# My Chromatography Has Changed: Steps for Effective Troubleshooting

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Agilent
Trusted Answers

# 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

# Steps For Effective Troubleshooting

## You've recognized that there is a problem

Ask questions:

- When did the system or chromatography last function properly?
- Has anything been changed?
- For the method, was the procedure followed correctly?
- Are the instrument settings correct?
- What exactly is the problem that is being seen?

# Steps For Effective Troubleshooting

## You've recognized that there is a problem

Considerations for where the problem might 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?

# First Step in Troubleshooting

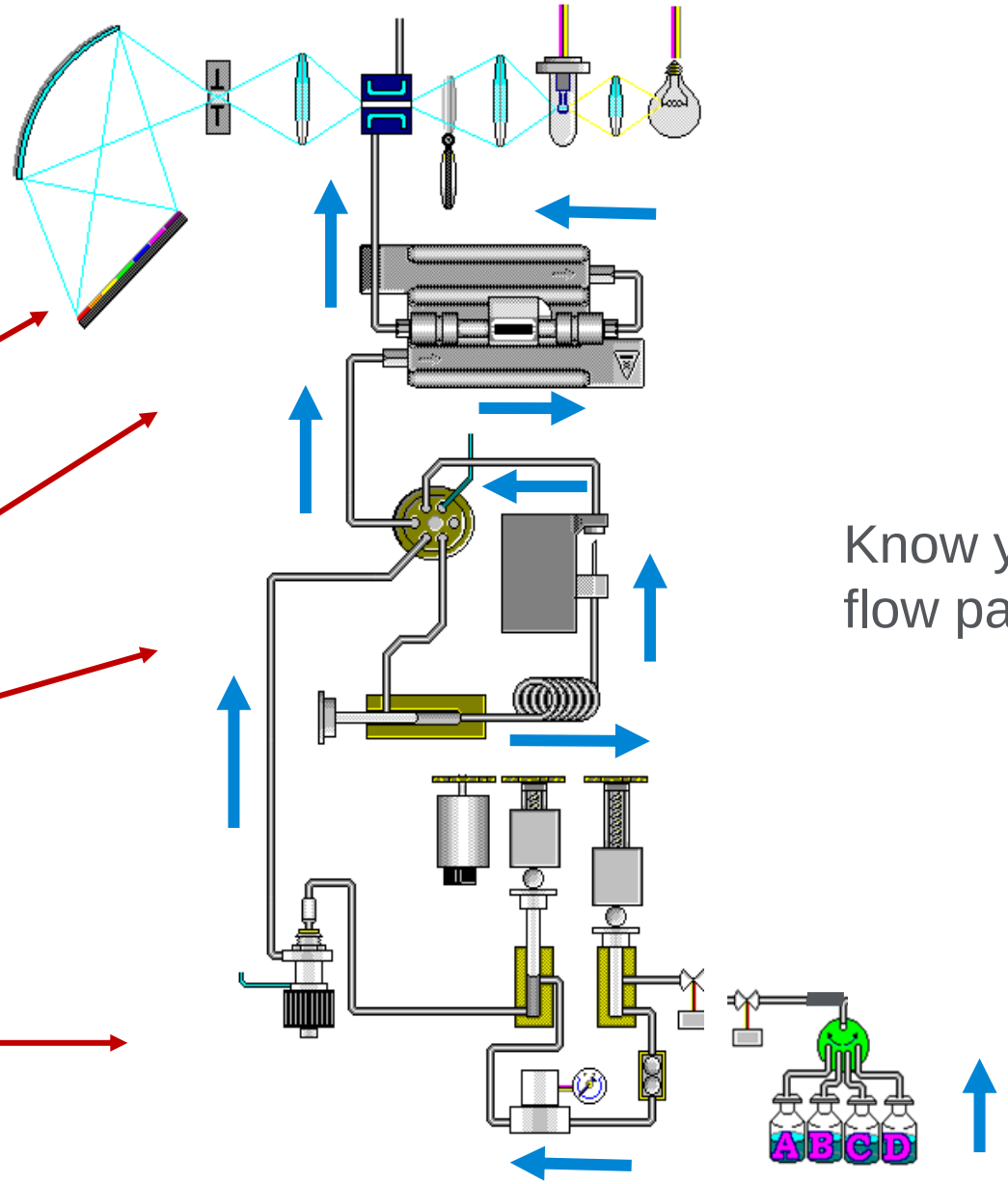
## Understand your HPLC system

Detector

Column compartment

Autosampler

Pump




Know your flow path

# Changes in System Pressure

# Causes of Increases in Back Pressure

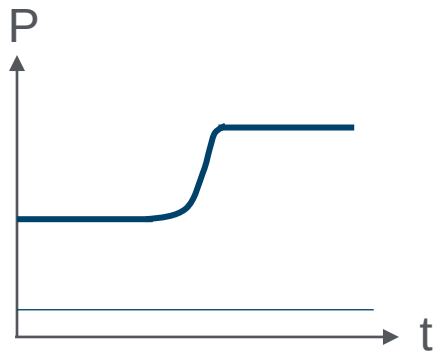
- Particles leading to blockage can come from sources located both *outside* and *inside* the LC system:
  - Solvent, buffer
  - Microbial growth in solvent reservoirs
  - The sample
  - Wear of LC components – piston seals, autosampler valve
- Debris will either be captured on the filter, frit, or inline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

Reduce LC problems by eliminating the most common sources of flow blockage that can cause increased pressure. Preventing this is the key.

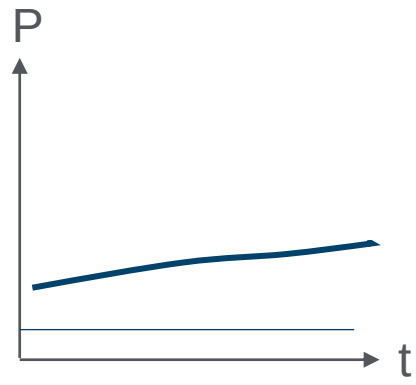
Filter, filter, filter

# 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 a 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<br>• Use a guard column |




**Blockages:** instant pressure increase step




**Clogging:** constant pressure increase over time

# Microbial Growth

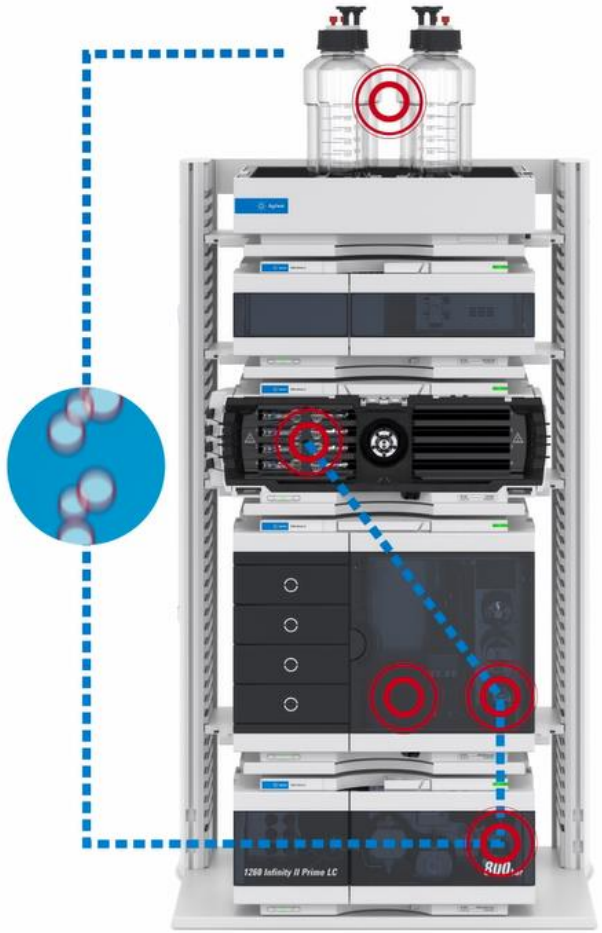
- Potential problems
  - **Increased system pressure or pressure fluctuations**
  - **Increased column pressure, premature column failure**
  - Can mimic application problems
  - Gradient inaccuracies
  - Ghost peaks
- Prevent or reduce microbial growth
  - Use freshly prepared mobile phase
  - Filter
  - Do not leave mobile phase in the 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, you can:
    - Add 5% organic to water – this can be used to reduce bacterial growth
    - Use a few mg/L of sodium azide

P/n 3150-0577
Solvent filter/degasser assembly

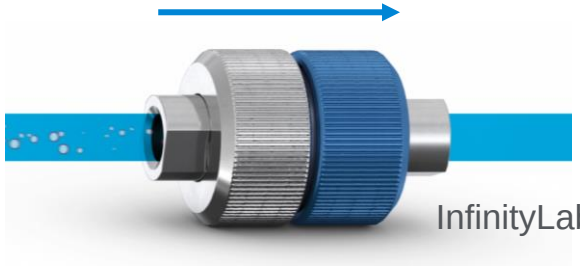
Glass solvent inlet filter (20 mm),
p/n 5041-2168
Stainless steel solvent inlet filter
p/n 01018-60028
Amber solvent bottle 1 L,
p/n 9301-6526
Clear solvent bottle 1 L
p/n 9301-6524

# Why Use an Inline Filter?

Particles lead to blockage



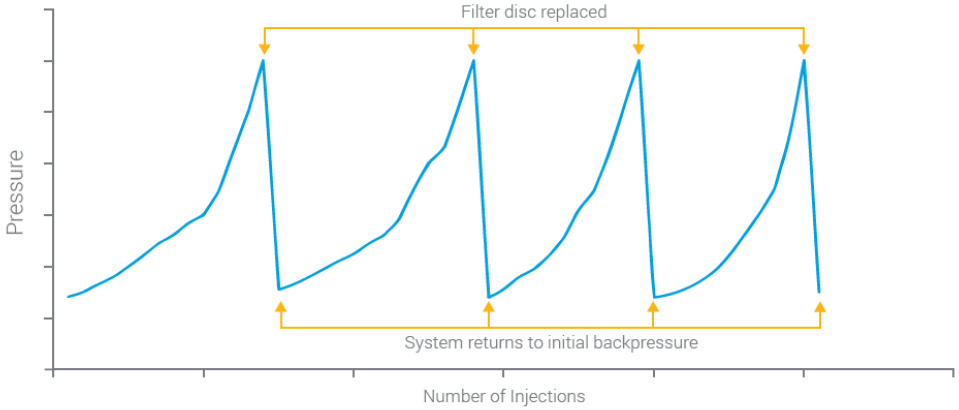
1290 inline filter, p/n 5067-6189



InfinityLab Quick Change inline filter

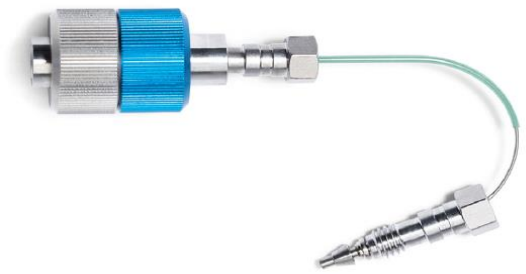
Filter particles to prevent column clogging

Extend column lifetime and reduce cost per sample




*Accelerated lifetime test shows how an inline filter removes particles*

# InfinityLab Quick Change Inline Filter and Filter Discs



## Ultimate ease-of-use

- **Finger-tight, tool-free** replacement of filter disc
- **Click and seal**: A click alerts users when the filter is tight up to 1300 bar, assuring no risk of over- or under-tightening

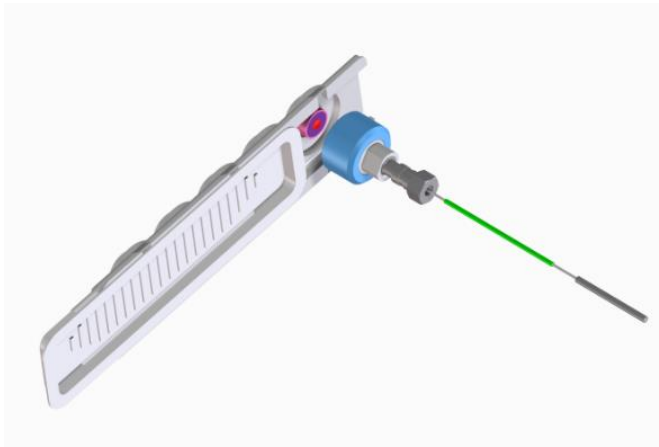
InfinityLab Quick Change Inline Filters

## High efficiency, easy-to-use filter discs

- **Various dimensions and porosities** – filter discs are available in 2.1 mm and 4.6 mm inner diameters with different pore sizes. The filter housing is compatible with all types of filter discs.
- **Touchless packaging to avoid potential contamination** – with specially designed packaging, you're able to insert the filter disc into the filter housing without touching it, to avoid potential contamination.
- **In situ replacement** of filter disc – no need to disconnect the inline filter from the system
- **Smart alert** to remind users when filter discs need replacing


