Care, Maintenance, and Troubleshooting of HPLC Columns

Part 2

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

1. HPLC System Pressure

2. Chromatogram - Peak Shape

3. Chromatogram - Peak Retention/Selectivity

2. Peak Shape Issues in HPLC

- **Split peaks**

- **Peak tailing**

- **Broad peaks**

- **Poor efficiency (low N)**

- **Inconsistent response**

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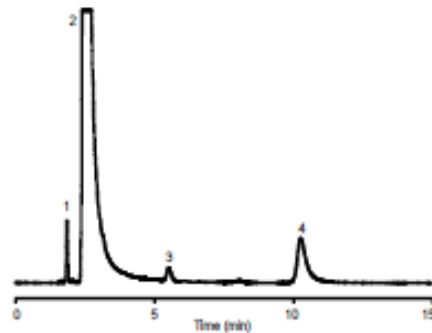
Split Peaks

Can be caused by:

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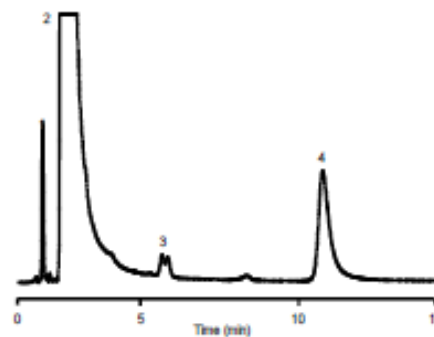- Injection solvent effects

Split Peaks
Column Contamination

Column: StableBond SB-C8, 4.6 x 150 mm, 5 μm   Mobile Phase: 60% 25 mM Na_2HPO_4, pH 3.0 : 40% MeOH   Flow Rate: 1.0 mL/min
Temperature: 35°C   Detection: UV 254 nm   Sample: Filtered OTC Cold Medication: 1. Pseudoephedrine   2. APAP   3. Unknown   4. Chlorpheniramine
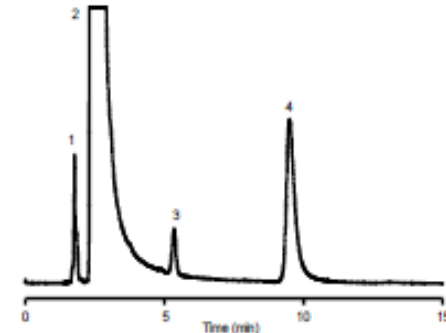
Injection 1 **Injection 30** **Injection 1 After Column Wash with 100% ACN**

• Column washing eliminates the peak splitting, which resulted from a contaminant on the column.
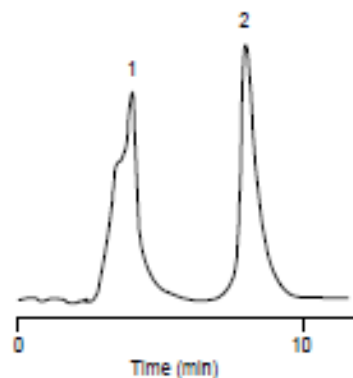
Split Peaks

Injection Solvent Effects

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

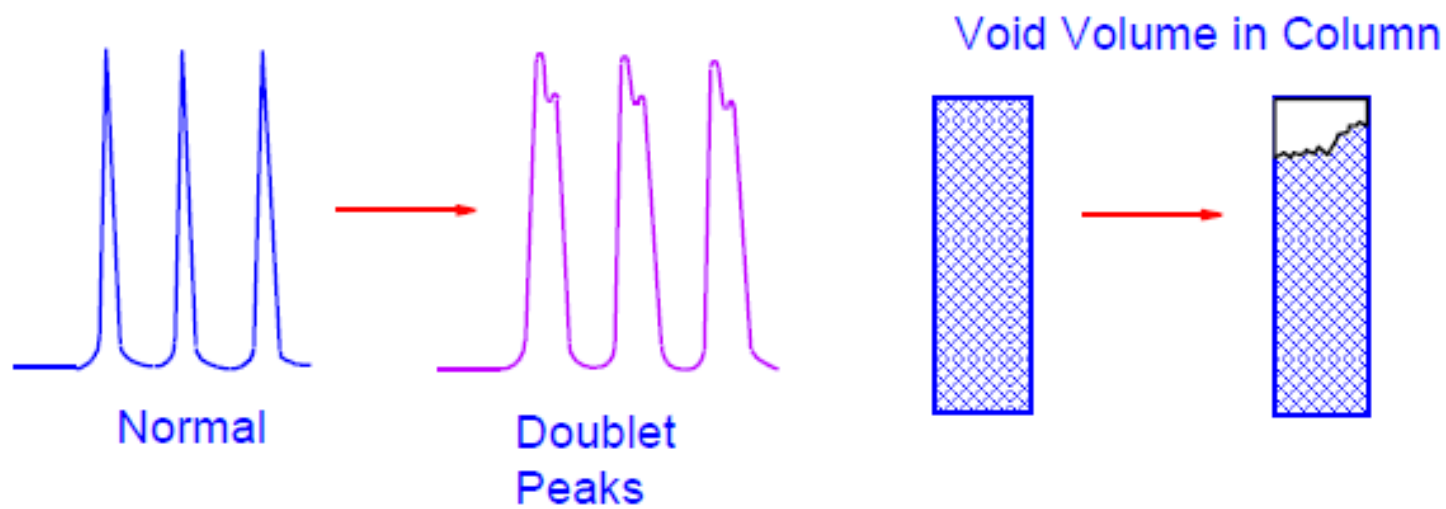**A. Injection Solvent 100% Acetonitrile**

