Treat Yourself to Good Chromatography

Tips and tricks

Rita Steed
LC Application Engineer
October 17, 2019

Agilent InfinityLab

Agilent

Column Related

Agilent InfinityLab

Column

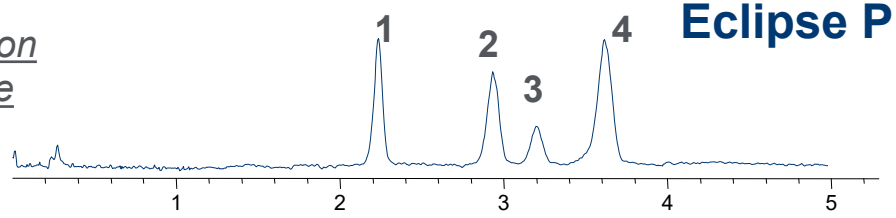
- Column Choices
- New Column
  - Save Performance Report
  - Test mix (PN 01080-68704); 65/35 ACN/H2O or MeOH
- Installation
- Appropriate conditions
- Column Lifetime
  - Conditions for bad lifetime
  - Keep record/Column History
  - Store properly
- Method parameters, flow, inj, etc.

Different ZORBAX RRHT C18 Bonded Phases

Agilent InfinityLab

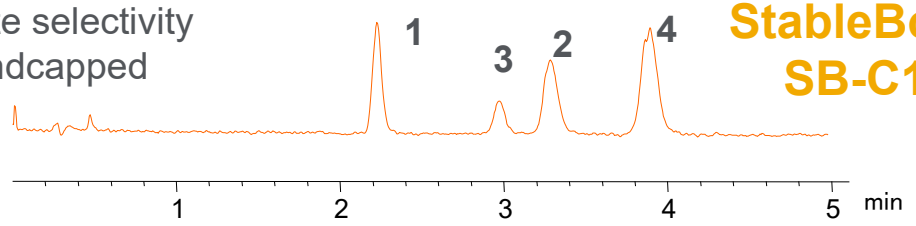
1st choice
Best Resolution & Peak Shape

Eclipse Plus C18



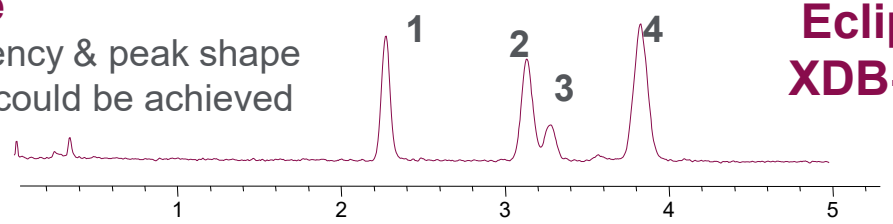
2nd choice
Good alternate selectivity due to non-endcapped

StableBond SB-C18



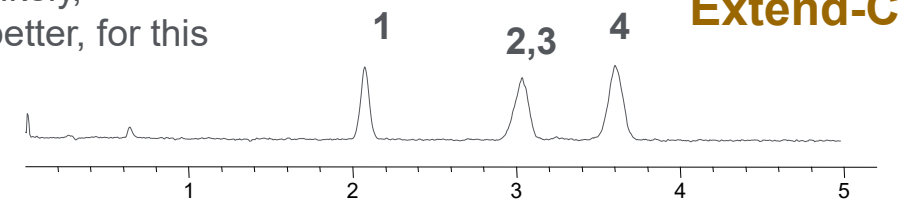
3rd choice
Good efficiency & peak shape
Resolution could be achieved

Eclipse XDB-C18



4th choice
Resolution not likely,
Other choices better, for this separation.

Extend-C18



Columns: RRHT, 4.6 x 50 mm 1.8 um
Mobile phase: (69:31) ACN: water
Flow 1.5 mL/min.
Temp: 30 °C
Detector: Single Quad ESI, pos scan mode

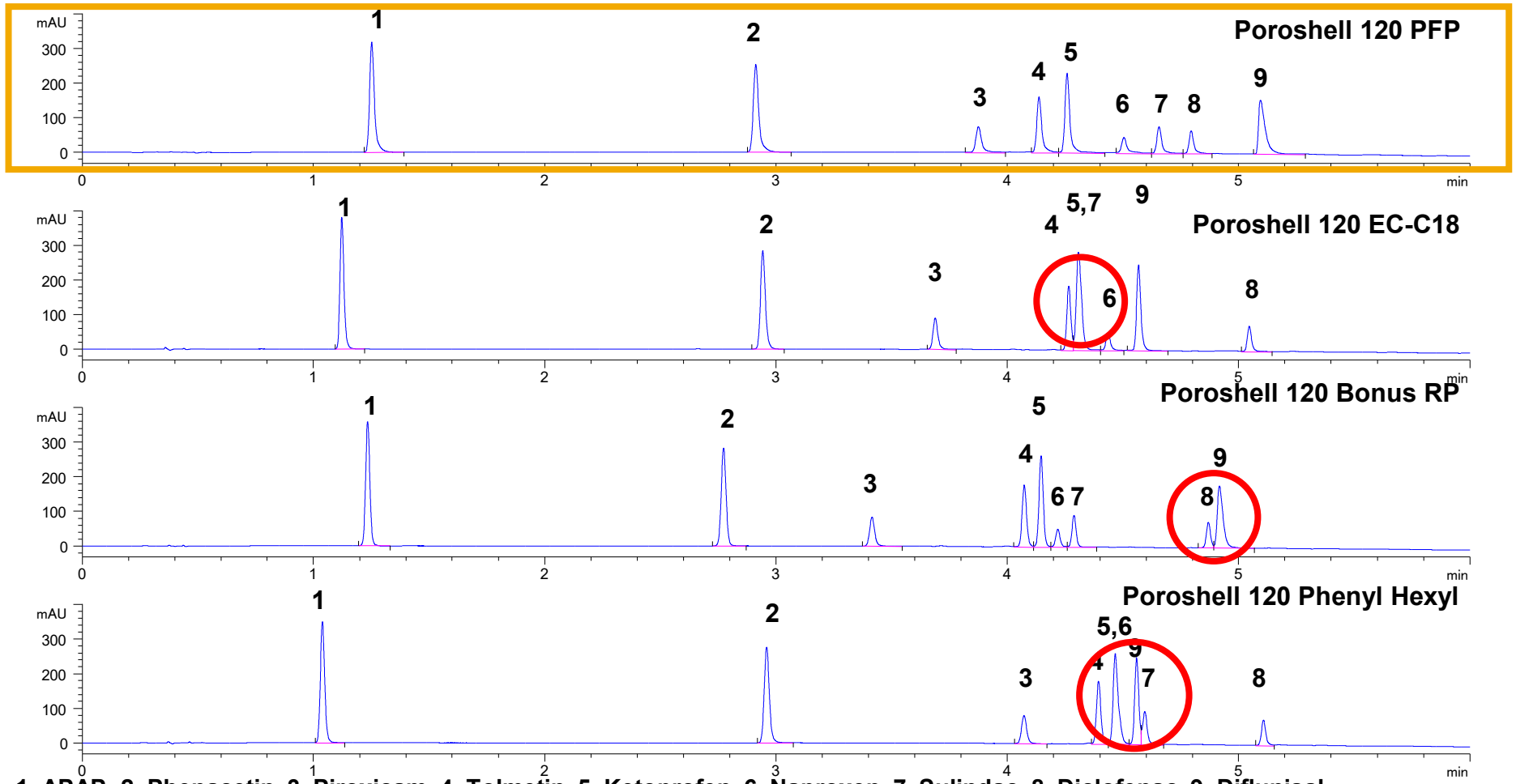
Sample:
1. anandamide (AEA)
2. Palmitoylethanolamide (PEA)
3. 2-arachinoylglycerol (2-AG)
4. Oleoylethanolamide (OEA)

Tip: Not all C18s are the same!
Trick: Test & find the best one for your application: Check out MD kits.

NSAID Separation with MeOH Gradient

Best resolution of all analytes with Poroshell 120 PFP

Time	% Organic
0	8
6	100
7	100
8	8
2 mL/min	254 nm



1. APAP, 2. Phenacetin, 3. Piroxicam, 4. Tolmetin, 5. Ketoprofen, 6. Naproxen, 7. Sulindac, 8. Diclofenac, 9. Diflunisal

Column Specifications

Agilent InfinityLab

InfinityLab Poroshell 120 column specifications

InfinityLab Poroshell Family		Pore Size	Temp. Limits	pH Range	Endcapped	Carbon Load	Surface Area
Best all around	EC-C18	120Å	60°C	2.0-8.0	Double	10%	130 m2/g
	EC-C8	120Å	60°C	2.0-8.0	Double	5%	130 m2/g
Best for low-pH mobile phases	SB-C18	120Å	90°C	1.0-8.0	No	9%	130 m2/g
	SB-C8	120Å	80°C	1.0-8.0	No	5.5%	130 m2/g
Best for high-pH mobile phases	HPH-C18	100Å	60°C	3.0-11.0	Double	Proprietary	95 m2/g
	HPH-C8	100Å	60°C	3.0-11.0	Double	Proprietary	95 m2/g
Best for basic compounds at low pH	CS-C18	120Å	60°C	2.0-8.0	Double	Proprietary	95 m2/g
Best for polar compounds (HILIC)	HILIC	120Å	60°C	0.0-8.0	N/A	N/A	130 m2/g
	HILIC-Z	120Å	80°C	3.0-11.0	Proprietary	Proprietary	130 m2/g
	HILIC-OH5	120Å	45°C	1.0-7.0	Double	Proprietary	130 m2/g
Best for alternative selectivity	Bonus-RP	120Å	60°C	2.0-9.0	Triple	9.5%	130 m2/g
	PFP	120Å	60°C	2.0-8.0	Double	5.1%	130 m2/g
	Phenyl-Hexyl	120Å	60°C	2.0-8.0	Double	9%	130 m2/g
	SB-Aq	120Å	80°C	1.0-8.0	No	Proprietary	130 m2/g
	EC-CN	120Å	60°C	2.0-8.0	Double	3.5%	130 m2/g
Best for Chiral separations	Chiral-T	120Å	45°C	2.5-7.0	Proprietary	Proprietary	130 m2/g
	Chiral-V	120Å	45°C	2.5-7.0	Proprietary	Proprietary	130 m2/g
	Chiral-CD	120Å	45°C	3.0-7.0	Proprietary	Proprietary	130 m2/g
	Chiral-CF	120Å	45°C	3.0-7.0	Proprietary	Proprietary	130 m2/g

Specifications represent typical values only

Particle	Pressure Limit
1.9 µm	1300 bar
2.7 µm	600 bar*
4 µm	600 bar

* unless otherwise noted

- Method development kits – Selectivity, pH
- Method validation kit – 3 different lots

Column Documentation

Agilent InfinityLab

Performance report

SERIAL NUMBER: USDAZ01333

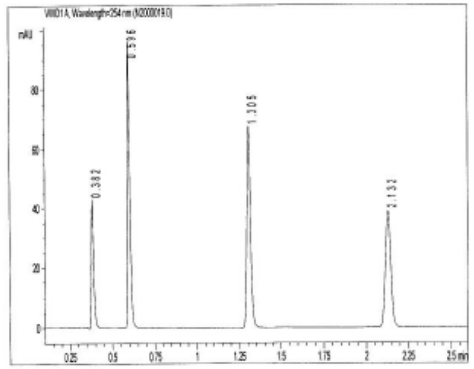
PART NUMBER: 959758-902
COLUMN TYPE: ZORBAX RRHD Eclipse Plus C18 2.1 x 100 mm, 1.8 µm
PACKING LOT #: B09089

TEST CONDITIONS

MOBILE PHASE	=	60% Acetonitrile / 40% Water
COLUMN PRESSURE	=	517.2 Bar
COLUMN FLOW	=	0.50 ml / min
LINEAR VELOCITY	=	0.436 cm / sec
TEMPERATURE	=	AMBIENT (Nominally 23 °C)
INJECTION VOLUME	=	1 µl

QUALITY CONTROL PERFORMANCE RESULTS FOR NAPHTHALENE

	TEST VALUES	SPECIFICATIONS
THEORETICAL PLATES =	22337	MIN = 21000
SELECTIVITY =	1.90	RANGE = 1.82 - 1.92
USP TAILING FACTOR = (@ 5% Peak Height)	1.08	RANGE = 0.98 - 1.20
k' =	4.58	



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

Data sheet or column guide

This booklet provides general information for all ZORBAX, Poroshell, Pursuit, and Polaris reversed-phase columns.