Different dimensions and porosities of filter discs

Filter discs in touchless packaging



No-touch insertion of filter disc into filter housing

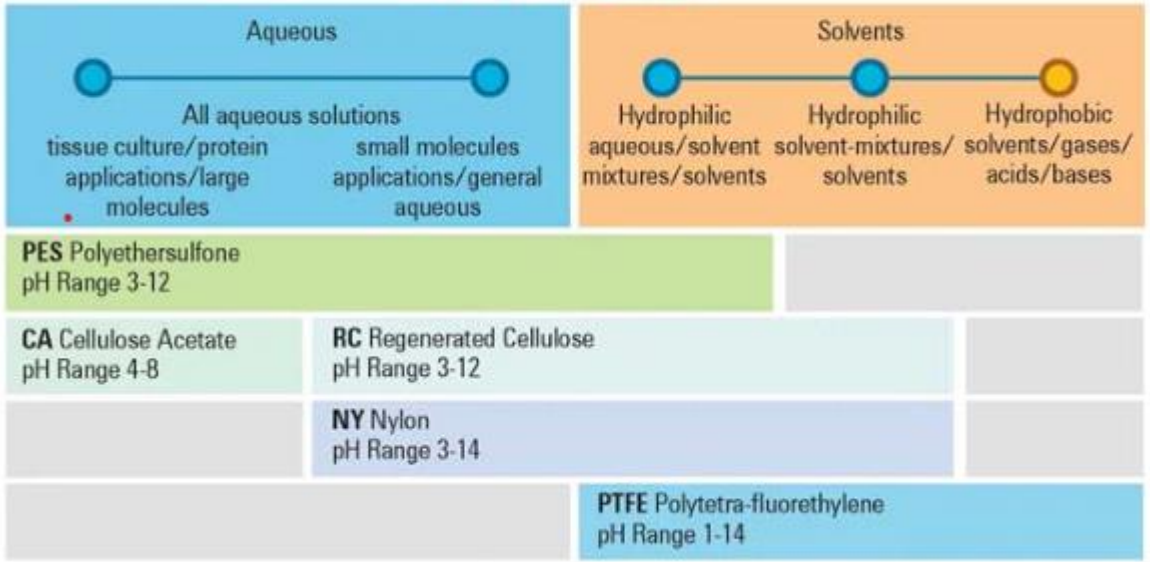
# Why Filter the LC Sample

- 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 |

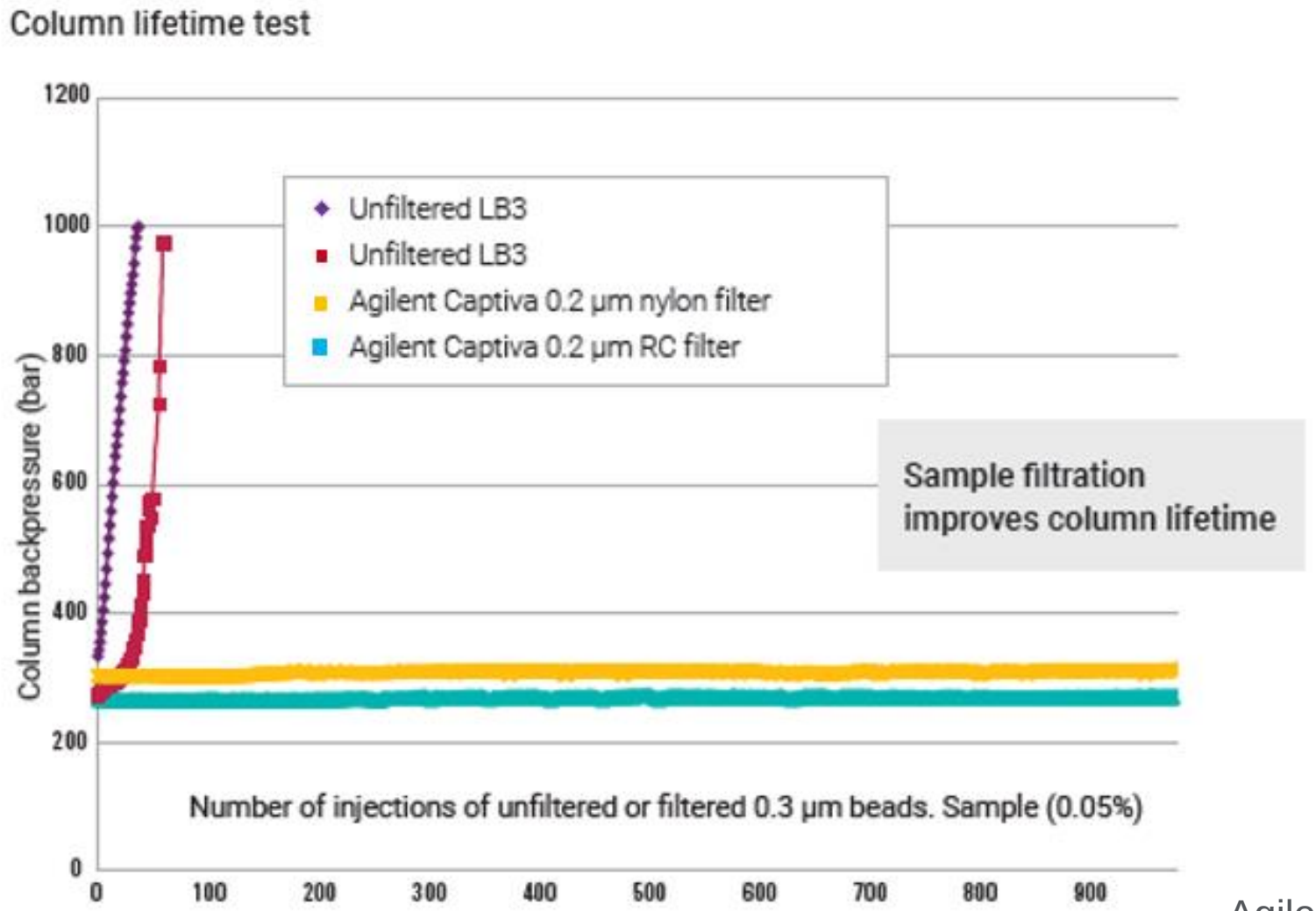
### Applications

| 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

Captiva syringe filters guide
Pub no: 5991-1230EN




The impact of filtering a 0.3 µm latex bead suspension on the lifetime of a sub-2 µm column

Filtering helps to reduce clogging, which can lead to increased pressure problems

Agilent technical note: 5994-1947EN

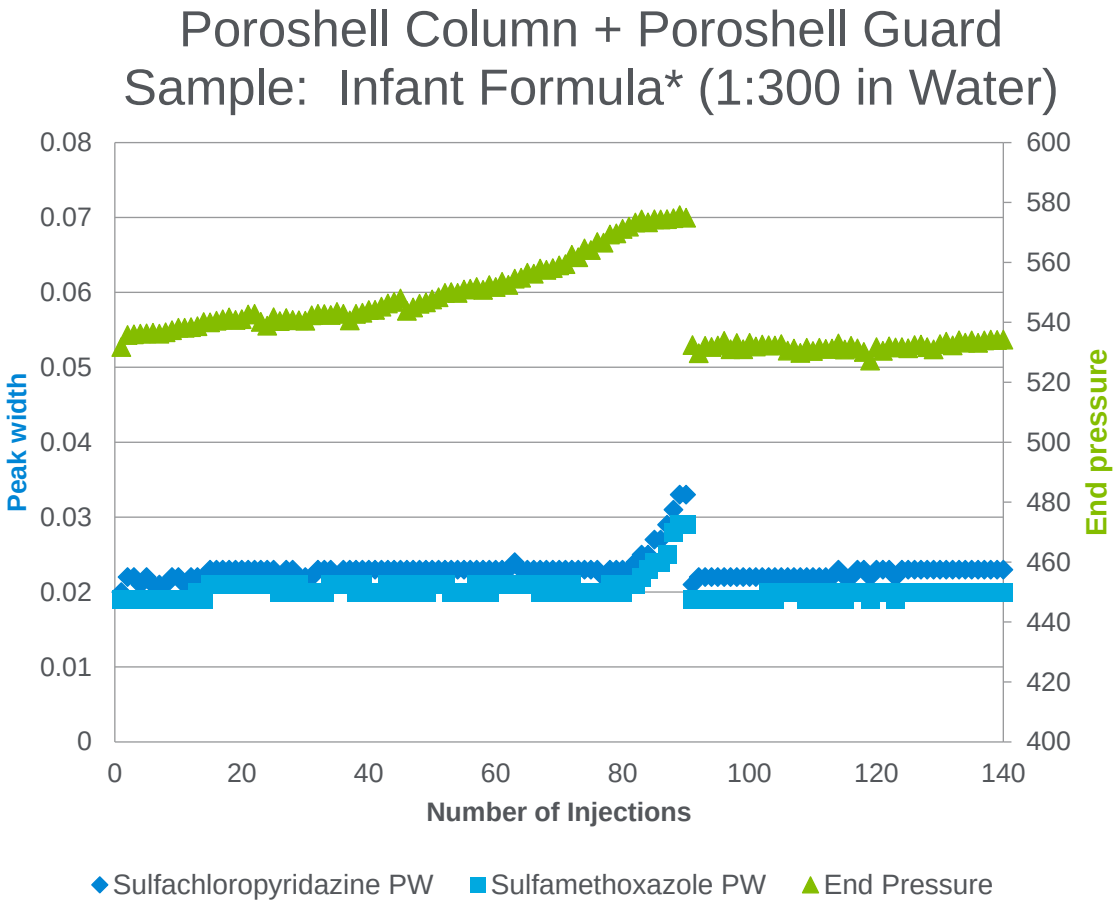
# Guard Columns

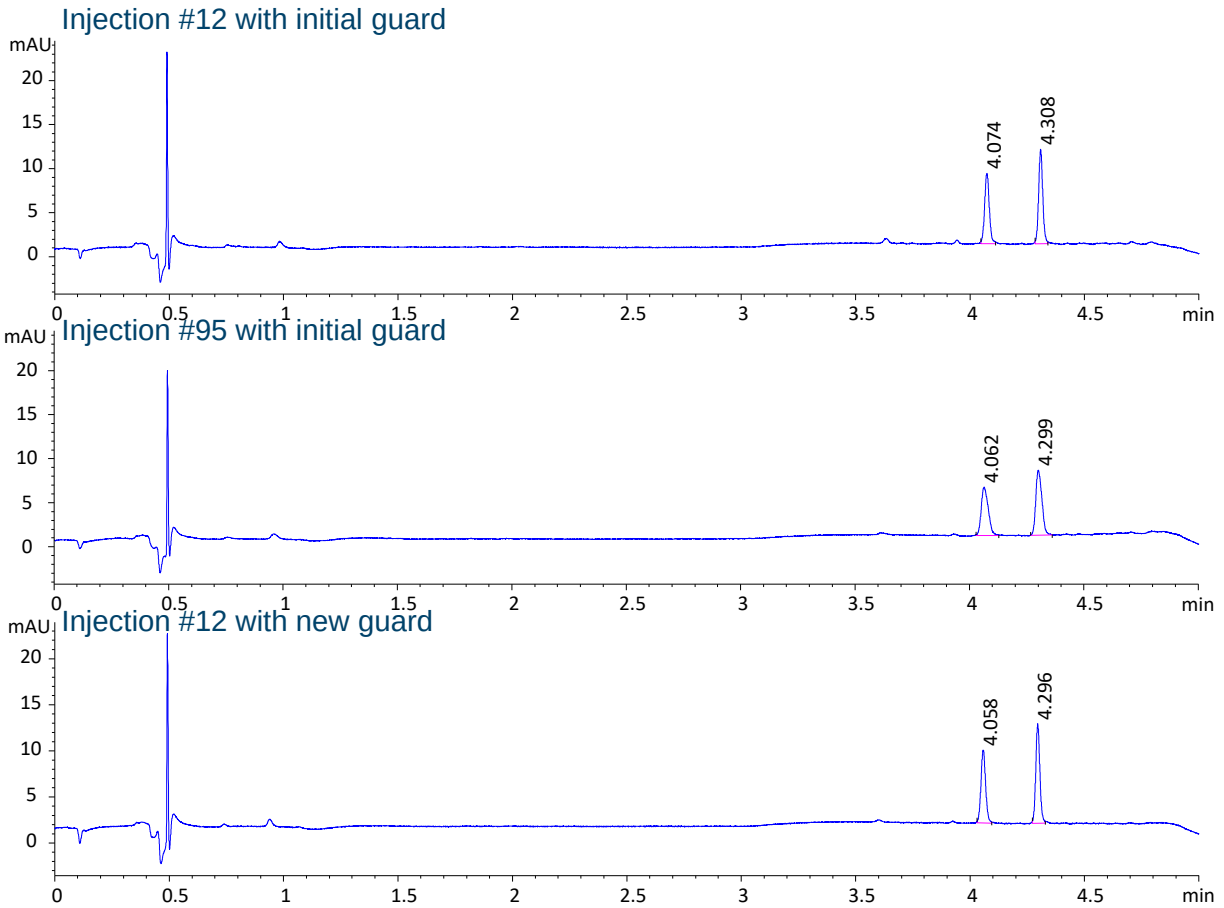
Agilent Guard Cartridge and holder

[Video: Fast Guards for HPLC and UHPLC | Agilent]

InfinityLab Fast Guard 

## Guards protect your column in many ways



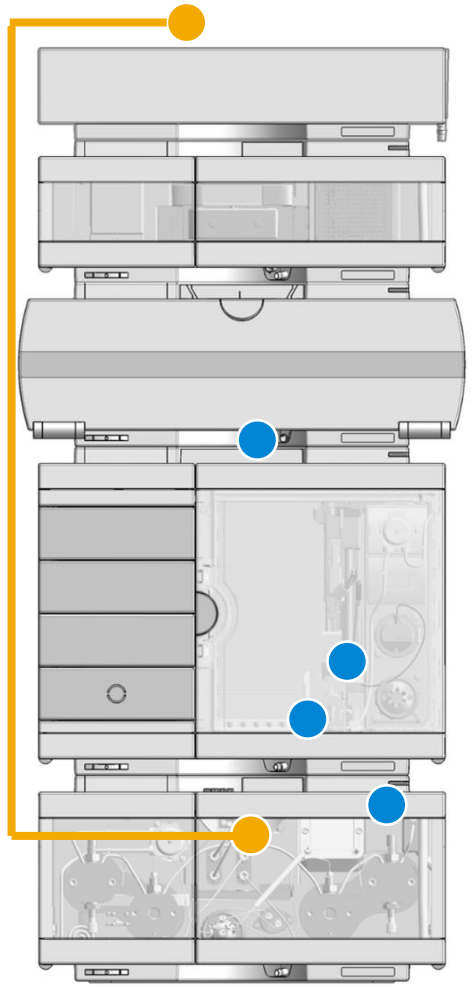




*Unfiltered infant formula including proteins and other precipitated ingredients.

