It’s Not All About the Column:

The Role of the Mobile Phase and Your Instrument

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June 14, 2021

Agilent InfinityLab

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DE443606314583333

Outline

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Mobile phase

- Aqueous
  - Buffers
  - Preparation
- Organic

Pre-column protection

- Filters
- Guard columns

Instrument

- Connections
- Dwell volume
- Extra column volume

Mobile Phase pH and Buffers

Why are they important in HPLC?

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pH

- Silica surface of column
- Sample components of interest

Buffers

- Resist changes in pH and maintain retention
- Improve peak shape for ionizable compounds

Column lifetime

- Low pH strips bonded phase
- High pH dissolves silica

“I Don’t Have Time to Make Buffers or Adjust pH…!”

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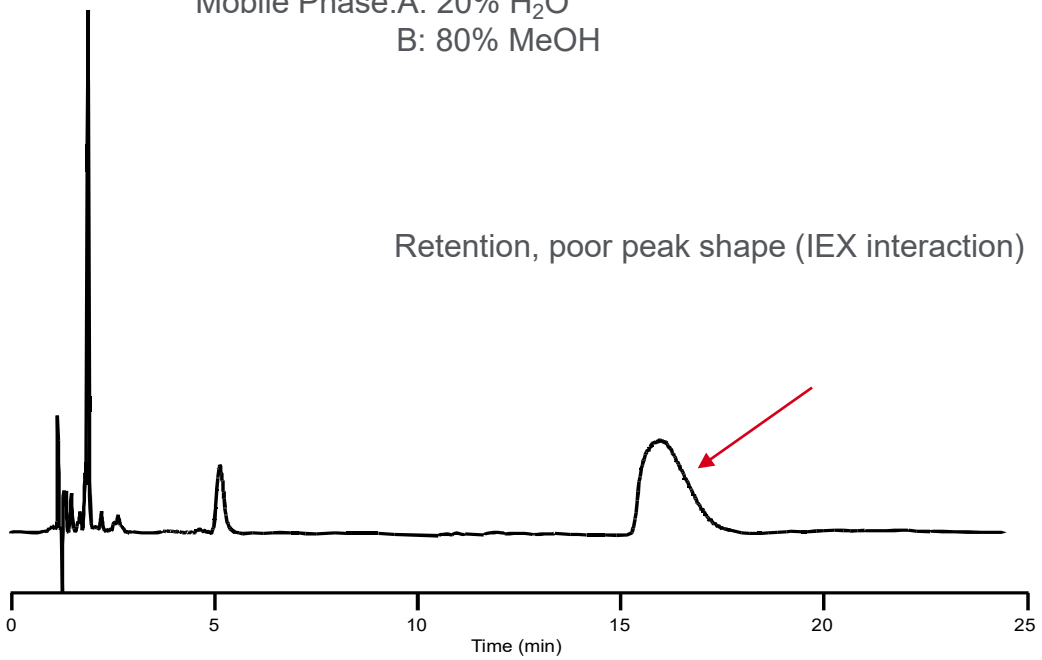
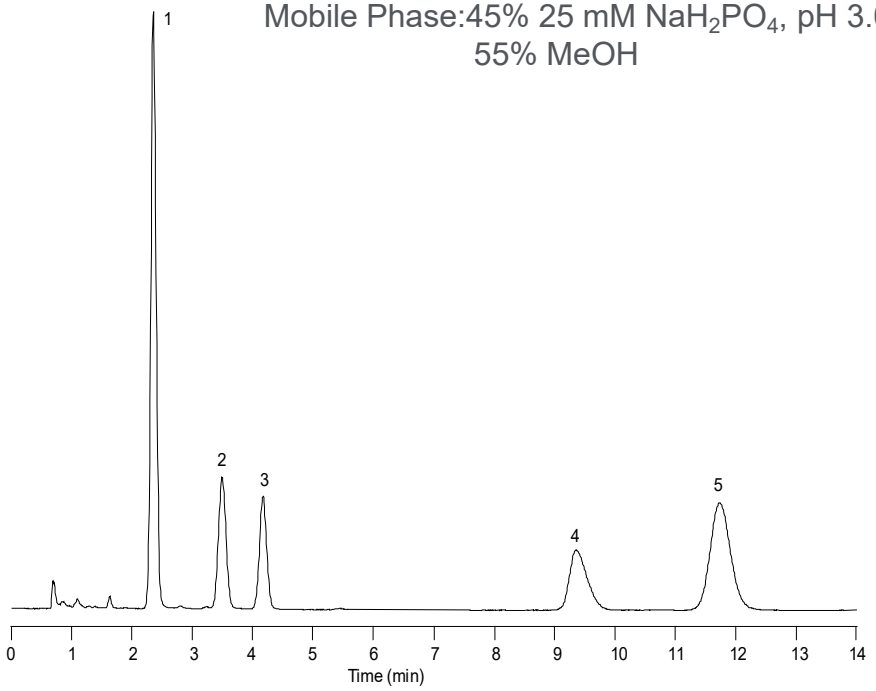
Mobile Phase:A: 20% H_2O
B: 80% MeOH

Retention, poor peak shape (IEX interaction)



Mobile Phase:45% 25 mM NaH_2PO_4, pH 3.0
55% MeOH



Column:	SB-C18, 4.6 x 150 mm, 5 mm
Flow rate:	1.0 mL/min.
Temperature:	35 °C
UV detection:	254 nm
Sample:	1. Diltiazem 2. Dipyridamole 3. Nifedipine 4. Lidoflazine 5. Flunarizine

Trick: Know your sample

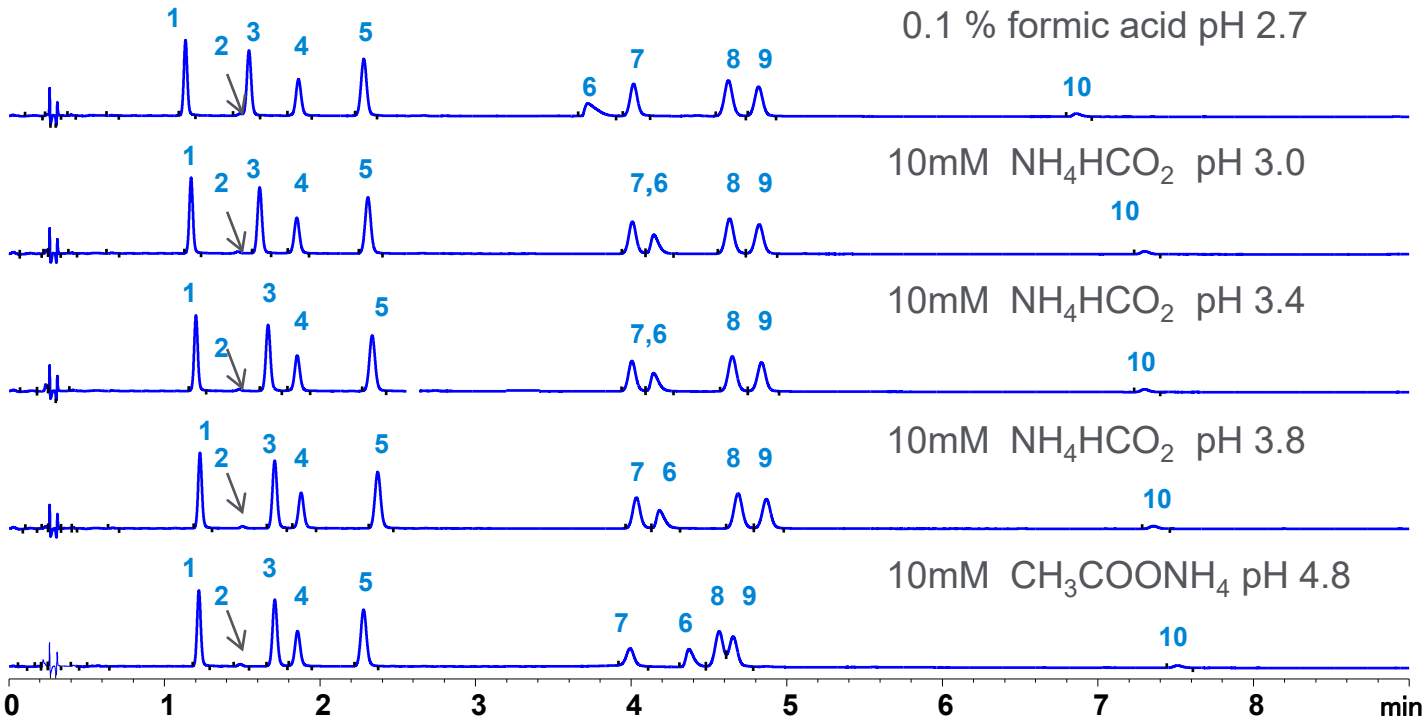
Tip: Know if your detector is compatible with the buffer you choose

Mobile Phase – Aqueous Buffer

Buffer type

- Can affect Rs and column lifetime
- Detector choice
  - DAD
  - MS

1. SMR
2. TPC
3. SDD
4. FZD
5. SMMX
6. PYM
7. OXA
8. SDMX
9. SQZ
10. DFZ



4.6 x 50 mm Poroshell 120 EC-C18; 205 Bar
10-40 %B (ACN)/12 min @ 2 mL/min 0.5 ul injection 0.1 mg/ml each

Buffer Options

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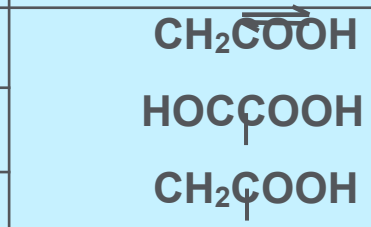
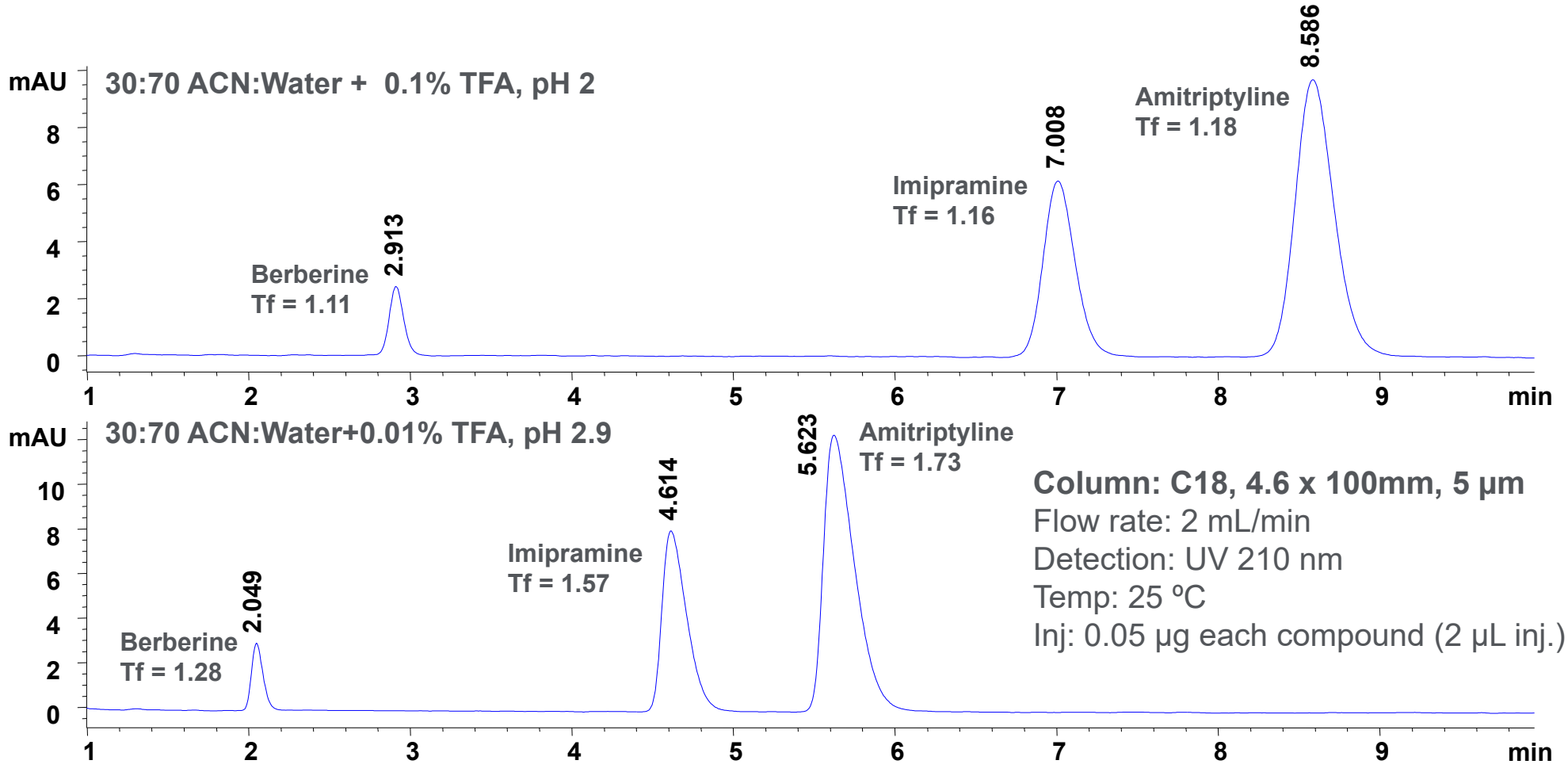
Nonvolatile		pK_a	Buffer range
Phosphate	H_3PO_4 $H_2PO_4^-$	$pK_1 = 2.1$	1.1–3.1
	$H_2PO_4^-$ ⇌ HPO_4^{-2}	$pK_2 = 7.2$	6.2–8.2
	HPO_4^{-2} ⇌ PO_4^{-3}	$pK_3 = 12.3$	11.3–13.3
Citrate		$pK_1 = 3.1$	2.1–4.1
		$pK_2 = 4.7$	3.7–5.7
		$pK_3 = 5.4$	4.4–6.4
Borate	H_3BO_3	$pK_1 = 9.2$	8.2–10.2
Volatile		pK_a	Buffer range
Trifluroacetate	F_3CCOOH	$pK_1 = 0.5$	xx–1.5
Formate	$HCOOH$	$pK_1 = 3.8$	2.8–4.8
Acetate	CH_3COOH	$pK_1 = 4.8$	3.8–5.8
Ammonium	NH_4^+	$pK_1 = 9.2$	8.2–10.2

Tip: Make sure you know the buffering range of your buffer!