For additional detailed information about your specific phase or family, see:
agilent.com/chem/columnchoices

Getting Started

A QC Column Performance Report, including a test chromatogram, is enclosed with every Agilent column. The QC test system has been modified from a standard system to minimize system dead volume, so it may vary from the system used in your lab. This allows a better evaluation of the column and assures a more consistent product. A properly configured LC system will generate similar results to the chromatogram on your QC Performance Report.

Modern columns are robust and are designed to operate for long periods under normal chromatographic conditions. You can maximize column performance by running it within specifications. Always review the specifications before putting in place a final method.

Manufacturing test chromatogram is done on a modified LC system to minimize ECV and will differ from a typical lab HPLC

- Don't expect to get exactly the same result as the performance report
- Test column performance on your instrument to have as a reference

Column Documentation – Benchmark

Agilent InfinityLab

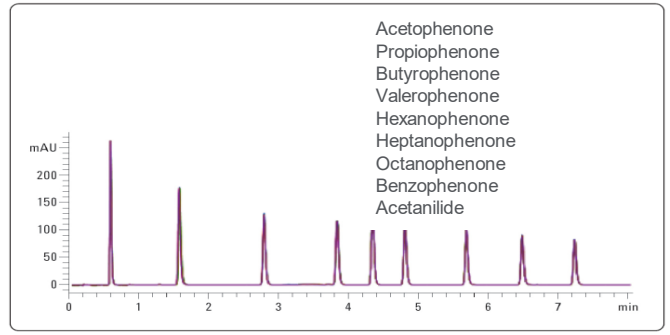
Benchmark new column on your system

1. Test mixes
   1) Isocratic Standard (01080-68704)
   2) Checkout sample (5188-6529)
   3) QC reference material
2. Criteria like retention time, peak area, peak tailing, resolution, response, system pressure, etc.
3. Theoretical plates
   - Monitor column over time
   - Troubleshoot

Trick: Know what your column looks like on your instrument when it's new.

Chromatographic conditions

Sample:	RRLC Checkout sample (p/n 5188-6529)
Column:	Agilent Poroshell 120 EC C18, 3 mm × 50 mm, 2.7 µm
Mobile phase:	A = Water B = Acetonitrile
Gradient:	0 min 20% B 8 min 80% B
Flow rate:	1.2 mL/min
Stop time:	8 min
Post time:	4 min
Injection volume:	1 µL
Column temperature:	30 °C
DAD:	245/10 nm Ref 400/100 nm
Flow cell:	10 mm
Peak width:	<0.025 min (10 Hz)



Sample	Isocratic standard sample (Agilent part number 01080-68704)*
Mobile phase A	water = 35 %
Mobile phase B	acetonitrile = 65 %
Flow rate	1.5 ml/min
Detection wavelength	210 nm
Stop time	6 min
Post time	1 min
Column compartment temperature	36 °C
Gradient	start with 65 % B go to 95 % B in 4 min go to 65 % B in 5 min
Injection volume	5 µl

*Isocratic standard sample contains 0.15 wt.% diethylphthalate, 0.15 wt.% diethylphthalate, 0.01 wt.% biphenyl and 0.03 wt.% o-terphenyl in methanol.

Column Installation Recommendations

Agilent InfinityLab

1. Purge the pumps (connections up to the column) of any buffered mobile phases. Flush at least 5 mL of solvent before attaching the column to instrument.

   Goal: Eliminate any dried out or precipitated buffer from the system so it doesn't wash onto the column and plug the frit.

2. Flush your new column with your mobile phase (compatible with the solvents the column was shipped in) at an appropriate flow rate – start slowly at 0.1 mL/min for a 2.1 mm id column, 0.2 mL/min for a 3.0 mm id column, and 0.4 mL/min for 4.6 mm id.

   Goal: Avoid a pressure spike when the new mobile phase reaches the column. This occurs when the different solvents mix. The low flow rate allows this to happen without causing an unanticipated pressure change.

3. Increase the flow rate to the desired flow over a couple of minutes.

   Goal: Reach final operating pressure

4. Once the pressure has stabilized, attach column to detector

5. Equilibrate column and detector with 10 column volumes of mobile phase prior to use

   Goal: Reproducible chromatography from the start

Initial Column and System Equilibration*

Using Buffers Successfully

Agilent InfinityLab

Insure HPLC has required mobile phase components

In appropriate vessel, test highest % organic/buffer ratio

- Verify buffer will not precipitate. With stirring, add organic to buffer first, not vice versa

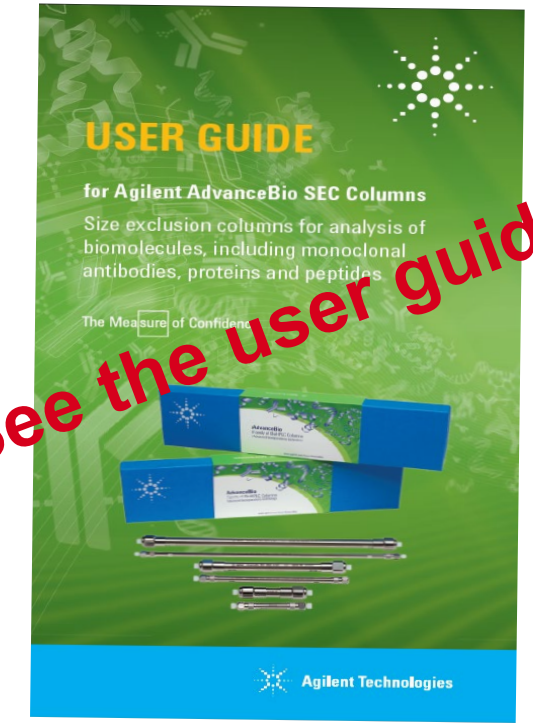
Install column – Make a good connection

Equilibrate column with, in order:

- 100% organic modifier (if brand new)
- Mobile phase minus buffer
- Buffered MP w/highest % organic modifier (gradient)
- Buffered MP w/lowest % organic modifier (gradient)

Inject standard or sample several times until RTs stable

- For gradient, precede former with 1-3 blank gradients



See the user guide!

*Check appendix for shutdown instructions

LC Columns Are Not Indestructible

Agilent InfinityLab

Columns are packed using hydraulic pressure and can be damaged by it.

