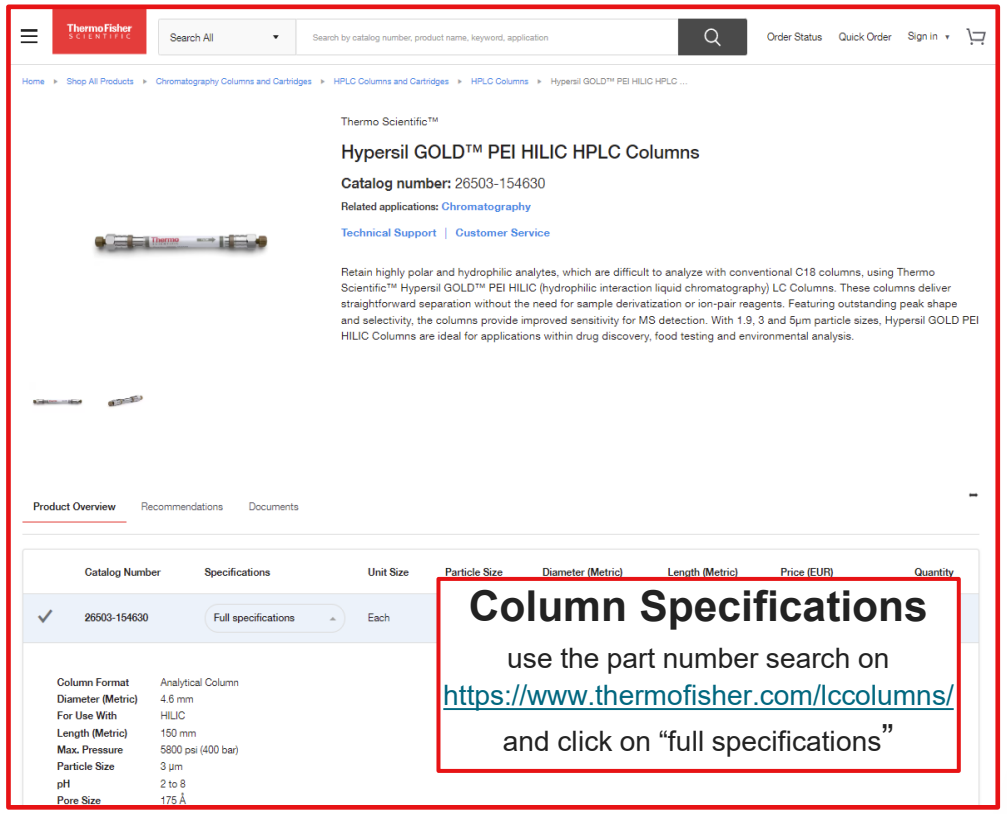
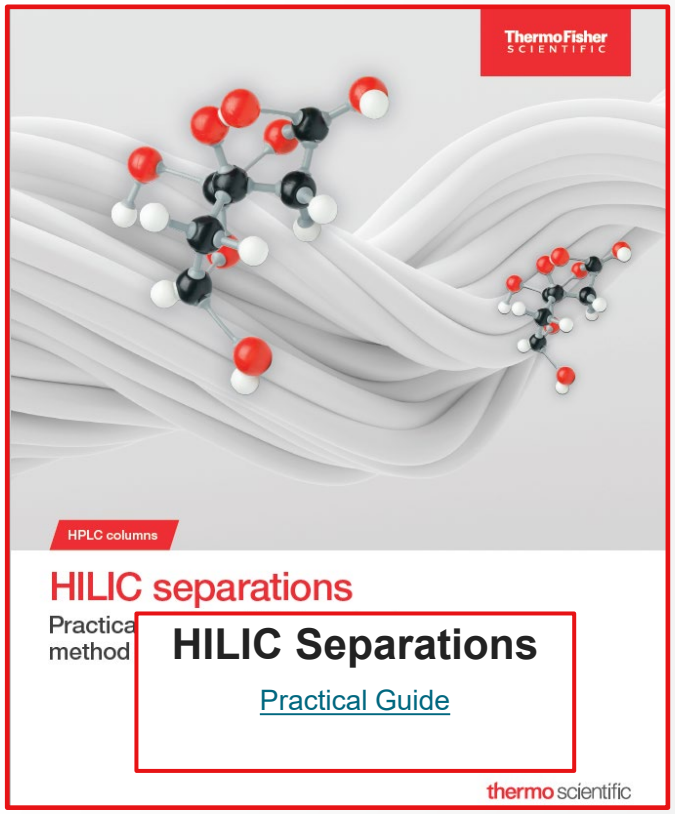
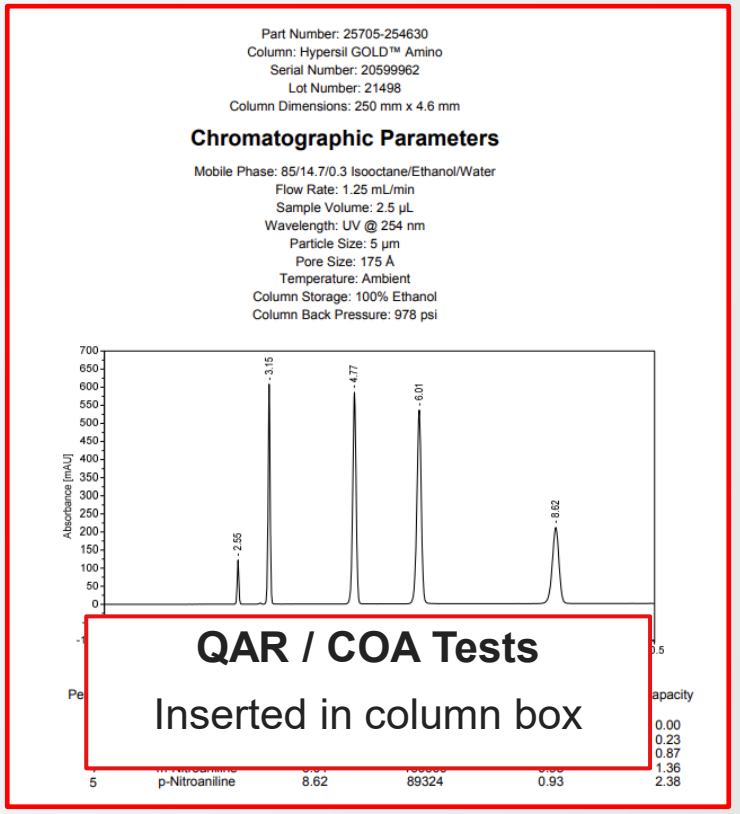
# General approach – Product specifications




Review the column specifications to determine if it has been used correctly.

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# General approach – Certificate of analysis (CoA)

Content:

- A certificate of analysis (CoA or QAR) is column specific

This provides details of the column specifications and performance, such as:

- Column name, dimensions, serial number and lot number
- Method and sample that the column was tested with
- Performance specifications, which the column must meet to be shipped to a customer
- Actual performance numbers of your column are listed as well as the chromatogram that gave these results. Users may see some slightly different performance numbers due to instrumentation
- You can use this method to verify that your column is meeting our shipping criteria as it left the factory. This may be useful in evaluating whether you need to purchase a new column

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# General approach – Instrument checks

Pump

- Have you checked the eluent bottles are filled correctly and there is no bubbles in the draw lines?
- Is the backpressure within the expected range and stable?
- If a gradient is in use, has it been changed?

Autosampler

- Is the autosampler injecting reliably?
- Has it been tested with a standard?
- Is there leaking at the needle seat?
- Is wash liquid available?
- Is the volume being picked up correct?

Detector

- Have you confirmed the detection method is suitable for your analyte? This could include things such as a chromophore for UV detection.
- Can the response of a standard be verified?
- Is the detector warmed up sufficiently?

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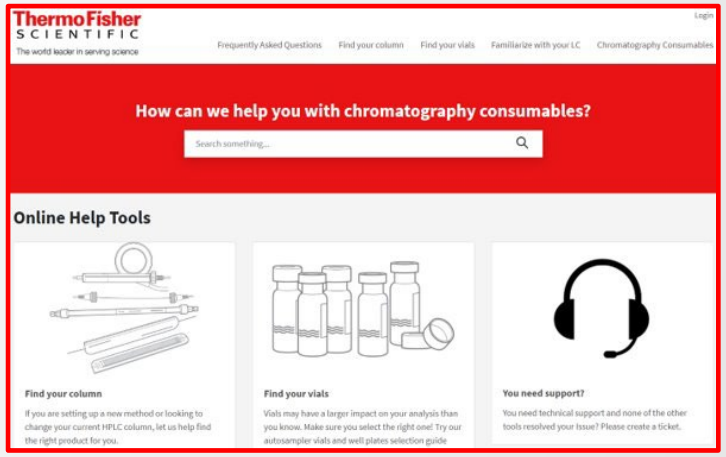
# Contact us for additional help

- We hope that this troubleshooting guide was able to solve your issue and includes the valuable information you were looking for
- If the issue could not be solved using this troubleshooting guide, please do not hesitate to contact us using the information below

Chromatography Consumables Support Page




- Using our webpage, you can report technical problems
- Access the support section and fill in the required information (e.g. part no., serial no., lot no., method and sample information, chromatograms showing the issue, Chromeleon CDS data)
- You can also access our FAQ section for further information. You just have to enter the keyword you are interested into the search field

