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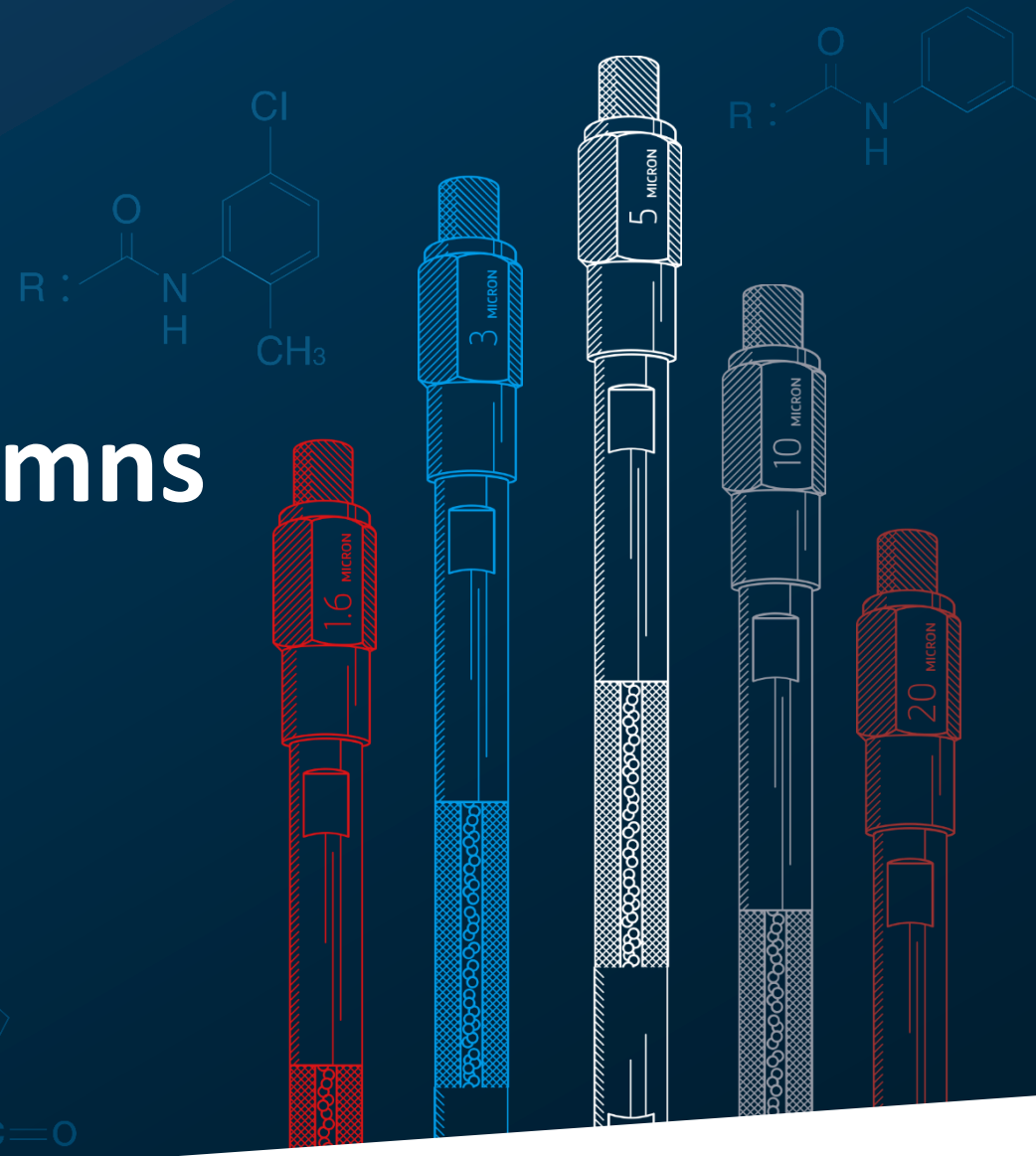
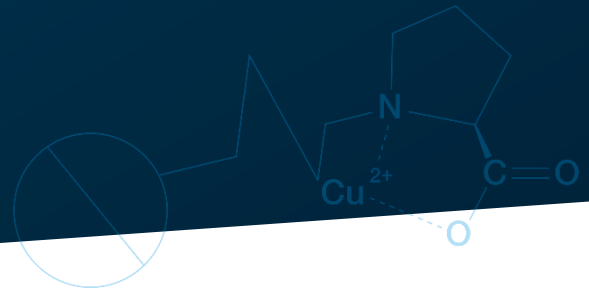
Chiral Technologies



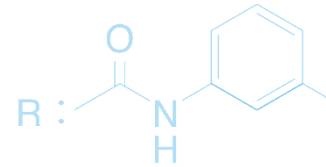
Protecting Your Chiral Columns for Optimal Performance

Dr. Weston Umstead

Technology and Business Development Manager



Overview



- Pressure Issues
- Peak Shape Issues
- Retention or Selectivity Issues
- How can we help?

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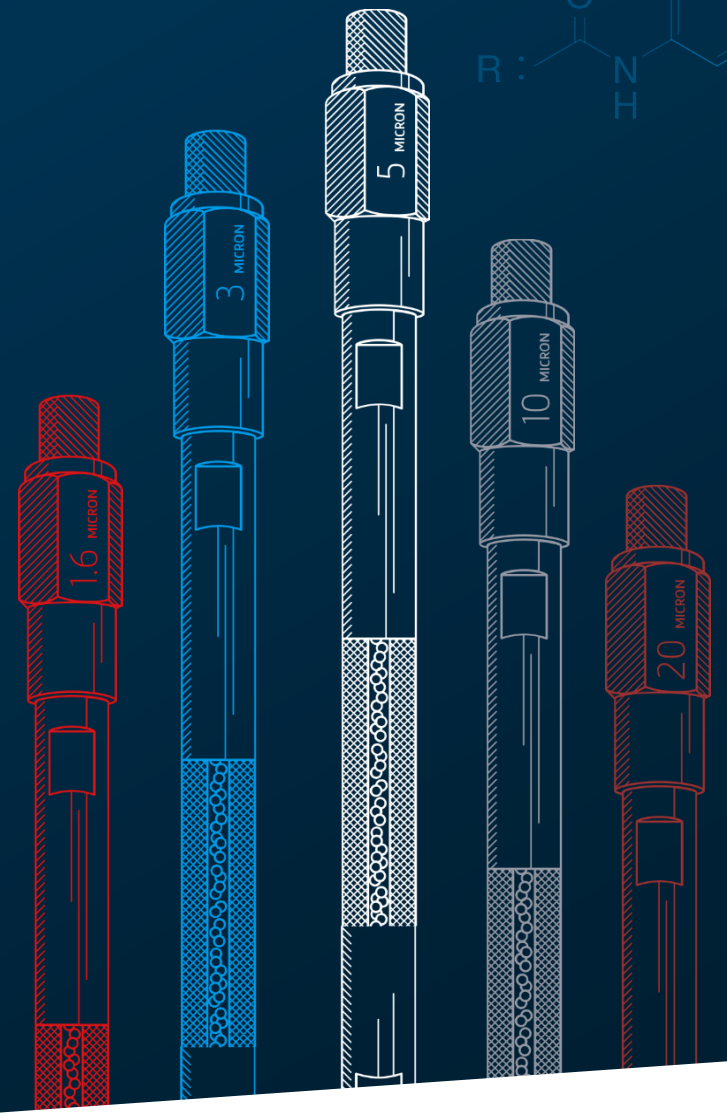
Issues with Pressure



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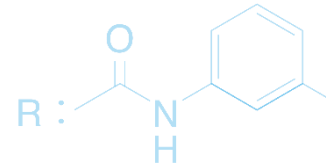
What Causes Pressure Issues?



