

# Don't Lose It: Getting Your Peaks in Shape

Choosing columns and conditions for the best peak shape

Golnar Javadi

Columns and Supplies Technical Support

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# Agenda

- What is a good peak shape and why is it important?
- How is peak shape measured?
- Problems with peak shape
- Factors affecting peak shape
- Examples of peak shape problems
- Guidelines for improved peak shape

# What is Good Peak Shape and Why is it Important?

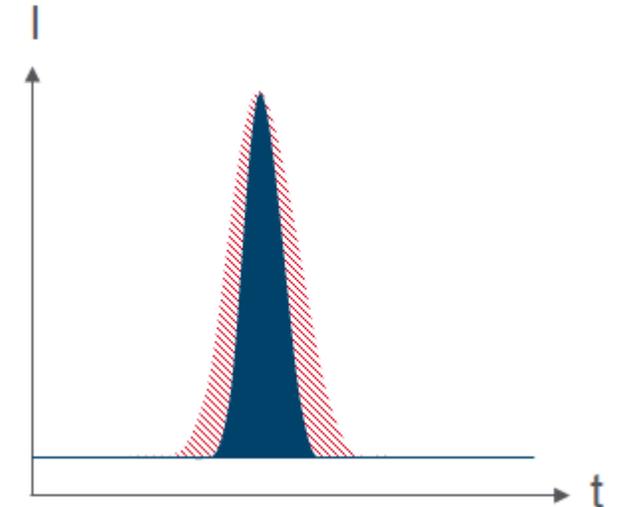
Good peak shape can be defined as symmetrical or Gaussian.

Good peak shape can be defined by:

- Tailing factor of 1.0
- High efficiency
- Narrow peak width

Good peak shape is important for:

- Improved resolution, sensitivity, and precision
- More accurate quantitation
- Longer usable column lifetime (based on system suitability criteria)



# How is Peak Shape Measured?

## Measures:

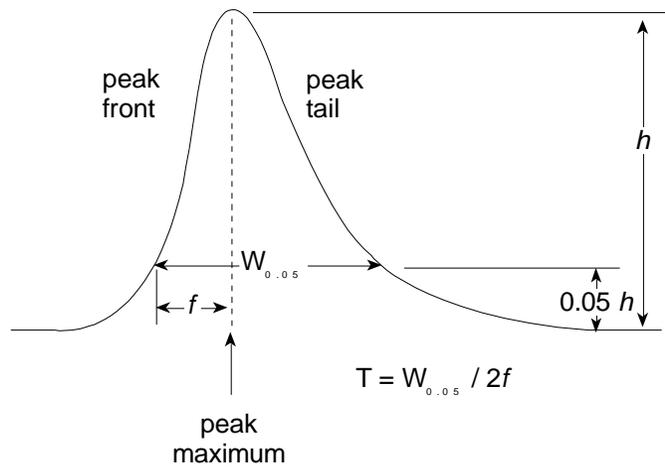
- USP tailing factor – at 5% of peak height
- Asymmetry factor – at 10% of peak height

## Indicators:

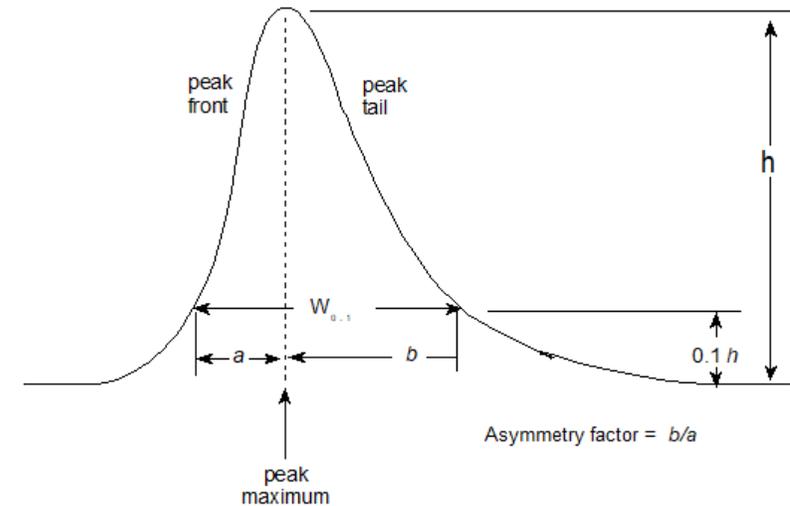
- Efficiency – plate number
- Peak width – peak width at  $\frac{1}{2}$  height

# How is Peak Shape Measured?

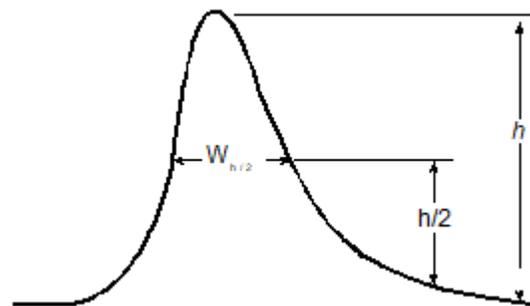
## USP tailing factor at 5% height



## Asymmetry factor at 10% height



## Peak width at 1/2 height



## Efficiency

$$N = 5.54 \frac{t_R}{W_{0.05}}$$

# How is Peak Shape Measured?

Column plate number as a function of experimental conditions

$$N(\text{plate number}) = L(\text{column length})/H(\text{plate height})$$

H varies with the linear velocity ( $u$ ) of the mobile phase as it passes through the column ( $u=L/t_0$ ).

$$H = A + \frac{B}{u} + Cu$$

Van Deemter equation

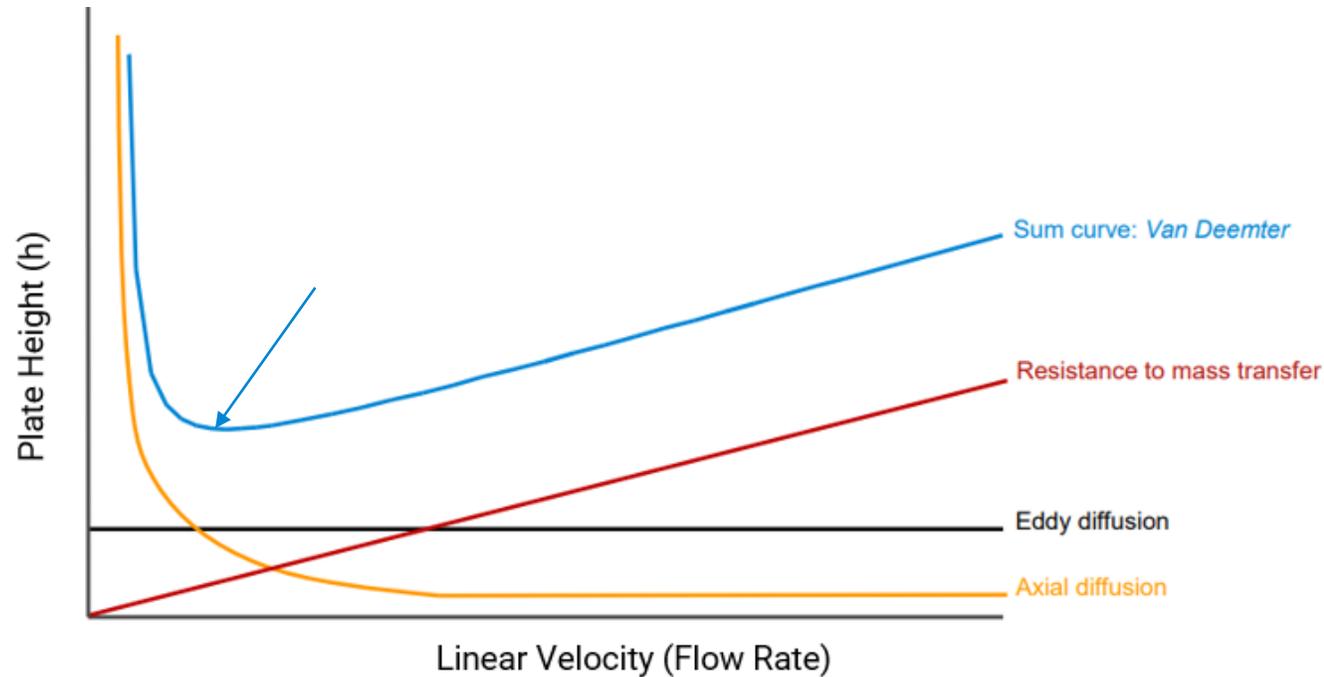
A, B, and C are constants for a particular compound and set of experimental conditions. Linear velocity ( $u$ ) is variable.

- A is eddy diffusion
- B is longitudinal (axial) diffusion
- C is resistance to mass transfer

# How is Peak Shape Measured?

## Van Deemter plot

A plot of plate height versus linear velocity



The point where minimum plate height is reached, is the “optimum” linear velocity at which the maximum plate number is reached.

Optimum flow rate =  $u$  (optimum linear velocity)  $\times$   $s$  (cross section area of the column)

# How is Peak Shape Measured?

Efficiency – column plate number as a function of experimental conditions

Column plate number (N) increases with:

- Column packing quality
- Column length
- Optimal flow rate
- Smaller particle size
- Use of superficially porous particles
- Appropriate pore size
- Lower mobile phase viscosity
- Higher temperature
- Minimized extra-column effect

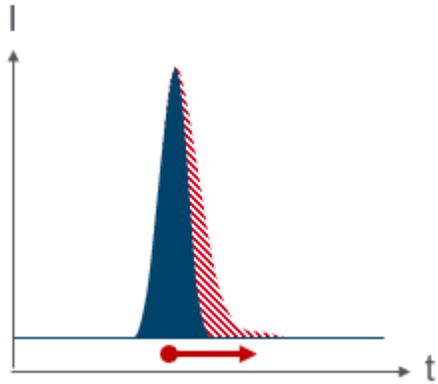
$$N = 5.54 \left( \frac{t_R}{W_{h/2}} \right)^2$$

$t_R$  = band retention time

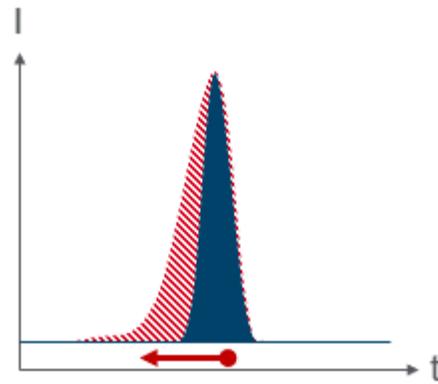
$W_{h/2}$  = bandwidth at half-height

# Problems with Peak Shape

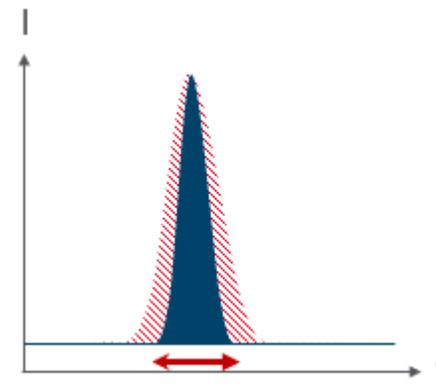
- Tailing
- Fronting
- Broadening
- Splitting/doubling



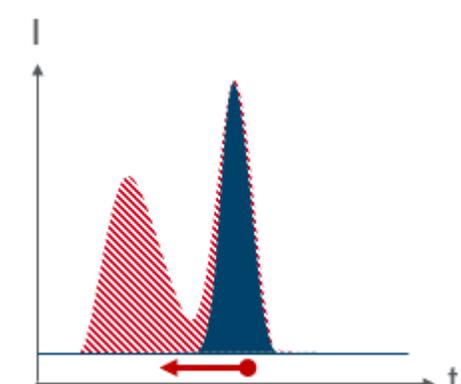
Tailing



Fronting



Broadening



Splitting/doubling

# Factors Affecting Peak Shape

- Column
- Mobile phase
- Connecting capillaries and fittings
- System
- Sample



# Factors Affecting Peak Shape

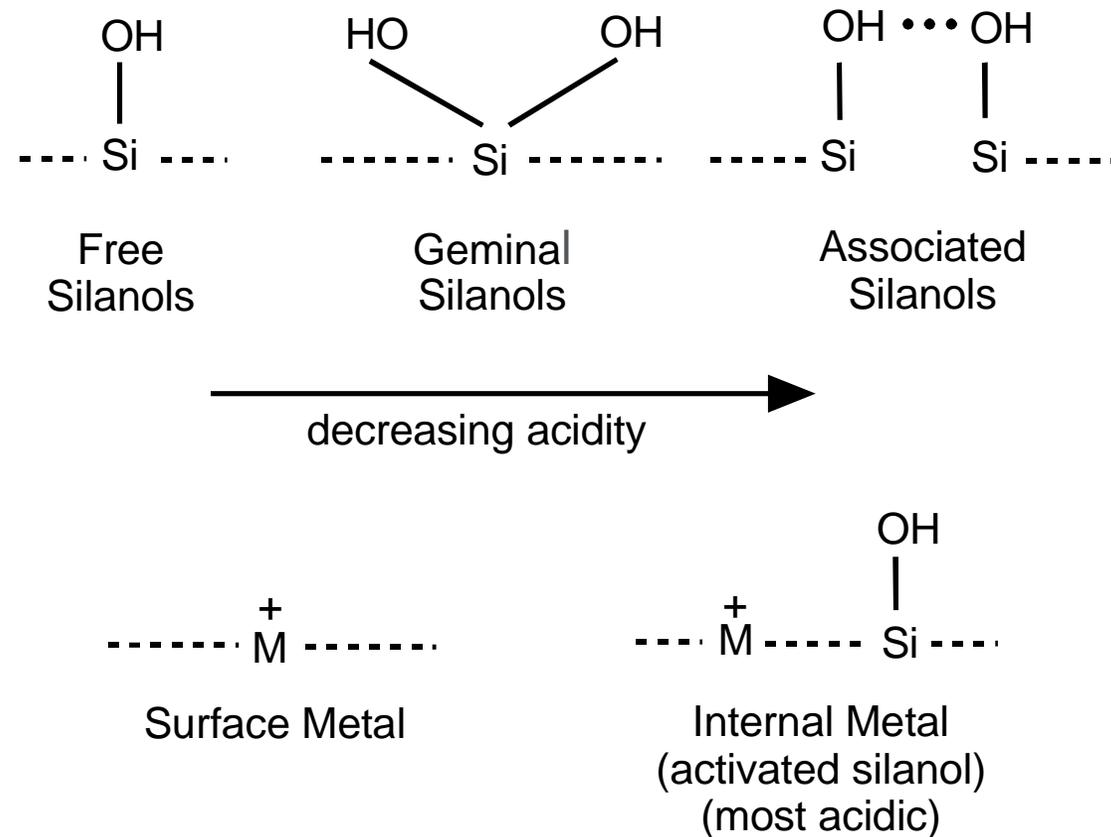
## Column related factors

- Silica type/acidity/metal content
- Column bonding and end capping
- Column packing
  - Pore size/particle size/particle morphology
  - Formation of voids in the packed bed



# Factors Affecting Peak Shape

## Column related factors – silica type



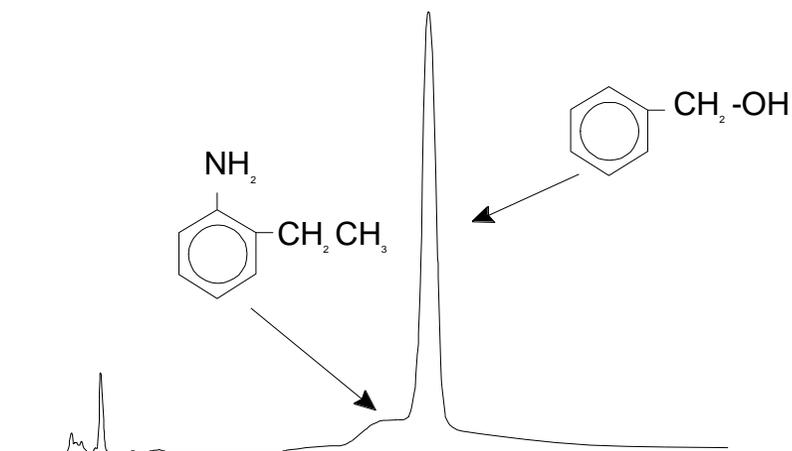
Fully hydroxylated and metal free silica reduces acidity

