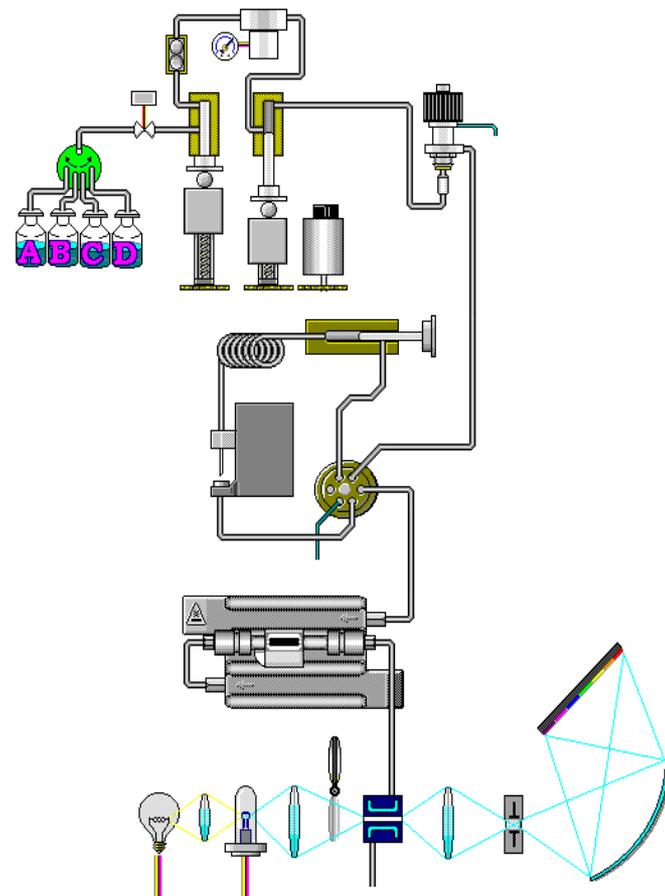
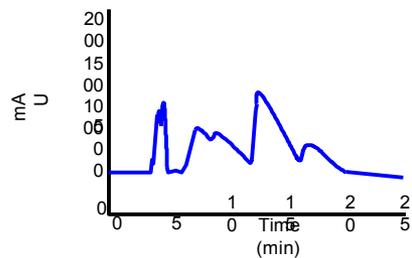
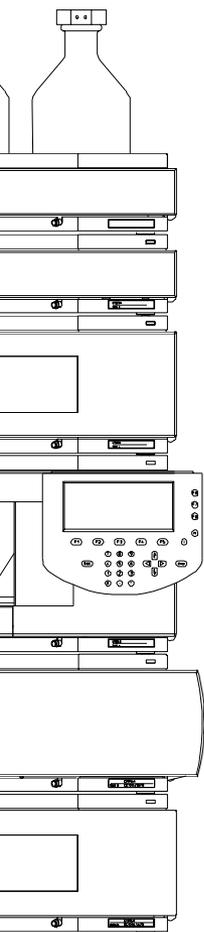


HPLC Column Troubleshooting:

Is It Really The Column?

Agilent Technologies, Inc.
Bill Champion
Application Engineer
February 23, 2011

Troubleshooting in HPLC



HPLC Components

- Pump
- Injector/Autosampler
- Column
- Detector
- Data System/Integrator

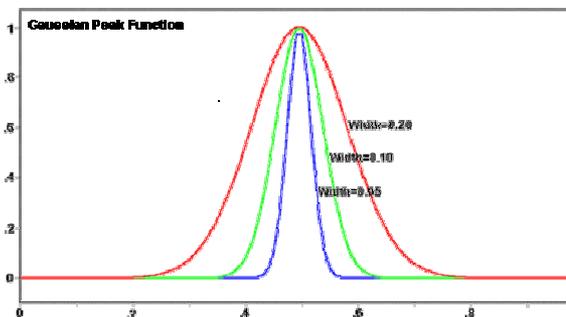
All of these components can have problems and require troubleshooting.

Categories of Column Problems

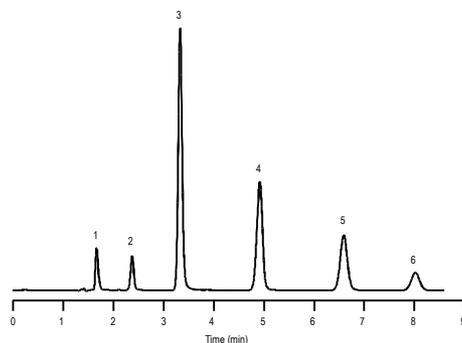
A. Pressure



B. Peak shape



C. Retention



Some Basic Chromatography Parameters

- Retention Factor (k), Capacity Factor (k')
- Selectivity or Separation Factor (α)
- Column Efficiency as Theoretical Plates (N)
- Resolution (R_s)

Retention Factor (k), Capacity Factor (k')

Chromatographic Separation is an Equilibrium Process

Sample Partitions between Stationary Phase and Mobile Phase

$$K = C_s / C_m$$

Compound moves through the column only while in mobile phase.

Separation occurs in Column Volumes.
(Flow is volume/time)

Retention Factor (k), Capacity Factor (k')

$$K = C_s/C_m \Rightarrow \Rightarrow \boxed{k = \frac{t_R - t_0}{t_0}}$$

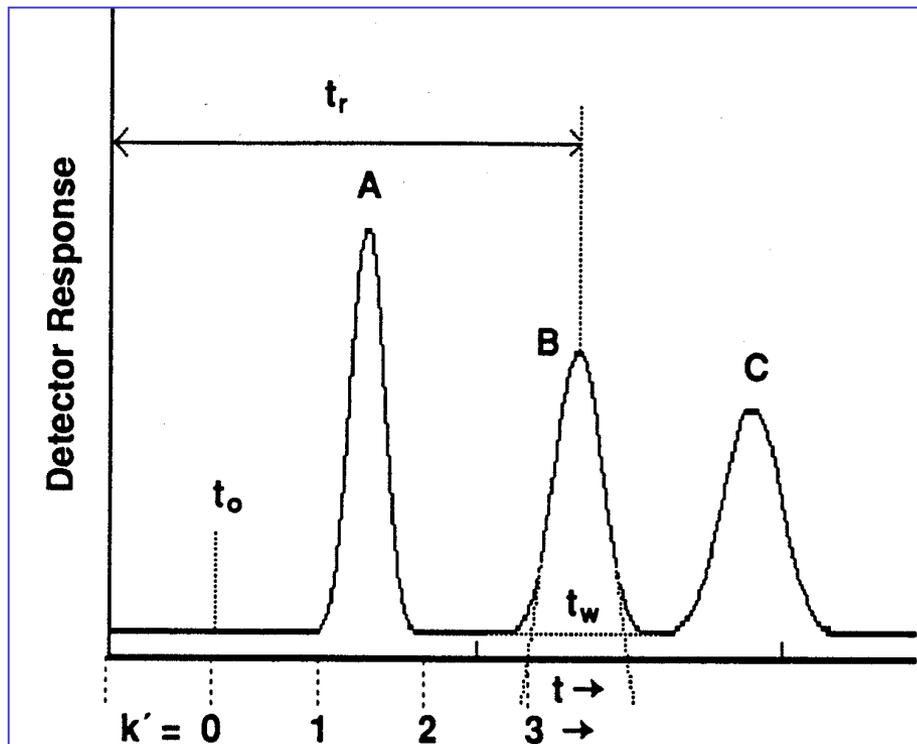
k is measure of number of column volumes required to elute compound.

Fundamental, dimensionless parameter that describes the retention.

$k = \underline{1 \text{ to } 20}$ - OK; $k = \underline{3 \text{ to } 10}$ - Better; $k = \underline{5 \text{ to } 7}$ - Ideal

Chromatographic Profile

Equations Describing Factors Controlling R_s



Retention Factor

$$k = \frac{(t_R - t_0)}{t_0}$$

Selectivity

$$\alpha = k_2 / k_1$$

Theoretical Plates-Efficiency

$$N = 16(t_R / t_w)^2$$
$$= 5.54(t_R / W_{1/2})^2$$

Test Chromatogram

LC Column Performance Report

SERIAL NUMBER: USUXC01613

PART NUMBER: 959963-902

COLUMN TYPE: ZORBAX Eclipse Plus C18 4.6 x 150 mm, 3.5 μ m

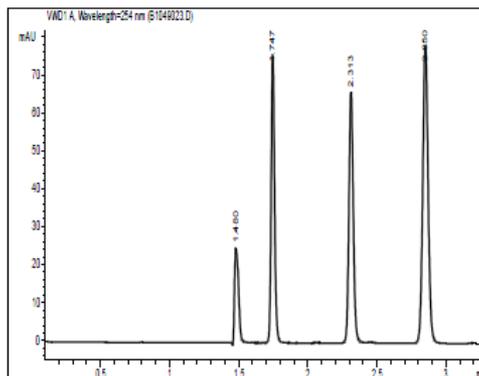
PACKING LOT #: B08022

TEST CONDITIONS

MOBILE PHASE = 85% Methanol / 15% Water
COLUMN PRESSURE = 126.4 Bar
COLUMN FLOW = 1.00 ml / min
LINEAR VELOCITY = 0.168 cm / sec
TEMPERATURE = AMBIENT (Nominally 23 °C)
INJECTION VOLUME = 5 μ l

QUALITY CONTROL PERFORMANCE RESULTS FOR TOLUENE

TEST VALUES	SPECIFICATIONS
THEORETICAL PLATES = 25116	MIN = 18000
SELECTIVITY = 1.65	RANGE = 1.61 - 1.71
USP TAILING FACTOR = 1.07 (@ 5% Peak Height)	RANGE = 0.98 - 1.20
k' = 0.93	



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

Peak #	Conc (ug/ml)	Sample Component
1	5	Uracil
2	200	Phenol
3	25	4-Chloro Nitrobenzene
4	850	Toluene

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

Column Data Sheet



Agilent Poroshell 120 SB-C18 Threaded Column

Data Sheet

General Description

Agilent Poroshell 120 SB-C18 is a superficially porous microparticle column packing. Superficially porous silica particles, such as Poroshell, have a solid silica core and a porous silica outer layer. A StableBond SB-C18 bonded phase is applied to the totally porous outer layer for this column. This type of particle provides high efficiency at lower pressures when compared to small, totally porous particles and is ideal for fast or high resolution separations of many types of analytes.