- Blockage within the system
- Plugged inlet frit
  - Insoluble material in the sample
  - Particulate from wearing pump seals or rotors
  - Mobile phase incompatibility with sample solubility
  - Mobile phase component crashes out inside the column
  - Bacterial growth
- Mobile phase – column dimension combinations

What Causes Pressure Issues?

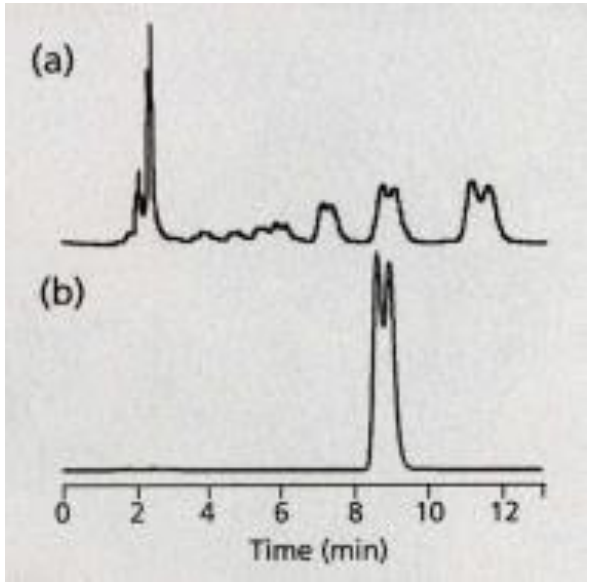


Figure 1: Peak splitting due to partial column blockage. Chromatograms: (a) sample and (b) analytical standard.

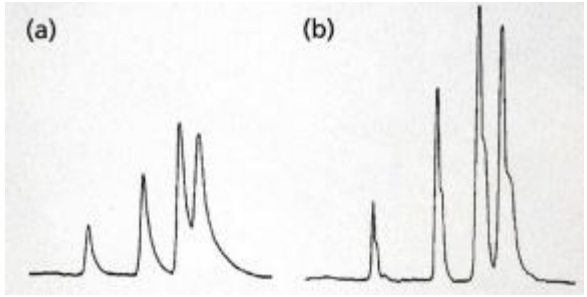


Figure 2: Characteristic peak shapes for (a, b) a partially blocked frit

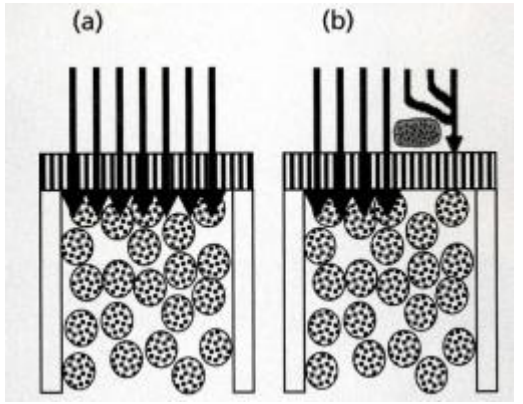


Figure 3: Schematic of flow path through column, where arrows represent sample before separation: (a) normal flow, (b) flow disturbance due to partially blocked frit,

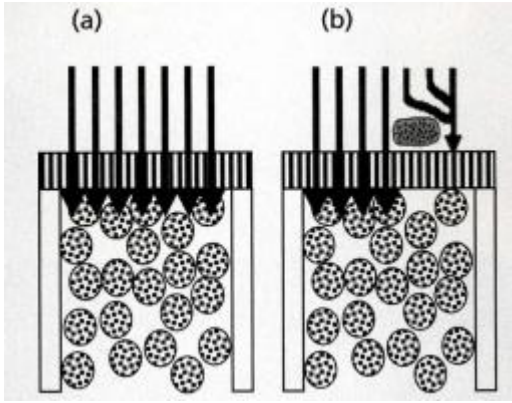
Peak Shape Problems, Dolan, LCGC North America, 2008, pg. 610-616

How Can I Diagnose This Issues?

- Reverse the column direction and inject a sample
  - If the chromatography is restored, it's likely a blocked inlet frit
  - If not, it's likely something else*

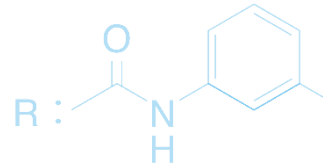
- QC the column and compare its performance to the test chromatogram that was provided with the column

- Try a different (larger i.d. or shorter length) column dimension.



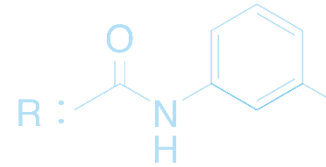
Peak Shape Problems, Dolan, LCGC North America, 2008, pg. 610-616

Preemptive Pressure Issue Solutions



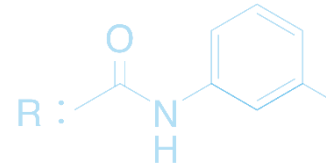
- Insoluble material in the sample
  - Filter sample with a 0.5 µm or 0.2 µm filter
- Particulate from wearing pump seals or rotors
  - Regular system maintenance
  - Use an in-line filter to catch particulate
- Mobile phase incompatibility with sample solubility
  - Ensure sample solvent/mobile phase match
  - Small solubility study to check the sample doesn’t crash out