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# Column troubleshooting guide – HILIC

Select topic

Baseline | Pressure | Resolution | Peak shape | Leaking | Retention

Contamination | Column lifetime | Recovery | Peak missing

How to contact us

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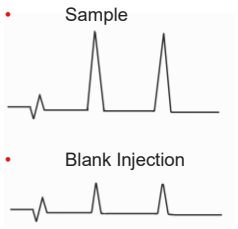
# Baseline – General

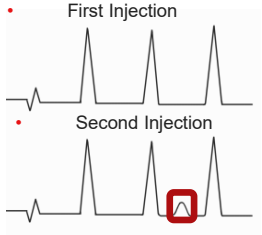
- **Instrument** – Make sure that your instrument is
  - Free of possible contamination
  - The detectors are sufficiently warmed up
  - Column is sufficiently equilibrated
- **Blank** – Perform a blank run. If the issue can be seen or even reproduced in the blank, a baseline issue could be considered
- **Mobile phase** – A common and general cause of baseline issues are contaminations of the mobile phase. The mobile phase can be responsible for an increased background, spikes, and noise. Make sure to use highly pure chemicals and at least HPLC grade eluents as mobile phase and for sample dilution (including salts, water, buffers, and any modifiers or other components added to the mobile phase) Mobile phases should be prepared fresh on a regular basis due to potential bacterial growth and should be filtered in order to avoid column contamination as well as an unstable baseline

## Select symptom

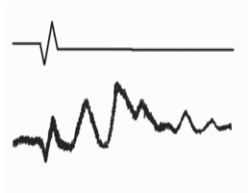
Carry Over



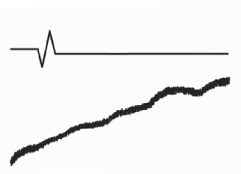

Ghost peaks




Noisy baseline



Drift



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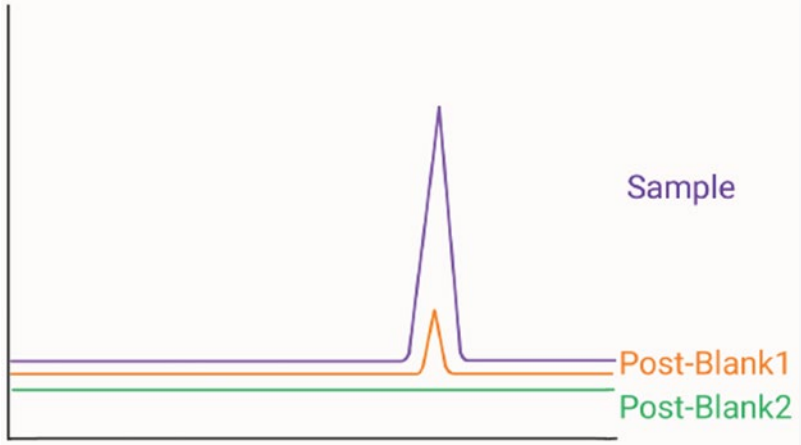
# Baseline – Carry over




Carry over is a special type of contamination and is described as the blank showing one or more peak(s) at the same retention time as the sample, which was injected before the blank. It can be caused by:

- **Lack of maintenance and cleaning** – Undesired interactions within the system can cause carry over. Regular maintenance and cleaning of the system is advised
- **Overloading** – This can cause sample to remain in the system and cause fouling of the column. It can be avoided by one reducing the injection volume and/or the concentration of the sample
- **Improper elution** – Your sample might not be properly eluted from the column. In order to properly elute your sample, increase the amount of aqueous solution, elongate the time of flushing at the end of the run or choose an alternative polar solvent. We also recommend implementing a column wash on a regular basis (See HILIC Wash)
- **Poor injection needle wash** – The injection needle or wash vial might be contaminated with sample. Make sure to choose a solvent that properly rinses off residual sample from the needle
- **Vial contamination** – Contaminations in the vial can lead to carry over. This can be caused e.g. by the injection needle or blank preparation. Improve your needle wash procedure and reprepare your blank

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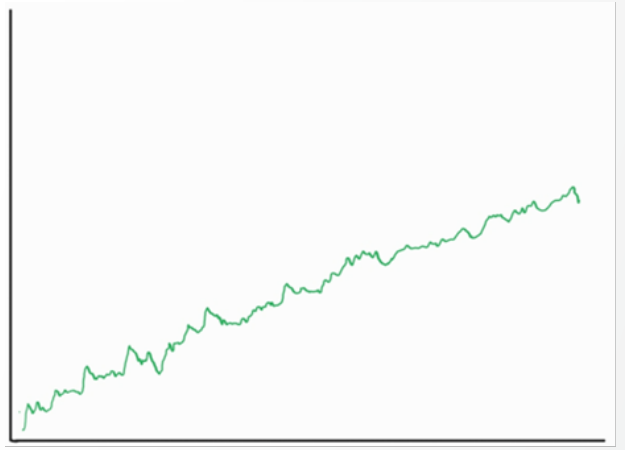
# Baseline – Drift

Normal Chromatography



Abnormal Chromatography

Baseline drifts can be normal when the mobile phase composition, flow rate, column, and temperature are changed. However, baseline drifts are not expected under isocratic conditions:

- **Baseline drift** - In case of a decreasing or increasing baseline: ensure that your instrument and detectors are properly warmed up, the column is sufficiently equilibrated, the column has had time for the temperature to stabilize, and your eluent and system are free of contamination. If the above conditions have been met, then review the UV cutoff of your mobile phase and ensure other environmental factors, such as ambient temperature and humidity, have been suitably controlled
- **Mobile phase composition** – Changes to the mobile phase composition can also contribute to a drift which is seen via the detection method. Check if the baseline is also drifting under isocratic conditions. Under isocratic conditions, we recommend premixing the mobile phase

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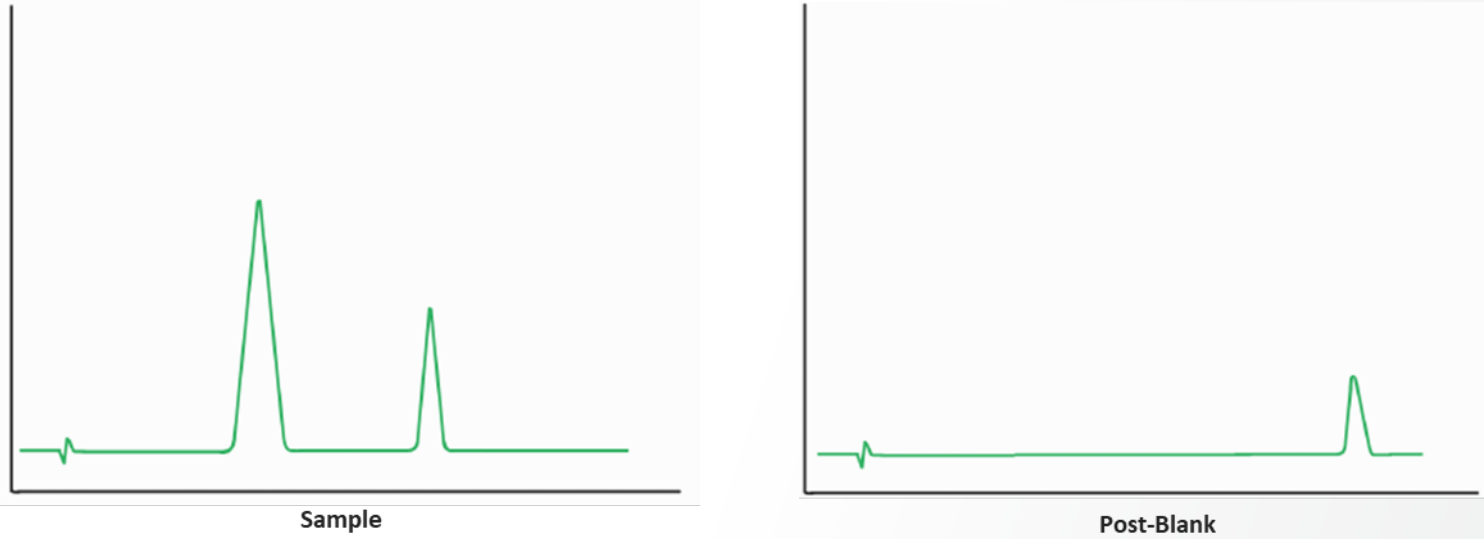
# Baseline – Ghost peaks




- **Air** – When air is present in the eluent or system might cause sharp peaks with a very small width. Degas your eluents and purge the instruments with eluent before connecting the column
- **Late eluting peak** - If a blank or a following run includes a broader, unexpected peak, it could be that the gradient is too weak or too short to elute all sample components properly. Then, a peak might be eluting in the next run, this is known as wrap-around peaks. Choose a longer gradient, increase the water percentage or add a washing step at end of each run to avoid this
- **Column bleed** – This can lead to an elevated baseline and continuous elution of background components. If an unexpected shark-fin shaped “peak” occurs during gradient elution, this may be column bleed. This can occur if a column is used out of its specifications, e.g. out of the recommended pH range or with incompatible eluents or detector. Thus, ensure to operate the column within the recommended specifications