The Poroshell 120 packing has a solid core of 1.7 μm in size with a porous outer layer 0.5 μm thick and a total particle size of 2.7 μm . The particles have a nominal surface area of 120 m^2/g and a controlled pore size of 120 \AA . The columns can be used up to an operating pressure of 600 bar (9000 psi). The uniform, spherical particles are ultrahigh purity (>99.995% SiO_2) silica. This high purity silica is designed to reduce or eliminate strong adsorption of basic and highly polar compounds.

The StableBond SB-C18 bonded phase is made by chemically bonding a sterically-protected C18 stationary phase to the porous shell of the Poroshell 120 silica support. The densely covered, sterically-protected, diisobutyl-n-octadecylsilane stationary phase is chemically

stable and gives long column life at low pH. Poroshell 120 SB-C18 is a reversed-phase packing that can be used for basic, neutral or acidic samples. It is particularly well suited for use with aggressive low pH mobile phases (for example, $\text{pH} < 2$, high ionic strength (> 25 mM), ion-pair additives, etc.) since the steric protection of the bonded phase resists degradation with such mobile phases. The recommended high temperature limit for this bonded phase is 90 $^\circ\text{C}$ at low pH.

Column Characteristics

A typical Quality Control test chromatogram for a Poroshell 120 SB-C18, 4.6 mm \times 50 mm, 2.7 μm threaded column is shown in Figure 1. The actual QC test and performance of your column is described on the Column Performance Report enclosed with your column. The efficiency reported on the Column Performance Report may be higher than the efficiency found in your laboratory. The QC test system may vary from the LC used in your lab and has been modified from a standard system to minimize system volume. This allows a better evaluation of the packed column and assures a more consistent product for the chromatographer.

Safety Considerations

All points of connection in liquid chromatographic systems are potential sources of leaks. Users of LCs and UHPLCs should be aware of the toxicity or flammability of their mobile phases.

These Poroshell 120 columns are mechanically stable and have been tested to very high pressures to assure safe lab operation on a variety of LC and UHPLC instruments. The operating pressure limit for all 2.1-, 3.0- and 4.6-mm id columns is 600 bar (9000 psi). While the 2.1- and 3.0-mm id columns are safe to 1300 bar (20,000 psi) and the 4.6-mm id columns are safe to 1000 bar (16,000 psi), chromatographic performance will be compromised if the 600 bar pressure limit is exceeded and the column may need to be replaced.

Because of its small particle size, dry Poroshell packings are respirable. Columns should only be opened in a well-ventilated area, and opening the column will compromise column performance.

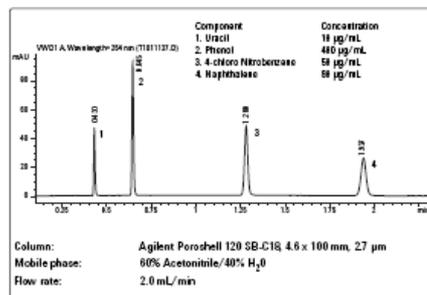


Figure 1. Agilent Poroshell 120 SB-C18 chromatogram.

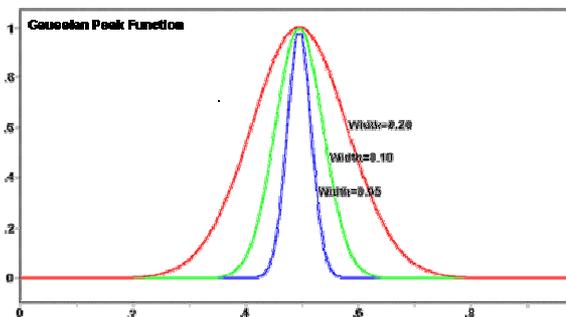


Categories of Column Problems

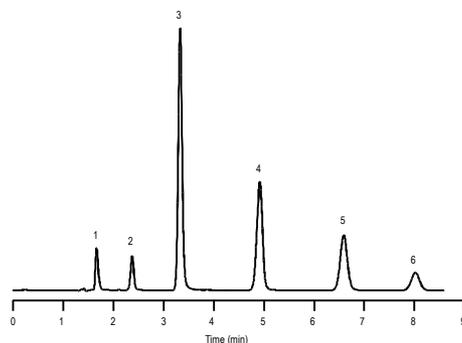
A. Pressure



B. Peak shape



C. Retention



What About Pressure?

Pressure Increases with Decreasing Particle Size

Equation For Pressure Drop Across an HPLC Column

$$\Delta P = \frac{\eta \cdot L \cdot v}{\theta \cdot d_p^2}$$

ΔP = Pressure Drop

η = Fluid Viscosity

L = Column Length

v = Flow Velocity

d_p = Particle Diameter

θ = Dimensionless Structural Constant of Order 600 For Packed Beds in LC

- ✓ Many parameters influence column pressure
- ✓ Particle size and column length are most critical
- ✓ Long length and smaller particle size mean more resolution and pressure
- ✓ We can now handle the pressure

Pressure Issues

Observation

Large pressure change

Potential Problems

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

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 $10 \times V_m$ 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*

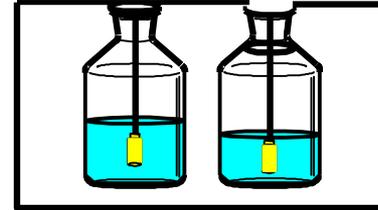
* When using either Hexane or Methylene Chloride the column must be flushed with Isopropanol before returning to your reversed-phase mobile phase.

Column Cleaning:

Normal Phase Solvent Choices In Order of Increasing Strength

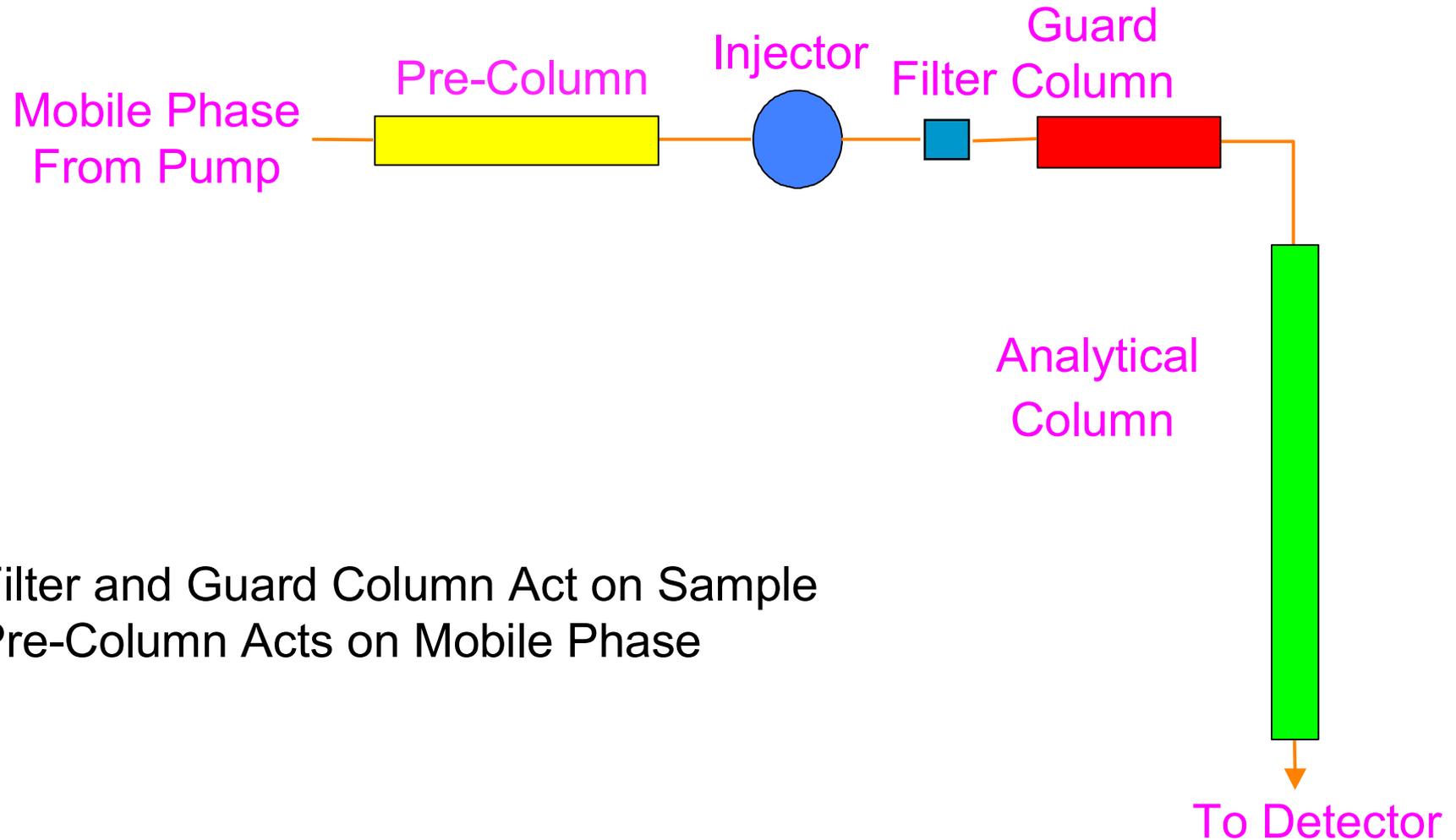
- Use at least 50 mL of each solvent
- 50% Methanol : 50% Chloroform
- 100% Ethyl Acetate

Preventing Column Back Pressure Problems



- Filter mobile phase:
 - Non-HPLC grade solvents
 - Buffer solutions
- Install an in-line filter between auto-sampler and column
 - Use 2 μm frit for 3.5 μm columns, use 0.5 μm frit for 1.8 μm columns.
- Filter all samples and standards
- Perform sample clean-up (i.e. SPE, LLE) on dirty samples.
- Appropriate column flushing –
 - Flush buffers from entire system at end of day with water/organic mobile phase
- Use Mobile Phase Miscible Sample Solvents

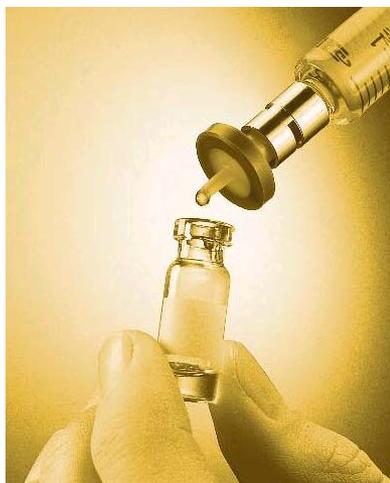
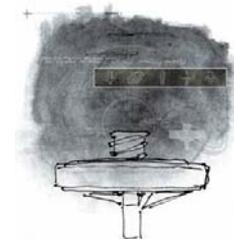
Preventing Back Pressure Problems: In-Line Devices



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

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

Mini-UniPrep Syringeless Filters

Mini-UniPrep Syringeless Filters are preassembled filtration devices for removing particulate matter from samples.