Column Storage Conditions



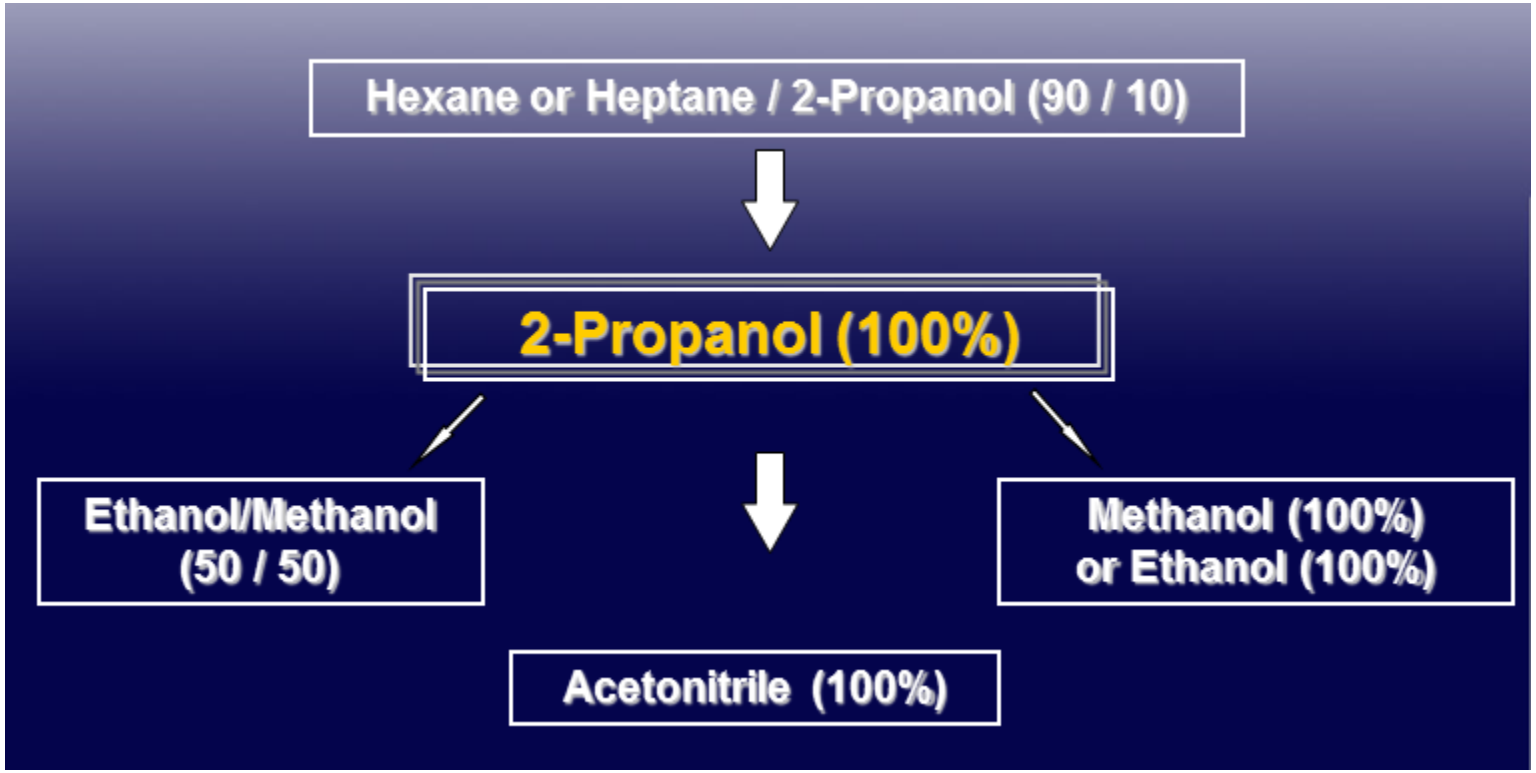
- Reversed Phase Short Term and Long Term
  - Okay to leave the column connected to the system (short term)
  - Flush the column with water/ACN (60:40) WITHOUT salts or buffer

Column Storage Conditions



- Normal Phase or Polar Organic Short Term (less than a few days)
  - Okay to leave the column connected to the system
  - Flush the column with Ethanol
- Normal Phase or Polar Organic Long Term (week or longer)
  - Flush the column with Ethanol
  - Store in 90:10 Hex/IPA
  - If the column was used for Polar Organic mode (especially 100% ACN or 100% MeOH, ethanol flush is essential!)

Column Storage Conditions



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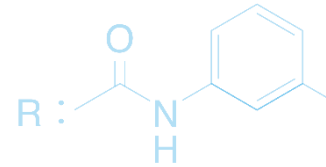
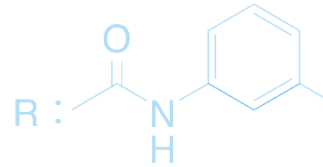
Column Storage Conditions



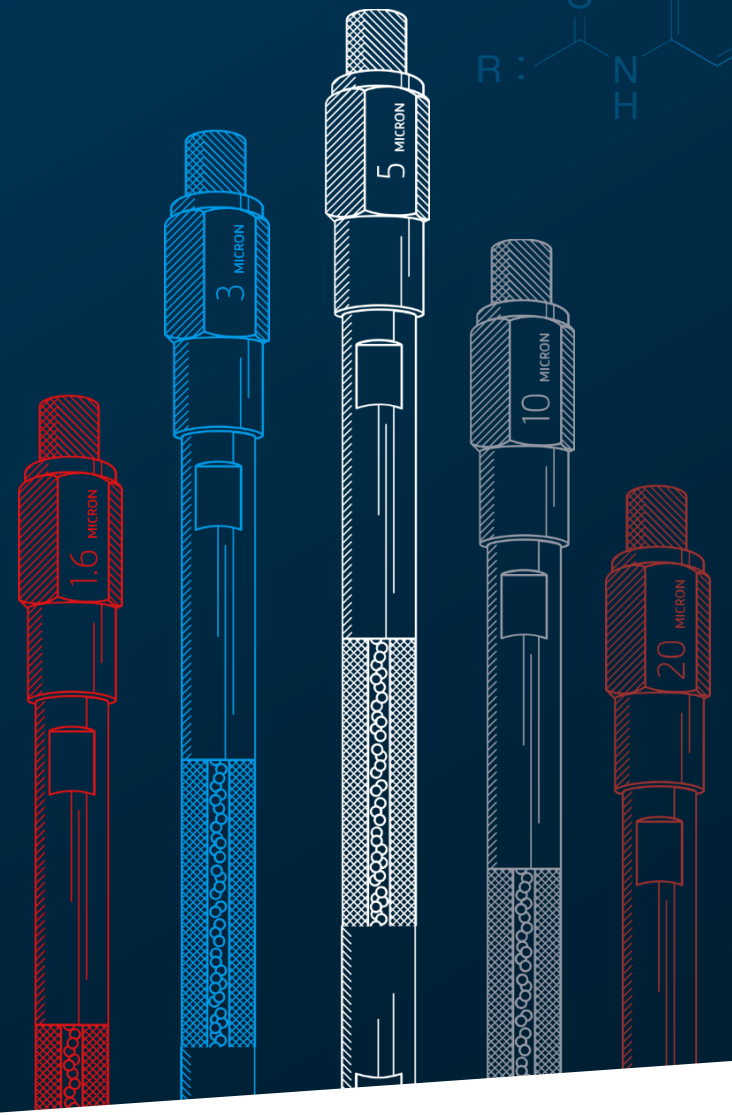
- Protein Columns Short Term (less than a few days)
  - Flush the column with mobile phase that doesn't contain any salt or buffer (water/IPA - 90:10)
  - Can be stored at ambient temperature in water/IPA
    - 85:15 for AGP and CBH
    - 90:10 for HSA/BSA/DSA/MSA/RSA
- Protein Columns Long Term (week or longer)
  - Same conditions as short term storage, except the column(s) should be stored in the refrigerator

After the Pressure Rises



- Particulate from wearing pump seals or rotors
  - Must replace the inlet frit
- Insoluble material from the sample or sample has crashed out of solution
  - Reverse flow of the column – disconnect the detector
  - Find what the insoluble material is soluble in
- Most of the time a plugged inlet frit requires replacement of the column

Peak Shape Issues



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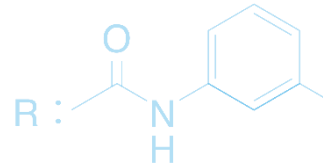
What Causes Peak Shape Issues?

