# It Isn’t Always the Column: Troubleshooting Your HPLC Separation

Jean Lane
Applications Engineer
LC Columns and Consumables Technical Support
July 25, 2023

Agilent
Trusted Answers

# You've Recognized that There Is a Problem

## Ask Questions:

When did the system and chromatography last function properly?

Has anything been changed?

For the method, was the procedure followed correctly?

Are the instrument settings correct?

What is the problem that is being seen?

## Where might the problem be?

- Pump
- Injector/autosampler
- Column
- Detector
- Data system
- Mobile phase
- Sample
- Tubing/fittings
- User

The problem could be one, some, many, or all of these independently or together.

How's that for a challenge?

# Common Symptoms and Problems

- Pressure
- Peak shape
- Retention
- Baseline


- Increased pressure
- Low pressure
- Leaks
- Pressure fluctuations
- Tailing
- Peak splitting and doubling
- Fronting
- Broadening
- Changing retention time
- Loss of resolution
- Noisy baseline
- Drifting baseline
- Reduced intensity or sensitivity

# Understand Your HPLC System

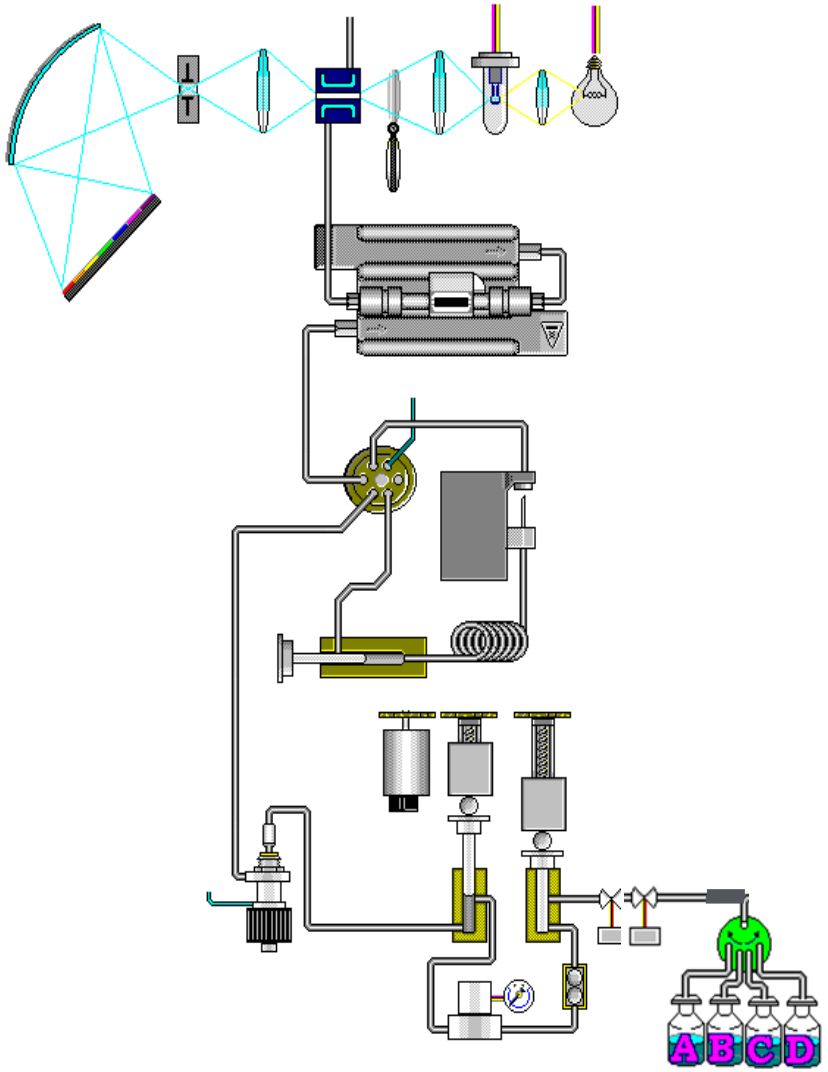
## Know your flow path

Detector

Column compartment

Autosampler

Pump and degasser



# Changes in System Pressure

# Causes of Increase in Back Pressure

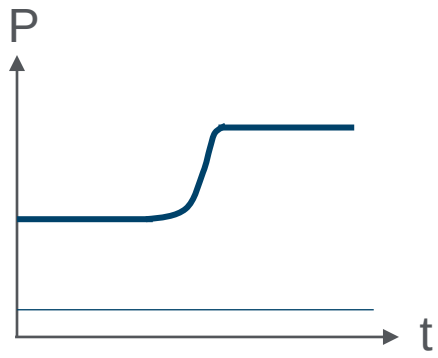
- Particles leading to blockage can come from sources located both *outside* and *inside* the LC system:
  - Solvent, buffer
  - Microbial growth in solvent reservoirs
  - The sample
  - Wear of LC components – piston seals, autosampler valve
- Debris will either be captured on the a filter, frit, or inline filter (inexpensive replaceable frit) or a column frit (column = expensive)

Reduce LC problems by eliminating most common sources of flow blockage:
Preventing this is the key

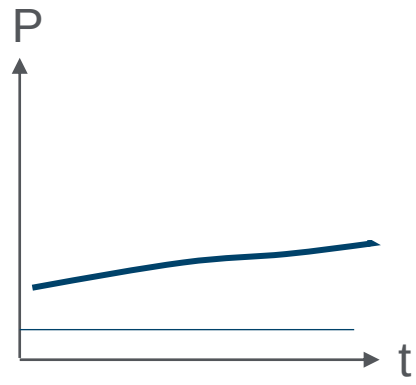
Filter, filter, filter

# Blockages and Clogging

| Characteristics | |
|---|---|
| Parts affected | Blockages:<br>• Capillaries, needle and needle seat<br>• Detector flow cells<br>Clogging:<br>• Filter frits (inline filter, column filter) |
| Characteristic | |
| Identification | • Start by disconnecting the capillary at the column inlet<br>• Install a test setup with restriction capillary<br>• Continue disconnecting capillaries, one-by-one, moving back toward the pump |
| Possible root cause | • Debris from mechanically worn parts (needle seat material, rotor seal at injection valve)<br>• Coring of vial septa material |
| Instant action/first aid | • Backflush affected part<br>• Replace part |
| Preventive measures | • Replace wear parts in time; apply proper preventive maintenance schedules<br>• Use high-quality septa<br>• Install inline filters |




**Blockages:** instant pressure increase step




**Clogging:** constant pressure increase over time

# Microbial Growth

- Potential problems
  - **Increased system pressure or pressure fluctuations**
  - **Increased column pressure, premature column failure**
  - Can mimic application problems
  - Gradient inaccuracies
  - Ghost peaks
- Prevent and/or Reduce Microbial Growth
  - Use freshly prepared mobile phase
  - Filter
  - Do not leave mobile phase in instrument for days without flow
  - Always discard “old” mobile phase
    - Do not add fresh mobile phase to old. No “topping off”
  - Use an amber solvent bottle for aqueous mobile phase
  - If possible, can add:
    - 5% organic added to water can be used to reduce bacterial growth
    - Use a few mg/L sodium azide

PN 3150-0577
Solvent filter/degasser assembly

Glass solvent inlet filter (20 mm), PN 5041-2168
Stainless Steel solvent inlet filter. PN 01018-60028
Amber solvent bottle 1L, PN 9301-6526

# Why Filter the LC Sample

Capillaries, frits, and the column inlet are less likely to end up with blockages

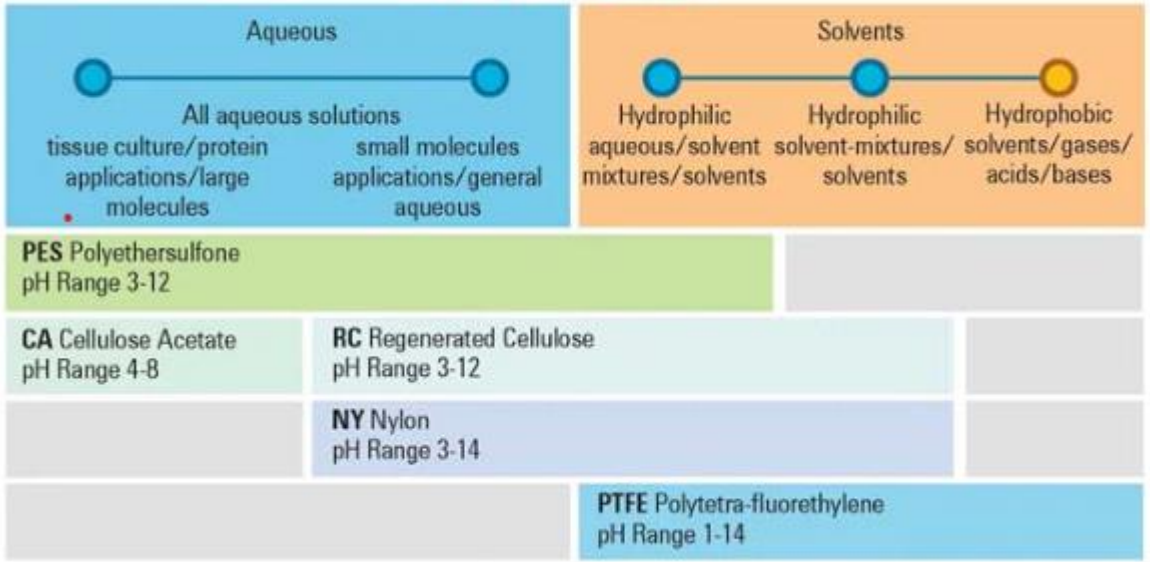
Less wear and tear of injection and switching valves

Less downtime

**Agilent Syringe Filter Selector tool**

[Captiva Syringe Filter Selector | Agilent]

### Sample Composition




### Sample Volume

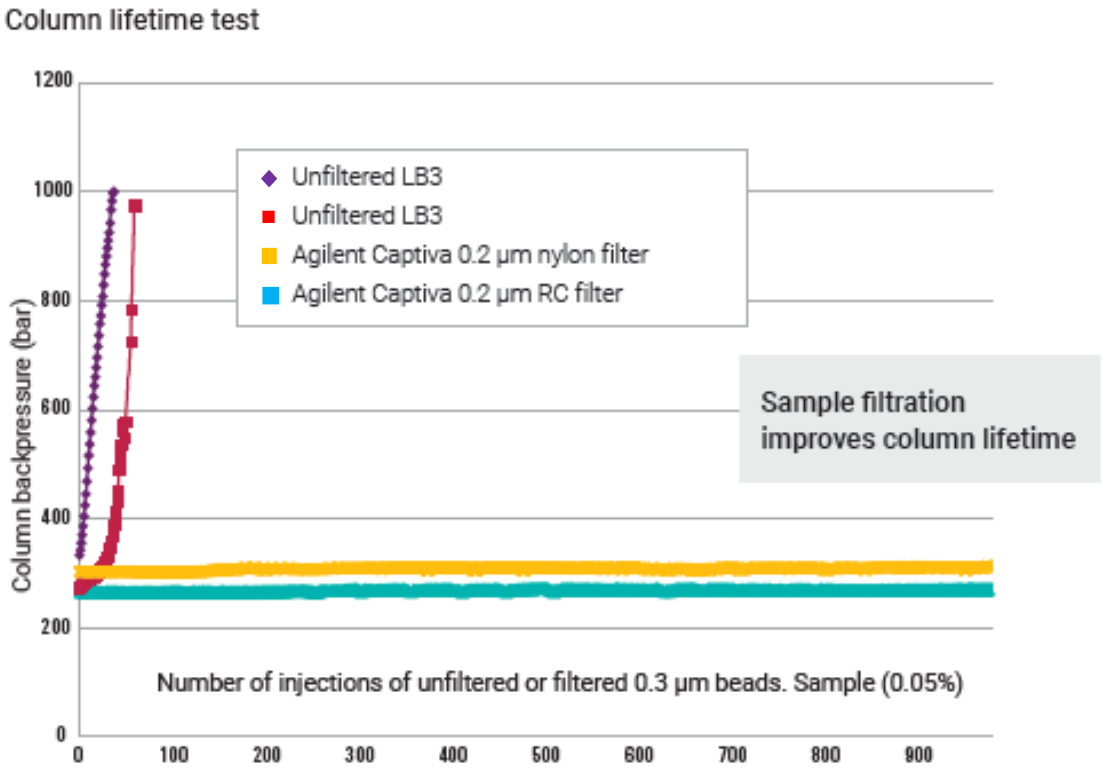

### What is the Particle Size of Your LC Column?

| Columns packed < 2 µm particles | Columns packed > 2 µm particles |
|---|---|
| **0.2 µm** UHPLC | **0.2 µm or 0.45 µm** HPLC |

