HPLC Column Troubleshooting:

Is It Really The Column?

LC Columns and Consumables

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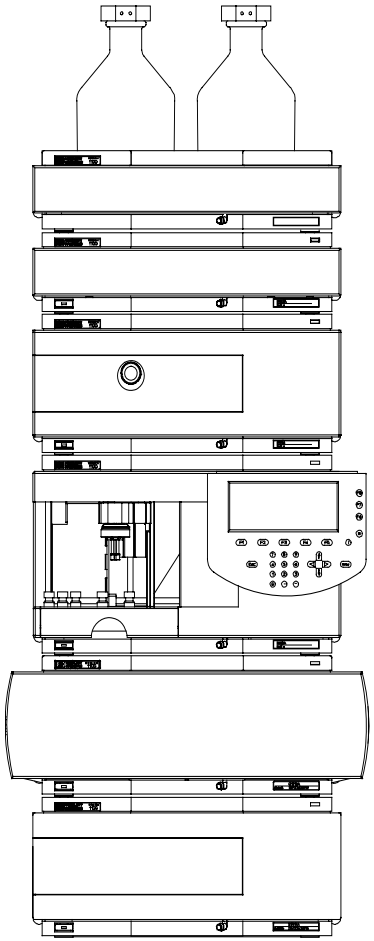
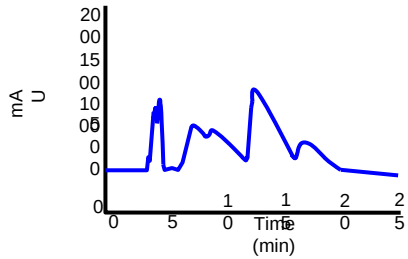
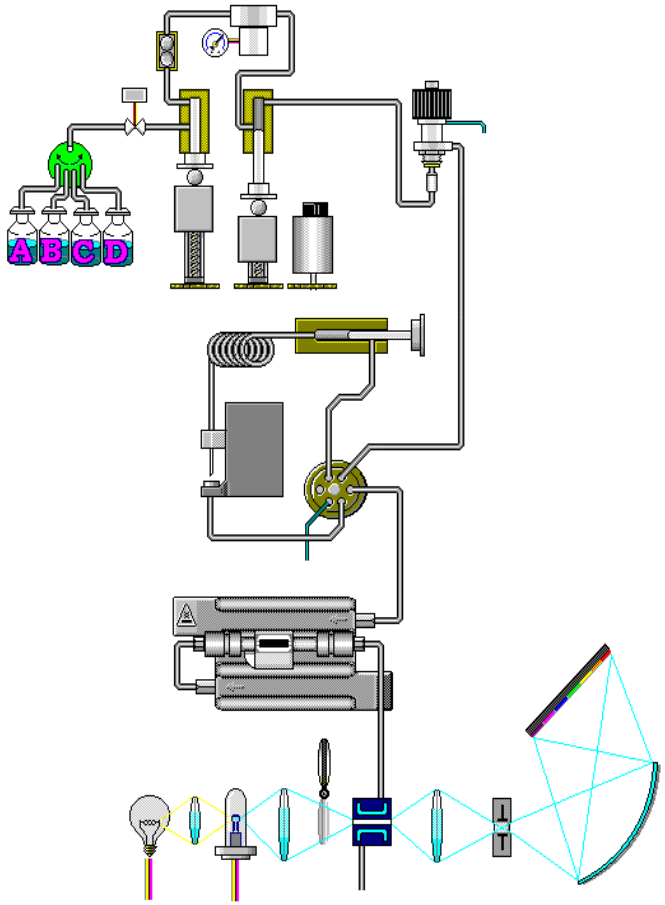
July 24, 2007

Troubleshooting in HPLC







Major Areas of Column Problems - Dramatic Changes in 3 Key Areas:

1. HPLC System Pressure
2. Chromatogram - Peak Shape
3. Chromatogram - Peak Retention/Selectivity

1. Pressure Issues

Column Observations	Potential Problems
Large pressure change	Plugged inlet frit Column contamination Plugged packing

Determining the Cause and Correcting High Back Pressure

- **Check pressure with/without column - many pressure problems are due to blockages elsewhere in the system.**

If Column pressure remains high:

- **Rinse column (remove detector from flow path!)**
  - **– Eliminate column contamination and plugged packing**
  - **– High molecular weight/adsorbed compounds precipitate from sample or buffer**
- **Back flush column – may clear plugged column inlet frit**
- **Install New column**

Eliminate pressure issues – add a disposable 0.5 or 2 um in-line filter to system.

Column Cleaning

Flush with stronger solvents than your mobile phase.
Make sure detector is taken out of flow path.

Reversed-Phase Solvent Choices

in Order of Increasing Strength

Use at least 10 x V_m of each solvent for analytical columns

1. Mobile phase without buffer salts (water/organic)
2. 100% Organic (MeOH or ACN)
3. Is pressure back in normal range?
4. If not, discard column or consider more drastic conditions:
   75% Acetonitrile:25% Isopropanol, then
5. 100% Isopropanol
6. 100% Methylene Chloride*
7. 100% Hexane*

*** When using either Hexane or Methylene Chloride the column must be flushed with Isopropanol before returning to your reversed-phase mobile phase.**

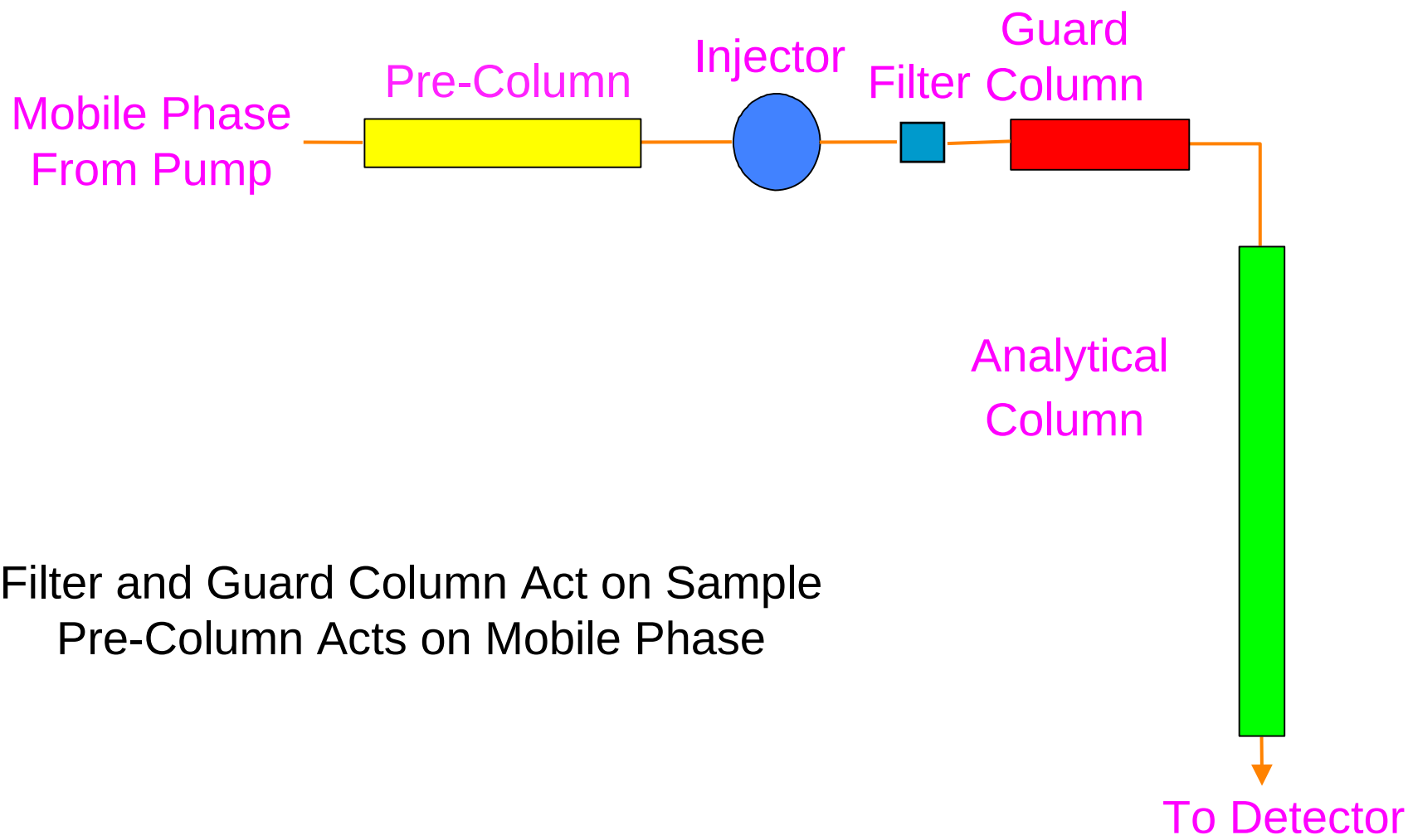
Column Cleaning

Normal Phase Solvent Choices

in Order of Increasing Strength

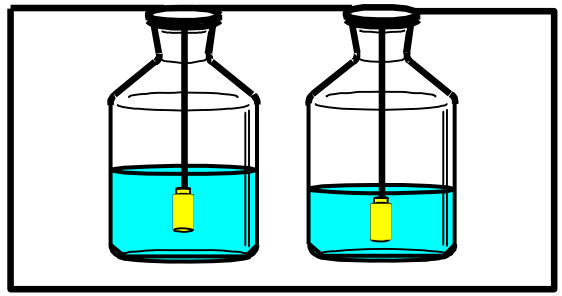
- Use at least 50 mL of each solvent
- 50% Methanol : 50% Chloroform
- 100% Ethyl Acetate

Preventing Back Pressure Problems: In-Line Devices



Filter and Guard Column Act on Sample
Pre-Column Acts on Mobile Phase

Preventing Column Back Pressure Problems



1. Filter mobile phase:
   - filter non-HPLC grade solvents
   - filter buffer solutions
   - Install an in-line filter between auto-sampler and column (removes pump seal debris, ALS rotor debris, and sample particulates). Use 2 um frit for 3.5 um columns, use 0.5 um frit for 1.8 um columns.
2. Filter all samples and standards
3. Perform sample clean-up (i.e. SPE, LLE) on dirty samples.
4. Appropriate column flushing – flush buffers from entire system at end of day with water/organic mobile phase.
5. Use Mobile Phase Miscible Sample Solvents

Filtration of the HPLC mobile phase helps prevent blockage and reduce wear of components along the flow pathway

Filtration is an easy but important step because it:

