HPLC Column Troubleshooting:

Is It Really The Column?

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Application Engineer
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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 \((\alpha)\)
• 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

$$K = \frac{C_s}{C_m}$$

Compound moves through the column only while in mobile phase.

Separation occurs in Column Volumes.
(Flow is volume/time)
Retention Factor \((k)\), Capacity Factor \((k')\)

\[
K = \frac{C_s}{C_m} \implies k = \frac{t_R - t_0}{t_0}
\]

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

Fundamental, dimensionless parameter that describes the retention.

\(k = 1\) to \(20\) - OK; \(k = 3\) to \(10\) - Better; \(k = 5\) to \(7\) - Ideal
Chromatographic Profile

Equations Describing Factors Controlling \( R_S \)

**Retention Factor**

\[ k = \frac{(t_R-t_0)}{t_0} \]

**Selectivity**

\[ \alpha = \frac{k_2}{k_1} \]

**Theoretical Plates-Efficiency**

\[ N = 16\left(\frac{t_R}{t_W}\right)^2 \]

\[ = 5.54\left(\frac{t_R}{W_{1/2}}\right)^2 \]
Test Chromatogram

LC Column Performance Report

SERIAL NUMBER: USUXC01613
PART NUMBER: 959603-902
COLUMN TYPE: ZORBAX Eclipse Plus C18 4.6 x 150 mm, 3.5 µm
PACKING LOT #: 800922

TEST CONDITIONS
- MOBILE PHASE = 65% Methanol / 15% Water
- COLUMN PRESSURE = 126.4 Bar
- COLUMN FLOW = 1.00 ml/min
- LINEAR VELOCITY = 0.168 cm/sec
- TEMPERATURE = AMBIENT (Nominally 23 ºC)
- INJECTION VOLUME = 5 µl

QUALITY CONTROL PERFORMANCE RESULTS FOR TOLUENE

<table>
<thead>
<tr>
<th>TEST VALUES</th>
<th>SPECIFICATIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>THEORETICAL PLATES = 25116</td>
<td>MIN = 18000</td>
</tr>
<tr>
<td>SELECTIVITY = 1.65</td>
<td>RANGE = 1.61 - 1.71</td>
</tr>
<tr>
<td>USP TAILING FACTOR = 1.07</td>
<td>RANGE = 0.99 - 1.20</td>
</tr>
<tr>
<td>(at 5% Peak Height)</td>
<td></td>
</tr>
<tr>
<td>k' = 0.93</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Peak</th>
<th>Conc (µg/ml)</th>
<th>Sample Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>Usual</td>
</tr>
<tr>
<td>2</td>
<td>200</td>
<td>Phenol</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>4-Chloro Nitrobenzene</td>
</tr>
<tr>
<td>4</td>
<td>550</td>
<td>Toluene</td>
</tr>
</tbody>
</table>

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

Agilent Technologies
Column Data Sheet

Agilent Poroshell 120 SB-C18 Threaded Column

Data Sheet

General Description
Agilent Poroshell 120 SB-C18 is a superbly porous monodisperse, late-bonded column packing. Silica-based particles, such as Poroshell, have a solid silica core and a porous silica outer layer. A SilicaBonded (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 solutes.

The Poroshell 120 packing has a solid core of 1.2 μm in size with a porous outer layer 0.6 μm thick and a total particle size of 2.7 μm. The particles have a normal surface area of 120 m²/g and a controlled pore size of 10 Å. The column can be used up to an operating pressure of 600 bar (8500 psi). The uniform spherical particles are a hexahedral (cubic) particle design with a high-purity silica core. The hexahedral silica is designed to reduce dead volume and improve separation of basic and highly polar compounds.

The SilicaBonded SB-C18 bonded phase is made by chemically bonding a sterically hindered C18 stationary phase to the porous shell of the Poroshell 120 silica support. The densely coated, sterically protected, dichlorophenylsilane 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 high-ph mobile phases (for example, pH < 2, high ionic strength (>0.25 M), surtide additives, etc.) due to the superior protection of the bonded phase versus degradation with such mobile phases. The recommended high temperature limit for the bonded phase is 80 °C at low pH.