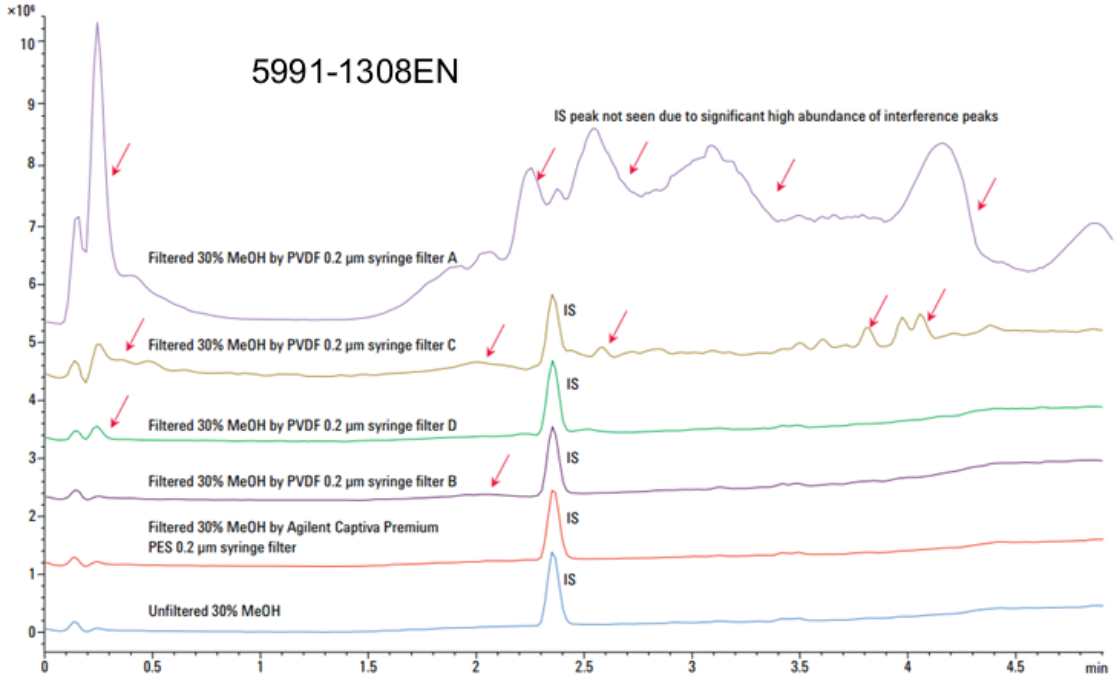
### Applications

| Type of Filtration | Recommended | Alternatives |
|---|---|---|
| HPLC • UHPLC • LC/MS • GC | RC | PTFE or Nylon |
| ICP-MS | PTFE | Glass Fiber/PTFE (High Particle Samples) |
| CE | RC | Nylon |
| Undiluted Organic Solvents | PTFE | Nylon |
| Protein Analysis • Samples with Biomolecules – Buffers | PES | RC or CA |
| Tissue Culture Media | PES | RC or CA |
| High Particle-Load Samples – Organic Solvents | Glass Fiber/PTFE | - |
| High Particle-Load Samples – Aqueous Solutions | Glass Fiber/Nylon | - |

# Filtration
## Captiva premium syringe filters




Impact of filtering a 0.3 µm latex-bead suspension on lifetime of a sub-2 µm column.



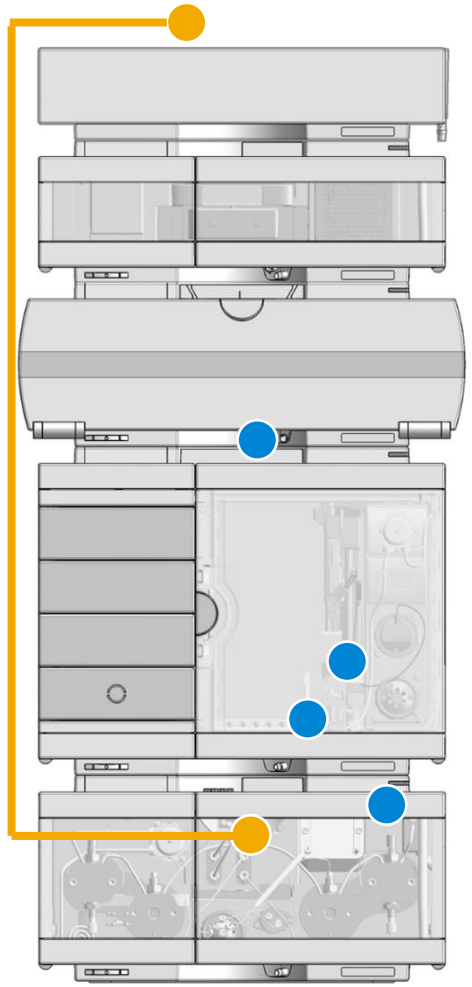

Filter cleanliness comparison of the Agilent Captiva Premium PES syringe filter with non-Agilent PVDF syringe filters using LC/MS under positive mode.

Captiva syringe filters guide: 5991-1230EN

# Changes in System Pressure

## Low pressure

| | Potential Cause | Recommended Action |
|---|---|---|
| ● | Leak in high-pressure flow path | • Visual inspection of the flow path<br>• Instrument diagnostic tests [LA] |
| ● | Wrong mobile phase | • Check for correct mobile phase<br>• Check solvent reservoir and tube connections |

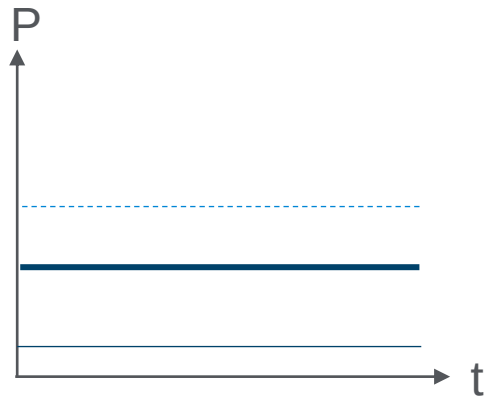


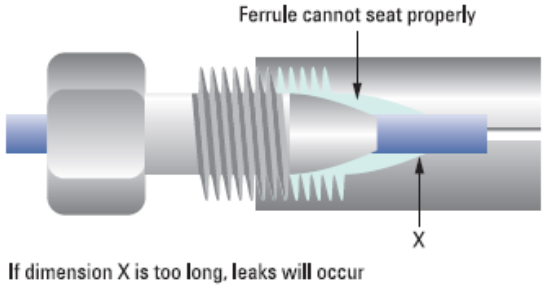
### Helpful Troubleshooting Tool

**LA**: With its advanced diagnostic and maintenance capabilities, **Agilent Lab Advisor SW** helps you to keep your Agilent analytical instruments in top condition. Agilent Lab Advisor is independent of the chromatography software you are using.

# Leaks

| Characteristics | |
|---|---|
| Parts affected | • Potentially all parts in the flow path<br>• High potential at frequently operated fitting connections (for example, column inlet) and parts with high mechanical stress (rotor seal, needle, and needle seat) |
| Characteristic | • Lower pressure<br>• Potentially impacting retention times and peak shape |
| Identification | • Drops of solvent or residues of salt<br>• System diagnostic tests LA |
| Possible root cause | • Loose or bad fitting connections<br>• Cracked capillaries<br>• Worn needle and needle seat |
| Instant action/first aid | • Replace affected parts<br>• Renew or redo fitting connections |
| Preventive measures | • Use proper fitting connections<br>• Replace fittings and wear parts in time |



If dimension X is too long, leaks will occur

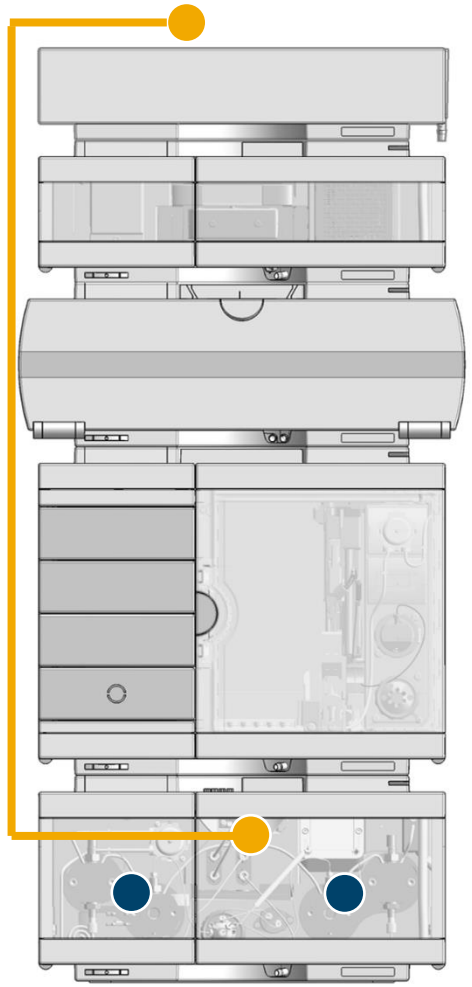
# Changes in System Pressure

## Pressure fluctuations

| | Potential Cause | Recommended Action |
|---|---|---|
| ● | Air in the system | • Prime and flush instrument<br>• Check for sufficient solvent supply<br>• Check for correct plumbing (SSV/MCGV)<br>• Check for correct degassing |
| ● | Malfunctions at pump head | • Perform pump head diagnostic tests LA<br>• Replace defective parts<br>• Implement proper maintenance schedule |
| ● | Cavitation effects | • Check for flow restrictions (solvent bottle to pump head inlet)<br>• Clean or replace parts<br>• Verify that solvent supply is positioned above the pump inlet |

### In addition

Pressure fluctuations will typically also impact the UV-signal due to refractive index effects.



# Peak Shape Changes

# Changes in Peak Shape

## What is typically seen

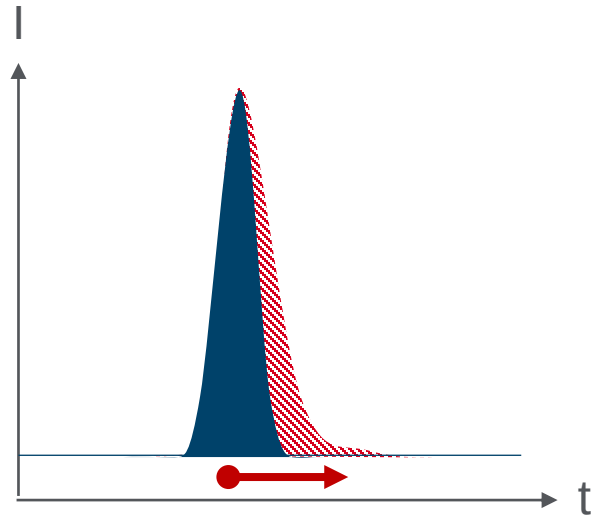
- Peak tailing
- Peak splitting/doublets
- Peak fronting
- Peak broadening

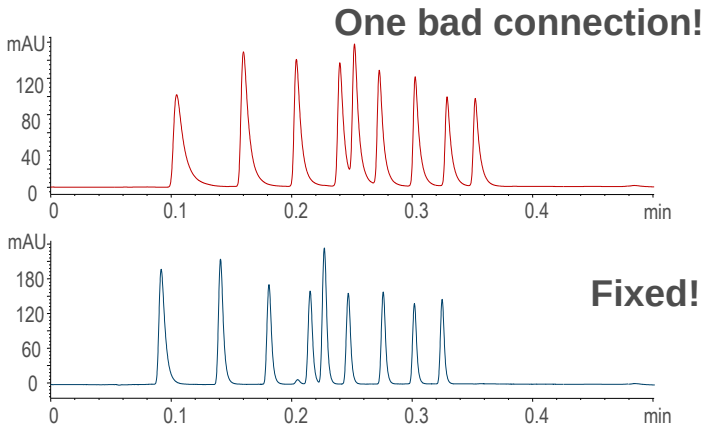
# Changes in Peak Shape

## Peak tailing

| If applicable to some peaks | Recommended Action |
|---|---|
| Secondary interactions | • Change pH<br>• Change stationary phase |
| Small peak eluting on tail of larger peak | • Change selectivity (column, mobile phase)<br>• Switch to methods with higher resolution (UHPLC, 2D-LC) |

| If applicable to all peaks | Recommended Action |
|---|---|
| Silica based – column degradation | • Use specialty, polymeric or sterically protected column |
| Silica based – basic interactions with stationary phase | • Use stronger mobile phase or add appropriate base (*e.g.* TEA) |
| Poor tubing connections; high dispersion volume | • Minimize number of connections<br>• check connections / fitting condition and proper seat of fittings<br>• use fittings with spring-load function |