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 Shape Problems - Doublets

Normal → Doublet Peaks

Void Volume in Column

- Void Volume in Column
- Partially Blocked Frit

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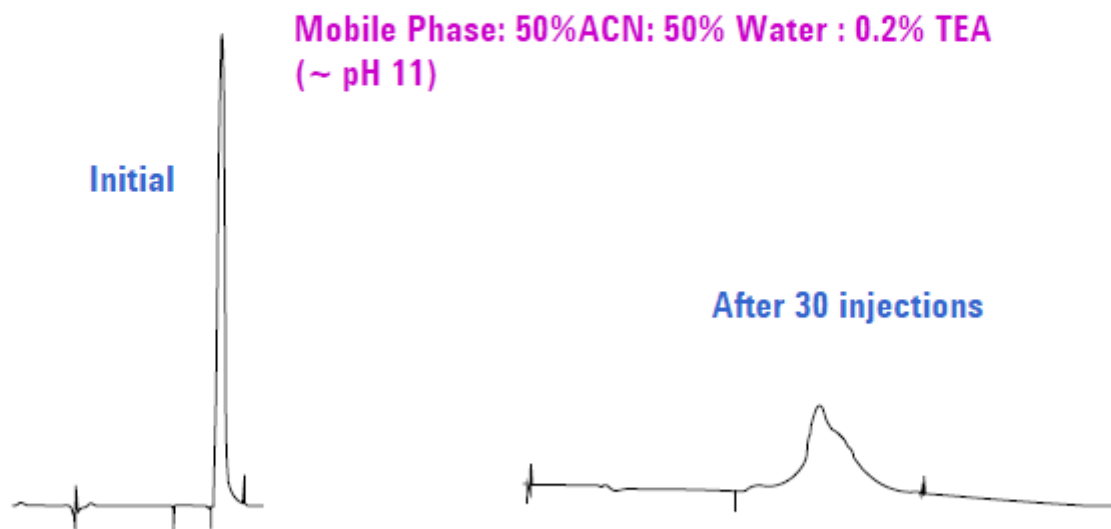
Peak Tailing, Broadening and Loss of Efficiency (N, plates)

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 Broadening, Splitting
Column Void

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

Initial

After 30 injections

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

Determining the Cause of Peak Tailing

- Evaluate mobile phase effects - alter mobile phase pH and additives to eliminate secondary interactions

- Evaluate column choice - try column with high purity silica or different bonding technology

- Reduce sample load – volume injection and concentration

- Eliminate extra-column effects – tubing, fittings, Uv cell

- Flush column and check for aging/void
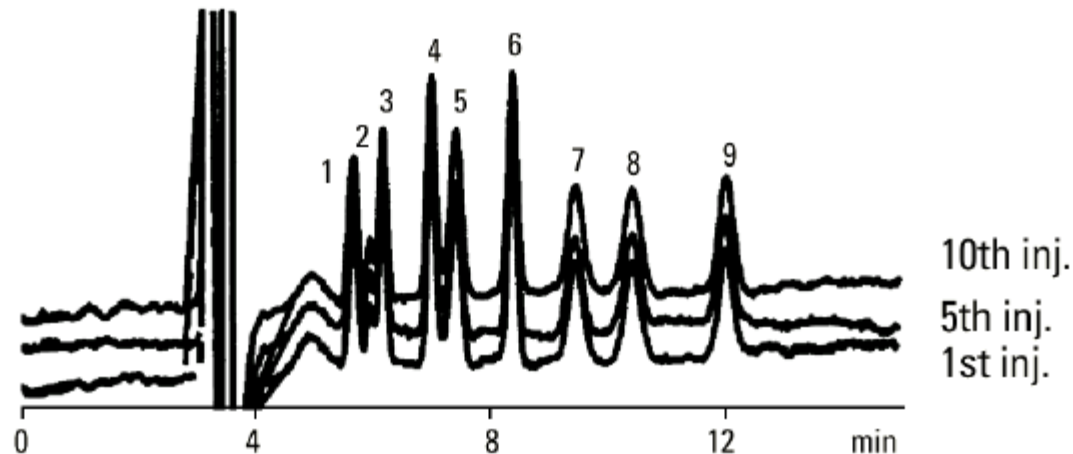
Reproducibility

Peak retention time precision:
$\Rightarrow$ with oven:_____ $< 0.3\%$
$\Rightarrow$ without oven: _____ $< 0.7\%$
Peak area precision: $\leq 1.5\%$

Typically,

- Area and Peak Height problems together point to the autosampler system

- Area and Retention Time problems together point to the pump

Problems with Reproducibility – Peak Areas
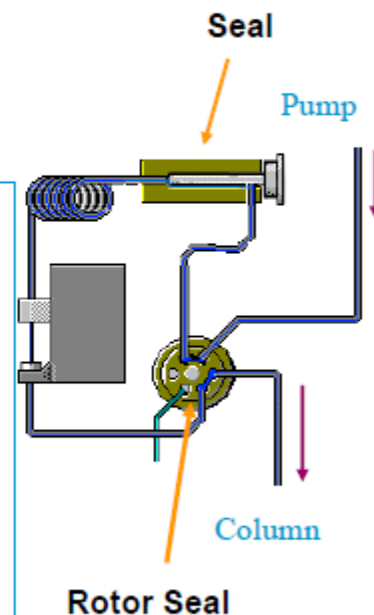
Peak Areas not Reproducible

With peak height
- Rotor seal cross-port leak or injection valve not tight
- Piston seal of metering unit leaking
- Needle partially blocked

With retention time
- Variable pump flow rate

Other
- Capillary from injector to detector not tight
- Detector equilibration problems

Seal

Pump

Column

Rotor Seal

Thank you

Will be continued in next presentation……..