A single disposable unit can replace the combination of syringe filters, syringes, auto-sampler vials, transfer containers, septa and caps.

Mini-UniPrep provides a quick, economical and environmentally conservative way to filter samples prior to HPLC analysis.

Manufactured by Whatman, a division of GE Healthcare



Key Reminders

1. As column particle size shrinks, column frit porosity is reduced
 - 5 μ m - 2 μ m frit \diamond 3-3.5 μ m - 0.5 μ m-2 μ m frit \diamond 1.8 μ m - 0.2 μ m frit
2. Mobile phase filtering reduces wear on instrument parts (Check valves, Piston seals, Autosampler)
3. Sample filtering reduces wear on instrument and prevents column plugging due to particulates

A Little Prevention Reduces Downtime and Maintenance Costs

Biological Samples

You should get many thousands of injections for a clean sample – for a “messy” sample you may be lucky to get a hundred

1. Can contain proteins, lipids
2. Components can foul column
3. Mandatory to remove these components from the sample
4. Requires routine and preventative column cleaning
5. Larger particles - 3.5 μm or 2.7 μm (Poroshell) – are more forgiving than sub-2 μm

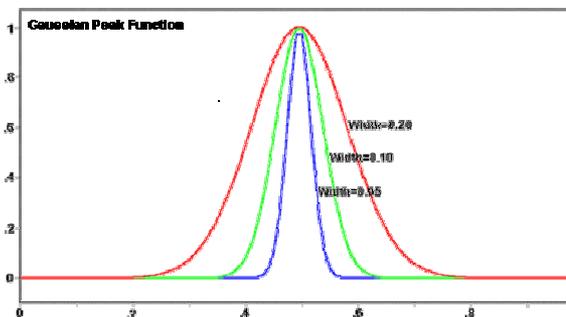
Ronald E. Majors , “The Cleaning and Regeneration of Reversed-Phase HPLC Columns”, LCGC Vol 21(1) p19, 2003.

Categories of Column Problems

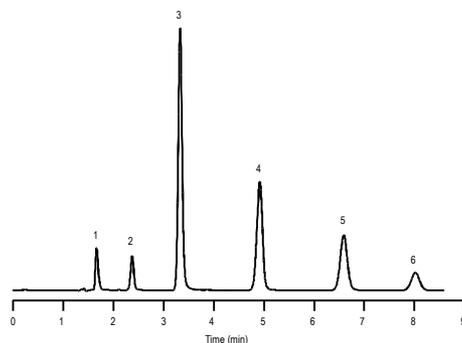
A. Pressure



B. Peak shape



C. Retention



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
- Detector/Data System Overload

Determining the Cause of Split Peaks

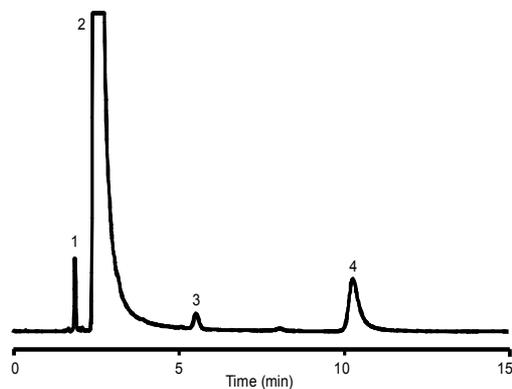
- 1. Complex sample matrix or many samples analyzed - likely column contamination or partially plugged column frit.**
- 2. Mobile phase pH > 7 - likely column void due to silica dissolution (unless specialty column used, Zorbax Extend-C18 stable to pH 11)**
- 3. Injection solvent stronger than mobile phase - likely split *and* broad peaks, shape dependent on injection volume and k value.**

Split Peaks

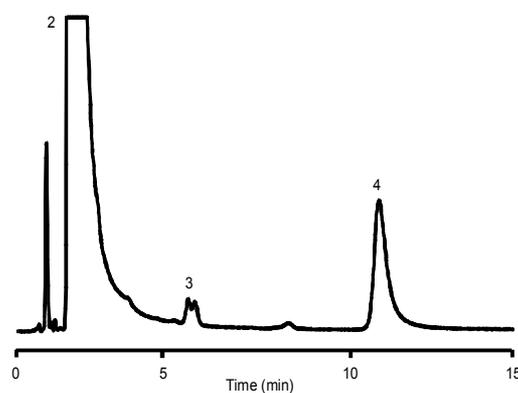
Column Contamination

Column: StableBond SB-C8, 4.6 x 150 mm, 5 μ m Mobile Phase: 60% 25 mM Na₂HPO₄, 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

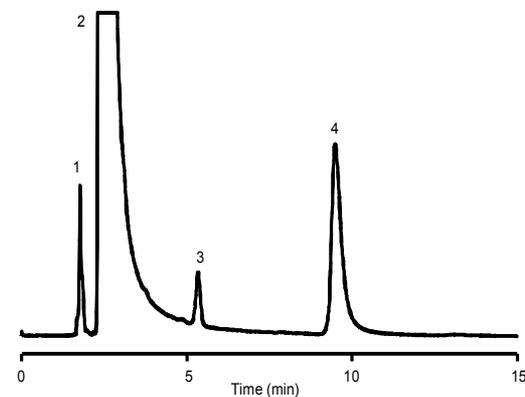
Injection 1



Injection 30



Injection 1 After Column Wash with 100% ACN



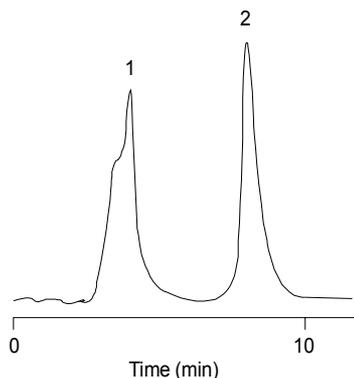
- Column washing eliminates the peak splitting, which resulted from a contaminant on the column

Split Peaks

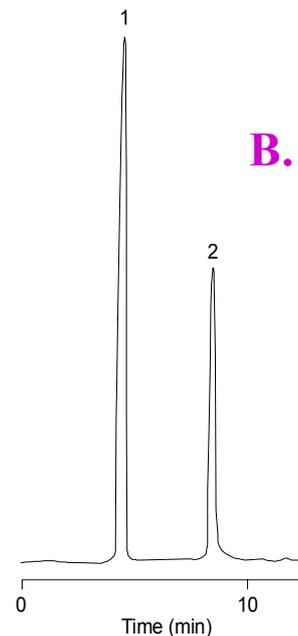
Injection Solvent Effects

Column: StableBond SB-C8, 4.6 x 150 mm, 5 μ m ; Mobile Phase: 82% H₂O :18% ACN;
Injection Volume: 30 μ L 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 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**

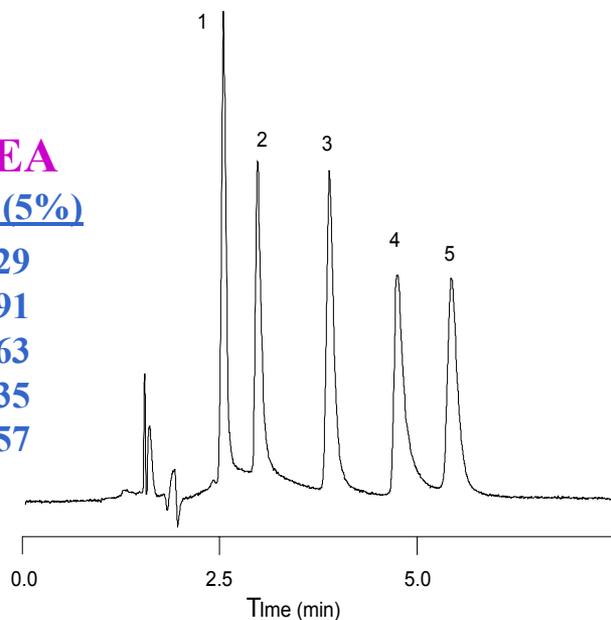
Peak Tailing

Column “Secondary Interactions”

Column: Alkyl-C8, 4.6 x 150 mm, 5 μ m Mobile Phase: 85% 25 mM Na₂HPO₄ pH 7.0 : 15% ACN
Flow Rate: 1.0 mL/min Temperature: 35°C Sample: 1. Phenylpropanolamine 2. Ephedrine 3. Amphetamine 4. Methamphetamine 5. Phenteramine

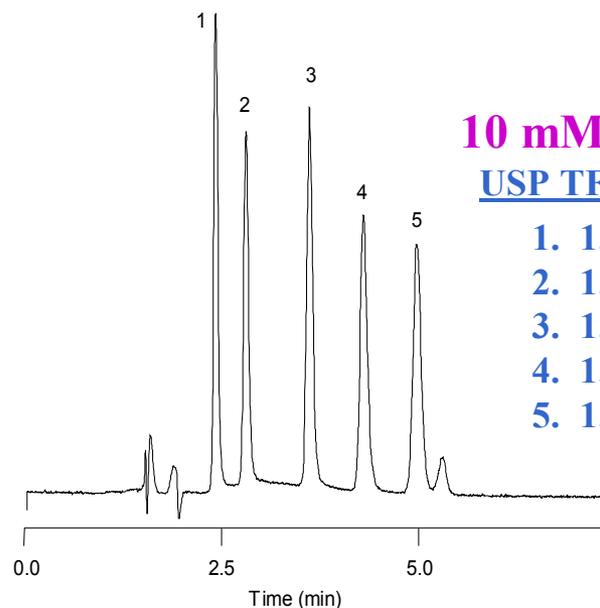
No TEA
USP TF (5%)