- **removes both microbial growth and particulates from the mobile phase …**
  - **Increasingly important as HPLC moves more towards smaller ID connecting capillaries and analytical columns, and smaller diameter silica particles within the columns**
- **degasses mobile phase solvents at the same time**
- **eliminates pump downtime caused by air locks and particulates in check valves**
- **decreases pump piston wear, while increasing lifetime of columns, ball valves, solvent and column inlet frits, and capillaries**
- **eliminates spurious peaks at the detector which interferes with the analysis**

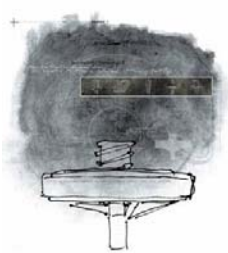
Part number 3150-0577

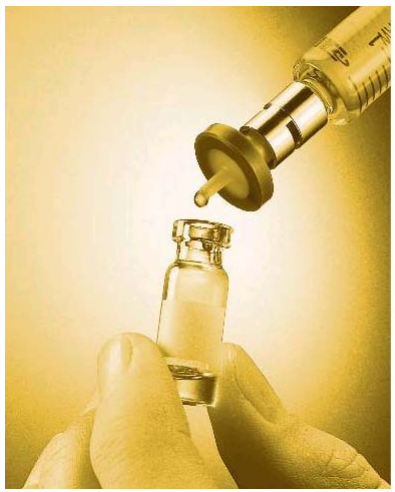
Preventing Back Pressure Problems: Sample Preparation

- **Solvent/Chemical Environment**
- **Particulate/Aggregate Removal**
  - Sample Filtration
  - Centrifugation
- **Solid Phase Extraction (SPE)**
  - Cartridges or Plates
  - Disks or Membranes

Why Filter the Sample?

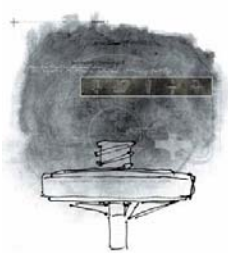
Extreme Performance Requires Better Sample “Hygiene”





- Prevents blocking of capillaries, frits, and the column inlet
- Results in less wear and tear on the critical moving parts of injection valves
- Results in less downtime of the instrument for repairs
- Produces improved analytical results by removing potentially interfering contamination

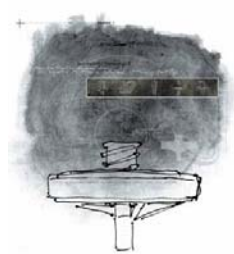
Suggested Choices of Sample Filters



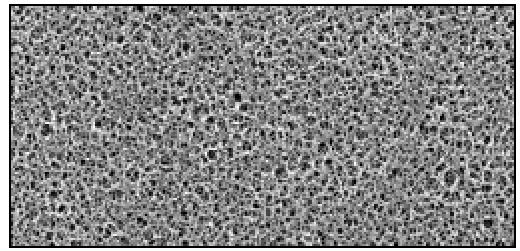
The Pore Sizes:

- 0.45 µm -Good for 3.5 and 5.0 Particle Columns
- 0.20 µm -Good for 1.8u Particles Columns
- 30, 25, and 13 mm diameter
- Best diameter to recommend is 13 or 25 mm

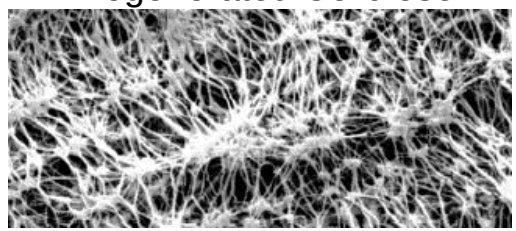
Sample Filtration: Choice of Membranes



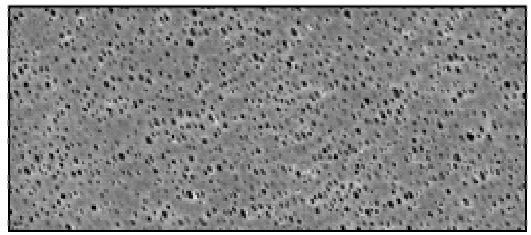
- Regenerated Cellulose (RC)
  - Universal hydrophilic membrane, compatible with most solvents - aqueous and organic
  - High purity, extremely low extractables and lowest protein binding
- PTFE
  - Good chemical compatibility with aqueous and organic solvents as well as for acids and bases
  - Hydrophobic
- Nylon
  - Universal filter for aqueous and organic solutions
  - Hydrophilic
- Cellulose Nitrate
  - Compatible with many, but not all, aqueous or non-aqueous solvents
  - Most commonly used as a pre-filter
- Cellulose Acetate (CA)
  - Only compatible with aqueous solvents
  - Used for proteins and protein-related samples



Regenerated Cellulose



PTFE



Nylon

Magnified views showing structural differences of some membrane types

Key Thing to Remember

- As Column Particle Size Shrinks, Column Frit Porosity is Reduced (5µm-2µm frit, 3.5µm-0.5µm frit, 1.8µm-0.2µm frit
- Mobile Phase Filtering Reduces Wear on Instrument Parts (Check Valves, Piston Seals, Autosampler)
- Sample Filtering Reduces Wear on Instrument and Prevents Column Plugging Due to Particulates
- A Little Prevention Reduces Downtime and Maintenance Costs

2. Peak Shape Issues in HPLC

- Split peaks
- Peak tailing
- Broad peaks
- Poor efficiency (low N)

- Many peak shape issues are also combinations - i.e. broad and tailing or tailing with increased retention

Split Peaks

Can be caused by:

- Column contamination
- Partially plugged frit
- Column void (gap in packing bed)
- Injection solvent effects

Determining the Cause of Split Peaks

1. **Complex sample matrix or many samples analyzed - likely column contamination or partially plugged column frit.**

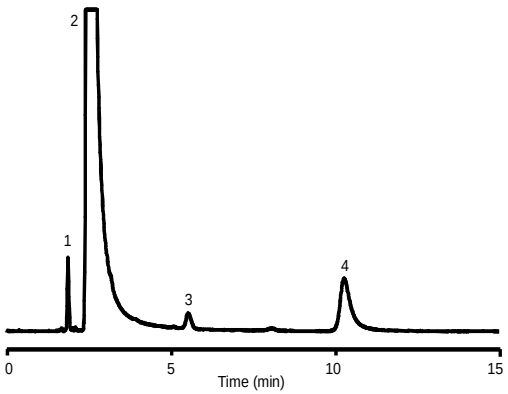
2. **Mobile phase pH > 7 - likely column void due to silica dissolution (unless specialty column used, Zorbax Extend-C18 stable to pH 11)**

3. **Injection solvent stronger than mobile phase - likely split *and* broad peaks, shape dependent on injection volume and k value.**

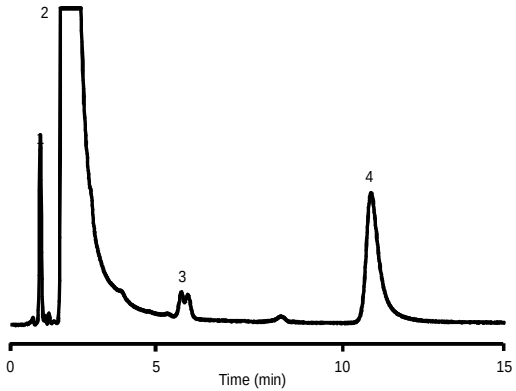
Split Peaks
Column Contamination

Column: StableBond SB-C8, 4.6 x 150 mm, 5 µm Mobile Phase: 60% 25 mM Na_2HPO_4, pH 3.0 : 40% MeOH Flow Rate: 1.0 mL/min
Temperature: 35°C Detection: UV 254 nm Sample: Filtered OTC Cold Medication: 1. Pseudoephedrine 2. APAP 3. Unknown 4. Chlorpheniramine

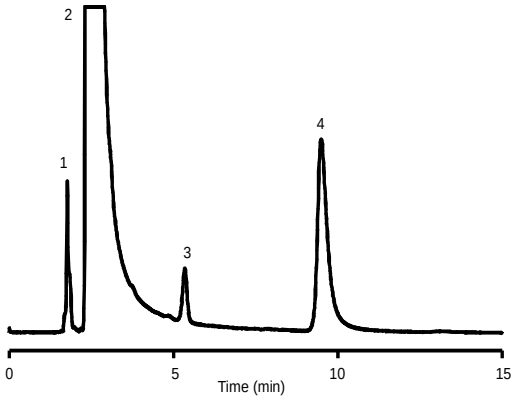
Injection 1



Injection 30



Injection 1
After Column Wash
with 100% ACN

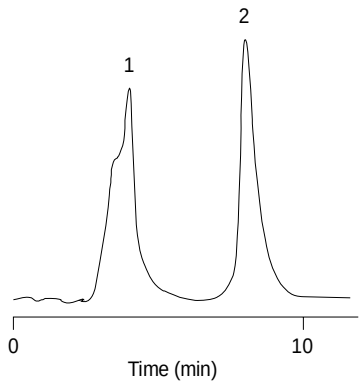


- Column washing eliminates the peak splitting, which resulted from a contaminant on the column.