# 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 |

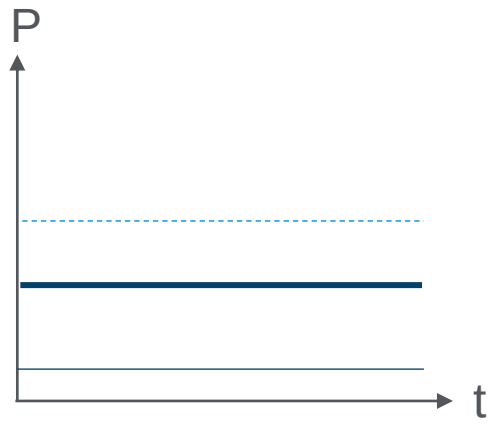


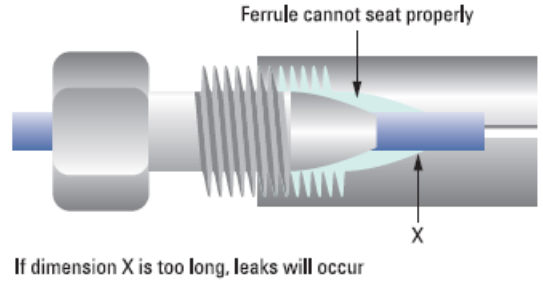
### Helpful Troubleshooting Tool

**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 | |
|---|---|
| Characteristics | • Lower pressure<br>• Potentially impacting retention times and peak shape |
| 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) |
| 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 |



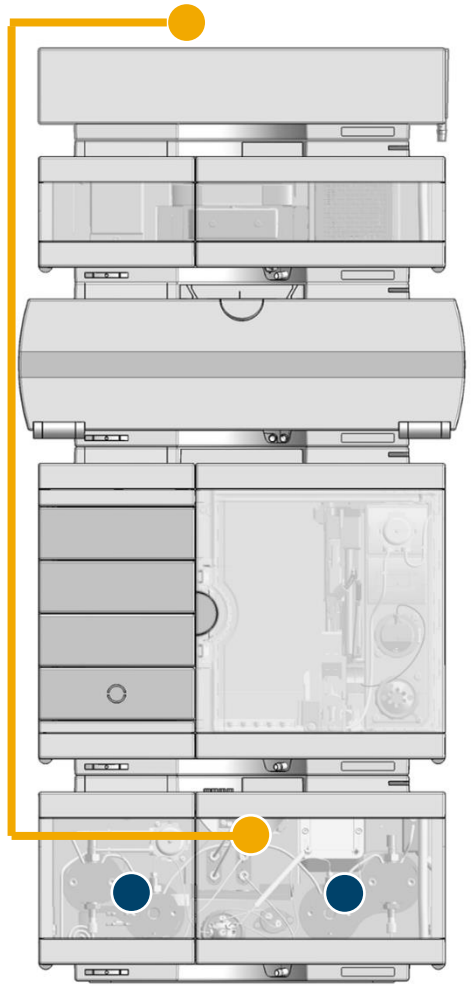



If dimension X is too long, leaks will occur

# Changes in System Pressure

## Pressure fluctuations

| | Potential Cause | Recommended Action |
|---|---|---|
| ● | Air in the system | • Prime and flush the 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 a proper maintenance schedule |
| ● | Cavitation effects | • Check for flow restrictions (solvent bottle to pump head inlet)<br>• Clean or replace parts<br>• Verify that the solvent supply is positioned above the pump inlet |



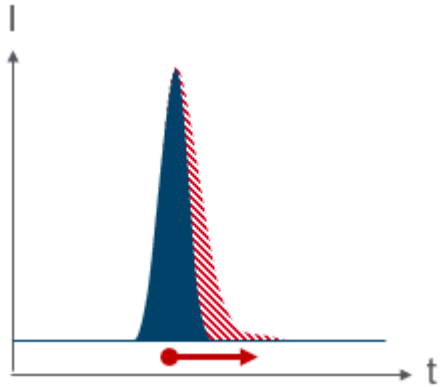
### In addition

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

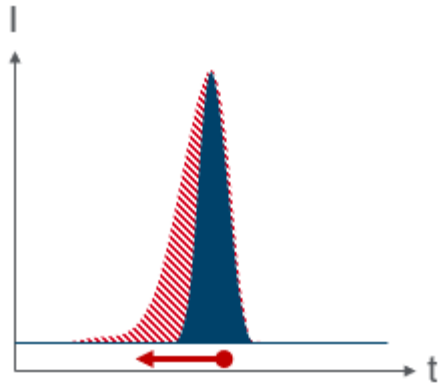
# Peak Shape Changes

# Problems with Peak Shape

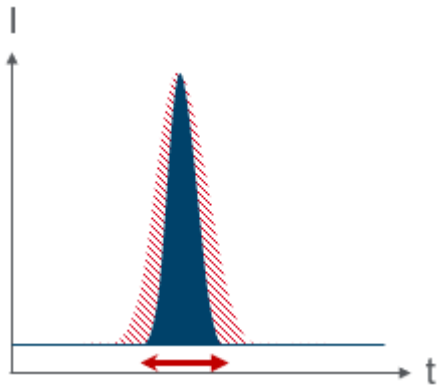
- Tailing
- Fronting
- Broadening
- Splitting/doubling



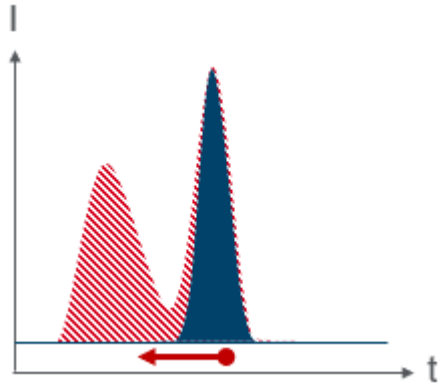

Tailing




Fronting




Broadening



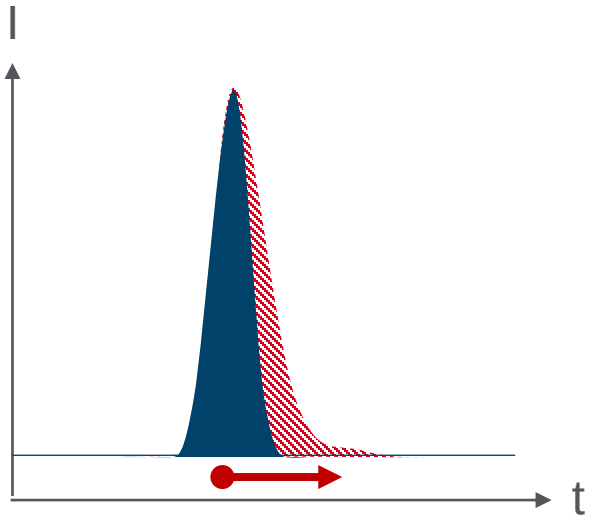

Splitting/doubling

# Changes in Peak Shape

## Peak tailing

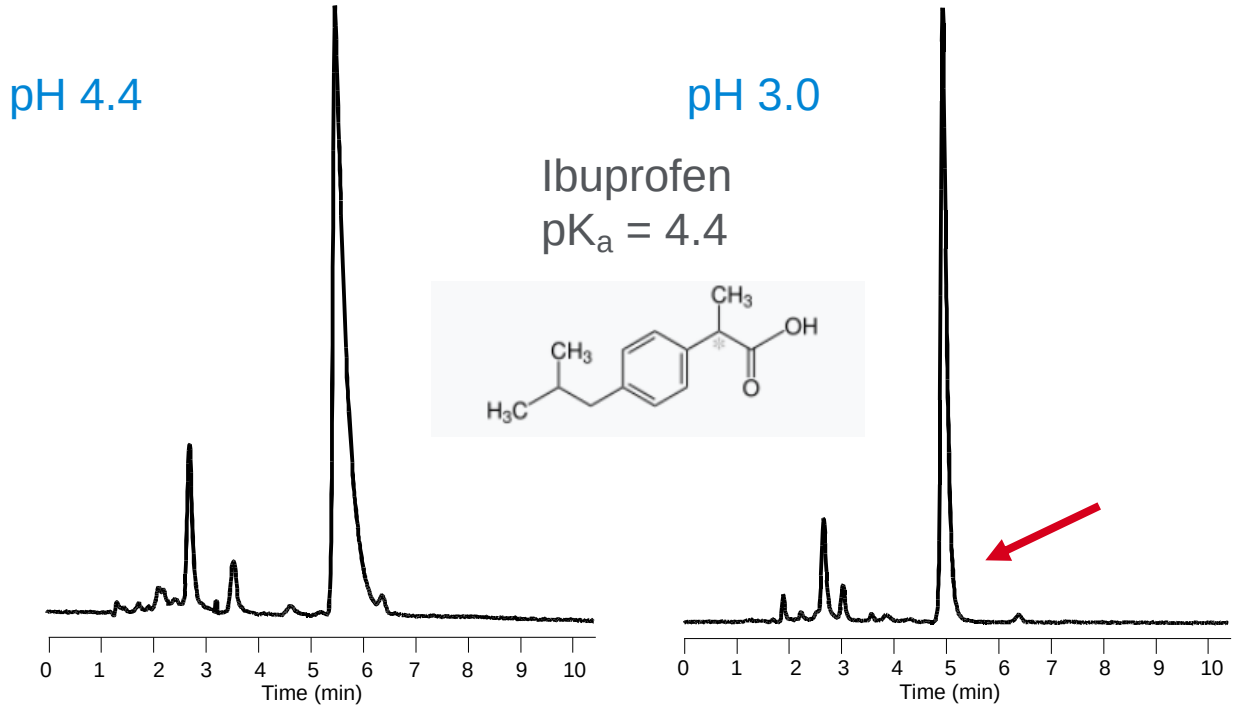
| If Applicable to Some Peaks | Recommended Action |
|---|---|
| Secondary interactions | • Change pH<br>• Change stationary phase |
| Small peak eluting on the tail of a larger peak | • Change selectivity (column, mobile phase)<br>• Switch to methods with higher resolution (UHPLC, 2D-LC) |

| 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 a stronger mobile phase or add an appropriate base (for example, TEA) |
| Poor tubing connections; high dispersion volume | • Minimize the number of connections<br>• Check the connections/fitting condition and proper seat of fittings<br>• Use fittings with spring-load function |