# Factors Affecting Peak Shape

## Column related factors – silica type

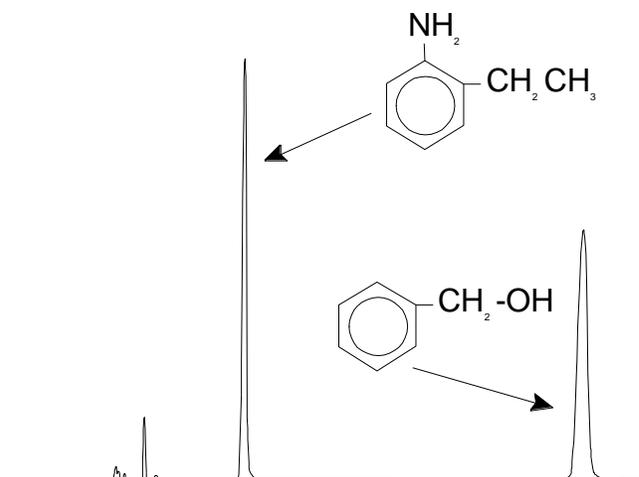
High purity, low acidity silica improves peak shape:

Type A Silica



Mobile Phase: 5% 2-Propanol in Heptane

High purity, low acidity  
ZORBAX Rx-SIL (Type B)



Flow Rate: 2.0 mL/min. Temperature: 35°C

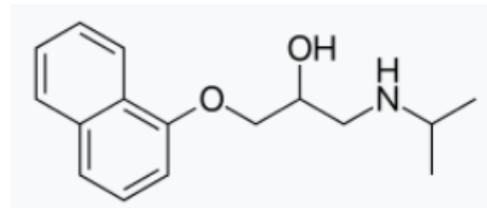
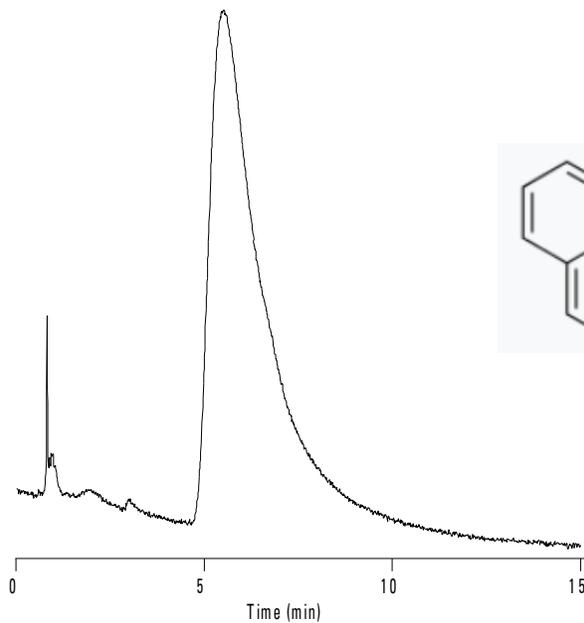
Improved peak shape for basic compounds with high purity, fully hydroxylated silica such as Rx-SIL

# Factors Affecting Peak Shape

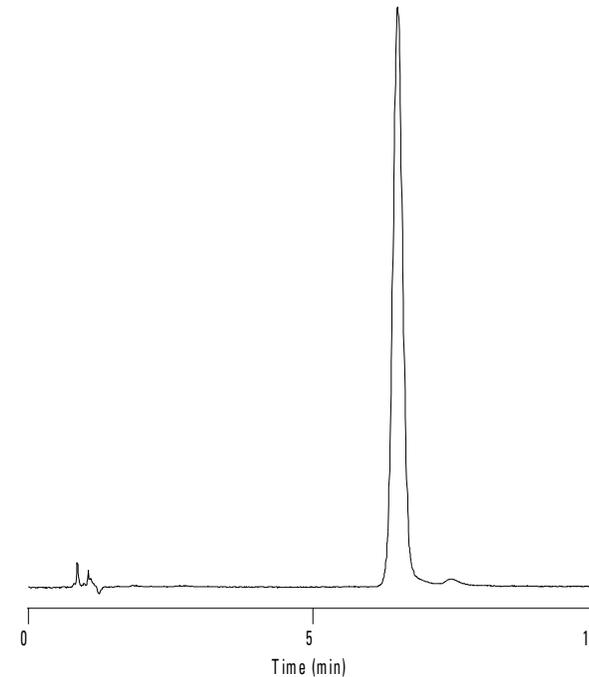
## Column related factors – silica type

Silica type – more acidic  
Column: ODS, 4.6 x 250 mm, 5  $\mu$ m  
Plates: 92  
USP Tf (5%): 2.90

Silica type – high purity, Rx-Sil  
Column: SB-C18, 4.6 x 150 mm, 5  $\mu$ m  
Plates: 6371  
USP Tf (5%): 1.09



Propranolol  
pKa 9.5

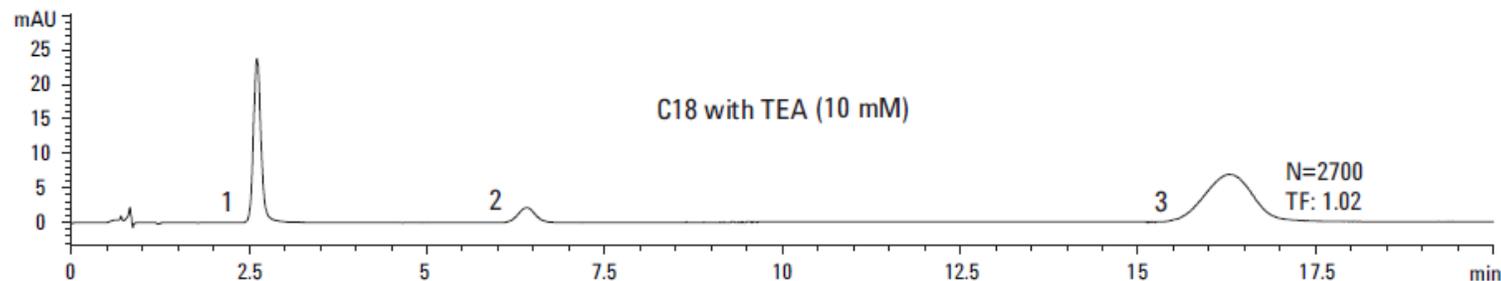
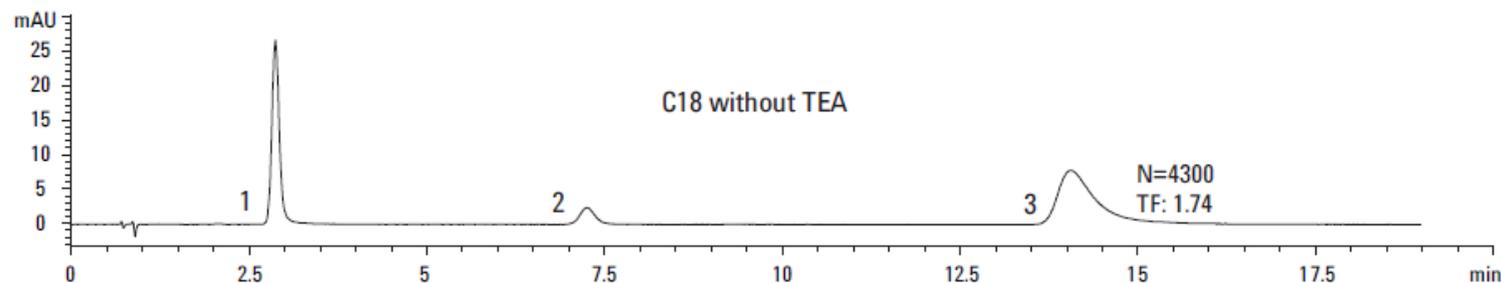
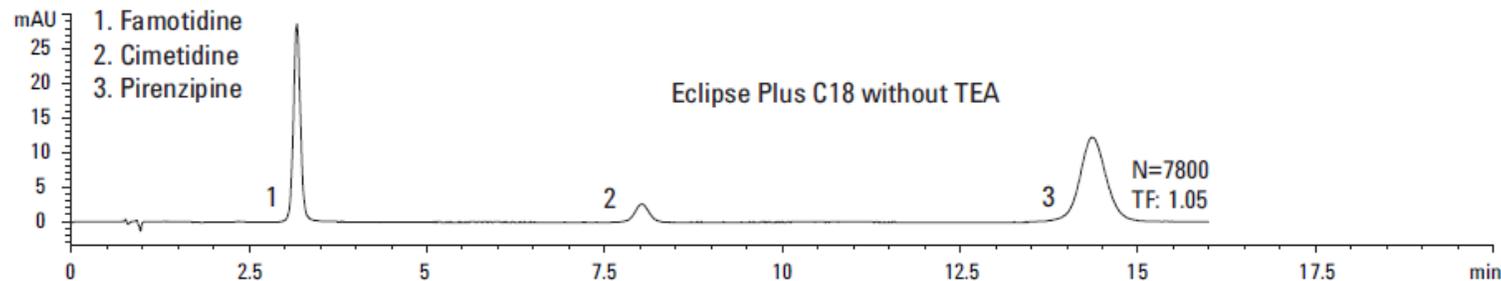


Mobile Phase: 75% 50 mM  $\text{KH}_2\text{PO}_4$ , pH 4.4 : 25% ACN Flow Rate: 1.5 mL/min

ZORBAX StableBond with Rx-SIL improves peak shape

# Factors Affecting Peak Shape

## Column related factors – silica type



Columns: 4.6 x 75 mm, 3.5  $\mu$ m  
Mobile phase:  
20% MeOH, 80% 20 mM phosphate pH 7.0  
Flow rate: 1 mL/min  
UV 254 nm  
Semi micro flow cell

Effect of ionized acidic silanols on peak shape of amine-containing ulcer medications. A comparison of Eclipse Plus C18 and another C18 column.

# Factors Affecting Peak Shape

## Column related factors – column bonding and endcapping

### Column bonding and endcapping

- Most Agilent RP columns are endcapped. Double and triple endcapping minimizes the number of unreacted silanols and potential peak tailing interactions
- Bonded phases such as StableBond (which is not endcapped but has bulky side chain groups sterically protect siloxane bonds from hydrolytic attack at low pH. SB columns are not endcapped to provide stability, lifetime, and reproducibility under acidic conditions.
- Bonded phases with embedded polar groups (Bonus RP, Polaris – Amide-C18) or endcapped with polar groups, provide unique silanol shielding, reducing peak tailing for basic compounds.
- Bonded phases that are stable at a high pH (Poroshell 120 HPH and ZORBAX Extend C18) minimize the interaction of basic compounds with free silanols, which reduces peak tailing.



# Factors Affecting Peak Shape

## Column related factors – column bonding and endcapping

InfinityLab Poroshell HPH-C18 with hybridized particle surface and double endcapping is designed to withstand high pH with good peak shape

### Conditions:

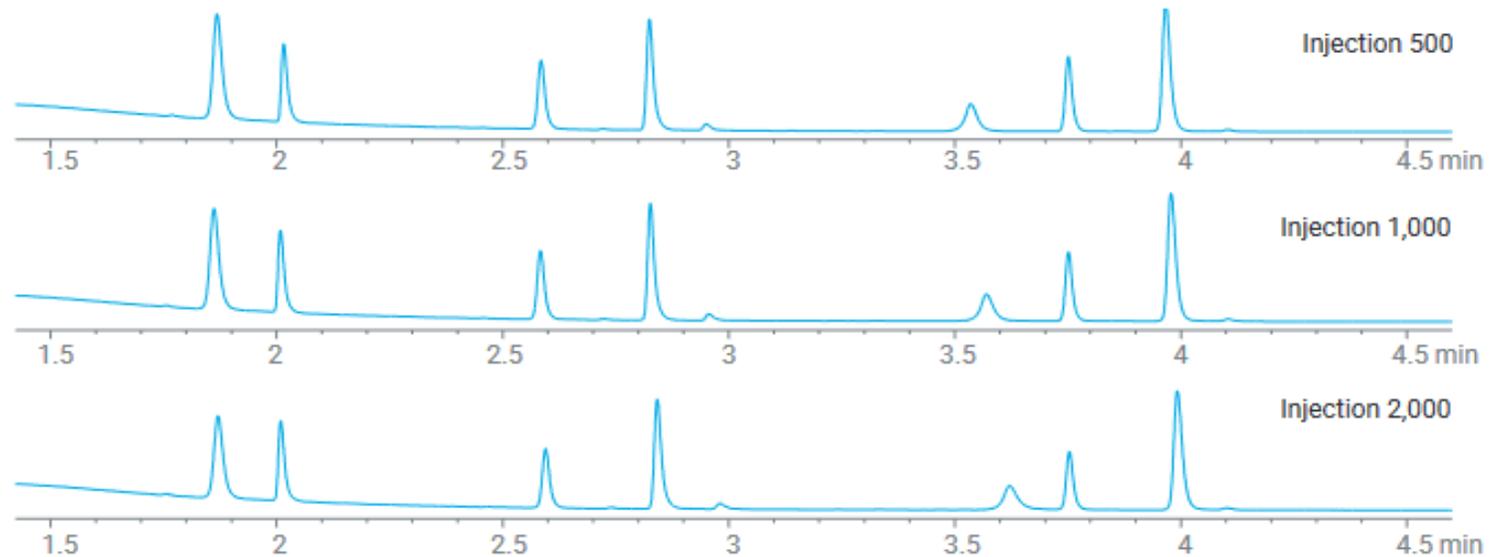
Instrument: 1260 Infinity II Binary LC  
Mobile phase: A: 10 mM Ammonium bicarbonate adjusted to pH 10.0 in water  
B: Acetonitrile  
Flow rate: 0.4 mL/min  
Gradient: 

| Time | %B |
|------|----|
| 0    | 5  |
| 5    | 95 |
| 5.1  | 5  |

### Sample:

1. Methyl salicylate
2. 4 Chlorocinnamic acid
3. Acetophenone
4. Quinine
5. Nortryptiline
6. Heptanophenone
7. Amitriptyline

### InfinityLab Poroshell HPH-C18, 2.1 x 50 mm, 2.7 µm

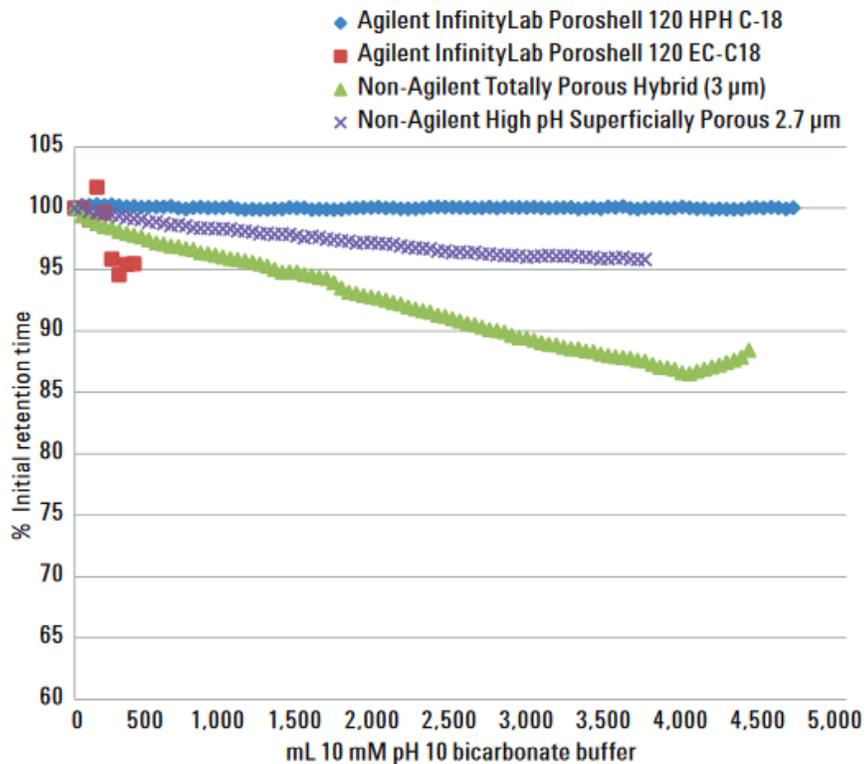


After 2,000 injections at pH 10, InfinityLab Poroshell 120 HPH-C18 showed no change in performance.