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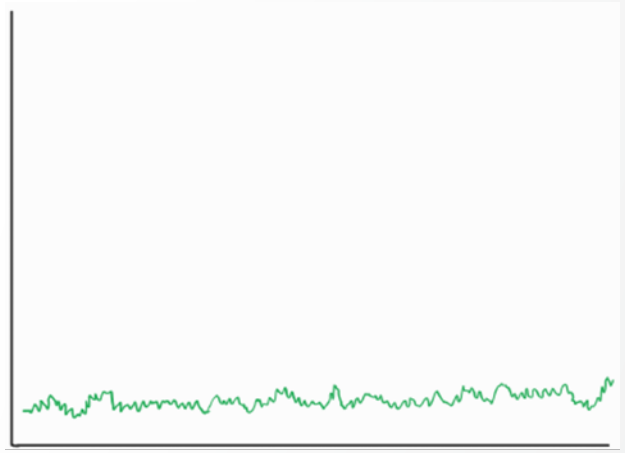
# Baseline – Noisy baseline

Normal Chromatography



Abnormal Chromatography

- **Pump pulsations** – Repetitive baseline noise can be caused by pump pulsations. Make sure the instrument is working properly and regular maintenance is done.
- **Proportioning** – Issues with eluent proportioning can contribute to a noisy baseline. In case of isocratic conditions, we recommend premixing the mobile phase instead of using the proportioning valve of the instrument.
- **Leak** – Check for leaks and if the pressure is below your regular level. Tighten all connections and see if this solves the issue.
- **Contaminated mobile phase** – This can also cause baseline fluctuations. Use only high-quality chemicals for your mobile phase and reprepare it on a regular basis.
- **Temperature effect** – If a column is heated to a high temperature and the detector is unheated, baseline noise can occur. Use a post-column cooler to reduce the temperature difference.

Link to Advanced Pump Troubleshooting Guide
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# Pressure – General

- Pressure is an important indicator for the state of your instrument and column. Thus, monitoring your pressure and comparing it regularly to the pressure of a well working system is recommended
- Pressure changes can be normal when the mobile phase composition, flow rate, column, and temperature are changed
- However, a lower, higher or fluctuating pressure compared to your regular pressure for the working conditions is often a sign of an issue. This can be sudden or gradually occurring. The speed of its occurrence can hint to the possible reason for the pressure change

**Select symptom**

No/Low pressure | Overpressure | Fluctuating pressure

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# Overpressure

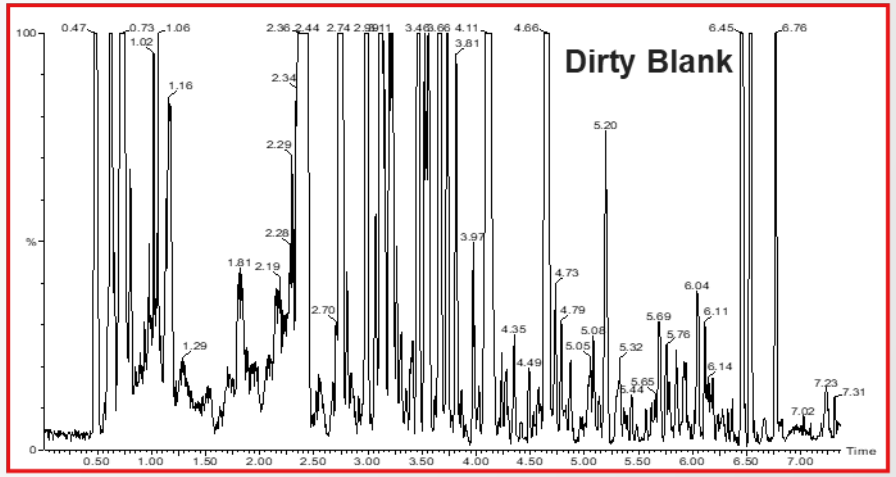
An increase or over pressuring often comes from these common sources:

| | |
|---|---|
| Sample preparation | Insufficient sample preparation leading to column contamination |
| Physical blockage | Physical blockage caused e.g. by larger particulates |
| Washing | Lack of prevention (washing, no guard or trap column) |

# Sample preparation

Pressure increases due to insufficient sample preparation can occur suddenly or gradually. The speed of the pressure increase can hint to its cause.

- **Gradual pressure increase** – Gradual pressure increase can be caused by the build up of substances from the samples on the column. Include blanks and washing steps during the run to avoid this and make sure blanks are showing no peaks or increased baseline. If something is seen in the blank, increase washing and/or switch to a stronger wash procedure. In addition, investigate a more efficient sample preparation, such as solid phase extraction (SPE) over liquid-liquid extraction. Preventative measures such as using a trap or guard column to keep samples from fouling the column is strongly advised
- **Sudden pressure increase** – A sudden pressure increase is usually due to particulates. Make sure samples are at least centrifuged or filtered. If the pressure increase continues, using SPE during sample preparation might be an option. We also recommend using inline filter, guard, or trap column to prevent large particulates from entering the column

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# Physical blockage

- A typical sign of physical blockage is a sudden pressure increase
- Physical blockage is often caused by larger particles stuck in the system or at the head of the column
- Incrementally go from mobile phase introduction to the column and break connections to see where the increase in pressure is coming from
  - If the tubing is causing a pressure change, replace the tubing
  - If the guard/column is the source of the pressure change, wash the guard/column (Lifetime → Washing)
  - If this should not fix the issue, replace guard/column
- Filter your samples, perform thorough sample preparation, and use a trap/guard column to prevent physical blockage
- Backflushing – Backflushing a column while washing can avoid contaminants to be pushed further down the column. It is important to ensure that washing is done to waste, this ensures that any post column fluidics does not become contaminated. Please note that sub-2 µm particle size columns cannot be backflushed

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# Washing

- Regular washing of your column and trap/guard will elongate its lifetime
- Washing in between samples, such as at the end of your separation gradient, is a good way to prevent build up of materials on the column that lead to an increase in pressure
- Reconditioning the column after a wash is important as to restabilize the aqueous layer on the column surface


| No | Time | Flow [ml/min] | %B | %C | %D | Curve |
|---|---|---|---|---|---|---|
| 1 | 0.000 | Run | | | | |
| 2 | 0.000 | 0.500 | 10.0 | 0.0 | 0.0 | 5 |
| 3 | 45.000 | 0.500 | 10.0 | 0.0 | 0.0 | 5 |
| 4 | 90.000 | 0.500 | 60.0 | 0.0 | 0.0 | 5 |
| 5 | 210.000 | 0.500 | 60.0 | 0.0 | 0.0 | 5 |
| 6 | 255.000 | 0.500 | 90.0 | 0.0 | 0.0 | 5 |
| 7 | *New Row* | | | | | |
| 8 | 300.000 | Stop Run | | | | |

Example of HILIC wash

General cleaning:

1. Slowly raise increase to 90% $H_2O$/10% ACN hold for 45 minutes Amide-HILIC columns show increased pressure for> 50% $H_2O$, so reduce the flow as needed
2. Then, over 45 minutes change gradient to 40% A/60% B
3. Hold this gradient step for 2 hours
4. Over next 45 minutes bring gradient back to starting mobile phase*

*This is typically 90% ACN

Wash procedures | Previous page | Back to start

# No/Low pressure

- **Purge valve** – An open purge valve leads to no pressure. Make sure that your purge valve is closed
- **Eluent lines** – Check that your eluent lines and reservoir are filled with eluent; followed by repurging the system to ensure no air is trapped inside the pumps
- **In-line filter** – Check that solvent is flowing out of the purge valve when opened. If not, clean or exchange the in-line filters
- **Leaking** – A leak in your system can also cause a lower pressure than expected. See Leaking

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# Fluctuating pressure

- **Solvent lines** – Make sure the solvent lines are properly filled with eluent and no air bubbles are present
- **Gas in eluent** – Not fully degassing your eluent can cause pressure fluctuations. Use a degaser and degas your eluent by using e.g. an ultrasonic bath, vacuum filtration or bubble with inert gas. Care should be taken not to sonicate unnecessarily long as to avoid the risk of partial evaporation of volatile mobile phase components
- **Pump** – Pressure fluctuations are more commonly a pump issue and can be e.g. caused by the check valves. Exchange the column for a different column

# Resolution – General

- Chromatographic resolution is mainly affected by selectivity. Please reprepare the mobile phase to check if the issue is not a result of a change in retention and/or selectivity
- Resolution issues can also be related to peak shape. For example, peak tailing may cause the resolution of two peaks to be decreased
- Please do not exceed the pH range of the column, this avoids damage that can cause a decrease of retention times and loss of resolution. pH monitoring during HILIC can be difficult due to the high organic content of mobile phases.