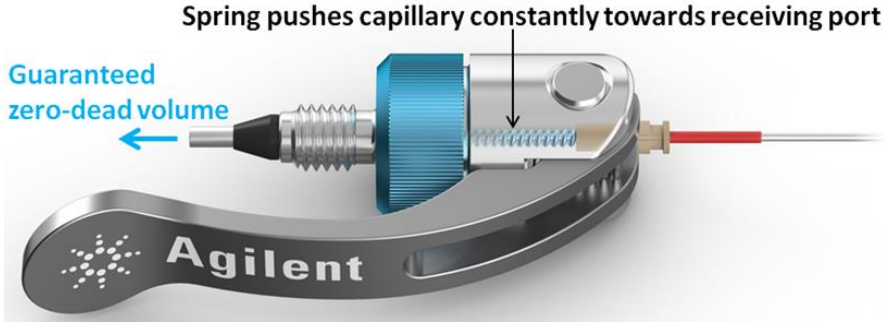



# InfinityLab Quick Connect and Quick Turn Fittings

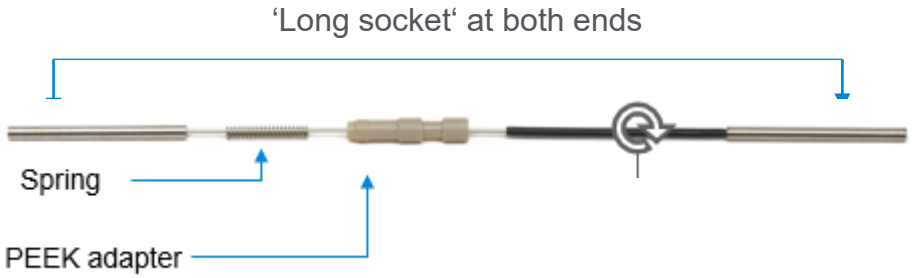
- Spring-loaded design
- Easy-to-use
- Works for all column types
- Reusable
- Consistent ZDV connection

**Quick Connect Fitting**
- Finger tight up to 1300 bar
- Hand tighten the nut, then depress the lever

**Quick Turn fitting**
- Finger tight up to 400 bar
- Up to 1300 bar with a wrench
- Compact design



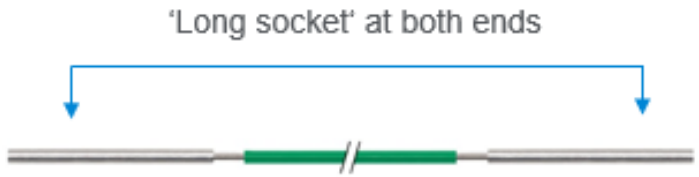

Capillary for Quick Connect fitting





InfinityLab Quick Turn fitting

Capillary for Quick Turn fitting

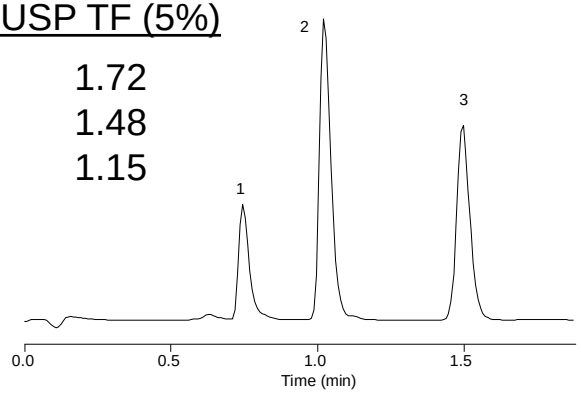



Brochure: 5991-5164EN

# Peak Tailing
## Injector seal failure

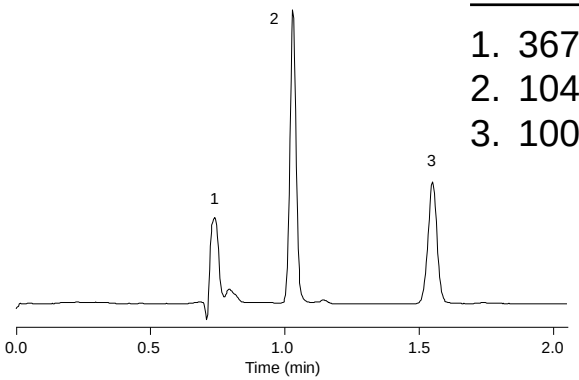
Column: Bonus-RP, 4.6 x 75 mm, 3.5 mm  Mobile phase: 30% $H_2O$ : 70% MeOH  Flow rate: 1.0 mL/min
Temperature: R.T.  Detection: UV 254 nm  Sample: 1. Uracil 2. Phenol 3. N,N-Dimethylaniline

| | Plates | USP TF (5%) |
|---|---|---|
| 1. | 2235 | 1.72 |
| 2. | 3491 | 1.48 |
| 3. | 5432 | 1.15 |




Before

| | Plates | USP TF (5%) |
|---|---|---|
| 1. | 3670 | 1.45 |
| 2. | 10457 | 1.09 |
| 3. | 10085 | 1.00 |

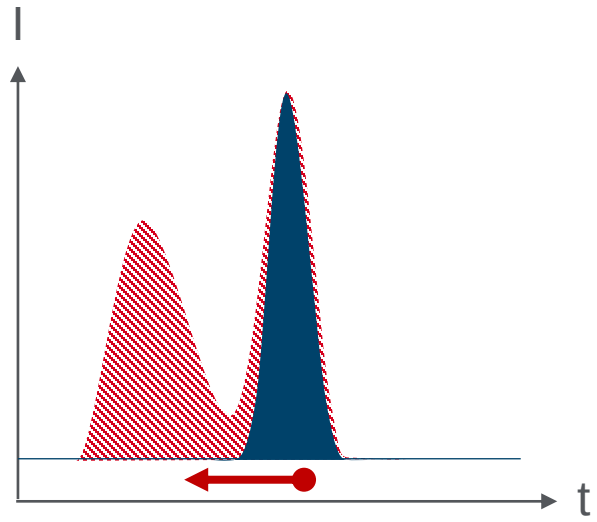



After replacing rotor seal and isolation seal

# Changes in Peak Shape

## Peak splitting/doubling

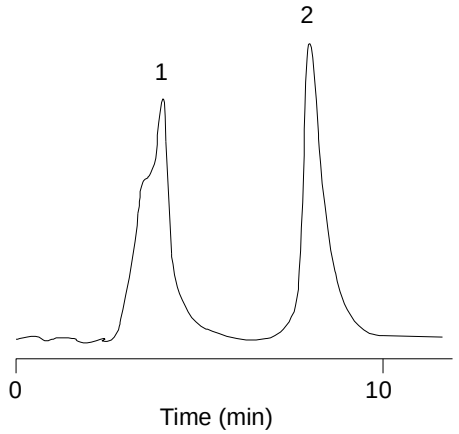
| | If Applicable to Some Peaks | Recommended Action |
|---|---|---|
| | Partially plugged column frit | • Backflush the column (if applicable)<br>• Use an inline filter<br>• Use a guard column |
| | Column void | • Use a guard column<br>• Use less aggressive mobile phase conditions<br>• Replace the column |
| | Sample volume overload | • Use a smaller injection volume |
| | Sample solvent incompatibility with mobile phase | • Use mobile phase or weaker miscible solvent as injection solvent |
| | Issues with injection valve | • Check injector valve parts<br>• Replace worn parts |



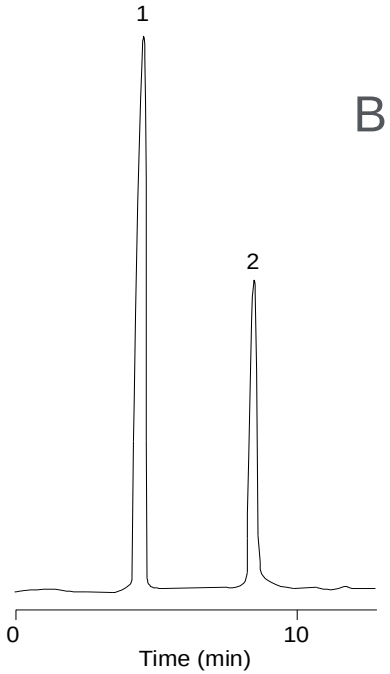
# Split Peaks from Injection Solvent Effects

Column: Agilent StableBond SB-C8, 4.6 x 150 mm, 5 mm  Mobile phase: 82% $H_2O$:18% ACN
Injection volume: 30 mL  Sample: 1. Caffeine 2. Salicylamide

A. Injection solvent
100% acetonitrile




B. Injection solvent
mobile phase

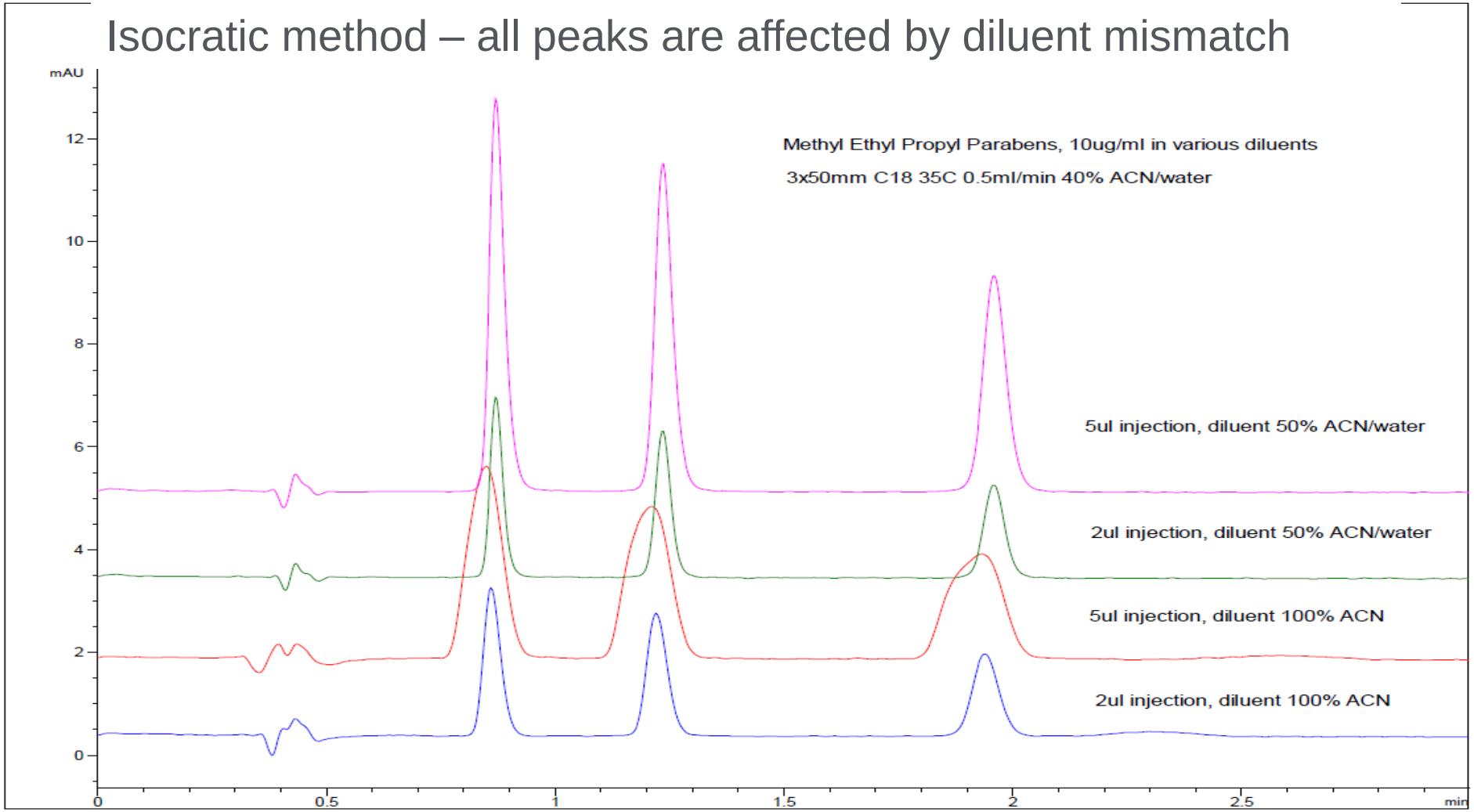



Injecting in a solvent stronger than the mobile phase can cause peak shape problems, such as peak splitting or broadening.