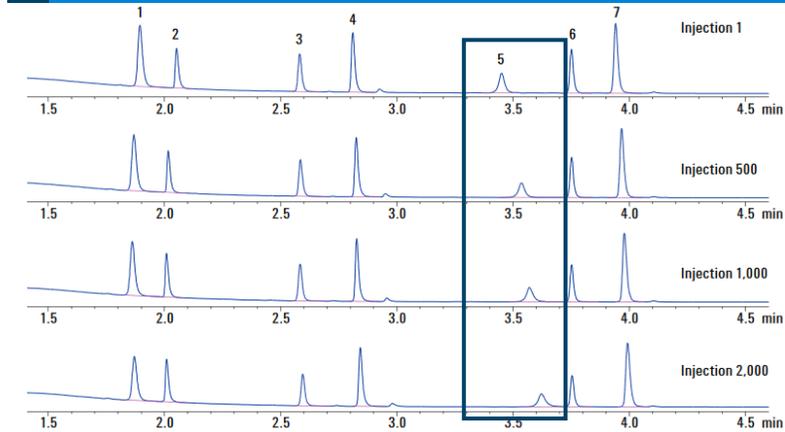
# Factors Affecting Peak Shape

## Column related factors – column bonding and endcapping

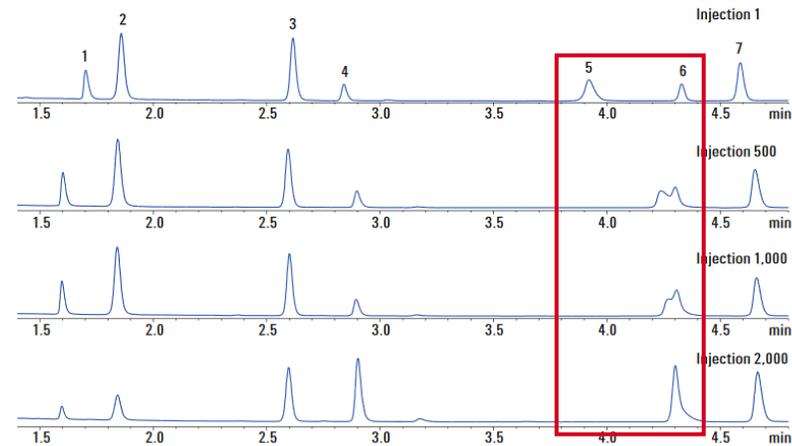
### High pH life time study



### Poroshell 120 HPH-C18



### Other hybridized surface column



Column: 2.1 x 50 mm, 2.7 μm  
Sample:

1. Methyl Salicylate
2. 4-Cholorcinnamic Acid
3. Acetophenone
4. Quinine
5. Nortriptyline
6. Heptanophenone
7. Amitriptyline

Instrument: 1260 Infinity II  
Binary LC

Mobile phase:

A: 10 mM ammonium bicarbonate in water pH10  
B: acetonitrile

Flow rate: 0.4 mL/min  
Gradient method:

Time %B

0 5

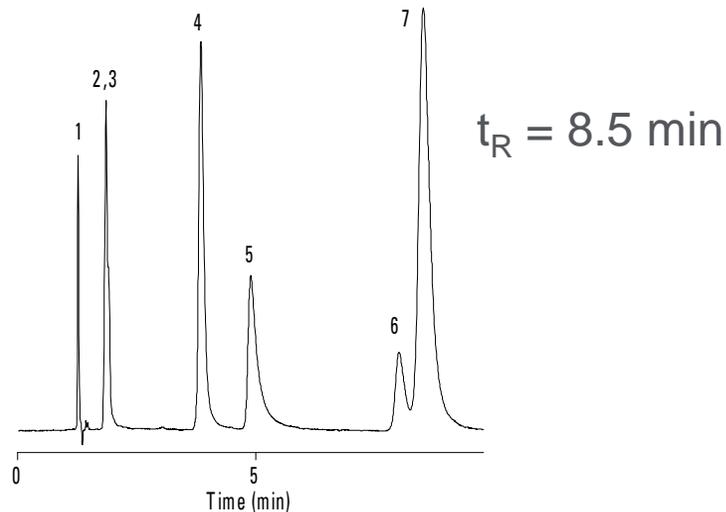
5 95

5.1 5

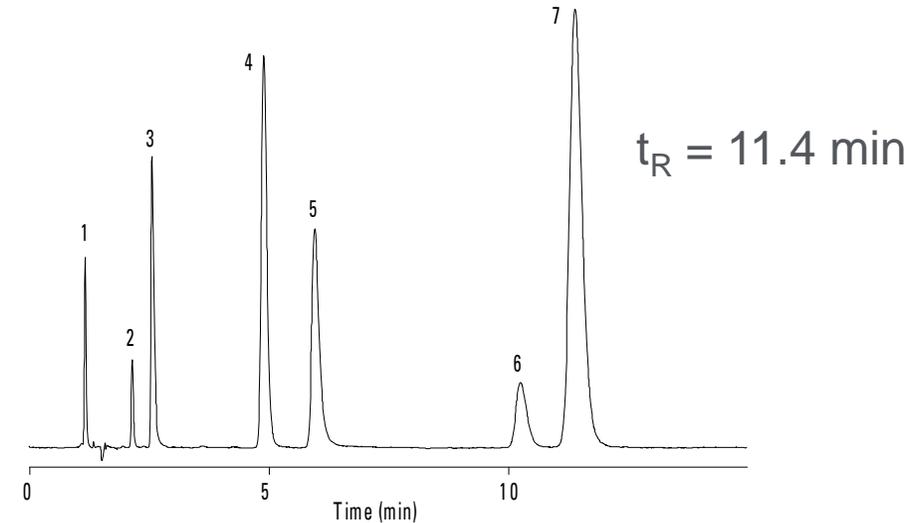
# Factors Affecting Peak Shape

## Column related factors – column bonding and endcapping

ZORBAX Extend-C18 at pH 7



ZORBAX Extend-C18 at pH 11



Mobile phase: 30% buffer: 70% MeOH; pH 7 buffer: 20 mM  $\text{Na}_2\text{HPO}_4$ ; pH 11 buffer: 20 mM TEA

Flow rate: 1.0 mL/min; Temperature: ambient; Detection: UV 254 nm

Sample: 1. Maleate 2. Scopolamine pKa 7.6 3. Pseudoephedrine pKa 9.8 4. Doxylamine pKa 9.2 5. Chlorpheniramine pKa 9.1

6. Triprolidine pKa 6.5 7. Diphenhydramine pKa 9.0

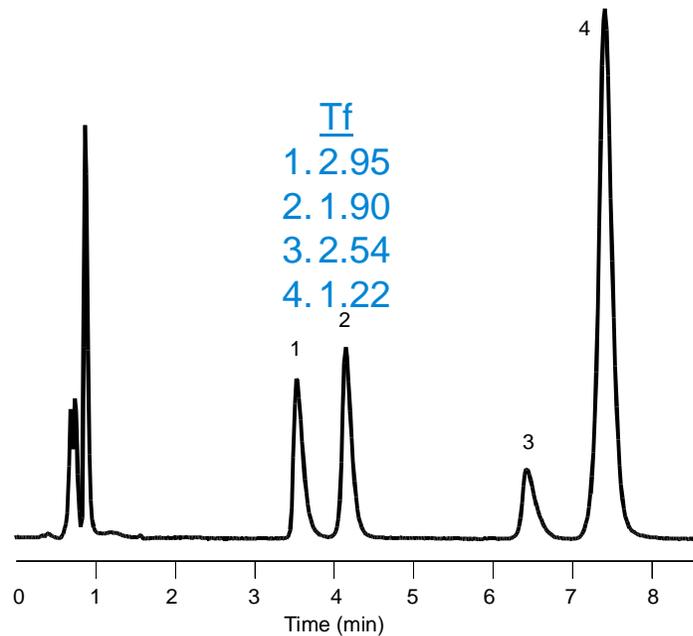
Column: ZORBAX Extend-C18, 4.6 x 150 mm, 5  $\mu\text{m}$

Retention and peak shape of basic compounds is improved at high pH on ZORBAX Extend-C18

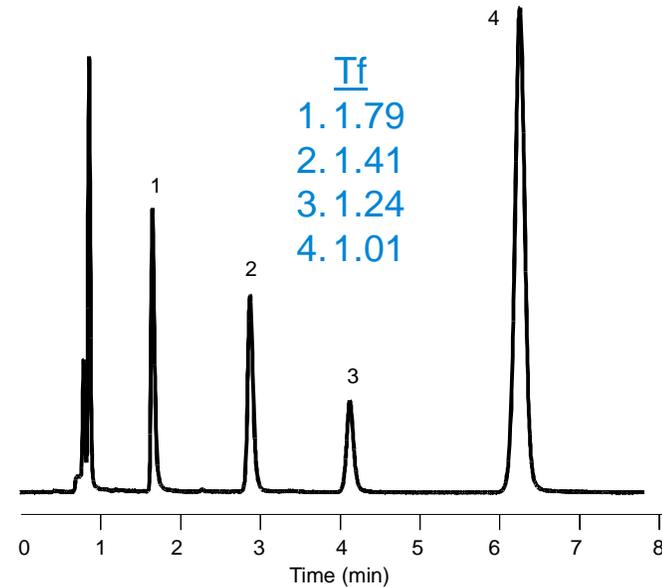
# Factors Affecting Peak Shape

## Column related factors – column bonding and endcapping

Single endcapped C18 from a different manufacturer



Double endcapped Eclipse XDB-C18

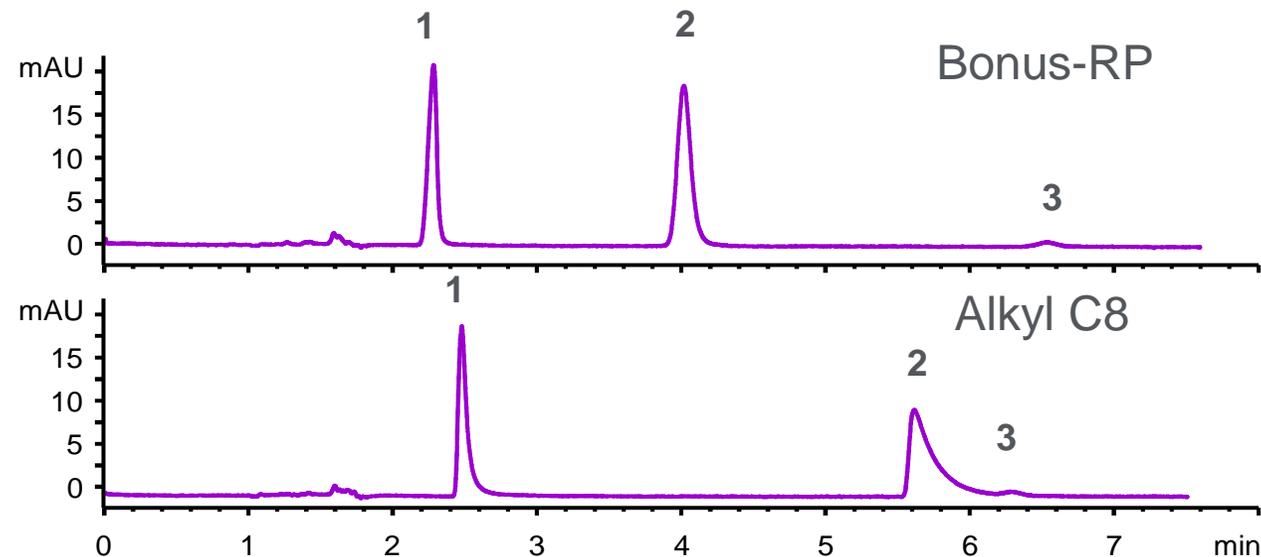
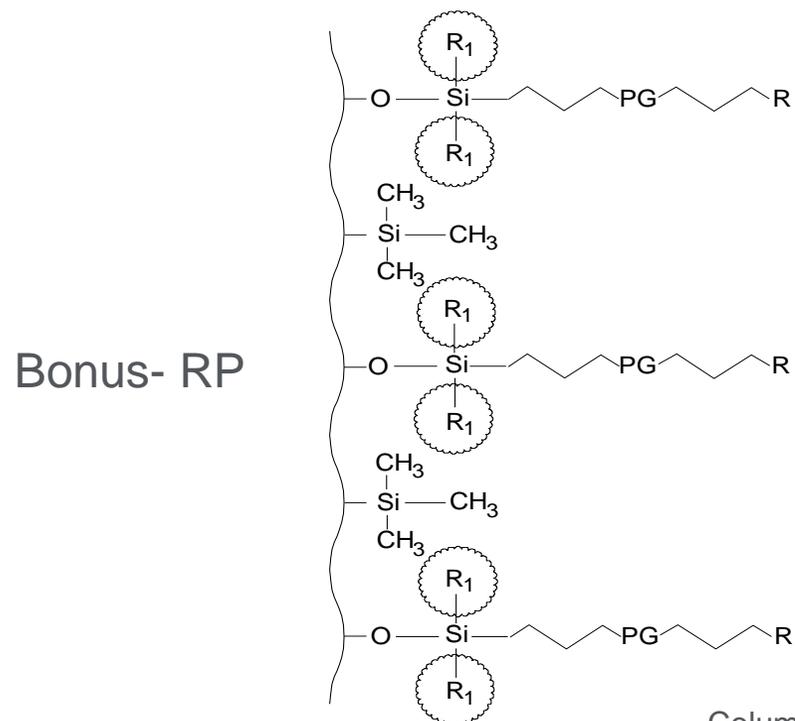


Columns: 4.6 x 150, 5  $\mu$ m    Mobile Phase: 60% ACN : 40% 10 mM phosphate buffer pH 7.0    Flow Rate: 1.5 mL/min.  
Temperature: 40°C    Sample: 1. Nortriptyline pKa 9.7 2. Doxepin pKa 9.0 3. Amitriptyline pKa 9.4 4. Trimipramine

Fewer silanol interactions on the double endcapped column reduce tailing of basic compounds

# Factors Affecting Peak Shape

## Column related factors – column bonding and endcapping



Columns: 4.6 x 150 mm; Mobile phase: 25 mM K<sub>2</sub>HPO<sub>4</sub>, pH 7.2 / (MeOH: ACN, 50:50), 45/55; low rate: 1 mL/min.  
Detection: UV 254 nm; Injection volume: 5  $\mu$ L  
Sample: Anorectics ("Fen-phen") 1. Phentermine pKa 10.1; 2. Fenfluramine pKa 9.1; 3. Impurity

Good peak shape of highly basic compounds is readily achieved on Bonus-RP

# Factors Affecting Peak Shape

## Column related factors – column packing

### Pore size/structure

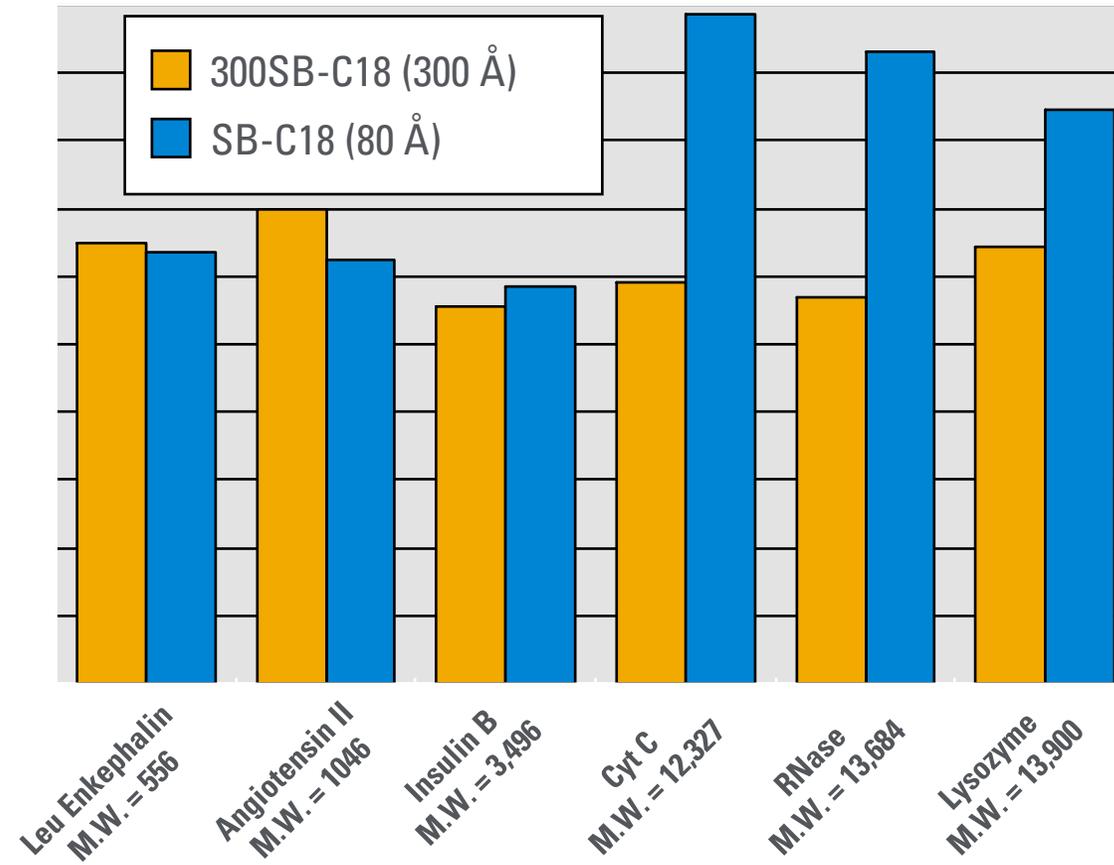
To get good peak shape, select column pore size according to the size of analyte molecules, large molecules need large pore sizes for good peak shape