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



Column: C18, 4.6 x 100mm, 5 µm
Flow rate: 2 mL/min
Detection: UV 210 nm
Temp: 25 ºC
Inj: 0.05 µg each compound (2 µL inj.)

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

How Low and High pH Can Cause Column Failure

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

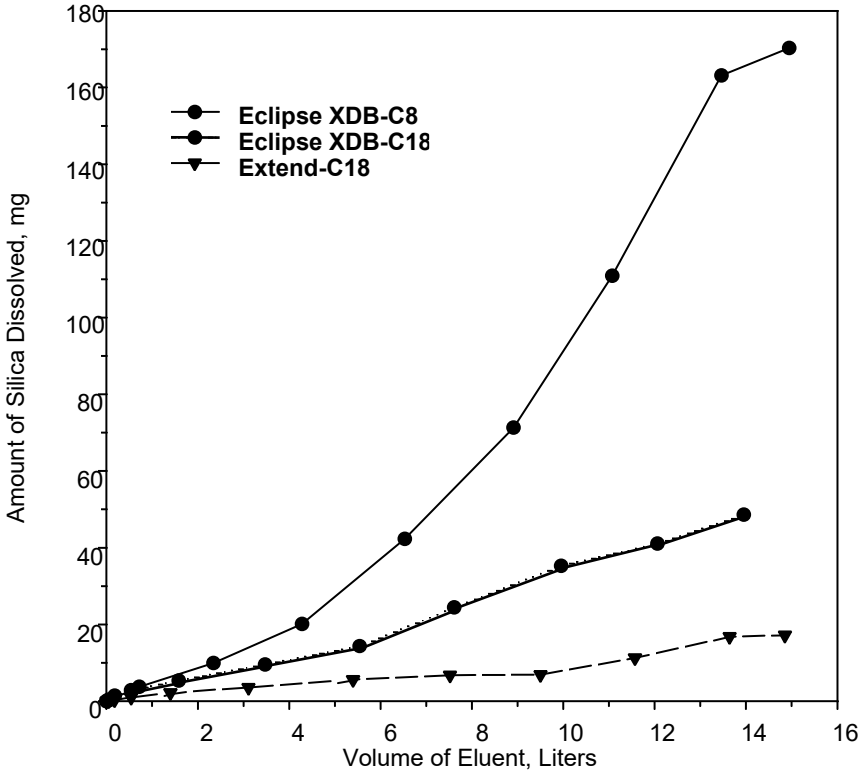
Best all around	Best for low pH mobile phases	Best for high pH mobile phases	Best for alternative selectivity	Best for more polar analytes	Chiral
EC-C18 1.9 µm, 2.7 µm, 4 µm	New! **SB-C18** New! 1.9 µm, 2.7 µm, 4 µm	**HPH-C18** 1.9 µm, 2.7 µm, 4 µm	**Bonus-RP** 2.7 µm	New! **SB-Aq** New! 1.9 µm, 2.7 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	**EC-CN** 2.7 µm	**Chiral-T** 2.7 µm
Phenyl-Hexyl 1.9 µm, 2.7 µm, 4 µm				**HILIC** 1.9µm, 2.7 µm, 4 µm	**Chiral- CD** 2.7 µm
				New! **HILIC-Z** New! 1.9 µm, 2.7 µm, 4 µm	**Chiral-CF** 2.7 µm
				HILIC- OH5 2.7 µm	

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



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

Trick: Choose a mobile phase that is right for your column

Tip: Keep record/history of your column

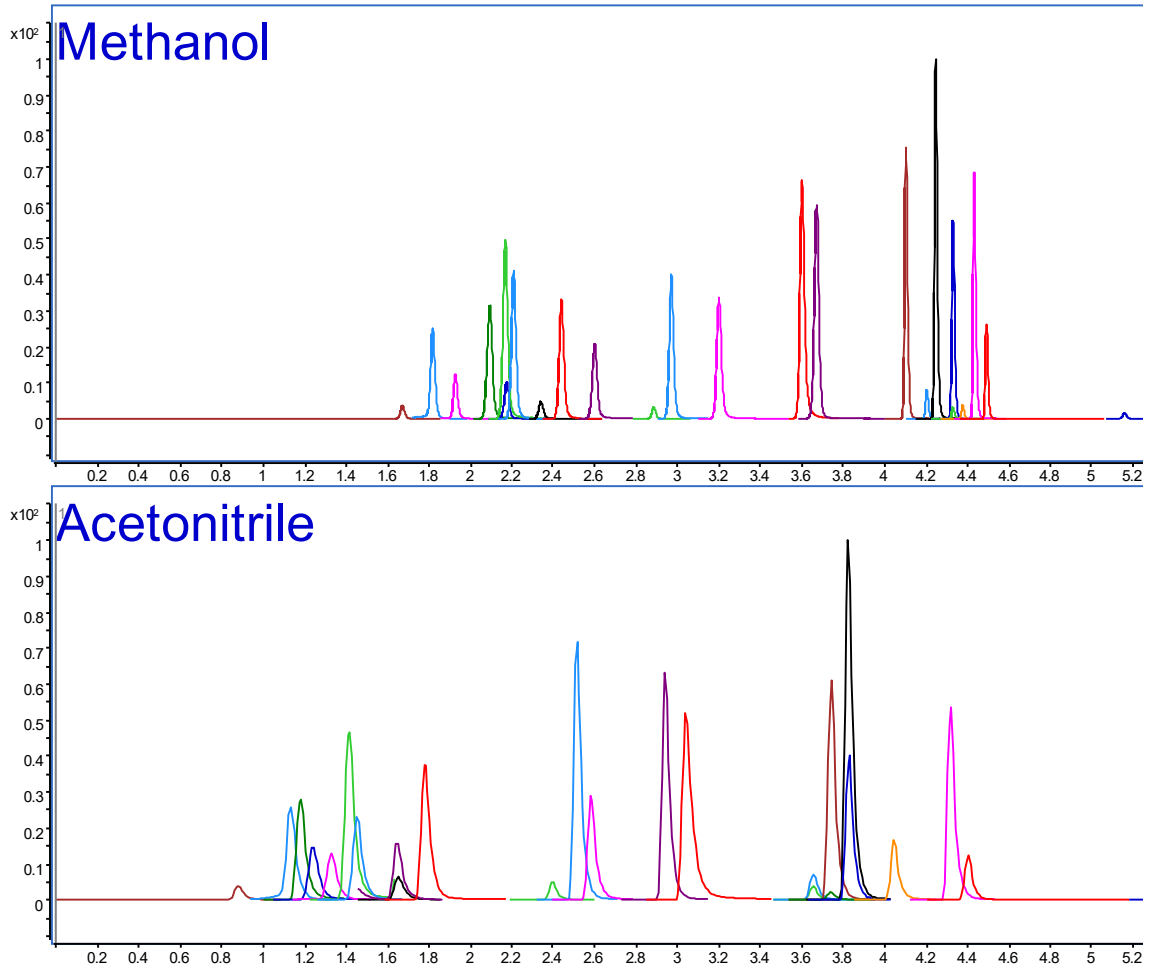
Mobile Phase – Explore Organic Options

Why?

- ✓ It's easy – ACN & MeOH are readily available
- ✓ Works on any bonded phase – optimize separation no matter the column choice

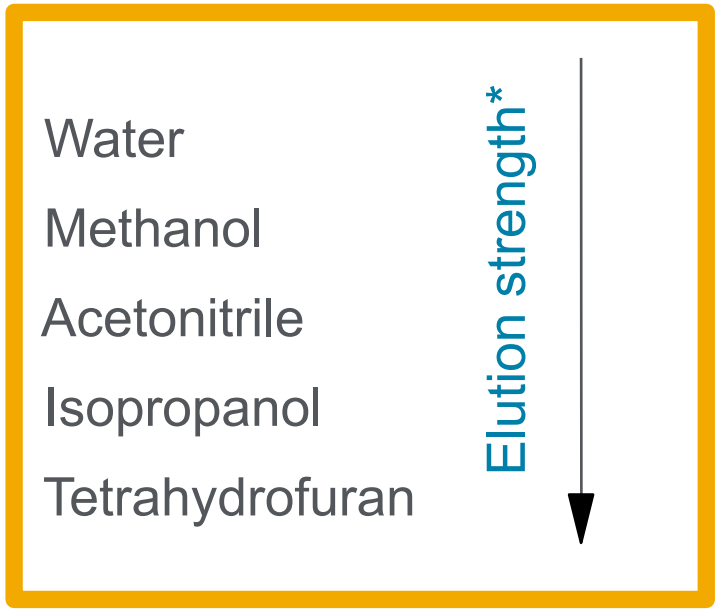
MeOH – Higher pressure, generally better peak shape with bases, protic solvent

Acetonitrile – Aprotic, wider UV window, stronger than MeOH



Common LC Solvents

Solvent	UV Cutoff (nm)*	Polarity
Acetonitrile	190	
Water	190	78.1
Cyclohexane	195	2.0
Hexane	200	1.9
Methanol	210	32.6
Acetone	331	20.7
Chloroform	240	4.8
Ethanol	210	24.3
Tetrahydrofuran	280	
Toluene	280	



*In HILIC water is the stronger solvent

Mobile Phase Preparation

- HPLC grade or better
- Buffer prep procedure
  - Be consistent
- Document process

Volume % of solvents can depend on preparation

Specified volume ACN added to a 1 L volumetric and made to volume with H_2O

≠

Specified volume H_2O added to a 1 L volumetric and made to volume with ACN

≠

500 mL H_2O added to 500 mL ACN

- Relative quantities of each affects degree of contraction
- Temperature

Tips

1. Small changes in mobile phase strength can have a large effect on retention
2. Immiscible solvent flow can cause high pressure and trigger system shutdown
3. Be aware of buffer solubility, e.g.
   a. Solubility of phosphate buffer, pH 7.0 is >50 mM in MeOH, ACN, and THF @10% organic
   b. At 70% organic, solubility of phosphate buffer, pH 7.0 is 35 mM in MeOH, 20 mM in ACN, and 10 mM in THF

Sample Considerations - Mobile Phase Diluents and Solubility

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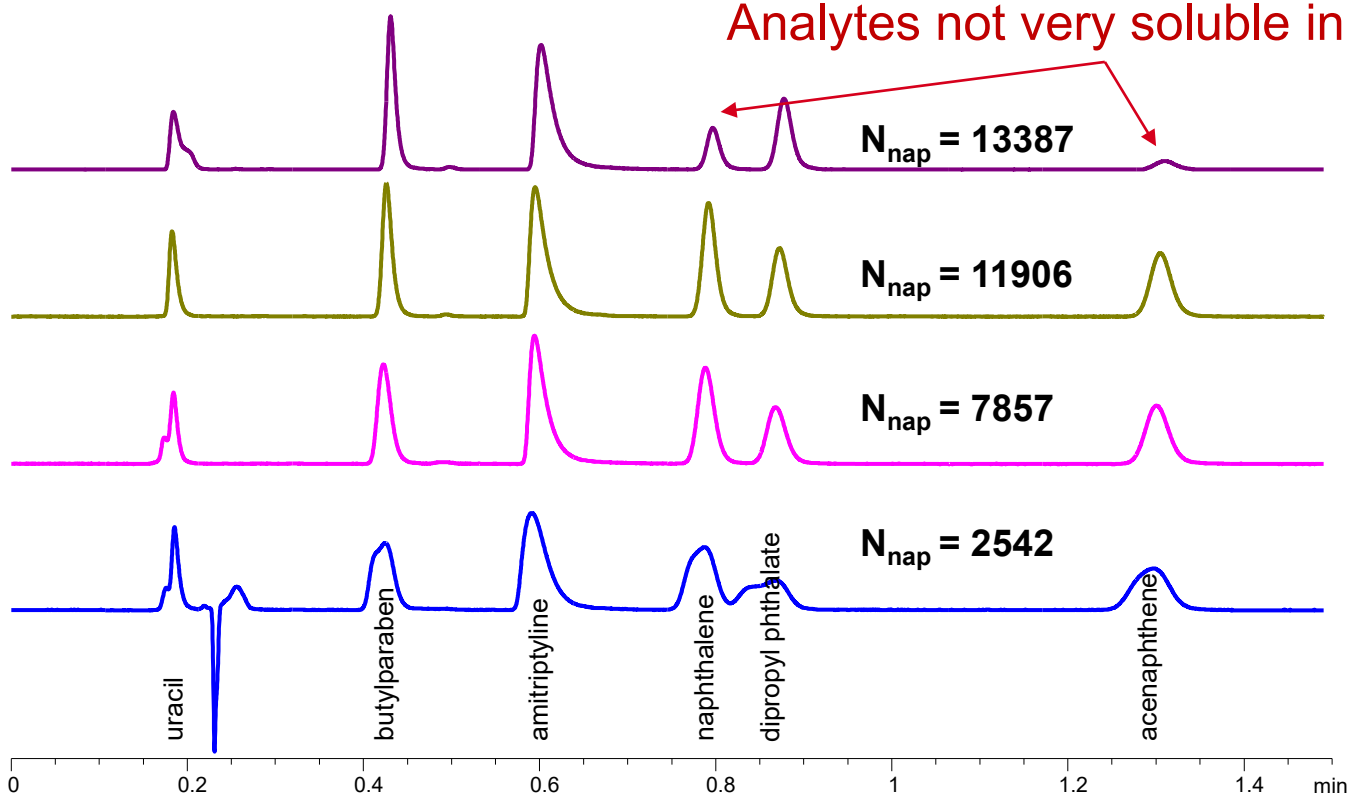
Increasing Sample Solvent Strength



