

## Tips and Tricks of HPLC System Troubleshooting



Agilent Technologies, Inc.  
LC Tips And Tricks Seminar Series

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### Trouble Shooting Steps

**You Have Recognized There is a Problem!**

**How Do You Fix It?**

- 1<sup>st</sup> Did System Suitability or Sample Fail?
- 2<sup>nd</sup> Review Method for Compliance
  - Is The Procedure Being Followed Properly?
  - Are Instrument Settings Correct?
- 3<sup>rd</sup> Ask More Questions!
  - When Did the System Last Function Properly?
  - Has Anything Been Changed?
- 4<sup>th</sup> Review ALL parameters!
  - The Obvious Is Not Always the Cause
  - Was There More Than One Change?

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## HPLC System Components

Pump  
Injector/Autosampler  
Column  
Detector  
Data System/Integrator

Problems Can Be Related to All Components in the System


## Categories of Column and System Problems

- A. Pressure
- B. Peak shape
- C. Retention

## Pressure Issues

<u>Column Observations</u>	<u>Potential Problems</u>
High pressure	- Plugged frit - Column contamination - Plugged packing
Low Pressure	- Leak - Flow Incorrect

## Determining the Cause and Correcting High Back Pressure

- Check pressure with/without column - many pressure problems are due to blockages in the system or guard col.
  - Remove Column - Pressure Still High?
  - Remove Guard – Pressure Still High?
- ***If Column pressure is high:***
  - Back flush column – Clear “dirty” frit surface
  - Wash column – Eliminate column contamination and plugged packing
    - high molecular weight/adsorbed compounds
    - precipitate from sample or buffer
-  Change frit – Clear plugged frit ***PREVENT THIS!***

## Column Cleaning

*Flush with stronger solvents than your mobile phase.*

### Reversed-Phase Solvent Choices in Order of Increasing Strength

Use at least 25 mL of each solvent for analytical columns

This Is Time Consuming  
Often Performed Offline

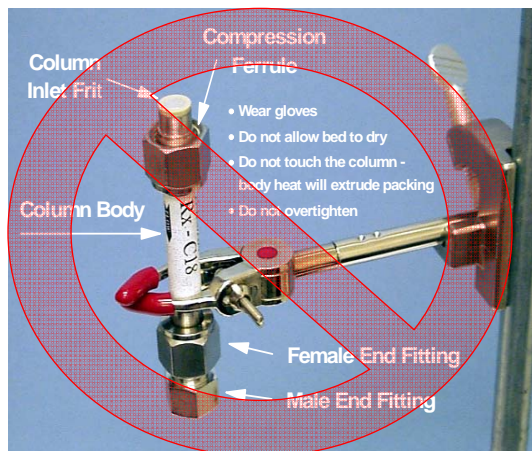
- Mobile phase without buffer salts
- 100% Methanol
- 100% Acetonitrile
- 75% Acetonitrile:25% Isopropanol
- 100% Isopropanol
- 100% Methylene Chloride\*
- 100% Hexane\*

Must Reverse  
to  
Re-Equilibrate

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

## Changing a Frit May Not Be a Good Idea

May not be possible with new generation columns  
May damage high performance columns



Tip: Prevention is a Much Better Idea!

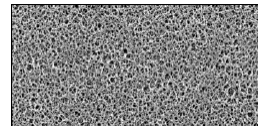
## The Trick: Prevention Techniques - A Better Choice!

- Use column protection
    - In-line filters
    - Guard columns
  - Filter samples
  - Filter buffered mobile phases
- } Easy
- Sample clean-up (i.e. SPE)
  - Appropriate column flushing
- } Not As Easy

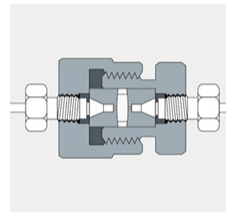
## Inexpensive Filters Prevent Column Frit Plugging



- Regenerated Cellulose (RC) **Recommended**
- **Universal hydrophilic membrane, compatible with most solvents - aqueous and organic**
  - **High purity, extremely low extractables and binding**
  - **More Uniform Surface**
  - **Different than Other Cellulose Filters!!**



- In-line Filters Easy to Use and replace**
- Frits Available in 0.2, 0.5 and 2.0 $\mu$  Porosity**
- Much Less expensive than a Column**
- Easier and Faster to Replace than a Column Frit**



## What Are Common Peak Shape Issues?

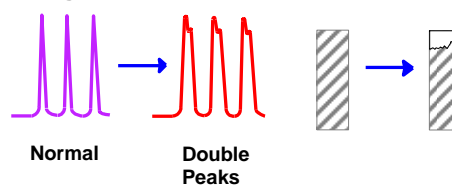
1. Split peaks
2. Peak tailing
3. Broad peaks

- Many peak shape issues are also combinations - i.e. broad and tailing or tailing with increased retention
- Symptoms do not necessarily affect all peaks in the chromatogram
- Each of these problems can have multiple causes

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## Peak Splitting Caused By Disrupted Sample Path

- Flow Path Disrupted by Void
- Sample Allowed to Follow Different Paths Through Column
- Poorly Packed Bed Settles in Use
- High pH Dissolves Silica



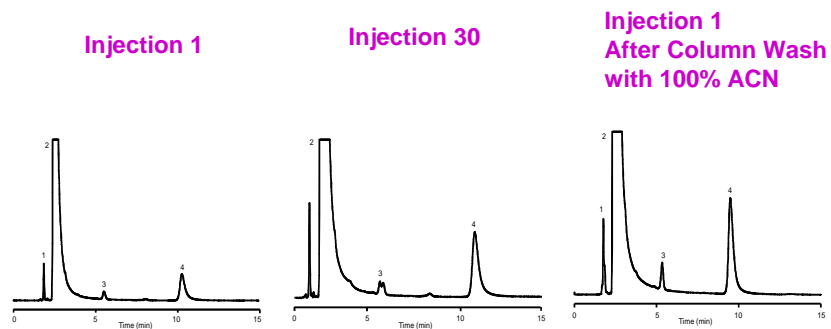
Split or Double Peaks

Tip: Similar Effect Can be Caused by Partially Plugged Frit

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## Split Peaks from Column Contamination