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

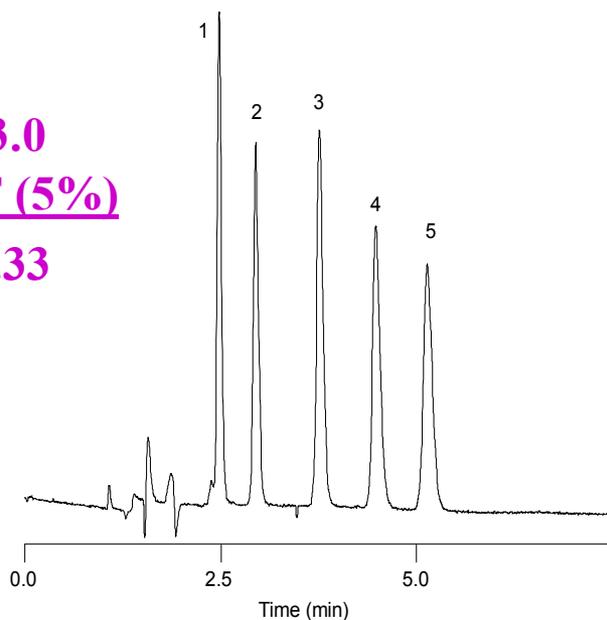
Peak Tailing

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Temperature: 35°C Sample: 1. Phenylpropanolamine 2. Ephedrine 3. Amphetamine 4. Methamphetamine 5. Phenteramine

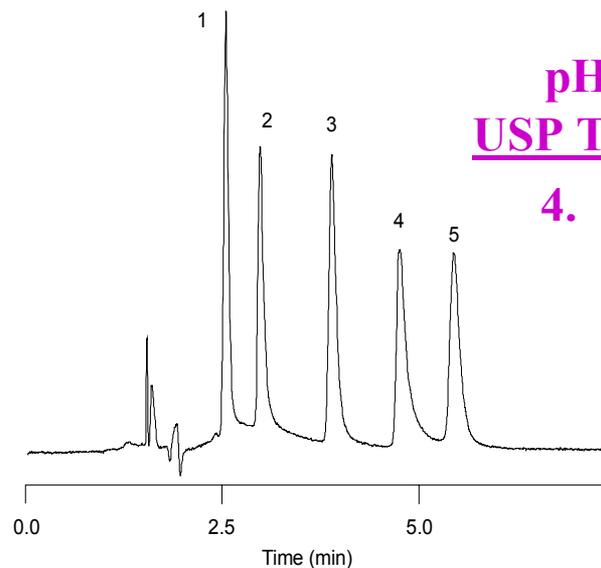
pH 3.0
USP TF (5%)

4. 1.33



pH 7.0
USP TF (5%)

4. 2.35



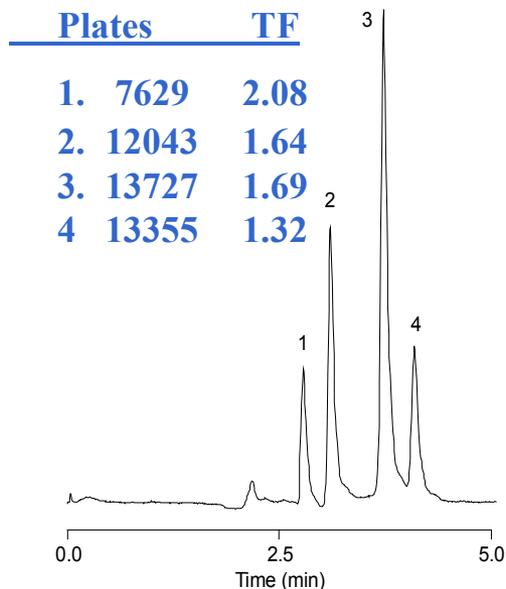
- Reducing the mobile phase pH reduces interactions with silanols that cause peak tailing. No TEA modifier required.

Peak Tailing

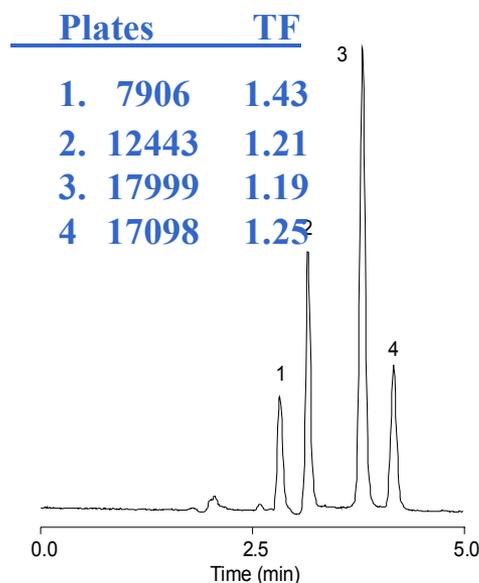
Column Contamination

Column: StableBond SB-C8, 4.6 x 250 mm, 5 μ m Mobile Phase: 20% H₂O : 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

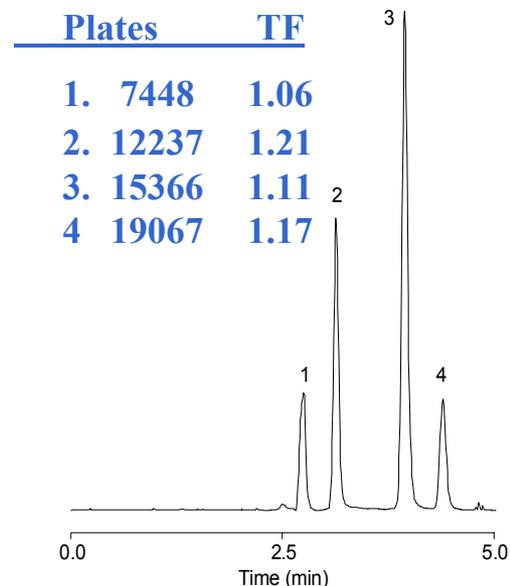
QC test forward direction



QC test reverse direction



QC test after cleaning 100% IPA, 35°C

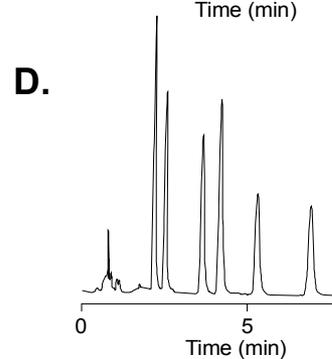
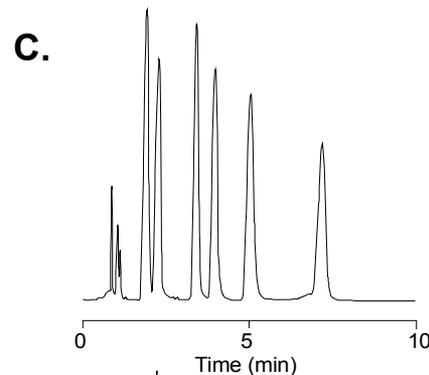
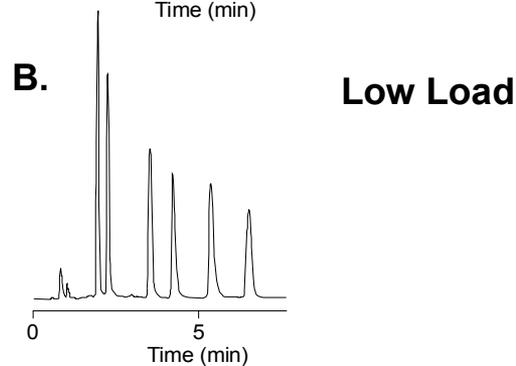
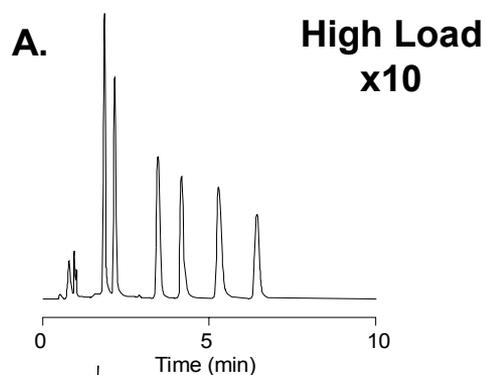


Peak Tailing/Broadening Sample Load Effects

Columns: 4.6 x 150 mm, 5 μ m Mobile Phase: 40% 25 mM Na₂HPO₄ 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%)

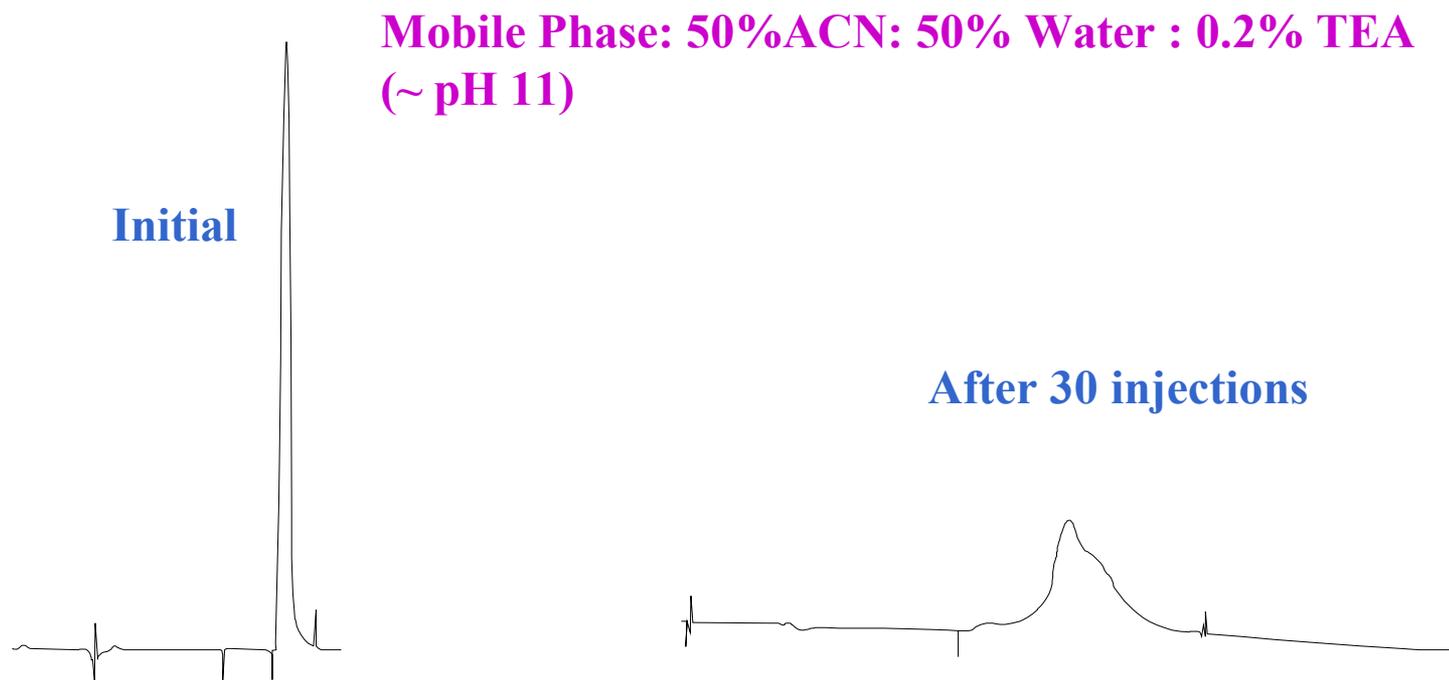
	<u>A</u>	<u>B</u>
1.	1.60	1.70
2.	2.00	1.90
3.	1.56	1.56
4.	2.13	1.70
5.	2.15	1.86
6.	1.25	1.25