- Wide-pore (300 Å and larger) columns can be selected for separating proteins and peptides
- Superficially porous Poroshell 300 columns can be used for more rapid mass transfer and improved efficiency of large peptides and proteins at higher flow rates
- Superficially porous Poroshell 120 columns can be used for small molecules as well as peptides for improved efficiency at higher flow rates
- Small-pore totally porous particle columns can be used for small molecules

# Factors Affecting Peak Shape

## Column related factors – column packing

Effect of pore size and molecular size on peak width – gradient separations



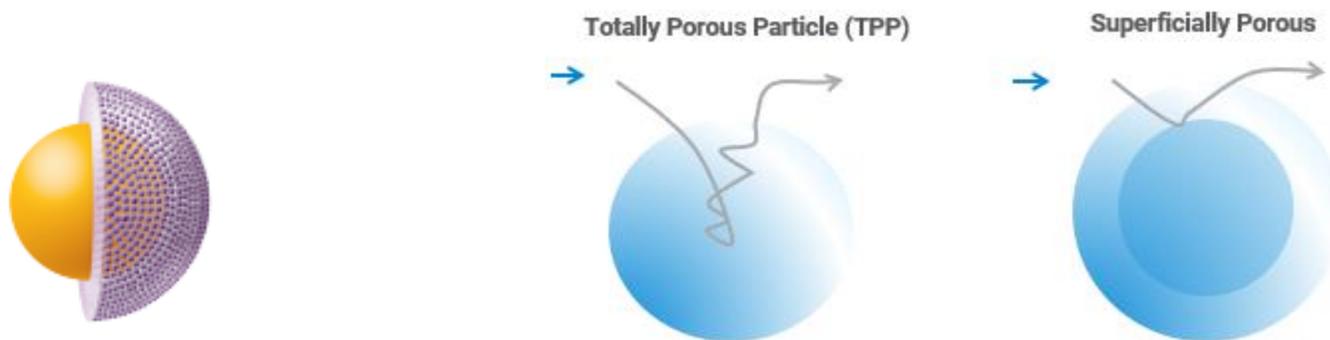
Proper pore size selection results in sharper peaks for large molecules

# Factors Affecting Peak Shape

## Column related factors – column packing

### Poroshell particle technology

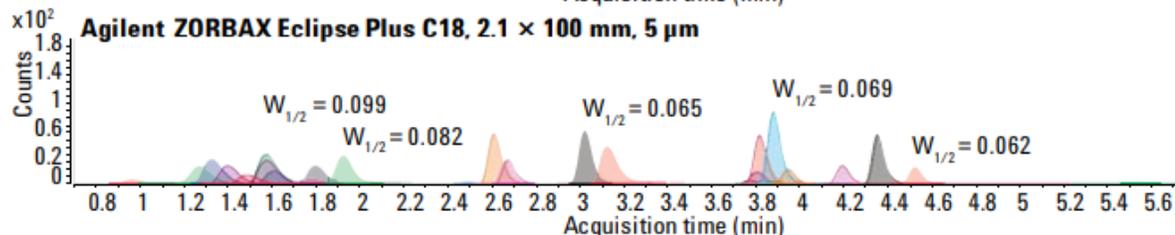
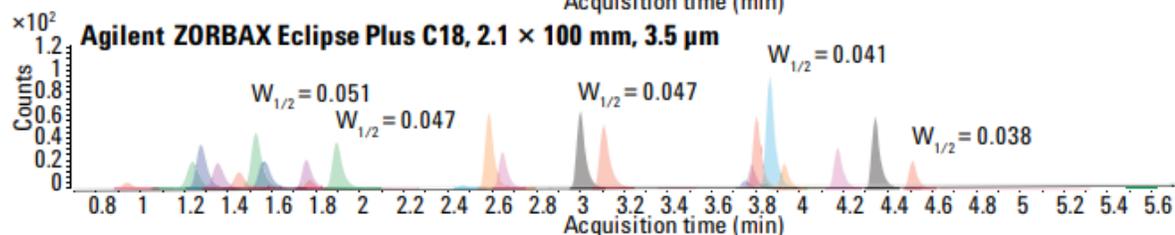
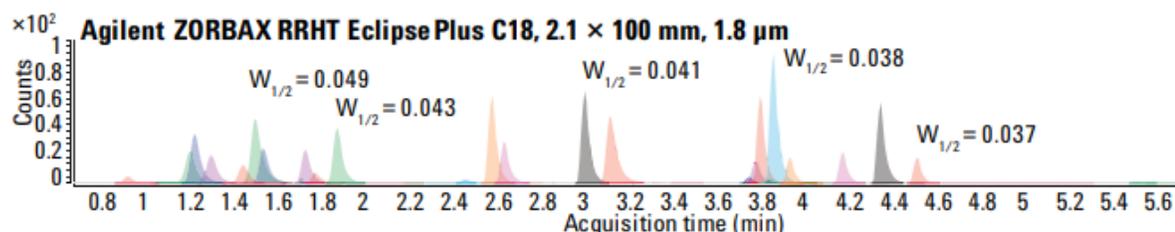
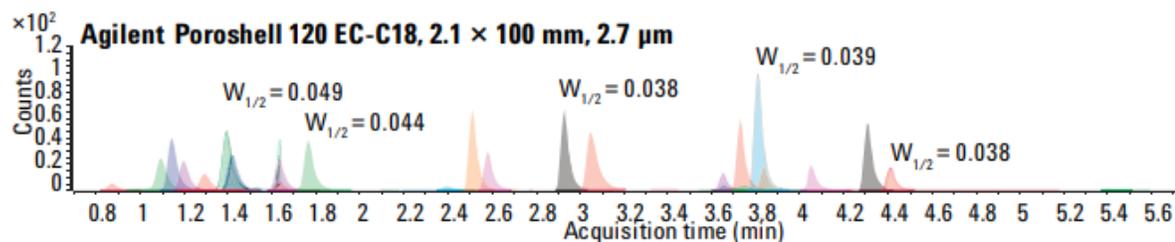
- Superficially porous, solid core particles with a porous outer layer – they provide both improved throughput and higher resolution
- Superior peak shapes for faster, more accurate, results due to high-purity silica and advanced bonding chemistries
- Poroshell 120, 4  $\mu\text{m}$  columns can provide higher efficiency at higher flow rates compared to 5  $\mu\text{m}$  totally porous columns
- Poroshell 120 2.7  $\mu\text{m}$  columns can achieve similar efficiencies as sub-2  $\mu\text{m}$  totally porous columns with substantially less pressure
- Poroshell 120 1.9  $\mu\text{m}$  columns can achieve superior efficiencies over totally porous sub-2  $\mu\text{m}$  columns



# Factors Affecting Peak Shape

## Column related factors – column packing

Smaller particles and superficially porous particles provide sharper peaks



Instrument: Agilent 1200/6410 LC/MS/MS

A: 5 mM ammonium formate with 0.01% formic acid in water

B: acetonitrile

Flow rate: 0.4 mL/min

Gradient method:

| Time | %B |
|------|----|
| 0    | 10 |
| 0.5  | 15 |
| 3.0  | 50 |
| 4.0  | 95 |
| 6.0  | 95 |

0 10

0.5 15

3.0 50

4.0 95

6.0 95

Stop time: 6 min

Post run time: 2 min

Temperature: 60°C

Injection volume: 5 μL

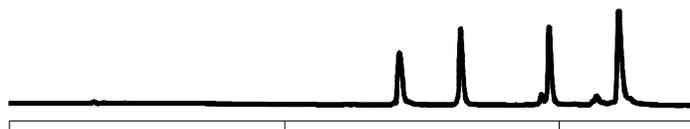
Sample: Agilent LC/MS test mix (p/n 5190-0470), diluted 1:10 in water

# Factors Affecting Peak Shape

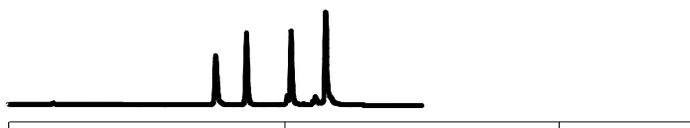
## Column related factors – column packing

High efficiency at high flow rates for ultra-fast protein analysis with Poroshell 300SB-C18

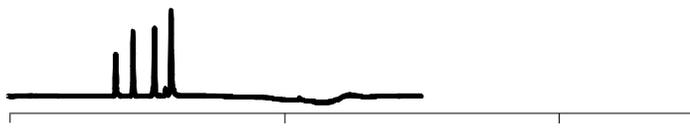
0.5 mL/min  
5–100%B in 4 min



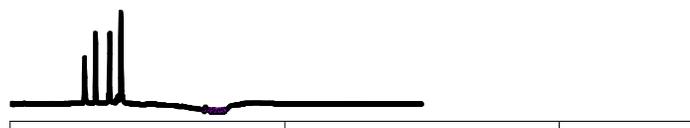
1 mL/min  
5–100%B in 2 min



2 mL/min  
5–100%B in 1 min



3 mL/min  
5–100%B in 0.67 min



4 mL/min  
5–100%B in 0.5 min



Column: Poroshell 300SB-C18  
2.1 x 75 mm, 5 μm

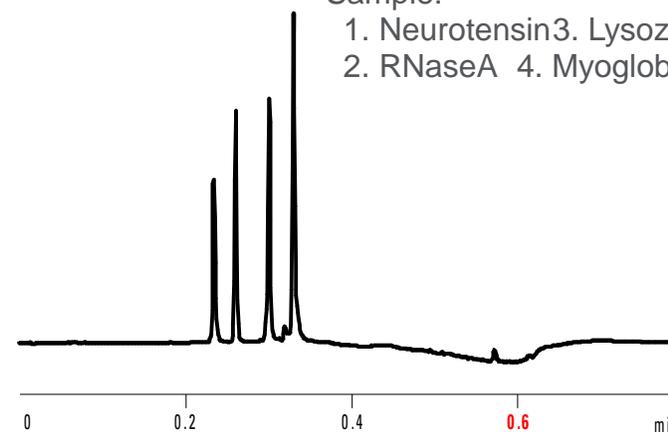
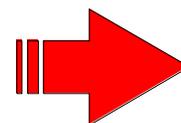
Agilent 1100 DAD  
Agilent 1100 WPS with ADVR

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

Temperature: 70°C

Detector: UV 215 nm

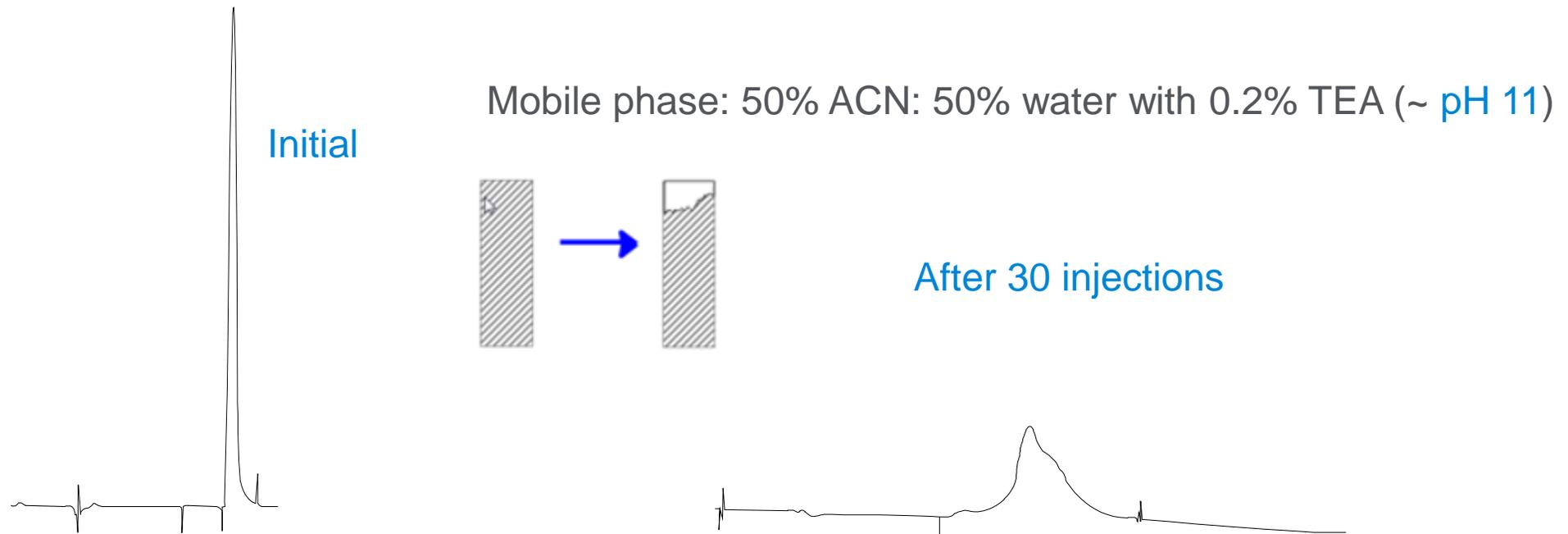
Sample:  
1. Neurotensin 3. Lysozyme  
2. RNaseA 4. Myoglobin



# Factors Affecting Peak Shape

## Column related factors – column packing

Formation of void in the column can result in bad peak shape



Multiple peak shape changes can be caused by presence of void in the column. In this case a void resulted from silica dissolved at high pH.

# Factors Affecting Peak Shape

## Mobile phase related factors

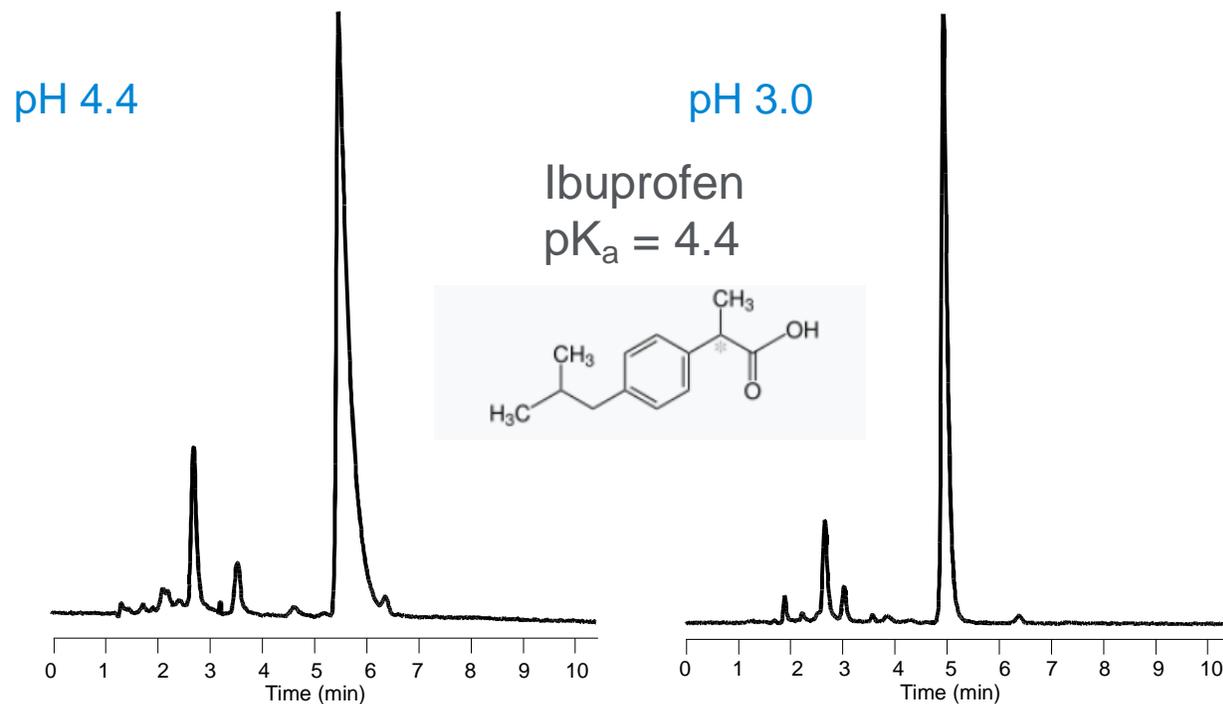
- pH
- Buffers
- Temperature
- Organic modifiers
- Mobile phase additives (TEA, TFA, etc.)