Silica dissolves (slowly)… higher pH

Acid hydrolysis of bonded phase at low pH

Column failure

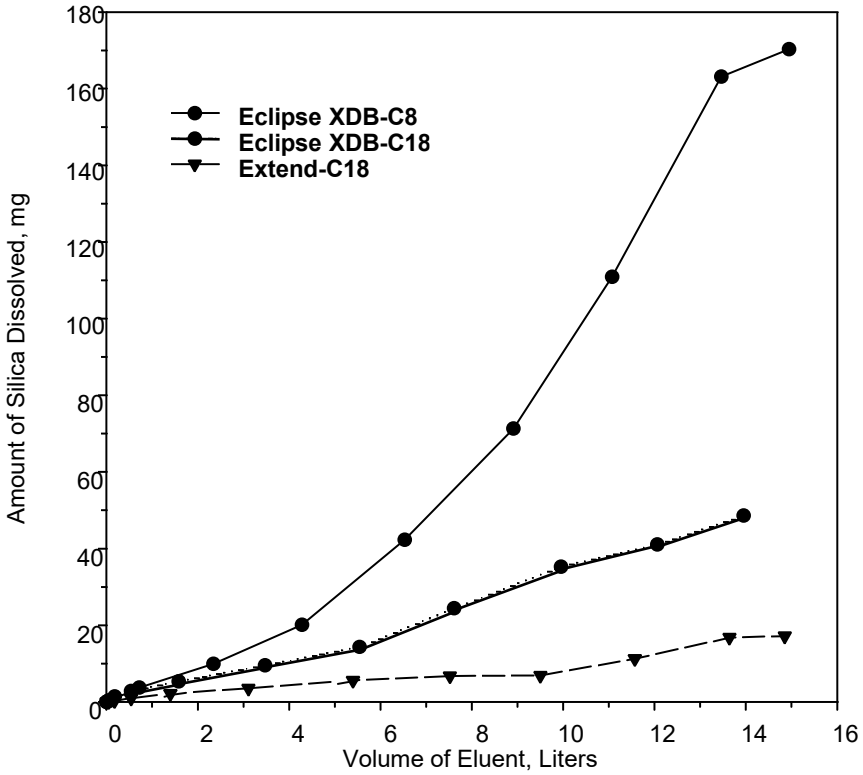
- Void
- Contamination

Columns must be stored properly

- Check your user guide

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

Tip: Keep record/history of your column



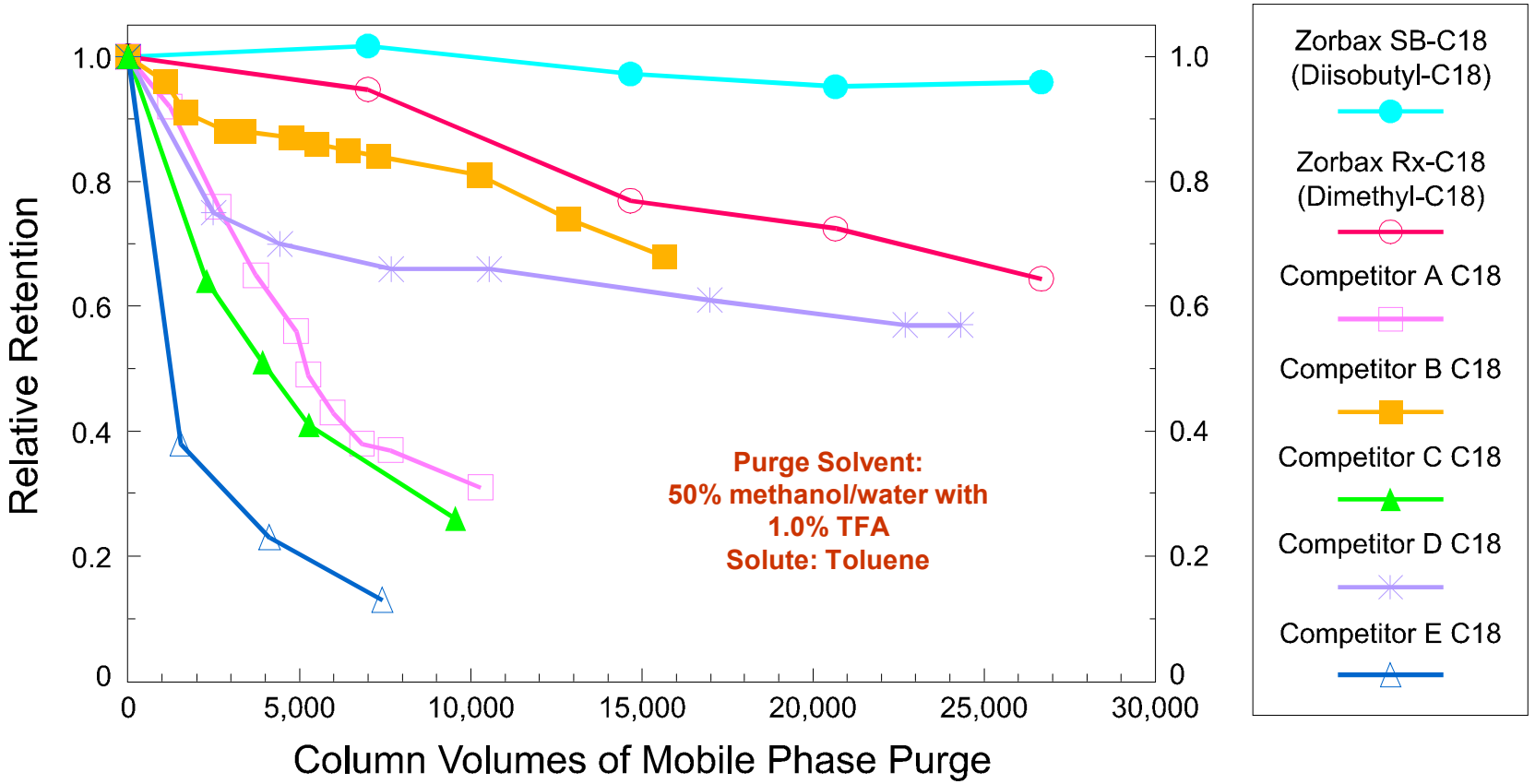
Columns:	4.6 x 150 mm, 5 µm
Purge:	50% ACN / 50% 0.02 M K_2HPO_4, pH 11
Flow Rate:	1.5 mL / min
Temperature:	25°C
Detection:	Silicate concentration by silicomolybdate color reaction

LC Columns Are Not Indestructible

For maximum column lifetime, match column to pH of mobile phase

Agilent InfinityLab

low pH and high temperature (pH 0.8, 90°C)



Kirkland, J.J. and J.W. Henderson, Journal of Chromatographic Science, 32 (1994) 473-480.

You Have Your Column, What's Next?

Agilent InfinityLab

Method conditions

- Flow rate
- Load
- Temperature
- Retention

What's in your mobile phase?

- Organic
- Buffers
  - pH
  - Tables
- How to make

Tip: For good chromatography the trick is to match your conditions to your column choice.

Column ID; Match Conditions

Agilent InfinityLab

Column id	Column volume	Peak volume, k=1	Typical injection volume.*	Typical injection volume range	Flow rate for equivalent ν**
4.6 mm	1500 µL	148 µL	20 µL	5–50 µL	1.0 mL/min
3.0 mm	640 µL	44 µL	10 µL	3–30 µL	0.42 mL/min
2.1 mm	320 µL	22 µL	2 µL	0.5–15 µL	0.21 mL/min
1.0 mm	70 µL	4 µL	0.5 µL	0.1–3 µL	47 µL/min
0.5 mm	15 µL	1 µL	150 nL	40–500 nL	12 µL/min
0.3 mm	6 µL	0.3 µL	50 nL	15–250 nL	4.2 µL/min
0.1 mm	700 nL	32 nL	10 nL	1–10 nL	472 nL/min
0.075 mm	400 nL	18 nL	2 nL	0.5–5 nL	266 nL/min

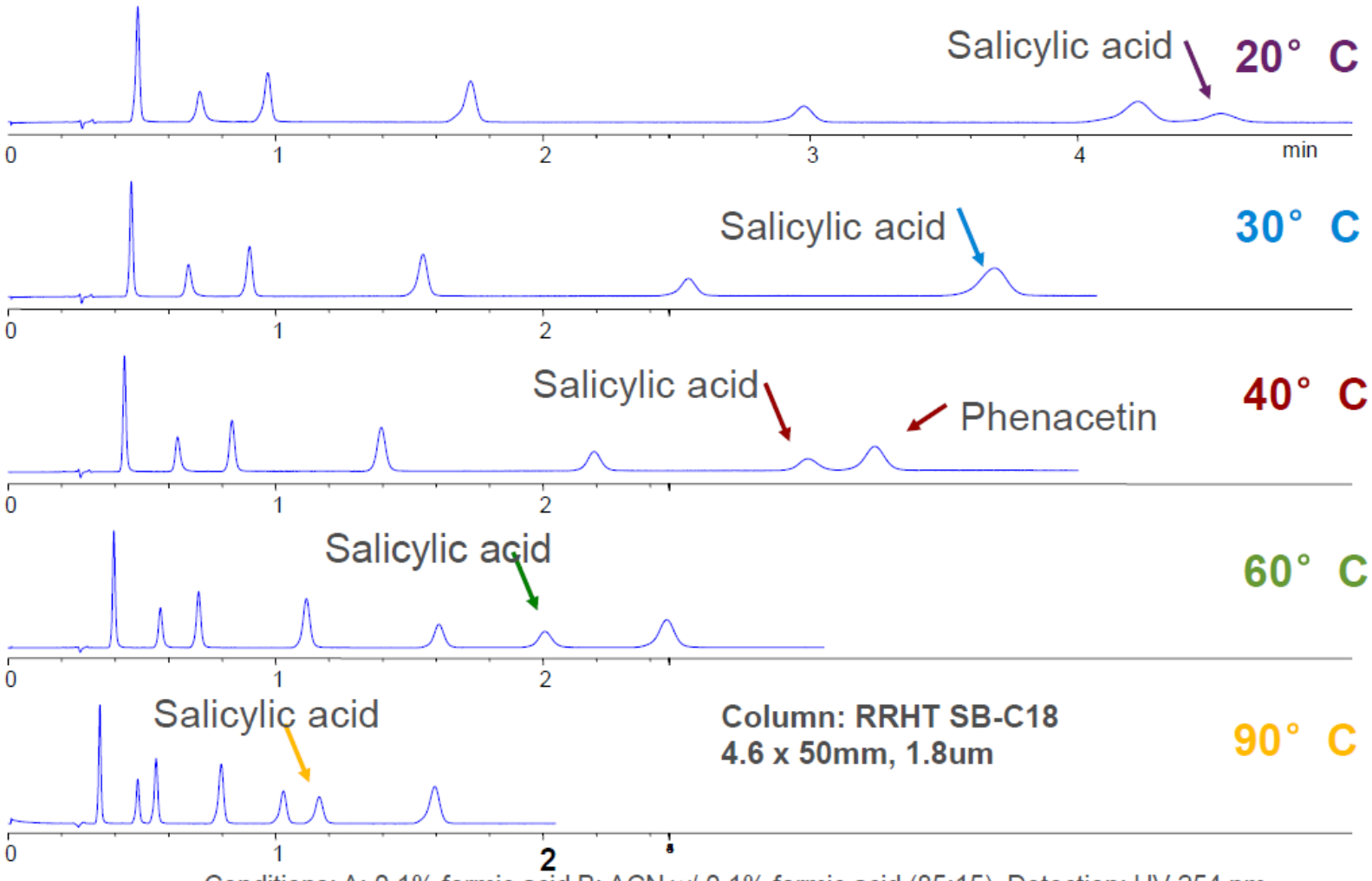
Column length = 150 mm, N =13,000

Tips

** Typical injection volume = 10–30% of peak volume of first eluting peak*

*** Maintain equivalent mobile phase linear velocity when scaling column diameter*

Effect of Temperature on Separation



Conditions: A: 0.1% formic acid B: ACN w/ 0.1% formic acid (85:15) Detection: UV 254 nm

Separation Conditions That Cause Changes in Retention*

Condition	Change	Retention	Change
Flow rate	+/- 1%	t_R	+/- 1%
Temperature	+/- 1 °C	t_R	+/- 1 to 2%
% Organic	+/- 1%	t_R	+/- 5 to 10%
pH	+/- 0.01%	t_R	+/- 0 to 1%

*excerpted from “Troubleshooting HPLC Systems”, J. W. Dolan and L. R. Snyder, p 442.

Tip: Minor changes in conditions can affect retention

Your Mobile Phase

- What's in your mobile phase?
  - Organic
  - Buffers
    - pH
    - Tables
- How to make
- Equilibration
- Shutdown

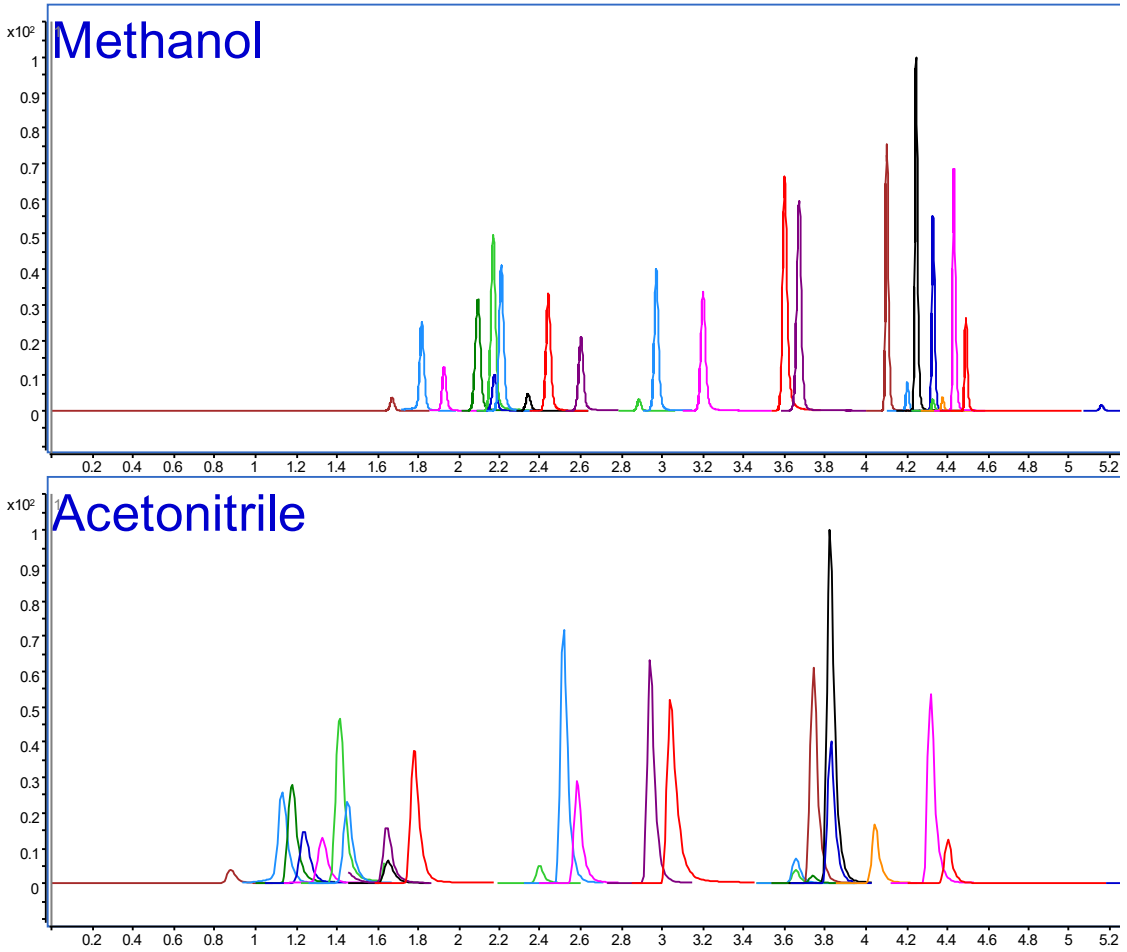
Comparison of 25 Component Mixture using Methanol or Acetonitrile as the Mobile Phase