Broadening
 Competitive C8
 Plates

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

Peak Broadening, Splitting Column Void

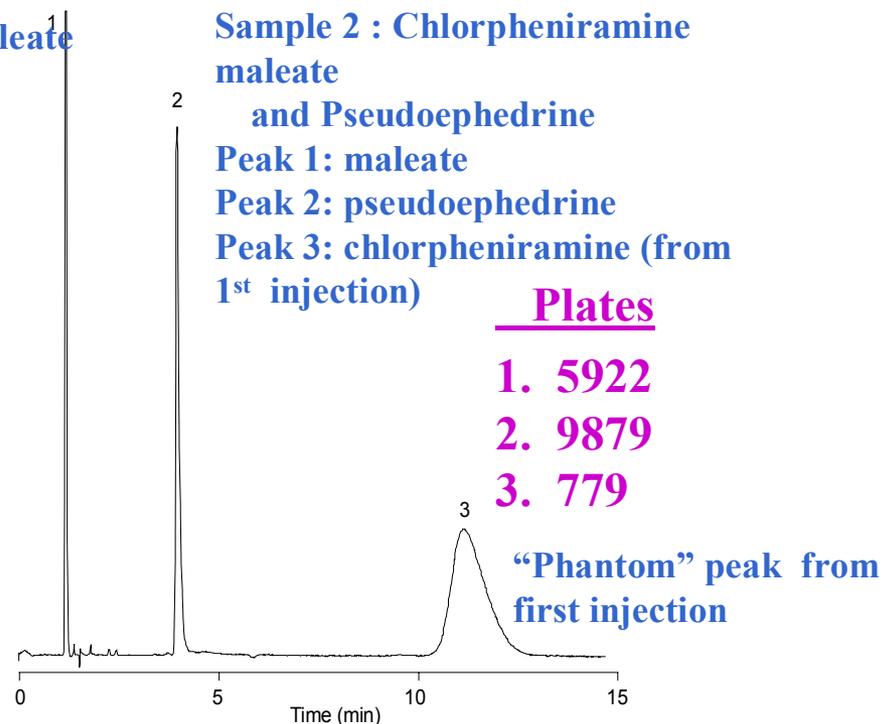
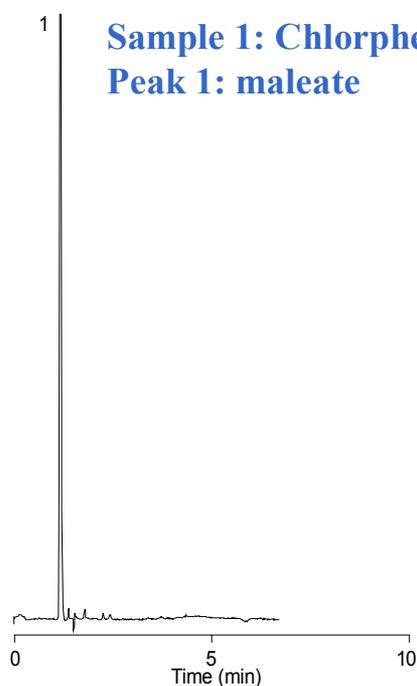


- Multiple peak shape changes can be caused by the same column problem. In this case a void resulted from silica dissolved at high pH.

Broad Peaks

Unknown “Phantom” Peaks

Column: Extend-C18, 4.6 x 150 mm, 5 μ m 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



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

Peak Tailing

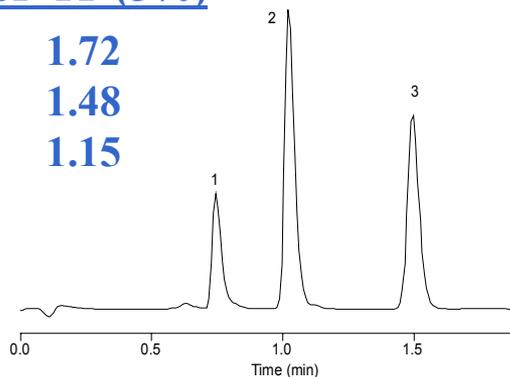
Injector Seal Failure

Column: Bonus-RP, 4.6 x 75 mm, 3.5 μ m Mobile Phase: 30% H₂O : 70% MeOH Flow Rate: 1.0 mL/min
Temperature: R.T. Detection: UV 254 nm Sample: 1. Uracil 2. Phenol 3. N,N-Dimethylaniline

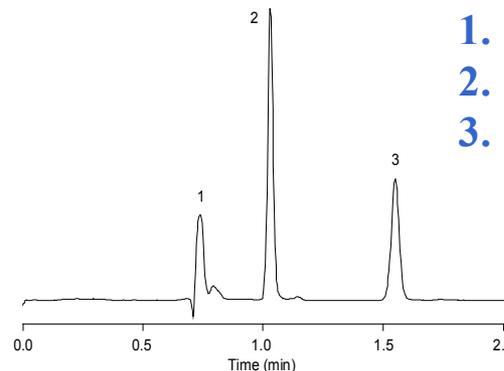
Before

After replacing rotor seal
and isolation seal

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

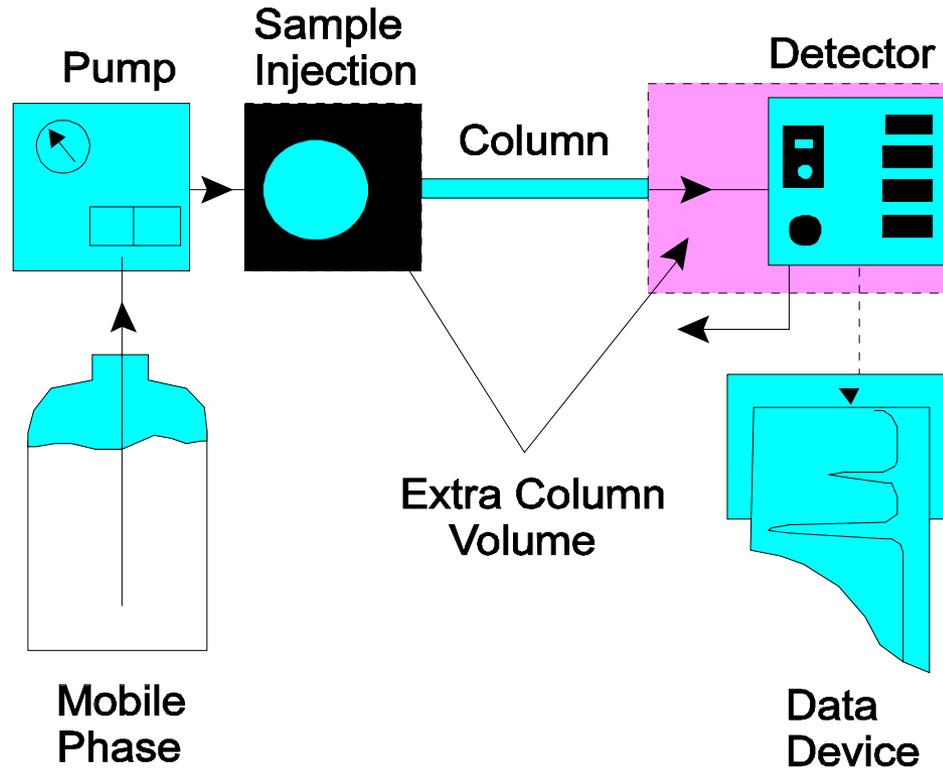


	<u>Plates</u>	<u>USP TF (5%)</u>
1.	3670	1.45
2.	10457	1.09
3.	10085	1.00



- Overdue instrument maintenance can sometimes cause peak shape problems.

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

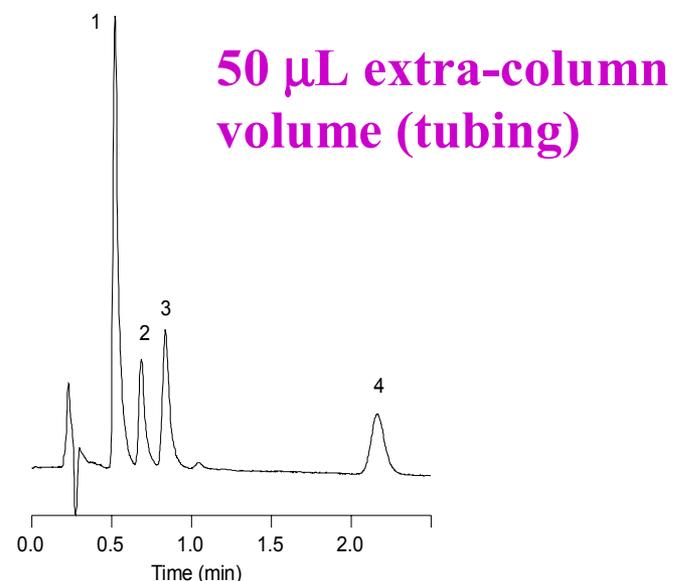
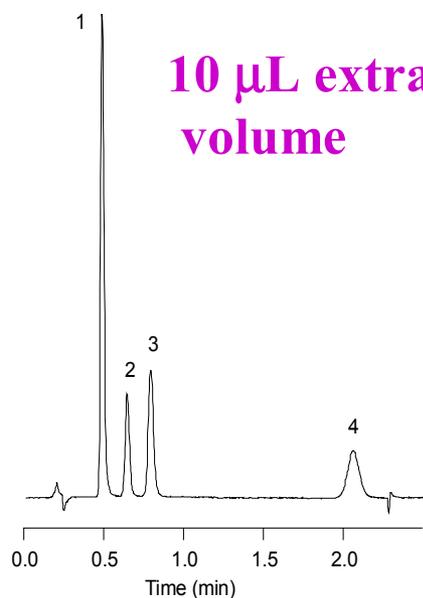
✓ Behaves as isocratic hold at the beginning of gradient

ECV= sample vol. + connecting tubing + fitting + detector cell

Peak Tailing

Extra-Column Volume

Column: StableBond SB-C18, 4.6 x 30 mm, 3.5 μ m Mobile Phase: 85% H₂O 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

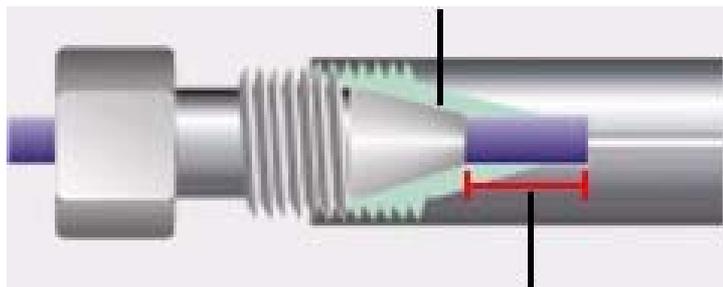


Peak tailing/fronting

What Happens If the Connections Poorly Made ?