Keep organic concentration in sample solvent ≤ mobile phase

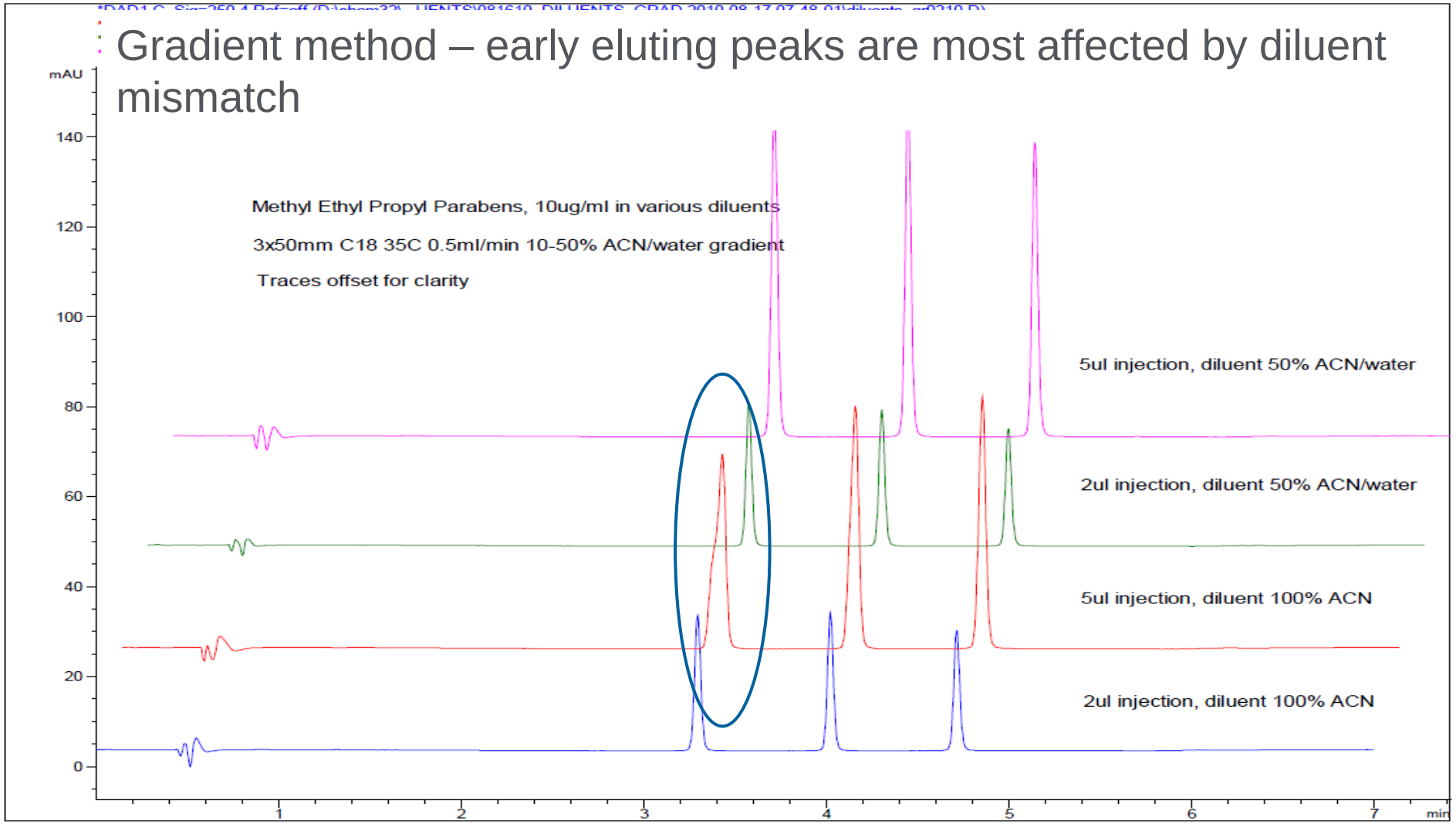
# Strong Sample Solvent Can Compromise Peak Shape

## Isocratic method



# Strong Sample Solvent Can Compromise Peak Shape

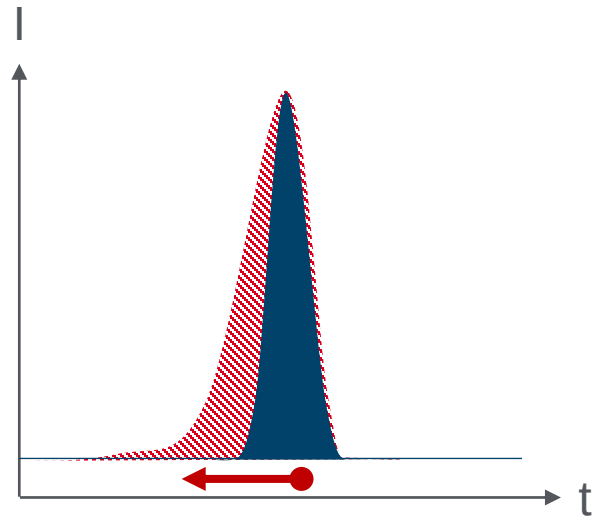
## Gradient analysis

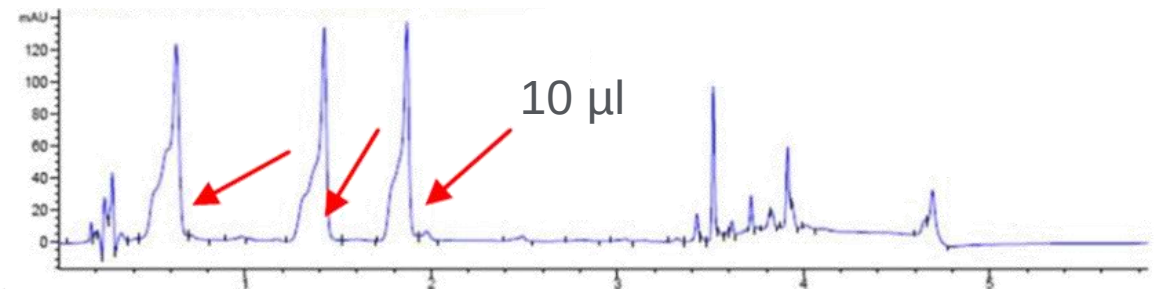


# Changes in Peak Shape

## Fronting

| | Potential Cause | Recommended Action |
|---|---|---|
| | Channeling in column | • Replace the column<br>• Use guard columns |
| | Column overload | • Use a higher capacity column (increase length, diameter or change to high-capacity material)<br>• Decrease sample amount |

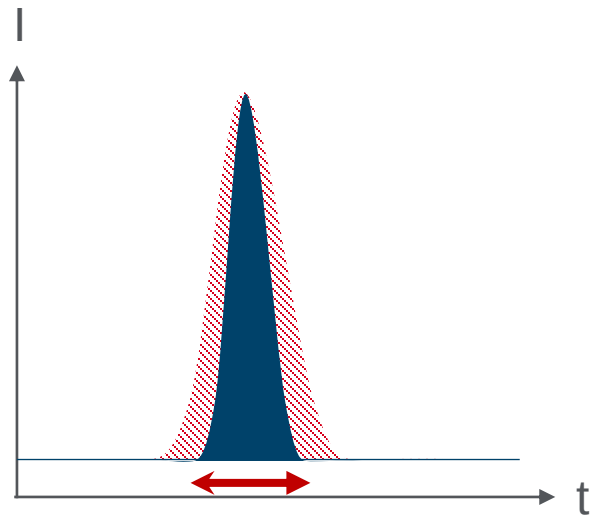



# Changes in Peak Shape

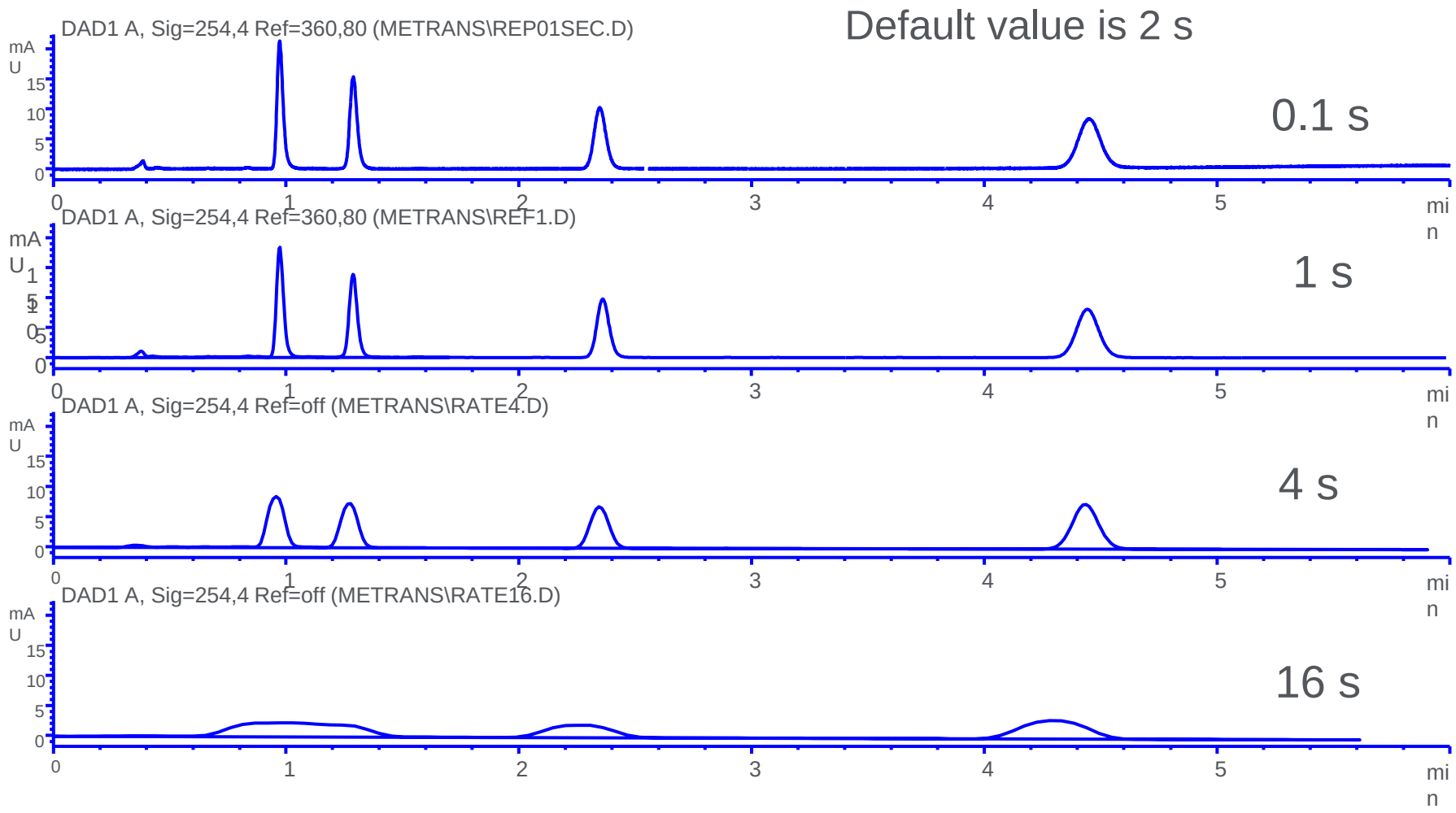
## Peak broadening

| | Potential Cause | Recommended Action |
|---|---|---|
| | Injection volume is too large | • Decrease the injection volume |
| | Long retention times | • Use gradient elution or stronger mobile phase |
| | System settings | • Check data collection rate:<br>• Adjust the detector setting or time constant to the fastest possible value without compromising signal-to-noise. |
| | Viscosity of the mobile phase is too high | • Increase the column temperature |
| | Detector cell volume is too large | • Use the smallest possible cell volume |
| | Improper fittings/connections | • Ensure that your fitting connections are correct |
| | Extra tubing volume on system | • Ensure that the tubing is narrow and as short as possible to avoid extra volume. |
| | Sample diluent too strong | • Reduce diluent strength |