InfinityLab Poroshell 120 EC-C18 - good 1st choice

Organic choice considerations:

- Selectivity differences
- Methanol –
  - Higher pressure
  - In general, better peak shape with bases
  - Generally more miscible
  - Protic solvent
  - Weaker than ACN
- Acetonitrile
  - Lower pressure
  - Aprotic solvent
  - Wider UV window than MeOH

Tip: Consider a blend



Mobile Phase pH and Buffers

Why are they important in HPLC?

Agilent InfinityLab

pH

- Silica surface of column
- Sample components of interest

Buffers

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

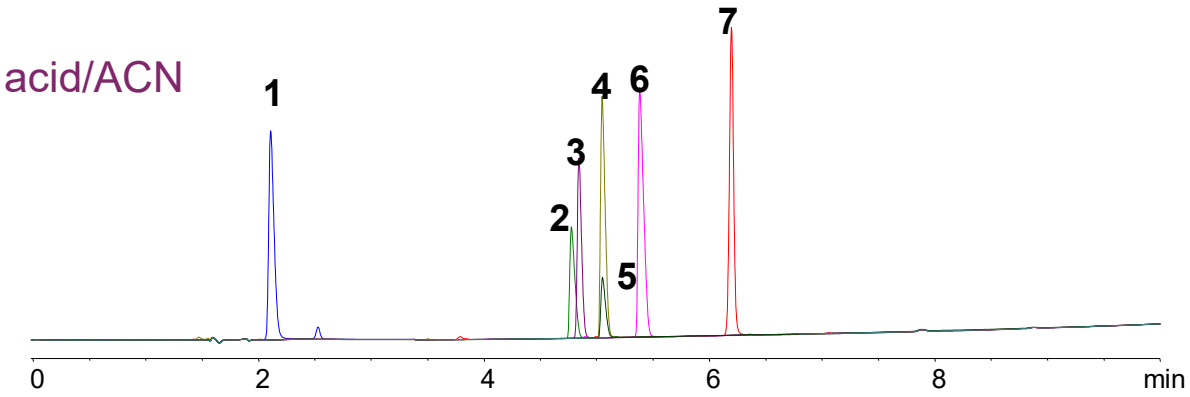
Column lifetime

- Low pH strips bonded phase
- High pH dissolves silica

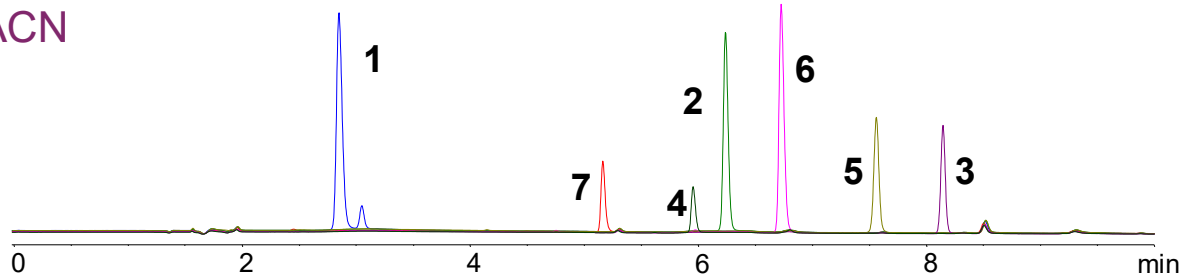
Selectivity and Resolution Can Change with pH

Agilent InfinityLab

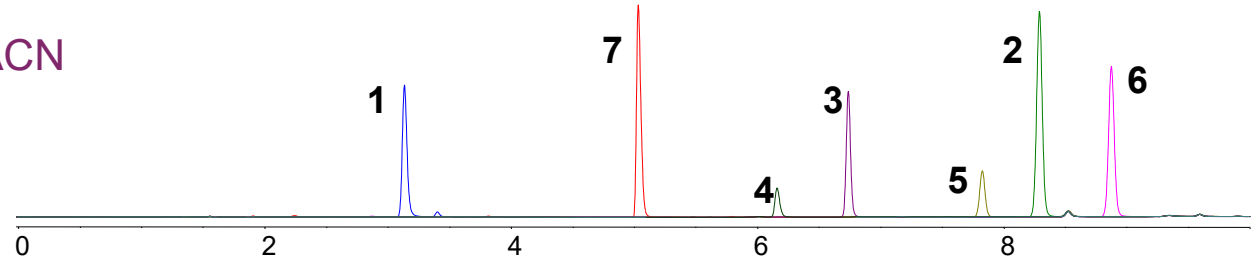
pH 2.7 0.1% Formic acid/ACN



pH 4.8 NH_4AC/ACN



pH 7 $NaPO_4$/ACN



1. procainamide
2. buspirone
3. pioglitazone
4. eletriptan
5. dipyridamole
6. diltiazem
7. furosemide

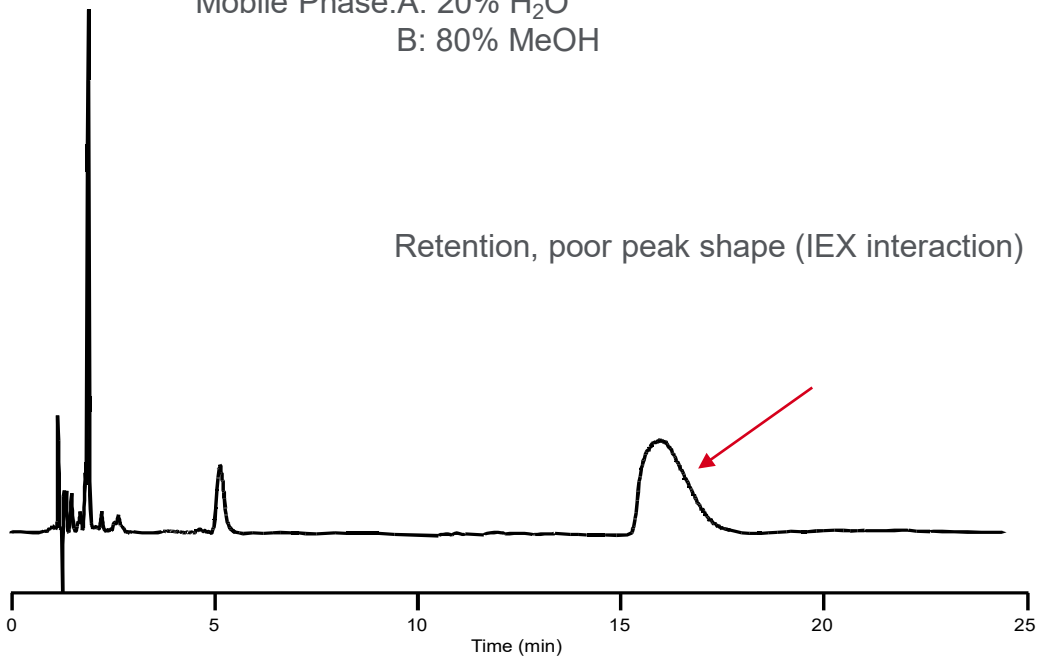
Conditions: Column: Eclipse Plus C18 4.6 x 100mm, 5um Gradient: 10 – 90% in 10 minutes Detection: UV 254 nm

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

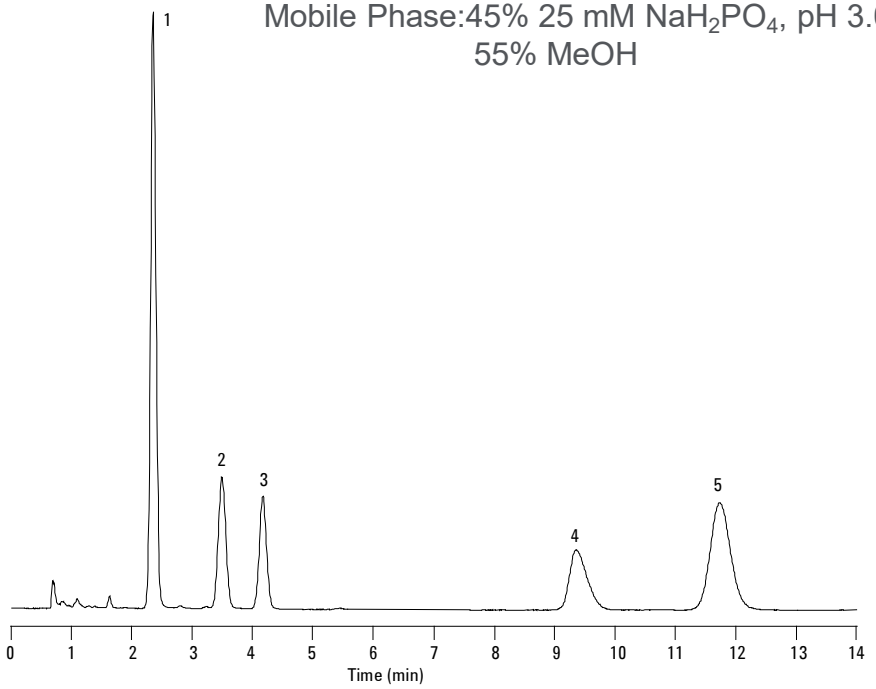
Agilent InfinityLab

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

Buffer Preparation
Does It Make a Difference?

Agilent InfinityLab

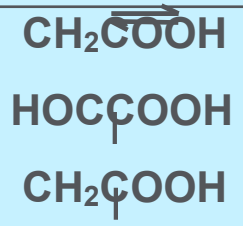
1. Dissolve salt in water using a 1 L or 2 L beaker. Use appropriate volume to leave space for pH adjustment. Equilibrate to RT for maximum accuracy.
2. Calibrate pH meter. Use 2-level calibration & bracket desired pH. Use appropriate audit solution to monitor statistical control (e.g., potassium hydrogen 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.