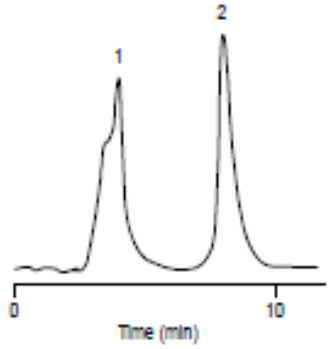


- Plugged Inlet Frit
  - Poor sample cleanup or particulate from the instrument
- Voids in the packing
  - Mechanical or thermal “shock”
- Chemical modifications
  - Biological samples – strongly adsorbing analytes or sample components
  - Column history or memory effect
- Won’t discuss method development issues – additives, sample overload, extra-column effects

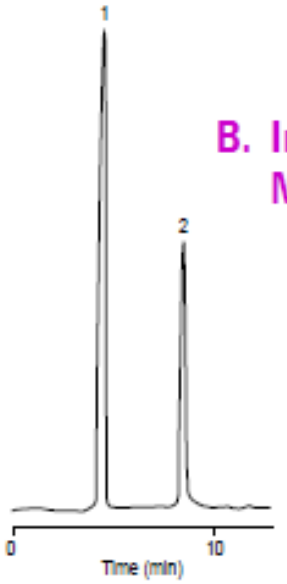
Injection Solvent/Mobile Phase Mismatch

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



Practical HPLC Method Development, Snyder and Kirkland, 1997, pg. 221

What Causes Peak Shape Issues?

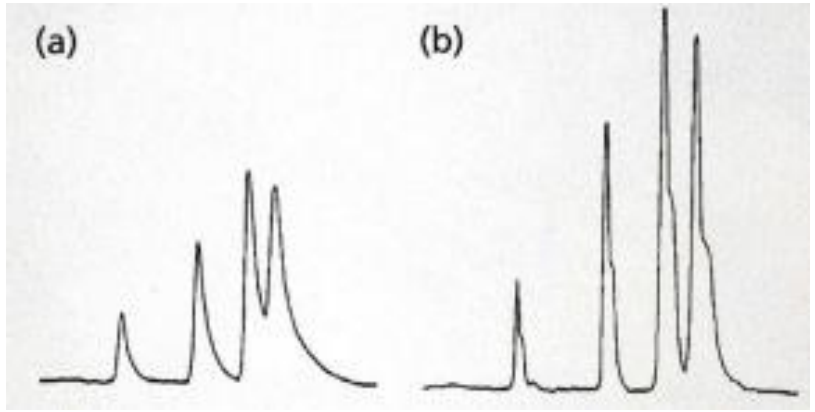
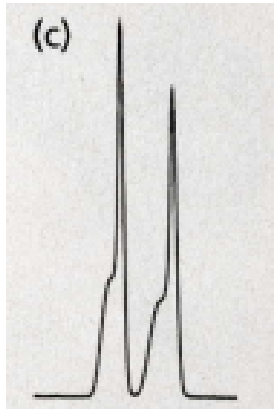
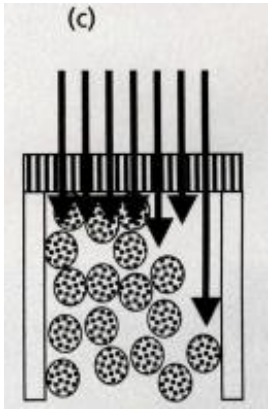


Figure 2: Characteristic peak shapes for (a, b) a partially blocked frit



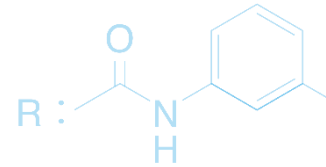
(c) void or column collapse.



(c) flow disturbance due to a column void.

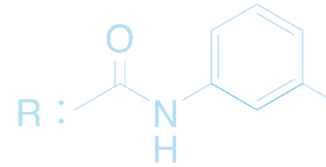
Peak Shape Problems, Dolan, LCGC North America, 2008, pg. 610-616

How Can I Diagnose This Issue?



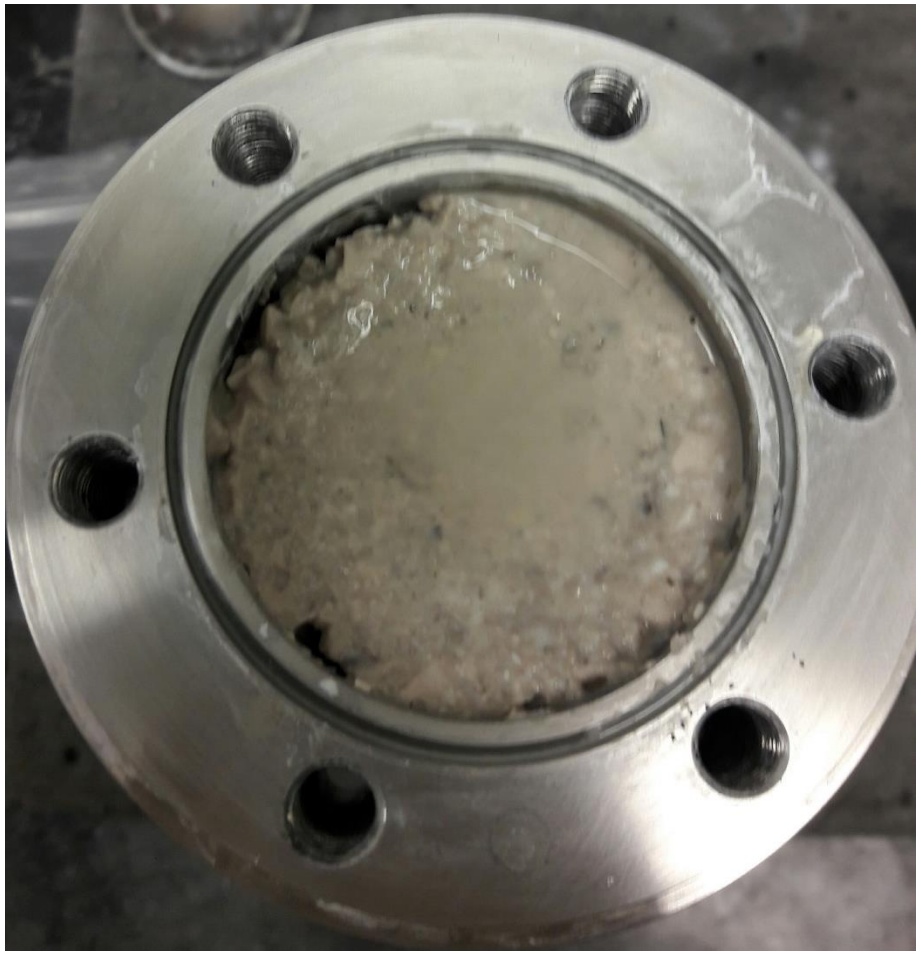
- Plugged Inlet Frit
  - QC the column and compare to the test chromatogram
- Void in the packing
  - Open the column and remove the frit
- Chemical modifications
  - Try a different column or a new column
  - QC the column and compare to the test chromatogram