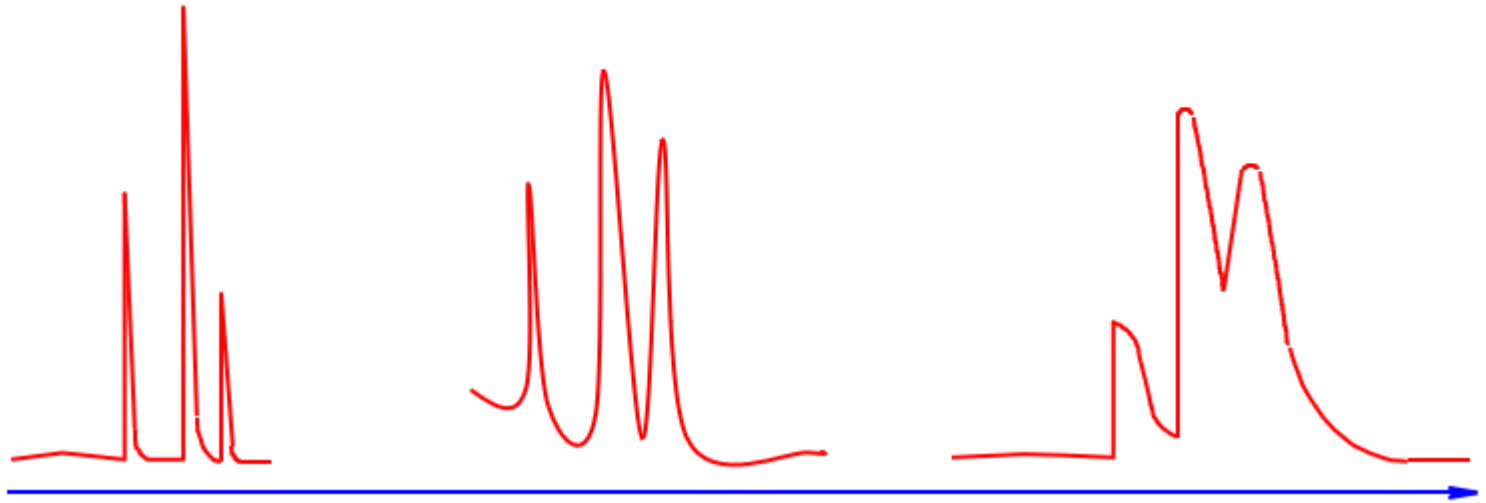
# Changes in Peak Shape

## Influence of data rate on appearance



# Peak Shape

## Extracolumn dispersion (Volume)

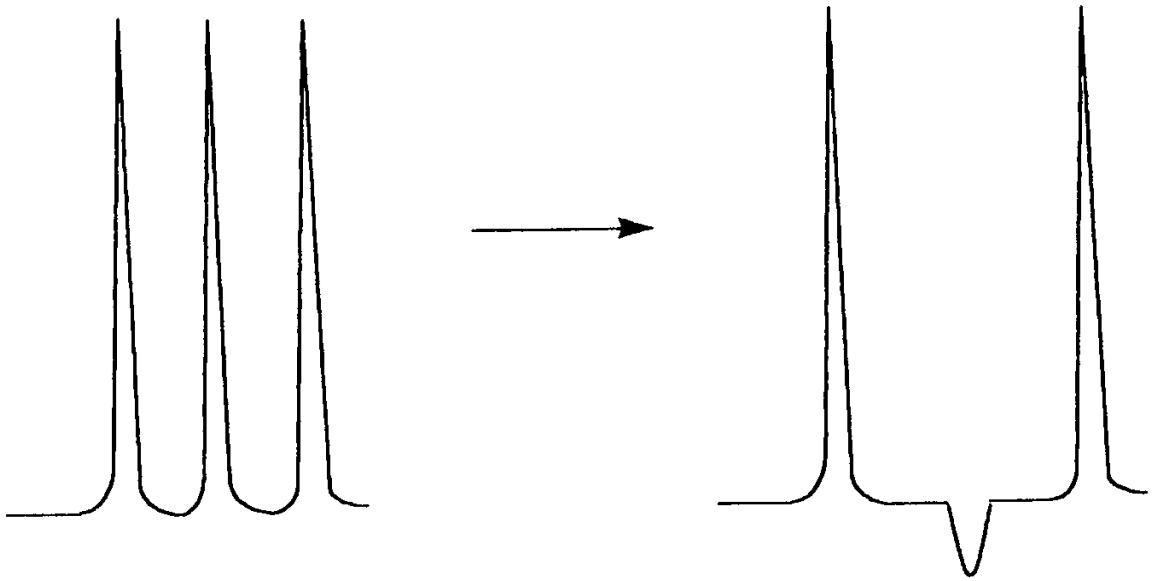


Increasing extracolumn volume

- Use short, small internal diameter tubing between the injector and the column and between the column and the detector.
- Make certain all tubing connections are made with matched fittings.
- Use a low-volume detector cell.
- Inject small sample volumes.

| Length | 10mm | 50mm | 100mm | 150mm |
|---|---|---|---|---|
| Tubing ID | Volume | Volume | Volume | Volume |
| 0.17mm (green) | 0.227 uL | 1.1uL | 2.27 uL | 3.3 uL |
| 0.12mm (red) | 0.113 uL | 0.55uL | 1.13 uL | 1.65 uL |

# Peak Shape
## Negative peaks



Normal

Negative

### Causes

- Absorbance of the sample is less than the mobile phase
- Equilibrium disturbance when the sample solvent passes through the column
- Normal with Refractive Index Detectors
- Indirect UV detection

# Retention

# What is the Specific Issue?

- Retention times of all peaks shift
- Retention time of only one peak shifts

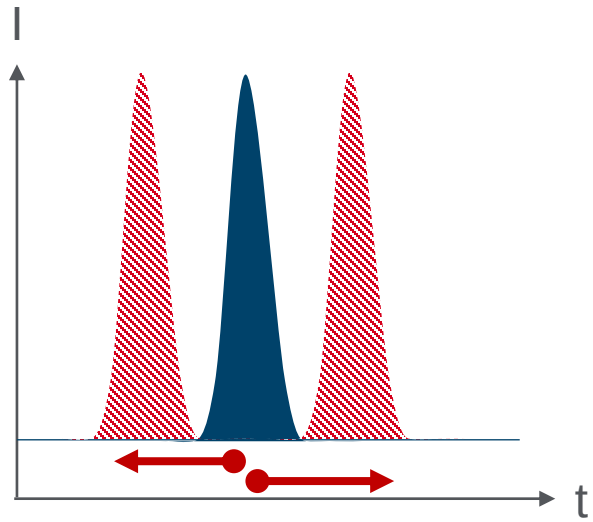
Increase the detail to be more specific:

- Retention time of all peaks shift
- Retention time of all peaks is shifting earlier
- Retention time of all peaks is shifting to earlier times and the extent of the shift appears to be the same

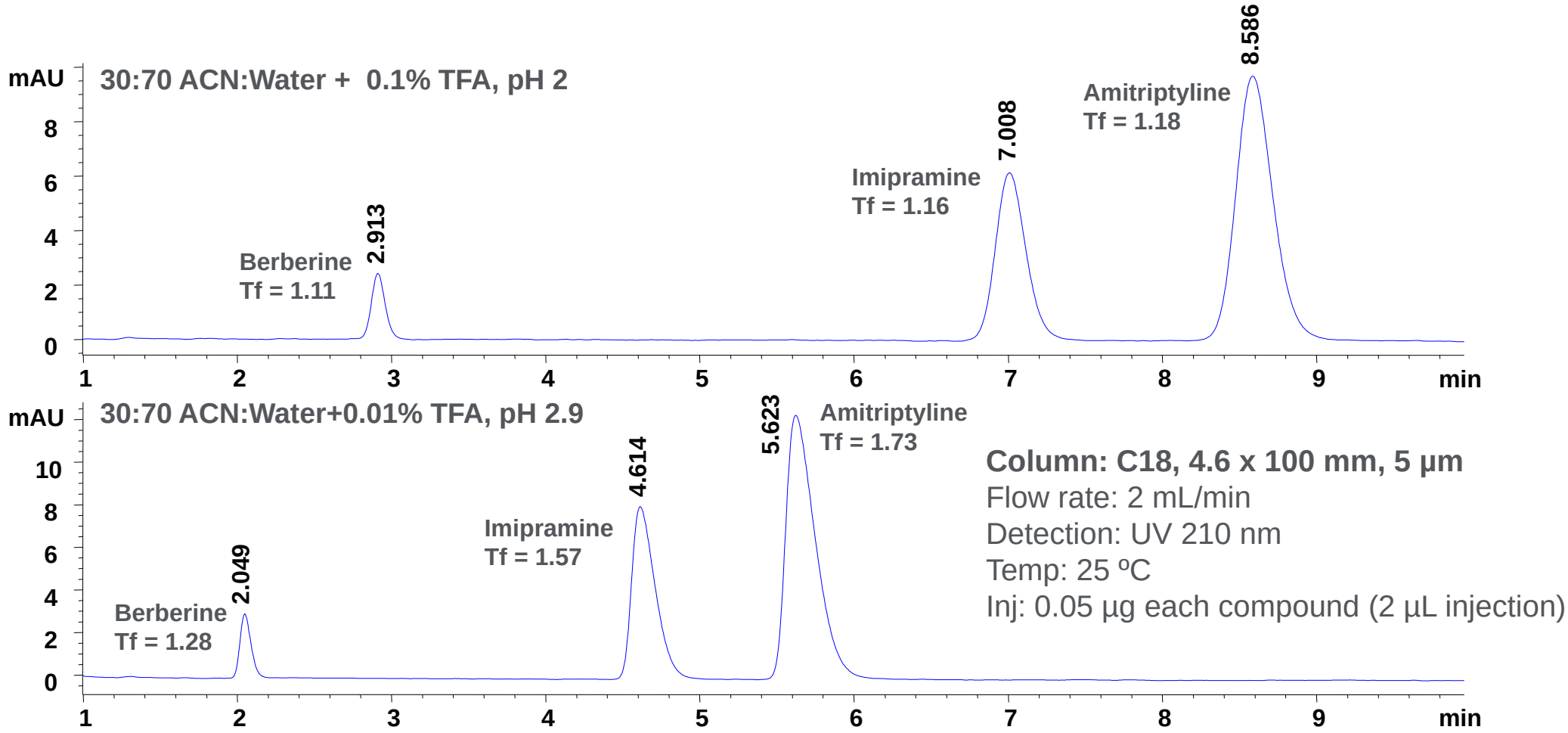
# Changes in Separation

## Retention time changing

| | Potential Cause | Recommended Action |
|---|---|---|
| | Flow rate changing | Check “Pressure fluctuation”, pump flow rate |
| | Inconsistent online mobile phase mixing | Ensure gradient system is delivering constant composition<br>check vs. manual preparation of mobile phase |
| | Column temperature varying | Thermostat column and<br>ensure constant lab temperature |
| | Equilibration time insufficient with gradient run or change in isocratic mobile phase | Flush with at least 10 column volumes after solvent change or gradient conclusion |
| | Selective evaporation of mobile phase component | Keep solvent reservoirs covered<br>Prepare fresh mobile phase |
| | Buffer capacity insufficient | Use >20 mM concentration of buffer |
| | Contamination buildup | Occasionally flush the column with strong solvent to remove contaminants |
| | First few injections – adsorption on active sites | Condition the column by initial injection of a concentrated sample |
| | Column overloaded with sample | Decrease injection volume or concentration |
| | Active sites on silica packing | Add competing base to mobile phase |
| | Mobile phase composition changing | Follow ‘best practices' |



# Change in Volatile Buffer Concentration and Shift in Retention Time and Peak Shape



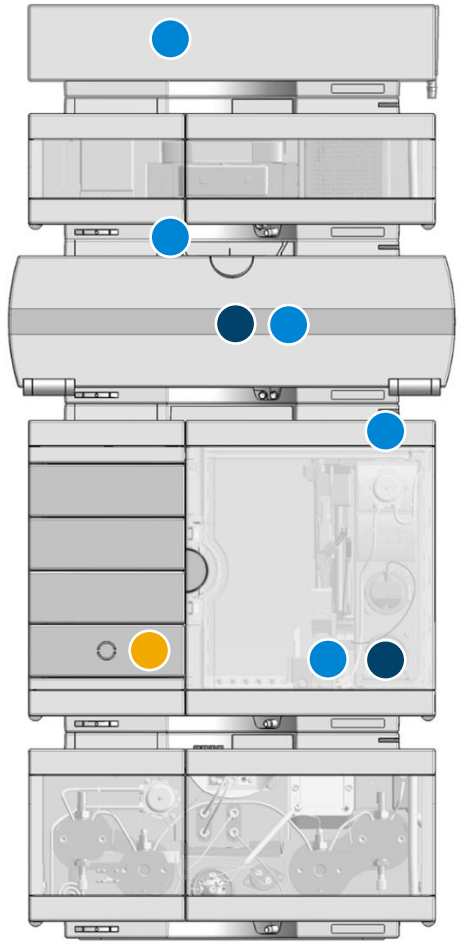

Tip: The definition of “volatile” is “evaporating rapidly” or “passing off rapidly in the form of vapor”