Column: StableBond SB-C8, 4.6 x 150 mm, 5  $\mu$ m    Mobile Phase: 60% 25 mM  $\text{Na}_2\text{HPO}_4$ , 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



**Tip:** Column washing eliminates the peak splitting, which resulted from a contaminant on the column  
 How could this be prevented? (Guard Column, SPE clean up of samples, Periodic column wash)

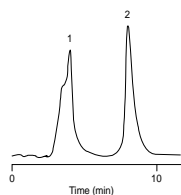
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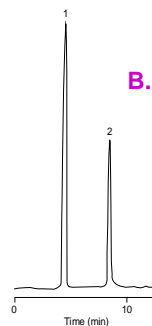
## Split Peaks from Injection Solvent Effects

Column: StableBond SB-C8, 4.6 x 150 mm, 5  $\mu$ m    Mobile Phase: 82%  $\text{H}_2\text{O}$  : 18% ACN  
 Injection Volume: 30  $\mu$ L    Sample: 1. Caffeine    2. Salicylamide

**A. Injection Solvent  
100% Acetonitrile**



**B. Injection Solvent  
Mobile Phase**



**Tip:** Injecting in a solvent stronger than the mobile phase can cause peak shape problems such as peak splitting or broadening  
**Trick:** Keep Organic Concentration in Sample Solvent  $\leq$  Mobile Phase

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Group/Presentation Title  
 Agilent Restricted  
 September 10, 2008 Month  
 ##.###

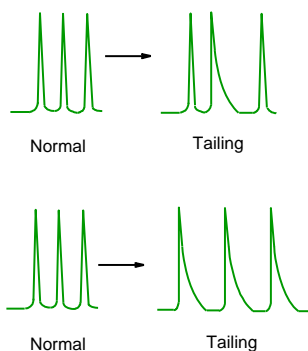
## Peak Tailing, Broadening and Loss of Efficiency

### May be caused by:

- Column “secondary interactions”
- Column contamination
- Column aging
- Column loading
- Extra-column effects

## Peak Shape: Tailing Peaks

Symmetry > 1.2



### Causes

#### Some Peaks Tail:

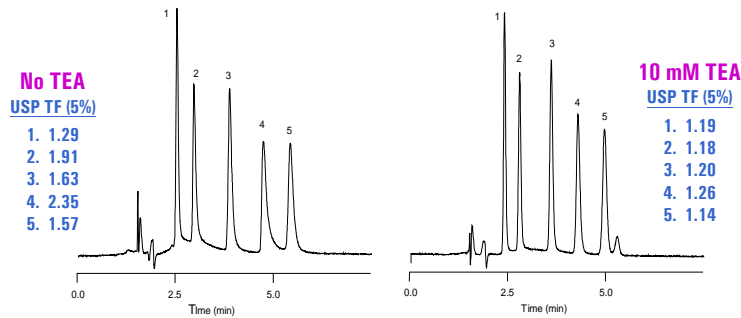
- Secondary - Retention Effects.
- Residual Silanol Interactions.
- Small Peak Eluting on Tail of Larger Peak.

#### All Peaks Tail:

- Extra-Column Effects.
- Build up of Contamination on Column Inlet.
- Heavy Metals.
- Bad Column.

## Peak Tailing Identifying Column “Secondary Interactions”

Column: Alkyl-C8, 4.6 x 150 mm, 5 $\mu$ m    Mobile Phase: 85% 25 mM Na<sub>2</sub>HPO<sub>4</sub> pH 7.0 : 15% ACN    Flow Rate: 1.0 mL/min  
Temperature: 35°C    Sample: 1. Phenylpropanolamine 2. Ephedrine 3. Amphetamine 4. Methamphetamine 5. Phentermine



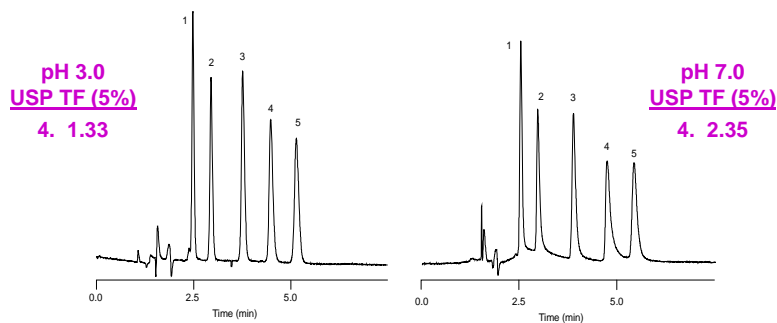
**Tip: Mobile phase modifier (TEA) competes with Sample for surface ion exchange sites at mid-range pH values**

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## Peak Tailing Low pH Minimizes “Secondary Interactions” for Amines

Column: Alkyl-C8, 4.6 x 150 mm, 5 $\mu$ m    Mobile Phase: 85% 25 mM Na<sub>2</sub>HPO<sub>4</sub> : 15% ACN    Flow Rate: 1.0 mL/min  
Temperature: 35°C    Sample: 1. Phenylpropanolamine 2. Ephedrine 3. Amphetamine 4. Methamphetamine 5. Phentermine



**Tip: Reducing mobile phase pH reduces interactions with silanols and peak tailing.**

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## Peak Tailing

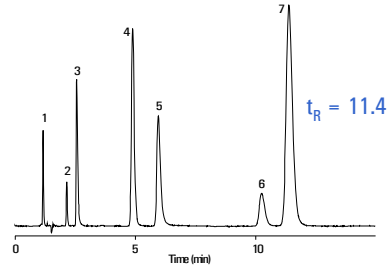
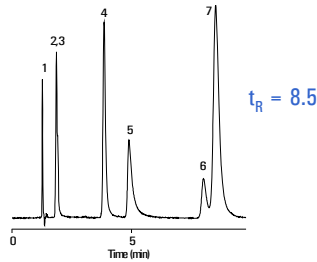
# High pH Eliminates “Secondary Interactions” for Amines