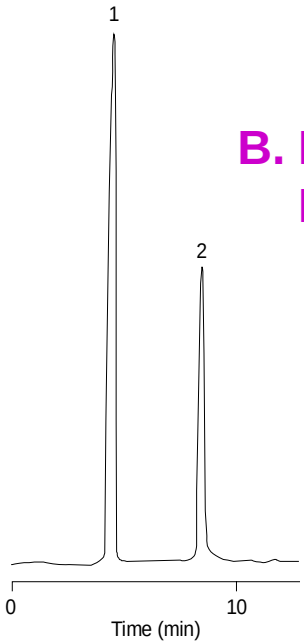
Split Peaks - Injection Solvent Effects

Column: StableBond SB-C8, 4.6 x 150 mm, 5 mm ; Mobile Phase: 82% H_2O :18% ACN;
Injection Volume: 30 mL Sample: 1. Caffeine 2. Salicylamide

A. Injection Solvent 100% Acetonitrile

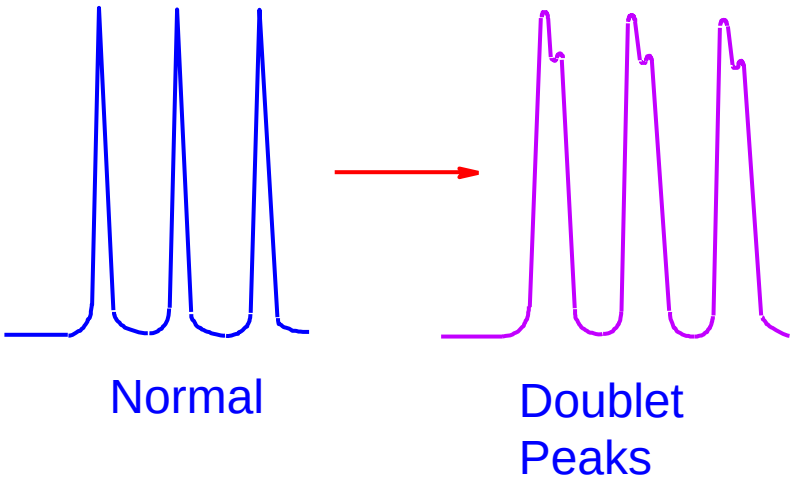


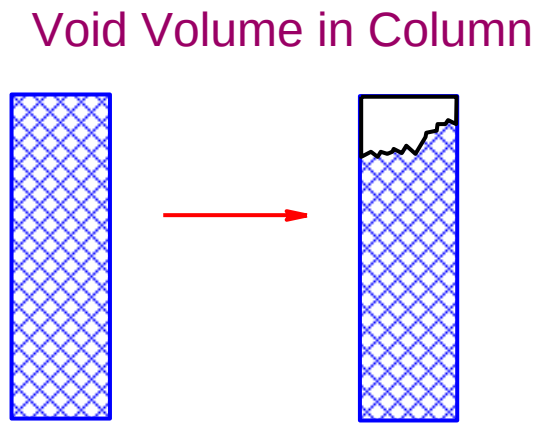
B. Injection Solvent Mobile Phase



- Injecting in a solvent stronger than the mobile phase can cause peak shape problems, such as peak splitting or broadening.
- Note: earlier peaks (low k) most affected

Peak Shape Problems - Doublets





- **Void Volume in Column**
- **Partially Blocked Frit**
- **Only One-Peak a Doublet- Coeluting Components**
- **Early (low k) peaks most affected**

Peak Tailing, Broadening and Loss of Efficiency (N, plates)

May be caused by:

1. Column “secondary interactions”
2. Column packing voids
3. Column contamination
4. Column aging
5. Column loading
6. Extra-column effects

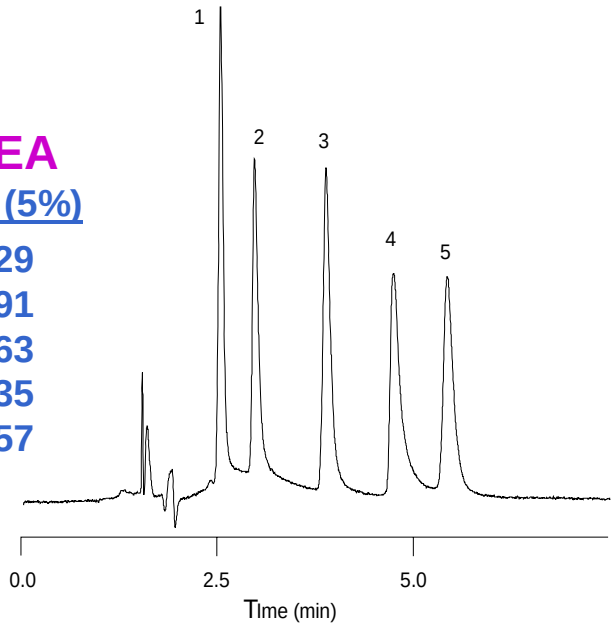
Peak Tailing
Column “Secondary Interactions”

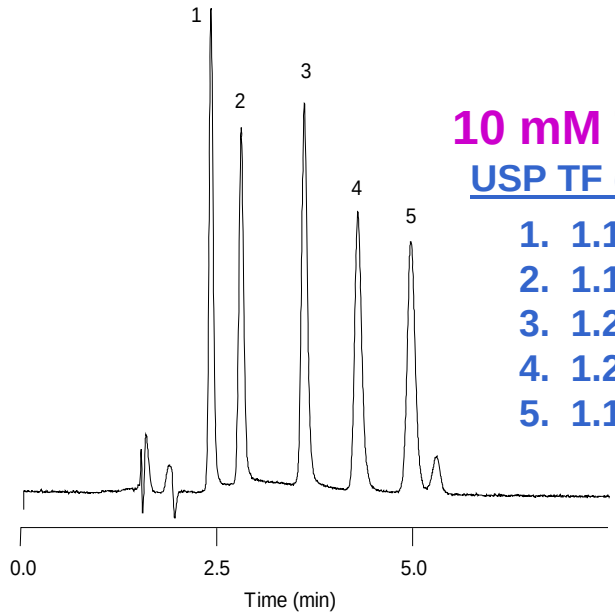
Column: Alkyl-C8, 4.6 x 150 mm, 5μm Mobile Phase: 85% 25 mM Na_2HPO_4 pH 7.0 : 15% ACN
Flow Rate: 1.0 mL/minTemperature: 35°C Sample: 1. Phenylpropanolamine 2. Ephedrine 3. Amphetamine 4. Methamphetamine 5. Phenteramine

No TEA

USP TF (5%)

1. **1.29**
2. **1.91**
3. **1.63**
4. **2.35**
5. **1.57**





10 mM TEA

USP TF (5%)

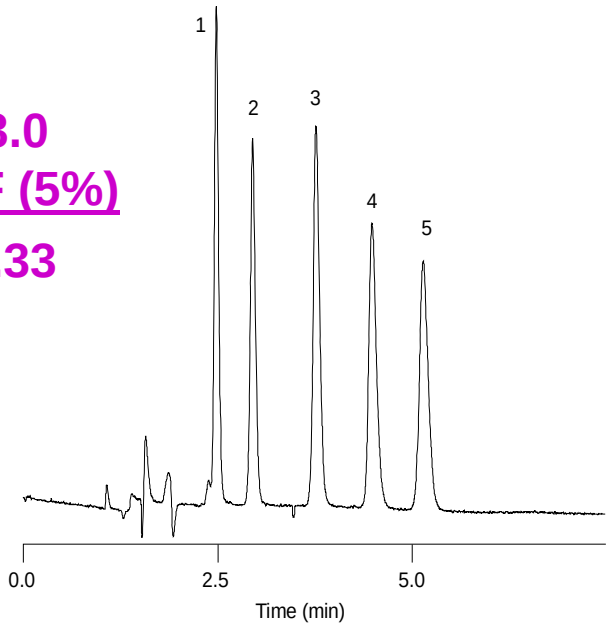
1. **1.19**
2. **1.18**
3. **1.20**
4. **1.26**
5. **1.14**

- Peak tailing of amine analytes eliminated with mobile phase modifier (TEA, triethylamine) at pH 7

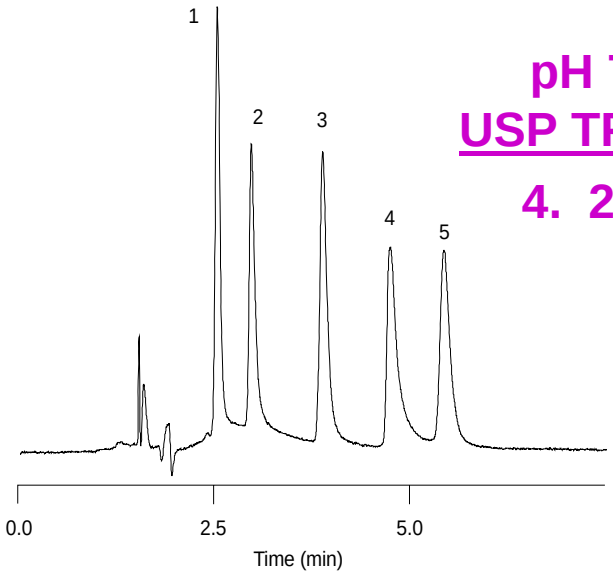
Peak Tailing
Column “Secondary Interactions”

Column: Alkyl-C8, 4.6 x 150 mm, 5mm Mobile Phase: 85% 25 mM Na_2HPO_4 : 15% ACN Flow Rate: 1.0 mL/min
Temperature: 35°C Sample: 1. Phenylpropanolamine 2. Ephedrine 3. Amphetamine 4. Methamphetamine 5. Phenteramine

pH 3.0
USP TF (5%)
4. 1.33



pH 7.0
USP TF (5%)
4. 2.35



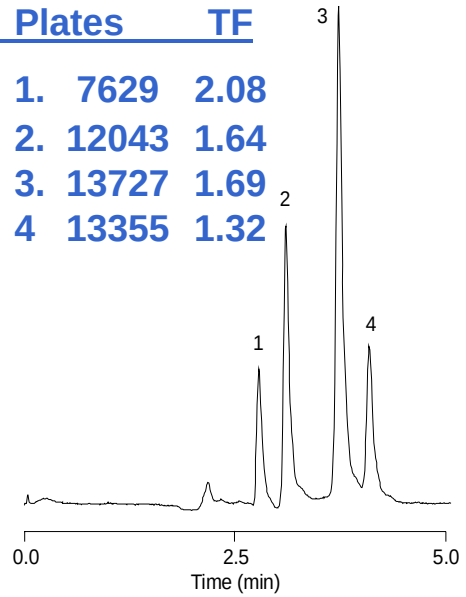
- Reducing the mobile phase pH reduces interactions with silanols that cause peak tailing. No TEA modifier required.