# Changes in Separation

## Ghost peaks, carry over

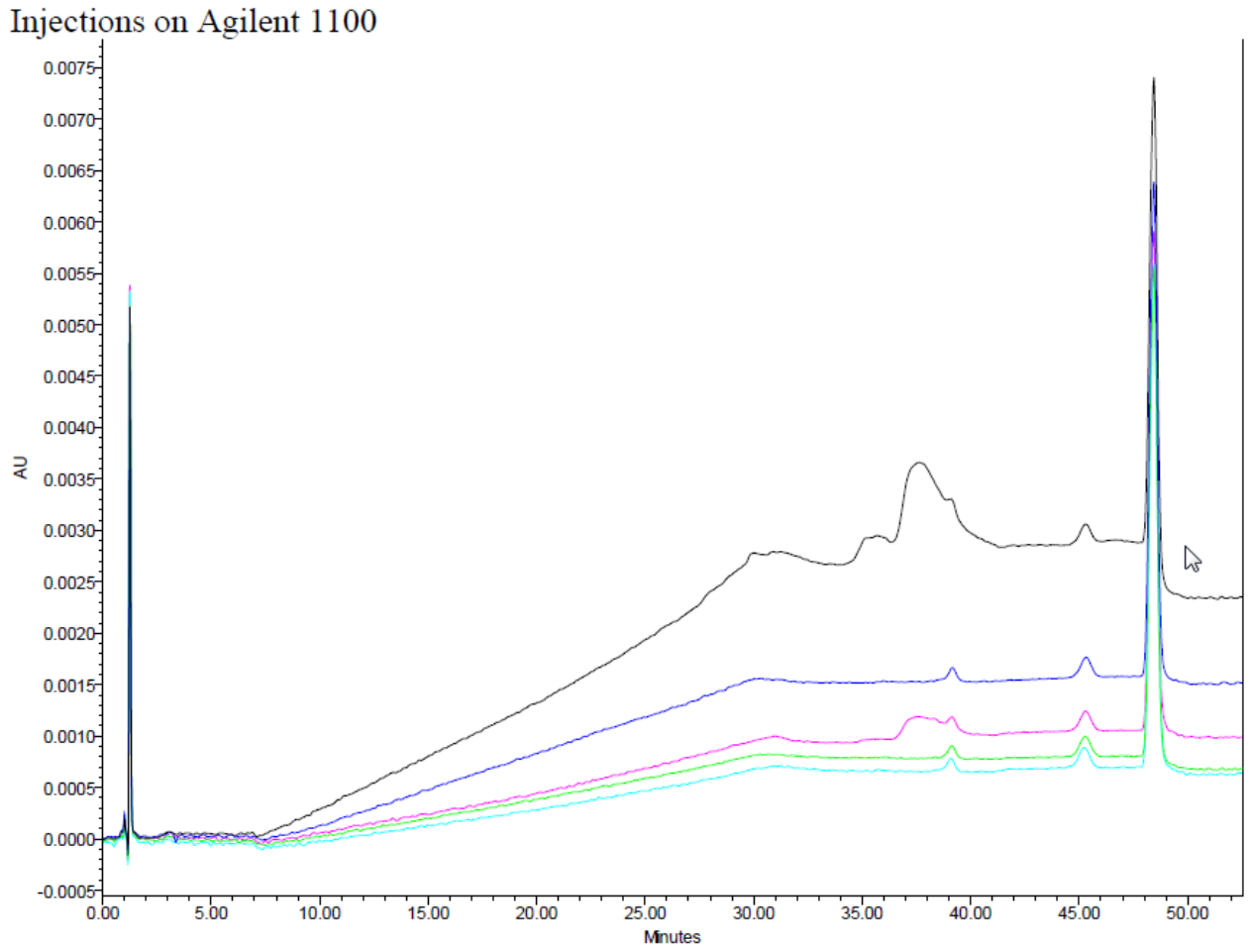
| | Potential Cause | Recommended Action |
|---|---|---|
| ● | Peaks from previous injections | • Flush the column to remove contaminants<br>• Check with blank injection |
| ● | Specific interaction with metal surfaces | • Passivate instrument<br>• Use InfinityLab Deactivator Additive<br>• Use bio-inert LC equipment |
| ● | Contamination or unknown interferences in samples | • Proper sample cleanup |
| ● | Ion pair – disequilibrium | • Prepare sample in actual mobile phase to minimize disturbance |

BIO INERT



[PN 5191-4506 | Deactivator Additive 50 ml]
[PN 5191-3940 | Deactivator Additive 25 ml]

# Solvent Contamination




Solvent: acetonitrile

Solvent source 1
Solvent source 2
Solvent source 3
Solvent source 4, lot 1
Solvent source 4, lot 2

# Column Cleaning

## Do what's recommended for your column

**Tips for cleaning columns:**

- Flush with stronger solvents than your mobile phase
- Make sure the detector is taken out of the flow path
- Do not add your organic solvent directly to the buffer, as this may cause the buffer salts to precipitate out and lead to more backpressure

**For reversed phase:**

Use at least 10 column volumes of each solvent for analytical columns

1. Start with your mobile phase without buffer salts (water/organic)
2. 100% organic (MeOH or ACN)
3. Check the pressure to see if it has returned to normal; if not, then
4. Discard the column or consider more drastic conditions: 75% acetonitrile/25% isopropanol
5. 100% isopropanol
6. 100% methylene chloride, solvent wash for very nonpolar compounds
7. Hexane

*Always see your specific column user guide for instructions

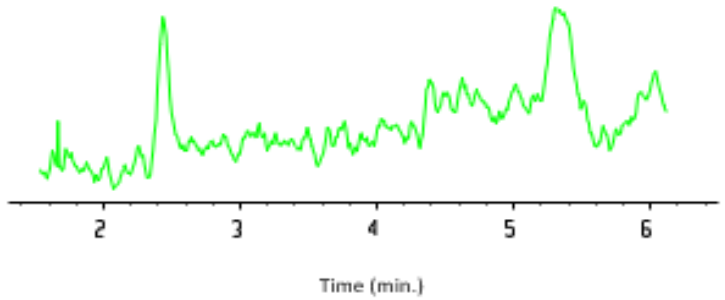
LC Column User Guides | Agilent

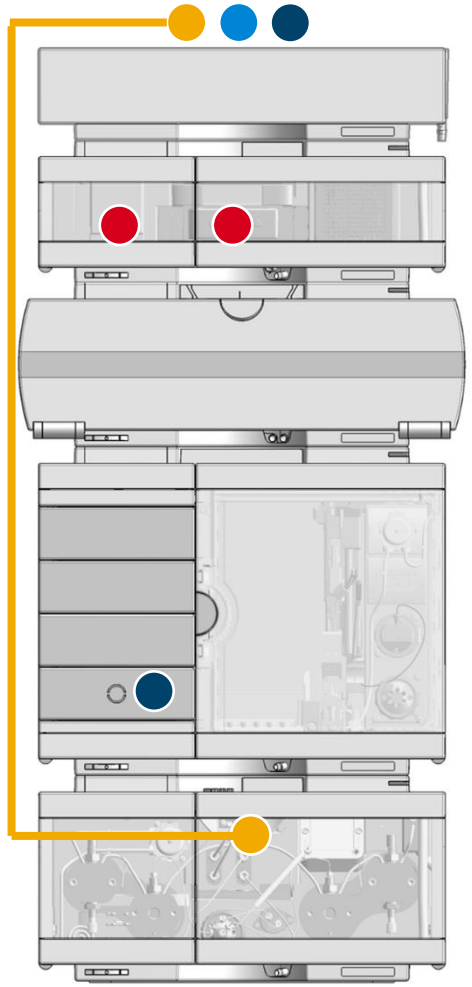
# Baselines and Detection

# Changes in Detection

## Noisy baseline

| | Potential Cause | Recommended Action |
|---|---|---|
| ● | Gas bubbles in the mobile phase | • Apply degassing<br>• Check degasser performance |
| ● | Low difference between sample and mobile phase absorbance | • Check absorbance values of sample vs. mobile phase |
| ● | Contamination | • Use degassed HPLC-grade solvents<br>• Flush system<br>• Clean up sample |
| ● | Detector optics | • Perform intensity test<br>• Check signal with flow cell removed if possible<br>• Replace lamp |
| | Pressure instability | • Check “Pressure fluctuation” |

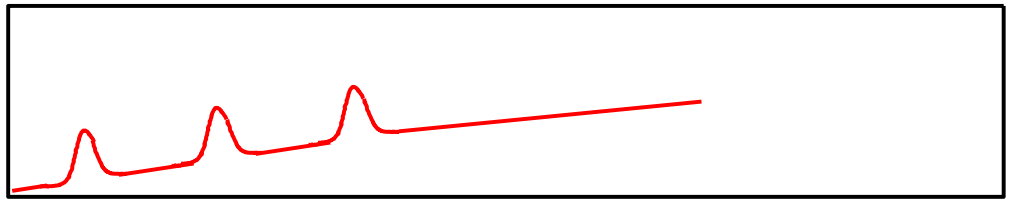


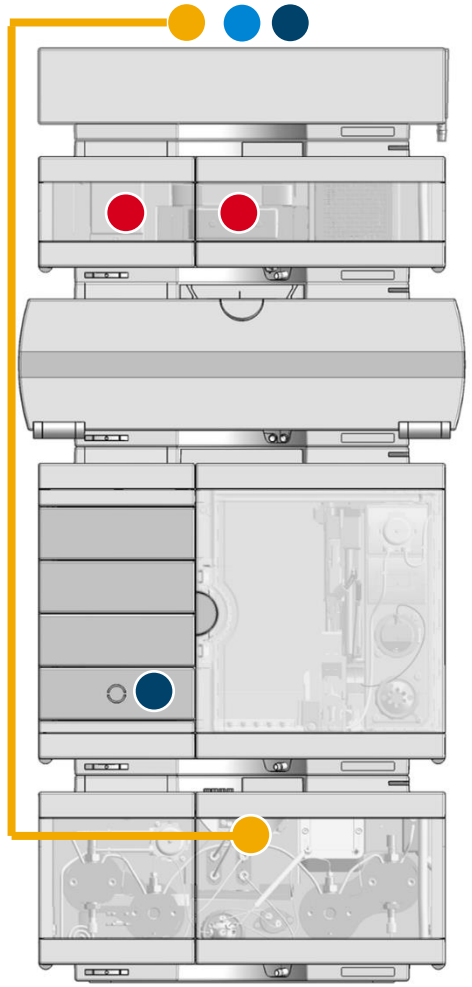



# Changes in Detection

## Drifting baseline

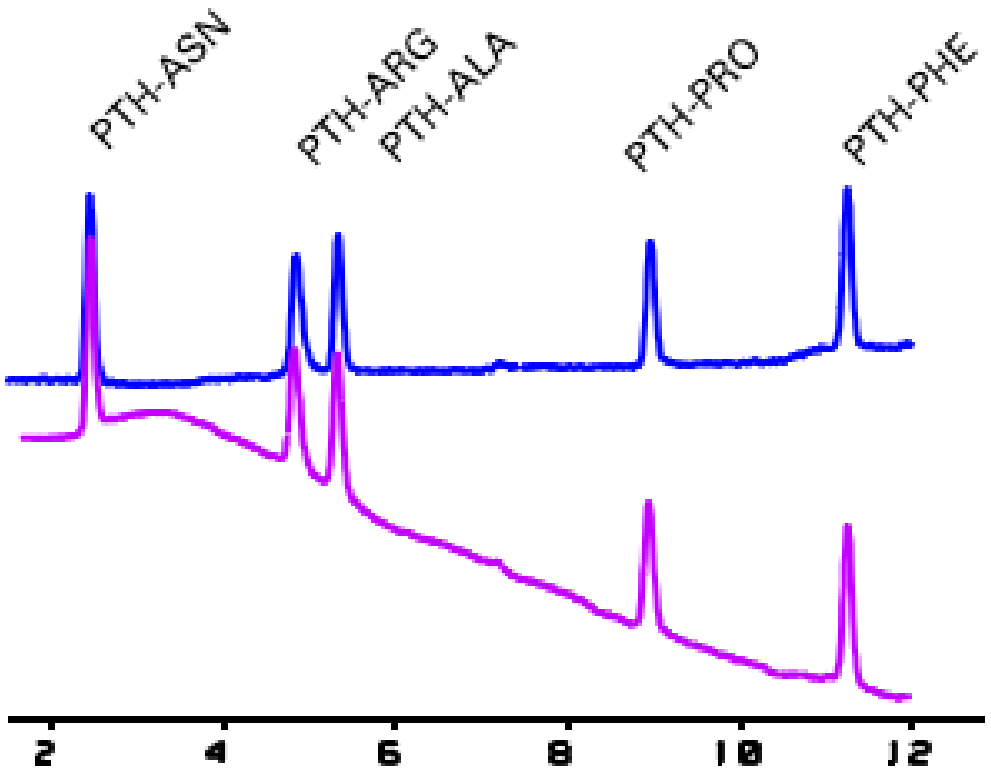
| | Potential Cause | Recommended Action |
|---|---|---|
| ● | Contamination in the mobile phase | • Make up new mobile phase<br>• If running a gradient, might need to adjust modifier |
| ● | Low difference between sample and mobile phase absorbance | • Check absorbance values of sample vs. mobile phase |
| ● | Contamination | • Use degassed HPLC-grade solvents<br>• Flush system<br>• Clean up sample |
| ● | Detector | • Check temperature stability<br>• Check for leaks<br>• Replace lamp |
| | Pressure instability | • Check “Pressure fluctuation” |