Column: ZORBAX Extend-C18, 4.6 x 150 mm, 5 μm Mobile Phase: See Below Flow Rate: 1.0 mL/min Temperature: RT  
 Detection: UV 254 nm

Sample 1. Maleate 2. Scopolamine 3. Pseudoephedrine 4. Doxylamine 5. Chlorpheniramine 6. Triprolidine 7. Diphenhydramine

**pH 7**  
 30% 20 mM Na<sub>2</sub>HPO<sub>4</sub>  
 70% MeOH

**pH 11**  
 30% 20 mM TEA  
 70% MeOH



Peak Shape and Retention of this sample of basic compounds improves at high pH where column has high IEX activity. Why?

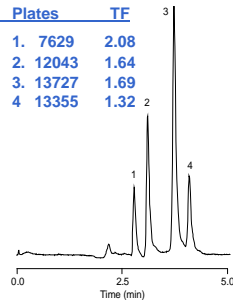
## Peak Tailing - Column Contamination

Tip: Quick Test to Determine if Column is Dirty or Damaged

Trick: Reverse Column and Run Sample –If Improved, Possible Cleaning Will Help -No improvement-Column Damaged and Needs to be Replaced

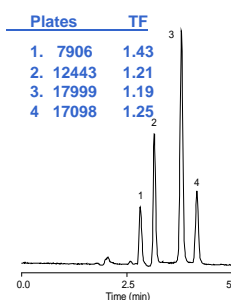
QC test forward direction

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



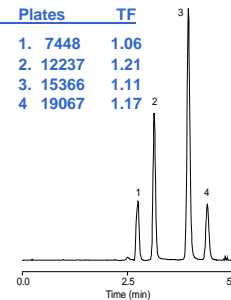
QC test reverse direction

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



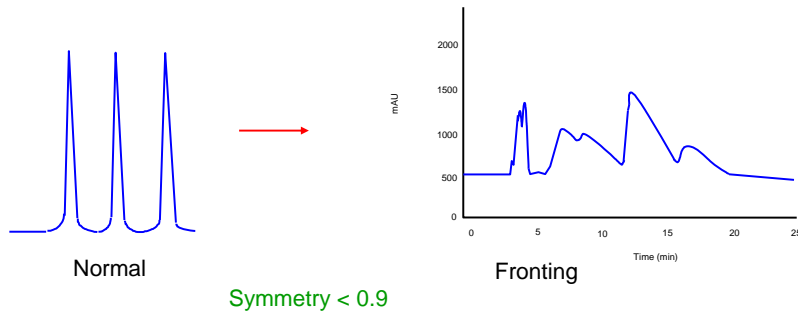
QC test after cleaning  
 100% IPA, 35°C

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



Column: StableBond SB-C8, 4.6 x 250 mm, 5 μm Mobile Phase: 20% H<sub>2</sub>O : 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

## Peak Shape: Fronting Peaks



### Causes:

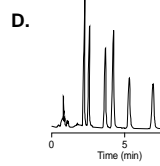
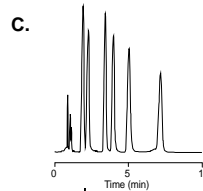
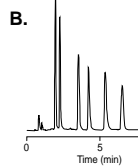
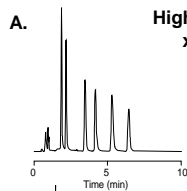
- Column Overload

## Peak Tailing/Broadening Sample Load Effects

Columns: 4.6 x 150 mm, 5 $\mu$ m    Mobile Phase: 40% 25 mM Na<sub>2</sub>HPO<sub>4</sub> 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%) i

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

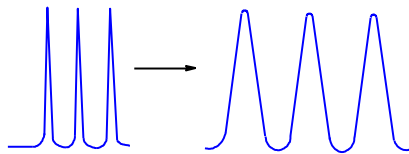


**Broadening**  
Competitive C8  
Plates

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

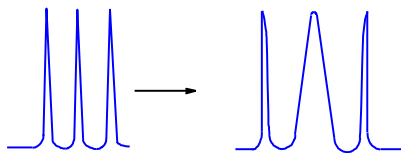
**Tip: Evaluate Both Volume and Mass Loading**

## Peak Shape: Broad Peaks



### All Peaks Broadened:

- Loss of Column Efficiency.
- Column Void.
- Large Injection Volume.

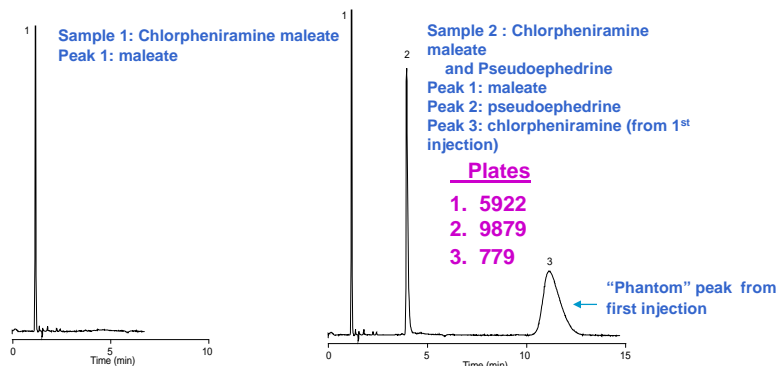


### Some Peaks Broadened:

- Late Elution from Previous Sample (Ghost Peak).
  - High Molecular Weight.
  - Sample - Protein or Polymer.