Tip: Filtering is important - small particles in MP can permanently block capillaries in degasser.

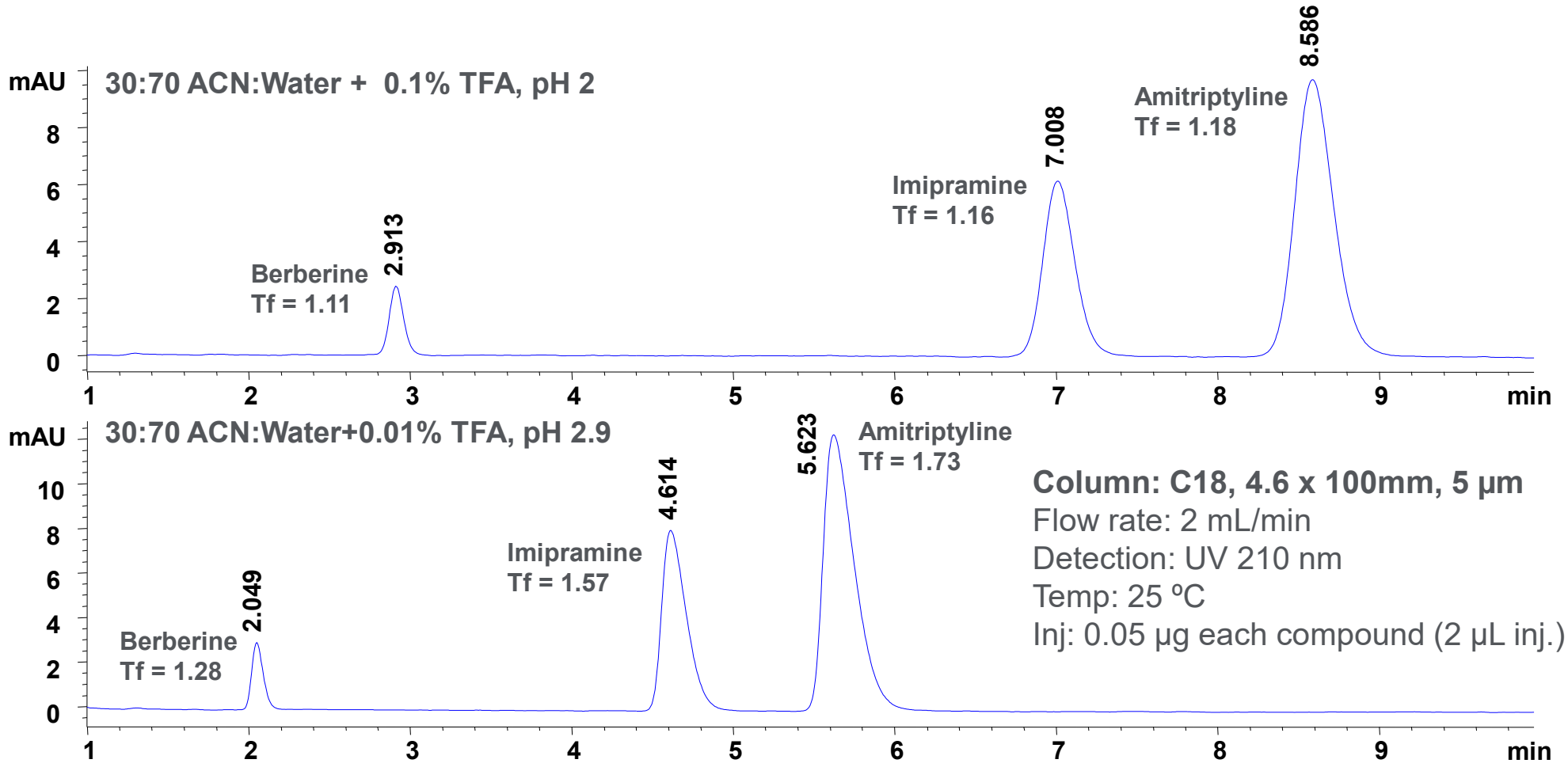
Buffer Options

Agilent InfinityLab

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



Tip: Volatile – It's definition is evaporating rapidly, passing off rapidly in the form of vapor…

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

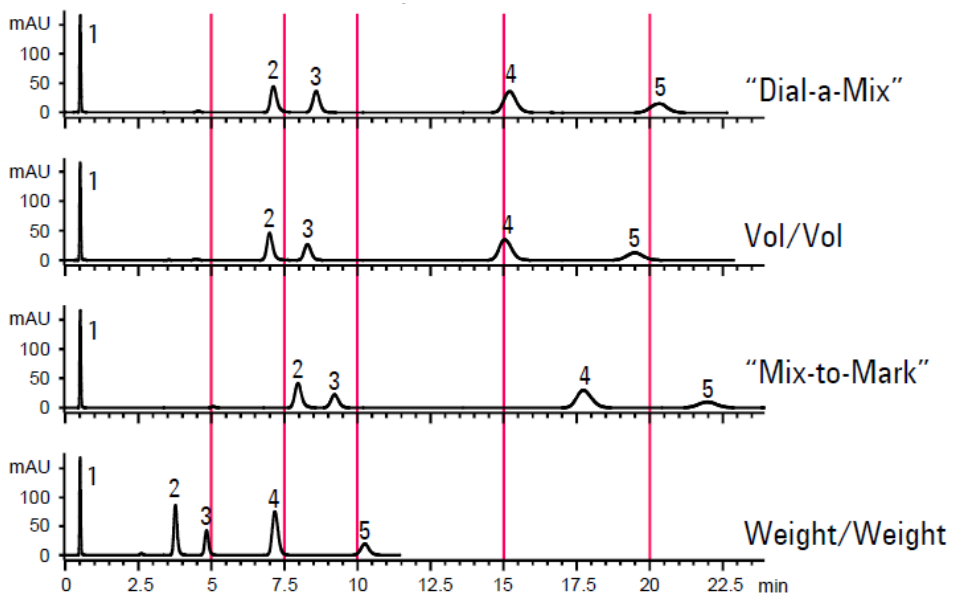
- Relative quantities of each affects degree of contraction
- Temperature

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.

Mobile Phase Preparation

Effect on chromatography



HPLC System:	Agilent 1100 with quaternary pump
Column:	ZORBAX Eclipse XDB-C8 Rapid-Resolution (3.5µm), 4.6 x 50 mm Agilent Part No. 935967-906
Mobile Phases:	Dial-a-Mix= A: water B: MeOH, pump 50% B Vol/Vol=250 mL water + 250 mL MeOH, pump 100% Mix-to-Mark = 250 mL MeOH, fill to 500 mL with water, pump 100% Premixed (w/w) = 200 g MeOH + 200 g water, pump 100%
Detection:	UV 254 nm
Flow:	1 mL/ min.
Temperature:	ambient

1. Uracil
2. Butylparaben
3. Naphthalene
4. Dipropylphthalate
5. Acenaphthene

Method used to prepare mobile phase can significantly affect elution pattern

Trick: Avoid this potential pitfall by being consistent.

Tip: W/w is more accurate than v/v.

Effect of Mobile Phase Preparation on Chromatography, Pub. No. 5988-6476EN

Consider Your Sample

Agilent InfinityLab

Type

- Ionizable?
- Compound type; acid, base, neutral

Solubility in mobile phase

- Test
  - If it's not aqueous soluble and your starting mobile phase is 90%H2O:10%ACN, precipitation likely on inlet frit of your column

Size/MW

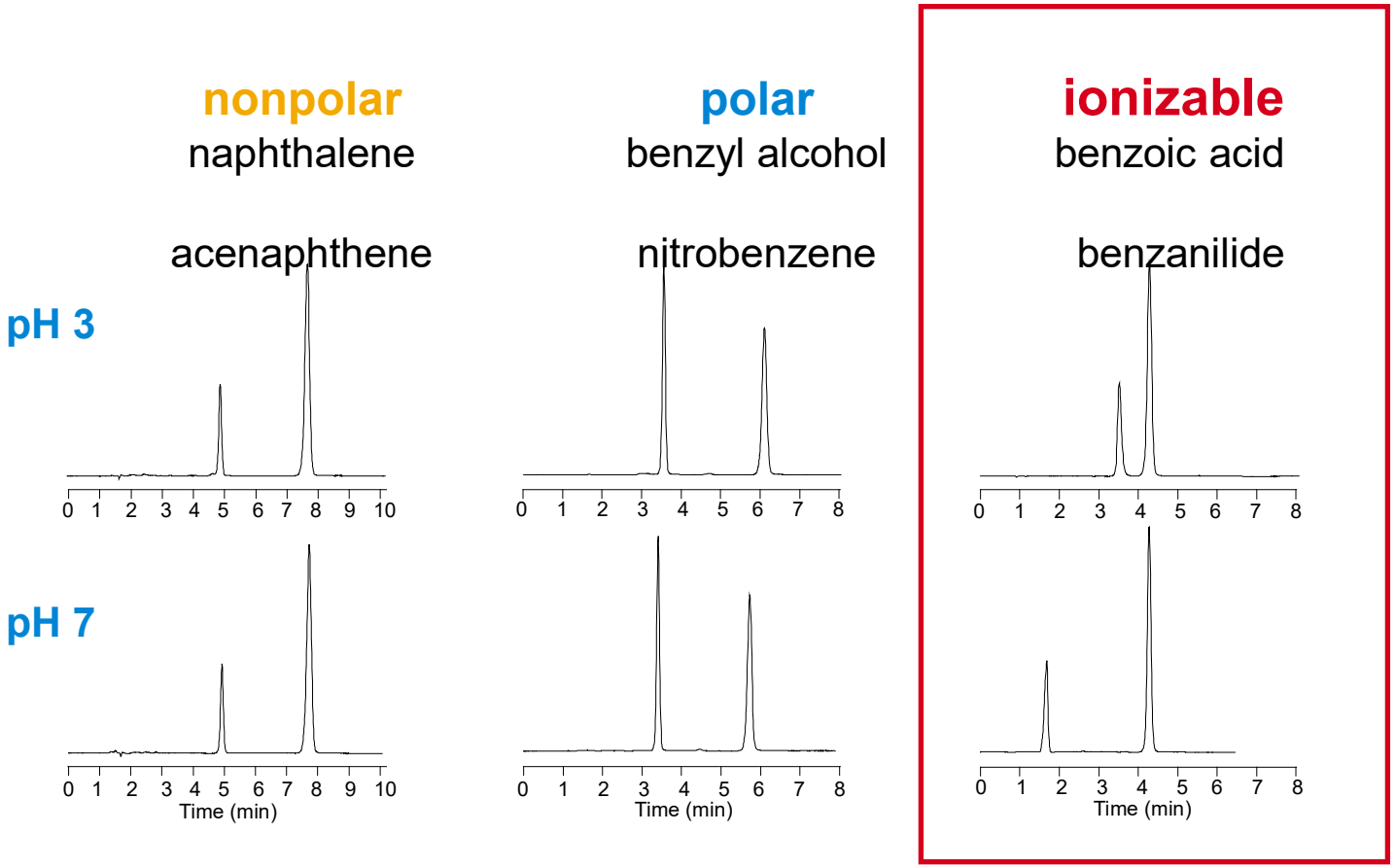
- Pore size

My sample is clean – or is it?