**Select symptom**

Retention/ Selectivity

Peak shape

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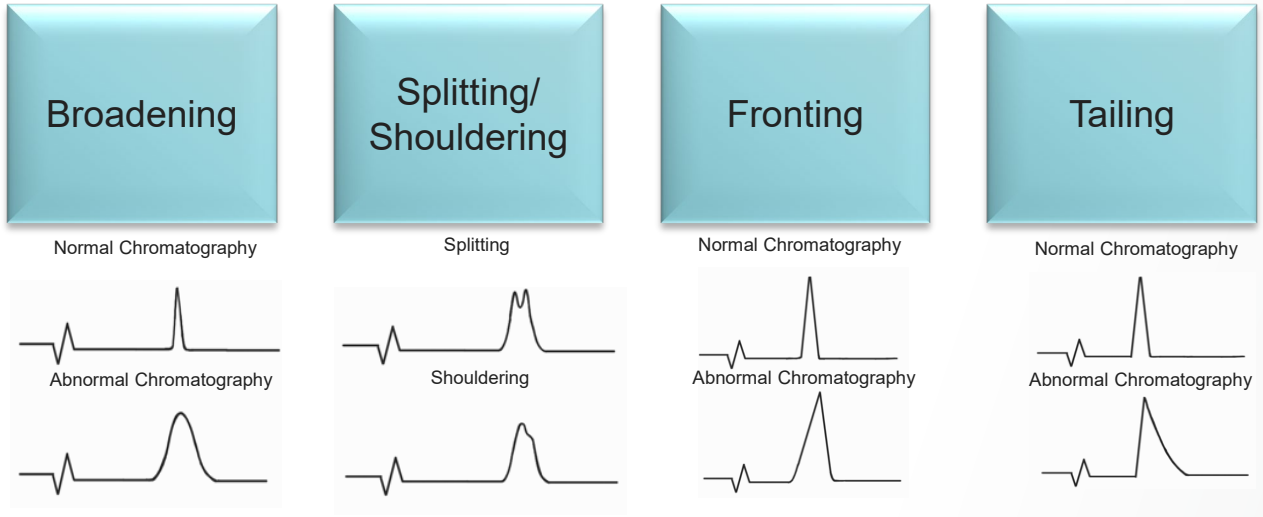
# Peak shape – General

- **Coelution of peaks** – Coelution can have the same appearance as a peak shape issue and look like a peak is broadening, shouldering, fronting or tailing. Thus, it is important to confirm, that you are observing a peak shape issue rather than two peaks coeluting, such as two distinct compounds or the target analyte in and its degradation product. Injecting a standard of only one compound is a good way to confirm a peak shape issue.
- **Check the connections to the column** – Changes to the tubing, such as poor connections, changes in length or internal diameter can cause performance effects.

**Select symptom**

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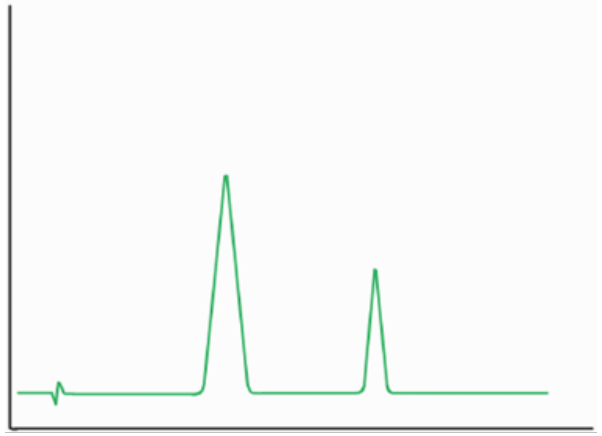
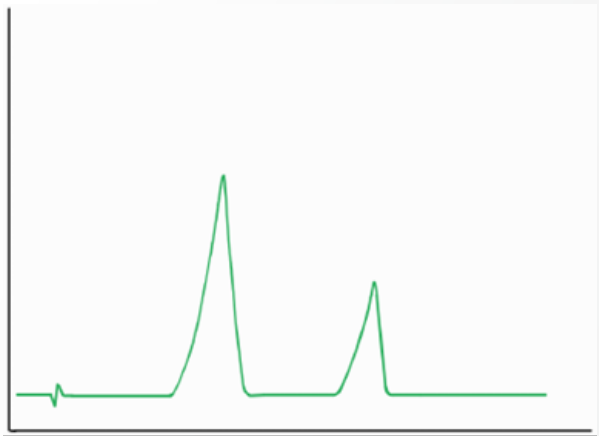
# Peak Shape – Fronting



Normal Chromatography



Abnormal Chromatography

- **Column overloading** – Injection volume overloading or mass overloading can result in fronting. To eliminate this, try lowering your injection volume or sample concentration to see if the peak shape improves
- **Injection solvent** – If the analytes has a higher affinity to the injection solvent than the mobile phase, peak fronting can occur. To avoid this, match your sample diluent to the starting mobile phase conditions
- **Physical damage** – It is possible that the column could be damaged, if you have attempted all above mentioned troubleshooting without success. Please contact us

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# Peak shape – Tailing

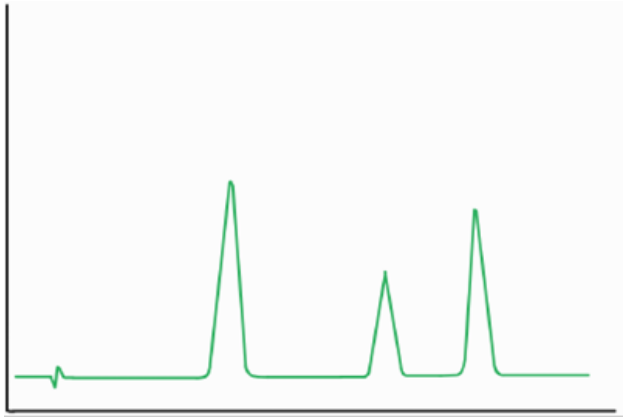
Single peak

Only a single peak in the chromatogram shows tailing issues

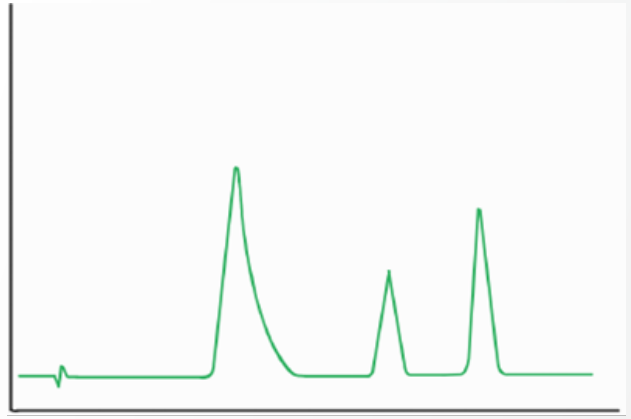
All peaks

All peaks in the chromatogram show tailing issues

# Peak shape – Tailing – Single peak



Normal Chromatography



Abnormal Chromatography

- **Partial separation** – It is possible that coelution of peaks can cause a peak to appear to be tailing, try injecting standards individually to confirm if this is the case
- **Temperature** – Column and mobile phase temperature can have a large effect on the pH, if an elevated column temperature is used ensure pre-column mobile phase heating is also implemented
- **pH related issues** – Tailing can be caused by secondary interactions of the stationary phase. Reducing silanol activity can be done by pH adjustment or buffering of the mobile phase. High buffer concentration are not always compatible with HILIC mobile phases, 10mM is generally a good starting concentration
- **Physical damage** – It is possible that the column could be damaged, if you have attempted all above mentioned troubleshooting without success. Please contact us

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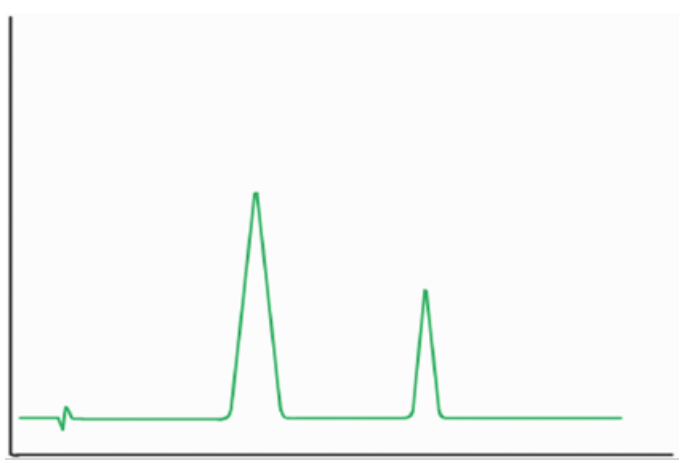
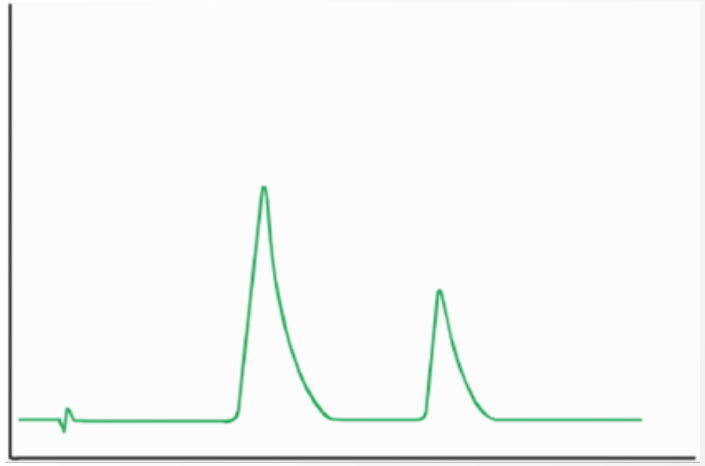
# Peak shape – Tailing – All peaks