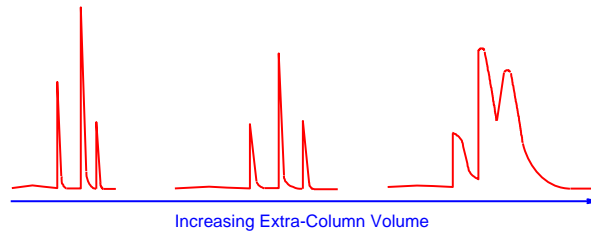
## Unknown “Phantom” Peaks

Column: Extend-C18, 4.6 x 150 mm, 5  $\mu$ 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



**Tip:** The extremely low plates for moderately retained peaks are an indication of a very late eluting peak from a preceding run.

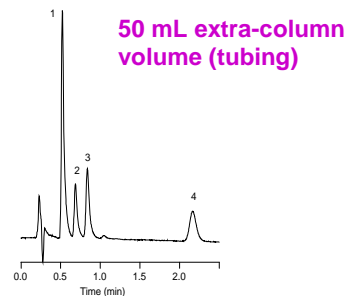
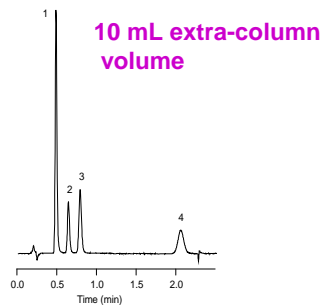
## Extra-Column Dispersion



- Use short, small internal diameter tubing between the injector and the column and between the column and the detector.
- Make certain all tubing connections are made with matched fittings.
- Use a low-volume detector cell.
- Inject small sample volumes.

## Peak Broadening Extra-Column Volume

Column: StableBond SB-C18, 4.6 x 30 mm, 3.5  $\mu$ m    Mobile Phase: 85% H<sub>2</sub>O 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



## Tip: Poorly Made HPLC System Connections Can Cause Peak Broadening

The System Has Been Optimized and :

- All Tubing Lengths Are Minimum
- Smallest Diameter Tubing Used
- Proper Flow Cell Volume

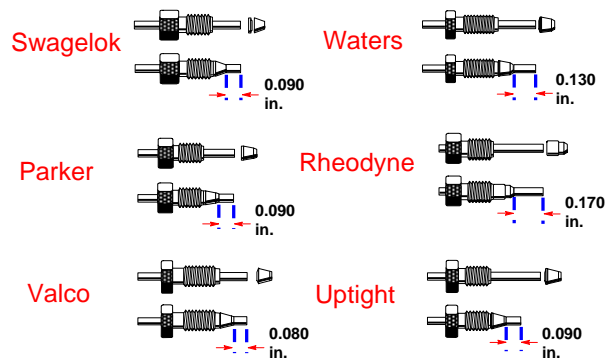
Symptom Still Seems to Have Too Much Extra-Column Volume

What Is Wrong?

Have You Made the Connections Properly?

## Column Connectors Used in HPLC

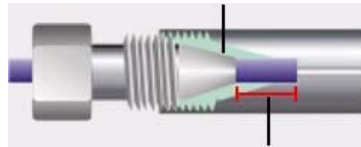
*Troubleshooting LC Fittings, Part II. J. W. Dolan and P. Upchurch. LC/GC Magazine 6:788 (1988)*



## What Happens If the Connections Poorly Made ?

Wrong ... too long

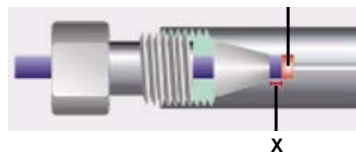
Ferrule cannot seat properly



If Dimension X is too long, leaks will occur

Wrong ... too short

Mixing Chamber



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

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## 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 all our LC systems



PEEK (<400b bar System Pressure) fittings are ideal where:

- Connections are changed frequently, i.e. connecting columns
- Pressure is less critical



PolyKetone

- Easy, hand tighten column connection
- **600 bar Pressure Rating** PN: 5042-8957 (10/pk)
- Fits to SS Tubing



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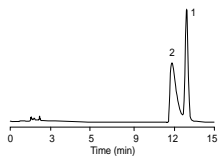
## Changes in Retention Can Be Chemical or Physical

### May be caused by:

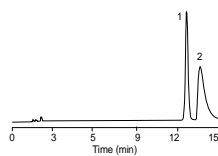
- Column aging
- Column contamination
- Insufficient equilibration
- Poor column/mobile phase combination
- Change in mobile phase
- Change in flow rate
- Different Gradient Delay Volumes

## Column Aging/Equilibration Causes Retention/Selectivity Changes

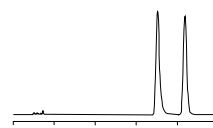
Column 1 - Initial



Column 1 - Next Day



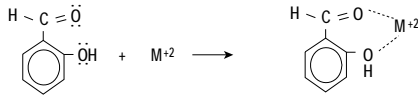
Column 1 - After Cleaning with 1% H<sub>3</sub>PO<sub>4</sub> /Equilibration



- 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

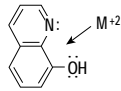
## Metal Sensitive Compounds Can Chelate

Hint: Look for Lone Pair of Electrons on  $\text{:O}$  or  $\text{N}$  Which Can Form 5 or 6 Membered Ring with Metal

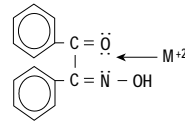


Salicylaldehyde

6-membered ring complex



8-hydroxyquinoline  
5-membered ring complex



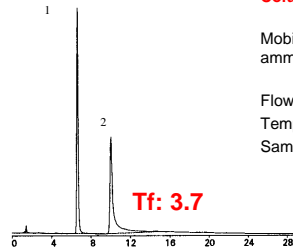
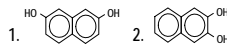
$\alpha$ -benzoinoximine  
5-membered ring complex

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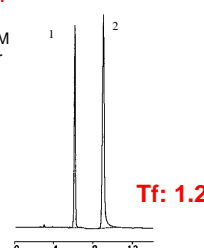
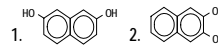
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## Acid Wash Can Improve Peak Shape

Before Acid Wash



After Acid Wash  
50 – 100 mLs 1%  $\text{H}_3\text{PO}_4$



Columns: ZORBAX SB-Phenyl  
4.6 x 150 mm

Mobile Phase: 75% ammonium phosphate buffer  
25% ACN

Flow Rate: 1.0 mL/min.