Is RP the best choice?

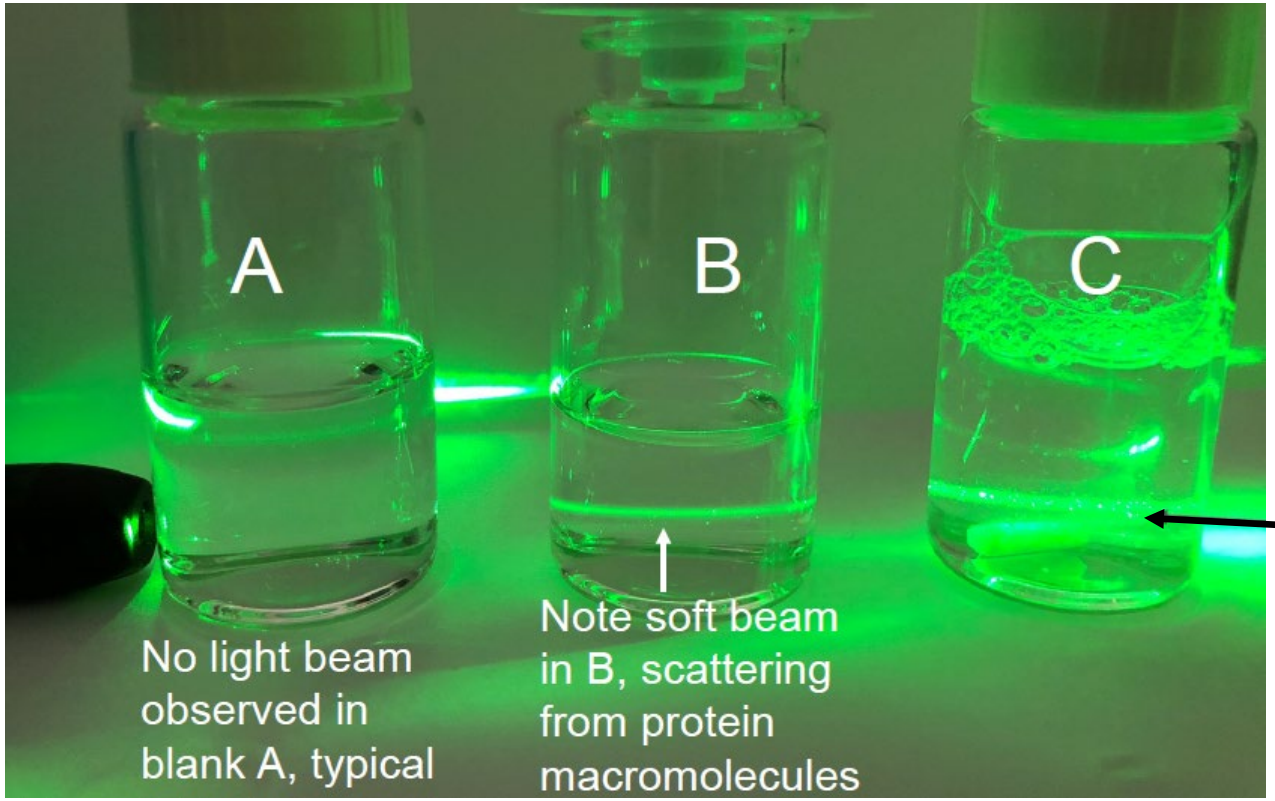
When Does pH Affect Resolution?

Compound type comparison



Tip: Ionizable compounds (acids and bases) can change retention and selectivity with changes in pH

I Don’t Have to Filter, My Sample Is Clean or… Is It?



A. PBS used as diluent

B. 2mg/ml egg albumin after GF filtration
   1) GF = glass over regen. cellulose

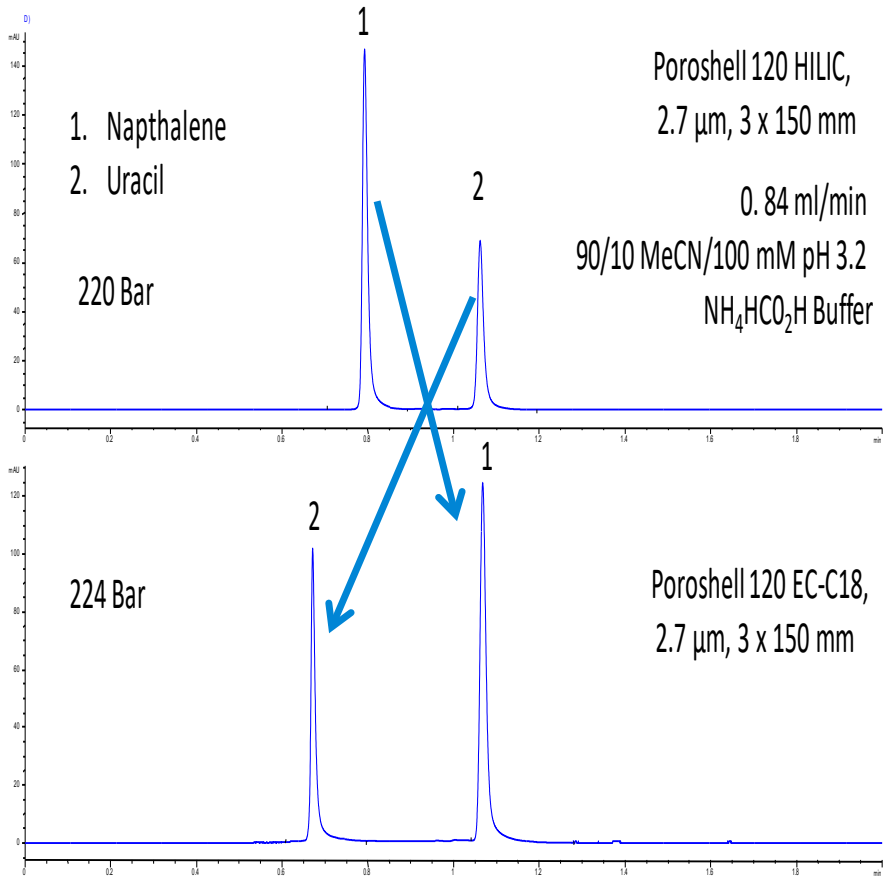
C. 2mg/ml egg albumin chilled overnight

Bright “sparkles” = insoluble debris and agglomerated proteins

CAUTION: Use appropriate safety precautions when using laser pointer for sample inspection!

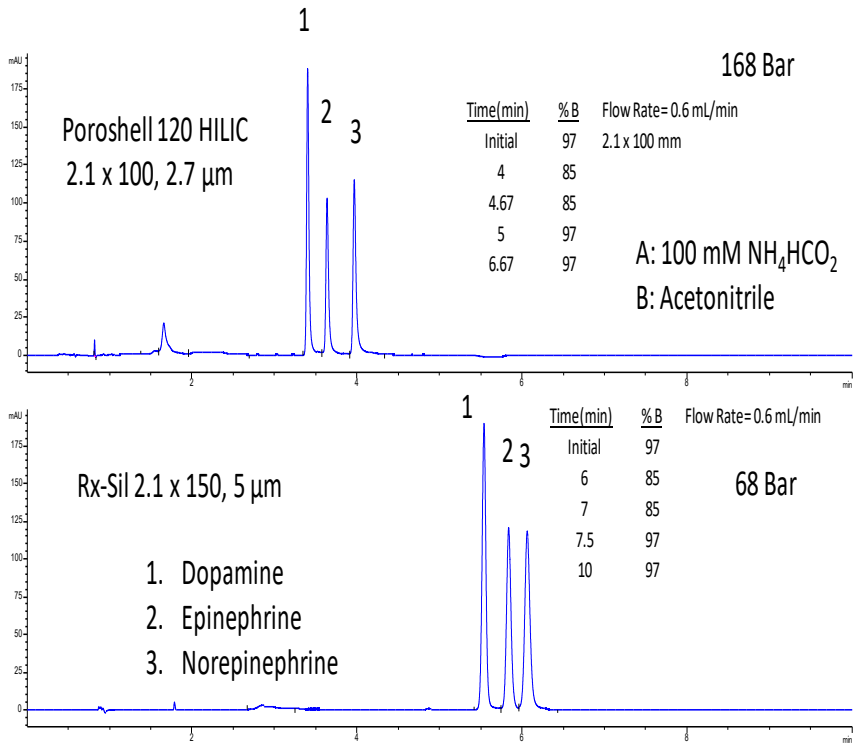
Trick: Use a laser pointer to check sample and blank quality.

Is Reversed Phase the Right Choice?



Overlay of HILIC Separation of Catecholamines

(Poroshell 120 2.1 x 100, 2.7 µm), (Rx-Sil 2.1 x 150, 5 µm)



"Hydrophobic Interaction Chromatography (HILIC) using Agilent Poroshell 120 HILIC"
William J. Long, Anne E. Mack, October 5, 2012, 5991-1242EN

Instrument

HPLC or UHPLC

- Pressure
- Particle size
- Column dimension

Dwell volume

Extra column volume

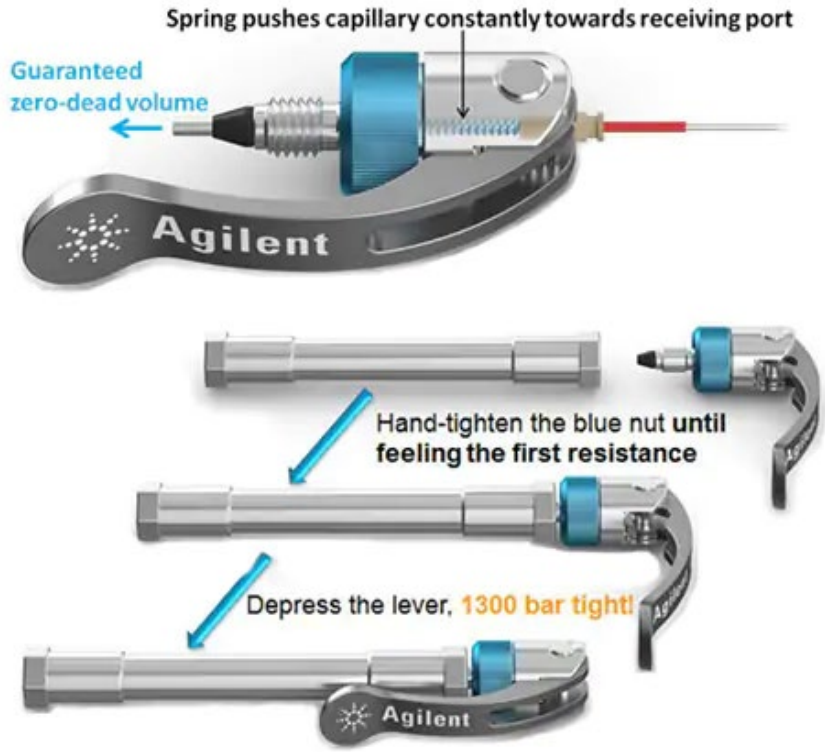
Column oven

Connections

- Use shortest length of tubing possible
- Quick connect and quick turn

Do you need an inline filter or guard column?