Peak Tailing
Column Contamination

Column: StableBond SB-C8, 4.6 x 250 mm, 5µm Mobile Phase: 20% H_2O : 80% MeOH Flow Rate: 1.0 mL/min
Temperature: R.T. Detection: UV 254 nm Sample: 1. Uracil 2. Phenol 3. 4-Chloronitrobenzene 4. Toluene

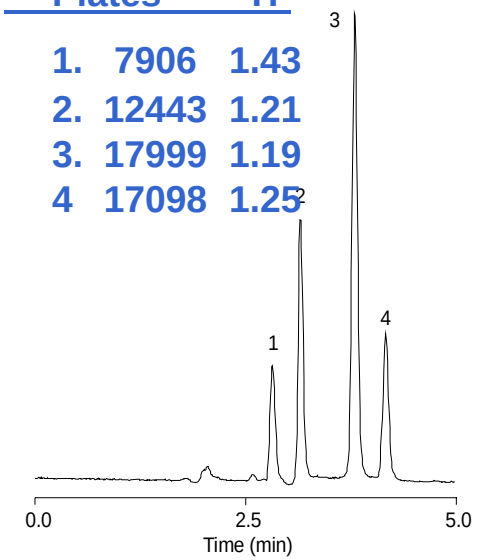
QC test forward direction

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



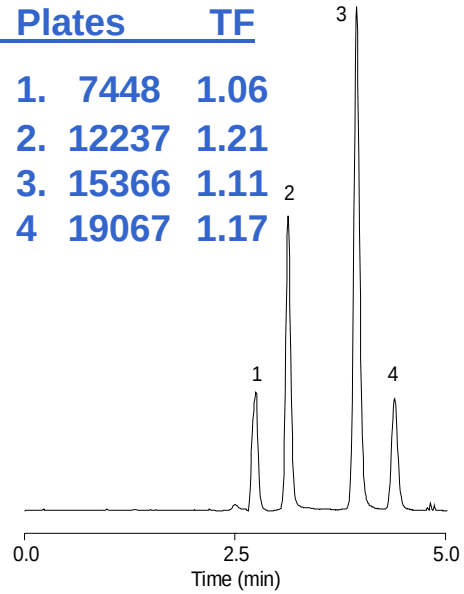
QC test reverse direction

	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/Broadening
Sample Load Effects

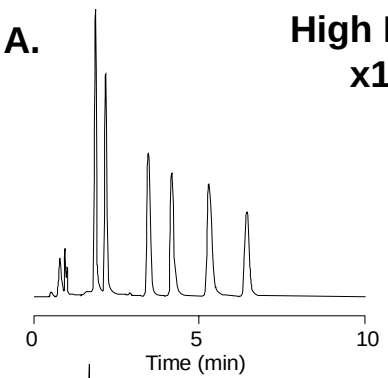
Columns: 4.6 x 150 mm, 5μm Mobile Phase: 40% 25 mM Na_2HPO_4 pH 7.0 : 60% ACN Flow Rate: 1.5 mL/min
Temperature: 40°C Sample: 1. Desipramine 2. Nortriptyline 3. Doxepin 4. Imipramine 5. Amitriptyline 6. Trimipramine

Tailing

Eclipse XDB-C8
USP TF (5%)

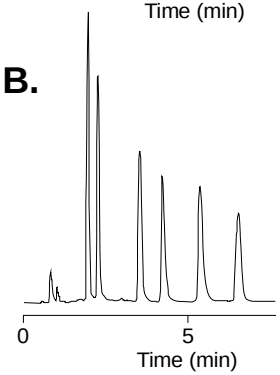
	A	B
1.	1.60	1.70
2.	2.00	1.90
3.	1.56	1.56
4.	2.13	1.70
5.	2.15	1.86
6.	1.25	1.25

A.



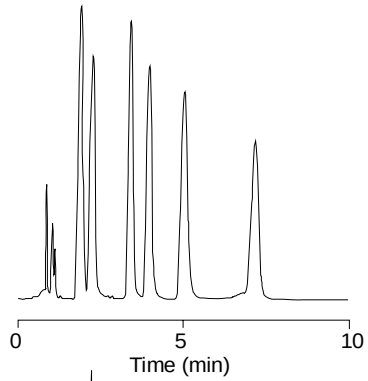
High Load x10

B.

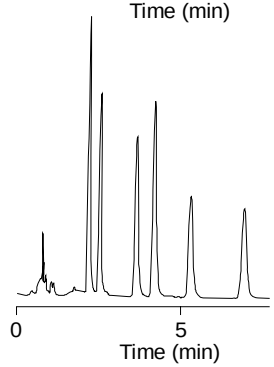


Low Load

C.



D.



Broadening

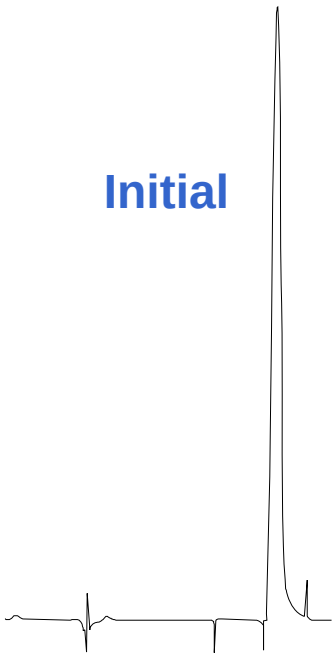
Competitive C8
Plates

	C	D
1.	850	5941
2.	815	7842
3.	2776	6231
4.	2539	8359
5.	2735	10022
6.	5189	10725

Peak Broadening, Splitting
Column Void

Mobile Phase: 50%ACN: 50% Water : 0.2% TEA (~ pH 11)

Initial



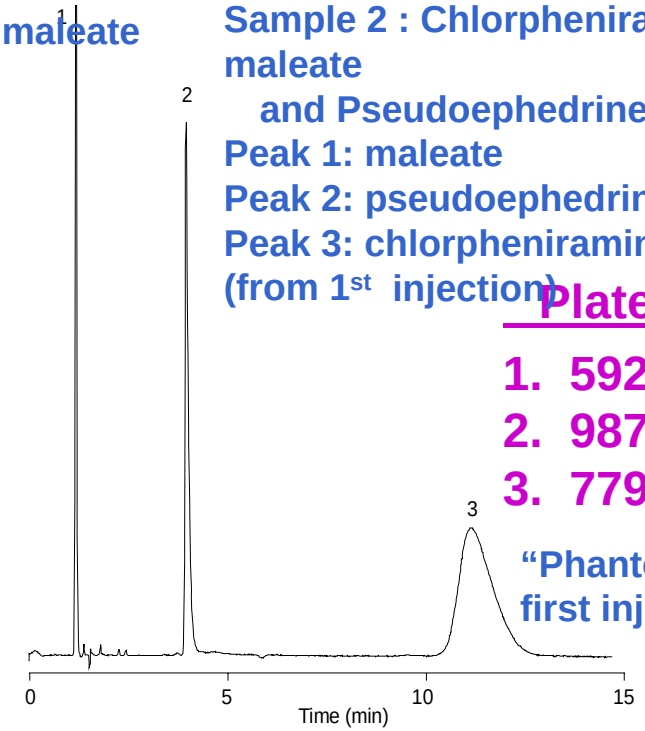
After 30 injections

- **Multiple peak shape changes can be caused by the same column problem. In this case a void resulted from silica dissolved at high pH.**

Broad Peaks
Unknown “Phantom” Peaks

Column: Extend-C18, 4.6 x 150 mm, 5 µm Mobile Phase: 40% 10 mM TEA, pH 11 : 60% MeOH Flow Rate: 1.0 mL/min
Temperature: R.T. Detection: UV 254 Sample: 1. Maleate 2. Pseudoephedrine 3. Chlorpheniramine

Sample 1: Chlorpheniramine maleate
Peak 1: maleate



Sample 2 : Chlorpheniramine maleate and Pseudoephedrine
Peak 1: maleate
Peak 2: pseudoephedrine
Peak 3: chlorpheniramine (from 1st injection)

Plates

1. **5922**
2. **9879**
3. **779**

“Phantom” peak from first injection

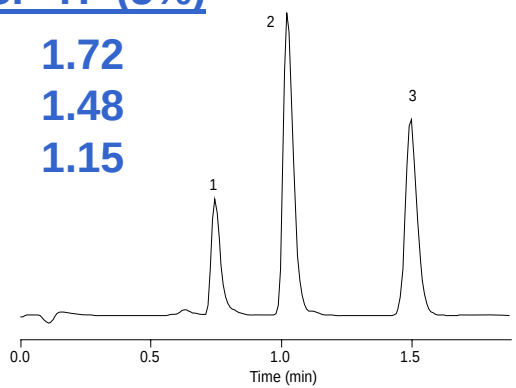
- **The extremely low plates are an indication of a very late eluting peak from the preceding run.**

Peak Tailing
Injector Seal Failure

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

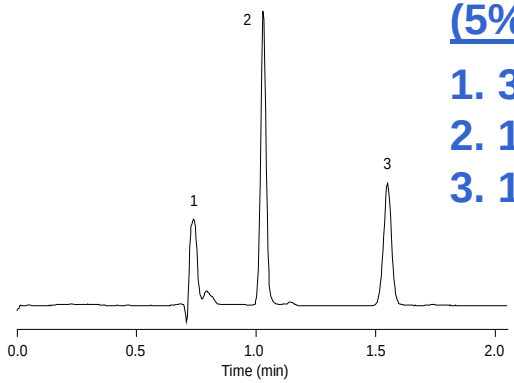
Before

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



After replacing rotor seal and isolation seal

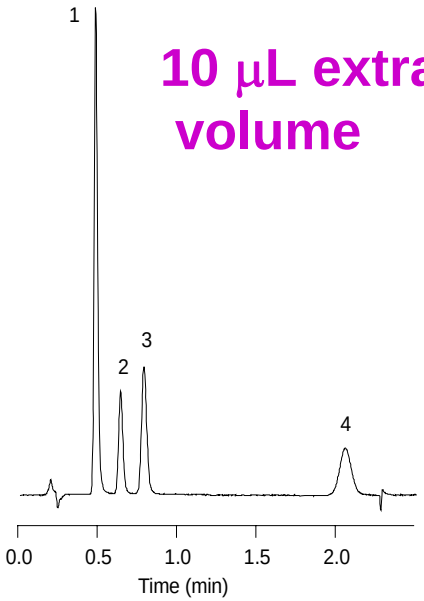
	Plates	USP TF (5%)
1.	3670	1.45
2.	10457	1.09
3.	10085	1.00



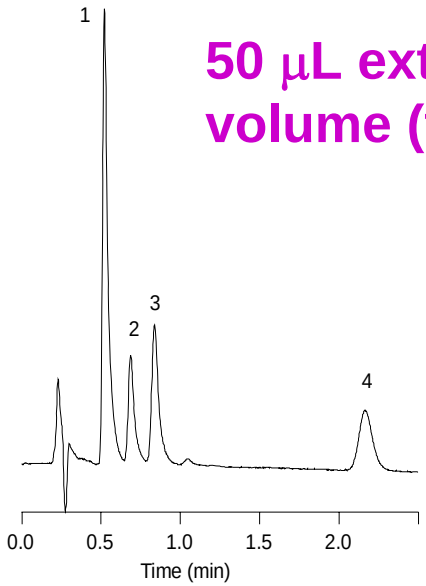
- **Overdue instrument maintenance can sometimes cause peak shape problems.**

Peak Tailing
Extra-Column Volume

Column: StableBond SB-C18, 4.6 x 30 mm, 3.5 µm Mobile Phase: 85% H_2O with 0.1% TFA : 15% ACN Flow Rate: 1.0 mL/min
Temperature: 35°C Sample: 1. Phenylalanine 2. 5-benzyl-3,6-dioxo-2-piperazine acetic acid 3. Asp-phe 4. Aspartame