Temperature: RT

Sample Size: 5 mL

• A 1%  $\text{H}_3\text{PO}_4$  solution is used on SB columns, 0.5 % can be used on endcapped columns.

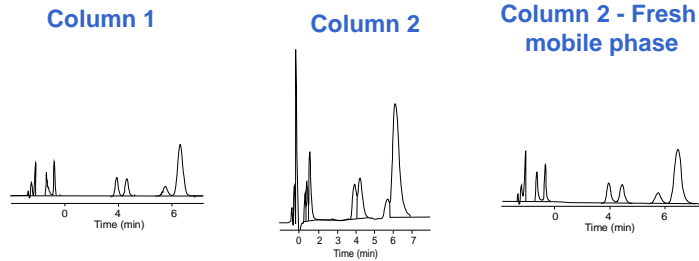
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## Example: Change in Retention/Selectivity

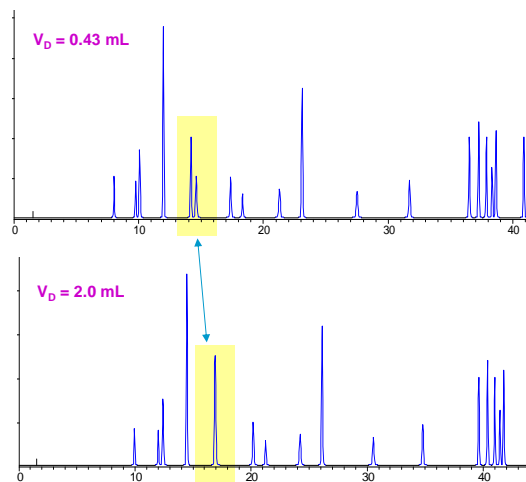
### Unintended Mobile Phase Variation

**Tip: The Source of the Problem is Often Not the Obvious Change**



*"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."*

## Tip: Dwell Volume Differences Between Instruments Can Cause Changes in Retention and Resolution



**Column:** ZORBAX Rapid Resolution Eclipse XDB-C8  
4.6 x 75 mm, 3.5  $\mu\text{m}$

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  $\mu\text{L}$

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  $V_D$

## Trick: Measure and Correct for Dwell Volume ( $V_D$ )

**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  $V_{D2} - \text{delay} = V_{D1}$**

## Mobile Phase pH and pH Buffers Why Are These So Important in HPLC?

- pH Effects Ionization
  - Silica Surface of Column
  - Sample Components of Interest
- Buffers
  - Resist Changes in pH and Maintain Retention
  - Improve Peak Shape for Ionizable Compounds
- Effects Column Life
  - Low pH strips Bonded Phase
  - High pH Dissolves Silica

## Minimize Change in Retention/Selectivity Lot-to-Lot

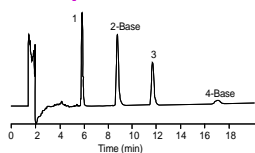
### Evaluate:

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

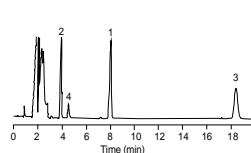
\*All causes of column-to-column change should be considered first, especially when only one column from a lot has been tested.

## Lot-to-Lot Selectivity Change Related to pH Choice

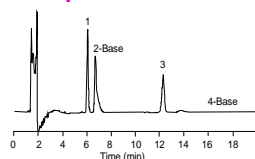
pH 4.5 - Lot 1



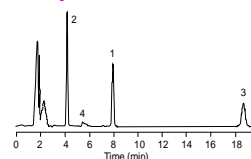
pH 3.0 - Lot 1



pH 4.5 - Lot 2

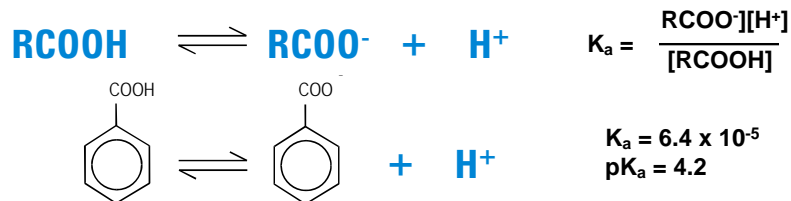


pH 3.0 - Lot 2



- pH 4.5 shows selectivity change from lot-to-lot for basic compounds
- pH 3.0 shows no selectivity change from lot-to-lot
- Indication of poorly controlled ionization

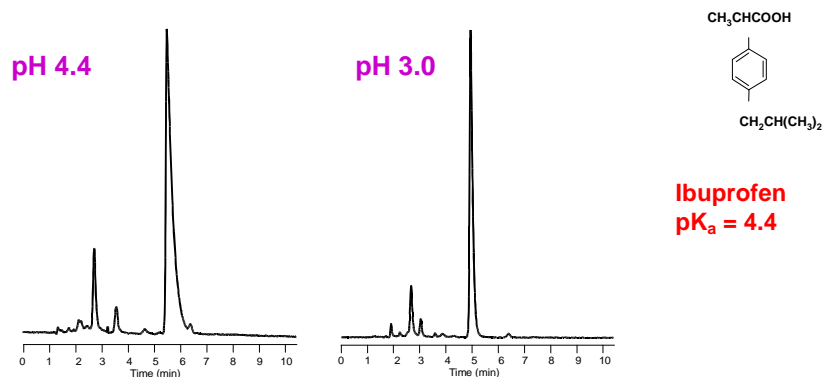
## Why Worry About pH? pH, pKa and Weak Acids



At pH 4.2 – the sample exists as benzoic acid and the benzoate ion in a ratio of 1:1. Peak shape can be poor  
 At pH 5.2 – 91% of the sample exists as the benzoate ion. RP retention decreases.  
 At pH 3.2 – 91% of the sample exists as benzoic acid. RP retention increases.