# Peak Tailing
## Mobile phase-related factors – pH

The effect of pH on peak shape at or near the sample pKa




Column: ZORBAX SB-C8, 4.6 x 150 mm, 5 µm
p/n: 883975-906

Mobile phase: 40% 5 mM $KH_2PO_4$, 60% ACN

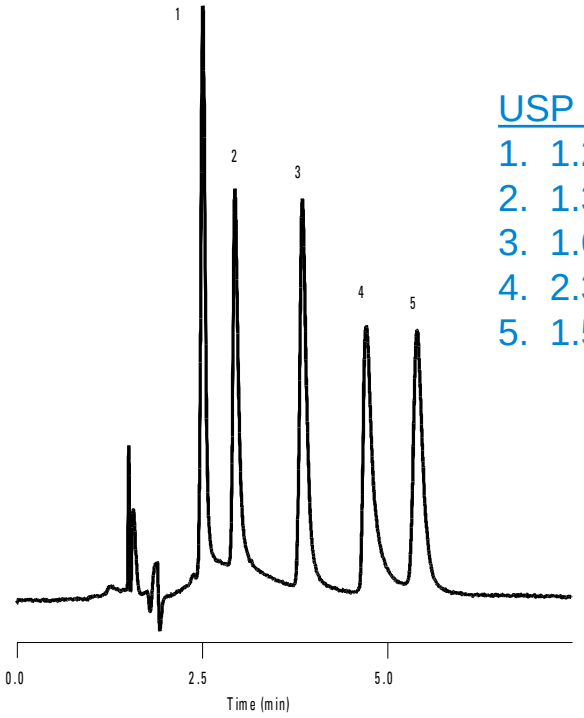
Flow rate: 1.0 mL/min

Temperature: Ambient

# Peak Tailing

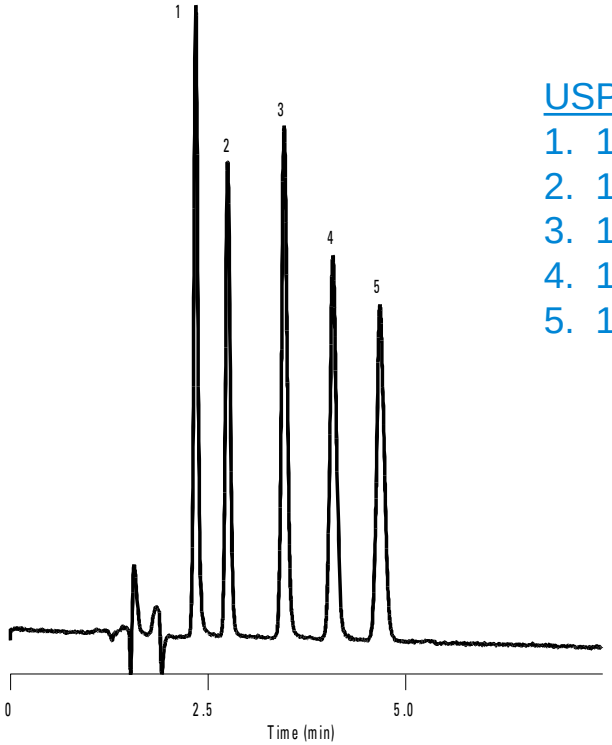
## Mobile phase-related factors – mobile phase additives

**No** TEA




USP Tf 5%
1. 1.29
2. 1.33
3. 1.63
4. 2.35
5. 1.57

**10 mM** TEA




USP Tf 5%
1. 1.19
2. 1.18
3. 1.20
4. 1.26
5. 1.14

Columns: Eclipse XDB-C8, 4.6 x 150 mm, 5 µm, p/n: 993967-906 Mobile phase: 85% 25 mM $Na_2HPO_4$ : 15% ACN pH: 7 Flow rate: 1.0 mL/min
Temperature: 35 °C Sample: Amphetamines 1. Phenylpropanolamine 2. Ephedrine 3. Amphetamine 4. Methamphetamine 5. Phenteramine

Incorrect concentration of TEA in mobile phase negatively effects peak shape of basic compounds

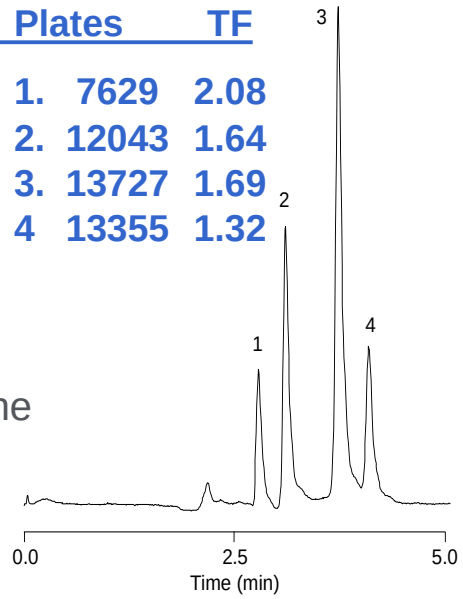
# Peak Tailing
## Column contamination

Column: StableBond SB-C8, 4.6 x 250 mm, 5 µm, p/n: 880975-902 Mobile phase: 20% $H_2O$ : 80% MeOH
Flow rate: 1.0 mL/min Temperature: RT Detection: UV 254 nm

Sample:
1. Uracil
2. Phenol
3. 4-Chloronitrobenzene
4. Toluene

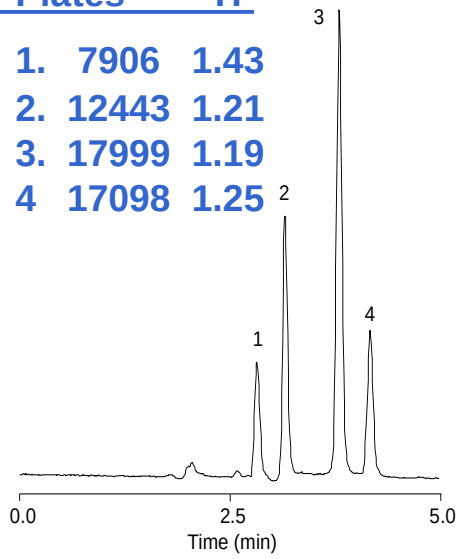
### QC test forward direction

| | Plates | TF |
|---|---|---|
| 1. | 7629 | 2.08 |
| 2. | 12043 | 1.64 |
| 3. | 13727 | 1.69 |
| 4 | 13355 | 1.32 |



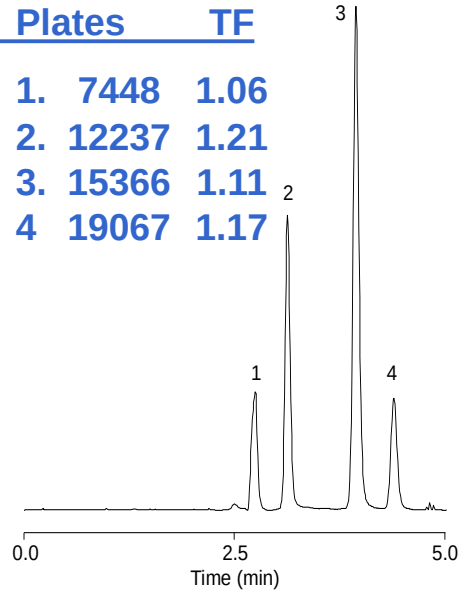

### QC test reverse direction

| | Plates | TF |
|---|---|---|
| 1. | 7906 | 1.43 |
| 2. | 12443 | 1.21 |
| 3. | 17999 | 1.19 |
| 4 | 17098 | 1.25 |



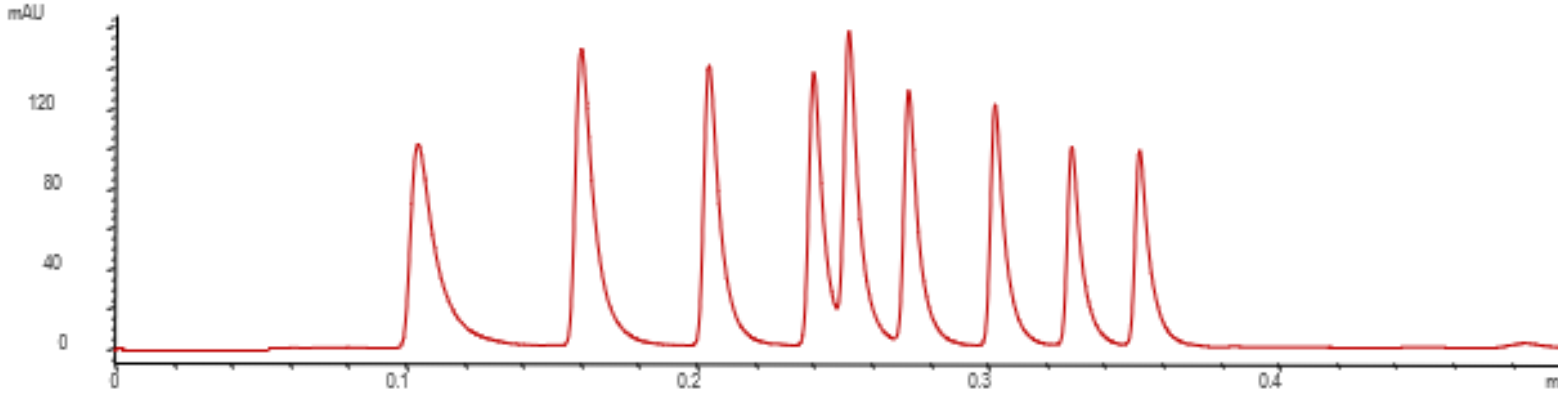

### QC test after cleaning 100% IPA, 35 °C

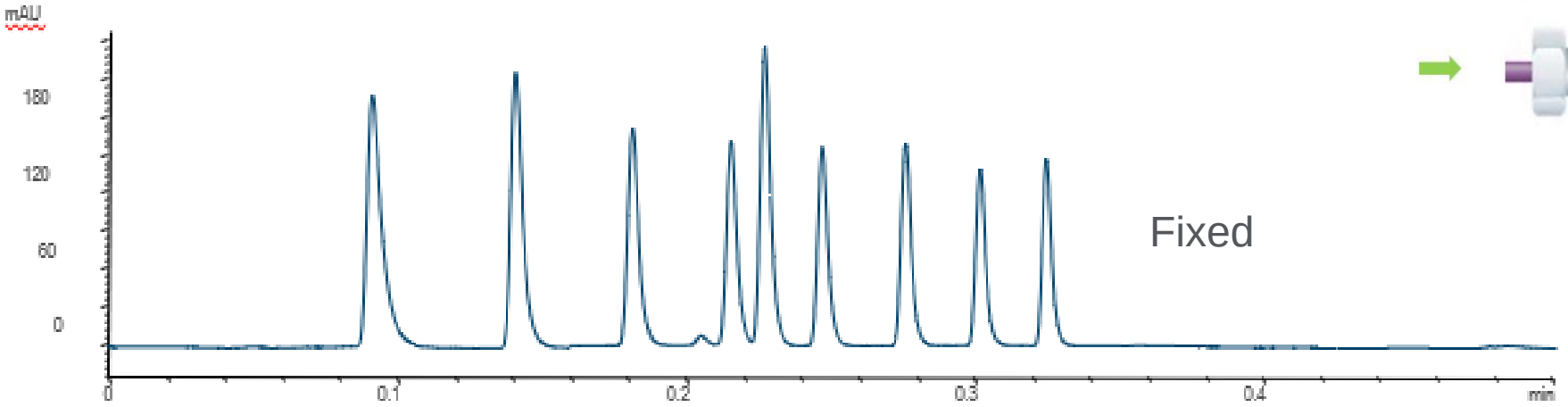
| | Plates | TF |
|---|---|---|
| 1. | 7448 | 1.06 |
| 2. | 12237 | 1.21 |
| 3. | 15366 | 1.11 |
| 4 | 19067 | 1.17 |



# Peak Tailing

## Importance of having correct connections




Fixed

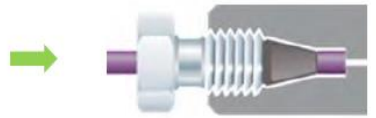
Connection problems can lead to:

Poor chromatography

- Broad or tailing peaks
- Loss of resolution

- Leak

- Peak shape problem



- No dead volume


InfinityLab Quick Connect fitting

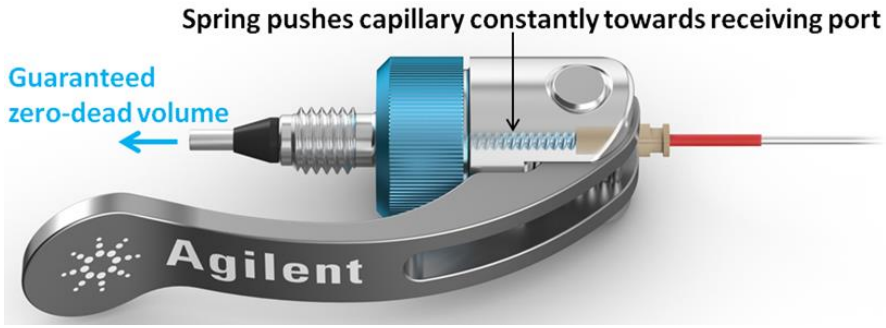
InfinityLab Quick Turn fitting

# InfinityLab Quick Connect and Quick Turn Fittings

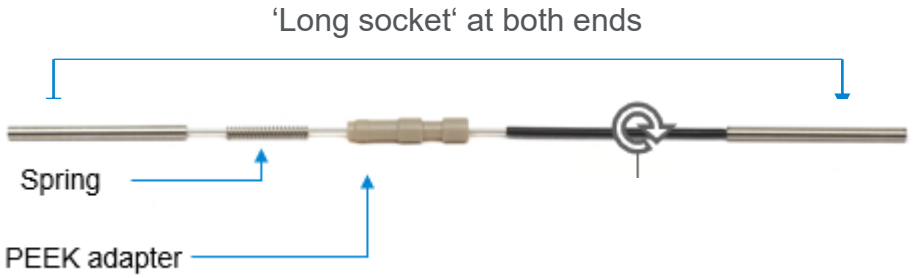
- Spring-loaded design
- Easy-to-use
- Works for all column types
- Reusable
- Consistent ZDV connection