Wrong ... too long

Ferrule cannot seat properly



If Dimension X is too long, leaks will occur

Wrong ... too short

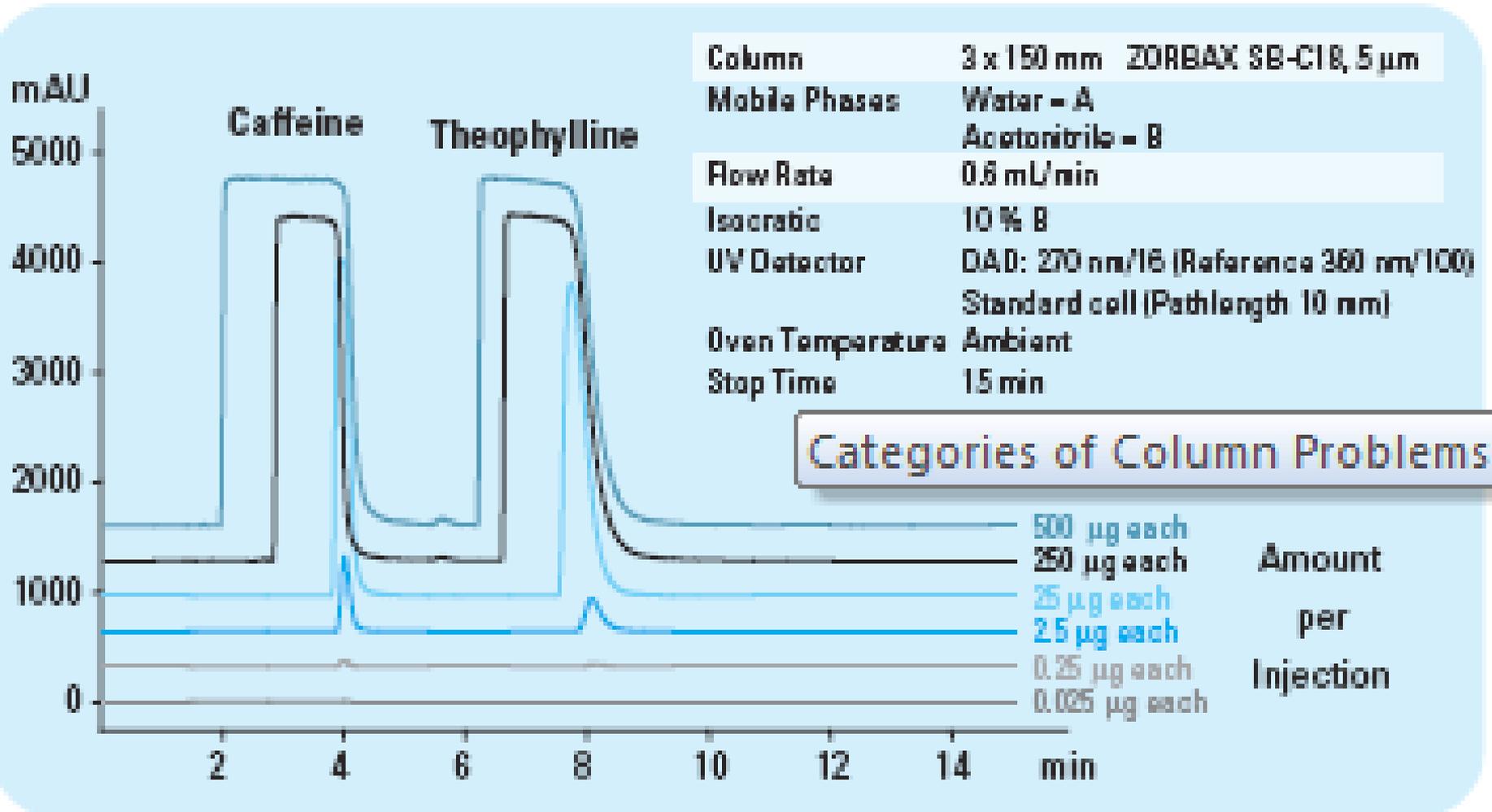


If Dimension X is too short, a dead-volume, or mixing chamber, will occur

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 – vol inj and concentration**
- **Eliminate extra-column effects**
 - tubing, fittings, UV cell
- **Flush column and check for aging/void**

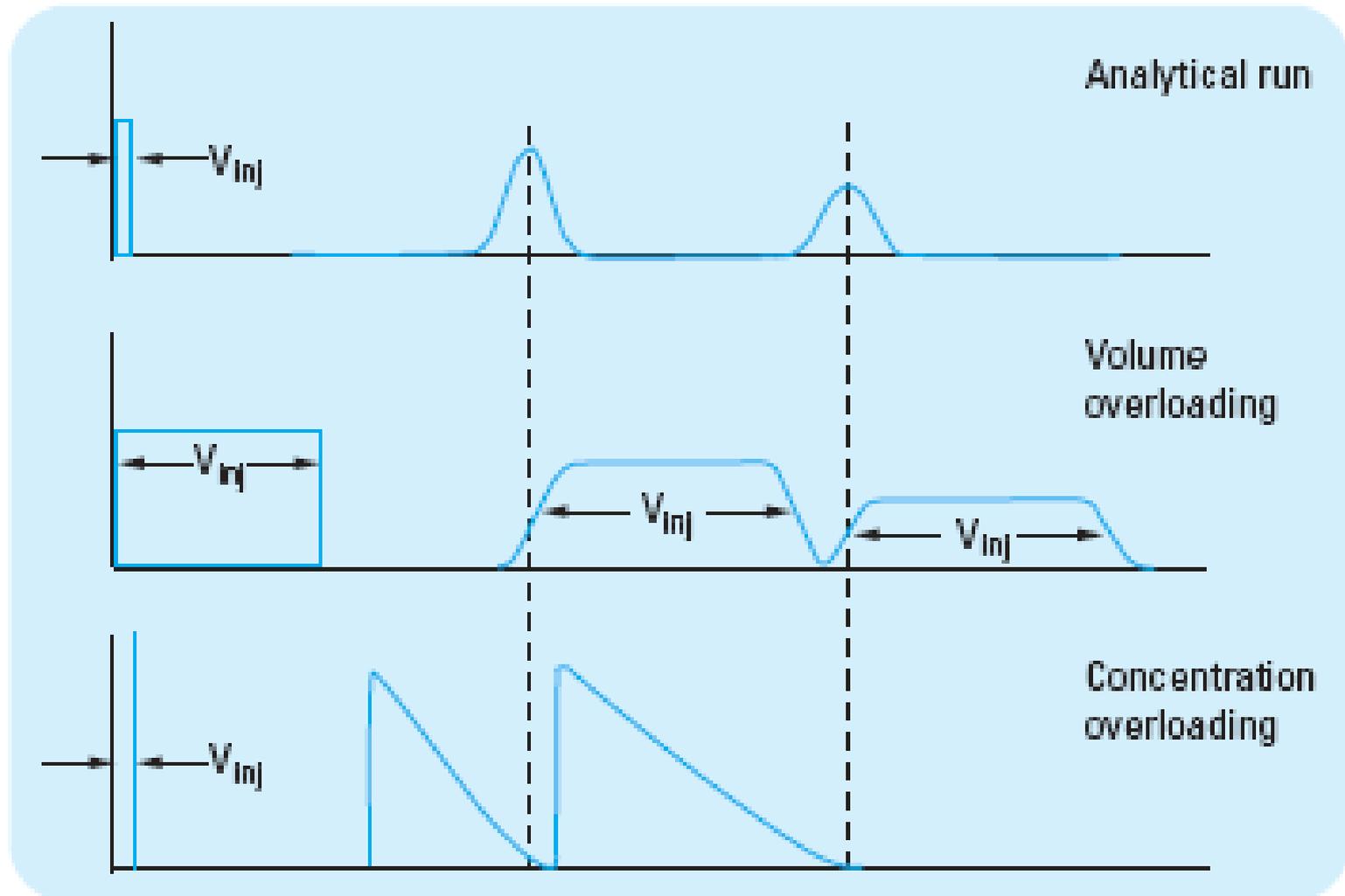
Column Overload



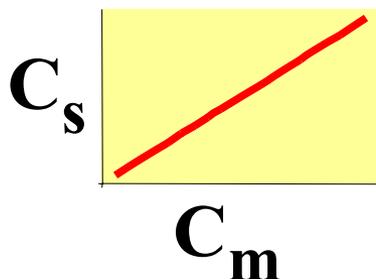
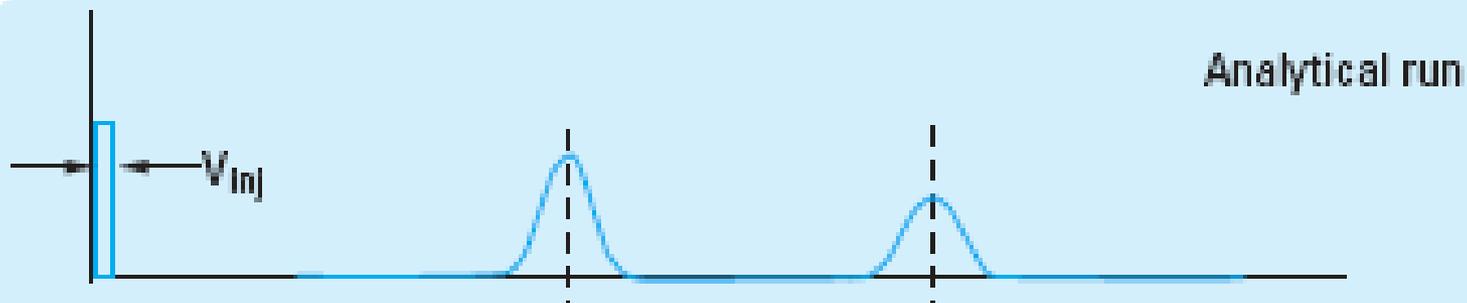
Column 3 x 150 mm ZORBAX SB-C18, 5 µm
Mobile Phases Water = A
 Acetonitrile = B
Flow Rate 0.6 mL/min
Isocratic 10% B
UV Detector DAD: 270 nm/16 (Reference 260 nm/100)
 Standard cell (Pathlength 10 nm)
Oven Temperature Ambient
Stop Time 15 min