## Effect of pH on Peak Shape at or Near the Sample pKa

Column: ZORBAX SB-C8 4.6 x 150 mm, 5 mm      Mobile Phase: 40% 5 mM KH<sub>2</sub>PO<sub>4</sub>: 60% ACN  
 Flow Rate: 1.0 mL/min.      Temperature: RT

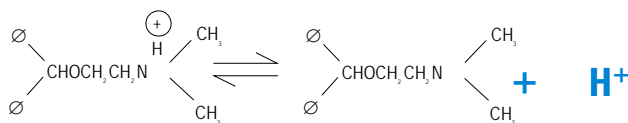


● Inconsistent and tailing peaks may occur when operating close to an analyte pKa and should be avoided.

## Why Worry About pH? pH, pKa and Weak Bases



$$K_a = \frac{[R_3N][H^+]}{[R_3NH^+]}$$



$$K_a = 1 \times 10^{-9}$$

$$pK_a = 9$$

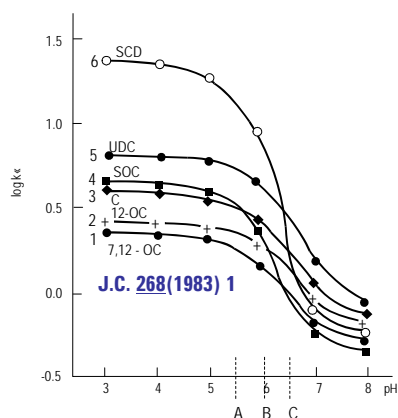
At pH 9 – the sample exists as protonated and unprotonated diphenhydramine in a ratio of 1:1. Peak shape can be poor.

At pH 10 – 91% of the sample exists as unprotonated diphenhydramine.

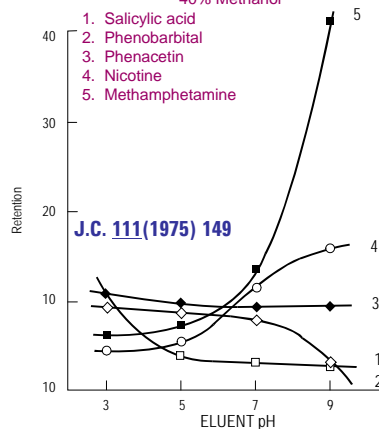
At pH 8 – 91% of the sample exists as protonated diphenhydramine.

## pH vs. Selectivity for Acids and Bases

Column: Nucleosil-C18  
Mobile Phase: 45% ACN/55% phosphate buffer  
Sample: Bile Acids



Column: mBondapak-C18  
Mobile Phase: 60% 25 mM phosphate buffer  
40% Methanol

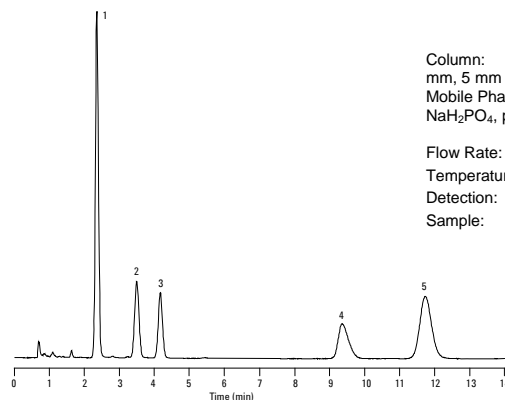


• Retention and selectivity can change dramatically when pH is changed.

## Importance of pH and Buffers A Practical Example

- Why the Sample Dictates Use
- What Happens When Buffer Used Effectively
- What Happens When Buffer Ignored or Used Improperly

## Importance of pH and Buffers - A Practical Example Optimized Isocratic Conditions for Cardiac Drugs

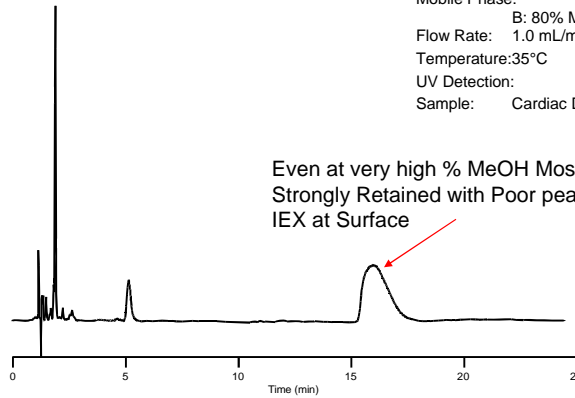


Column: StableBond SB-C18, 4.6 x 150 mm, 5 mm  
Mobile Phase: 45% 25 mM NaH<sub>2</sub>PO<sub>4</sub>, pH 3.0, 55% MeOH  
Flow Rate: 2.0 mL/min.  
Temperature: 35°C  
Detection: UV 254 nm  
Sample: Cardiac Drugs  
1. Diltiazem  
2. Dipyridamole  
3. Nifedipine  
4. Lidoflazine  
5. Flunarizine

## I Don't Have Time to Make Buffers or Adjust pH ...

Column: **StableBond SB-C18**  
4.6 x 150 mm, 5 mm

Mobile Phase: A: 20% MeOH  
B: 80% MeOH  
Flow Rate: 1.0 mL/min.  
Temperature: 35°C  
UV Detection: 254 nm  
Sample: Cardiac Drugs



• Buffers are critical to good retention and peak shape in many separations.

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## What If You Work Outside the Buffer Range?