1.5 µL injection of sample diluted 1:10 in **water**

1.5 µL injection of sample diluted 1:10 in **mobile phase**

1.5 µL injection of sample diluted 1:10 in **acetonitrile**

1.5 µL injection of sample diluted 1:10 in **tetrahydrofuran**

Sample solvents should be of equal or lesser strength than the mobile phase, otherwise poor peak shape can occur, resulting in poor efficiency

Precolumn protection

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Filters

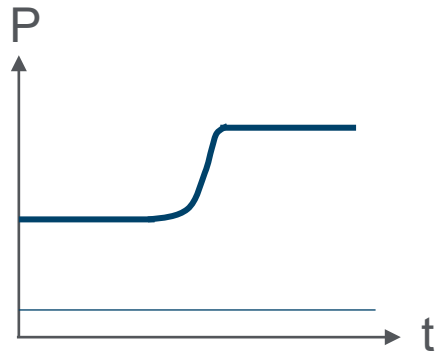
- Solvent Filters
- Sample filters
- Inline filters

Guard columns

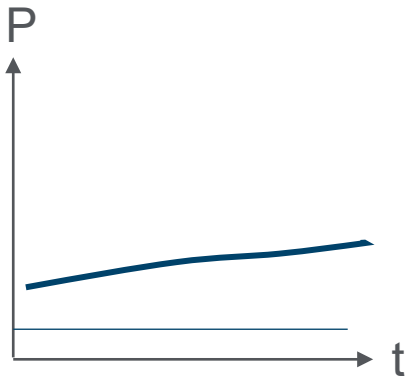
- Examples

Protect your Column Before a Run

How to protect your column from all sources of particulates



Blockages: instant pressure increase step



Clogging: constant pressure increase over time

Instrument and solvents as a source of particulates

- Use Agilent inline filters in the pump to remove pump seal wear
- Filter buffered LC solvents with Agilent Solvent Filtration equipment to remove precipitated / unresolved salts

InfinityLab Solvent Filtration Assembly

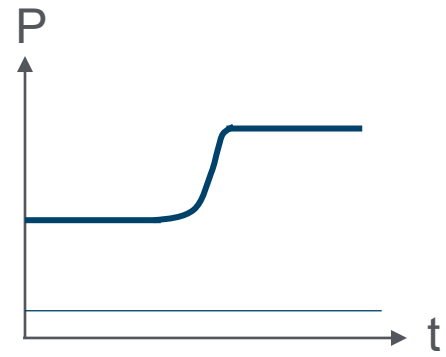
Sample as a source of particulates

- Filter sample with Agilent syringe filters to remove particulates
- Use Agilent inline filters and / or guard columns to protect from injector seal wear and sample compounds precipitating in gradient starting conditions

Agilent Inline Filters

Blockages and Clogging

Characteristics	
Parts affected	Blockages: • Capillaries, needle and needle seat • Detector flow cells Clogging: • Filter frits (inline filter, column filter)
Characteristic	
Identification	• Start by disconnecting the capillary at the column inlet • Install test setup with restriction capillary • 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) • Coring of vial septa material
Instant action / First aid	• Backflush affected part • Replace part
Preventive measures	• Replace worn parts in time; apply proper preventive maintenance schedules • Use high quality septa • Install inline filters



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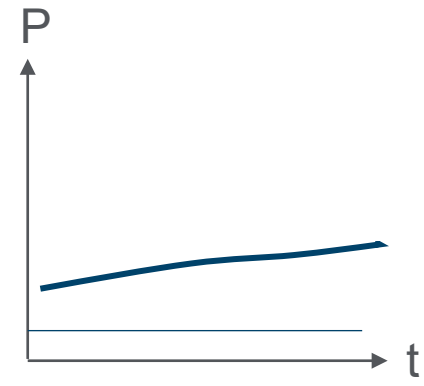

Clogging: constant pressure increase over time

Protect your Column Before a Run

Why it is important to avoid solvent contamination?

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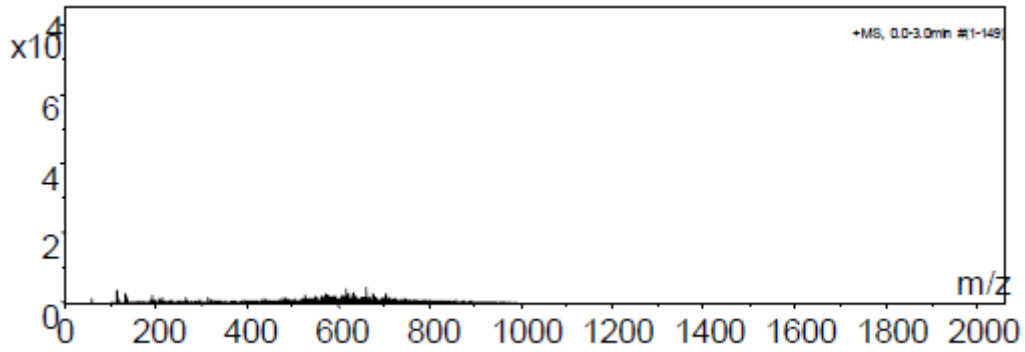
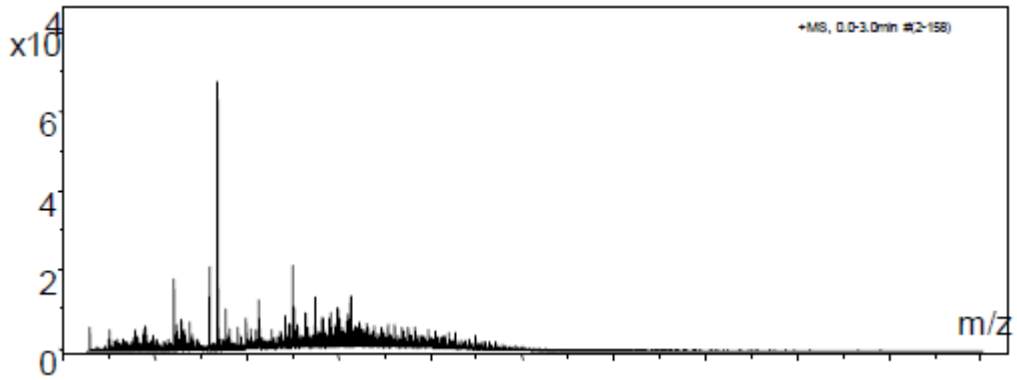
Example: Water from Lab Purification System

Purified water after discarding several liters



Purified water after one weekend



Contaminated solvents can lead to:

- Partially blocked frits and filters causing
  - Increased column backpressure
  - Increased pump backpressure
  - Peak splitting and broadening
- Change in column selectivity and performance

!

Important to know

To prevent microbial growth in your aqueous mobile phase, prepare, filter, and degas mobile phases on a daily basis.

If the instrument is not used over a longer period of time, properly flush the instrument first with water to remove buffer residues, then with at least 10% IPA (or MeOH, ACN) in water.

Sample Filtration

Captiva premium syringe filters

- Certified to be free of UV-detectable extractables on HPLC. PES and glass fiber also certified for LC/MS.
- Color-coded boxes for easy identification
- Comprehensive portfolio to meet all customers' needs

Premium Syringe Filters						
Membrane	Diameter/Pore Size					
	4 mm		15 mm		25 mm (28 mm)	
	0.2 µm	0.45 µm	0.2 µm	0.45 µm	0.2 µm	0.45 µm
PTFE	♦	♦	♦	♦	♦	♦
Nylon			♦	♦	♦	♦
PES	♦	♦	♦	♦	♦	♦
Regenerated cellulose	♦	♦	♦	♦	♦	♦
Cellulose acetate					♦	♦
Glass microfiber			♦		♦	
Depth filters: glass/PTFE			♦	♦	♦	♦
Depth filters: glass/nylon			♦	♦	♦	♦

Sample Filtration

Captiva filter vials

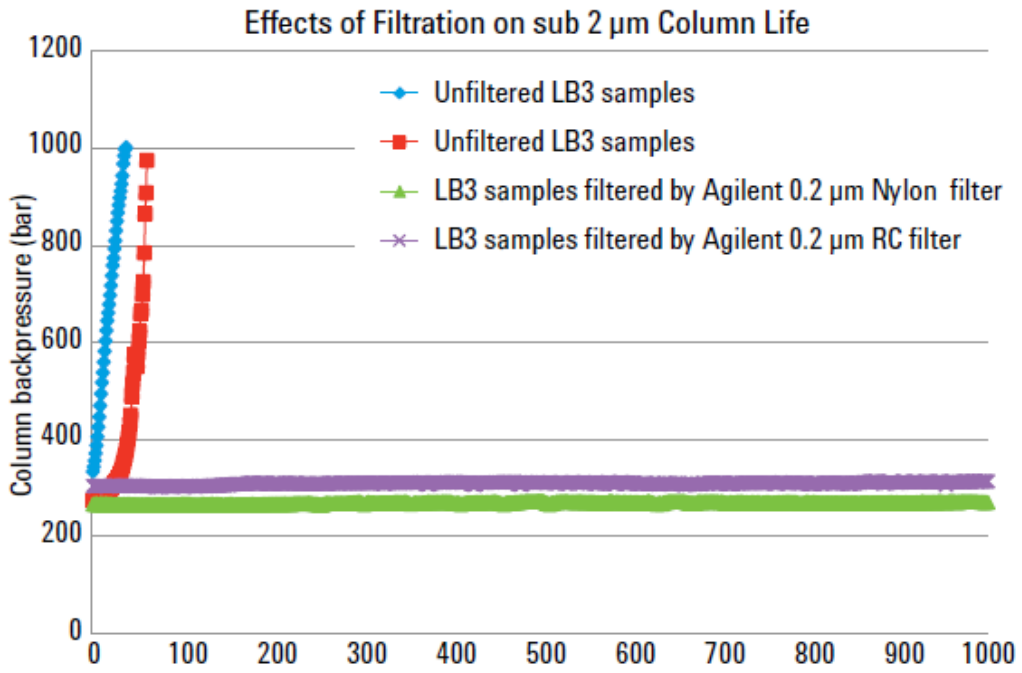
Description	Part No.
0.45 µm PTFE filter vial, 100/pack	5191-5933
0.20 µm PTFE filter vial, 100/pack	5191-5934
0.45 µm Nylon filter vial, 100/pack	5191-5935
0.20 µm Nylon filter vial, 100/pack	5191-5936
0.45 µm RC filter vial, 100/pack	5191-5939
0.20 µm RC filter vial, 100/pack	5191-5940
0.45 µm PES filter vial, 100/pack	5191-5941
0.20 µm PES filter vial, 100/pack	5191-5942
Vial closure tool	5191-5943

www.agilent.com/chem/filtervials

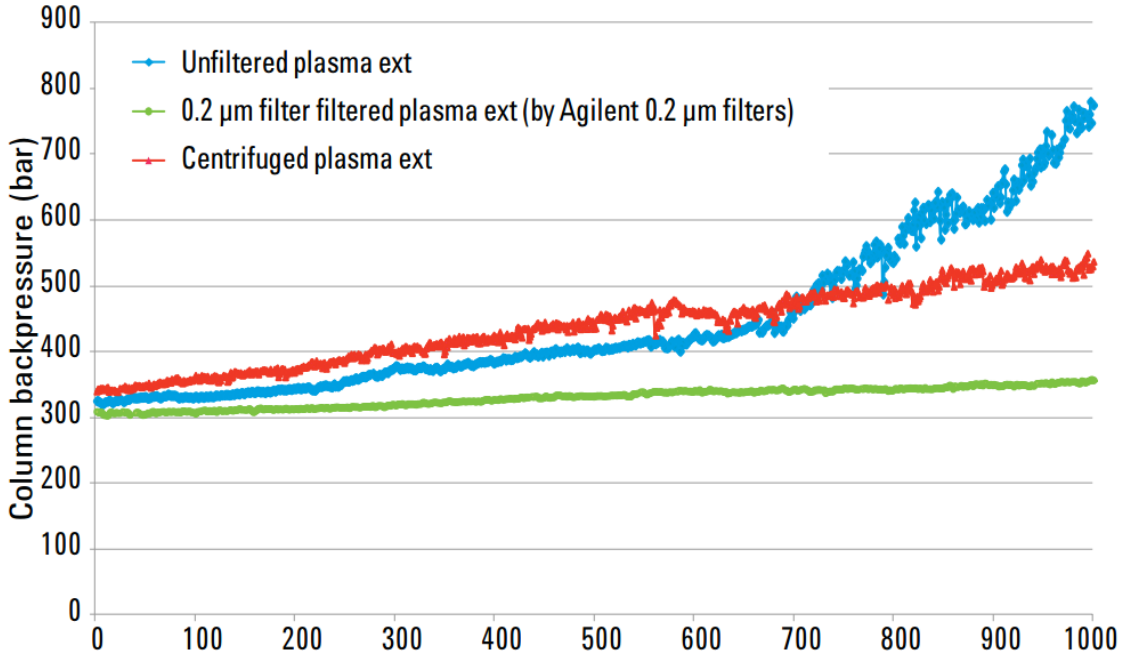
Filter vials user guide: 5994-0814EN

Protect your Column Before a Run

How particle and matrix components can block your LC column



Number of injections of unfiltered or filtered 0.3 µm latex beads (0.05% stock).