**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



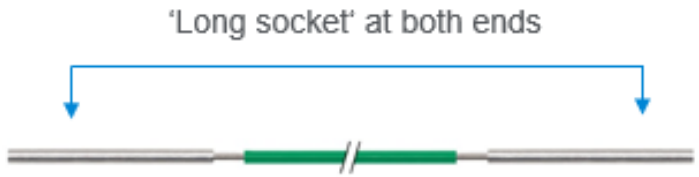

Capillary for Quick Connect fitting





InfinityLab Quick Turn fitting

Capillary for Quick Turn fitting

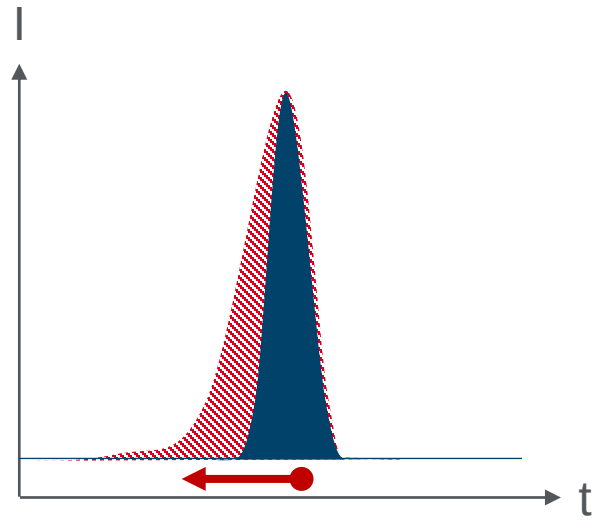


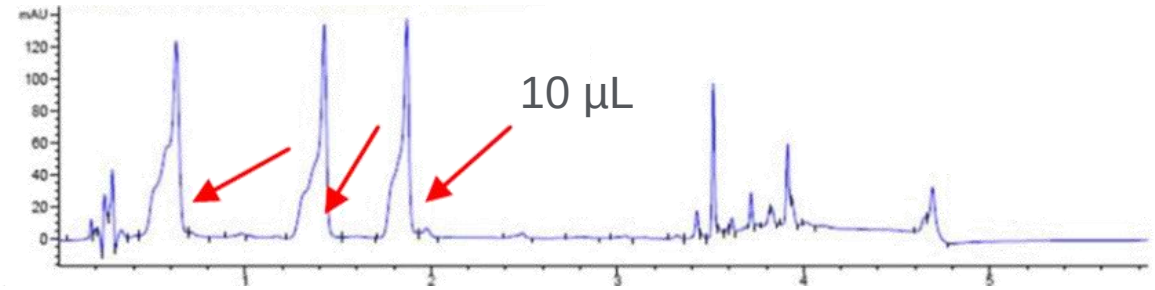

Brochure: 5991-5164EN

# Changes in Peak Shape

## Fronting

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

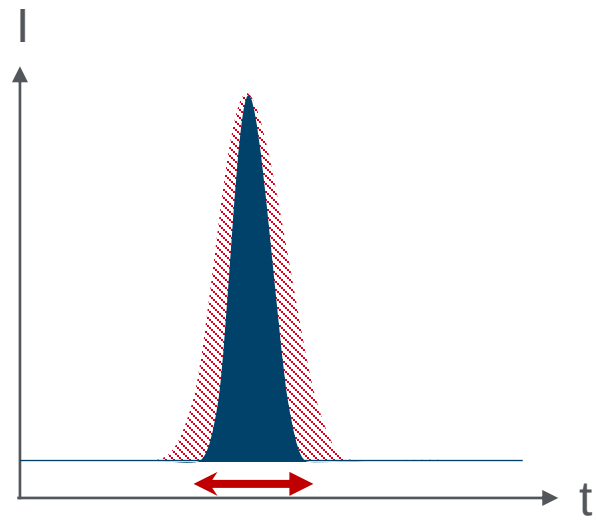



# Changes in Peak Shape

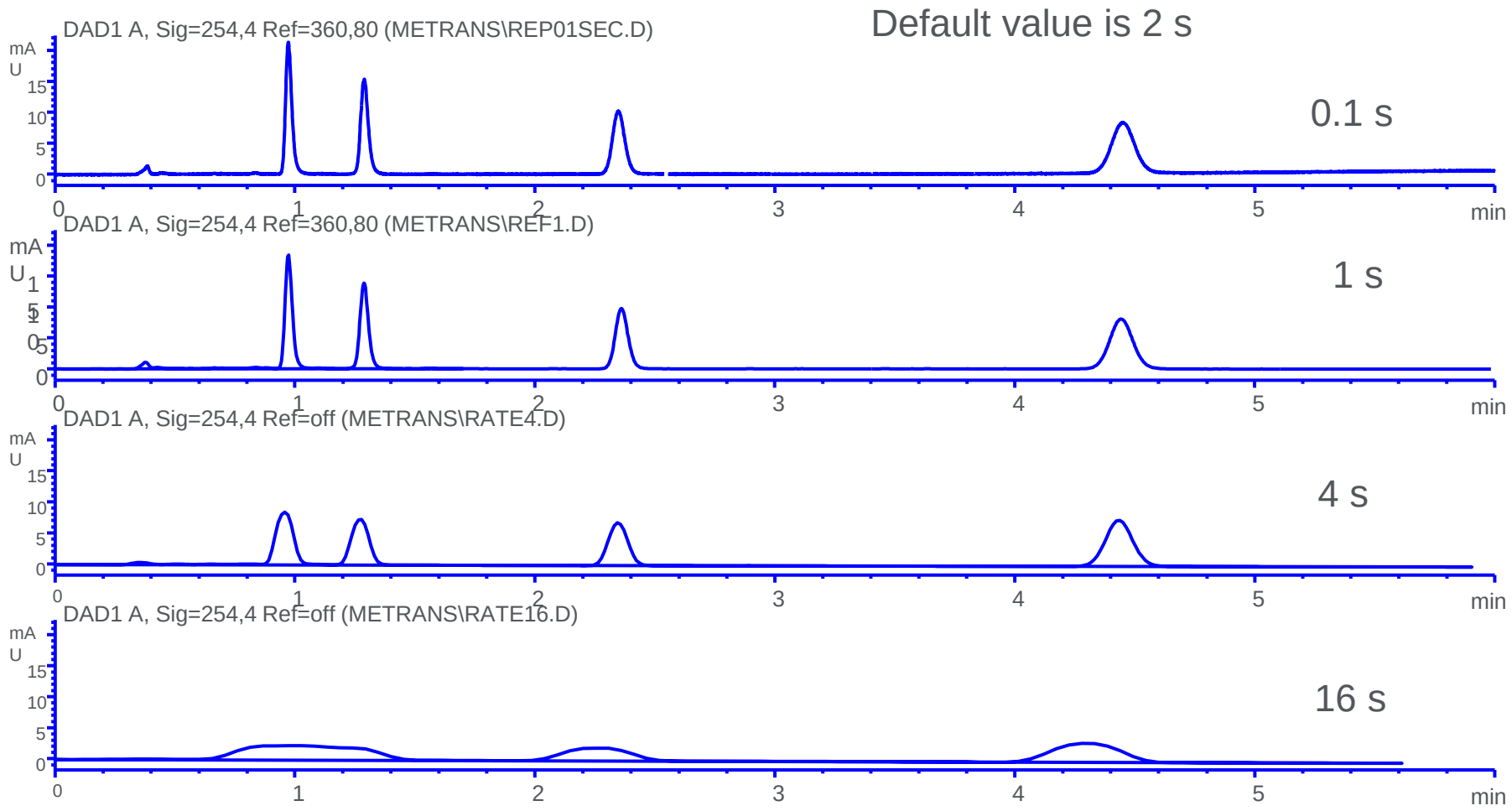
## Peak broadening

| | Potential Cause | Recommended Action |
|---|---|---|
| | Injection volume is too large | • Decrease the injection volume |
| | Long retention times | • Use gradient elution or a stronger mobile phase |
| | System settings | • Check the 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 the system | • Ensure that the tubing is narrow and as short as possible to avoid extra volume |
| | Sample diluent is too strong | • Reduce diluent strength |



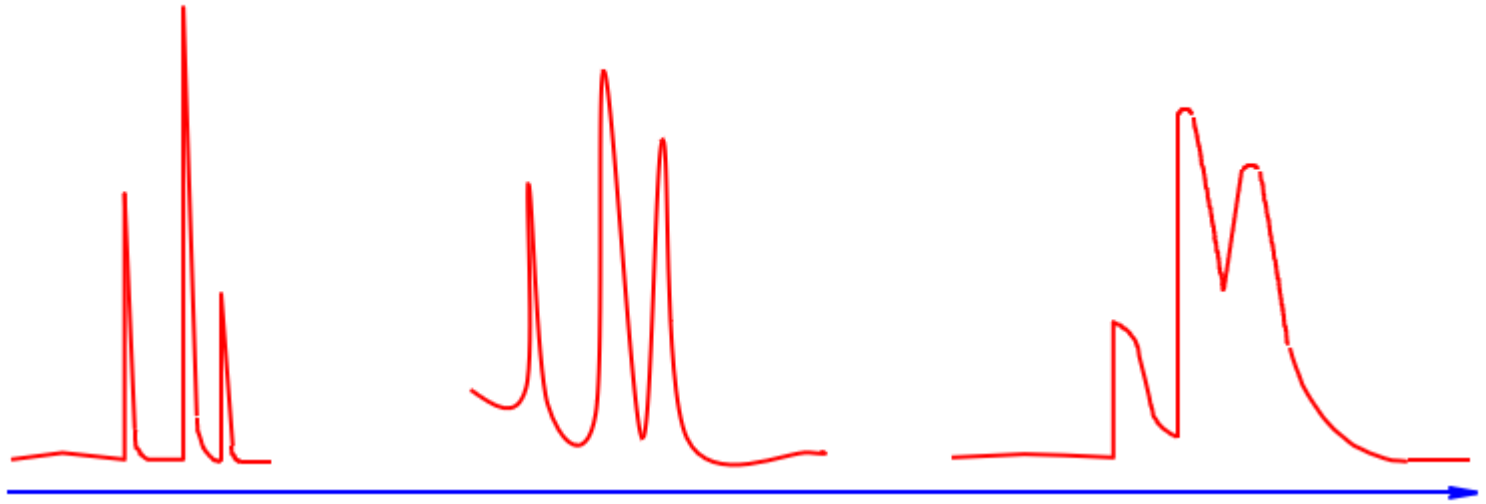
# Peak Broadening

## Influence of data rate



# Peak Broadening

## Extracolumn dispersion (Volume)



Increasing extracolumn volume

For minimizing dispersion:

- 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 |

# Retention

# What Is the Specific Issue?

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

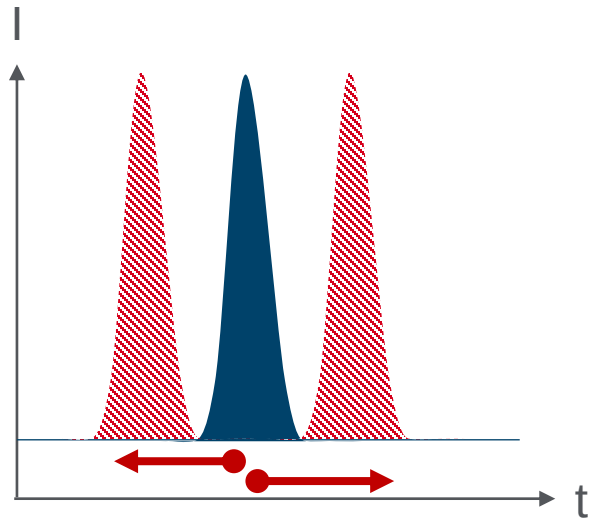
Increase the detail to be more specific:

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

# Changes in Separation

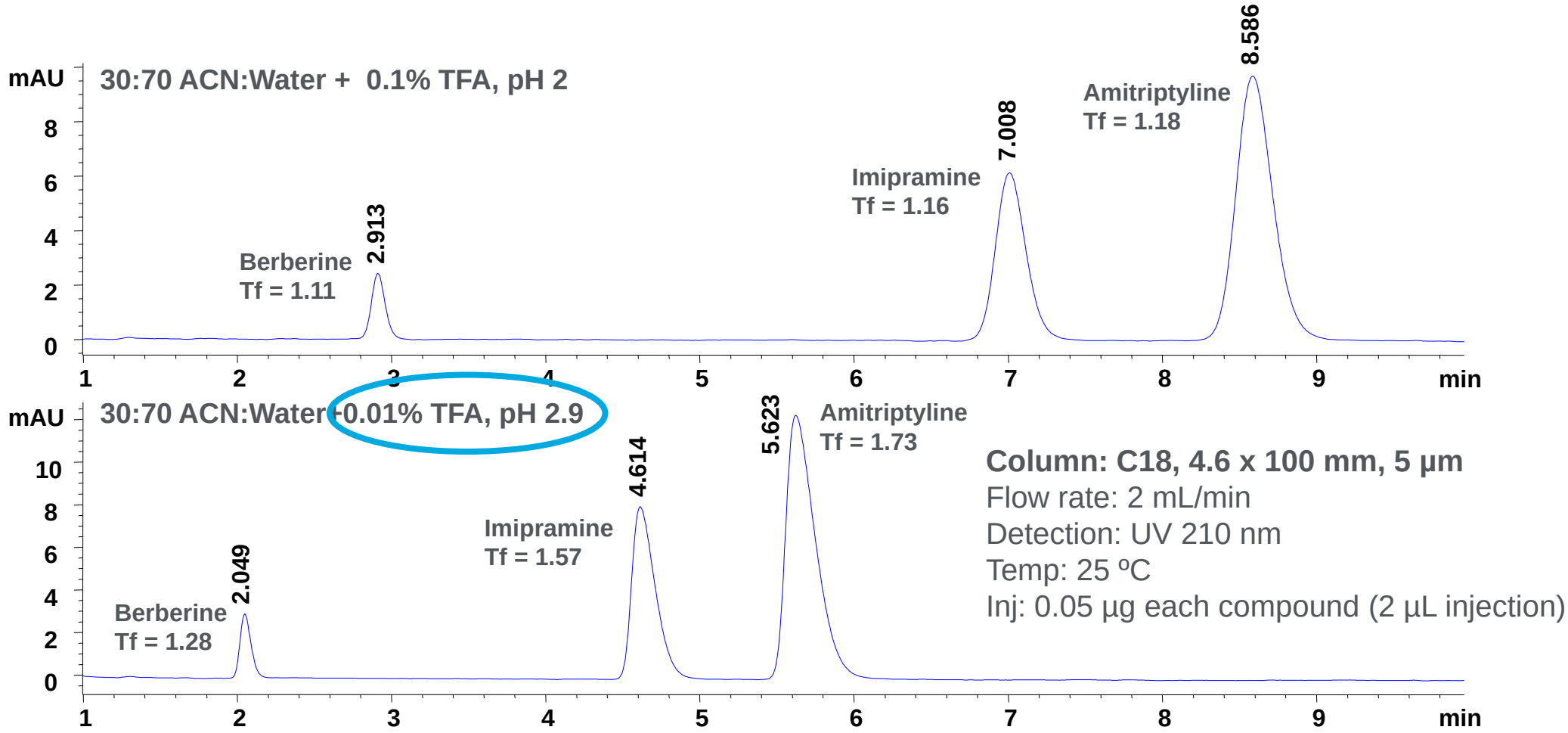
## Retention time changing

| | 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 check vs. manual preparation of mobile phase |
| | Column temperature varying | Thermostat column and ensure constant lab temperature |
| | Equilibration time insufficient with the gradient run or a 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 a strong solvent to remove contaminants |
| | First few injections – adsorption on active sites | Condition the column using an initial injection of a concentrated sample |
| | Column overloaded with sample | Decrease the injection volume or concentration |
| | Active sites on silica packing | Add a competing base to mobile phase |
| | Mobile phase composition is changing | Follow the ‘best practices' |



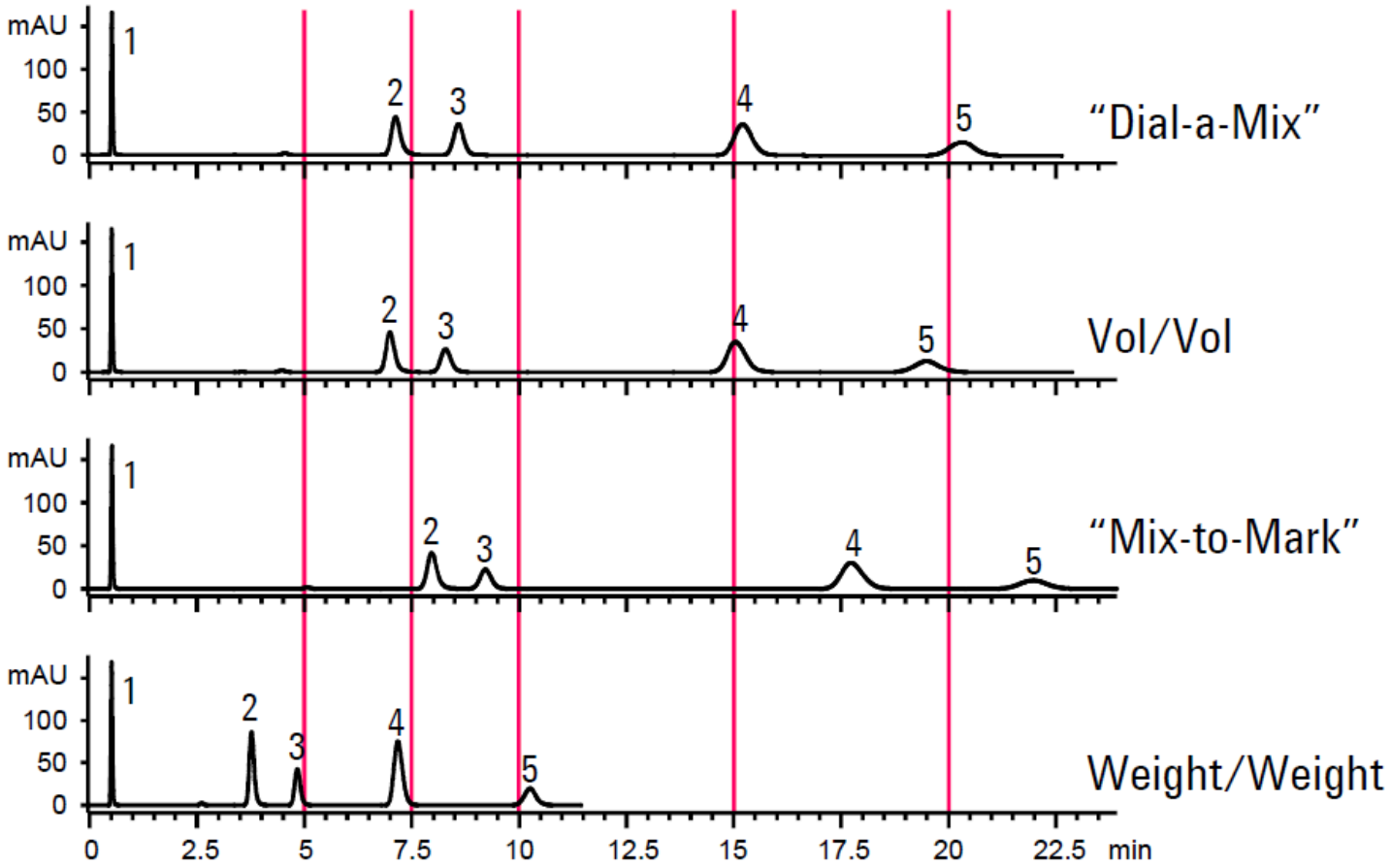
# Change in Volatile Buffer Concentration

## Shift in Retention Time and Peak Shape



# Mobile Phase Preparation

Agilent 1100 with quaternary pump
Column: Zorbax Eclipse XDB-C8 RR 3.5um, 4.6 x 50mm
p/n 935967-906




**Dial-a-Mix** = A: water B: MeOH, pump 50% B

**Vol/Vol** = 250mL water + 250mL MeOH, pump 100%

**Mix-to-Mark** = 250mL MeOH, fill to 500 mL with water, pump 100%

**Premixed (w/w)** = 200g MeOH + 200g water, pump 100%

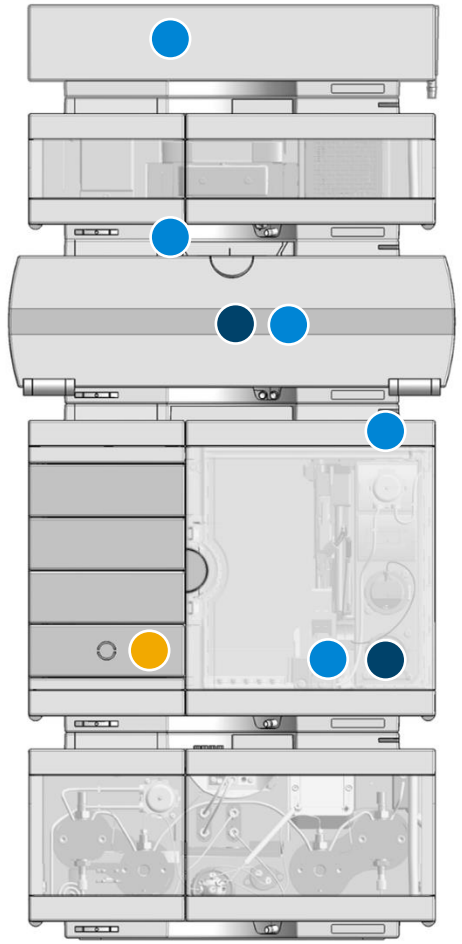
Detection: UV 254nm
Flow rate: 1 ml/min

# Changes in Separation

## Ghost peaks, carryover

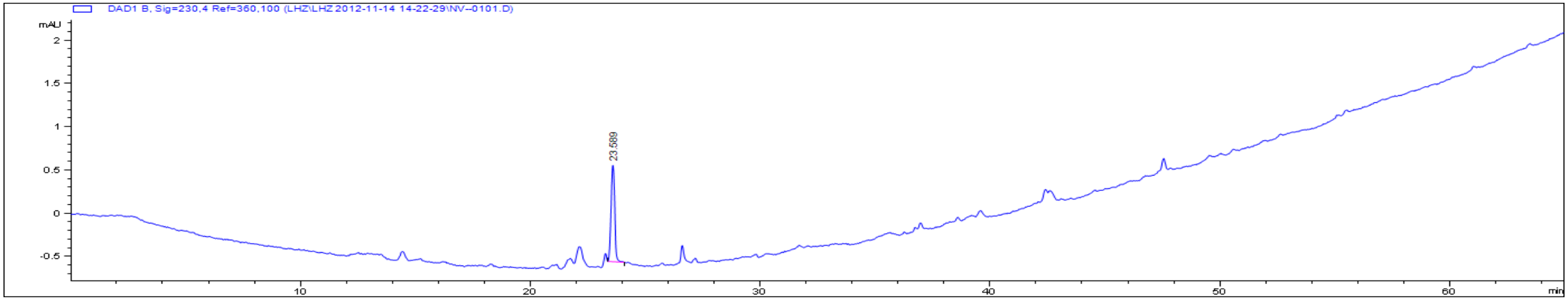
| | Potential Cause | Recommended Action |
|---|---|---|
| ● | Peaks from previous injections | • Flush the column to remove contaminants<br>• Check with blank injection |
| ● | Specific interaction with metal surfaces | • Passivate instrument<br>• Use InfinityLab deactivator additive<br>• Use bio-inert 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

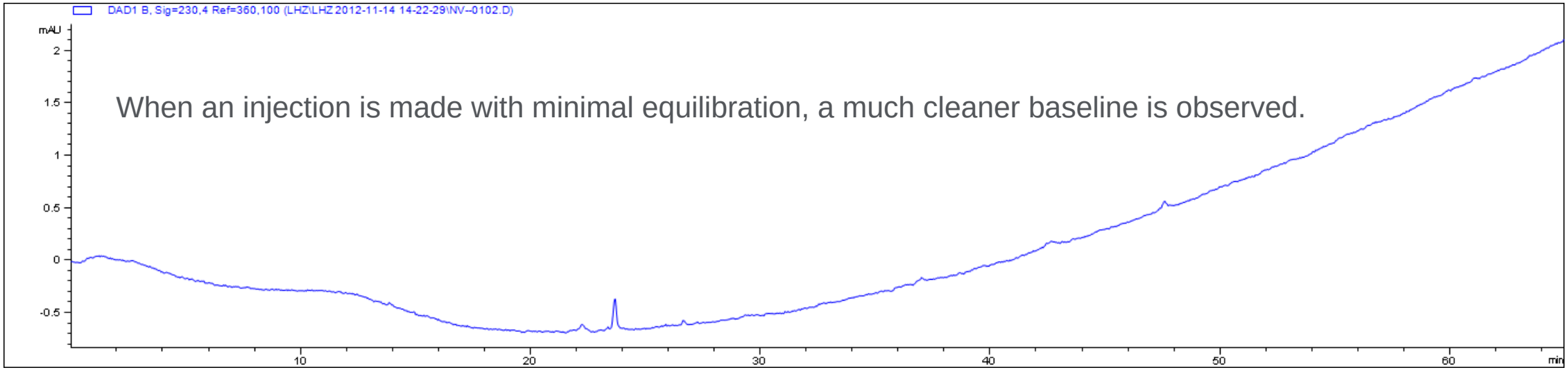


[P/n 5191-4506 | Deactivator additive 50 mL]
[P/n 5191-3940 | Deactivator additive 25 mL]

# Ghost Peaks




The LC system was equilibrated using starting conditions for 30 minutes, then a gradient run was made. Impurities were trapped and eluted out with the gradient.