10 µL extra-column volume

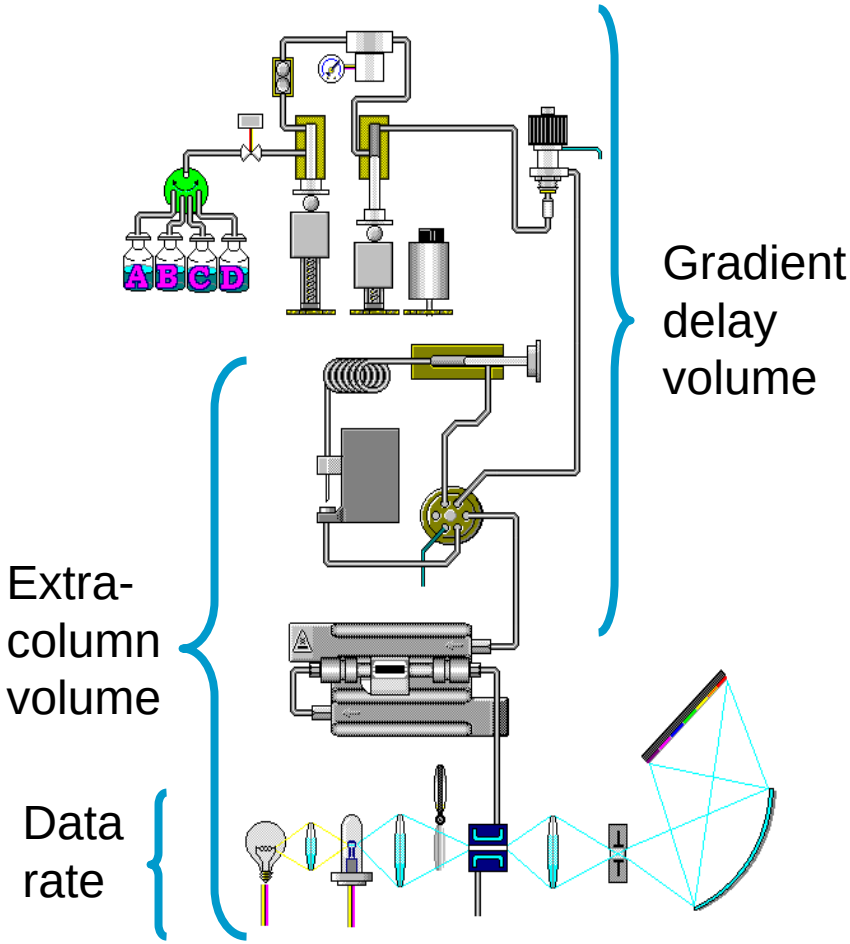


50 µL extra-column volume (tubing)

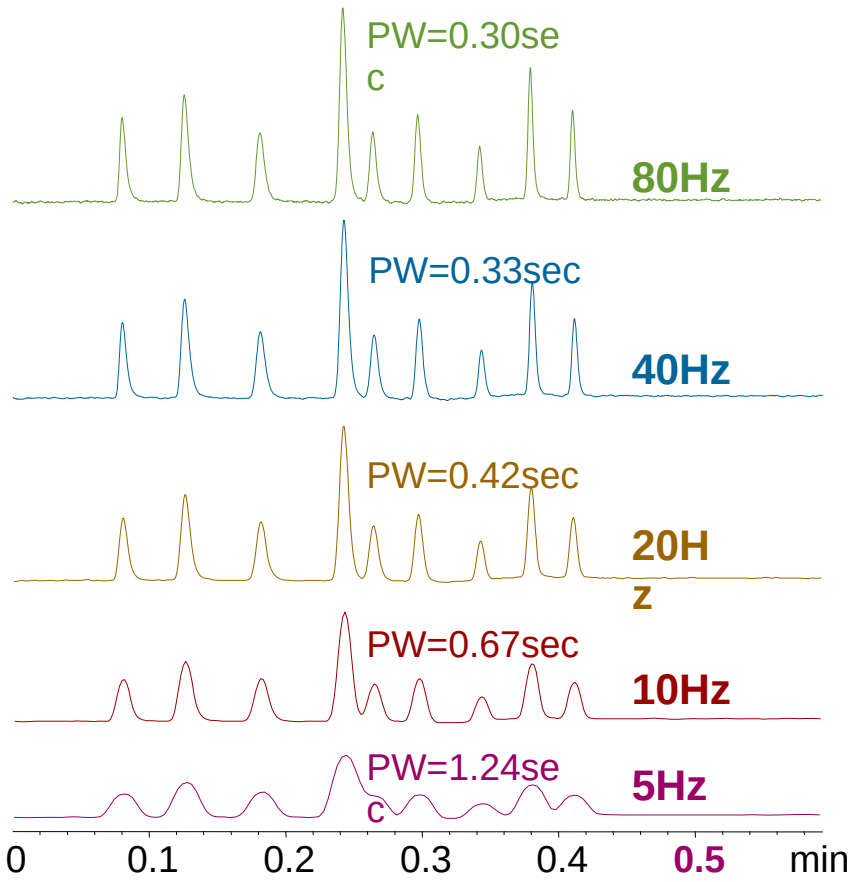
Peak Tailing
Extra-Column Volume

Things to consider on your system:

- **Gradient delay volume** – effects column re-equilibration and gradient profile
- **Extra-column volume** – effects peak dispersion and peak width for Isocratic separations
- **Data Rate** – match to expected peak widths-If rate is too slow sensitivity and peak detection suffer in Iso and Gradient separations



Peak Shape Issues: Broad Peaks



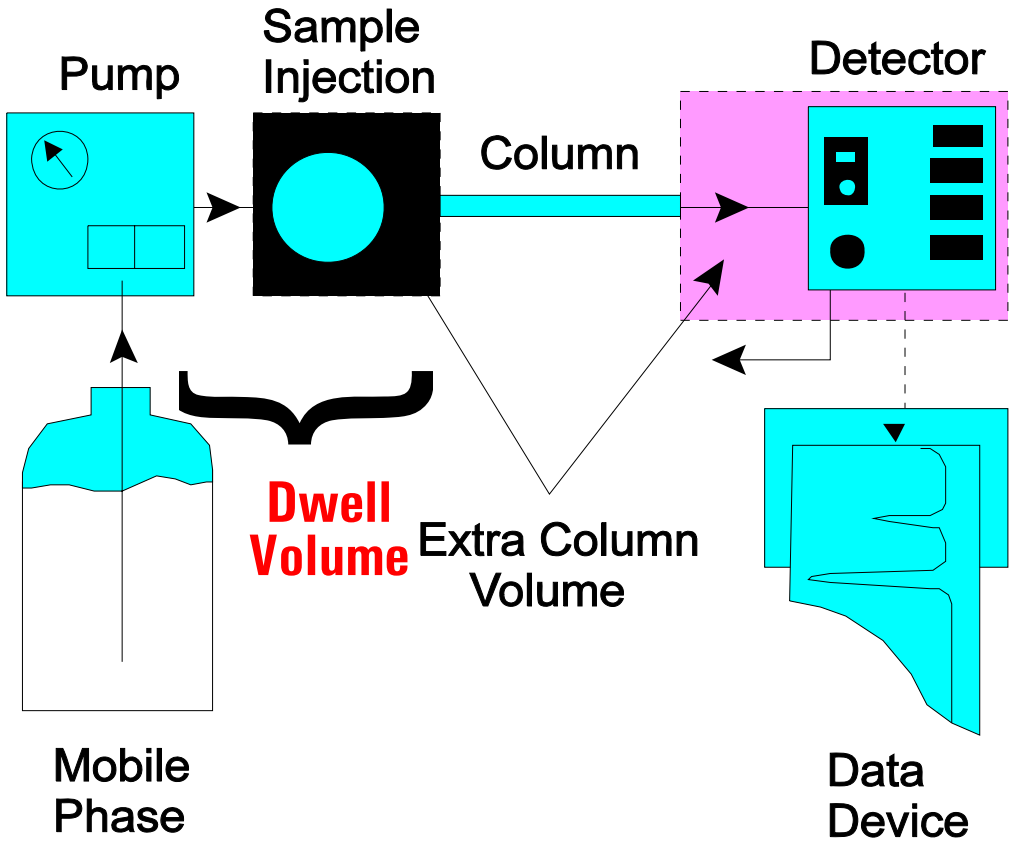
80Hz versus 10Hz (20Hz) Data Rate

- Peak Width: – 55% (– 30%)
- Resolution: + 90% (+ 30%)
- Peak Capacity: + 120% (+ 40%)
- App. Column Eff.: + 260% (+ 70%)

Data Rate	Peak Width	Resolution	Peak Capacity
80 Hz	0.300	2.25	60
40 Hz	0.329	2.05	55
20 Hz	0.416	1.71	45
10 Hz	0.666	1.17	29
5 Hz	1.236	0.67	16

Sample: Phenones Test Mix
Column: Zorbax SB-C18, 4.6x30, 1.8um
Gradient:: 50-100%ACN in 0.3min
Flow Rate: 5ml/min

What is Dwell Volume & Extra Column Volume?