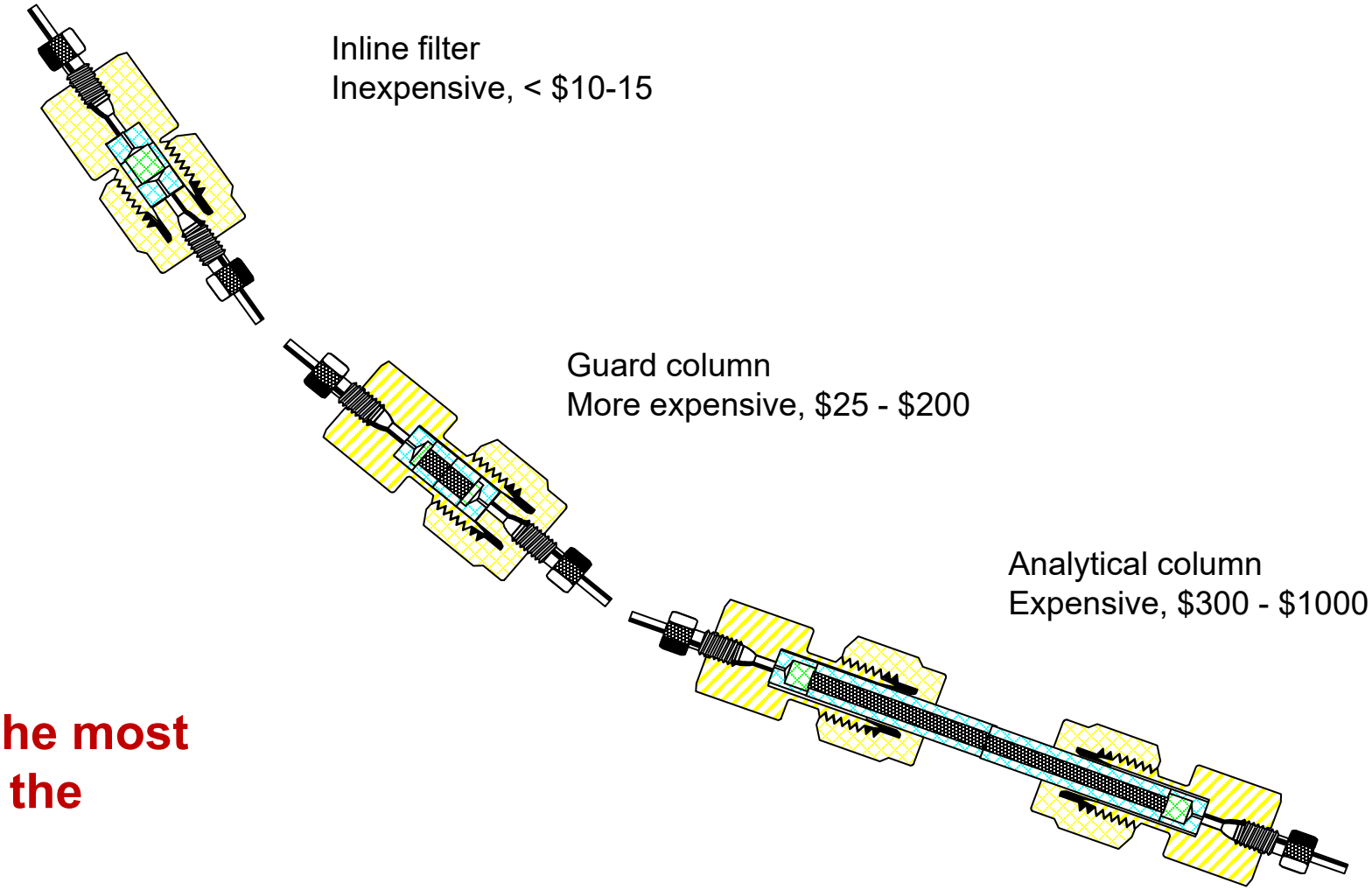


Number of injections of unfiltered, centrifuged or filtered human plasma after protein precipitation.

More information: Agilent application note 5994-1947EN

Prevention

Ways to protect your column



Inline filter
Inexpensive, < $10-15

Guard column
More expensive, $25 - $200

Analytical column
Expensive, $300 - $1000

Your column is the most effective filter in the whole LC!

Column Protection

Inline Filters

- Extend column life
- Easy to change
- Not intended to replace sample cleanup

UHPLC options

RRLC, 0.2 µm, max 600 bar
- 4.6mm frit id, 5067-1553
- 2.1mm frit id, 5067-1551

1290 Infinity LC, 0.3 µm, max 1200 bar
- 5067-4638, replacement frits 5023-0271

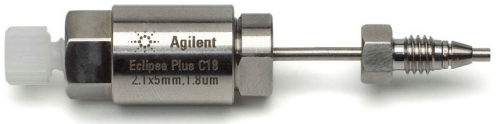
1290 Infinity II, 0.3 µm, max 1200 bar
- 5067-6189, replacement frits 5023-0271

Guard columns

Extend column life
Less expensive than analytical column
Match analytical column packing material
- Traps material that could bind strongly or irreversibly to analytical column

Inlet frit traps particulates

Cartridge format
340 bar, 200 bar
w/PEEK fitting

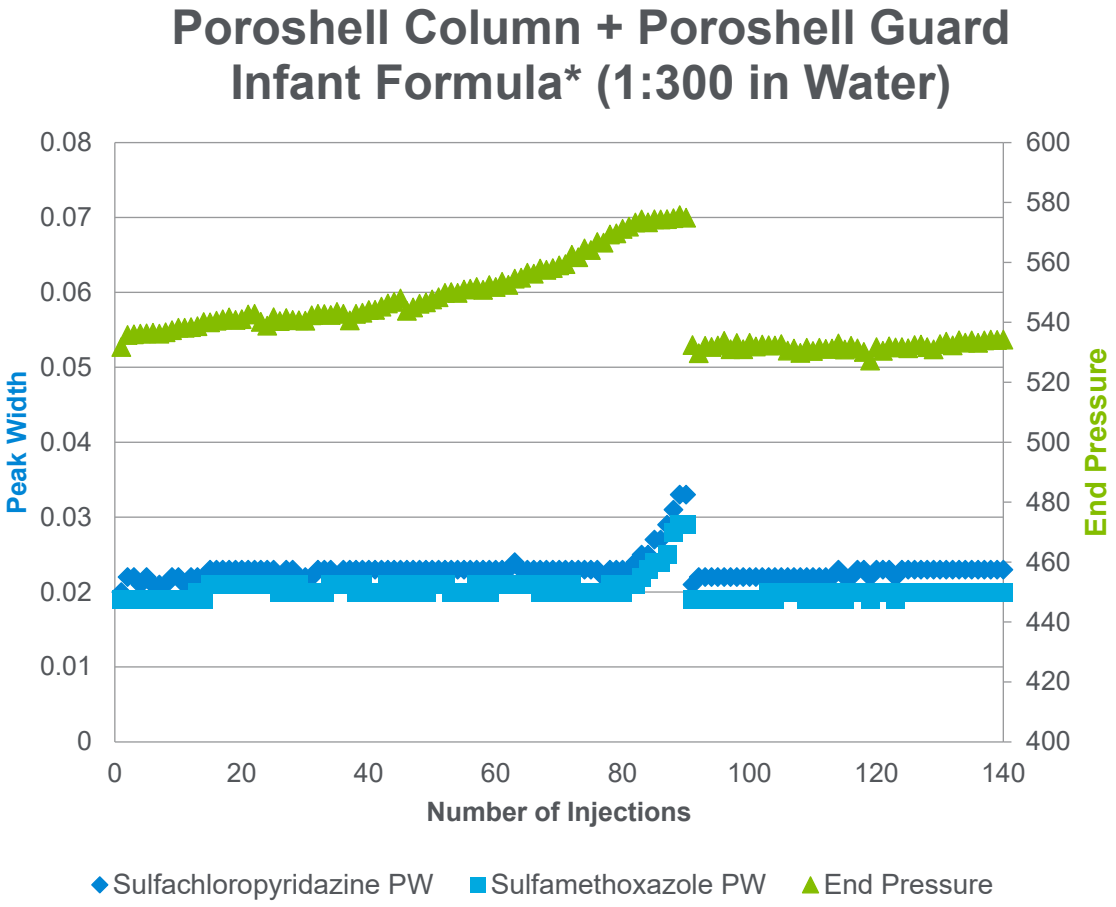


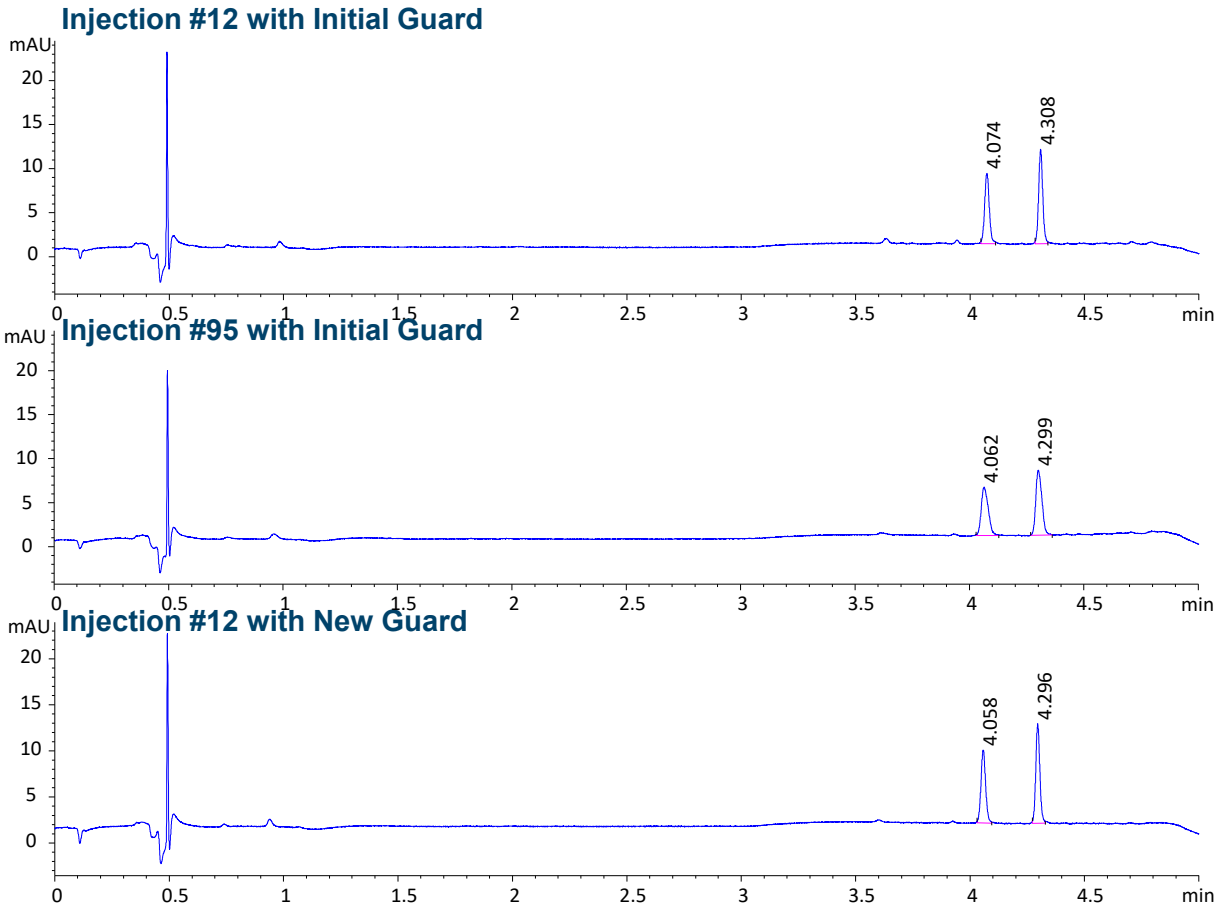
Individual guard column
600-1300 bar

Tip: Consider the cost versus the benefit

Guard columns/chemical modification

Guards protect your column in many ways





*Unfiltered infant formula including proteins and other precipitated ingredients.