# Factors affecting Peak Shape

## Mobile phase related factors – pH

Effect of pH on peak shape at or near the sample pKa

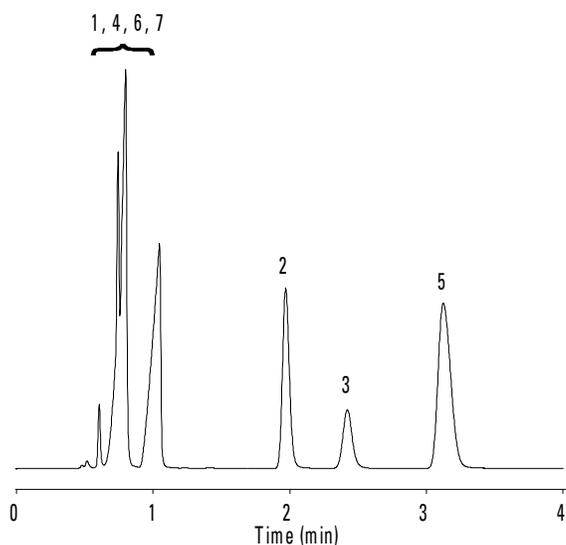


Column: ZORBAX SB-C8 4.6 x 150 mm, 5 μm  
 Mobile phase: 40% 5 mM KH<sub>2</sub>PO<sub>4</sub>, 60% ACN  
 Flow rate: 1.0 mL/min.  
 Temperature: ambient

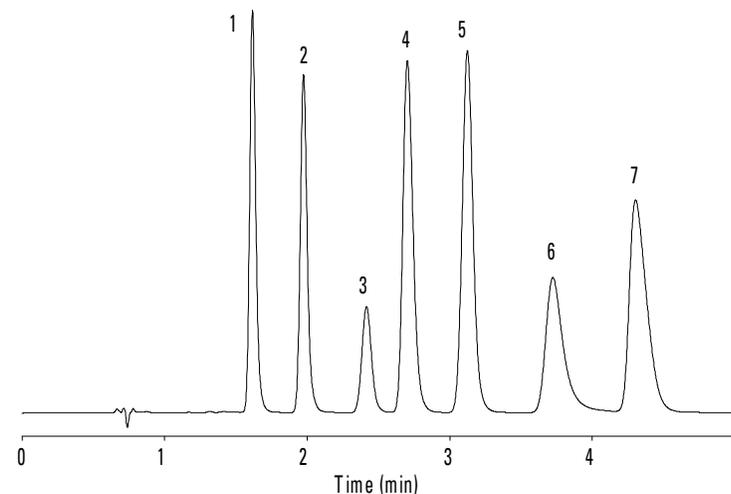
# Factors Affecting Peak Shape

## Mobile phase related factors – buffer

A = pH 7.0 water



A = pH 7.0, 25 mM phosphate buffer



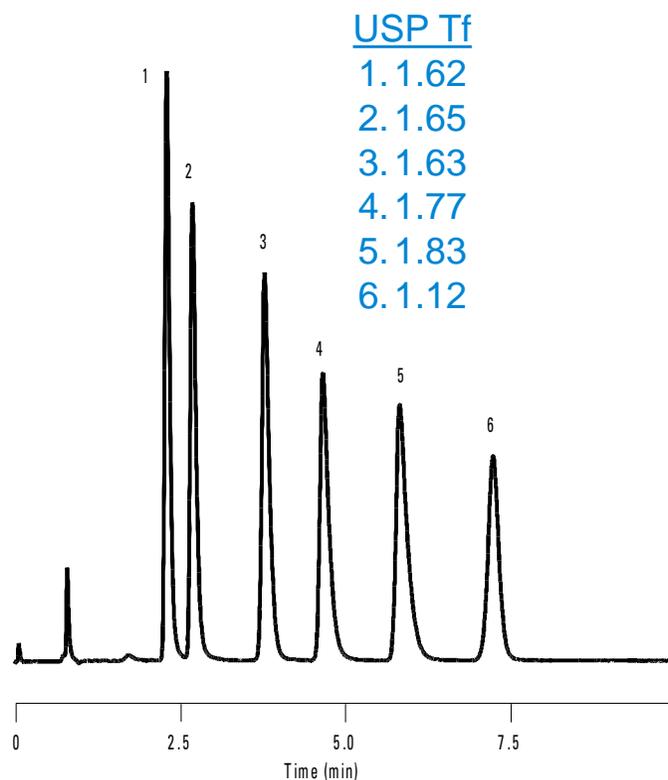
Column: ZORBAX Rapid Resolution Eclipse XDB-C8, 4.6 x 75 mm, 3.5  $\mu$ m      Mobile phase: 44% A : 56% methanol  
 Flow rate: 1.0 mL/min      Temperature: 25°C      Detection: UV 250 nm  
 Sample: 1. ketoprofen 2. ethyl paraben 3. hydrocortisone pKa 5.1 4. Fenoprofen pKa 4.5 5. propyl paraben 6. Propranolol pKa 9.5 7. Ibuprofen pKa 4.4

Buffered mobile phases enhance retention, resolution, and peak shape

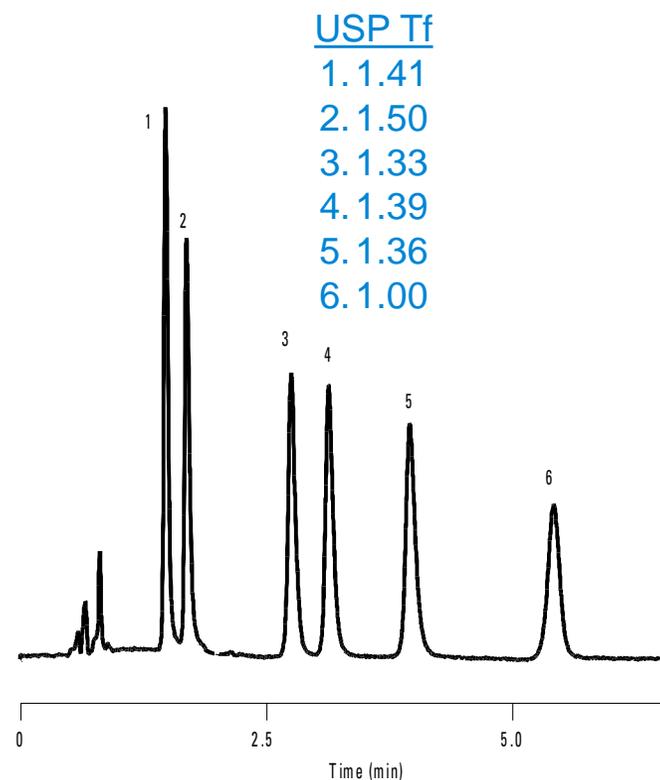
# Factors Affecting Peak Shape

## Mobile phase related factors – buffer

10 mM Phosphate, pH 7.0



25 mM Phosphate, pH 7.0

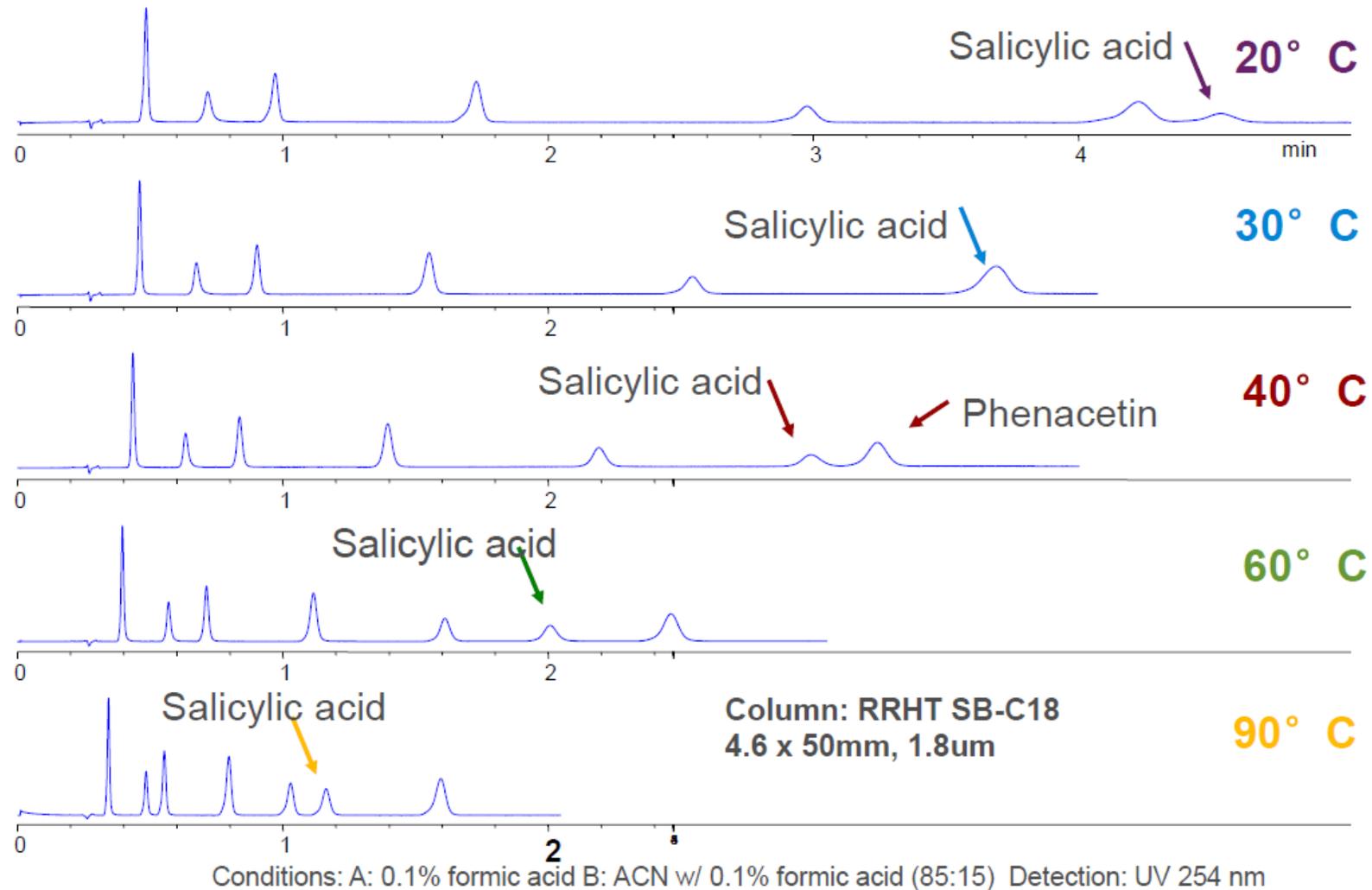


Columns: Eclipse XDB-C8  
4.6 x 150 mm, 5  $\mu$ m  
Mobile phase: 40% phosphate buffer, 60% ACN  
Flow rate: 1.5 mL/min.  
Temperature: 40°C  
Sample: Tricyclic Antidepressants  
1. Desipramine  
2. Nortriptyline  
3. Doxepin  
4. Imipramine  
5. Amitriptyline  
6. Trimipramine

Increasing buffer concentration decreases tailing factor (Tf)

# Factors Affecting Peak Shape

## Mobile phase related factors – temperature

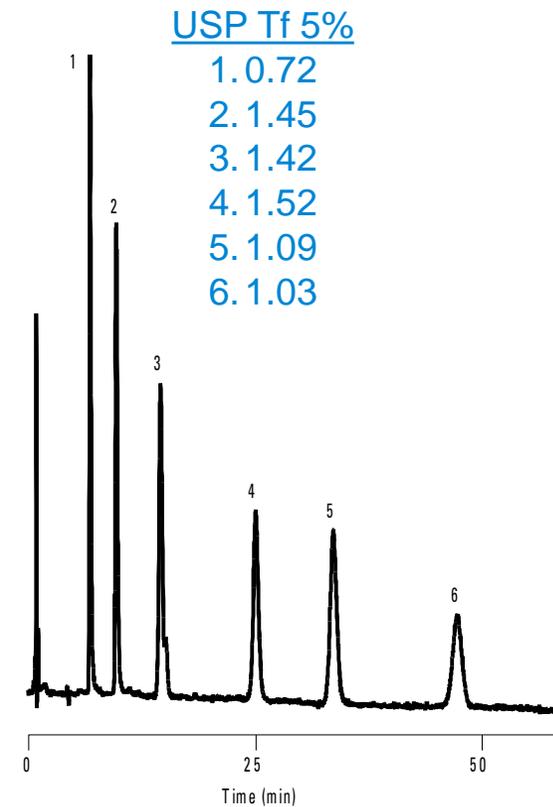
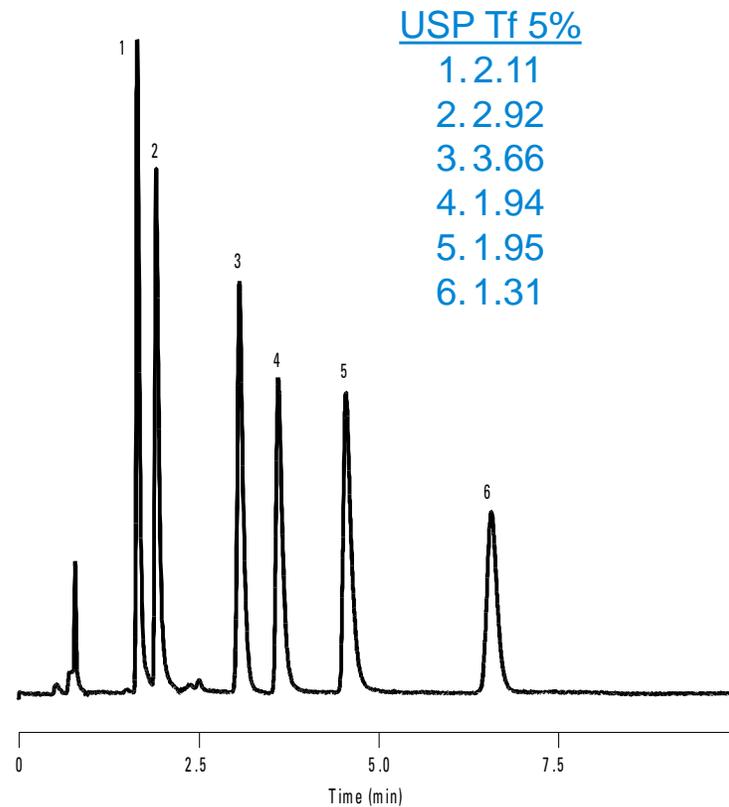


# Factors Affecting Peak Shape

## Mobile phase related factors – organic modifier

40% 25 mM Na<sub>2</sub>HPO<sub>4</sub> pH 7.0  
60% **ACN**

40% 25 mM Na<sub>2</sub>HPO<sub>4</sub> pH 7.0  
60% **MeOH**

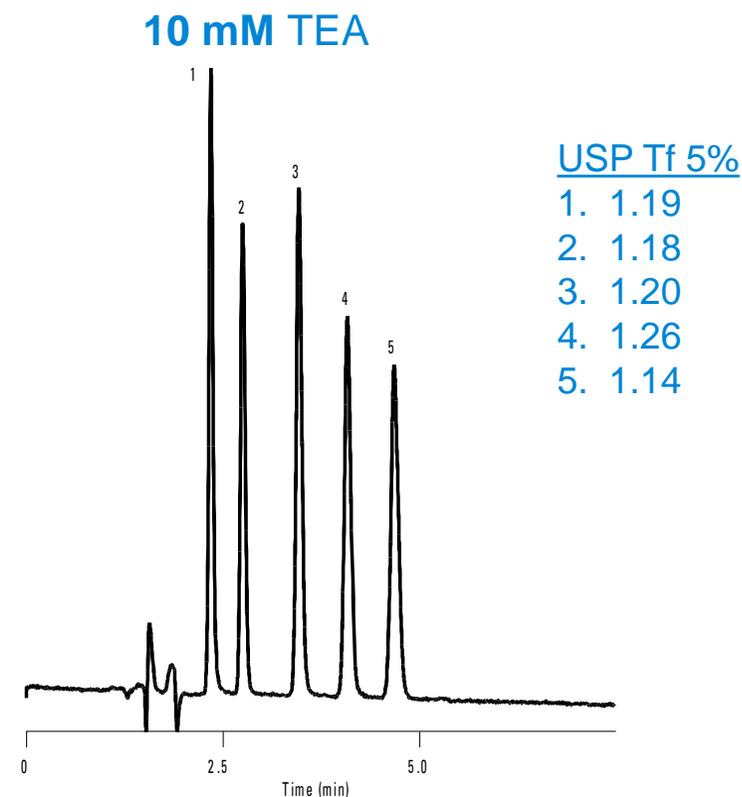
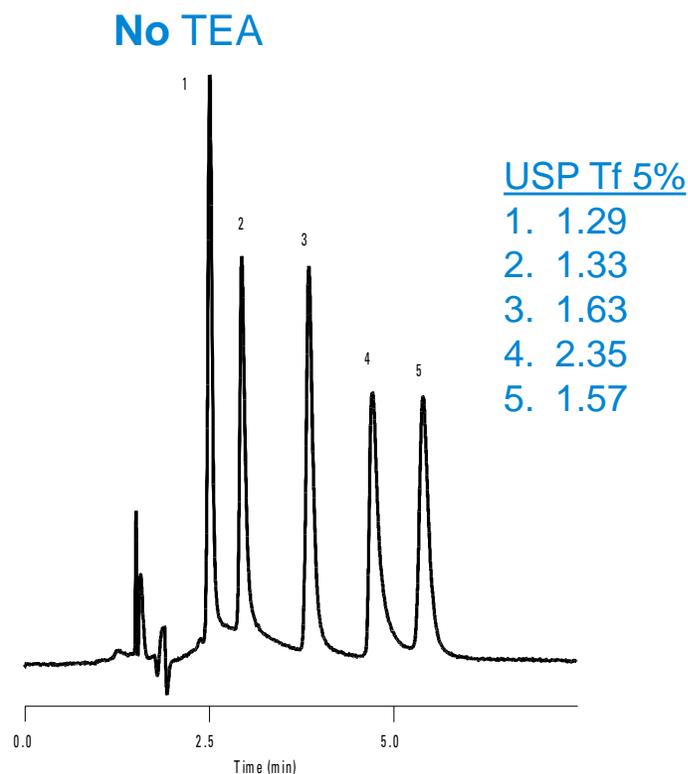