# Reference Wavelength

## How settings can affect the baseline




1 pmol each

λ 267 nm

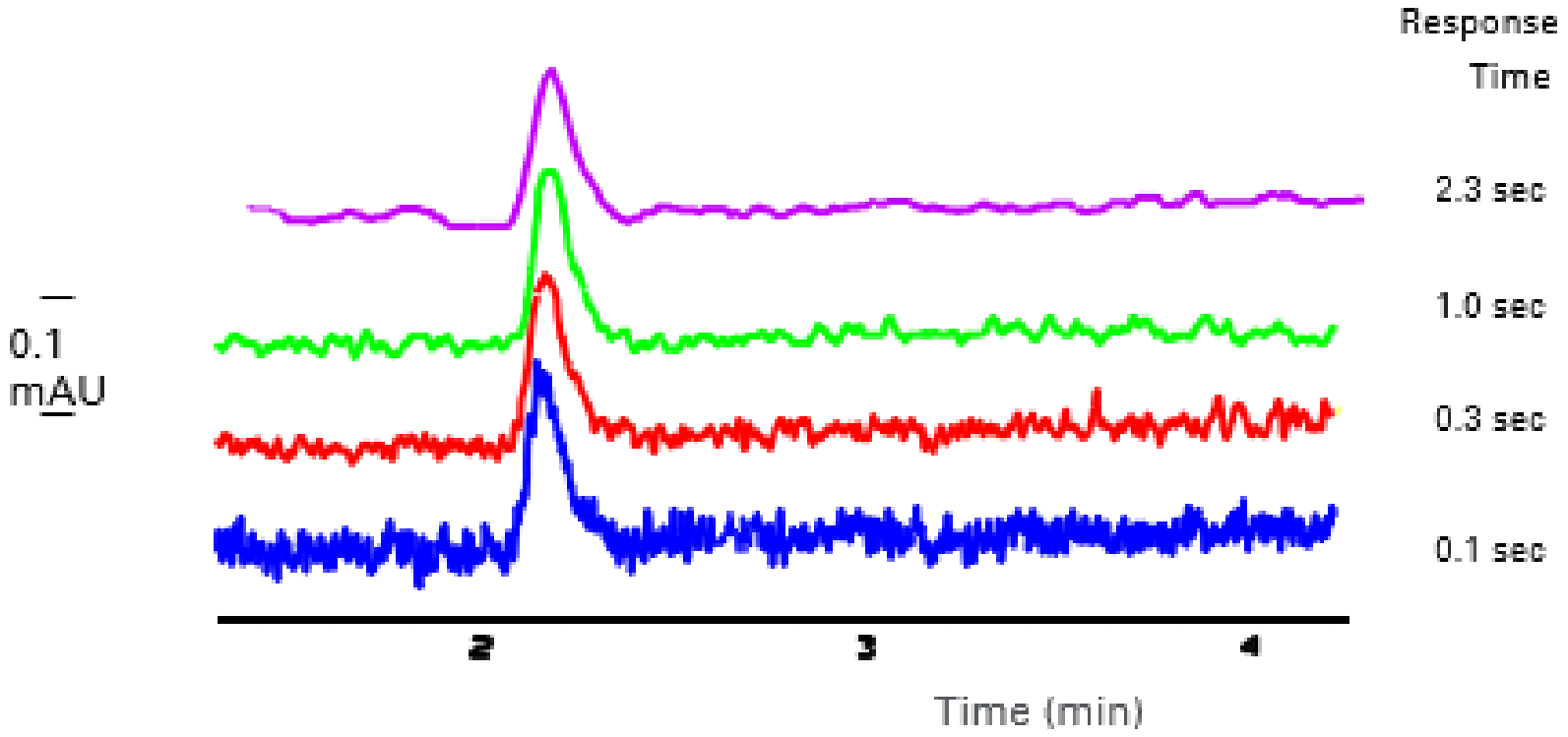
ref 380 nm

λ 267 nm

No reference

Gradient: 0.02 m $KH_2PO_4$/ACN, from 12% ACN to 45% ACN in 12 min

# Influence of Data Collection Rate on Noise



# Column Considerations

# LC Columns Are Not Indestructible

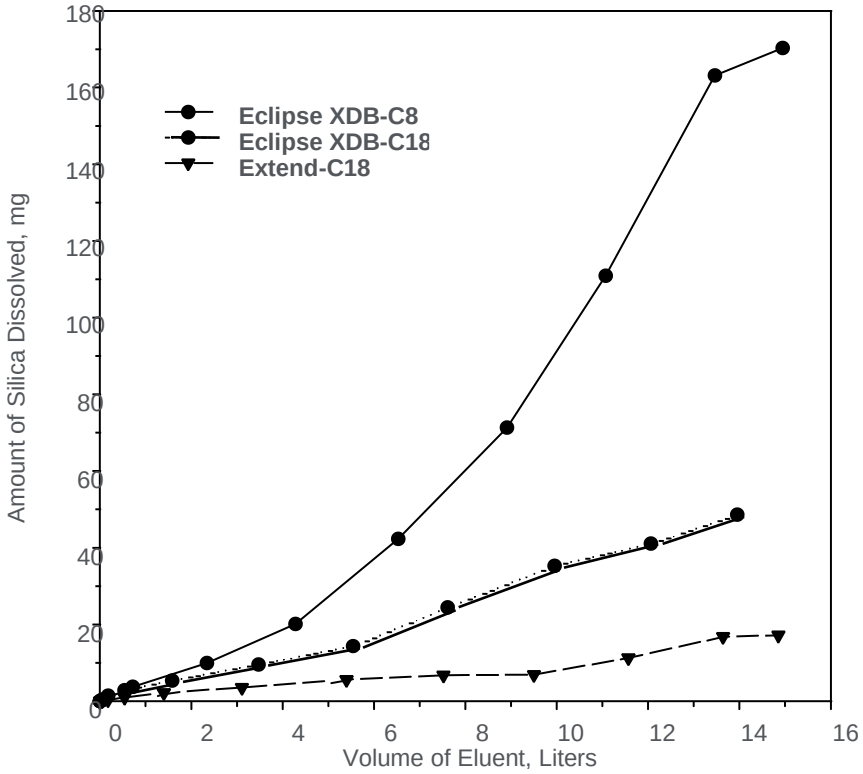
- Columns are packed using hydraulic pressure and can be damaged by it.
- Silica dissolves (slowly) at higher pH
- Acid hydrolysis of bonded phase can occur at low pH
- Column failure
  - Void
  - Contamination

Columns must be stored properly

- Check your user guide

Important to Do:

- Know the technical specifications for your column
- Choose a mobile phase that is right for your column
- Keep record/history of your column




| | |
|---|---|
| Columns: | 4.6 x 150 mm, 5 µm |
| Purge: | 50% ACN / 50% 0.02 M $K_2HPO_4$, pH 11 |
| Flow Rate: | 1.5 mL / min |
| Temperature: | 25°C |
| Detection: | Silicate concentration by silicomolybdate color reaction |

[LC Column User Guides | Agilent]

# Choice of Your Column

## Low and high pH can cause column failure

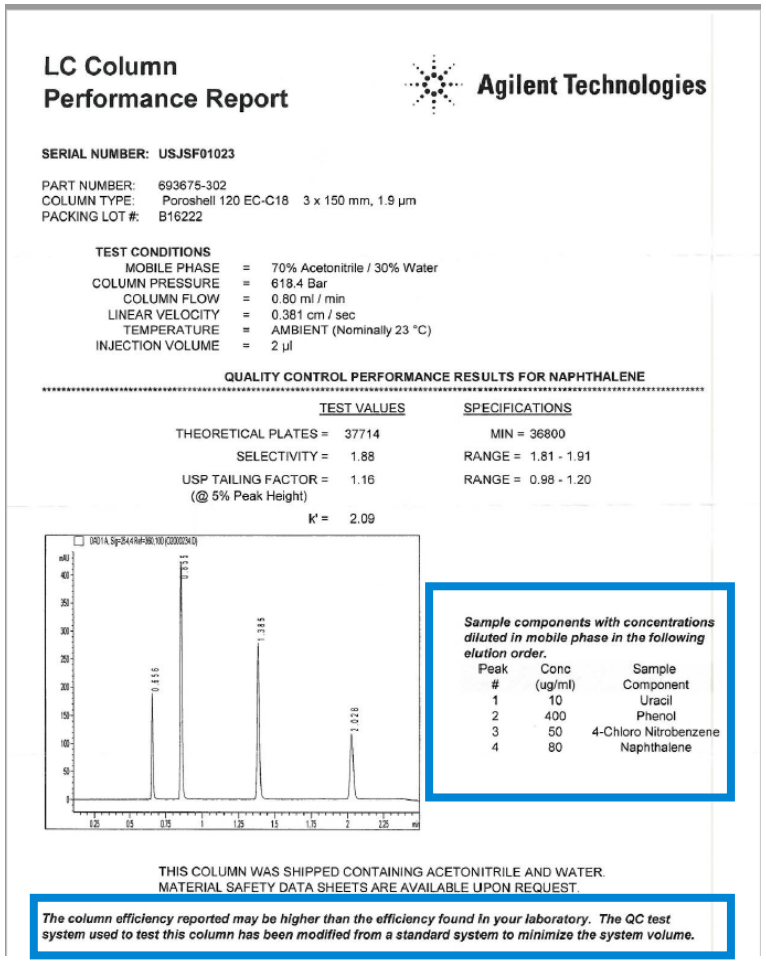
**The InfinityLab Poroshell 120 portfolio offers choices for low and high pH**

| Best all around | Best for low pH mobile phases | Best for high pH mobile phases | Best for alternative selectivity | Best for more polar analytes | Chiral |
|---|---|---|---|---|---|
| **EC-C18**<br>1.9 µm, 2.7 µm, 4 µm | **SB-C18**<br>1.9 µm, 2.7 µm, 4 µm | **HPH-C18**<br>1.9 µm, 2.7 µm, 4 µm | **Bonus-RP**<br>2.7 µm | **SB-Aq**<br>1.9 µm, 2.7 µm, 4 µm<br>**AQ-C18**<br>2.7 µm | **Chiral-V**<br>2.7 µm |
| **EC-C8**<br>1.9 µm, 2.7 µm, 4 µm | **SB-C8**<br>2.7 µm | **HPH-C8**<br>2.7 µm, 4 µm | **PFP**<br>1.9 µm, 2.7 µm, 4 µm | **EC-CN**<br>2.7 µm | **Chiral-T**<br>2.7 µm |
| **Phenyl-Hexyl**<br>1.9 µm, 2.7 µm, 4 µm | | **CS-C18**<br>2.7 µm | | **HILIC**<br>1.9 µm, 2.7 µm, 4 µm | **Chiral- CD**<br>2.7 µm |
| | | | | **HILIC-Z**<br>1.9 µm, 2.7 µm, 4 µm | **Chiral-CF**<br>2.7 µm |
| | | | | **HILIC- OH5**<br>2.7 µm | |

# Benchmark Your Column

Agilent InfinityLab

## Every new column should be tested on your instrument



LC Column
Performance Report

Agilent Technologies

SERIAL NUMBER: USJSF01023

PART NUMBER: 693675-302
COLUMN TYPE: Poroshell 120 EC-C18 3 x 150 mm, 1.9 µm
PACKING LOT #: B16222

TEST CONDITIONS
MOBILE PHASE = 70% Acetonitrile / 30% Water
COLUMN PRESSURE = 618.4 Bar
COLUMN FLOW = 0.80 ml / min
LINEAR VELOCITY = 0.381 cm / sec
TEMPERATURE = AMBIENT (Nominally 23 °C)
INJECTION VOLUME = 2 µl

QUALITY CONTROL PERFORMANCE RESULTS FOR NAPHTHALENE

| | TEST VALUES | SPECIFICATIONS |
|---|---|---|
| THEORETICAL PLATES = | 37714 | MIN = 36800 |
| SELECTIVITY = | 1.88 | RANGE = 1.81 - 1.91 |
| USP TAILING FACTOR = (@ 5% Peak Height) | 1.16 | RANGE = 0.98 - 1.20 |
| k' = | 2.09 | |



*Sample components with concentrations diluted in mobile phase in the following elution order.*

| Peak # | Conc (ug/ml) | Sample Component |
|---|---|---|
| 1 | 10 | Uracil |
| 2 | 400 | Phenol |
| 3 | 50 | 4-Chloro Nitrobenzene |
| 4 | 80 | Naphthalene |

THIS COLUMN WAS SHIPPED CONTAINING ACETONITRILE AND WATER.
MATERIAL SAFETY DATA SHEETS ARE AVAILABLE UPON REQUEST.