Column Characteristics
A typical Quality Control test chromatogram for a Poroshell 120 SB-C18 4.6 mm x 50 mm, 2.7 μm packed column is shown in Figure 1. The actual GC 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 GC test system may vary from the GC used in your lab and has been modified from a standard system to include system volume. This allows a better evaluation of the packed column and ensures a more consistent product for your chromatograph.

Safety Considerations
All parts of connection in liquid chromatographic systems are potential sources of leaks. Users of LCs and UPLCs 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 UPLC systems. The operating pressure limit for all 1.4- and 3.6-mm columns is 600 bar (8500 psi). While the 2.1- and 3.0-mm columns are safe to 1000 bar (14500 psi) the 4.0-mm columns are safe to 600 bar (8500 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, the Poroshell packing is 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

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

- \( \Delta P \) = Pressure Drop
- \( \eta \) = Fluid Viscosity
- \( L \) = Column Length
- \( v \) = Flow Velocity
- \( d_p \) = Particle Diameter
- \( \theta \) = 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 x $V_m$ of each solvent for analytical columns

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

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

**Normal Phase Solvent Choices**

*In Order of Increasing Strength*

- Use at least 50 mL of each solvent
- 50% Methanol : 50% Chloroform
- 100% Ethyl Acetate
Preventing 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 um frit for 3.5 um columns, use 0.5 um frit for 1.8um 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

Mobile Phase From Pump → Pre-Column → Injector → Guard Column → Analytical Column → To Detector

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
   - 5µm - 2µm frit ▽ 3-3.5µm - 0.5µm-2um frit ▽ 1.8µm - 0.2µm 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

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 µm
Mobile Phase: 60% 25 mM Na₂HPO₄, pH 3.0 : 40% MeOH
Flow Rate: 1.0 mL/min
Temperature: 35°C
Detection: UV 254 nm

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 µm; Mobile Phase: 82% H₂O : 18% ACN;
Injection Volume: 30 µL  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, 5µm  Mobile Phase: 85% 25 mM Na₂HPO₄ pH 7.0 : 15% ACN

- Peak tailing of amine analytes eliminated with mobile phase modifier (TEA, triethylamine) at pH 7
Peak Tailing
Column “Secondary Interactions”

Column: Alkyl-C8, 4.6 x 150 mm, 5µm  
Mobile Phase: 85% 25 mM Na₂HPO₄ : 15% ACN  
Flow Rate: 1.0 mL/min  
Temperature: 35°C  

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

pH 3.0  
USP TF (5%)  
4. 1.33

pH 7.0  
USP TF (5%)  
4. 2.35
Peak Tailing
Column Contamination

Column: StableBond SB-C8, 4.6 x 250 mm, 5µm
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

<table>
<thead>
<tr>
<th>Plates</th>
<th>TF</th>
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<tbody>
<tr>
<td>1.</td>
<td>7629    2.08</td>
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<tr>
<td>2.</td>
<td>12043   1.64</td>
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<tr>
<td>3.</td>
<td>13727   1.69</td>
</tr>
<tr>
<td>4.</td>
<td>13355   1.32</td>
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QC test reverse direction

<table>
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<th>TF</th>
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<td>7906    1.43</td>
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<tr>
<td>2.</td>
<td>12443   1.21</td>
</tr>
<tr>
<td>3.</td>
<td>17999   1.19</td>
</tr>
<tr>
<td>4.</td>
<td>17098   1.25</td>
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</tbody>
</table>

QC test after cleaning
100% IPA, 35°C

<table>
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<th>TF</th>
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<td>1.</td>
<td>7448    1.06</td>
</tr>
<tr>
<td>2.</td>
<td>12237   1.21</td>
</tr>
<tr>
<td>3.</td>
<td>15366   1.11</td>
</tr>
<tr>
<td>4.</td>
<td>19067   1.17</td>
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</tbody>
</table>
Peak Tailing/Broadening
Sample Load Effects

Columns: 4.6 x 150 mm, 5µm
Mobile Phase: 40% 25 mM Na₂HPO₄ pH 7.0 : 60% ACN
Flow Rate: 1.5 mL/min
Temperature: 40°C

Tailing
Eclipse XDB-C8
USP TF (5%)

A. High Load x10

B. Low Load

C. Broadening
Competitive C8 Plates

D.