Normal Chromatography



Abnormal Chromatography

- **Physical obstructions** – A blocked or contaminated guard column or inlet frit can cause tailing, refer to our column care guides to perform a suitable washing procedure and replace the guard cartridge
- **pH related issues** – Tailing can be caused by secondary interactions of the stationary phase. Reducing silanol activity can be done by pH adjustment or buffering of the mobile phase
- **Connections** - Increases in peak tailing can also be caused by damage caused to fittings or connections, ensure these have been tightened or replaced
- **Physical damage** – It is possible that the column could be damaged, if you have attempted all above mentioned troubleshooting without success. Please contact us

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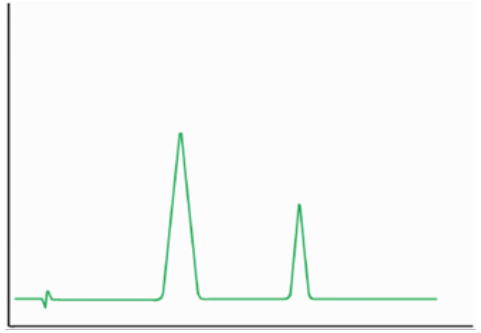
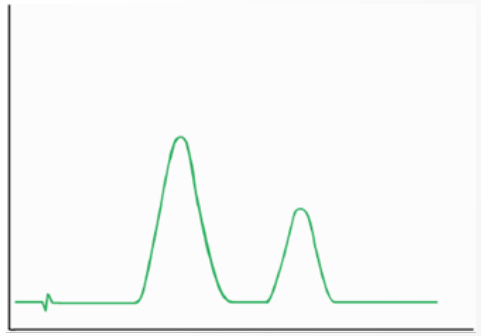
# Peak shape – Broadening



Normal Chromatography



Abnormal Chromatography

- **Injection solvent** – Enabling the sample to partition correctly into the mobile phase is particularly important for HILIC separations, using an injection solvent which does not closely match the initial mobile phase conditions can cause peak broadening and other issues
- **Column age** – Loss of efficiency is common in columns as they age; below are some different routes to take if the column is showing peak broadening prematurely

Follow our column care guide to ensure you are extending your column lifetime as much as possible.

- If broadening is accompanied by retention issues, then please check you have the correct temperature and flow rate. Please see Retention
- If peak broadening occurs on a new column, ensure that you are following the best practices to enable narrow peaks. This includes reducing system dead volume by ensuring you have the correct length and diameter capillaries and are using zero dead volume connections where possible

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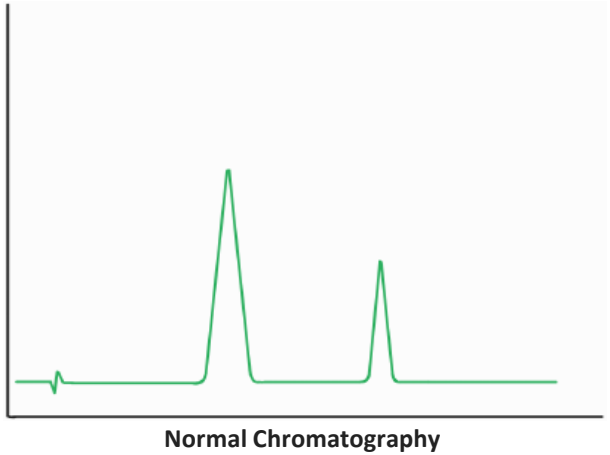
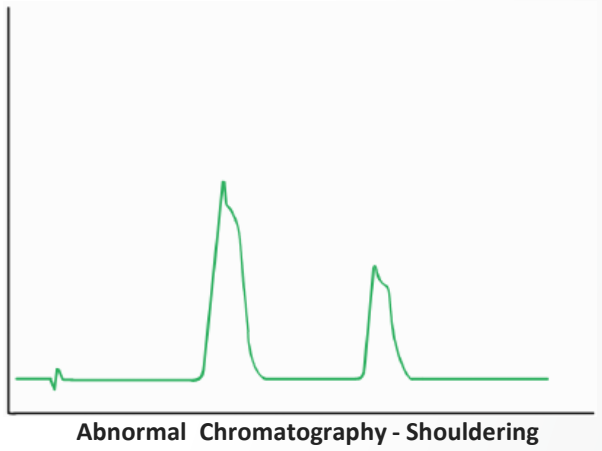
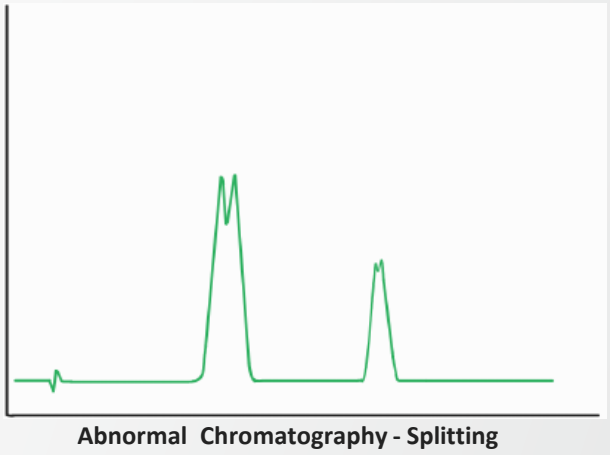
# Peak shape – Shouldering/Splitting


Normal Chromatography


Abnormal Chromatography - Shouldering


Abnormal Chromatography - Splitting

- **Physical blockages** – Splitting or shouldering peaks can be caused by a physical blockage at the column inlet, try backflushing the column if you are using a column with a particle size larger than 2 µm. Unfortunately, columns with particle size smaller than 2 µm cannot be backflushed
- **Extreme pH** – If you are using the column at or close to the pH limit of the column, you will cause degradation of the silica at the head of the column. If you are using elevated temperature the effect will be even more pronounced
- **Physical damage** – It is possible that the column could be damaged. If you have attempted all above mentioned troubleshooting, then please contact us

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# Leaking – General

- Compression and finger tight fittings have a finite lifetime. Eventually, they will have to be replaced with new ones
- The ferrule of the worn fitting will be swaged to the tubing; i.e. it will be irremovable (stainless steel) or will be removable but will leave an indentation around the tubing (PEEK).This section of tubing cannot be used again, as it would not seal properly and would leak
- Make sure that connections have been retightened
- Check that the system, connections, and column are used within their pressure limits and specifications

**Select symptom**

Column

Connections

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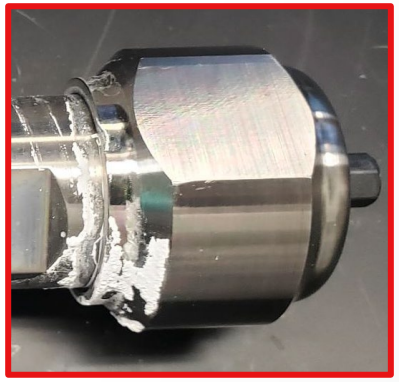
# Leaking – Column



- **Position** – Visual inspection of the column body to determine if mobile phase is coming out. Check the exact position (inlet/outlet)
- **Pressure** – Make sure the column is used under pressure, which is within the column specifications. A pressure above this limit can cause the column to start leaking. The pressure limits of each column can be found on Thermofisher.com/lccolumns
- If the column is leaking from the body and has been used at a pressure within the column specifications, then please contact us

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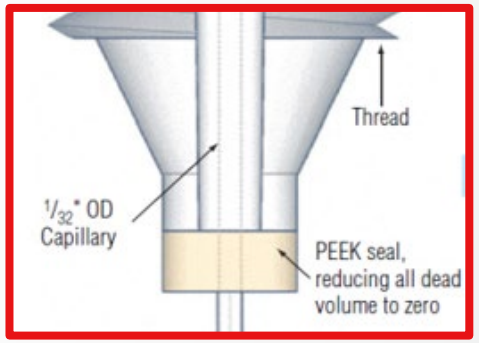
# Leaking – Connections




- As a symptom of leaking, the pressure is often not as expected or there is a lack of signal
- Follow connections to determine where the pressure drop is occurring
- Tighten connections at each step and see if pressure increases to the expected level
- Unscrew and inspect Thermo Scientific™ Viper™ capillaries visually to make sure that the PEEK tip is intact
- Replace unsuitable Viper capillaries

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# Retention – General

- Small differences in retention time from column to column can be expected
- When larger retention time difference between analyses or a previous column are detected, make sure your system and column are fully equilibrated to the starting conditions of your gradient before the analysis. As a rule of thumb, 20 column volumes should be sufficient. It is important to ensure that both the system pressure and the baseline are steady prior to injection
- Retention time issues can occur to a single peak of an analysis, while the other peaks show reproducible retention times, or to multiple or even all peaks in a chromatogram