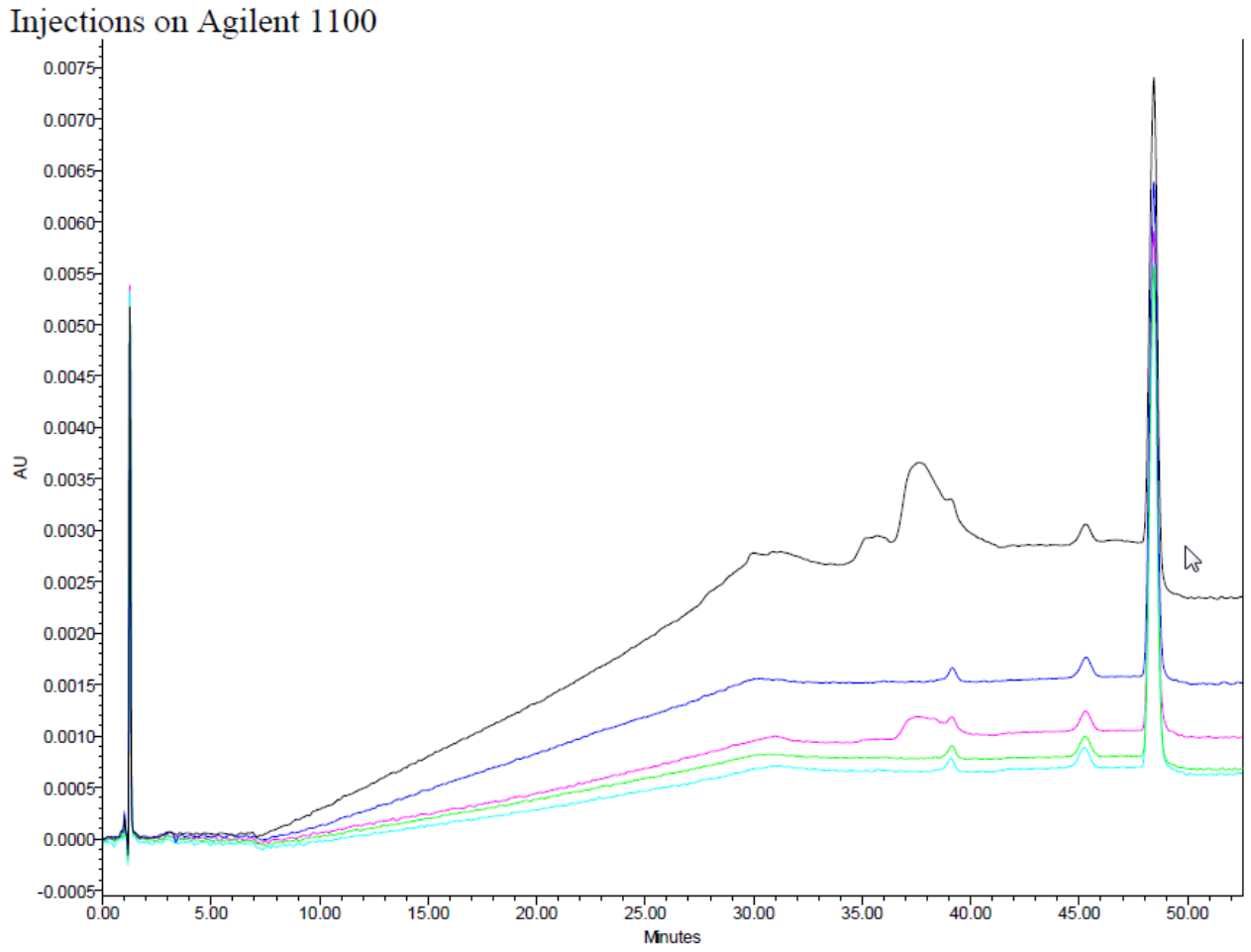

When an injection is made with minimal equilibration, a much cleaner baseline is observed.

# Ghost Peaks

## Where do they come from

- Organic
- Additives
  - TFA
  - Salts
- $H_2O$
- Sample or from a previous run
- Other
  - Glassware
  - pH meter
  - Filters

# Unknown Phantom Peaks
## 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

# LC Columns Are Not Indestructible

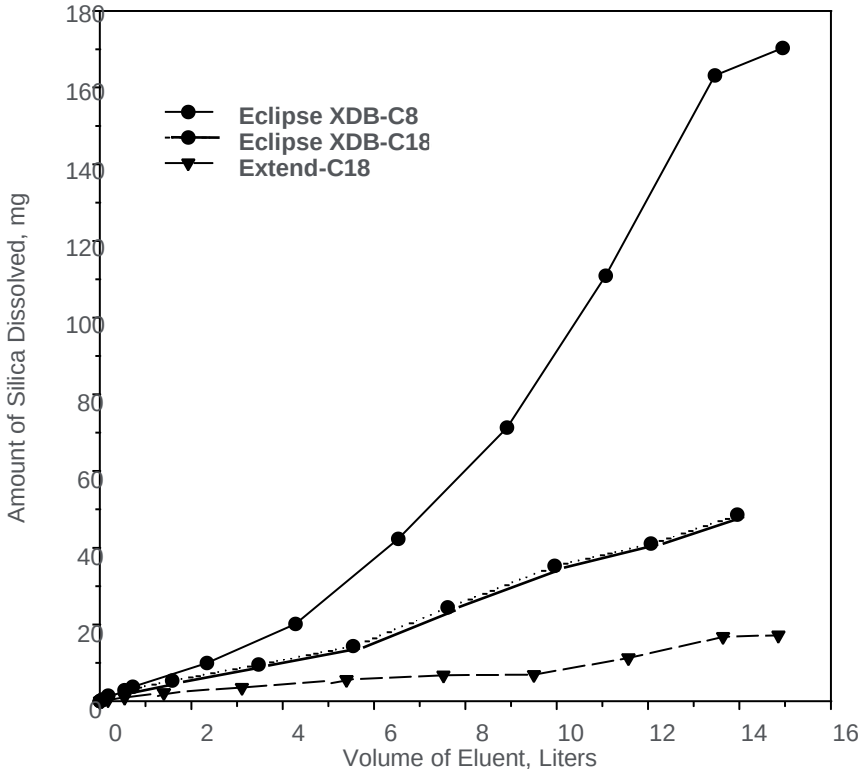
- Columns are packed using hydraulic pressure and can be damaged by excess pressure
- Silica dissolves (slowly) at higher pH
- Acid hydrolysis of bonded phase can occur at low pH
- Column failure
  - Void
  - Contamination

Columns must be stored properly

  - Check your user guide

It's important to:

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

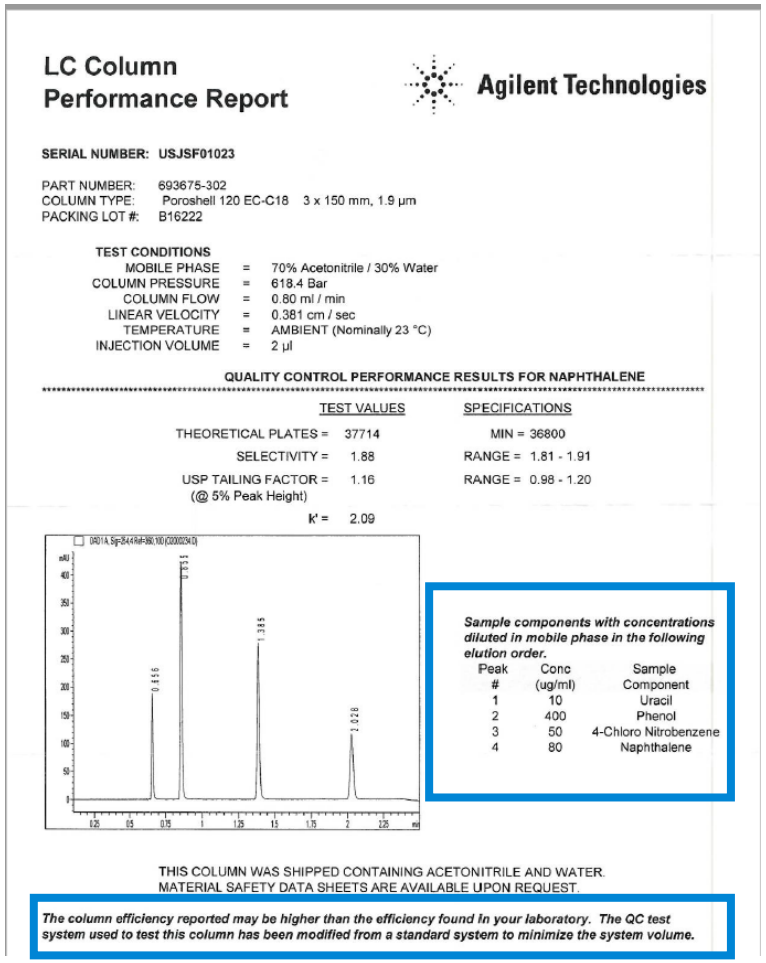
**The InfinityLab Poroshell 120 portfolio offers choices for low and high pH**

| Best All Around | Best for Low pH Mobile Phases | Best for High pH Mobile Phases | Best for Alternative Selectivity | Best for More Polar Analytes | HILIC for polar analytes | Chiral |
|---|---|---|---|---|---|---|
| **EC-C18** 1.9 µm, 2.7 µm, 4 µm | **SB-C18** 1.9 µm, 2.7 µm, 4 µm | **HPH-C18** 1.9 µm, 2.7 µm, 4 µm | **Bonus-RP*** 2.7 µm | **Aq-C18*** 2.7 µm | **HILIC** 1.9µm, 2.7 µm, 4 µm | **Chiral-V** 2.7 µm |
| **EC-C8** 1.9 µm, 2.7 µm, 4 µm | **SB-C8** 2.7 µm | **HPH-C8** 2.7 µm, 4 µm | **PFP*** 1.9 µm, 2.7 µm, 4 µm | **SB-Aq*** 1.9 µm, 2.7 µm, 4 µm | **HILIC-Z** 1.9 µm, 2.7 µm, 4 µm | **Chiral-T** 2.7 µm |
| **Phenyl-Hexyl*** 1.9 µm, 2.7 µm, 4 µm | | ← **CS-C18** 2.7 µm → | | **EC-CN*** 2.7 µm | **HILIC-OH5** 2.7 µm | **Chiral-CD** 2.7 µm |
| | | | | | | **Chiral-CF** 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 the difference between your instrument and the performance report (use as a base value)
- Run again if the column seems to lose performance
  - Compare with the results from first run

### Performance verification based on in-house checkout

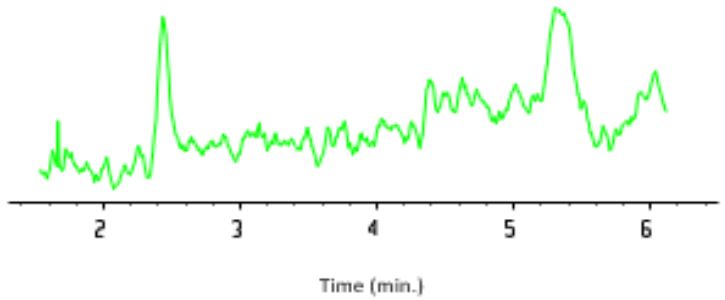
- Run in house checkout before use
  - Record key specifications, such as tailing factor, plates, and backpressure
- Run again if the column seems to lose performance
  - Compare with the results from first run