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>D</th>
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<tr>
<td>1</td>
<td>850</td>
<td>5941</td>
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<td>2</td>
<td>815</td>
<td>7842</td>
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<tr>
<td>3</td>
<td>2776</td>
<td>6231</td>
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<td>4</td>
<td>2539</td>
<td>8359</td>
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<tr>
<td>5</td>
<td>2735</td>
<td>10022</td>
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<tr>
<td>6</td>
<td>5189</td>
<td>10725</td>
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<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
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<tbody>
<tr>
<td>1</td>
<td>1.60</td>
</tr>
<tr>
<td>2</td>
<td>2.00</td>
</tr>
<tr>
<td>3</td>
<td>1.56</td>
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<tr>
<td>4</td>
<td>2.13</td>
</tr>
<tr>
<td>5</td>
<td>2.15</td>
</tr>
<tr>
<td>6</td>
<td>1.25</td>
</tr>
</tbody>
</table>
Peak Broadening, Splitting
Column Void

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

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

**Unknown “Phantom” Peaks**

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

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

Sample 1: Chlorpheniramine maleate  
Peak 1: maleate

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

Plates

1. 5922  
2. 9879  
3. 779  

“Phantom” peak from first injection
Peak Tailing
Injector Seal Failure

Column: Bonus-RP, 4.6 x 75 mm, 3.5 µm
Mobile Phase: 30% H₂O : 70% MeOH
Flow Rate: 1.0 mL/min
Temperature: R.T.
Detection: UV 254 nm

Before

<table>
<thead>
<tr>
<th>Plates USP TF (5%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 2235 1.72</td>
</tr>
<tr>
<td>2. 3491 1.48</td>
</tr>
<tr>
<td>3. 5432 1.15</td>
</tr>
</tbody>
</table>

After replacing rotor seal and isolation seal

<table>
<thead>
<tr>
<th>Plates USP TF (5%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 3670 1.45</td>
</tr>
<tr>
<td>2. 10457 1.09</td>
</tr>
<tr>
<td>3. 10085 1.00</td>
</tr>
</tbody>
</table>

• 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 µm
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

10 µL extra-column volume

50 µL extra-column volume (tubing)
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

Mixing Chamber

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 Overload

Analytical run

Volume overloading

Concentration overloading

$V_{inj}$
Column Overload - Isotherms

Linear isotherm,

\[ K = \frac{C_s}{C_m} \]
Column Overload - Isotherms

Analytical run

Column overload

Concentration overloading

$V_{\text{inj}}$

$C_s$

$C_m$
Categories of Column Problems

A. Pressure

B. Peak shape

C. Retention
Retention Issues

• Retention time changes ($t_r$)
• Retention factor changes ($k'$)
• Selectivity changes ($\alpha$)
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

60% MeOH: 40% 0.1% TFA

Fresh TFA Added to Mobile Phase

- 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*

<table>
<thead>
<tr>
<th>Condition</th>
<th>Variation</th>
<th>Relative Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow Rate</td>
<td>+/- 1%</td>
<td>+/- 1% tr</td>
</tr>
<tr>
<td>Temp</td>
<td>+/- 1 deg C</td>
<td>+/- 1 to 2% tr</td>
</tr>
<tr>
<td>%Organic</td>
<td>+/- 1%</td>
<td>+/- 5 to 10% tr</td>
</tr>
<tr>
<td>pH</td>
<td>+/- 0.01%</td>
<td>+/- 0 to 1% tr</td>
</tr>
</tbody>
</table>

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

- 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-hydroxyquinolone

5-membered ring complex

α-benzoinoxomine

5-membered ring complex
Acid Wash Can Improve Peak Shape

Before Acid Wash

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: 3.7

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

Column 1

Column 2

Column 2 - Fresh mobile phase
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 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!