**Select symptom**

Single peak

Multiple peaks

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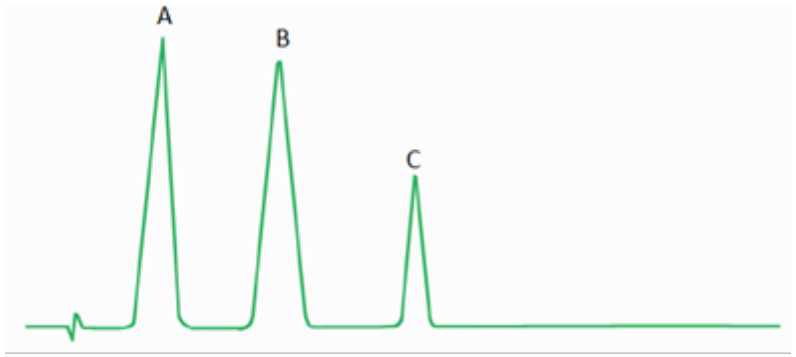
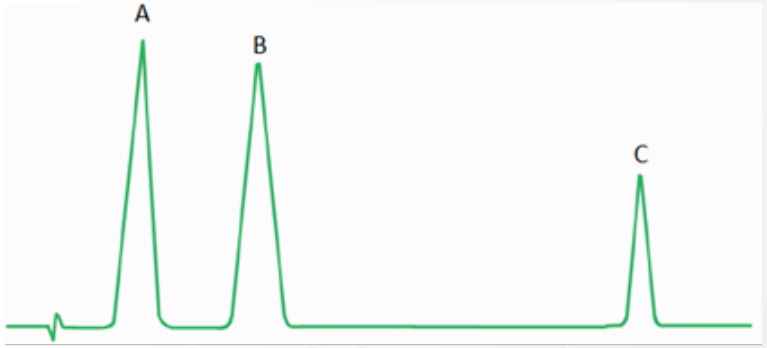
# Retention – Single peak



Normal Chromatography



Abnormal Chromatography

If you find that only certain peaks are changing retention or that certain peaks are shifting by different degrees, then please investigate the following:

- **Contamination** – If this issue is accompanied with other effect, such as high pressure, poor peak shape or other effects, then it is possible that the column may have become contaminated (Contamination)
- **Sample matrix effects** – Retention time differences could be due to differences in the sample matrix composition or the pH. Try injecting a standard mixed in pH matched solution of the starting mobile phase conditions. If this resolves the issue, then additional sample preparation may be required

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# Retention – Multiple peaks

| | |
|---|---|
| Early/Late eluting peaks | All of your analytes elute earlier or later compared to previous analyses |
| Elution order | You are not able to obtain the same retention time for consecutive injections of the same sample |
| Loss of retention | All of your analytes have lost retention and are not resolved anymore |

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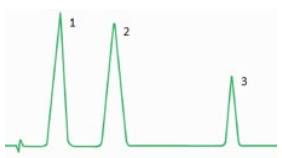
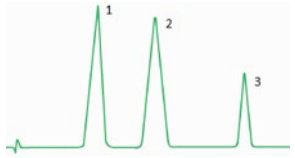
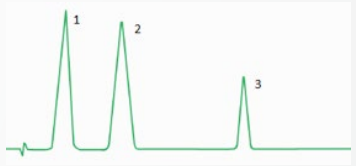
# Retention – Early/Late eluting peaks



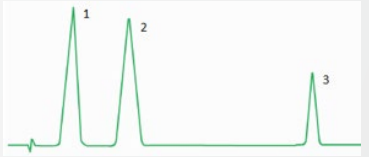

Normal Chromatography




Abnormal Chromatography




Normal Chromatography




Abnormal Chromatography

- **Injection peak** – If the “injection peak” does not match previous analyses, then a difference in flow rate is likely the cause
- **Peak shape** – Visually check if the peak shape is suitable. If it is not, refer to Peak Shape
- **Temperature** – A higher or lower column temperature can lead to early or late eluting peaks. Make sure to use the temperature control in the column oven. If the system temperature is different from the ambient temperature, consider using a pre column heater/cooler
- **Mobile phase** – Incorrect preparation of your mobile phase, e.g. a higher/lower organic content, changes in the buffer concentration or pH can lead to early or late eluting peaks. Reprepare your mobile phase
- **Conditioning** – Insufficient conditioning of the column to your mobile phase might result in shorter or longer retention times. Make sure that your column has been conditioned properly and run a blank injection before your first standard or sample. HILIC methods require longer column conditioning to achieve stable retention times
- **Leaks** – Depending on the location of a leak, leaks can also cause your analytes to be detected at a faster or slower retention time. See the Leaking section
- **Equilibration** – Insufficient equilibration to the starting conditions of your analysis can lead to mostly early eluting peaks. Ensuring a stable water partition on the phase surface requires longer equilibration and a minimum 3% water concentration
- **Contamination** – Contamination in the column can cause peaks to elute early. See Contamination to find out more about column contamination
- **Modification –** Has the stationary phase of your previously used column been permanently modified

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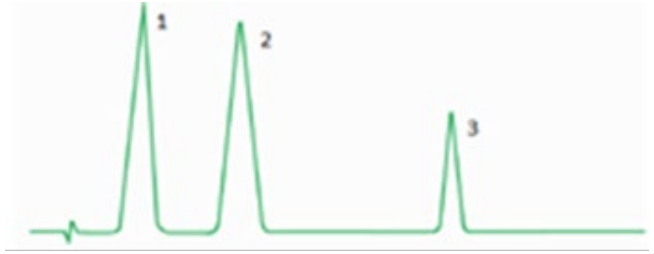
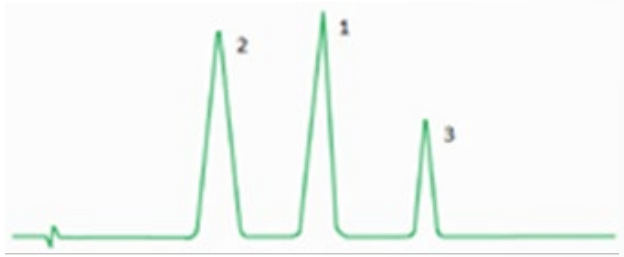
# Retention – Elution order



Normal Chromatography



Abnormal Chromatography

Abnormal Chromatography

- If the peaks vary with some having shorter and some longer retention times, then investigate the pH of the eluent or its composition. Many columns such as mixed-mode phases can operate in both HILIC and RP modes, ensuring the column is equilibrated in HILIC conditions will ensure that the elution order changes are not caused by switching between operating modes
- **Buffer Choice** – If using a buffer, ensure that it has the capacity to buffer at your desired pH
- Ensure you are following the “2 pKa rule”, meaning that the pH of your mobile phase is >2 pH above or below the pKa of your analytes, as this should ensure the complete ionization of >99% of your analytes.

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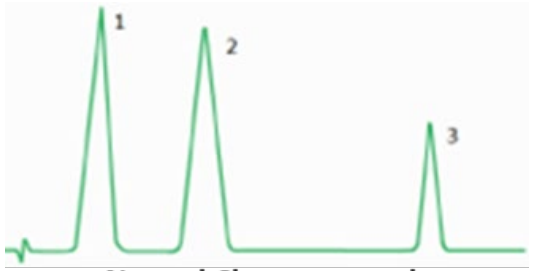
# Retention – Loss of retention



Normal Chromatography

Abnormal Chromatography

- **Mobile phase composition** – If you observe a complete loss of retention of your peaks in the chromatogram with all eluting at or close to the dead volume, the first step is to check the mobile phase composition. HILIC mobile phases use >60% organic solvent, typically acetonitrile. In general, it is recommended to use aprotic solvents, alcohols can cause a decrease in the strength of the partition
- **Equilibration** – Ensure the column has been adequately conditioned and equilibrated. Conditioning of HILIC columns typically takes between 20-40 column volumes, much longer than reversed phase methods. If the method utilizes a gradient, a minimum 20 column volume equilibration time should be implemented between injections
- **Injection solvent** – It is recommended that the injection solvent is as close to the starting mobile phase conditions as possible. If the aqueous concentration of the injection solvent is too high this can cause issues with the partitioning into the mobile phase
- **Shipping solvent** – Some columns which are traditionally used for normal phase chromatography are shipped in normal phase solvents, these column require additional conditioning

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# Contamination – General

- **Blank runs** – Comparing the blank run of your column to the blank run of a new column can help you identify if your column or your system/mobile phase is contaminated. Consider the advices on this page and refer to Wash Procedures for column washes.
- **Particles** – Particles and larger substances can contaminate your column and even create physical blockage increasing the column pressure. Use a syringe filter to remove larger particles from your sample to avoid clogging your column. A guard column can prevent contamination of your analytical column and elongate its lifetime. Make sure to replace the guard regularly.
  See Physical Blockage
- **Mobile phase** - A too low quality of your solvents, water, and additives (e.g. buffer, salts) in your mobile can lead to column contamination over time. Symptoms include a higher background signal and pressure. Use high quality mobile phases to avoid this.
  See Baseline → General
- **Samples** – Matrix molecules present in your sample can cause column contamination as these might not be properly eluted from your column, increase your column pressure, cause a higher background signal or interfere with your analyte. Use thorough sample preparation e.g. SPE, liquid extraction or filtration to remove as much matrix from your sample as possible. See Sample Preparation
- **Sample concentration** – A too high sample concentration can cause overloading effects and contamination. A reduction of the injected sample concentration is advised. See Baseline → Carry-Over
- **System contamination** – This can originate from different sources e.g. non-biocompatible systems, the solvent filters, the injection needle or the dead volume of connections. Implementing regular system maintenance and cleaning as well as a needle wash procedure and using Viper fittings can significantly reduce the risk for system contamination