U. Huber and R. E. Majors "Principles in preparative HPLC", (2007) 5989-6639EN

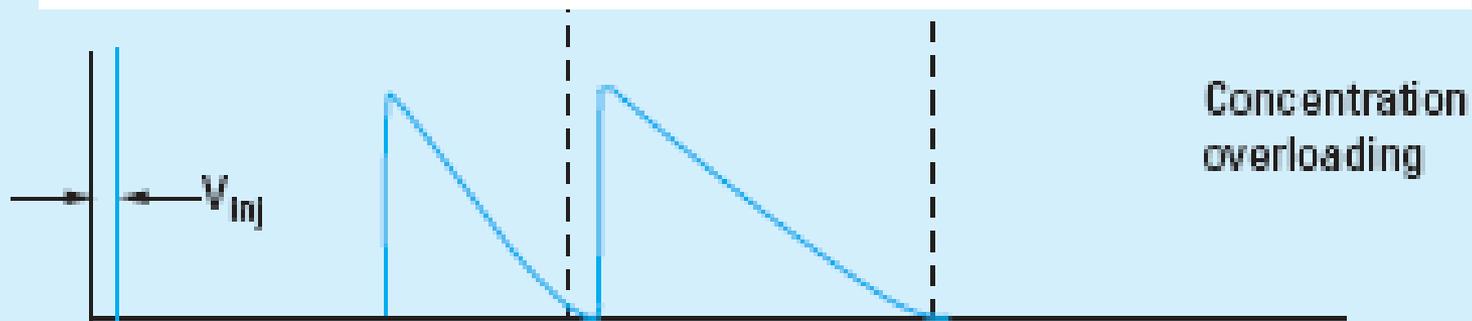
Column Overload



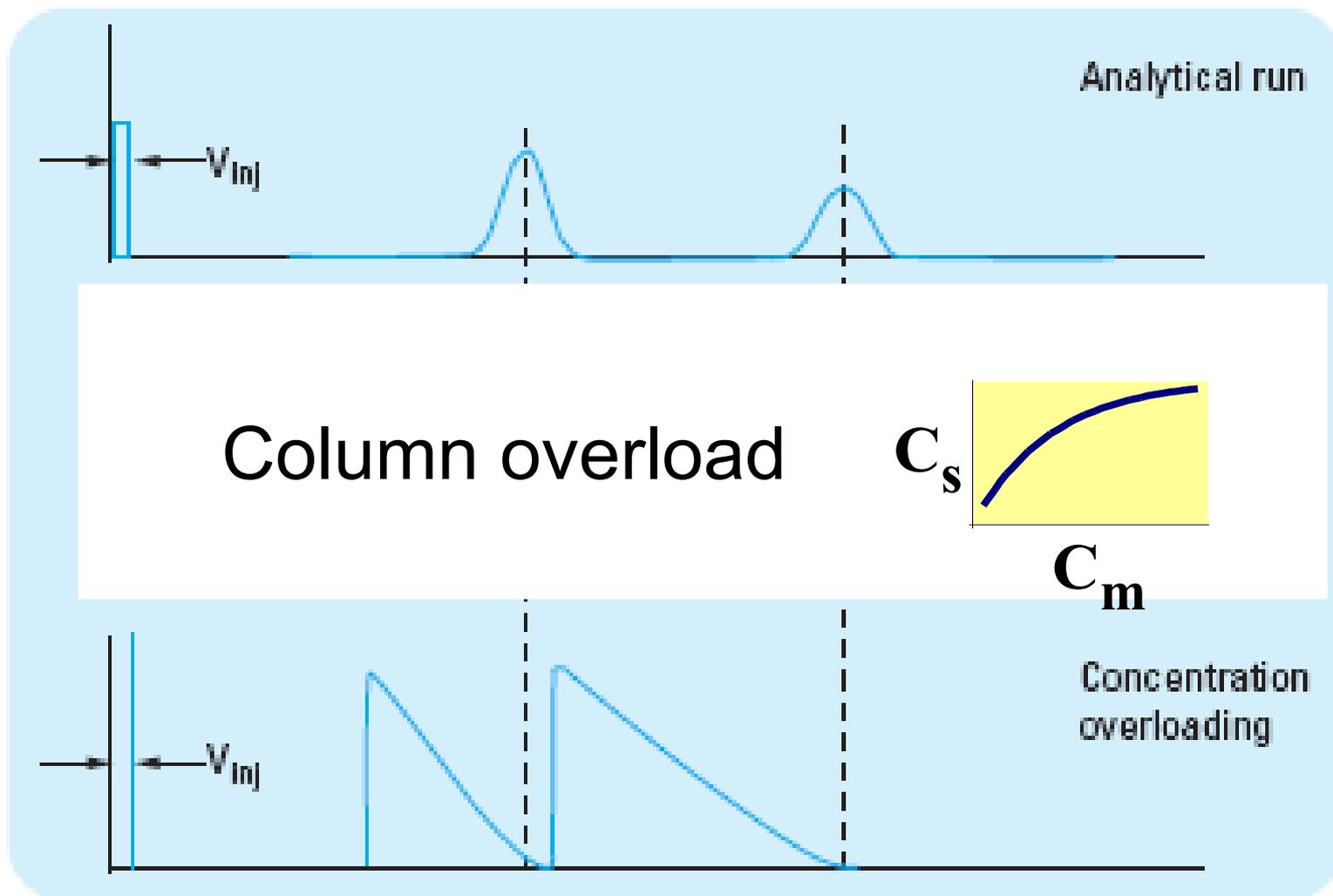
Column Overload - Isotherms



linear isotherm,
 $K = C_s / C_m$



Column Overload - Isotherms

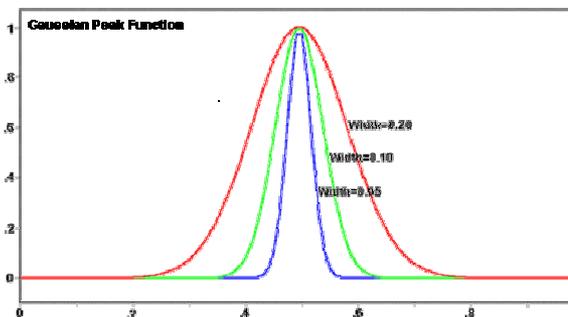


Categories of Column Problems

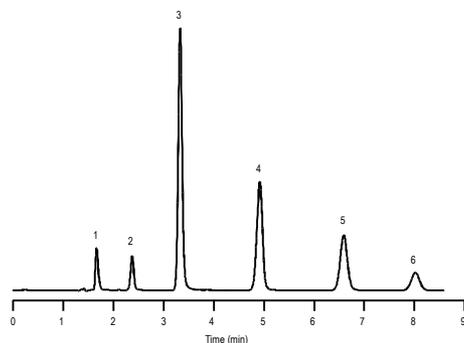
A. Pressure



B. Peak shape



C. Retention



Retention Issues

- Retention time changes (t_r)
- Retention factor changes (k')
- Selectivity changes (α)

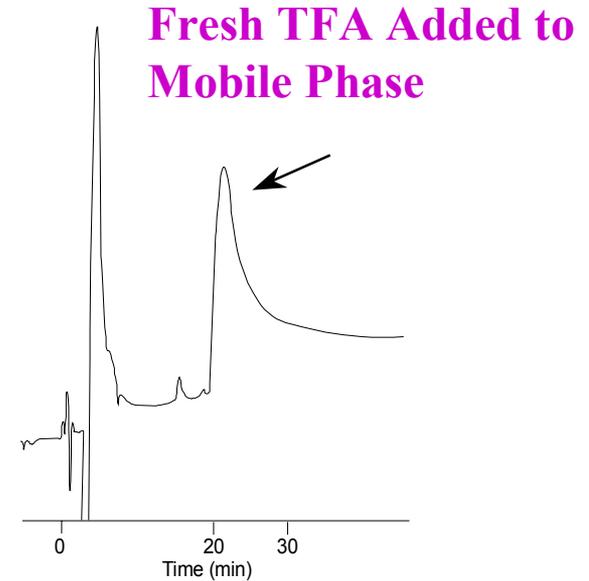
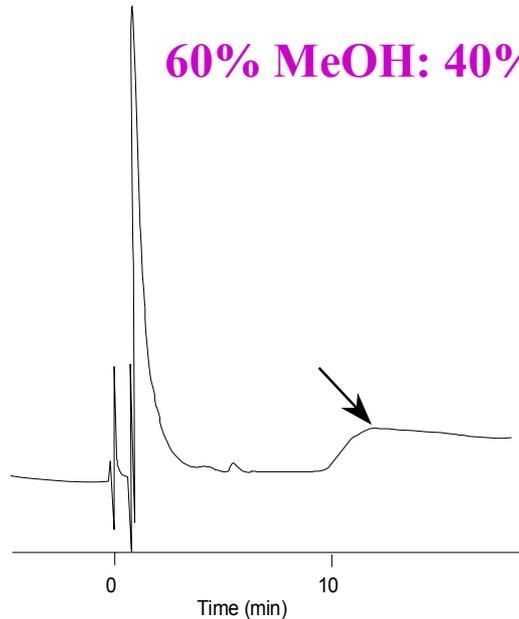