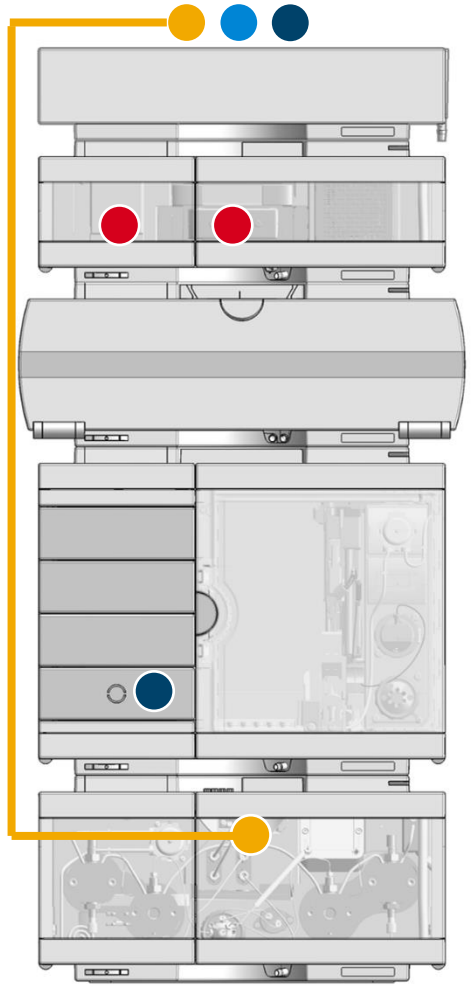
# Baselines and Detection

# Changes in Detection

## Noisy baseline

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

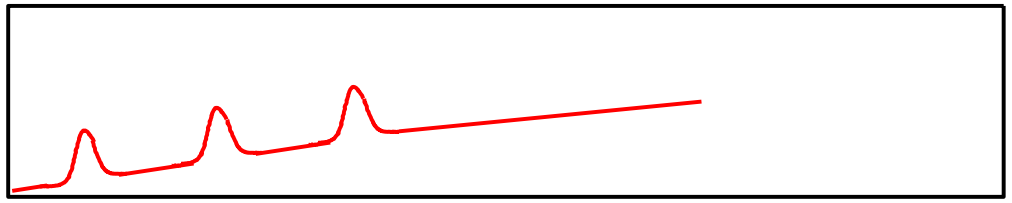


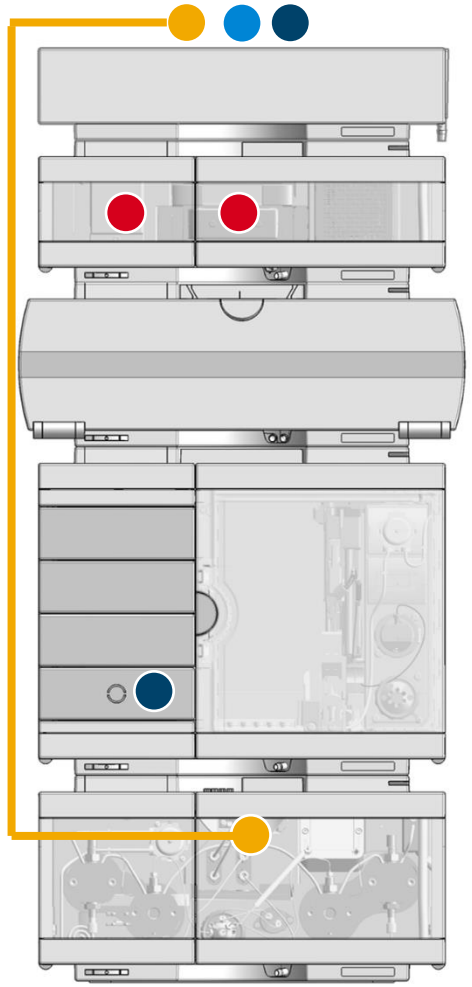



# Changes in Detection

## Drifting baseline

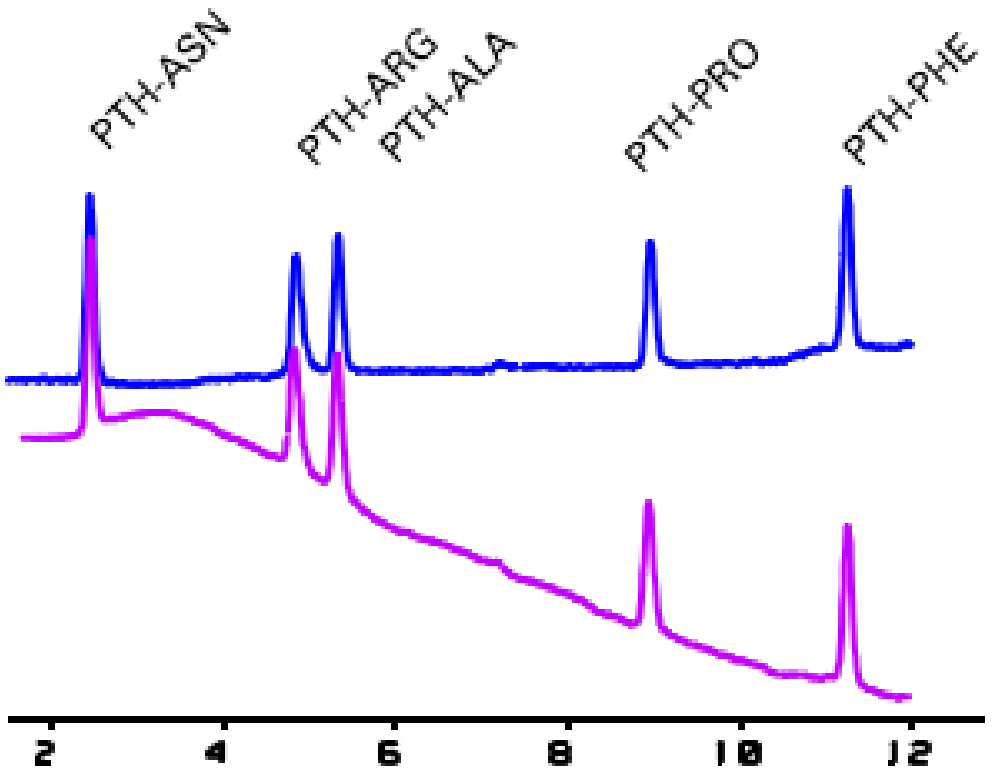
| | Potential Cause | Recommended Action |
|---|---|---|
| ● | Contamination in the mobile phase | • Make up new mobile phase<br>• If running a gradient, you might need to adjust the modifier |
| ● | Low difference between the sample and the mobile phase absorbance | • Check absorbance values of the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





# Reference Wavelength

## How settings can affect the baseline




1 pmol each

λ 267 nm

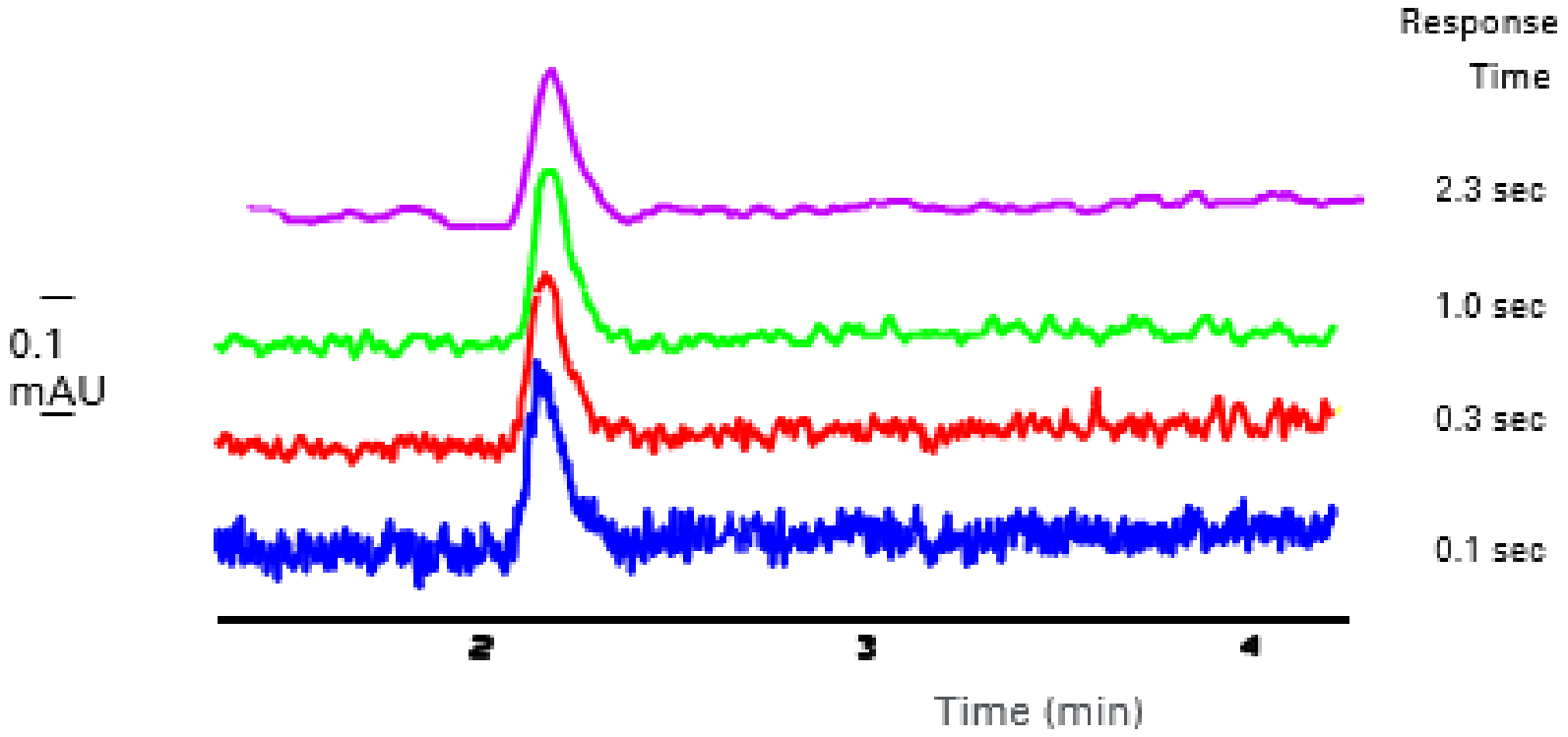
Ref 380 nm

λ 267 nm

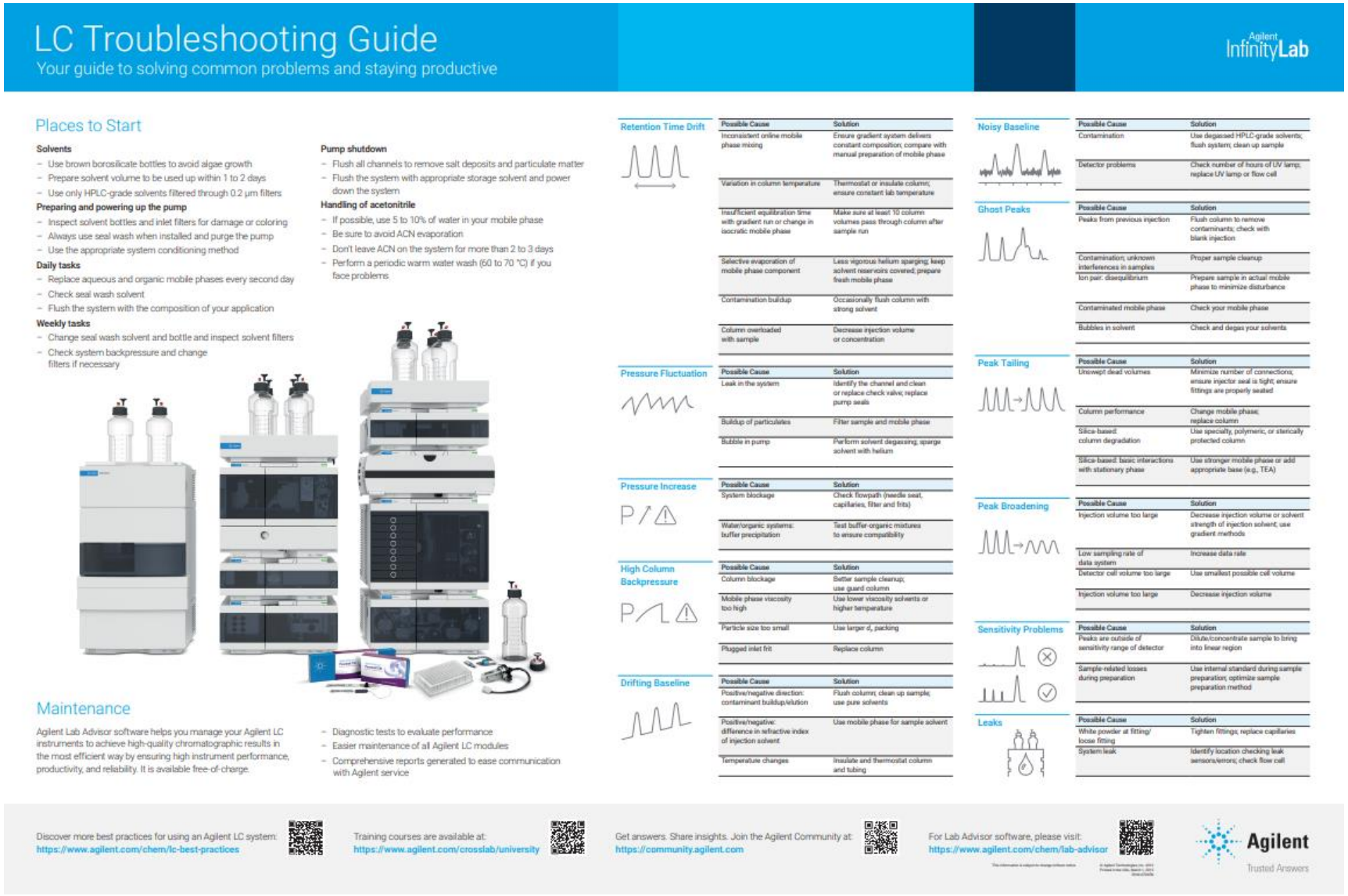
No reference

Gradient: 0.02 m KH2PO4/ACN, from 12% ACN to 45% ACN in 12 min

# Influence of Data Collection Rate on Noise



# 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

Download 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 LC 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 and 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

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