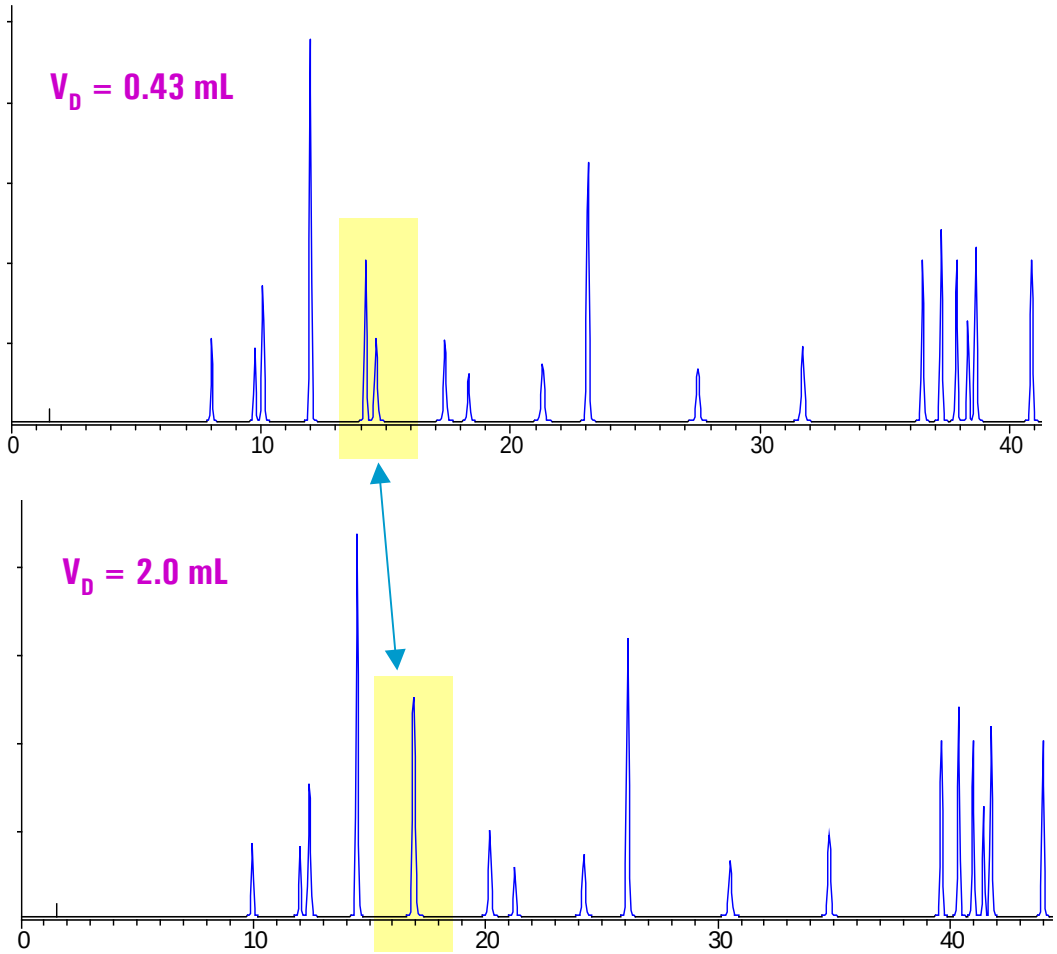


Dwell Volume = Volume of the Instrument before the column inlet

- High Pressure Mixing: V_D = mixing chamber + connecting tubing + injector
- Low Pressure Mixing:V_D = the above + pump heads + associated tubing

Behaves as isocratic hold at the beginning of gradient.

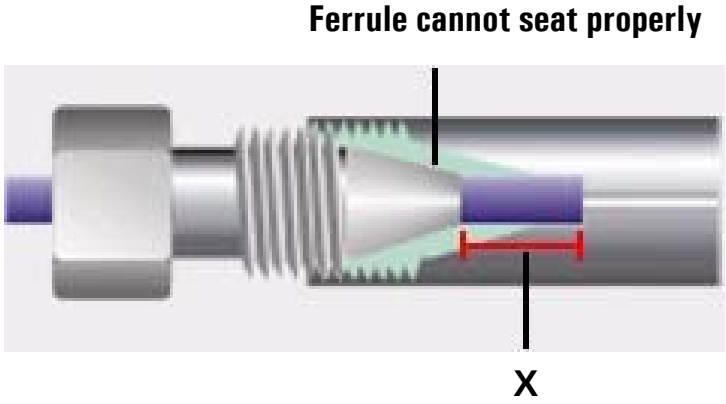
Minor Dwell Volume Differences Can Change Resolution



Column:	**ZORBAX Rapid Resolution Eclipse XDB-C8 4.6 x 75 mm, 3.5 μm**
Mobile Phase:	Gradient, 0 - 100 %B in 52.5 min.
	A: 5/95 methanol/ 25 mM phosphate pH 2.50 B: 80/20 methanol/25 mM phosphate pH 2.50
Flow Rate:	0.5 mL/min
Temperature:	25°C
Injection:	5 µL
Detection:	250 nm
Sample:	Mixture of antibiotics and antidepressants
	Upper trace simulates actual run data entered into DryLab® 3.0 software
	Lower trace is simulated chromatogram for larger V_D

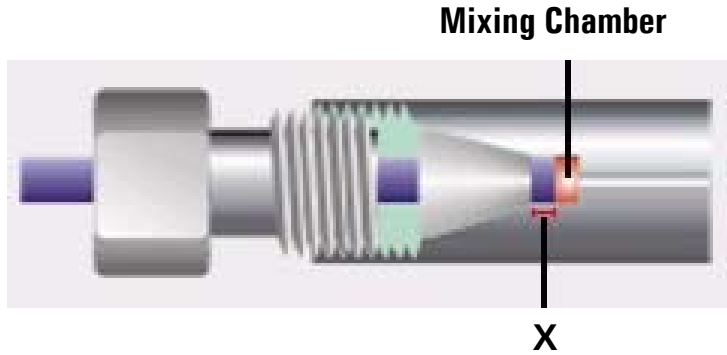
Peak Tailing/Fronting
What Happens If the Connections Poorly Made ?

Wrong … too long



If Dimension X is too long, leaks will occur

Wrong … too short

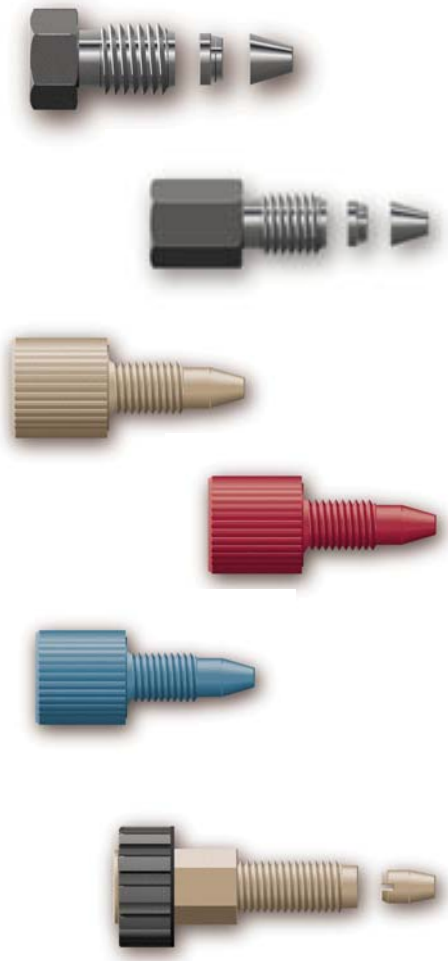


If Dimension X is too short, a dead-volume, or mixing chamber, will occur

Stainless Steel and Polymer Fittings

Which type is used and when?

- Stainless Steel (SS) fittings are the best choice for reliable high pressure sealing
  - Agilent uses Swagelok type fittings with front and back ferrules – which give best sealing performance – throughout our LC systems
- PEEK (<400b bar System Pressure) fittings are ideal where:
  - Connections are changed frequently, i.e. connecting columns
  - Bio-compatibility is needed
  - Pressure is less critical

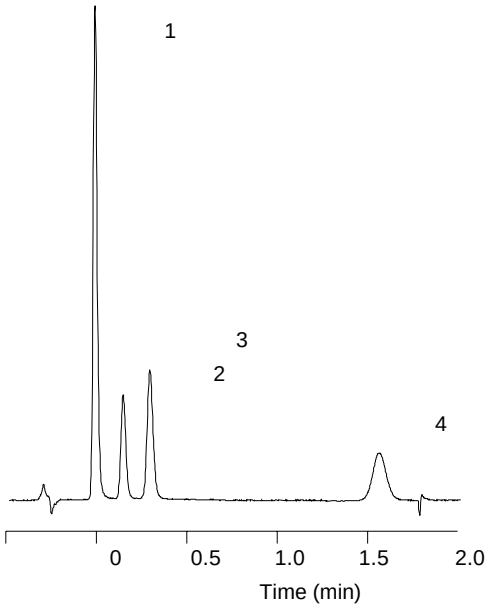


Some typical column connectors shown here

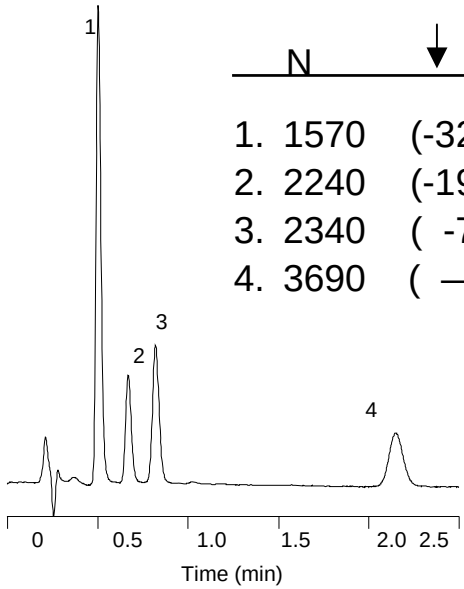
Minimum Tubing Volume for Maximum Efficiency 4.6 x 30 mm, 3.5 µm

Column: StableBond SB-C18 Mobile Phase: 85% H_20 with 0.1% TFA : 15% CH3CN, Flow Rate: 1.0 mL/min Temperature: 35°C
Sample: 1. Phenylalanine, 2. 5-benzyl-3,6-dioxo-2-piperazine acetic acid, 3. Asp-phe, 4. Aspartame

No extra tubing

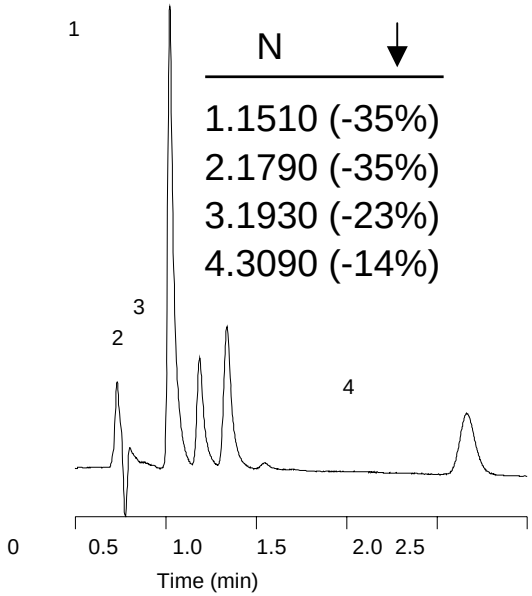


35" of 0.007" i.d. tubing
Volume = 22 µL



	N	
1.	1570	(-32%)
2.	2240	(-19%)
3.	2340	(-7%)
4.	3690	(—)