Consider Your Instrument

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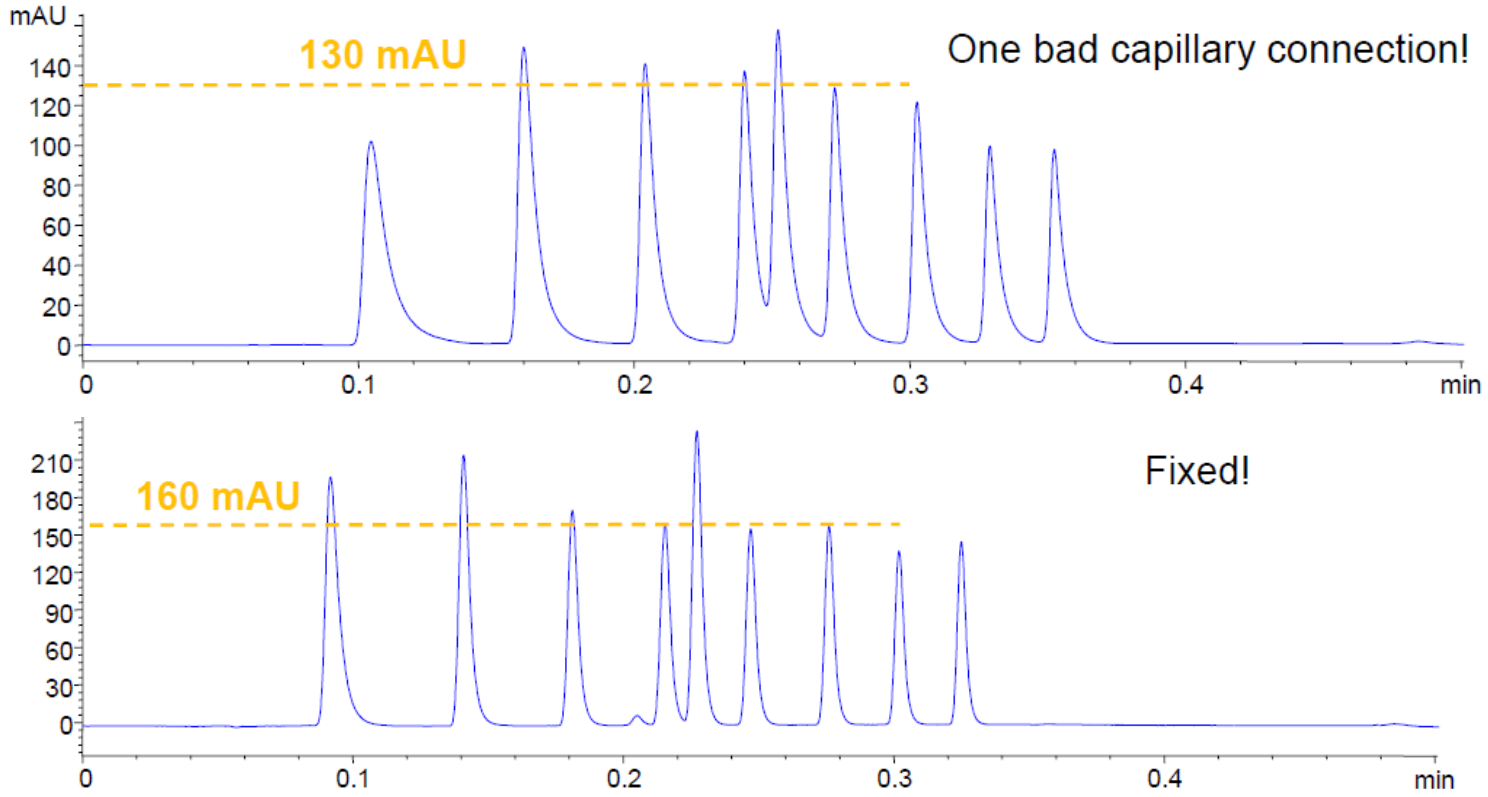
Instrument

- Connections
- Tubing
- Dwell volume
- Extracolumn volume

Modules

- Pump
- Column oven
- Detector

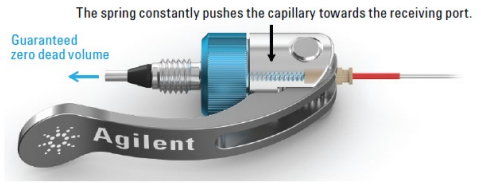
Importance of Correct Connections

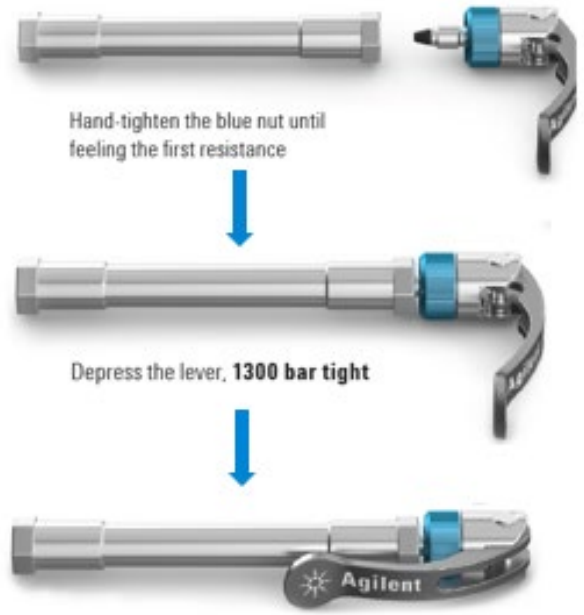


Quick Turn

Agilent Technical Note: Agilent InfinityLab Fittings
Pub No. 5991-5525EN

Correct connection every time



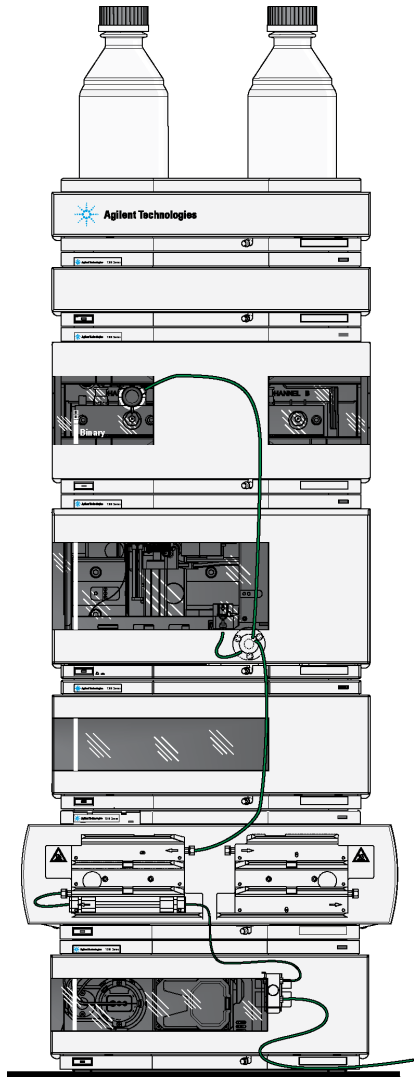


Quick Connect

1100/1200/1260 Series System Capillaries

Connection	p/n	Description
Solvent bottle to vacuum degasser	G1311-60003	Bottle head assembly for screw bottle (GL45), with glass filter 20 µm, (5041-2168)
Degasser to pump	G1322-67300	Tubing kit degasser, 300 mm tubing, 4 each
Pump to autosampler	G1312-87303	Capillary, 0.17 mm x 400 mm
Pump (purge valve) to waste	5062-2461	PTFE tube, 5000 mm
Autosampler to column compartment	G1313-87305 G1313-87304	Capillary, 0.17 mm x 180 mm Capillary, 0.12 mm x 180 mm
Thermostatted ALS to column compartment	01090-87309 01090-87610	Capillary, 0.17 mm x 380 mm Capillary, 0.12 mm x 280 mm
Column compartment to column	G1316-87300 01090-87611	Capillary, 0.17 mm x 90 mm Capillary, 0.12 mm x 105 mm
Column to VWD (standard flow cell)	5062-8522	Inlet Tubing Assembly PEEK, 0.17 mm 600 mm (see 'Specials' slide for additional flow cells)
Column to DAD/MWD	G1315-87311 G1315-87312	Capillary, 0.17 mm x 380 mm (S/S, ps/ns) Capillary, 0.12 mm x 150 mm
VWD to waste	5062-8535	Waste accessory kit
DAD to waste	5062-2462	PTFE tubing 0.7 mm id, 1.6 mm od, 5 m

0.17 mm id capillaries	Standard Setup
0.12 mm id capillaries	Rapid Resolution LC Setup



Instrument Considerations

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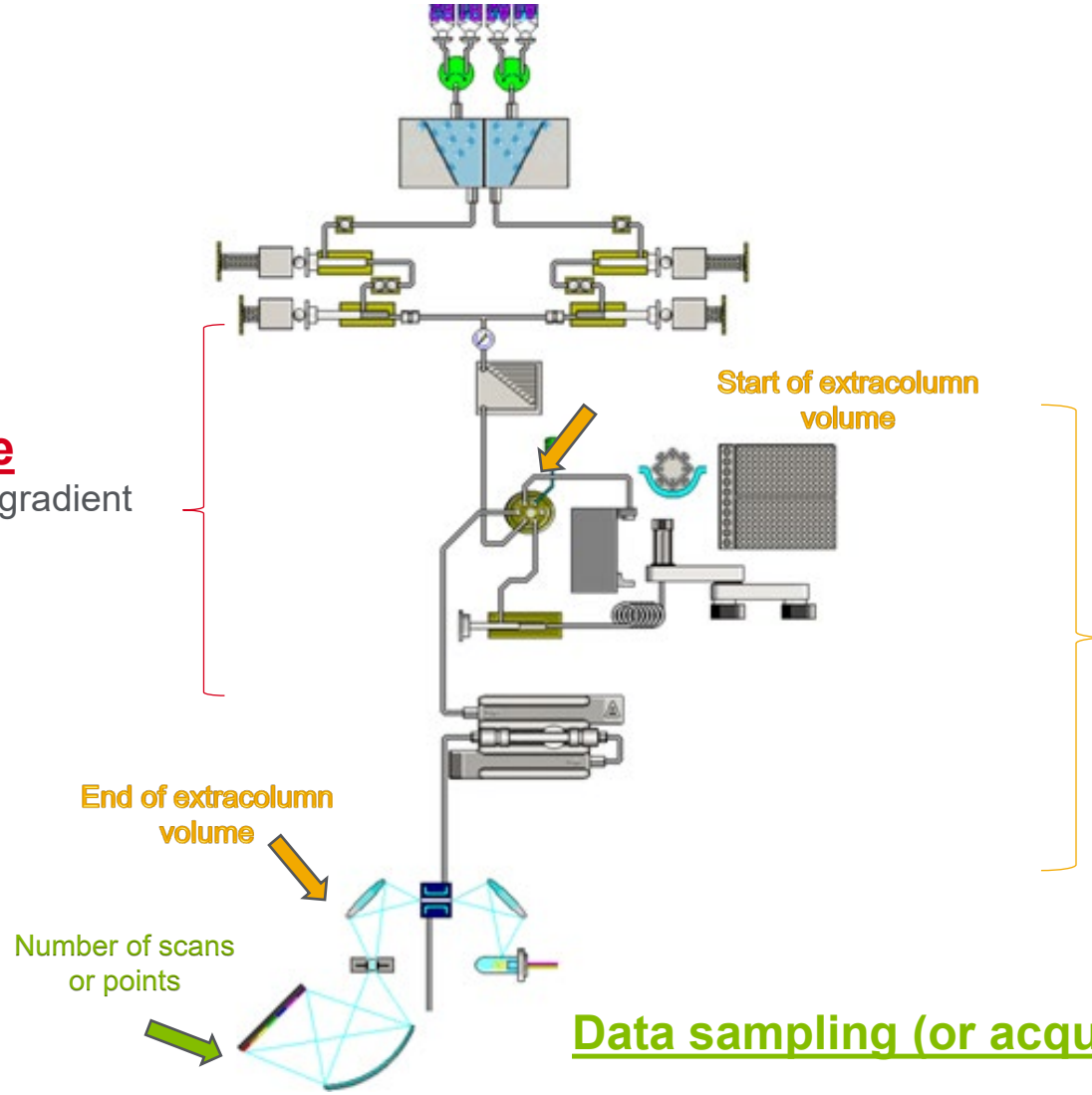
Dwell/delay volume
Volume from formation of gradient to the column



Extracolumn volume
From injection to detection
Sample flow path outside column and sample volume