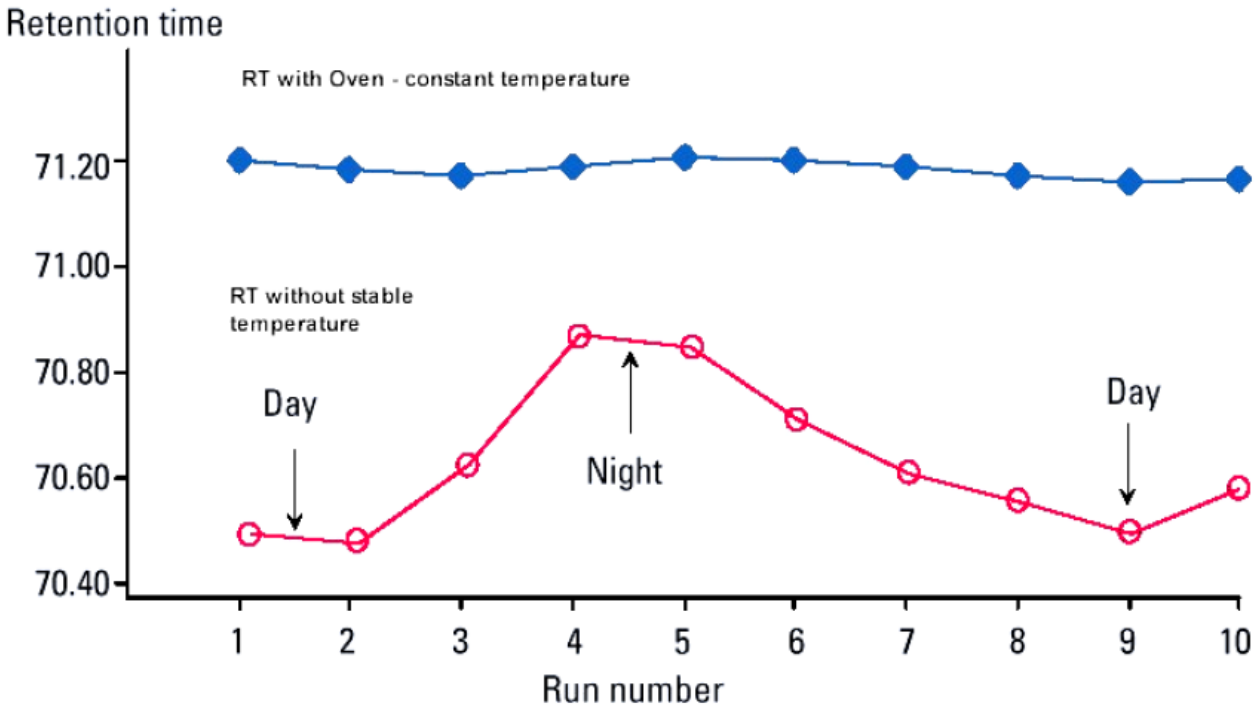
InfinityLab Quick Connect fitting



InfinityLab Quick Turn fitting

Tip: Attend upcoming webinar, November 17th @1 EST: *All About LC Connections – The Importance of Making a Great One*

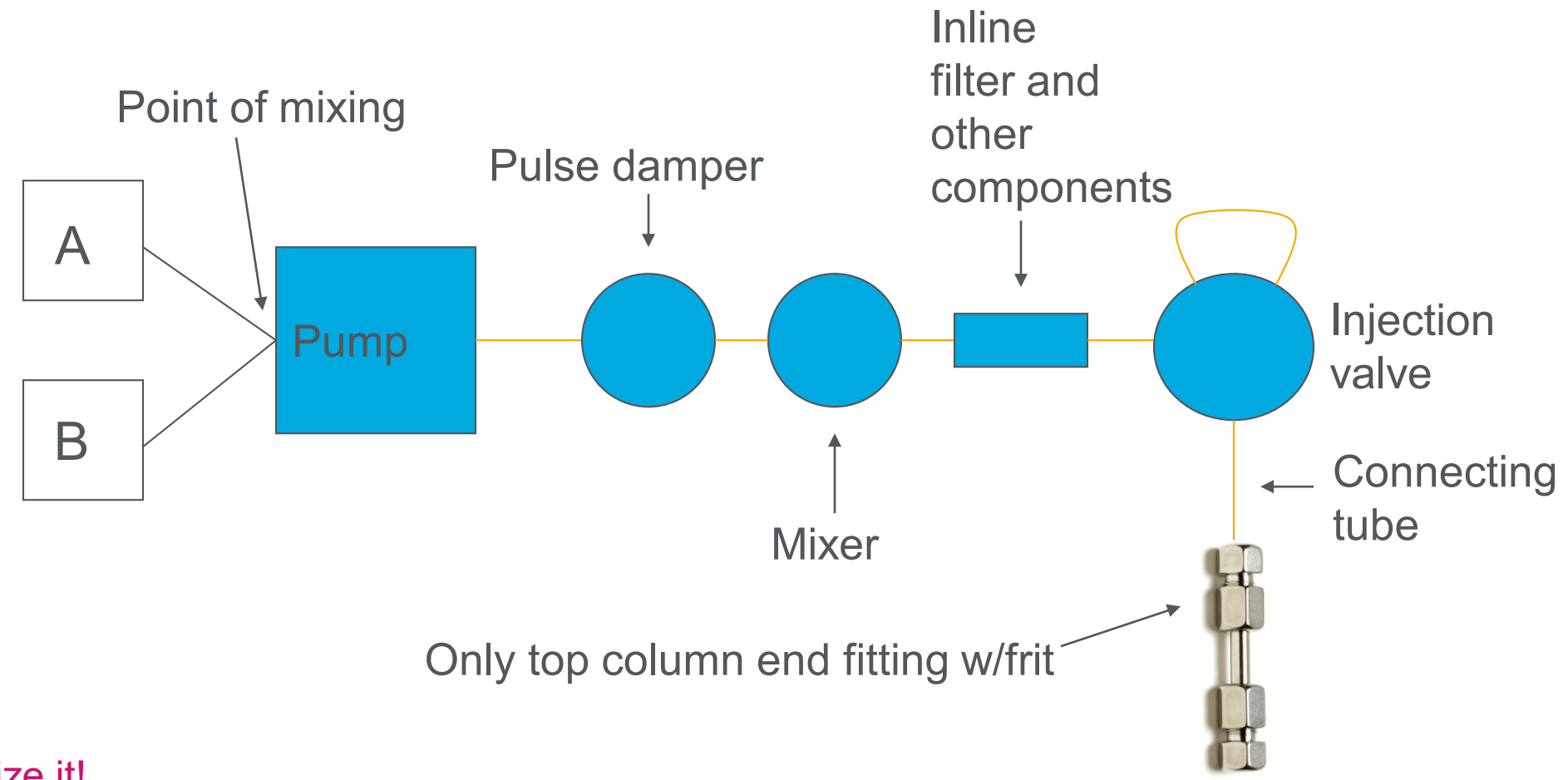
Column Oven
Control the Temperature



Tip: Constant temperature = constant retention

Dwell Volume of HPLC System with Low Pressure Mixing

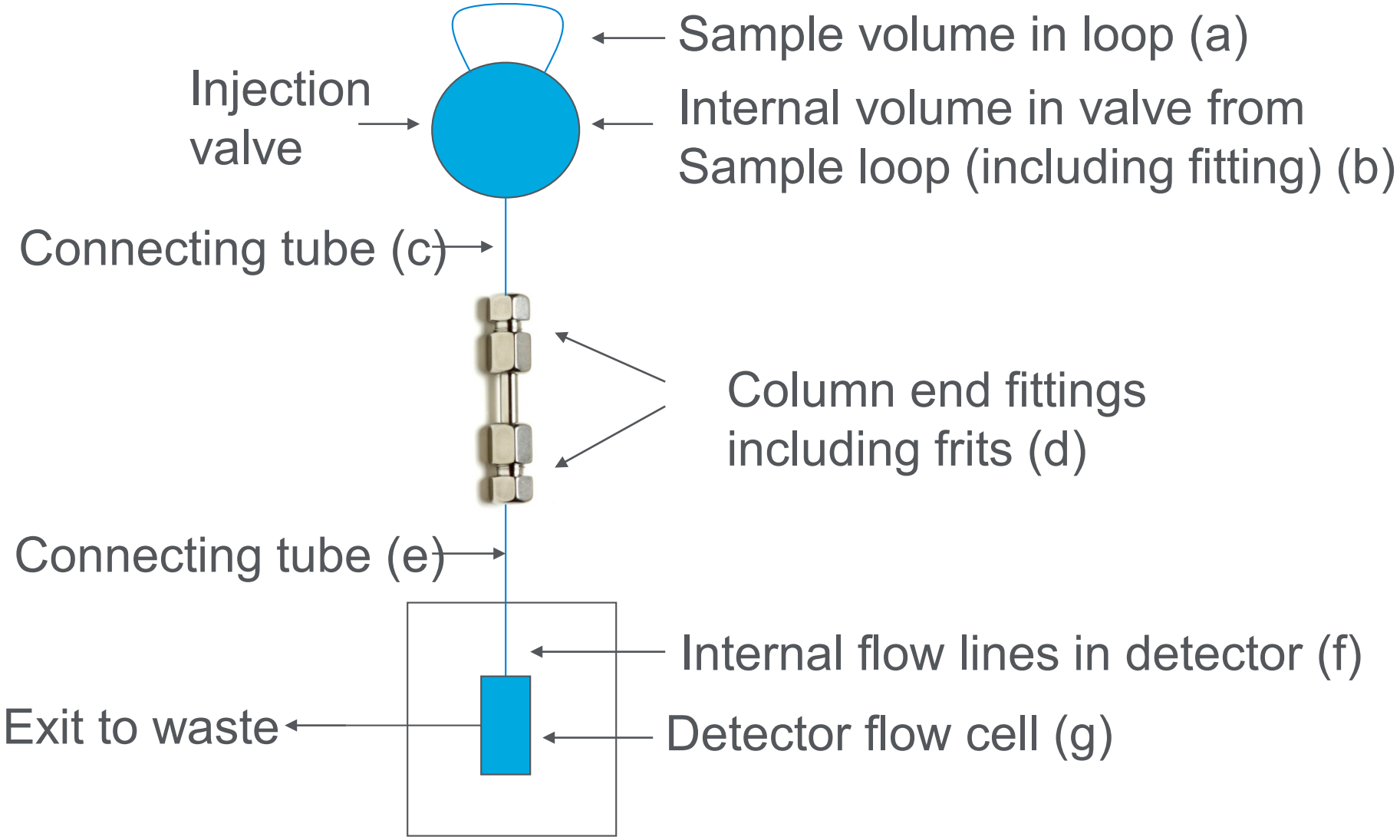
aka Gradient Delay Volume



Trick: Minimize it!

Tip: Check the appendix for instructions on determining your system's dwell volume and document it.

Extracolumn Volumes in HPLC Sample Flow System



Tip: Effect of ECV – “Throw away plates!”