Columns: **StableBond SB-C18**  
4.6 x 150 mm, 5 mm

Mobile Phase: A: 30% 25 mM NaH<sub>2</sub>PO<sub>4</sub>, pH 4.8 unbuffered  
B: 70% MeOH

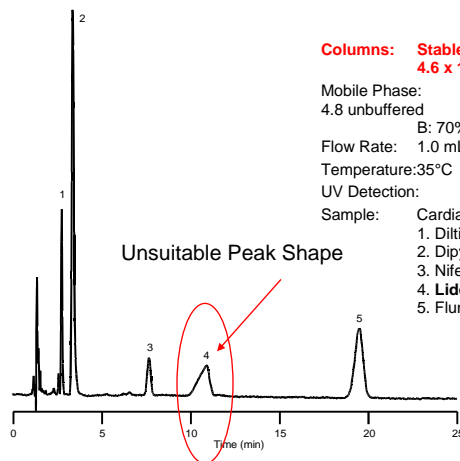
Flow Rate: 1.0 mL/min.

Temperature: 35°C

UV Detection: 254 nm

Sample: Cardiac Drugs

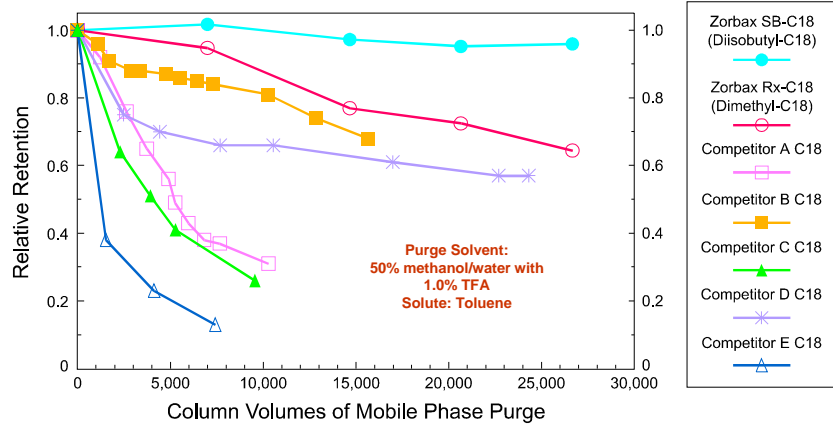
1. Diltiazem
2. Dipyridamole
3. Nifedipine
4. **Lidoflazine**
5. Flunarizine



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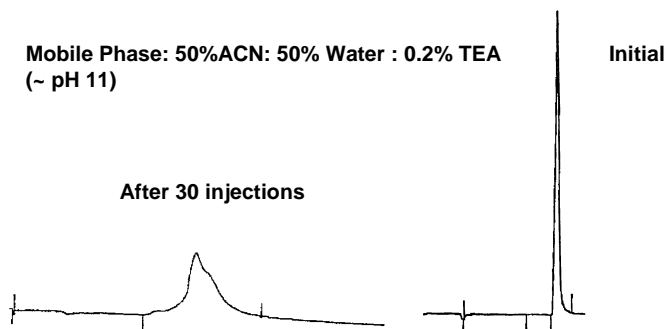
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**Don't Forget - Match Column to pH of Mobile Phase  
for Maximum Column Lifetime**  
low pH and high temperature (pH 0.8, 90°C)



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

**Don't Forget - Match Column to pH of Mobile Phase  
for Maximum Column Lifetime**  
High pH and Room Temperature (pH 11 RT)



**Tip: Use Columns Designed for chosen pH**

## Detection Issues

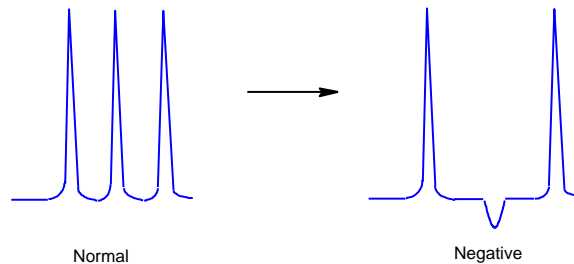
Recognize Where the Problem Originates

- Is it a consequence of technique?
- Is It expected due to use of certain mobile phase components?
- Can it be corrected by adjusting detector parameters?
- Answers Will Help Find a Solution!

Let's Explore Some Problems and Solutions



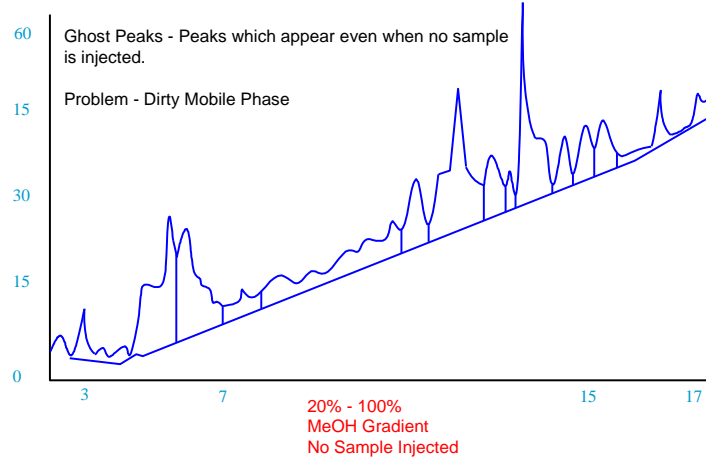
## Peak Shape: Negative Peaks



### Causes:

- Absorbance of sample is less than the mobile phase.
- Equilibrium disturbance when sample solvent passes through the column.
- Normal with Refractive Index Detectors.