Preemptive Peak Shape Issue Solutions



- Plugged Inlet Frit
  - Filtering the sample – good sample cleanup
  - Inline filter
  - Guard column
- Voids in the packing
  - Do not drop or hit the column on a hard surface
  - Operating at reasonable pressure and flow rate
    - Prep columns – bent inlet frits
  - Ensure proper equilibration when changing conditions
  - Quick rotation of sample-injection valve

Preemptive Peak Shape Issue Solutions



Preemptive Peak Shape Issue Solutions

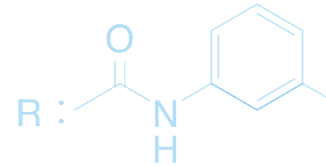
Preemptive Peak Shape Issue Solutions

- Plugged Inlet Frit
  - Filtering the sample
  - Inline filter
  - Guard column

- Voids in the packing
  - Do not drop or hit the column on a hard surface
  - Operating at reasonable pressure and flow rate
    - Prep columns – bent inlet frits
  - Ensure proper equilibration when changing conditions
  - Quick rotation of sample-injection valve

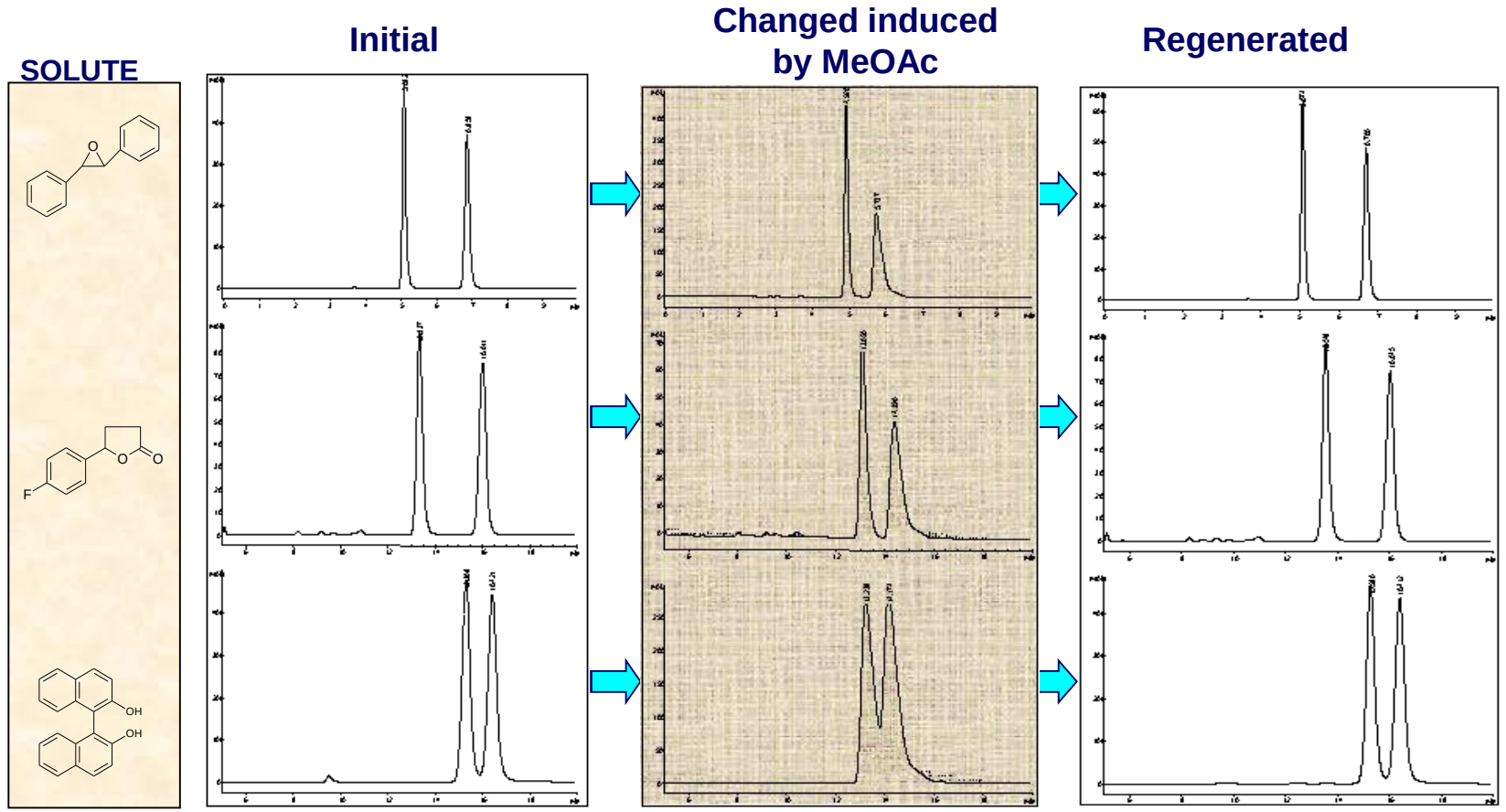
- Chemical modifications
  - Good sample clean up
  - Dedicate columns to specific conditions i.e. acidic OD-H, neutral OD-H, and a basic OD-H
  - Dedicate columns to specific mobile phases i.e. once a coated phase is put in reversed phase mobile phase, it must stay reversed phase
    - Even immobilized phases shouldn't be moved back and forth on a regular basis

After Poor Peak Shape Occurs



- Plugged Inlet Frit
  - Washing the column in reverse
  - Replace the inlet frit
- Voids in the packing
  - Repack the column with new CSP
- Chemical modifications
  - Recondition or repack
  - Replace the column