Data sampling (or acquisition) rate

Gradient Delay Volume

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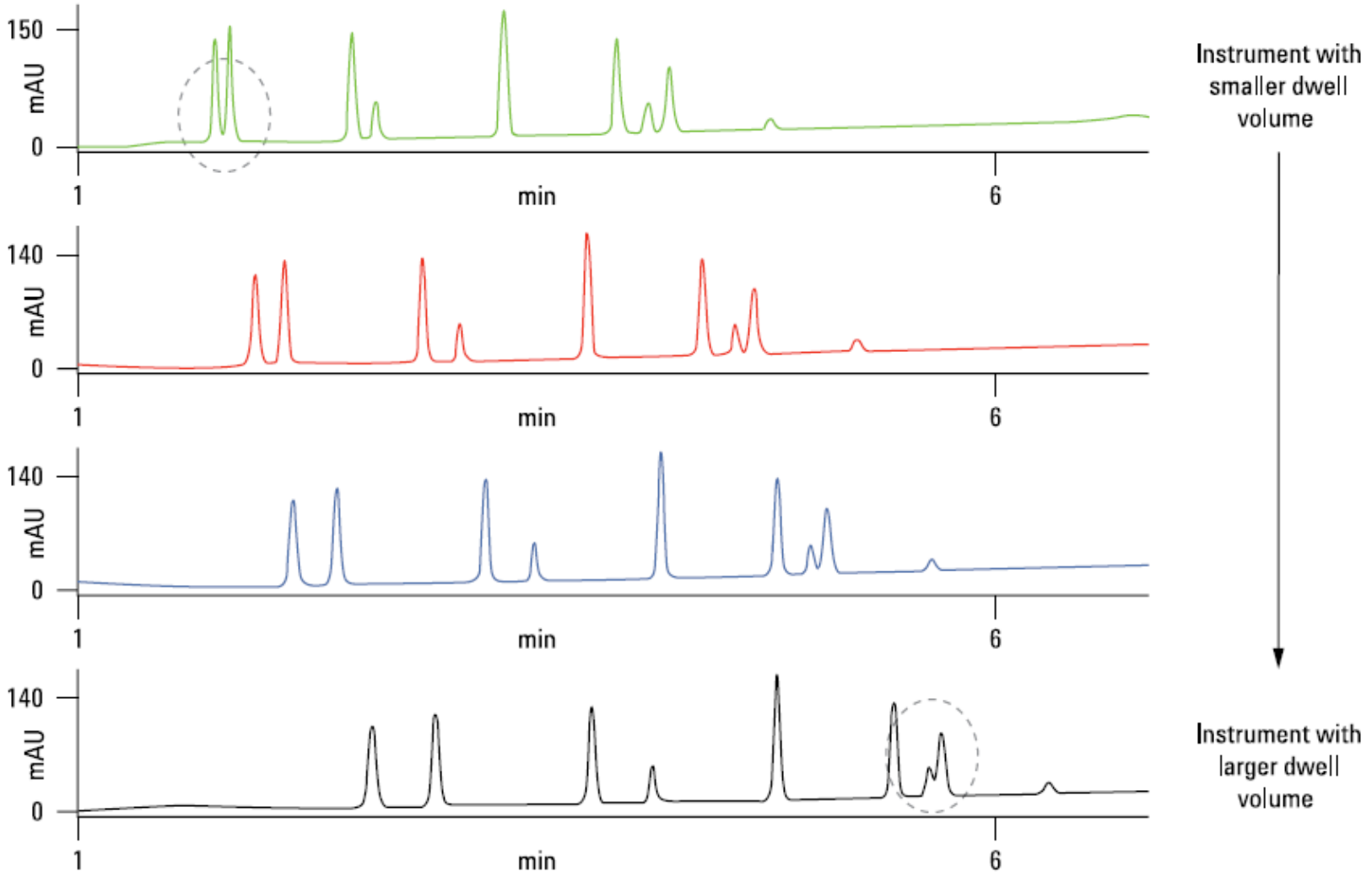
Key parameter that can cause major chromatography differences between systems

Why is delay/dwell volume important?

1. Different dwell volumes result in a RT shift
   - Definition: The time (or volume) for mobile phase from point of mixing to reach the column head
2. Different dwell volume could affect resolution
   - Peaks spends different time under isocratic/gradient conditions
3. Dwell volume effects on gradient shape
   - Dispersion effects => the programmed gradient becomes deteriorated
4. Same “delay” volume – chromatograms could look different on different systems
5. Big impact for narrow bore applications, especially when combined with fast gradient

Chromatographic Test Result; Different Delay Volumes



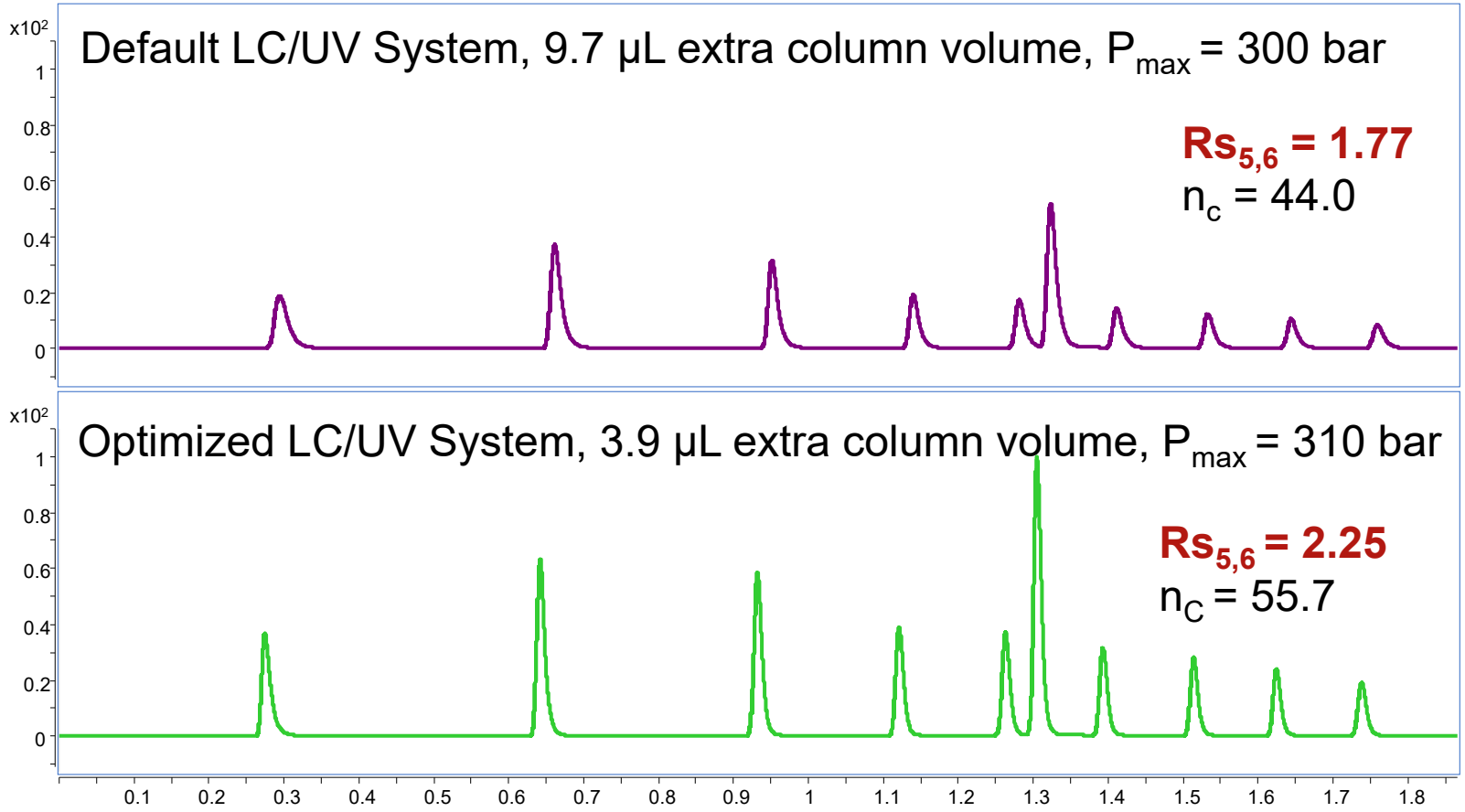
Dispersion
Extracolumn volume

- ECV – System volume between point of injection and detector outlet
- ECV major contributors
  - Capillaries, length & id
  - Heat-exchangers
  - Connectors and fittings
  - Flow cell
- Large ECV causes sample dispersion and band broadening of analytes
  - Result – Decreased resolution and less sensitivity
- Take special care with capillary connectors or when mounting columns into a system.
- Remember: Diluent strength and injection volume contribution
- Small id columns, ≤ 2.1 mm

Optimized LC Volume Improves Gradient Resolution

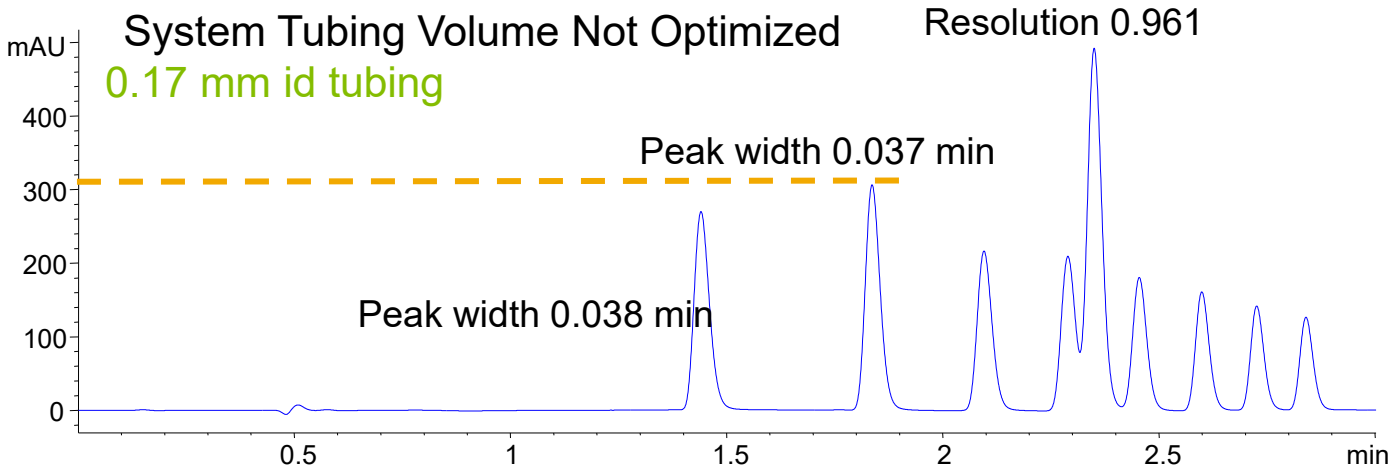
Agilent InfinityLab

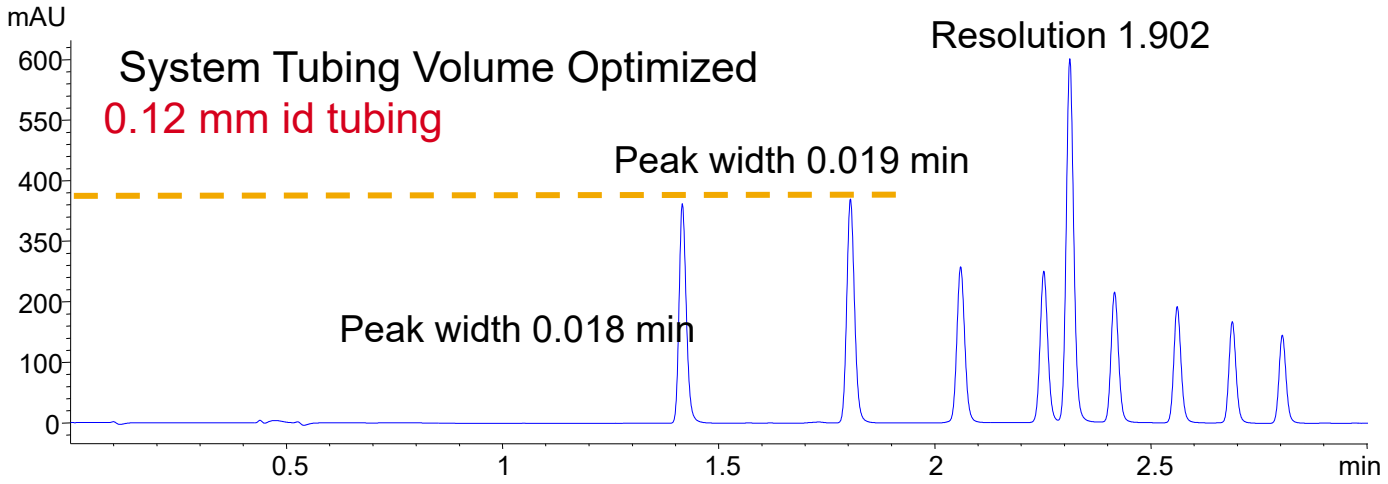
Column: RRHD Eclipse Plus C18, 2.1 x 50 mm, 1.8 µm Gradient: 25-95% CH_3CN in 1.2 min, Flow Rate: 0.4 mL/min, LC: Agilent 1290 Infinity, Sample: Alkylphenones



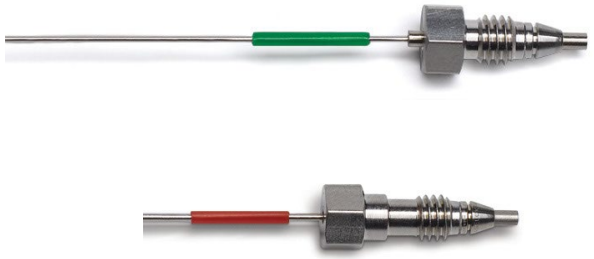
>20% improvement in gradient Rs and peak capacity with optimized LC