Column Protection

Inline Filters

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

UHPLC options

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

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

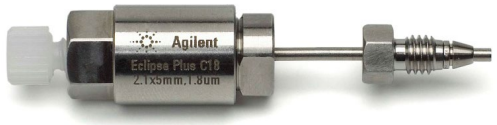
1290 Infinity II, 0.3µm, max 1300 bar
- 5067-6189, replacement frits 5023-0271

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 vs. benefit

Remember Your

Agilent InfinityLab

Column Choice

Method conditions

Mobile phase

Sample

Instrument

… all contribute to good chromatography. The more you know about each of them and how they can affect your chromatography, the better your chances of success are.

Exclusive Offer for Attending Agilent Chemistries Webinars

Agilent InfinityLab

Receive a 25% discount off any Agilent J&W GC Columns, Agilent LC Columns, Sample Prep Products, Chemical Standards and your most often used GC and LC supplies*

- Discount applies on future purchase of up to $3,000 (USD)
- Offer good 30 days from the Agilent webinar
- Use promotion code 9969

Some restrictions apply on supplies

Contact Agilent Chemistries and Supplies Technical Support

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

- Option 1 for GC/GCMS Columns and Supplies
- Option 2 for LC/LCMS Columns and Supplies
- Option 3 for Sample Preparation, Filtration and QuEChERS
- Option 4 for Spectroscopy Supplies
- Option 5 for Chemical Standards

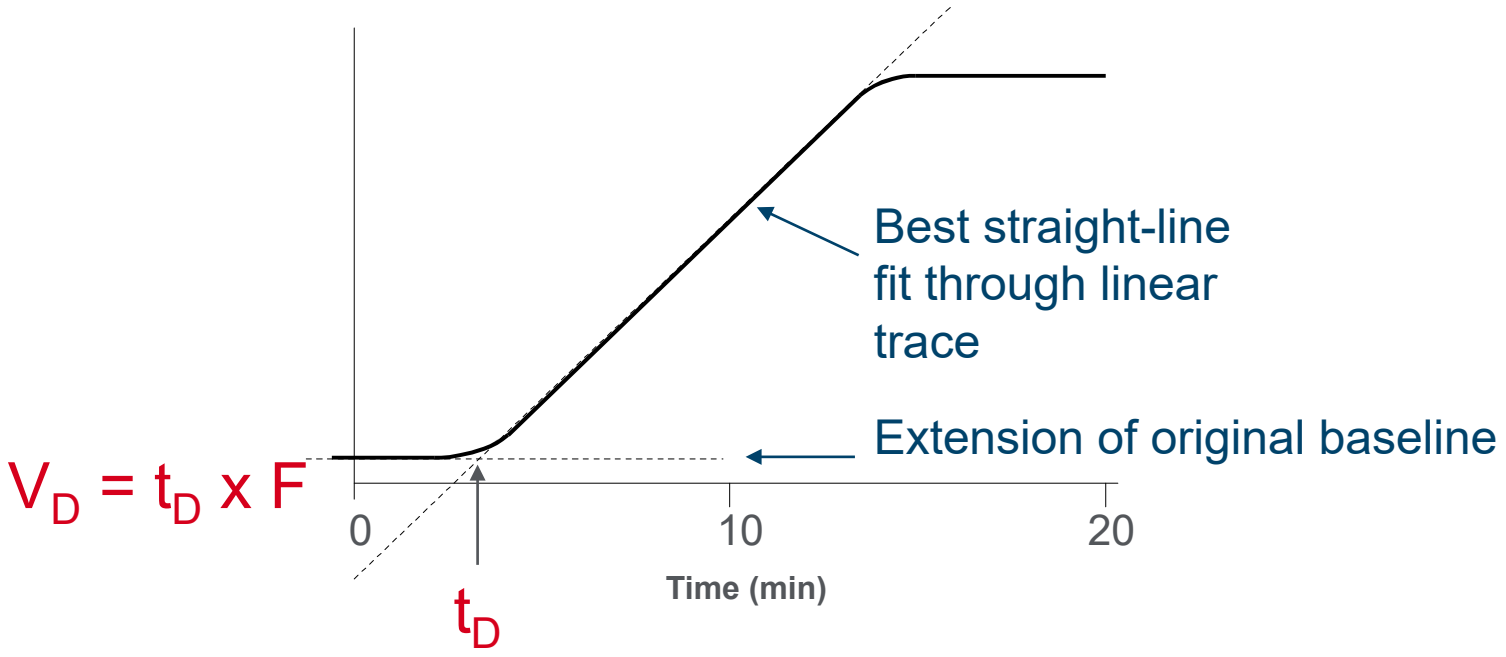
- gc-column-support@Agilent.com
- lc-column-support@agilent.com
- spp-support@agilent.com
- spectro-supplies-support@agilent.com
- chem-standards-support@agilent.com

Appendix

Determining the Dwell Volume of Your System

- 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 to 100% B/10 min at 1.0 mL/min
- Record

Measuring Dwell Volume (V_D)



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

Measure and Correct for Dwell Volume (V_D)

Agilent InfinityLab

If $V_{D1} > V_{D2}$

Compensate for longer V_{D1} by adding an isocratic hold to V_{D2}, such that Hold + V_{D2} = V_{D1}

If $V_{D1} < V_{D2}$

Delay injection, such that V_{D2} - delay = V_{D1}

001014P1.PPT

How to Estimate the Extracolumn Volume of Your System

One way:

- Remove HPLC column from instrument
- Join injector and detector tubing with zero-dead-volume (ZDV) union
- Inject (0.5–2 μL) of toluene in 100% acetonitrile
- Determine width of peak at base ($W_{instrument}$)
- Peak bandwidth follows:

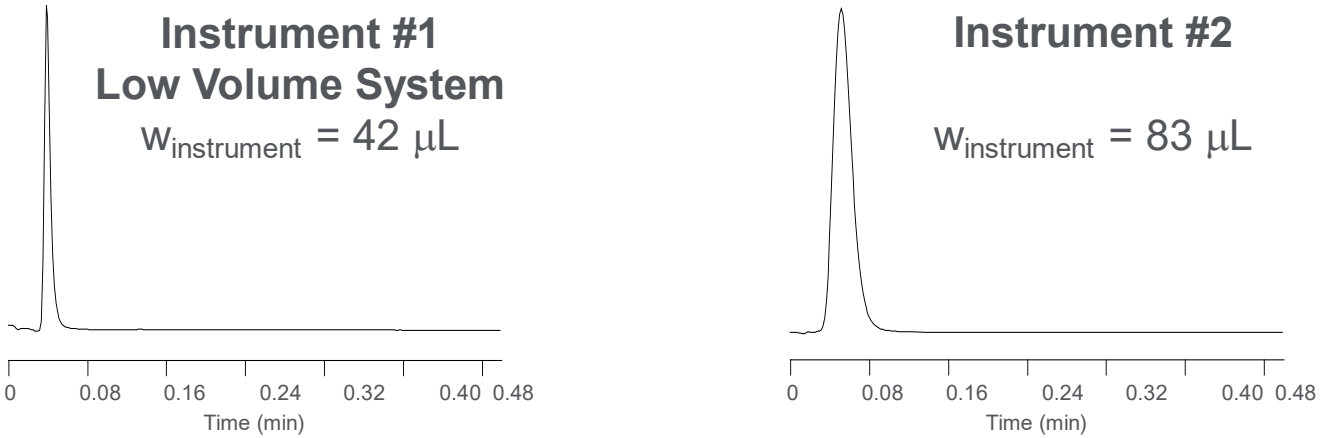
Make concentration about 1–5 mg/mL

$$W^2_{tot} = W^2_{col} + W^2_{instrument}$$

Extra Column Volume and Peak Shape

Agilent InfinityLab

Toluene in Acetonitrile



$$w^2_{tot} = w^2_{col} + w^2_{instrument}$$

For peak having a k′ = 2

4.6 x 150 mm, 5 µm

$w^2_{tot} = (180)^2 + (42)^2$
w_{tot} = 185 µL

↑ 3%

$w^2_{tot} = (180)^2 + (83)^2$
w_{tot} = 198 µL

↑ 10%

4.6 x 50 mm, 3.5 µm

$w^2_{tot} = (73)^2 + (42)^2$
w_{tot} = 84 µL

↑ 15%

$w^2_{tot} = (73)^2 + (83)^2$
w_{tot} = 110 µL

↑ 51%

Method Development Kits

Part No.	Kits	Description
5190-6160	**P120, USP method development kit, 3.0 x 100 mm**	InfinityLab Poroshell 120 EC-C18, EC-C8, EC-CN columns, 3.0 x 100 mm
5190-6159	**P120, USP method development kit, 4.6 x 100 mm**	InfinityLab Poroshell 120 EC-C18, EC-C8, EC-CN columns, 4.6 x 100 mm
5190-6155	**P120, Selectivity method development kit, 2.1 x 50 mm**	InfinityLab Poroshell 120 EC-C18, Phenyl-Hexyl, Bonus RP columns, 2.1 x 50 mm
5190-6156	**P120, Selectivity method development kit, 4.6 x 50 mm**	InfinityLab Poroshell 120 EC-C18, Phenyl-Hexyl, Bonus RP columns, 4.6 x 50 mm
5190-6157	**P120, Aqueous method development kit, 2.1 x 50 mm**	InfinityLab Poroshell 120 SB-Aq, Phenyl-Hexyl, Bonus RP columns, 2.1 x 50 mm
5190-6158	**P120, Aqueous method development kit, 4.6 x 50 mm**	InfinityLab Poroshell 120 SB-Aq, Phenyl-Hexyl, and Bonus RP columns, 4.6 x 50 mm
5190-6153	**RRHD Eclipse Plus method development kit, 2.1 mm id**	RRHD Eclipse Plus C18, Eclipse Plus C8, Eclipse Plus Phenyl-Hexyl, 2.1 x 50 mm columns
5190-6154	**RRHD Aqueous method development kit, 2.1 mm id**	RRHD SB-Aq, Bonus RP, and Eclipse Plus Phenyl-Hexyl columnc 2.1 x 50 mm
5190-6152	**RRHD pH method development kit, 2.1 mm id**	RRHD StableBond SB-C18, Eclipse Pluse C18, and Extend-C18 column, 2.1 x 50 mm
5190-6160	**P120, USP method development kit, 3.0 x 100 mm**	InfinityLab Poroshell 120 EC-C18, EC-C8, EC-CN columns, 3.0 x 100 mm
5190-6159	**P120, USP method development kit, 4.6 x 100 mm**	InfinityLab Poroshell 120 EC-C18, EC-C8, EC-CN columns, 4.6 x 100 mm
5190-6155	**P120, Selectivity method development kit, 2.1 x 50 mm**	InfinityLab Poroshell 120 EC-C18, Phenyl-Hexyl, Bonus RP columns, 2.1 x 50 mm
5190-6156	**P120, Selectivity method development kit, 4.6 x 50 mm**	InfinityLab Poroshell 120 EC-C18, Phenyl-Hexyl, Bonus RP columns, 4.6 x 50 mm
5190-6157	**P120, Aqueous method development kit, 2.1 x 50 mm**	InfinityLab Poroshell 120 SB-Aq, Phenyl-Hexyl, Bonus RP columns, 2.1 x 50 mm

Shutdown State and Instrument Flushing

Shutdown state

Next day use—using same buffers:

- Pump the mobile phase slowly (for example, 0.01–0.1 mL/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.