Columns: Eclipse XDB-C8  
4.6 x 150 mm, 5 μm  
Flow Rate: 1.5 mL/min.  
Temperature: 40°C  
Sample: Tricyclic  
Antidepressants  
1. Desipramine  
2. Nortriptyline  
3. Doxepin  
4. Imipramine  
5. Amitriptyline  
6. Trimipramine

Acetonitrile versus methanol

# Factors Affecting Peak Shape

## Mobile phase related factors – mobile phase additives



Columns: Eclipse XDB-C8, 4.6 x 150 mm, 5  $\mu$ m    Mobile Phase: 85% 25 mM Na<sub>2</sub>HPO<sub>4</sub> : 15% ACN    pH: 7    Flow Rate: 1.0 mL/min.  
Temperature: 35°C    Sample: Amphetamines 1. Phenylpropanolamine 2. Ephedrine 3. Amphetamine 4. Methamphetamine 5. Phenteramine

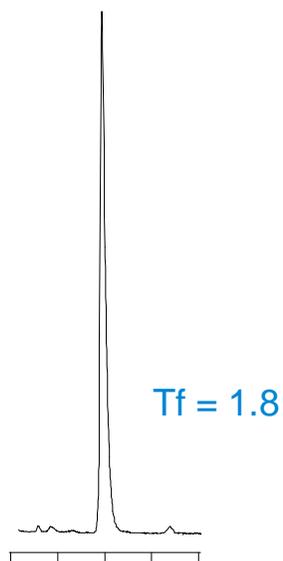
Effect of TEA on peak shape of basic compounds

# Factors Affecting Peak Shape

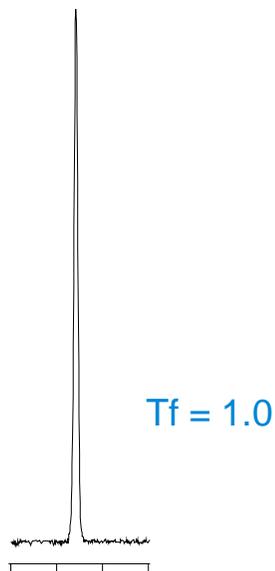
Mobile phase related factors –mobile phase additives

Mobile phase A:

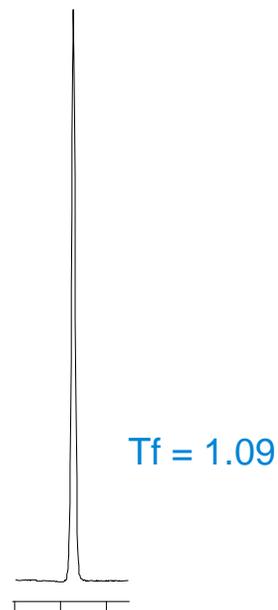
pH 3  
5 mM NaH<sub>2</sub>PO<sub>4</sub>



pH 3  
5 mM NaH<sub>2</sub>PO<sub>4</sub>  
**1% acetic acid**



pH 2.5  
5 mM NaH<sub>2</sub>PO<sub>4</sub>  
**0.1% TFA**



Column: StableBond SB-C18  
4.6 x 150 mm, 5 μm  
Mobile Phase: 40% A: 60% ACN  
Flow Rate: 1.0 mL/min.  
Temperature: Ambient  
Sample: Ibuprofen, pKa 4.4

Effect of competing acids on the peak shape of acidic compounds

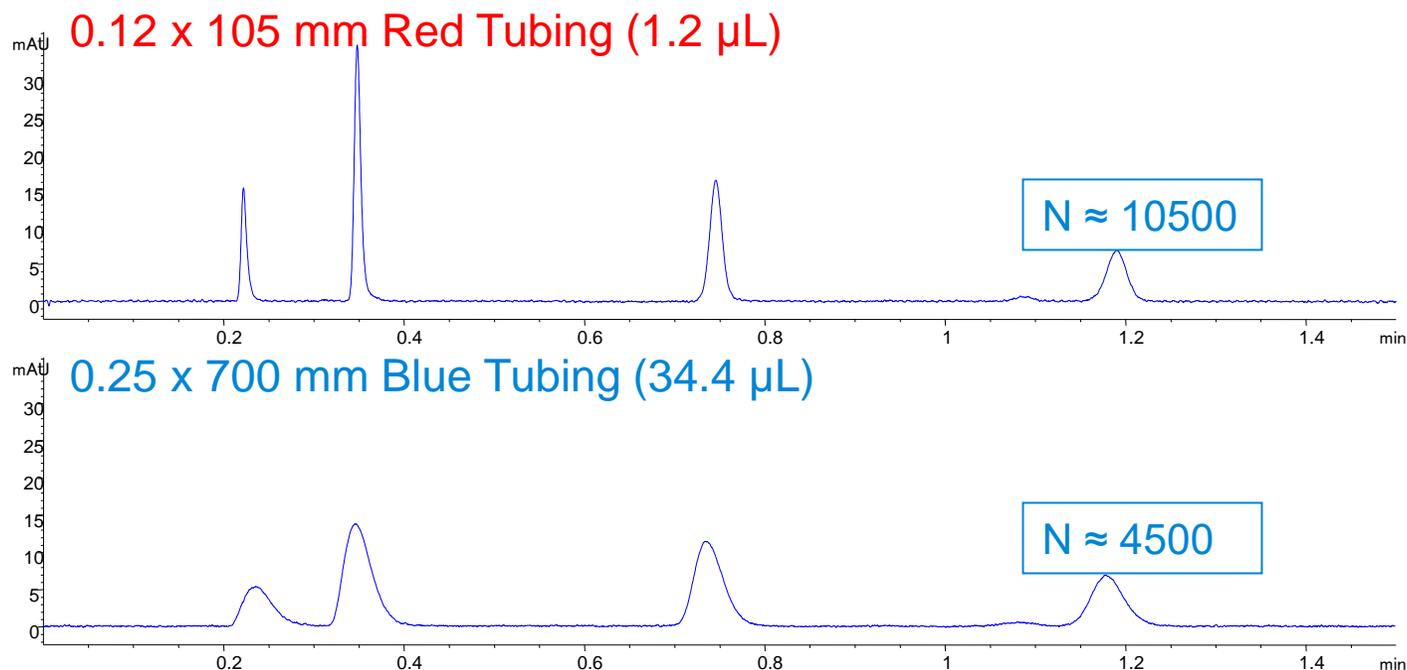
# Factors Affecting Peak Shape

## Connecting capillaries and tubing

Capillary tubing dimensions can affect peak shape



Agilent  
InfinityLab



QC Test Conditions:

55% ACN

45% H<sub>2</sub>O

Isocratic, 0.6 mL/min

1 µL injection of QC Mix

23°C

254 nm

QC Mix (in elution order):

1. 5 µg/mL uracil

2. 200 µg/mL phenol

3. 25 µg/mL 4-chloro-nitrobenzene

4. 40 µg/mL naphthalene

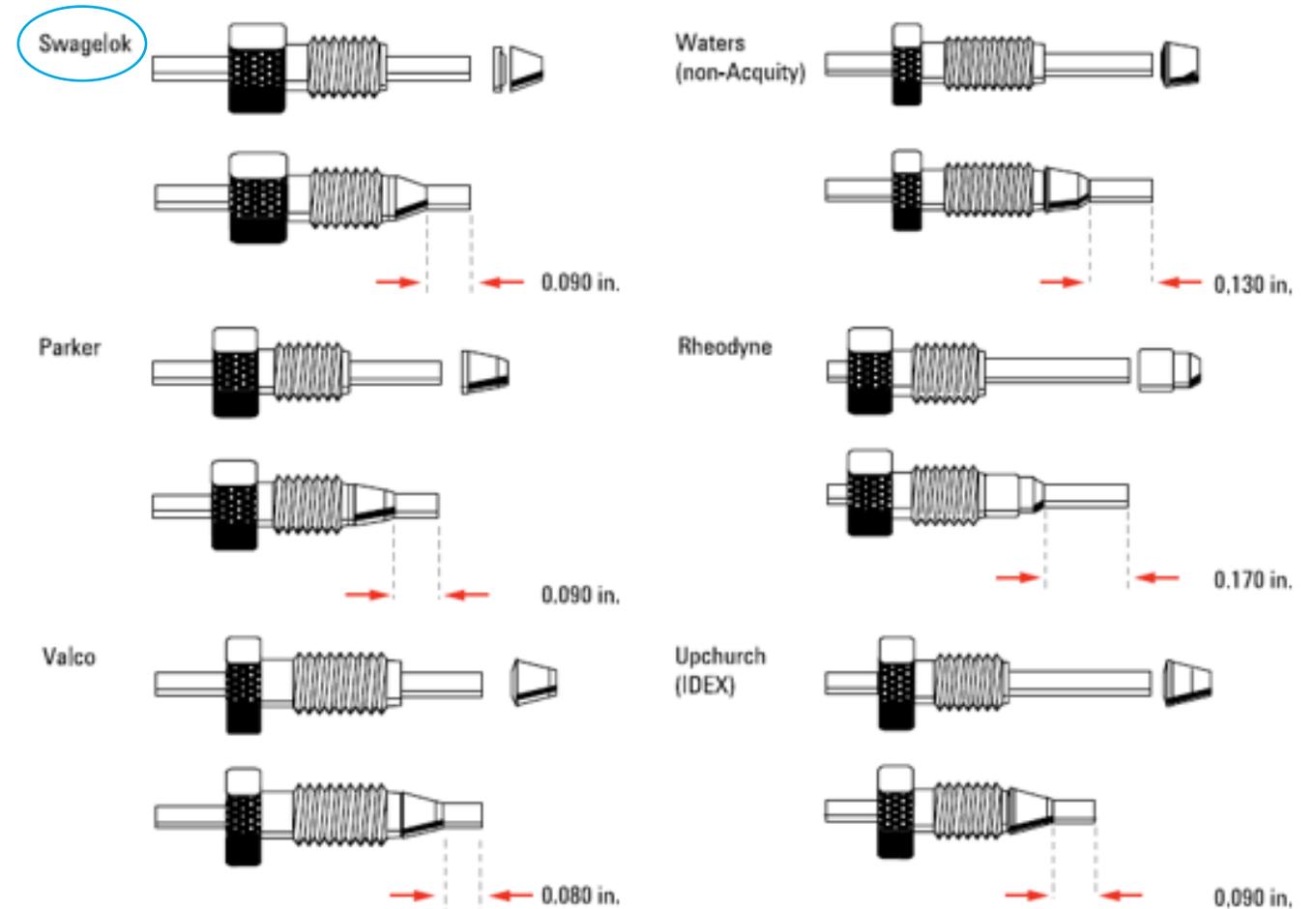
In 50/50 ACN/Water

- 2.1 x 50 mm, 1.8 µm Eclipse Plus C18
- Peak broadening when larger volume tubing installed between autosampler & column
- 43% of the efficiency is lost with too much extra column volume

# Factors Affecting Peak Shape

## Fittings

- Improper fittings can lead to broad, split, and tailing peaks
- Different manufacturers supply different types of fittings
- Use the fittings recommended for your system
- Agilent LC systems use Swagelok-type fittings for many instrument connections



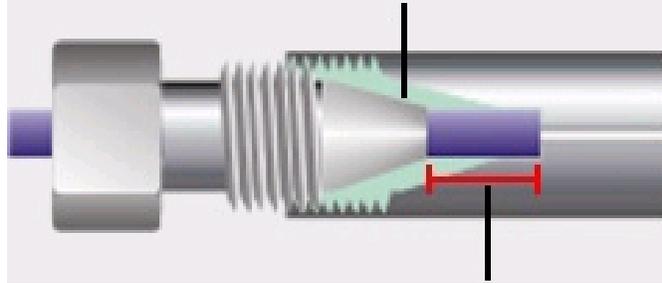
*Different fitting types have different stem lengths*

# Factors Affecting Peak Shape

## Fitting connections

**Bad**

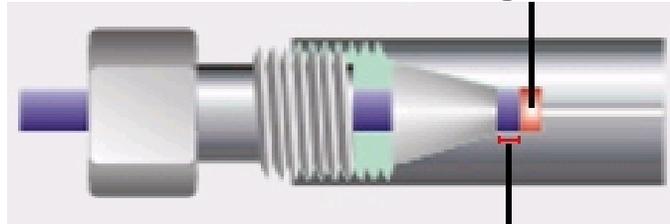
Ferrule cannot seat properly



Too long

**Bad**

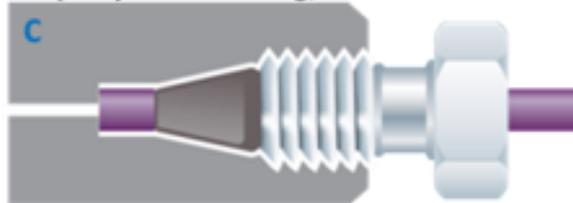
Mixing chamber



Too short

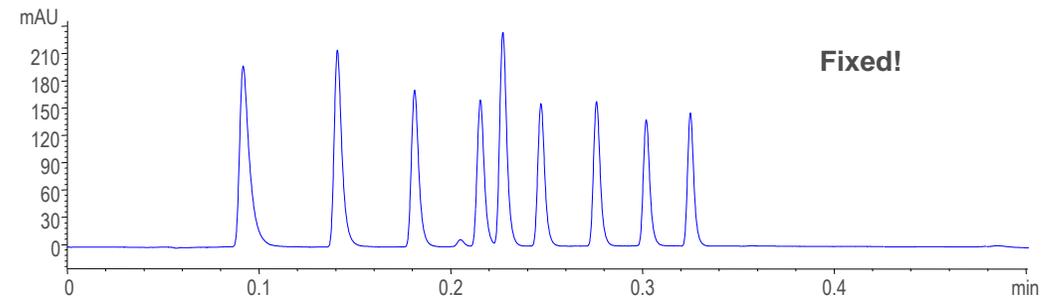
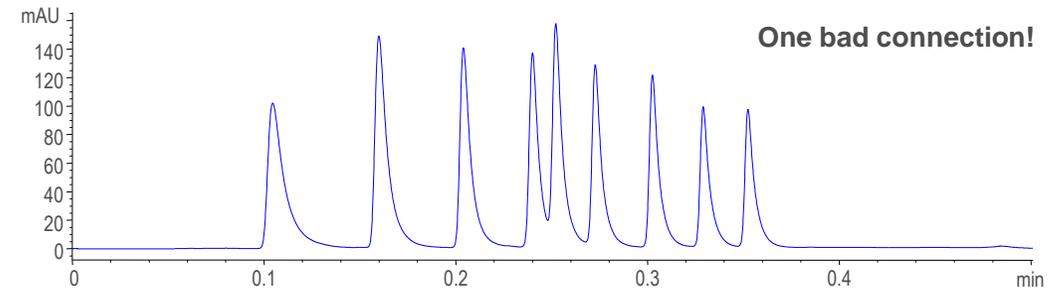
**Good**

Properly fitted tubing, no dead volume



## Poor-fitting connections

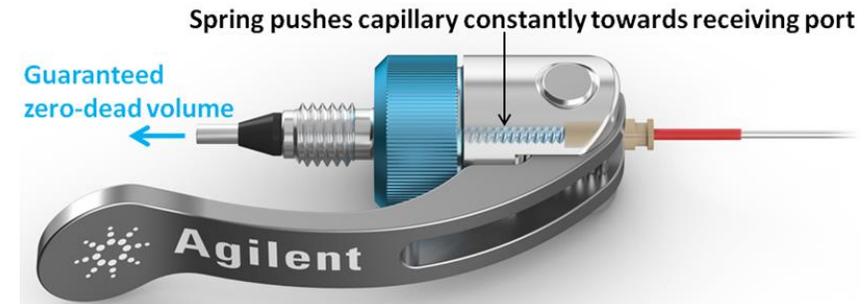
- Will broaden or split peaks or cause tailing
- Will typically affect all peaks, but especially early eluting peaks
- Can cause of carry-over



# Factors Affecting Peak Shape

## Fittings – InfinityLab Quick Connect and Quick Turn

- Spring loaded design
- Easy; no tools needed!
- Works for all column types
- Reusable
- Consistent ZDV connection