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# Lifetime – General

- **Clean system** – A clean system contributes to a long column lifetime.
- **Eluents** – Make sure to use fresh solvents and buffers to avoid particles and bacterial growth.
- **Filters** – Blocked or saturated filters can decrease the column lifetime significantly. Make sure to exchange your inline filters, pre-columns, guard columns, and guard cartridges on a regular basis. We recommend exchanging the pre-columns, guard columns, and guard cartridges at least every six months or when exchanging the analytical column.
- **Column specifications** – Only use the column within its specifications to avoid irreversible column damage, such as column bleed caused by dissolution of the column material or surface modification.
- **Sample load** – Overloading a column can result in a shorter column lifetime. Avoid overloading by reducing the injection volume and choosing the right detector for your analysis.
- **Guard column** – The use of a guard column can extend your column lifetime significantly. More information can be found here: guard column

**Select symptom**

Sample preparation | Washing | Storage

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# Lifetime – Column storage

- **Short term storage** – For short-term (< 3 days), it is acceptable to leave the column installed on the LC and filled with mobile phase at room temperature. Maintaining a low flow rate while the column is on the system can help with avoiding the need to recondition the column before using it again.
- **Long term storage** – During long term storage, buffer/salt might precipitate in the column and bacterial growth could occur. Thus, we recommend to thoroughly flush the column with unbuffered mobile phase to remove any non-volatile salts and acids. In a next step, flush the column with 90 % acetonitrile and then use the storage solution listed on the CoA or QAR
- See the Column Care Guide

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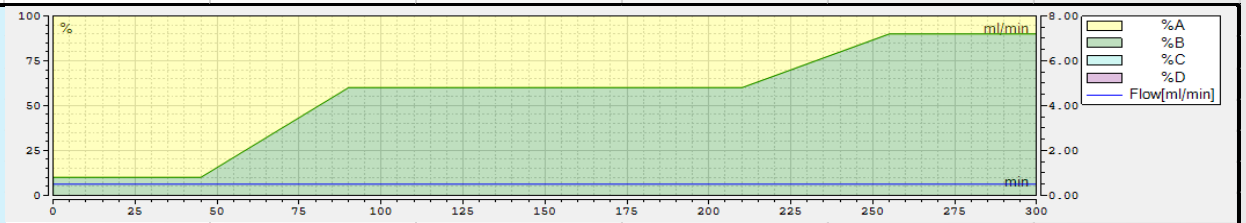
# Lifetime – Wash procedures

- **Backflushing** – Backflushing a column while washing can avoid contaminants to be pushed further down the column. Please note that sub-2 µm particle size columns cannot be backflushed.
- **HILIC wash** – Our most recommended wash (HILIC wash)
- **HILIC wash 2** – This is a more aggressive HILIC clean utilizing buffers (HILIC wash 2)
- **Biological wash** – Wash procedure for the Thermo Scientific™ Acclaim™ HILIC-1 Mixed Mode column (Acclaim HILIC wash)
- More information on washes forspecific issues can be found in the Column Care Guide

Acclaim HILIC wash | HILIC wash | HILIC wash 2 | Previous page | Back to start

# Lifetime – HILIC wash

| | | | |
|---|---|---|---|
| Name | HILIC Column Wash | | |
| Time Taken | Minimum: 5 hours | Recommended: 5 Hours+ | |
| Solvents Required | A: Deionised Water<br>B: Acetonitrile<br>C:<br>D: | | |
| Description | This procedure is for the cleaning of HILIC columns.<br>It is important to understand that the HILIC mechanism requires significantly more time to equilibrate than other chromatographic modes. | Flow rate: | The flow rate for this wash should match that of the method as to cause as little distruption to the water layer as possible. |
| Suitable for: | Any column being used in HILIC mode | Notes: | Please ensure that all buffers have been carefully removed, if not click here.<br>Make sure that all washes the linear velocity is started lower and slowly moved up as to monitor back pressure |
| Procedure | 1) 90% A / 10% B - 45 minutes<br>2) Over 45 minutes change gradient to 40% A / 60% B<br>3) Hold this gradient for 2 hours<br>4) Over next 45minutes bring gradient back to starting mobile phase<br>*This is typically 90%+ B | | |




| No | Time | Flow [ml/min] | %B | %C | %D | Curve |
|---|---|---|---|---|---|---|
| 1 | 0.000 | Run | | | | |
| 2 | 0.000 | 0.500 | 10.0 | 0.0 | 0.0 | 5 |
| 3 | 45.000 | 0.500 | 10.0 | 0.0 | 0.0 | 5 |
| 4 | 90.000 | 0.500 | 60.0 | 0.0 | 0.0 | 5 |
| 5 | 210.000 | 0.500 | 60.0 | 0.0 | 0.0 | 5 |
| 6 | 255.000 | 0.500 | 90.0 | 0.0 | 0.0 | 5 |
| 7 | *New Row* | | | | | |
| 8 | 300.000 | Stop Run | | | | |

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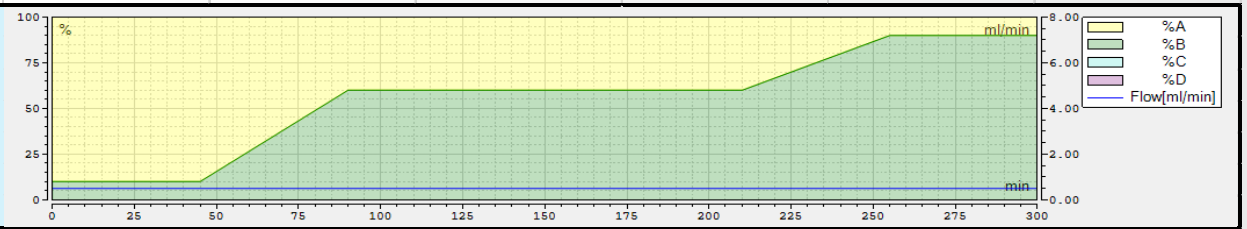
# Lifetime – HILIC wash 2

| | |
|---|---|
| Name | HILIC Column Wash 2 |
| Time Taken | Minimum: 5 hours — Recommended: 5 Hours+ |
| Solvents Required | A: 0.1M Ammonium Acetate (pH 4 to 5)<br>B: Acetonitrile<br>C:<br>D: |
| Description | This procedure is for the cleaning of HILIC columns.<br>It is important to understand that the HILIC mechanism requires significantly more time to equilibrate than other chromatographic modes.<br>This is a more aggresive HILIC clean utilising buffers. |
| Suitable for: | Any column being used in HILIC mode |
| Procedure | 1) 90% A / 10% B - 45 minutes<br>2) Over 45 minutes change gradient to 40% A / 60% B<br>3) Hold this gradient for 2 hours<br>4) Over next 45minutes bring gradient back to starting mobile phase<br>*This is typically 90%+ B |




Flow rate: The flow rate for this wash should match that of the method as to cause as little distruption to the water layer as possible.

Notes: Make sure that all washes the linear velocity is started lower and slowly moved up as to monitor back pressure

| No | Time | Flow [ml/min] | %B | %C | %D | Curve |
|---|---|---|---|---|---|---|
| 1 | 0.000 | Run | | | | |
| 2 | 0.000 | 0.500 | 10.0 | 0.0 | 0.0 | 5 |
| 3 | 45.000 | 0.500 | 10.0 | 0.0 | 0.0 | 5 |
| 4 | 90.000 | 0.500 | 60.0 | 0.0 | 0.0 | 5 |
| 5 | 210.000 | 0.500 | 60.0 | 0.0 | 0.0 | 5 |
| 6 | 255.000 | 0.500 | 90.0 | 0.0 | 0.0 | 5 |
| 7 | *New Row* | | | | | |
| 8 | 300.000 | Stop Run | | | | |