*The column efficiency reported may be higher than the efficiency found in your laboratory. The QC test system used to test this column has been modified from a standard system to minimize the system volume.*

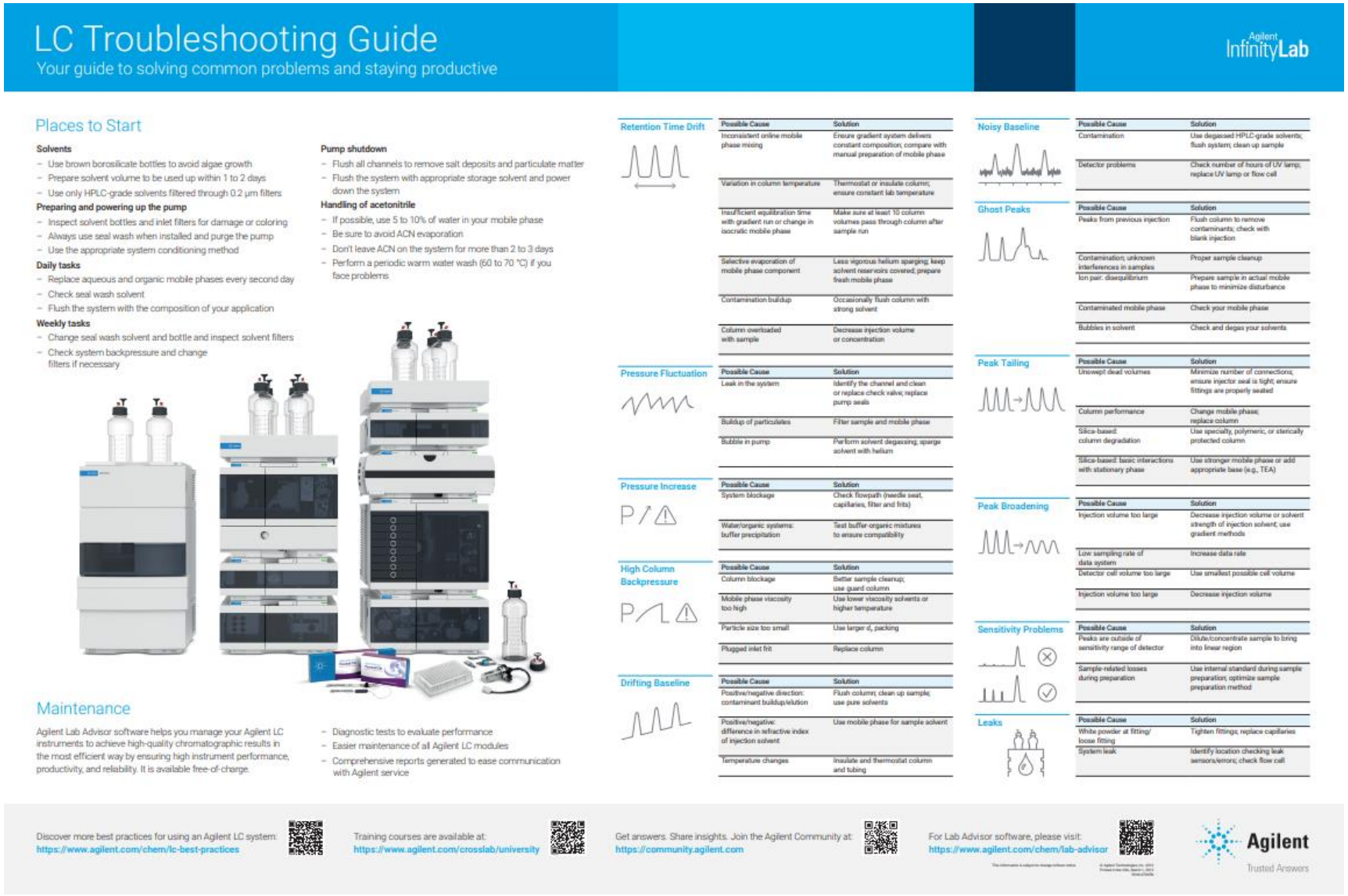
### Performance verification based on Agilent checkout

- Run Agilent checkout before use
  - Record difference between your instrument and performance report (use as base value)
- Perform again if column seems to lose performance
  - Compare with results from first run

### Performance verification based on in-house checkout

- Run in house checkout before use
  - Record key specifications such as tailing factor, plates, and backpressure
- Perform again if column seems to lose performance
  - Compare with results from first run

# LC Troubleshooting Poster Available



## LC Troubleshooting Guide

Your guide to solving common problems and staying productive

Agilent InfinityLab

### Places to Start

**Solvents**
- Use brown borosilicate bottles to avoid algae growth
- Prepare solvent volume to be used up within 1 to 2 days
- Use only HPLC-grade solvents filtered through 0.2 µm filters

**Preparing and powering up the pump**
- Inspect solvent bottles and inlet filters for damage or coloring
- Always use seal wash when installed and purge the pump
- Use the appropriate system conditioning method

**Daily tasks**
- Replace aqueous and organic mobile phases every second day
- Check seal wash solvent
- Flush the system with the composition of your application

**Weekly tasks**
- Change seal wash solvent and bottle and inspect solvent filters
- Check system backpressure and change filters if necessary

**Pump shutdown**
- Flush all channels to remove salt deposits and particulate matter
- Flush the system with appropriate storage solvent and power down the system

**Handling of acetonitrile**
- If possible, use 5 to 10% of water in your mobile phase
- Be sure to avoid ACN evaporation
- Don't leave ACN on the system for more than 2 to 3 days
- Perform a periodic warm water wash (60 to 70 °C) if you face problems

### Maintenance

Agilent Lab Advisor software helps you manage your Agilent LC instruments to achieve high-quality chromatographic results in the most efficient way by ensuring high instrument performance, productivity, and reliability. It is available free-of-charge.

- Diagnostic tests to evaluate performance
- Easier maintenance of all Agilent LC modules
- Comprehensive reports generated to ease communication with Agilent service

**Retention Time Drift**

| Possible Cause | Solution |
|---|---|
| Inconsistent online mobile phase mixing | Ensure gradient system delivers constant composition; compare with manual preparation of mobile phase |
| Variation in column temperature | Thermostat or insulate column; ensure constant lab temperature |
| Insufficient equilibration time with gradient run or change in isocratic mobile phase | Make sure at least 10 column volumes pass through column after sample run |
| Selective evaporation of mobile phase component | Less vigorous helium sparging; keep solvent reservoirs covered; prepare fresh mobile phase |
| Contamination buildup | Occasionally flush column with strong solvent |
| Column overloaded with sample | Decrease injection volume or concentration |

**Pressure Fluctuation**

| Possible Cause | Solution |
|---|---|
| Leak in the system | Identify the channel and clean or replace check valve; replace pump seals |
| Buildup of particulates | Filter sample and mobile phase |
| Bubble in pump | Perform solvent degassing; sparge solvent with helium |

**Pressure Increase**

| Possible Cause | Solution |
|---|---|
| System blockage | Check flowpath (needle seat, capillaries, filter and frits) |
| Water/organic systems: buffer precipitation | Test buffer-organic mixtures to ensure compatibility |

**High Column Backpressure**

| Possible Cause | Solution |
|---|---|
| Column blockage | Better sample cleanup; use guard column |
| Mobile phase viscosity too high | Use lower viscosity solvents or higher temperature |
| Particle size too small | Use larger $d_p$ packing |
| Plugged inlet frit | Replace column |

**Drifting Baseline**

| Possible Cause | Solution |
|---|---|
| Positive/negative direction: contaminant buildup/elution | Flush column; clean up sample; use pure solvents |
| Positive/negative: difference in refractive index of injection solvent | Use mobile phase for sample solvent |
| Temperature changes | Insulate and thermostat column and tubing |

**Noisy Baseline**

| Possible Cause | Solution |
|---|---|
| Contamination | Use degassed HPLC-grade solvents; flush system; clean up sample |
| Detector problems | Check number of hours of UV lamp; replace UV lamp or flow cell |

**Ghost Peaks**

| Possible Cause | Solution |
|---|---|
| Peaks from previous injection | Flush column to remove contaminants; check with blank injection |
| Contamination; unknown interferences in samples | Proper sample cleanup |
| Ion pair: disequilibrium | Prepare sample in actual mobile phase to minimize disturbance |
| Contaminated mobile phase | Check your mobile phase |
| Bubbles in solvent | Check and degas your solvents |

**Peak Tailing**

| Possible Cause | Solution |
|---|---|
| Unswept dead volumes | Minimize number of connections; ensure injector seal is tight; ensure fittings are properly seated |
| Column performance | Change mobile phase; replace column |
| Silica-based: column degradation | Use specialty, polymeric, or sterically protected column |
| Silica-based: basic interactions with stationary phase | Use stronger mobile phase or add appropriate base (e.g., TEA) |

**Peak Broadening**

| Possible Cause | Solution |
|---|---|
| Injection volume too large | Decrease injection volume or solvent strength of injection solvent; use gradient methods |
| Low sampling rate of data system | Increase data rate |
| Detector cell volume too large | Use smallest possible cell volume |
| Injection volume too large | Decrease injection volume |

**Sensitivity Problems**

| Possible Cause | Solution |
|---|---|
| Peaks are outside of sensitivity range of detector | Dilute/concentrate sample to bring into linear region |
| Sample-related losses during preparation | Use internal standard during sample preparation; optimize sample preparation method |

**Leaks**

| Possible Cause | Solution |
|---|---|
| White powder at fitting/ loose fitting | Tighten fittings; replace capillaries |
| System leak | Identify location checking leak sensors/errors; check flow cell |

Discover more best practices for using an Agilent LC system: https://www.agilent.com/chem/lc-best-practices

Training courses are available at: https://www.agilent.com/crosslab/university

Get answers. Share insights. Join the Agilent Community at: https://community.agilent.com

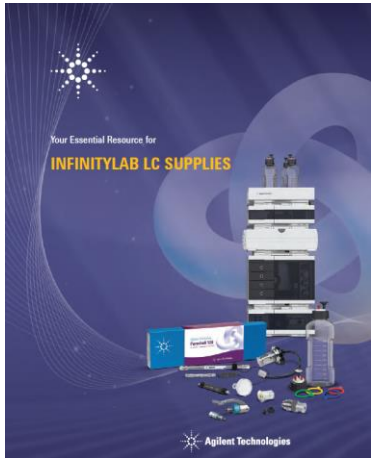
For Lab Advisor software, please visit: https://www.agilent.com/chem/lab-advisor

Agilent Trusted Answers

Request yours today at [www.agilent.com/chem/troubleshootLC](www.agilent.com/chem/troubleshootLC)

# Resources for Support


- Column user guides: LC Column User Guides | Agilent
- LC Troubleshooting poster: LC Troubleshooting Guide 5994-0709EN
- Resource page: http://www.agilent.com/chem/agilentresources
  - Quick reference guides
  - Catalogs, column user guides
  - Online selection tools, how-to videos
- InfinityLab Supplies catalog: 5991-8031EN
- LC handbook: 5990-7595EN
- YouTube – Agilent channel (maintenance videos)
- Consumables Community: Agilent Collection of Columns, Supplies, and Standards Resources - Consumables - Agilent Community
- App finder: Application Finder | Agilent
- Agilent University: Agilent University
- Your local product specialists
- Agilent Peak Tales podcasts: peaktales.libsyn.com
- Webinars, upcoming and recorded: LC & LC/MS Column Webinars | Agilent




# Contact Agilent Chemistries and Supplies Technical Support

Available in the U.S. and Canada, 8-5 all time zones

1-800-227-9770 option 3, option 3:

Option 1 for GC and GC/MS columns and supplies

Option 2 for LC and LC/MS columns and supplies

Option 3 for sample preparation, filtration, and QuEChERS

Option 4 for spectroscopy supplies

Option 5 for chemical standards

Option 6 for Prozyme products

gc-column-support@agilent.com

lc-column-support@agilent.com

spp-support@agilent.com

spectro-supplies-support@agilent.com

chem-standards-support@agilent.com

pzi.info@agilent.com

# Agilent

Trusted Answers