8" of 0.02" i.d. tubing
Volume = 40.5 µL



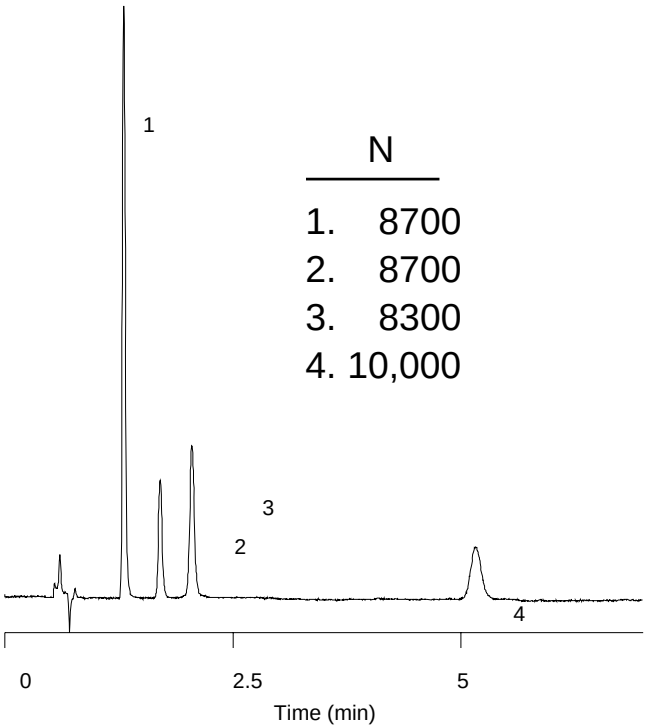
	N	
1.	1510	(-35%)
2.	1790	(-35%)
3.	1930	(-23%)
4.	3090	(-14%)

Effect of Detector Cell Volume on Peak Width

4.6 x 75 mm, 3.5 µm

Column: StableBond SB-C18 : Mobile Phase: 85% H_20 with 0.1% TFA : 15% ACN; Flow Rate: 1.0 mL/min
Temperature: 35°C
Sample: 1. Phenylalanine 2. 5-benzyl-3,6-dioxo-2-piperazine acetic acid 3. Asp-phe 4. Aspartame

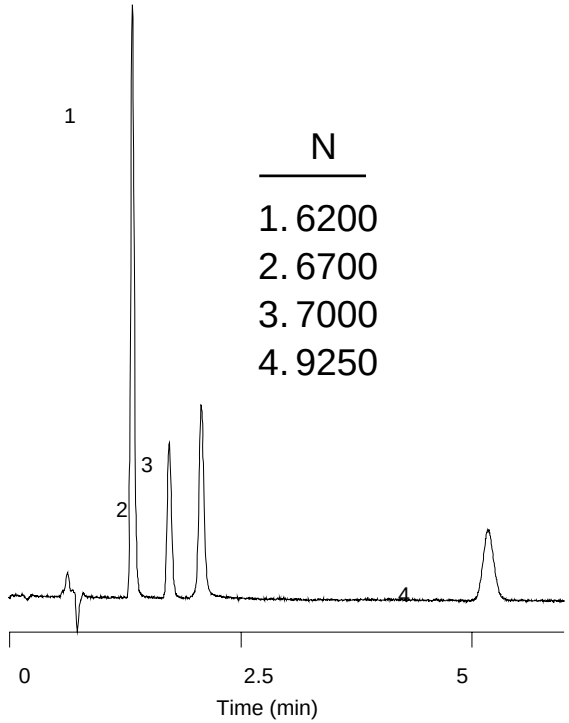
Microflow Cell - 2.5 µL



	N
1.	8700
2.	8700
3.	8300
4.	10,000

1.	-29%
2.	-23%
3.	-16%
4.	-7%

Standard Flow Cell - 8 µL



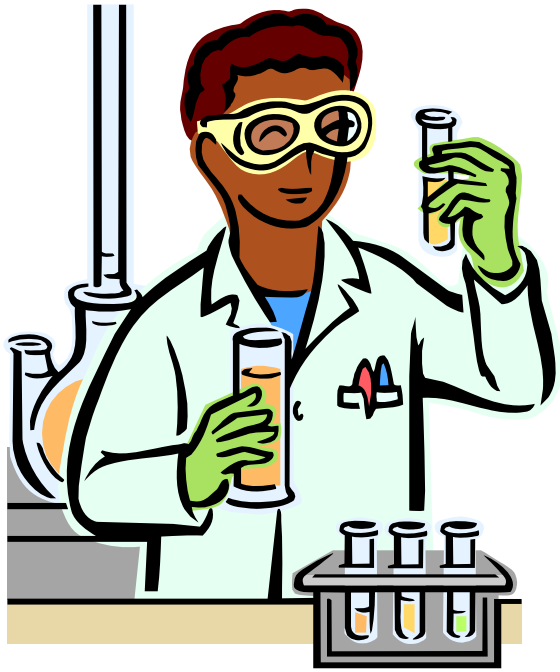
	N
1.	6200
2.	6700
3.	7000
4.	9250

Determining the Cause of Peak Tailing

- Evaluate mobile phase effects - alter mobile phase pH and additives to eliminate secondary interactions
- Evaluate column choice - try column with high purity silica or different bonding technology
- Reduce sample load – volume injection and concentration
- Eliminate extra-column effects – tubing, fittings, Uv cell
- Flush column and check for aging/void

3. Retention Issues

- **Retention time changes (t_r)**
- **Retention factor changes (k')**
- **Selectivity changes (a)**

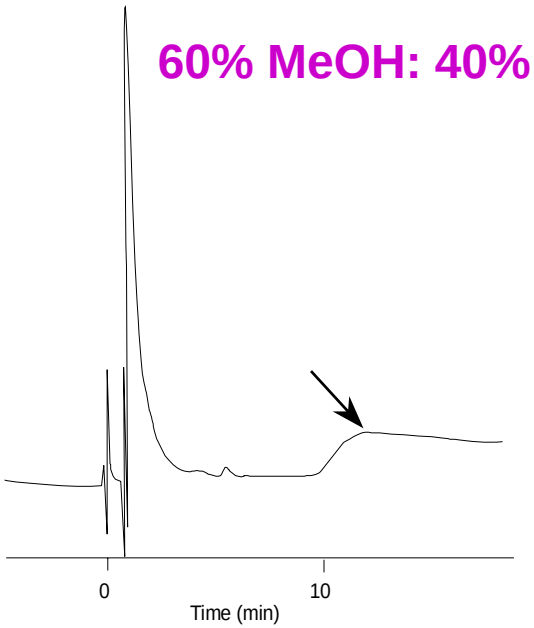


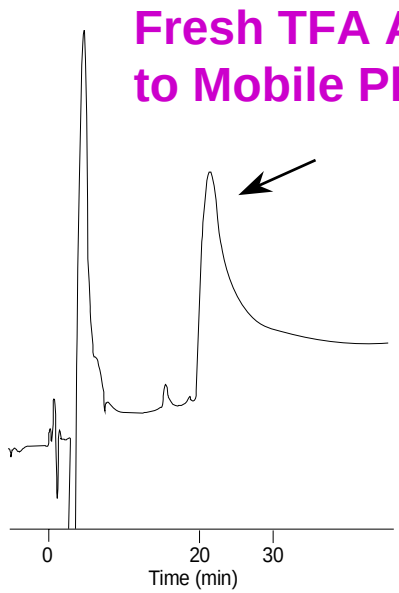
Changes in Retention (k) - Same Column, Over Time

May be caused by:

1. Column aging
2. Column contamination
3. Insufficient column equilibration
4. Poor column/mobile phase combination
5. Change in mobile phase
6. Change in flow rate
7. Change in column temperature
8. Other instrument issues

Mobile Phase Change Causes Change in Retention





- Volatile TFA evaporated/degassed from mobile phase. Replacing it solved problem.
- Chromatography is from a protein binding study and peak shape as expected.

Separation Conditions That Cause Changes in Retention*

Flow Rate	+/- 1%	+/- 1% Tr
Temp	+/- 1 deg C	+/- 1 to 2% Tr
%Organic	+/- 1%	+/- 5 to 10% Tr
pH	+/- 0.01%	+/- 0 to 1% Tr

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

Determining the Cause of Retention Changes
Same Column

1. Determine k', a, and t_r for suspect peaks
2. Wash column
3. Test new column - note lot number
4. Review column equilibration procedures
5. Make up fresh mobile phase and test
6. Check instrument performance

Change in Retention/Selectivity
Column-to-Column

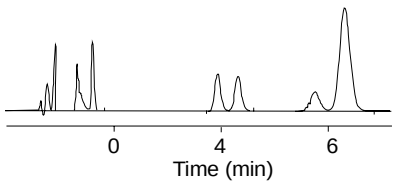
1. Different column histories (aging)
2. Insufficient/inconsistent equilibration
3. Poor column/mobile phase combination
4. Change in mobile phase
5. Change in flow rate
6. Other instrument issues
7. Slight changes in column bed volume (t_r only)

Example Change in Retention/Selectivity

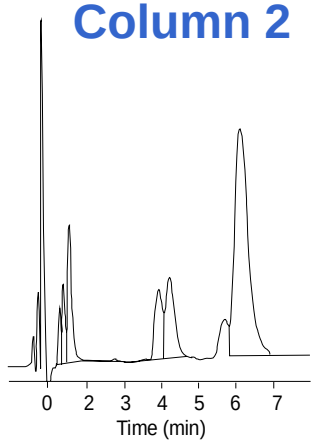
Column-to-Column

Mobile Phase Variation

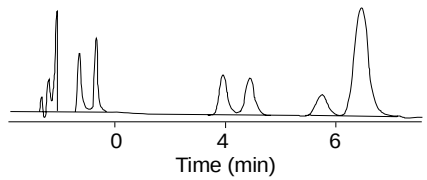
Column 1



Column 2



Column 2 - Fresh mobile phase



"I have experimented with our mobile phase, opening new bottles of all mobile phase components. When I use all fresh ingredients, the problem ceases to exist, and I have narrowed the problem to either a bad bottle of TEA or phosphoric acid. Our problem has been solved."

Determining the Cause of Retention Changes Column-to-Column

1. Determine k', a, and t_r for suspect peaks
2. Test new column - note lot number
3. Determine column history of all columns
4. Review column equilibration procedures
5. Make up fresh mobile phase and test
6. Check instrument performance

Minimize Change in Retention/Selectivity Lot-to-Lot

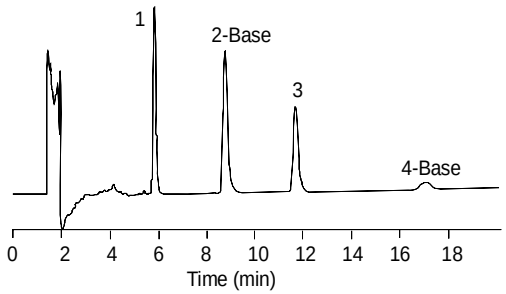
Evaluate:

1. All causes of column-to-column change*
2. Method ruggedness (buffers/ionic strength)
3. pH sensitivity (sample/column interactions)

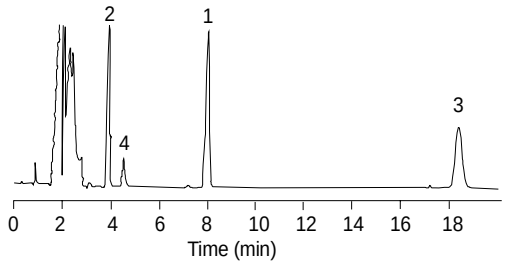
*All causes of column-to-column change should be considered first, especially when only one column from a lot has been tested.