Regeneration Examples



Regeneration Examples

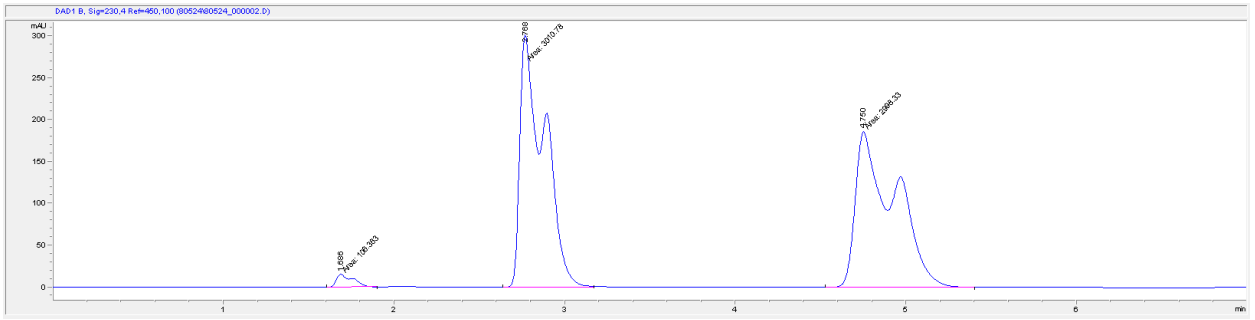


Figure 1: Initial Performance Check of CHIRALPAK® IA-3 before Backflushing and Regeneration

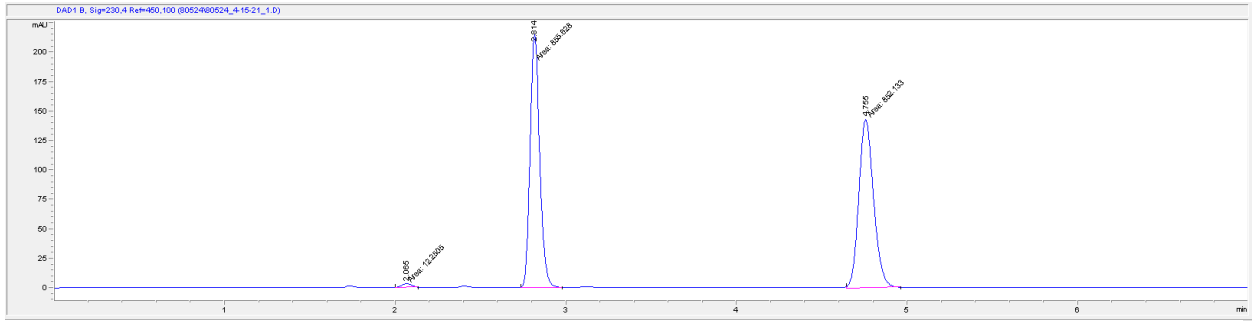


Figure 2: Performance Check of CHIRALPAK® IA-3 after Backflushing and Regeneration

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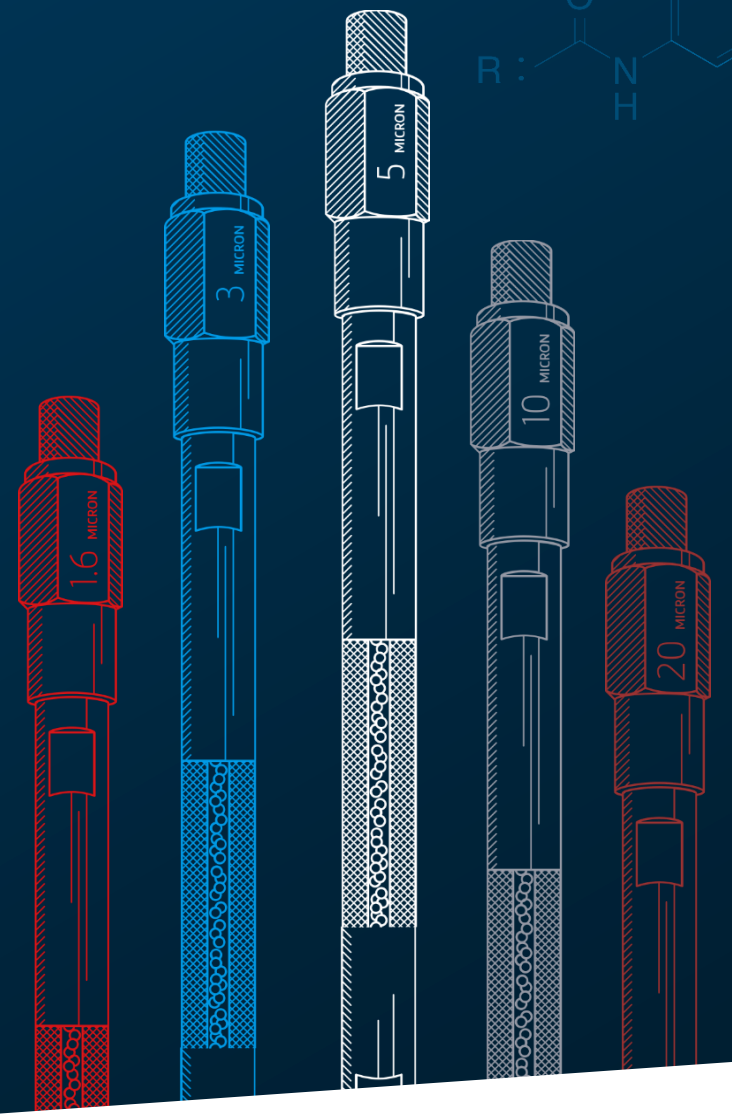
Retention/Selectivity Issues



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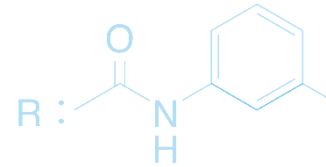
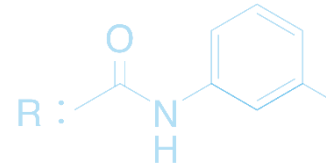
What Causes Changes in Retention or Selectivity?



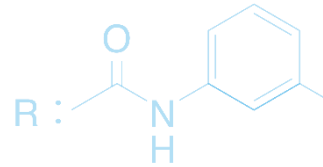
- Adsorbed sample
  - Biological samples, petroleum-based samples
- Chemical Attack
  - Incompatible additives or solvents
  - Too high/low pH
- Won’t discuss method development issues – scaling from smaller to larger particles/columns, instrument differences, method robustness

How Can I Diagnose This Issue?