Changes in Retention (k) Same Column, Over Time

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

***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' , α , 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**

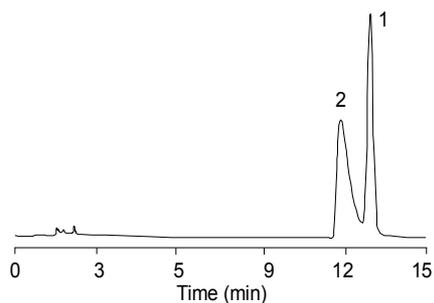
Change in Retention/Selectivity

Column-to-Column

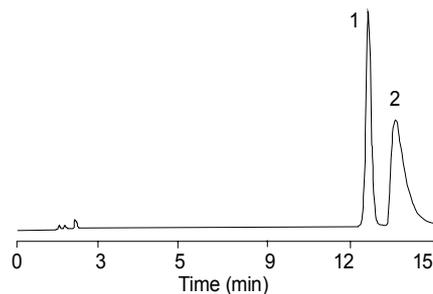
1. Different column histories (aging)
2. Insufficient/inconsistent equilibration
3. Poor column/mobile phase combination
4. Change in mobile phase
5. Change in flow rate
6. Other instrument issues
7. Slight changes in column bed volume (t_r only)

Column Aging/Equilibration Causes Retention/Selectivity Changes

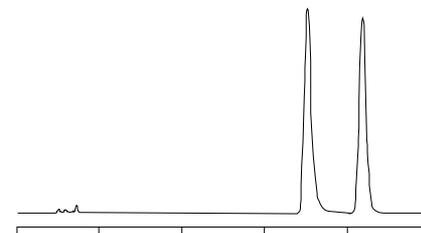
Column 1 - Initial



Column 1 - Next Day



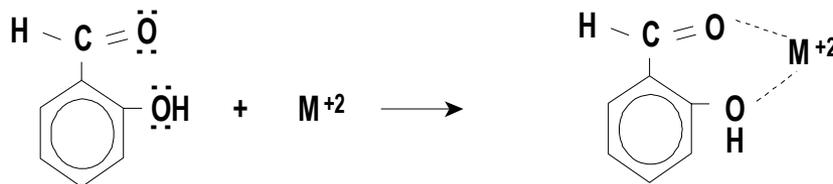
Column 1 - After wash with 1% H₃PO₄/Equilibration



- The primary analyte was sensitive to mobile phase aging/conditioning of the column
- The peak shape was a secondary issue (metal chelating compound) resolved by “de-activating” the active metal contamination

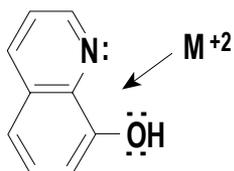
Metal Sensitive Compounds Can Chelate

Hint: Look for O or N Which Can Form 5 or 6 Membered Ring with Metal

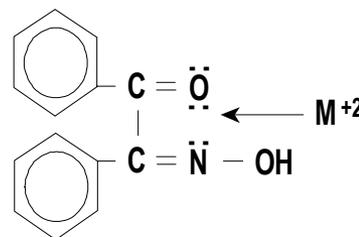


Salicylaldehyde

6-membered ring complex



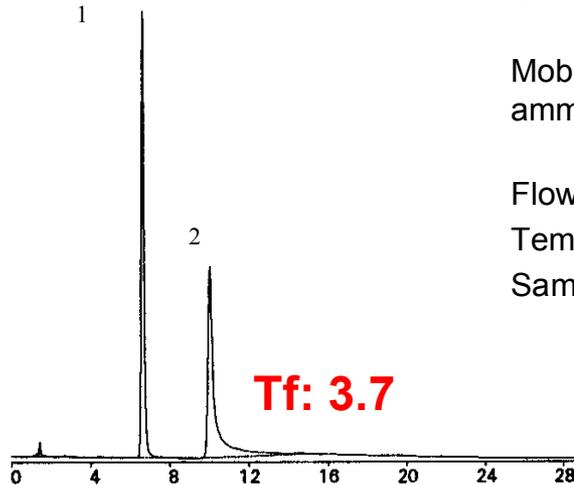
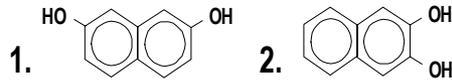
8-hydroxyquinoline
5-membered ring complex



α -benzoinoxime
5-membered ring complex

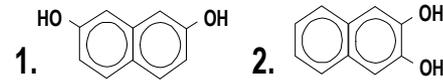
Acid Wash Can Improve Peak Shape

Before Acid Wash



Tf: 3.7

After Acid Wash
50 – 100 mLs 1% H₃PO₄



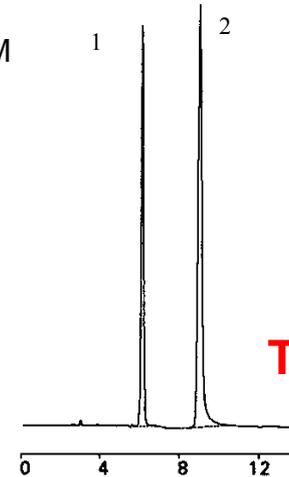
Columns: **ZORBAX SB-Phenyl**
4.6 x 150 mm

Mobile Phase: 75% 25 mM
ammonium phosphate buffer
25% ACN

Flow Rate: 1.0 mL/min.

Temperature: RT

Sample Size: 5 mL

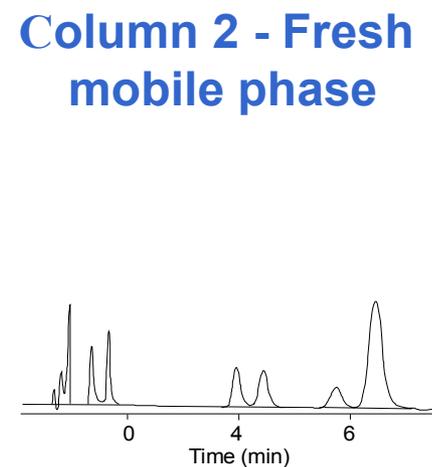
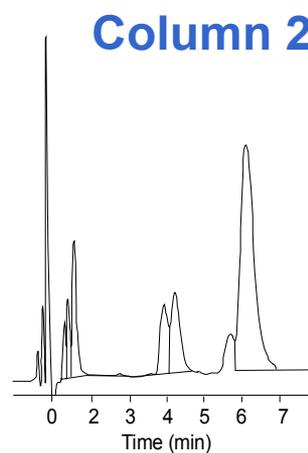
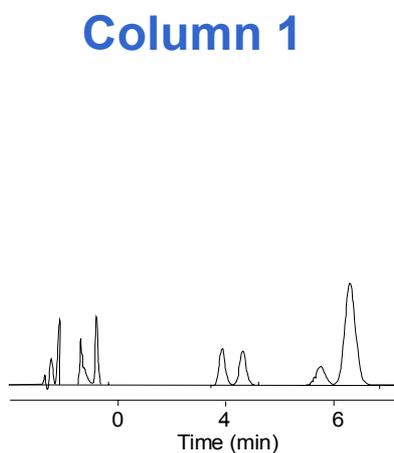


Tf: 1.2

- A 1% H₃PO₄ solution is used on SB columns, 0.5 % can be used on endcapped columns.

Example Change in Retention/Selectivity

Column-to-Column Mobile Phase Variation



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

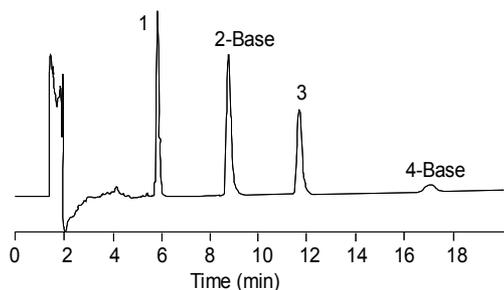
Evaluate:

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

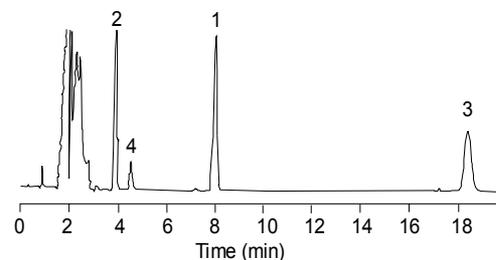
*All causes of column-to-column change should be considered first, especially when only one column from a lot has been tested.

Lot-to-Lot Selectivity Change - pH

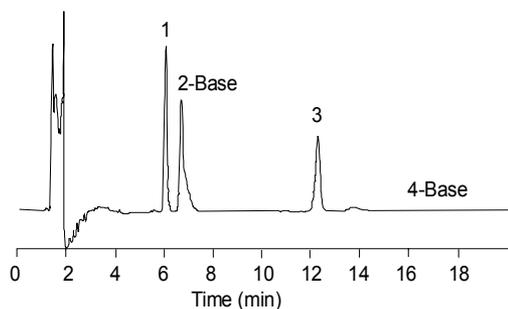
pH 4.5 - Lot 1



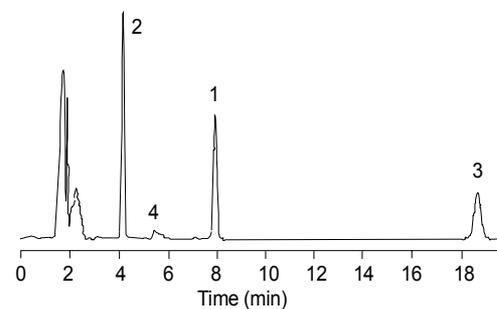
pH 3.0 - Lot 1



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**

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

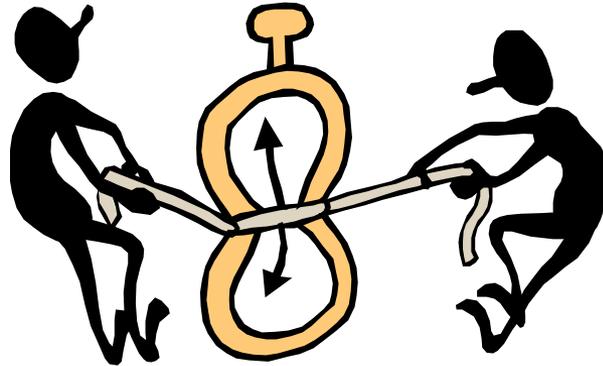
LC or GC Column Support

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

Select opt. 3, opt. 3, then option 1 for GC or option 2 for LC.

www.agilent.com/chem





The End – Thank You!