### Quick Connect fitting

- Finger tight up to 1300 bar
- Hand tighten the nut, then depress the lever



### Quick Turn fitting

- Finger tight up to 400 bar
- Up to 800 bar with mounting tool
- Up to 1300 bar with a wrench
- Compact design, fits everywhere



# Factors Affecting Peak Shape

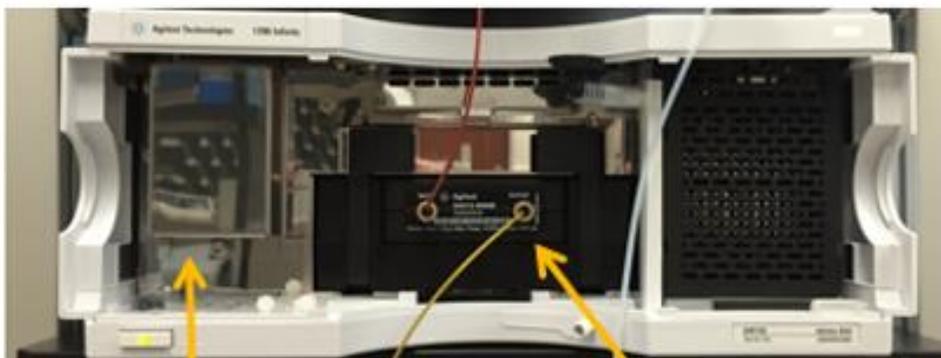
## System related factors

### Detector

- Lamp
- Detector setting – response time/data collection rate
- Flow cell

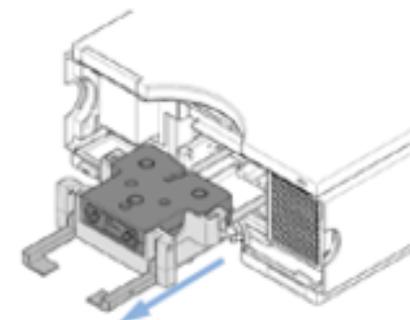


Max-Light cartridge



Lamp

Flow Cell



1290 and some 1260 systems

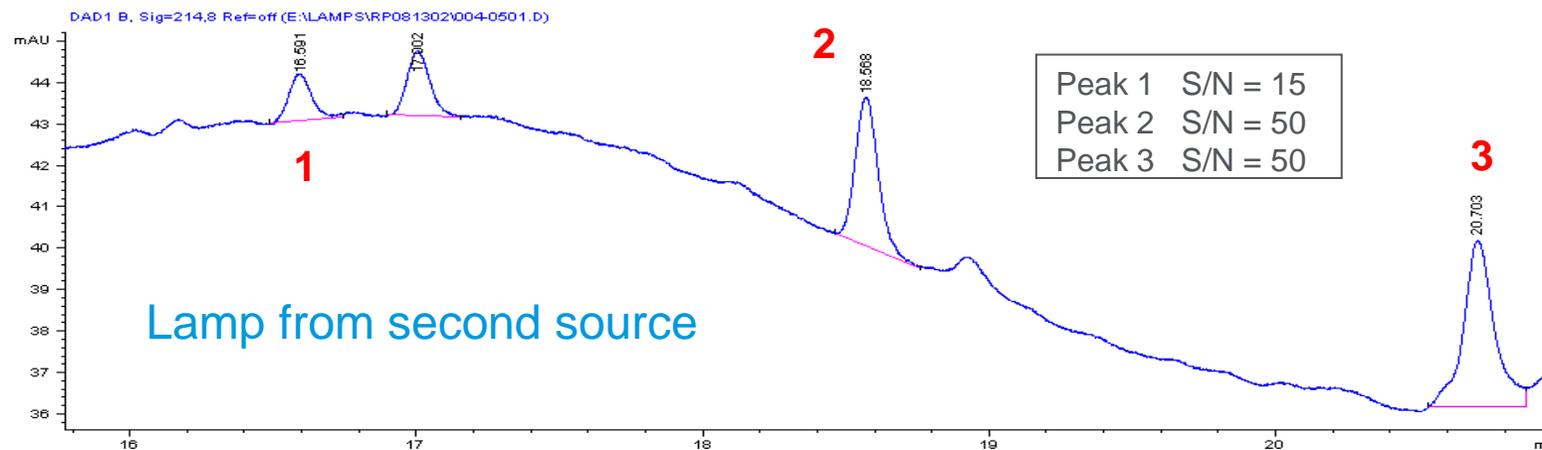
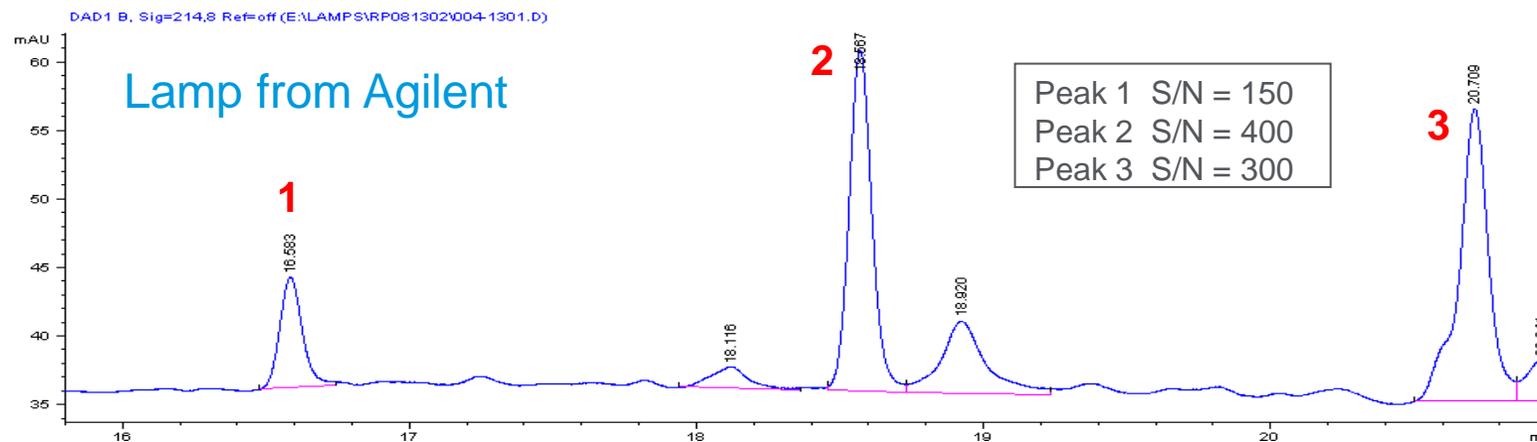
Lamp and flow cells are available with RFID to track usage and to predict replacement

# Factors Affecting Peak Shape

## System related factors – detector lamp



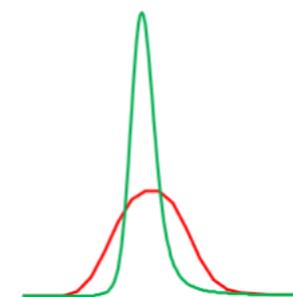
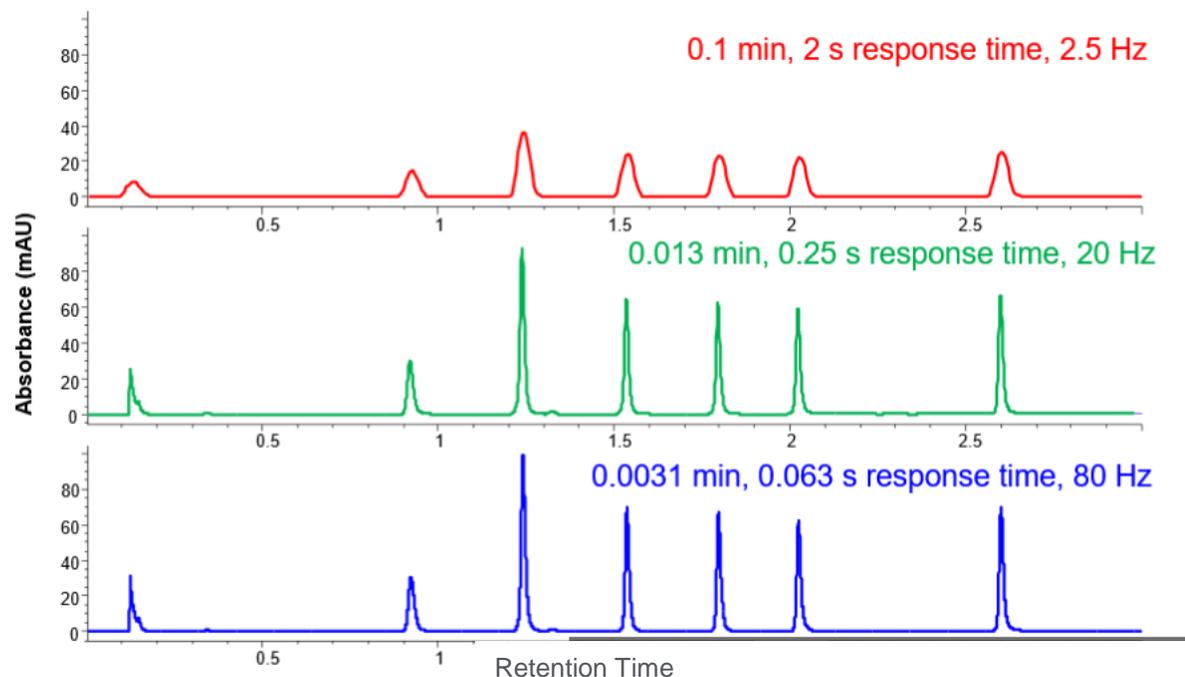
### Detector lamp performance



# Factors Affecting Peak Shape

## System related factors – detector setting

### DAD setting – choose the right sampling rate



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

Column: ZORBAX Eclipse Plus C18, 2.1x 50 mm, 1.8  $\mu$ m  
Column temperature: 35°C  
Flow rate: 1 mL/min  
Gradient: 10-100% acetonitrile in 3 min  
Signal: 254 nm, band width: 4 nm  
Reference: 360 nm, band width: 100 nm

# Factors Affecting Peak Shape

## System related factors – flow cells

Match flow cell volume to chromatographic peak widths



| Flow Cell Volume/Pathlength | Uv Signal /Noise | Chrom. Resolution* |
|-----------------------------|------------------|--------------------|
| 13 µl / 10 mm               | +++              | +                  |
| 5 µl / 6 mm                 | ++               | ++                 |
| 1.7 µl / 6 mm               | +                | +++                |

\* Depends on analytical conditions and column dimension

### 13 µl Standard Flow Cell:

For highest sensitivity and linearity  
4.6-3 mm ID, 2.7, 3.5, 5 µm columns

### 1.7 µl Micro Flow Cell:

For highest resolution  
UHPLC, 1.8, 2.7 µm  
2.1-1 mm ID columns

### 5 µl Semi-micro Flow Cell:

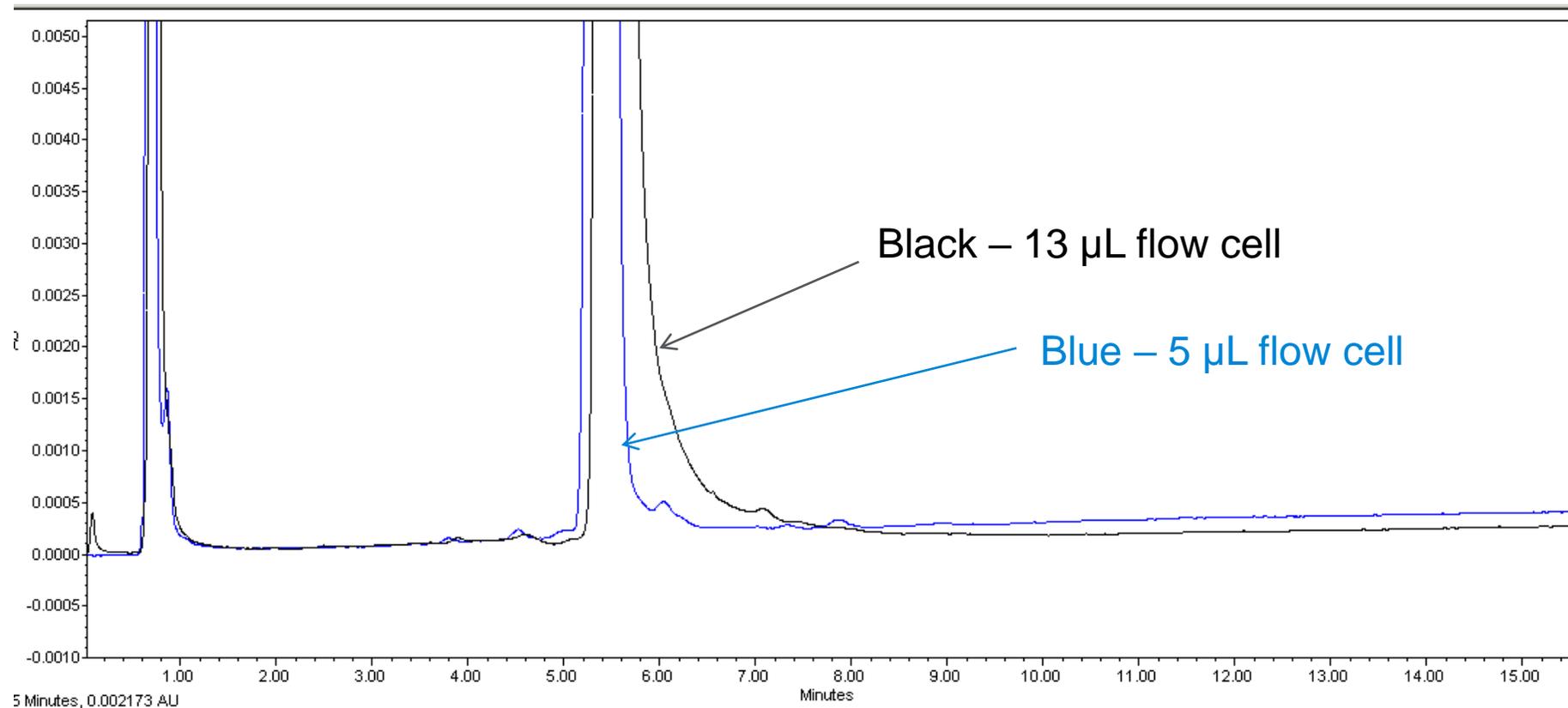
Best compromise of sensitivity & selectivity  
HPLC/UHPLC, 1.8 to 5 µm  
4.6 – 1 mm ID columns

Other flow cells include  
Max-Light Cartridge cells for Infinity DAD  
500 nL for capillary LC  
80 nL for nano LC  
0.6 mm for Prep LC

# Factors Affecting Peak Shape

## System related factors – flow cell

To get good peak shape, match flow cell volume to column



3 x 100 mm, 1.8 µm column

# Factors Affecting Peak Shape

## Sample related factors

- Sample load
- Sample solvent strength
- Sample cleanliness
- Metal complexation

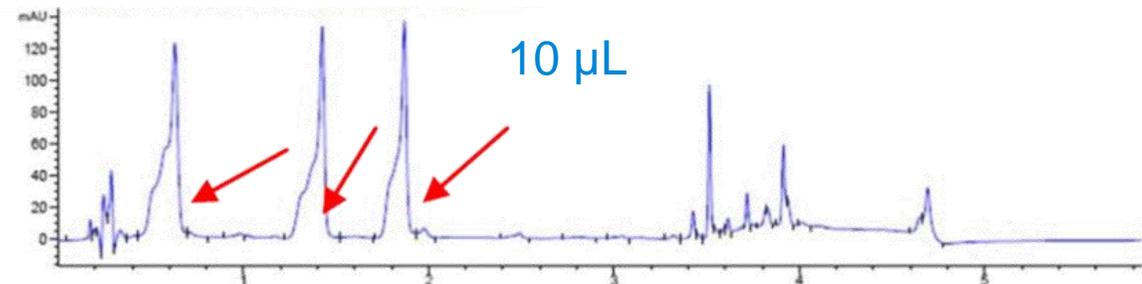
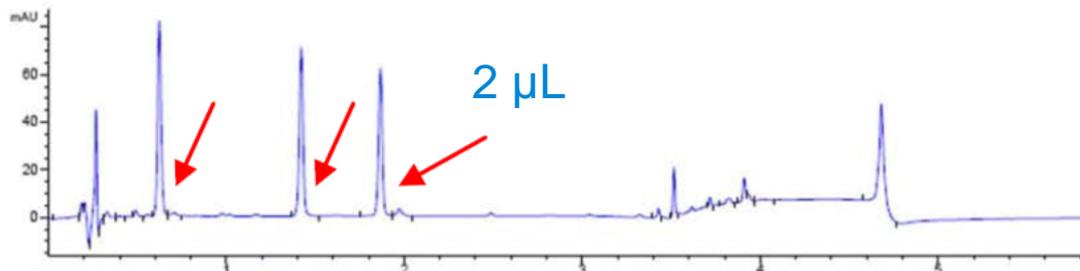


# Factors Affecting Peak Shape

## Sample related factors – sample load

Sample overload may cause peak fronting/broadening/splitting/doubling.