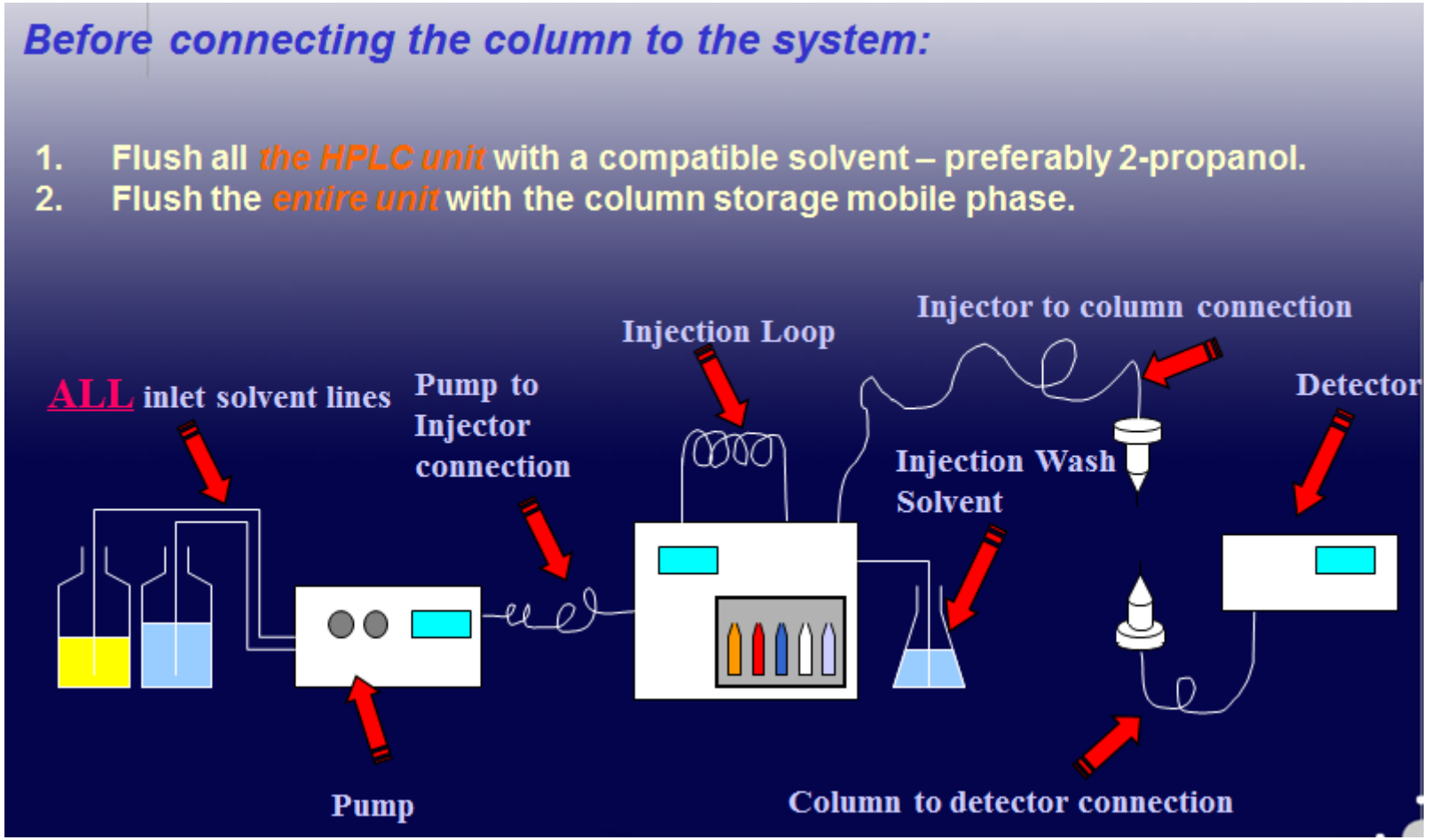
- Adsorbed sample
  - QC the column and compare to the test chromatogram
  - Open the column and inspect the CSP
- Chemical Attack
  - Presence of a white powdery solid exiting the column

Preemptive Retention or Selectivity Issue Solutions



- Adsorbed sample
  - Good sample clean up
    - SPE cartridges, achiral C18 column, membrane filtration, liquid extraction
- Chemical Attack
  - Develop methods with a pH high than 2, but lower than 8 (or 9 with the correct buffer)
    - Ligand cleavage or silica degradation
  - Avoid harsh acidic additives like HCl or harsh basic additives like NaOH
    - Preferred options for acids = TFA, Formic acid, Acetic acid
    - Preferred options for bases = DEA, TEA, buffered phosphate or bicarbonate
  - Incompatible solvents – flush the system well!
    - Avoid using DCM, Ethyl Acetate, DMSO, THF, MtBE and Chloroform with coated CSPs.
      - Okay with immobilized

Preemptive Retention or Selectivity Issue Solutions



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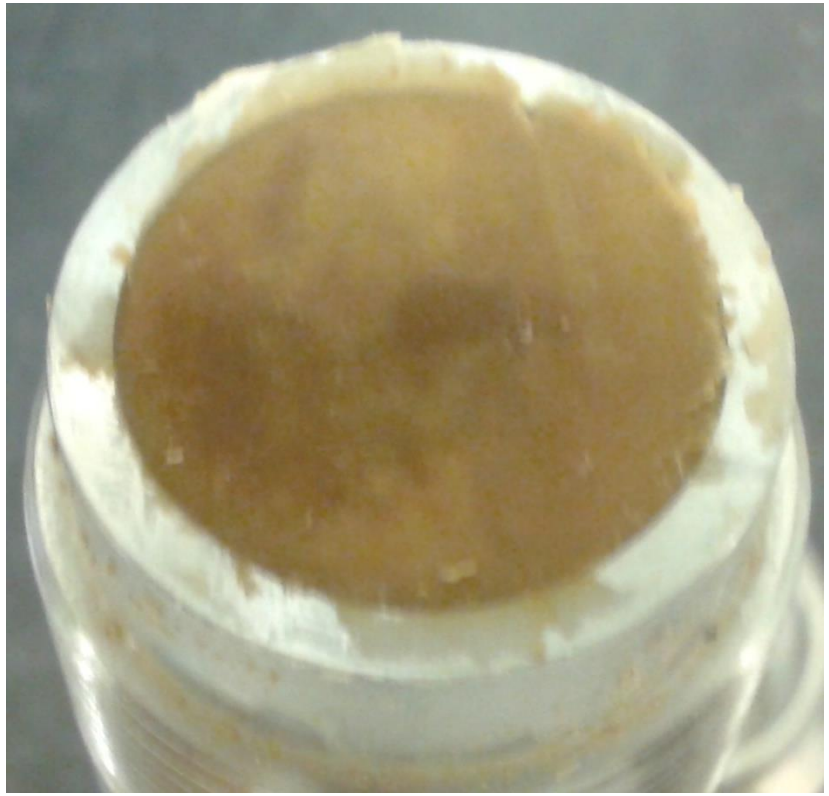
Preemptive Retention or Selectivity Issue Solutions