Optimize Tubing Volume for Small Volume Columns



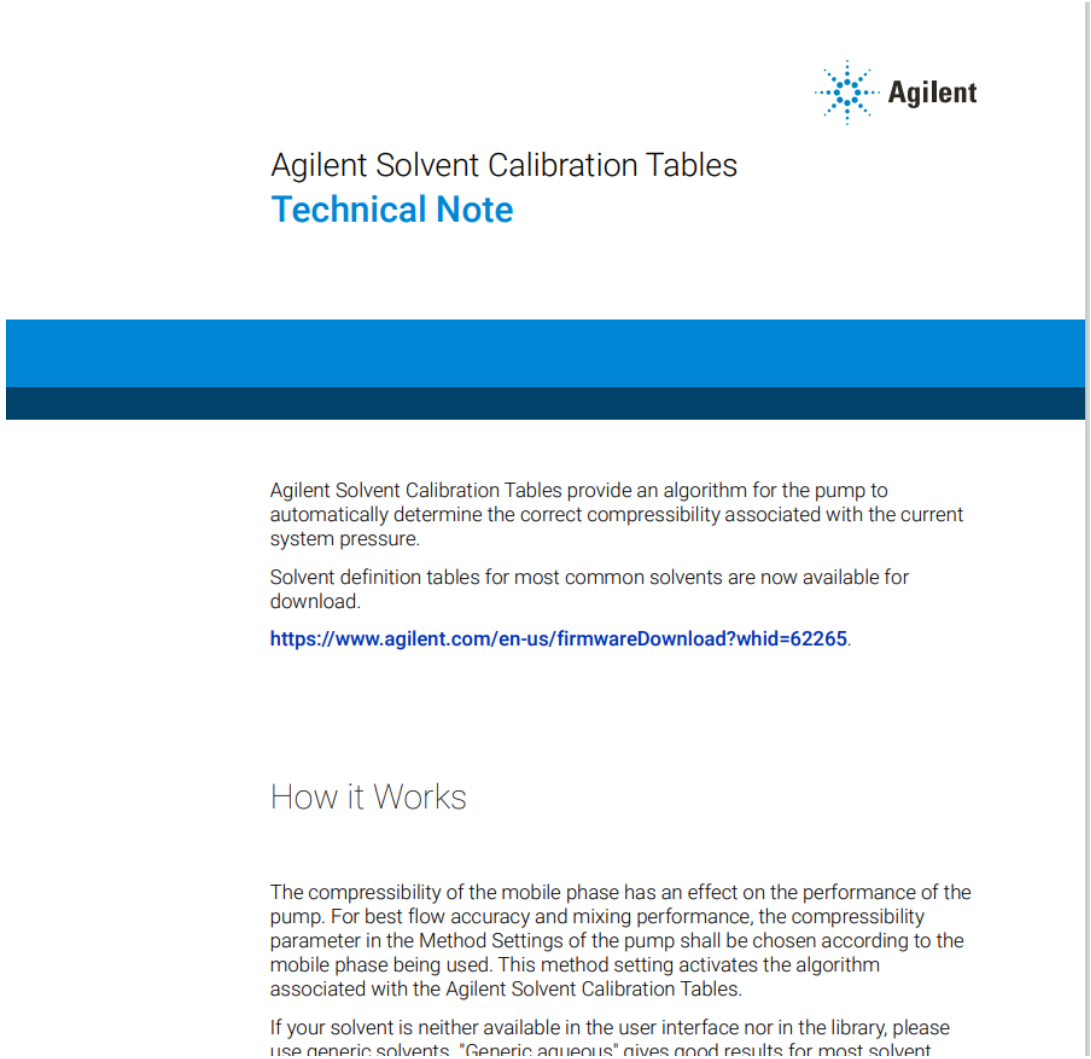


Length	10 mm	50 mm	100 mm	150 mm
Tubing id	Volume	Volume	Volume	Volume
0.17mm (green)	0.227 µL	1.1 µL	2.27 µL	3.3 µL
0.12mm (red)	0.113 µL	0.55 µL	1.13 µL	1.65 µL



Instrument

Compressibility tables: Agilent Solvent Calibration Tables Technical Note



Agilent

Agilent Solvent Calibration Tables

Technical Note

Agilent Solvent Calibration Tables provide an algorithm for the pump to automatically determine the correct compressibility associated with the current system pressure.

Solvent definition tables for most common solvents are now available for download.

https://www.agilent.com/en-us/firmwareDownload?whid=62265.

How it Works

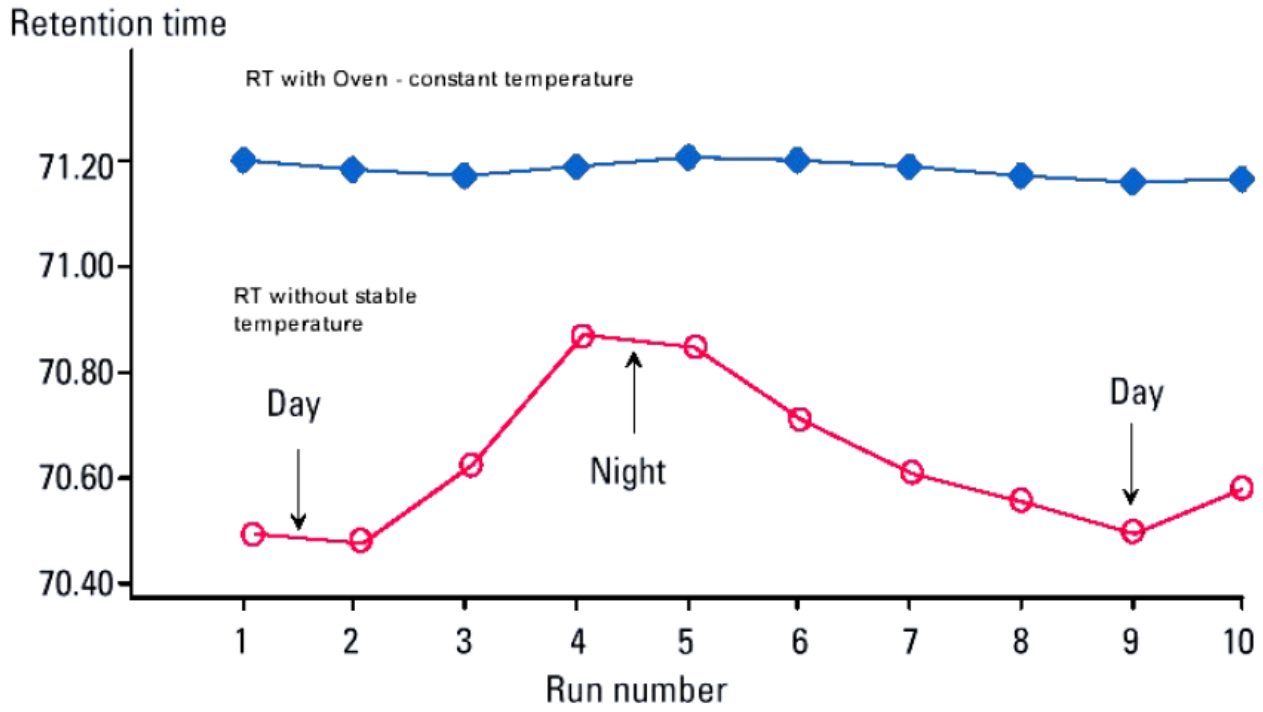
The compressibility of the mobile phase has an effect on the performance of the pump. For best flow accuracy and mixing performance, the compressibility parameter in the Method Settings of the pump shall be chosen according to the mobile phase being used. This method setting activates the algorithm associated with the Agilent Solvent Calibration Tables.

If your solvent is neither available in the user interface nor in the library, please use generic solvents. "Generic aqueous" gives good results for most solvent

Solvent	Compressibility (10^{-6} per bar)*
Water	46
Acetonitrile	96
Methanol	120
Isopropanol	100
Tetrahydrofuran	97

*Values are approximate at 20 °C

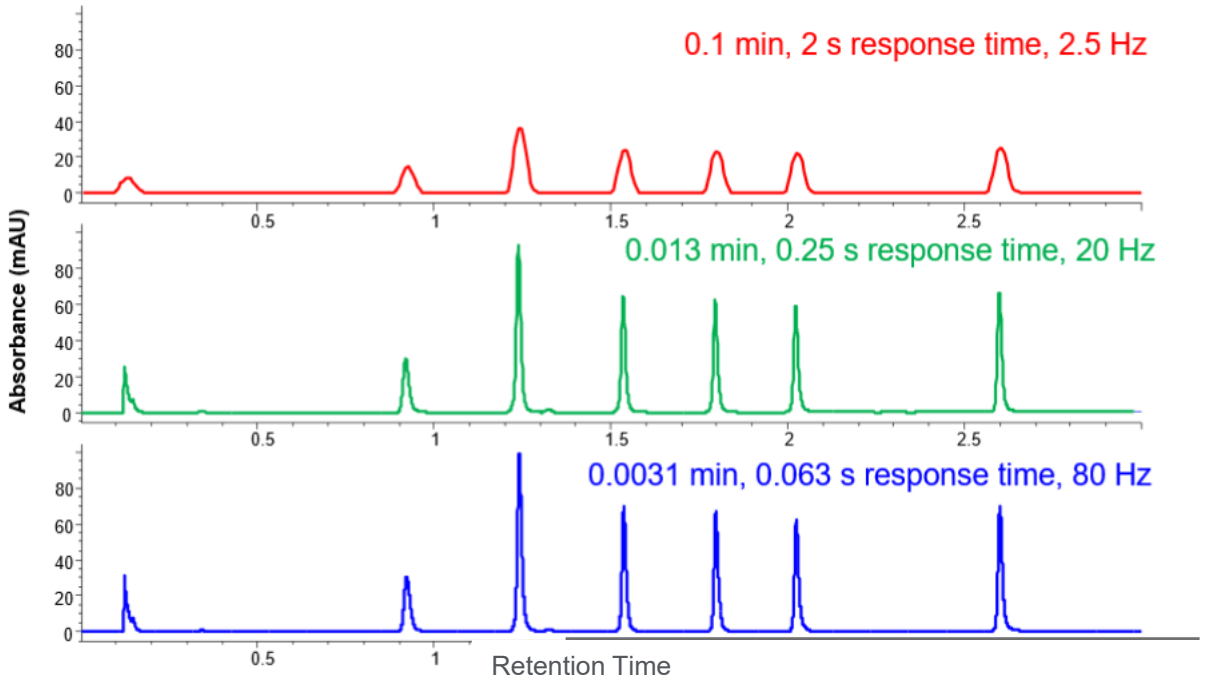
Column Oven
Control the temperature

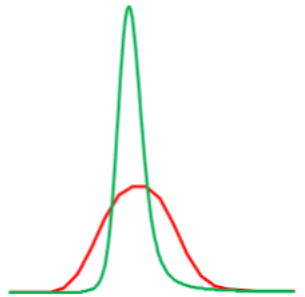


Tip: Constant temperature = constant retention

Detector

DAD Setting — Choose the right sampling rate

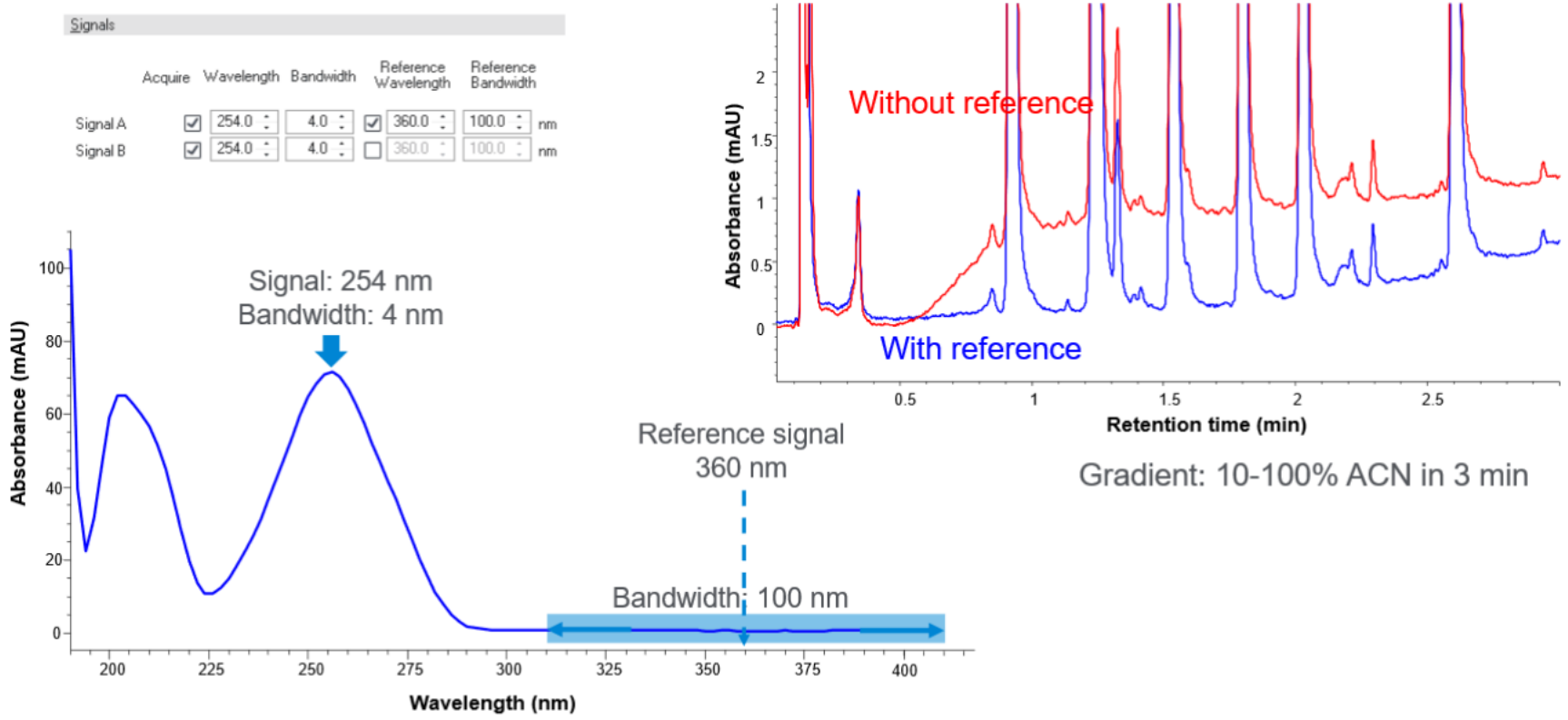




Changes in **Peak Width** and **Resolution**

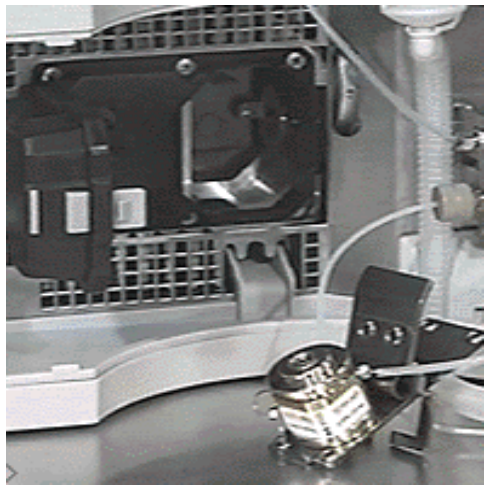
Column: ZORBOX Eclipse Plus C18, 2.1x50 mm, 1.8 µm
Column temperature: 35 °C; Flow rate: 1 mL/min
Gradient: 10-100% ACN in 3 min
Signal: 254 nm, Bandwidth: 4 nm
Reference: 360 nm, Bandwidth: 100 nm