Lot-to-Lot Selectivity Change - pH

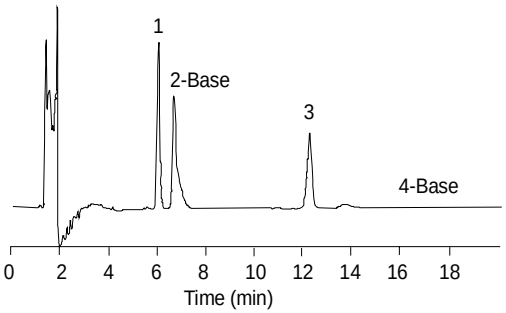
pH 4.5 - Lot 1



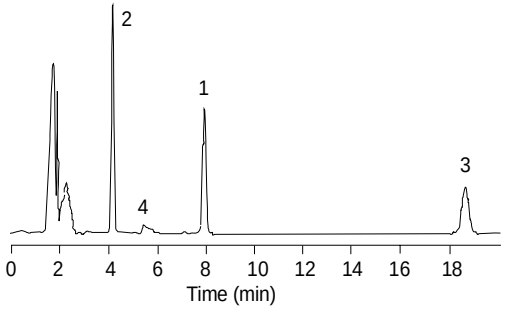
pH 3.0 - Lot 1



pH 4.5 - Lot 2



pH 3.0 - Lot 2



- **pH 4.5 shows selectivity change from lot-to-lot for basic compounds**
- **pH 3.0 shows no selectivity change from lot-to-lot, indicating silanol sensitivity at pH 4.5**
- **Evaluate several pH levels to establish most robust choice of pH**

Evaluate Retention Changes Lot-to-Lot

1. Eliminate causes of column-to-column selectivity change
2. Re-evaluate method ruggedness - modify method
3. Determine pH sensitivity - modify method
4. Classify selectivity changes
5. Contact manufacturer for assistance*

*Agilent Column Support:
800-227-9770, option 4, option 2 (LC columns)

Conclusions:

HPLC column problems are evident as:

1. High pressure
2. Undesirable peak shape
3. Changes in retention/selectivity

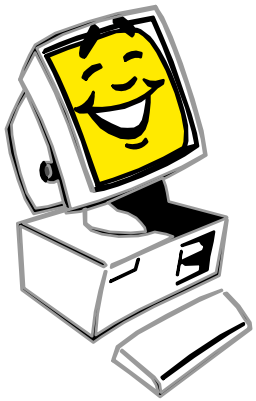
These problems are not always associated with the column and may be caused by instrument and experimental condition issues.

Agilent Technical Support

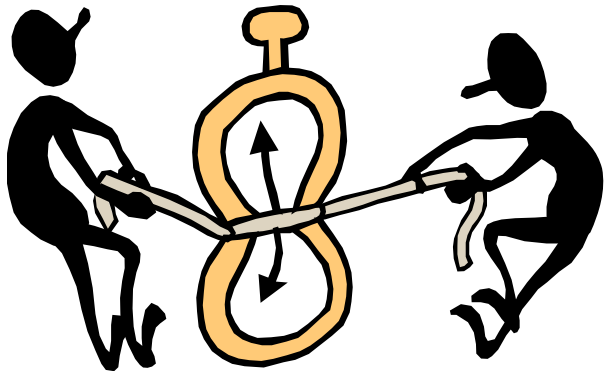
LC or GC Column Support

800-227-9770 (phone: US & Canada)

Select option 4, then option 1 for GC or option 2 for LC.



www.agilent.com/chem



The End – Thank You!

Agilent LC Column Tech Support:
800-227-9770 #4, #2

Appendix

Separation Ruggedness
Buffer Preparation

Dissolve salt in organic-free water in 1- or 2-L beaker. Use appropriate volume to leave room for pH adjustment solution. Equilibrate solution to room temperature for maximum accuracy.

Calibrate pH meter. Use 2-level calibration and bracket desired pH. Use appropriate audit solution to monitor statistical control (for example, potassium hydrogen tartrate, saturated solution, pH = 3.56).

Adjust salt solution to desired pH. Minimize amount of time electrode spends in buffer solution (contamination). Avoid overshoot and readjustment (ionic strength differences can arise).

Transfer pH-adjusted buffer solution quantitatively to volumetric flask, dilute to volume, and mix.

Filter through 0.45 µm filter. Discard first 50 – 100 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

Using Buffers Successfully
Initial Column and System Equilibration

In an appropriate vessel, test highest % organic/buffer ratio to verify that buffer will not precipitate. With stirring, add organic to buffer first, not vice versa.

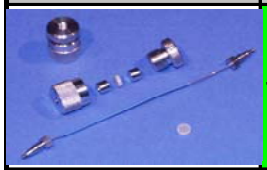
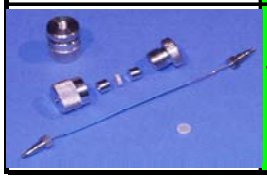
Equilibrate column with, in order:

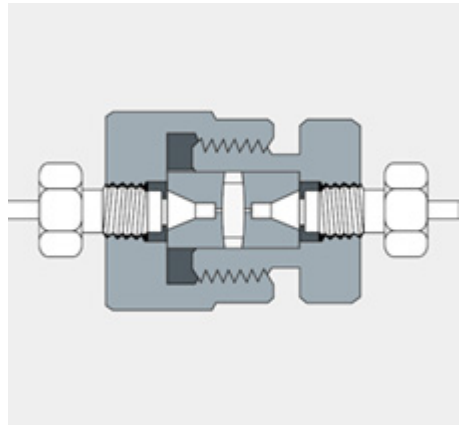
- 100% organic modifier (if brand new)
- mobile phase minus buffer
- buffered mobile phase containing highest % organic modifier (gradient high end)
- buffered mobile phase containing lowest % organic modifier (gradient low end).

Inject standard or sample several times until RTs stable, or for gradient methods, precede former with 1 or 2 blank gradients.

In-Line Filters Provide Good Insurance Against System OverPressure

NEW RRLC In-line filter and fitting – max 600 bar

	Description	Part number	Porosity	Frit diameter	Flow rate	Part number Replacement Frits
	**RRLC In-line filter, 2.1 mm, max 600 bar**	**5067-1551**	0.2 µm	2.1 mm	<1 mL/min	**5067-1555 (10/pk)**
	**RRLC In-line filter, 3.0 & 4.6 mm, max 600 bar**	**5067-1553**	0.2 µm	4.6 mm	1 - 5 mL/min	**5067-1562 (10/pk)**



Protect RRHT columns with efficient in-line filter with 0.2 µm pore size frits

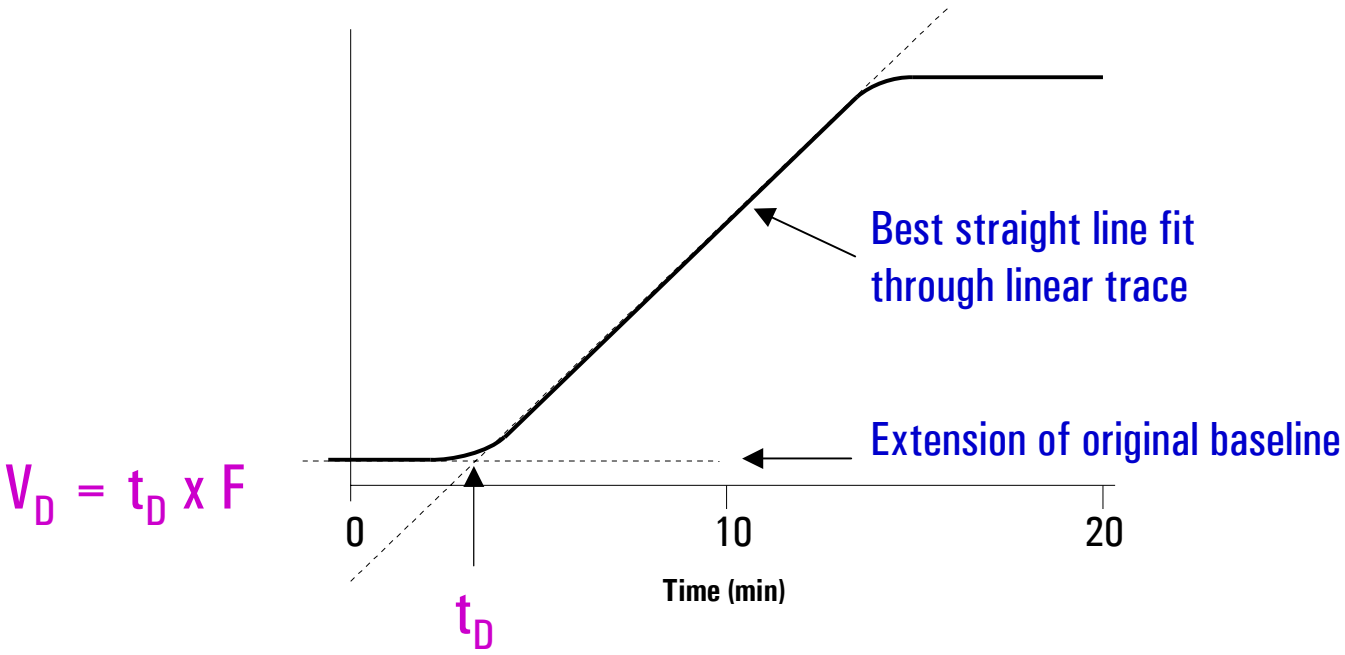
Determining the Dwell Volume of Your system

- ✓ 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 such 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.

Resolution Equation

$$R_S = \frac{\sqrt{N}}{4} \frac{(\alpha - 1)}{\alpha} \frac{k}{(k+1)}$$

α = **Selectivity** – influenced by mobile and stationary phase

N = **Column Efficiency** – influenced by length and particle size

k = **Capacity Factor** (retention) – influenced by stationary and mobile phase, gradient slope and dwell volume (gradients)