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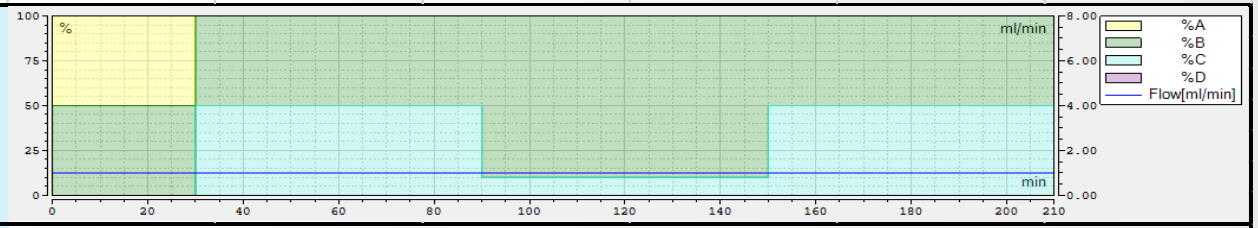
# Lifetime – Acclaim HILIC column wash

| | | | |
|---|---|---|---|
| Name | Acclaim HILIC-1 Mixed Mode Wash | | |
| Time Taken | Minimum: 210 Minutes | Recommended: 210 Minutes | |
| Solvents Required | A: Deionised Water<br>B: Acetonitrile<br>C: 0.1M ammonium acetate pH 4 to 5<br>D: | | |
| Description | Wash procedure for the Acclaim HILIC-1 Mixed Mode column. | Flow rate: | 1.0ml/min |
| Suitable for: | Acclaim HILIC-1 Mixed Mode | Notes: | For a 2.1mm I.D column, the flow rate should be reduced to 20% of that for 4.6mm I.D column |
| Procedure | 1) 50% A / 50% B for 30 minutes<br>2) 50% B / 50% C for 60 minutes<br>3) 90% B / 10% C for 60 minutes<br>4) 50% B / 50% C for 60 minutes<br>5) Re-equilibrate with mobile phase | | |




| No | Time | Flow [ml/min] | %B | %C | %D | Curve |
|---|---|---|---|---|---|---|
| 1 | 0.000 | Run | | | | |
| 2 | 0.000 | 1.000 | 50.0 | 0.0 | 0.0 | 5 |
| 3 | 30.000 | 1.000 | 50.0 | 0.0 | 0.0 | 5 |
| 4 | 30.000 | 1.000 | 50.0 | 50.0 | 0.0 | 5 |
| 5 | 90.000 | 1.000 | 50.0 | 50.0 | 0.0 | 5 |
| 6 | 90.000 | 1.000 | 90.0 | 10.0 | 0.0 | 5 |
| 7 | 150.000 | 1.000 | 90.0 | 10.0 | 0.0 | 5 |
| 8 | 150.000 | 1.000 | 50.0 | 50.0 | 0.0 | 5 |
| 9 | *New Row* | | | | | |
| 10 | 210.000 | Stop Run | | | | |

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# Lifetime – Sample preparation

- Sample preparation is the most important factor to increase the lifetime of your column. While a thorough sample preparation can be time consuming, it has the biggest influence on any column issues, especially column lifetime
- **Matrix** – Sample preparation steps, which remove large parts of the sample matrix increase the column lifetime. These include e.g. SPE, liquid-liquid extraction, and solid-liquid-extraction (SLE)
- **Filtration** – Using syringe filters to filter your sample before the analysis removes particles, which can decrease the column lifetime
- We have linked some useful documentation for sample preparation - SPE Method Development Guide, SPE Application Notebook, Sample Prep Selection Guide, and Sample Preparation Catalog



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# Recovery – General

- **Sample and standard preparation** – Errors during the sample preparation can lead to both too high and too low recovery rates. Reprepare your sample and evaluate again
- **Matrix effects** – Matrix present in complex samples might influence your recovery significantly. Improve your sample preparation in order to avoid injecting too much matrix into your LC system. If this should not be possible or not successful, try to match the matrix of the sample in the standards. Alternatively, use standard addition or isotope dilution as calibration methods instead of an external calibration
- **Standards** – Use certified reference standards if possible or determine the amount of analyte present in your standard. Be aware that standards might degrade over time and should be replaced on a regular basis

**Select symptom**

High Recovery

Low Recovery

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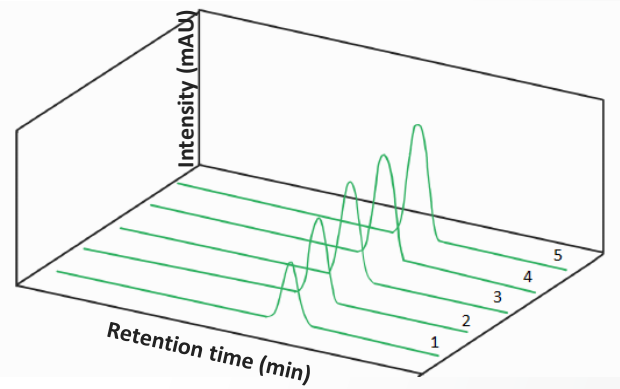
# Recovery – High recovery




- **Carry over** – Carry over can lead to higher recovery rates than expected. To read more about it and find out how to avoid this, see Baseline → Carry over
- **Evaporation of the solvent** – If the solvent/diluent of the sample has partially evaporated, higher recovery rates might be detected. Make sure to analyze your sample right after the sample preparation, do not reuse already pierced caps, cool the autosampler if this should be compatible with the sample e.g. there is no precipitation risk, and switch to a less volatile sample diluent, if possible

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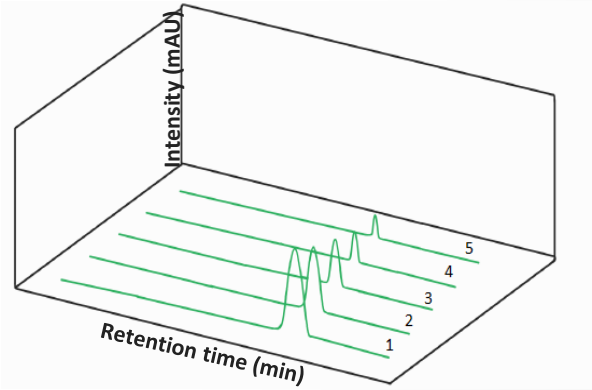
# Recovery – Low recovery




- **Insufficient elution** – If the elution solvent is not strong enough to completely elute the analyte, low recovery might occur. Make sure to fully elute your sample. In HILIC separations, water has greater elution strength when compared to acetonitrile.
- **Loss of retention** – When the percentage of water exceeds a certain percentage typically around 40%, the stationary phase will be unable to maintain the adsorbed aqueous layer on the surface, losing retention. Please see Retention
- **Conditioning of new columns** – HILIC methods require extended conditioning periods to ensure stable retention times. We recommend a minimum of 20 column volumes before first use. It is common for insufficiently conditioned columns to have analytes elute in dead volume
- **Column contamination** – If the sample is not completely eluted from the column, it might modify the column surface and cause further sample components such as the analyte to bond to the column, which might not be properly elute from the column. Make sure to perform a washing step on a regular basis or even at the end of each run in order to avoid column contamination. Please see → Carry over and Contamination for more information

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# Peak missing – General

- **Standard** – If one of your peaks is missing in your chromatogram, inject a standard of this analyte, dissolved in the corresponding diluent, on its own and check if the peak appears. If yes, reprepare your sample and have a closer look at your sample preparation method. If it does not appear, check your method and see Retention.
- **Coelution** – If one of your analyte peaks has a larger peak area than usual and maybe even a peak shoulder, analyze standards of your missing analyte and the analyte with the larger peak area on their own. If they are eluting at the same or very similar retention times, they will be coeluting in your sample analysis. See Contamination and Retention. We also recommend washing your column ( → See Column Washing)
- **Detection window** – Broaden your MS detection window in case the retention time of your analyte has increased or decreased. If this applies, see Retention
- **Wavelength** – Review that your UV detector is set to the correct wavelength under which your analyte can be detected.
- **Dead volume** – Check if your analyte elutes in the dead volume. If so, see Retention
- **Run time** – Double the length of your run time to see if your analyte elutes later. Please see Retention
- **Baseline** – If you should detect a higher baseline as usual and expect a small analyte peak, your analyte might be hidden underneath the baseline. Inject a higher concentration of your analyte in order to confirm this. See Chapter Baseline
- **Leaking** – A leak in your system can avoid your analytes to reach the detector. Please see Leaking

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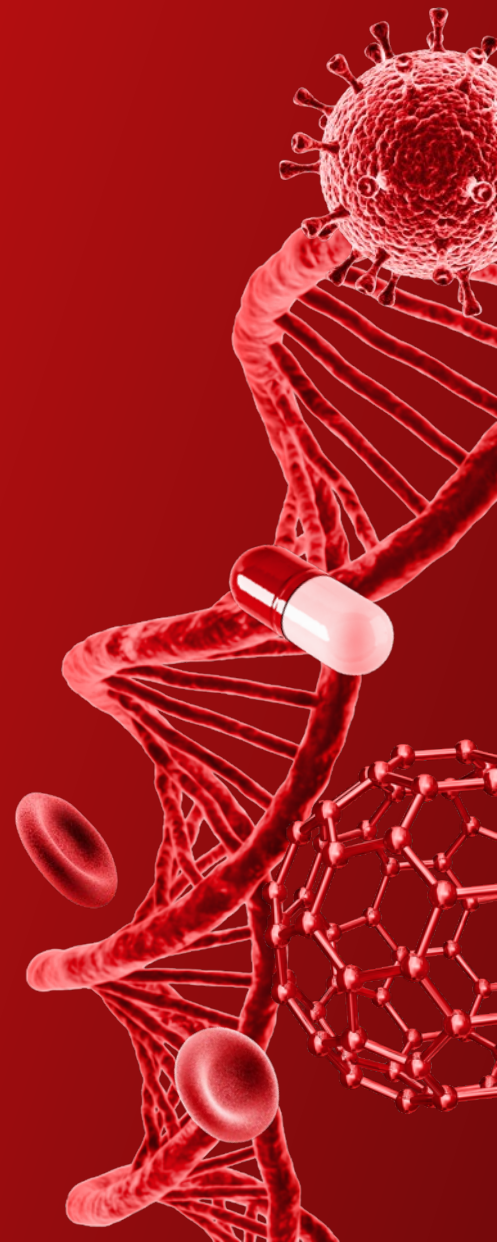

**General Laboratory Equipment – Not For Diagnostic Procedures**