- Peak fronting from sample overload – more sample than can effectively partition, results in some sample preceding the rest of the peak
- Reduce sample load to eliminate the problem.

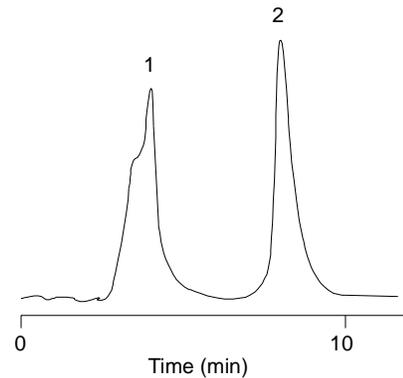


# Factors Affecting Peak Shape

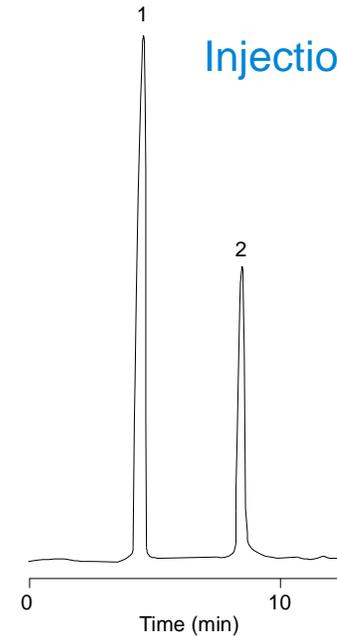
## Sample related factors – sample solvent strength

Strong injection solvent may cause poor peak shape

Injection solvent: **100% Acetonitrile**



Injection solvent: **mobile phase**

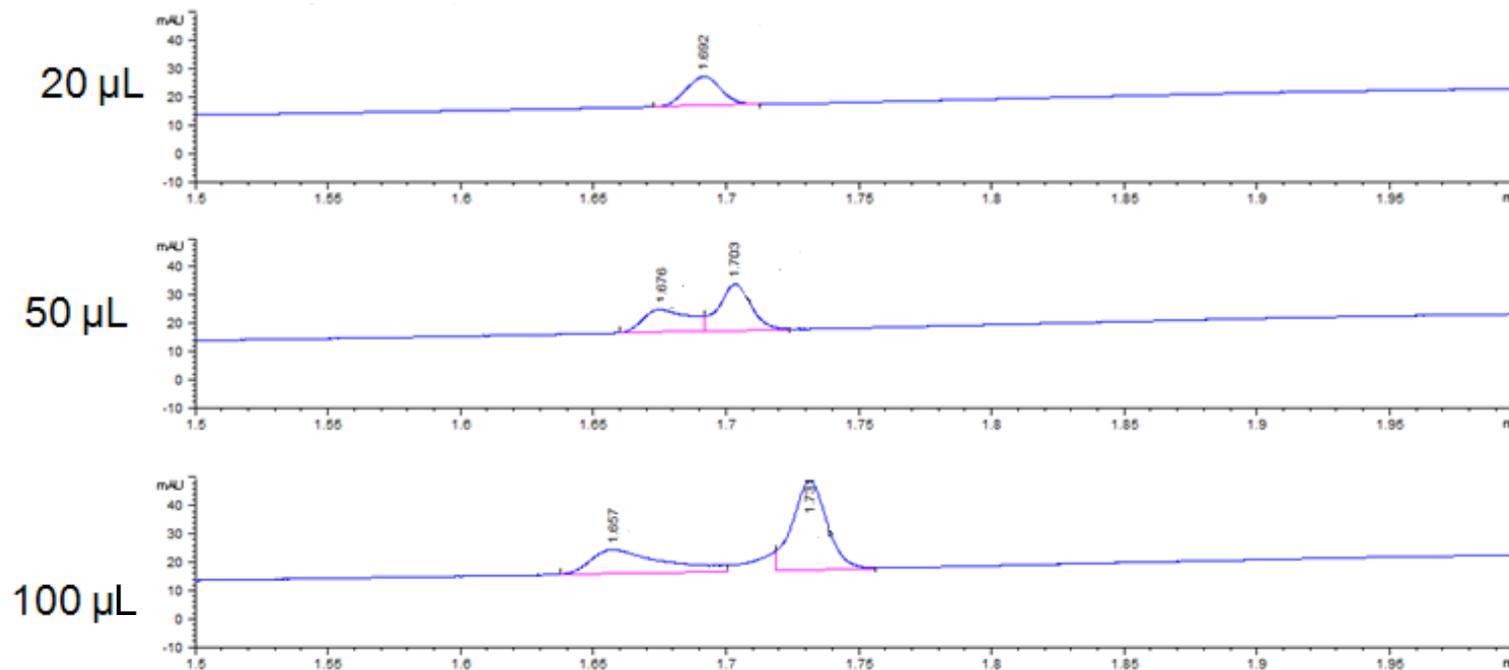


Column: StableBond SB-C8, 4.6 x 150 mm, 5  $\mu$ m  
Mobile phase: 82% H<sub>2</sub>O : 18% ACN  
Injection volume: 30  $\mu$ L  
Sample: 1. Caffeine 2. Salicylamide

# Factors Affecting Peak Shape

## Sample related factors – sample solvent strength

Peak splitting when injecting a large volume of sample in a solvent stronger than the mobile phase



**ZORBAX SB-C18, 4.6 x 50 mm, 1.8 µm**  
Mobile phase: 80% H<sub>2</sub>O with 0.1% TFA; 20% ACN  
Injection solvent; 40% H<sub>2</sub>O, 60% ACN

# Factors Affecting Peak Shape

## Sample related factors – sample cleanliness

- Dirty samples can partially clog the column inlet frit, causing split peaks.
- Chemical contamination from the sample can reside on the column and cause secondary interactions with analytes, resulting in peak tailing and broad peaks.



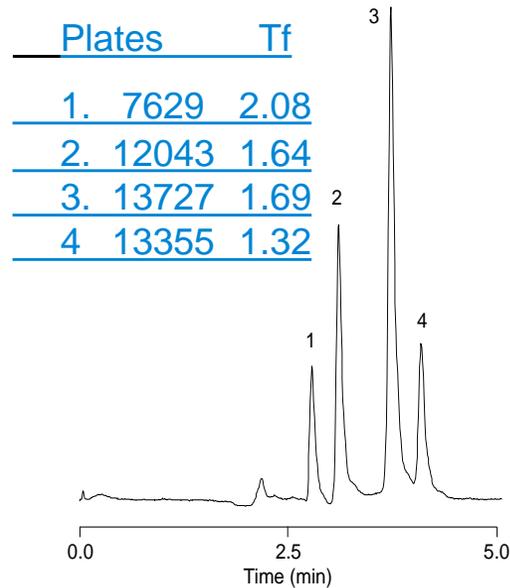
Physical and chemical filtration can minimize these problems



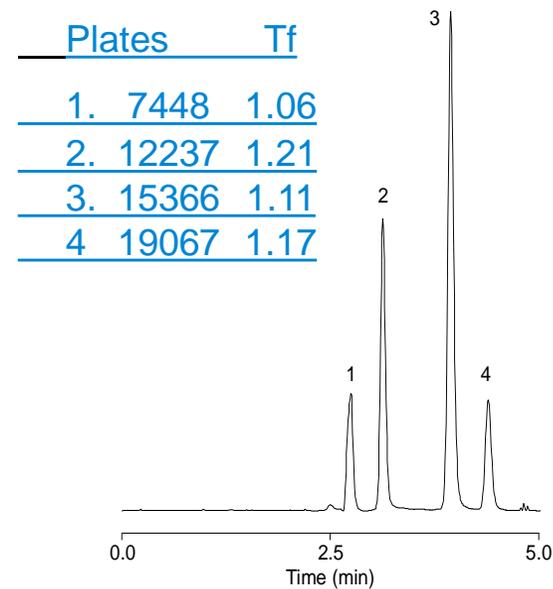
# Factors Affecting Peak Shape

## Sample related factors – sample cleanliness

Column contamination from the samples causing peak tailing



QC test, contaminated column



QC test after cleaning the column

Column: StableBond SB-C8, 4.6 x 250 mm, 5  $\mu$ m    Mobile phase: 20% H<sub>2</sub>O : 80% MeOH    Flow rate: 1.0 mL/min  
Temperature: ambient    Detection: UV 254 nm    Sample: 1. Uracil    2. Phenol    3. 4-Chloronitrobenzene    4. Toluene

# Factors Affecting Peak Shape

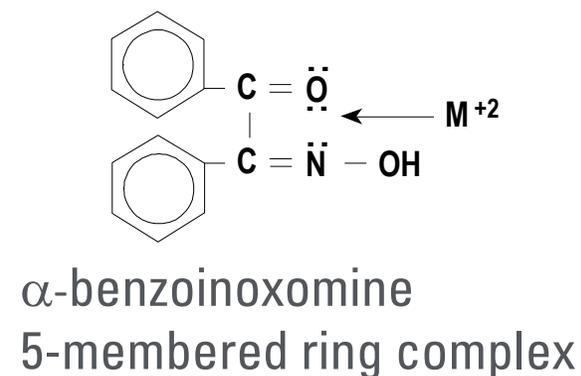
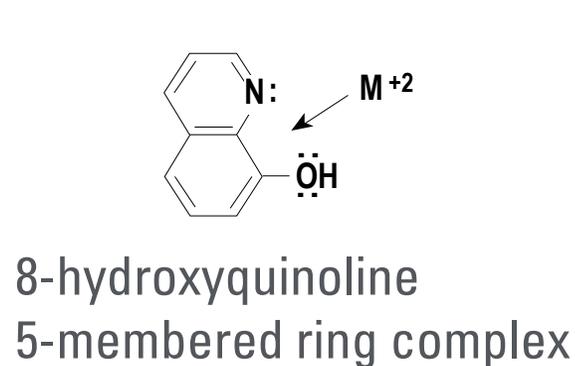
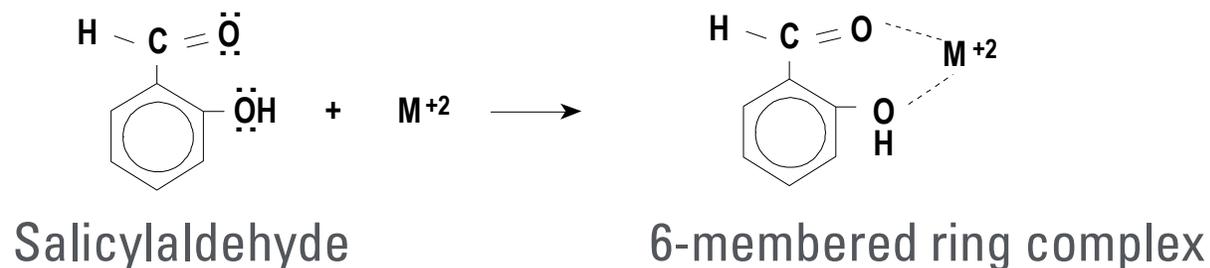
## Sample related factors – metal complexation

- Analytes that can complex with metals may show poor peak shape
- Both tailing and fronting may result from metal complexation
- Metals are present in LC system, column, tubing, fitting ferrules, frits, etc.
- Column packed with high purity silica eliminates silica as a source of metals

# Factors Affecting Peak Shape

## Sample related factors – metal complexation

Metal sensitive compounds can chelate



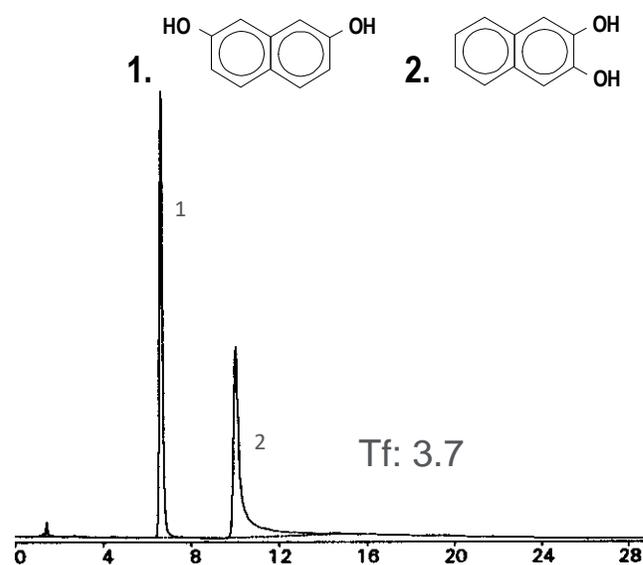
**Hint:** Look for lone pair of electrons on oxygen or nitrogen which can form 5 or 6-membered ring with metal

# Factors Affecting Peak Shape

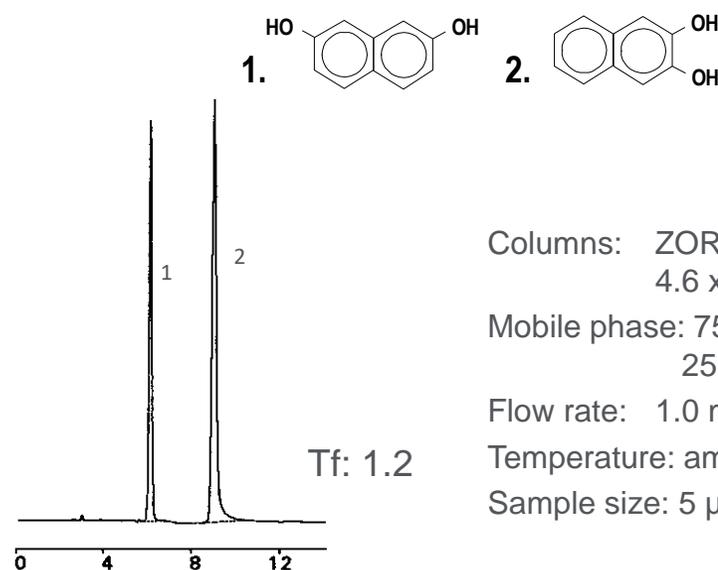
## Sample related factors – metal complexation

Acid wash can improve peak shape

Before acid wash



After acid wash with 50–100 mL 1% H<sub>3</sub>PO<sub>4</sub> in 10% acetonitrile



Columns: ZORBAX SB-Phenyl  
4.6 x 150 mm  
Mobile phase: 75%, 25 mM ammonium phosphate buffer,  
25% acetonitrile  
Flow rate: 1.0 mL/min.  
Temperature: ambient  
Sample size: 5  $\mu$ L

1% H<sub>3</sub>PO<sub>4</sub> in 10% acetonitrile solution is used on SB columns

0.5 % H<sub>3</sub>PO<sub>4</sub> in 10% acetonitrile solution can be used for endcapped columns

# Guidelines for Improved Peak Shape

- Select columns based on high purity fully hydroxylated silica such as InfinityLab Poroshell line of columns as well as ZORBAX Eclipse Plus, StableBond, Eclipse XDB, Bonus-RP and Extend-C18
- Select double or triple endcapped columns for mid pH or difficult basic compounds
- Select special bonded phases (InfinityLab Poroshell 120 HPH, ZORBAX Bonus-RP, ZORBAX Extend-C18) for better peak shape at mid and high pH
- Select wide-pore columns for high molecular weight analytes
- Use spring loaded fittings such as InfinityLab Quick Connect and Quick Turn together with appropriate size connecting capillaries
- Use buffered low pH mobile phases to reduce secondary interactions
- Use 20 – 50 mM buffered mobile phases at every pH
- Use mobile phase additives when needed
- Do sample cleanup
- Check sample solvent and its strength
- Use optimized flow rate and data collection rate



# Contact Agilent Chemistries and Supplies Technical Support



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

Option 1 for GC and GC/MS columns and supplies

**Option 2 for LC and LC/MS columns and supplies**

Option 3 for sample preparation, filtration and QuEChERS

Option 4 for spectroscopy supplies

Option 5 for chemical standards

**Available in the USA & Canada 8-5 all time zones**

[gc-column-support@agilent.com](mailto:gc-column-support@agilent.com)

**[lc-column-support@agilent.com](mailto:lc-column-support@agilent.com)**

[spp-support@agilent.com](mailto:spp-support@agilent.com)

[spectro-supplies-support@agilent.com](mailto:spectro-supplies-support@agilent.com)

[chem-standards-support@agilent.com](mailto:chem-standards-support@agilent.com)

[advancebio.glycan@Agilent.com](mailto:advancebio.glycan@Agilent.com)

WebChat: product pages of [agilent.com](http://agilent.com)

# Appendix

# Column Cleaning Procedure for Reversed Phase Columns

Flush with stronger solvents than your mobile phase

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

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

Use at least 10 column volumes of each solvent for analytical columns

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