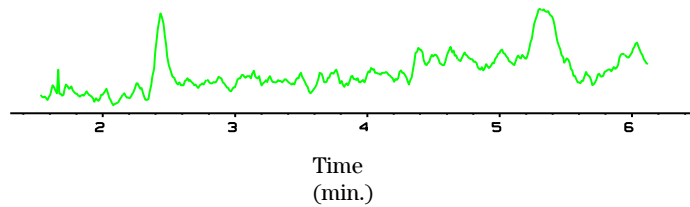
## Ghost Peaks



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## Noisy Baselines



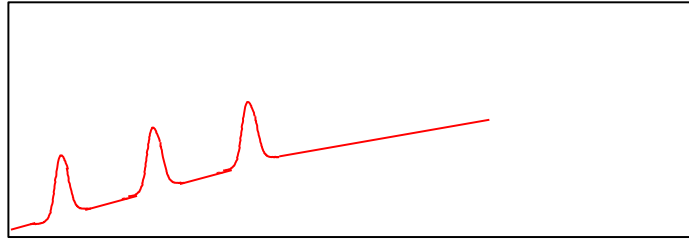
### Possible Causes:

- Dirty Flow Cell
- Detector Lamp Failing
- Pulses from Pump if Periodic
- Temperature Effects on Detector
- Air Bubbles passing through Detector

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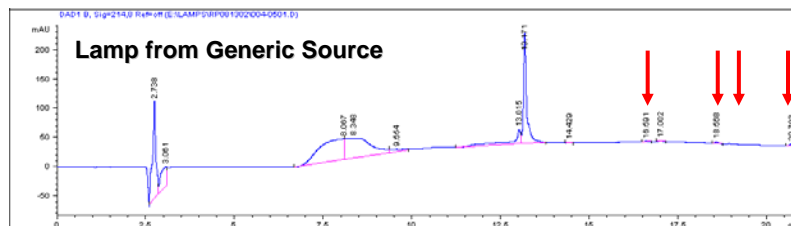
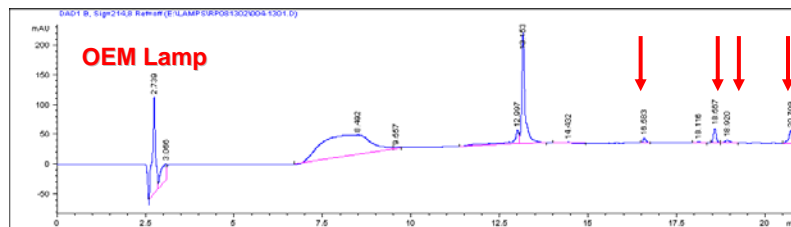
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## Drifting Baselines



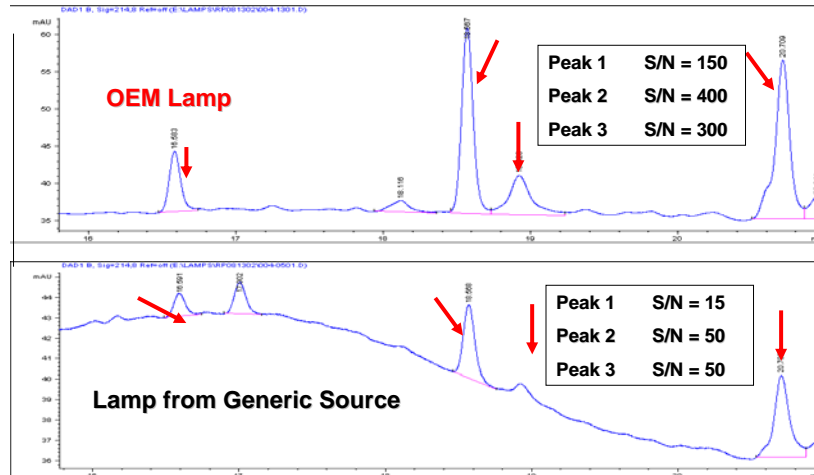
- Gradient Elution
- Temperature Unstable (Refractive Index Detector)
- Contamination in Mobile Phase
- Mobile Phase Not in Equilibrium with Column
- Contamination Bleed in System

## Chromatographic Results with “Wrong” Lamp at 214 nm Wavelength



Tip: Could also be a symptom of aging lamp

## Expanded View of Chromatographic Results Generic Source Lamp at 214 nm Wavelength



Tip: Poor S/N makes it difficult to detect low level impurities

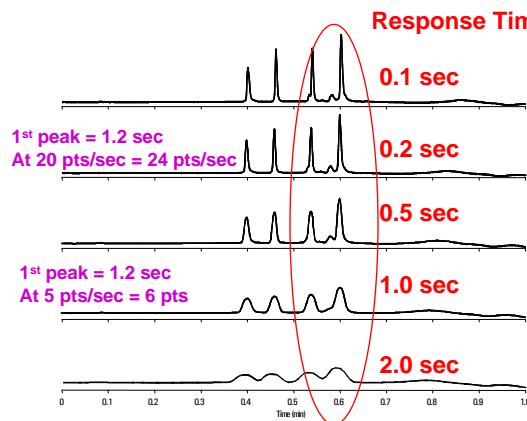
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## Effect of Detector Response Time

The System is operating well-the settings were poorly made!

Slow Data Rates Can Hinder Impurity Detection and Reduce Sensitivity



Agilent 1100 DAD  
Agilent 1100 WPS with ADVR  
Column: Poroshell 300SB-C18  
2.1 x 75 mm, 5 mm  
Mobile Phase:  
A: 95% H<sub>2</sub>O, 5% ACN with 0.1% TFA  
B: 5% H<sub>2</sub>O, 5% ACN with 0.1% TFA  
Flow Rate: 2 mL/min  
Temperature: 70°C  
Detector: UV 215 nm  
Piston stroke: 20  
Sample:  
1. Neurotensin3. Lysozyme  
2. RNaseA 4. Myoglobin

• Tip: Adjust the response rate of your detector for best peak detection.

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## Conclusions

HPLC column problems are evident as

- High pressure (prevention better than the cure)
- Undesirable peak shape
- Changes in retention/selectivity

Often these problems are not associated with the column and may be caused by instrument and chemistry issues.

- pH of mobile Phase
- Instrument Connections
- Detector Settings
- Metal Contamination

Start With the Correct Questions

- Find the Answers
- The Answers will Lead to Solutions