DAD Settings
Choose the right signal and reference



Flow Cell

Match volume to chromatographic peak widths



Flow Cell Volume/Pathlength	UV Signal /Noise	Chrom. Resolution*
13 µL / 10 mm	+++	+
5 µL / 6 mm	++	++
1.7 µL / 6 mm	+	+++

* Depends on analytical conditions and column dimension

13 µL Standard Flow Cell:

For highest sensitivity and linearity
4.6 – 3 mm id, 3.5 to 5 µm columns

1.7 µL Micro Flow Cell:

For highest chrom resolution
UFLC 1.8 µm
2.1 – 1 mm id columns

5 µL Semi-micro Flow Cell:

Best compromise of sensitivity and selectivity
HPLC/UHPLC, 1.8 to 5 µm
4.6 – 1 mm id columns

Summary

Agilent InfinityLab

It’s not always about the column

- Mobile phase
  - Aqueous
  - Organic

Column protection

- Filters
  - Offline
  - Inline
- Guard columns

Instrument

- Connections
- System volume
- Modules

Resources for Support

- LC troubleshooting poster (5994-0709EN)
- Tech support www.agilent.com/chem/techsupport
- Resource page www.agilent.com/chem/agilentresources
  - Quick reference guides
  - Catalogs, column user guides
  - Online selection tools, how-to videos
  - Application workflows (such as cannabis, PFAS, and more)
- InfinityLab LC Supplies catalog (5991-8031EN)
- LC handbook (5990-7595EN)
- Best practices for using an Agilent LC system (01200-90090)
- Your local FSE and specialists
- Agilent University www.agilent.com/crosslab/university
- YouTube – Agilent Channel (maintenance videos)
- Agilent service contracts

Contact Agilent Chemistries and Supplies Technical Support

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 former Prozyme products

Available in the USA and Canada 8–5 all time zones

gc-column-support@agilent.com

lc-column-support@agilent.com

spp-support@agilent.com

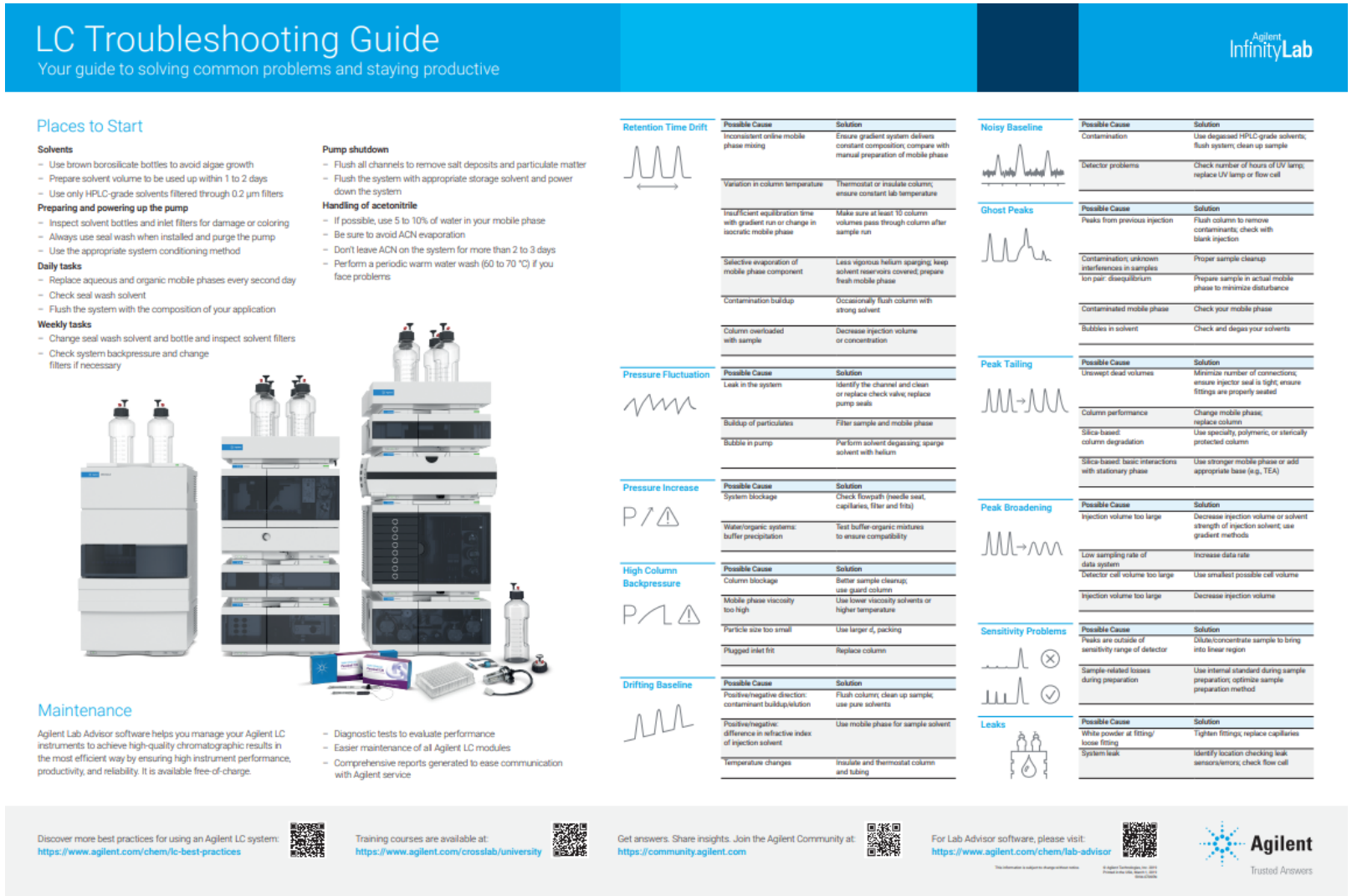
spectro-supplies-support@agilent.com

chem-standards-support@agilent.com

advancebio.glycan@agilent.com

Web Chat: Product pages of agilent.com

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

Resources for Support

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  - Quick Reference Guides
  - Catalogs, Column User guides
  - Online Selection Tools, How-to Videos
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- LC handbook (5990-7595EN)
- Your local FSE and Specialists
- Youtube – Agilent Channel (maintenance videos)
- Agilent Service Contracts

Appendix

Buffer Preparation
Does it make a difference?

Agilent InfinityLab

1. Dissolve salt in water using a 1 L or 2 L beaker. Use appropriate volume to leave space for pH adjustment. Equilibrate to RT for maximum accuracy.
2. Calibrate pH meter. Use 2-level calibration & bracket desired pH. Use appropriate audit solution to monitor statistical control (e.g., potassium hydrogen tartrate, saturated solution, pH = 3.56).
3. Adjust salt solution to desired pH. Minimize amount of time electrode spends in buffer solution (contamination). Avoid overshooting and re-adjustment (ionic strength differences can arise).
4. Transfer pH-adjusted buffer solution quantitatively to volumetric flask, dilute to volume, and mix.
5. Filter through 0.45 µm filter (discard first ~50 mL filtrate). Rinse solvent reservoir with small volume of filtrate and discard. Fill reservoir with remaining filtrate or prepare premix with organic modifier.
   - Agilent solvent filtration kit, 250 mL reservoir, 1000 mL flask, P/N 3150-0577
   - Nylon filter membranes, 47 mm, 0.45 µm pore size, P/N 9301-0895 (not for proteins!)

Trick: For gradient methods, avoid buffer precipitation by testing the solubility of buffered mobile phase component with highest % organic used. Always add organic to buffer with stirring, not vice versa.

Tips: Small particles in MP can permanently block capillaries in degasser.

Using Buffers Successfully
Shutdown State and Instrument Flushing

Agilent InfinityLab

Shutdown State

Next day use—using same buffers

- Pump mobile phase very slowly (for example, 0.01 – 0.1mL/min).

When flushing column or for longer term column storage

- Flush with 20/80 organic/water, then 80/20 organic/water or 100% organic.

Instrument flushing

Replace column with capillary tubing. Leave disconnected from detector.

Flush pumps with water, then connect capillary tubing to detector.

Inject water 2-3 times at maximum injection volume setting.

Flush all pumps with 100% organic for long term storage.

Determining the Dwell Volume of Your System

Agilent InfinityLab

Replace column with short piece of HPLC stainless steel tubing

Prepare mobile phase components
- A. Water -UV-transparent
- B. Water with 0.2% acetone - UV-absorbing

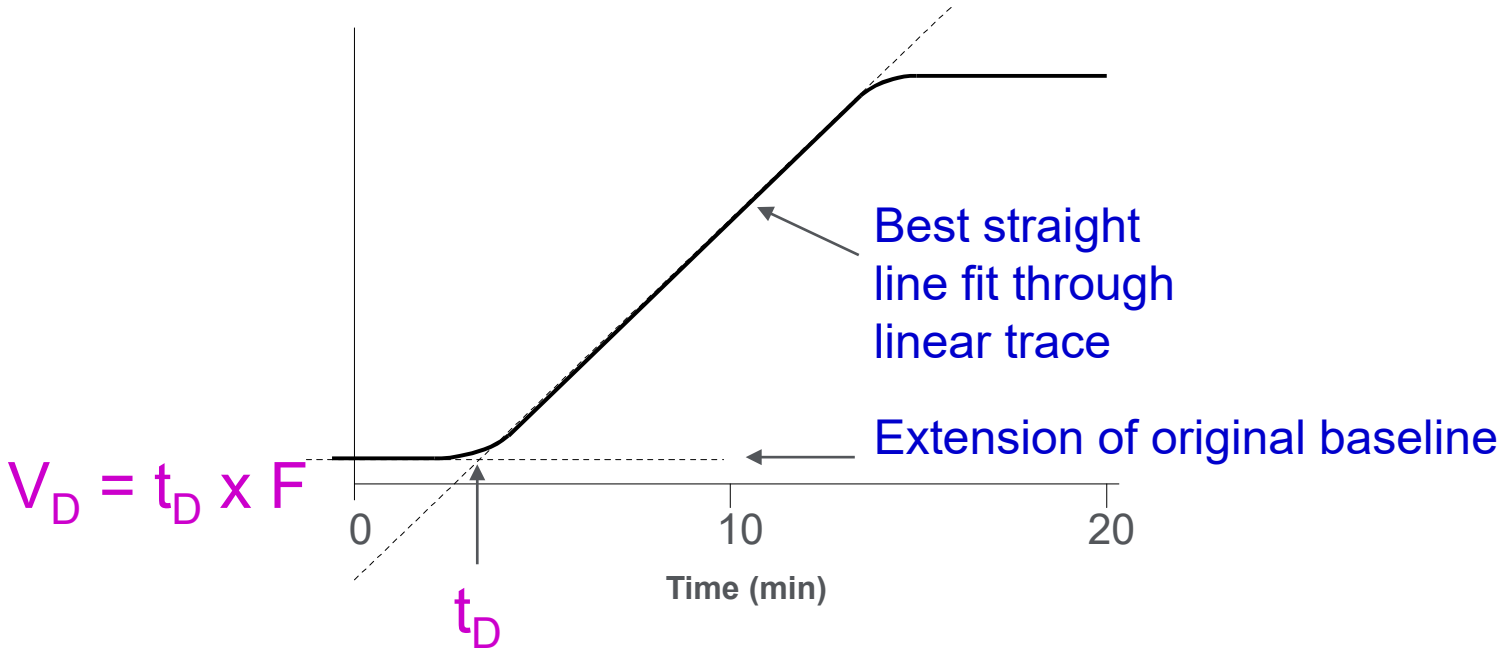
Monitor at 265 nm

Adjust attenuation so that both 100% A and 100% B are on scale

Run gradient profile 0 - 100% B/10 min at 1.0 ml/min

Record

Measuring Dwell Volume (V_D)



- Intersection of the two lines identifies dwell time (t_D)
- Dwell volume is equal to product of the flow rate and the dwell time.

Useful parts

Parts that address potential issues and help to ease your daily tasks

Part Description	Information	Part number
InfinityLab Stay Safe Caps	Prevents solvent evaporation; changes in mobile phase concentration	Various; www.agilent.com/chem/staysafecaps
InfinityLab Quick Connect and Quick Turn Fittings	with spring-load function for optimized dead volume reduction	Various; www.agilent.com/chem/InfinityLabFittings
Blank nut long 10-32	Blank nut PEEK with steel core; for system diagnostic tests; finger tight up to 1300bar, easy to use and gentle to receiving port	5043-0277
Agilent Captiva Syringe Filters	Solve issues like inlet clogging, increased backpressure, and retention time shift by filtering your samples	Various; www.agilent.com/chem/filtration
InfinityLab Poroshell 120 Columns	High efficiency and high resolution; available in 18 chemistries	Various, www.aglient.com/chem/discoverporoshell

InfinityLab Poroshell 120 Columns

InfinityLab Stay Safe Cap on solvent bottle

InfinityLab Quick Connect Fitting

InfinityLab Quick Turn Fitting

Blank nut, long, 10-32, PEEK with stainless steel core, 5043-0277