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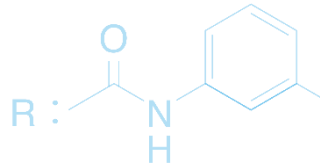
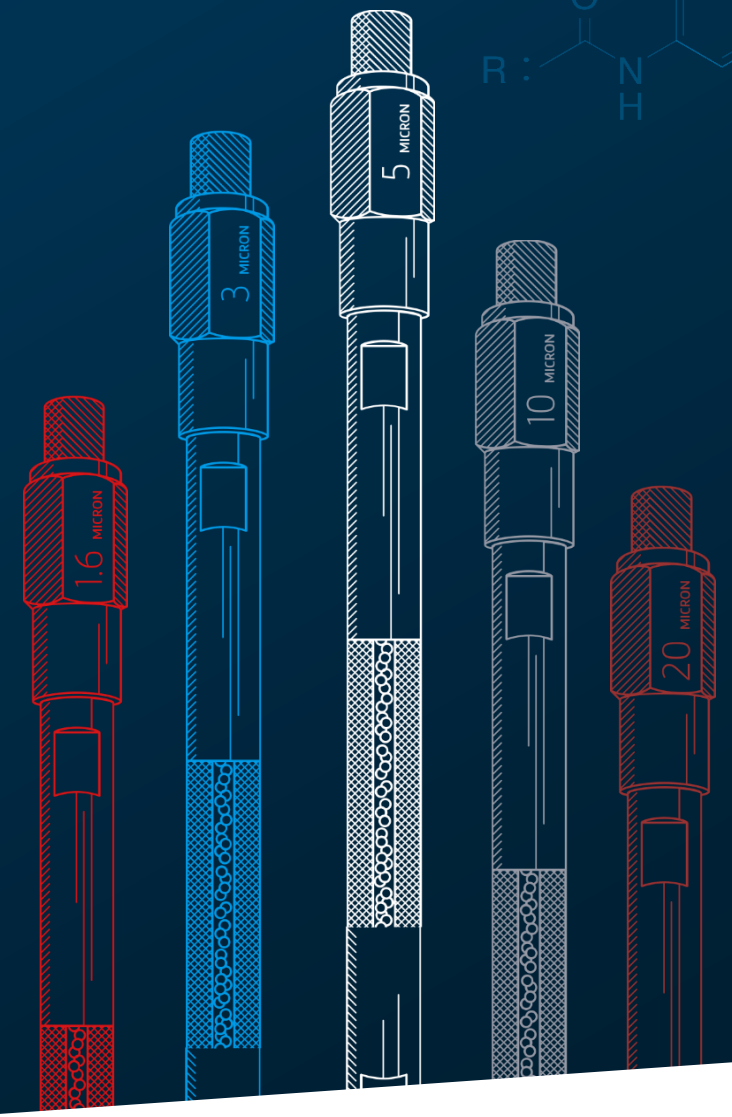
After Retention or Selectivity Changes Have Occurred



- Adsorbed sample
  - Regeneration or column flushing
  - Repack the column with new CSP
- Chemical Attack
  - Replace the column

How Can We Help You?



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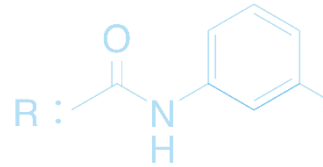


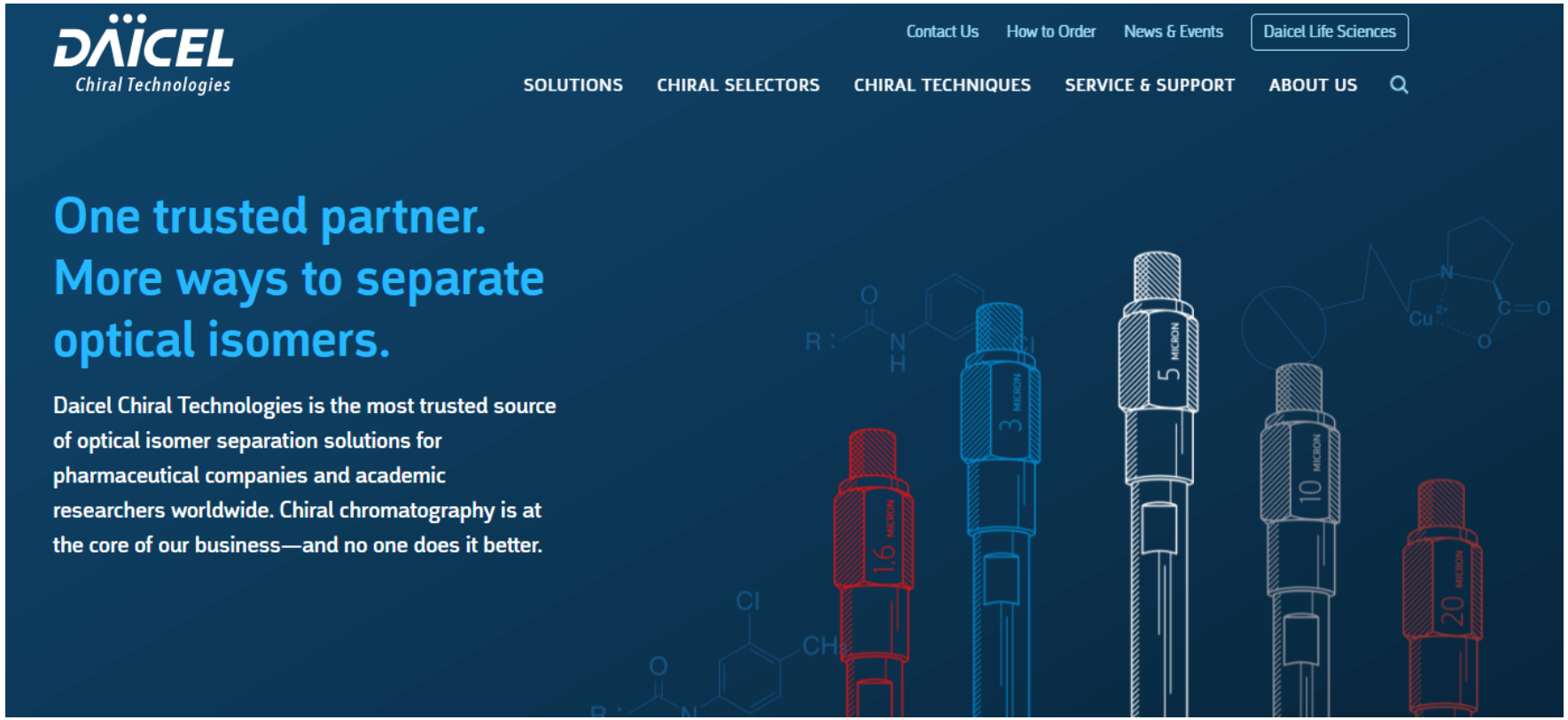
Services for Prep Columns

- Column Hardware Replacement
  - Open column, replace frit, ensure the CSP bed is not damaged
- Column Recondition
  - Take the column through a regeneration sequence – remove and clean-up CSP, add in new CSP, repack, and QC

Less cost than purchasing a new column

How Can We Help You?

www.chiraltech.com



Pressure | Peak Shape | Retention/Selectivity Issues | Help?

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How Can We Help You?

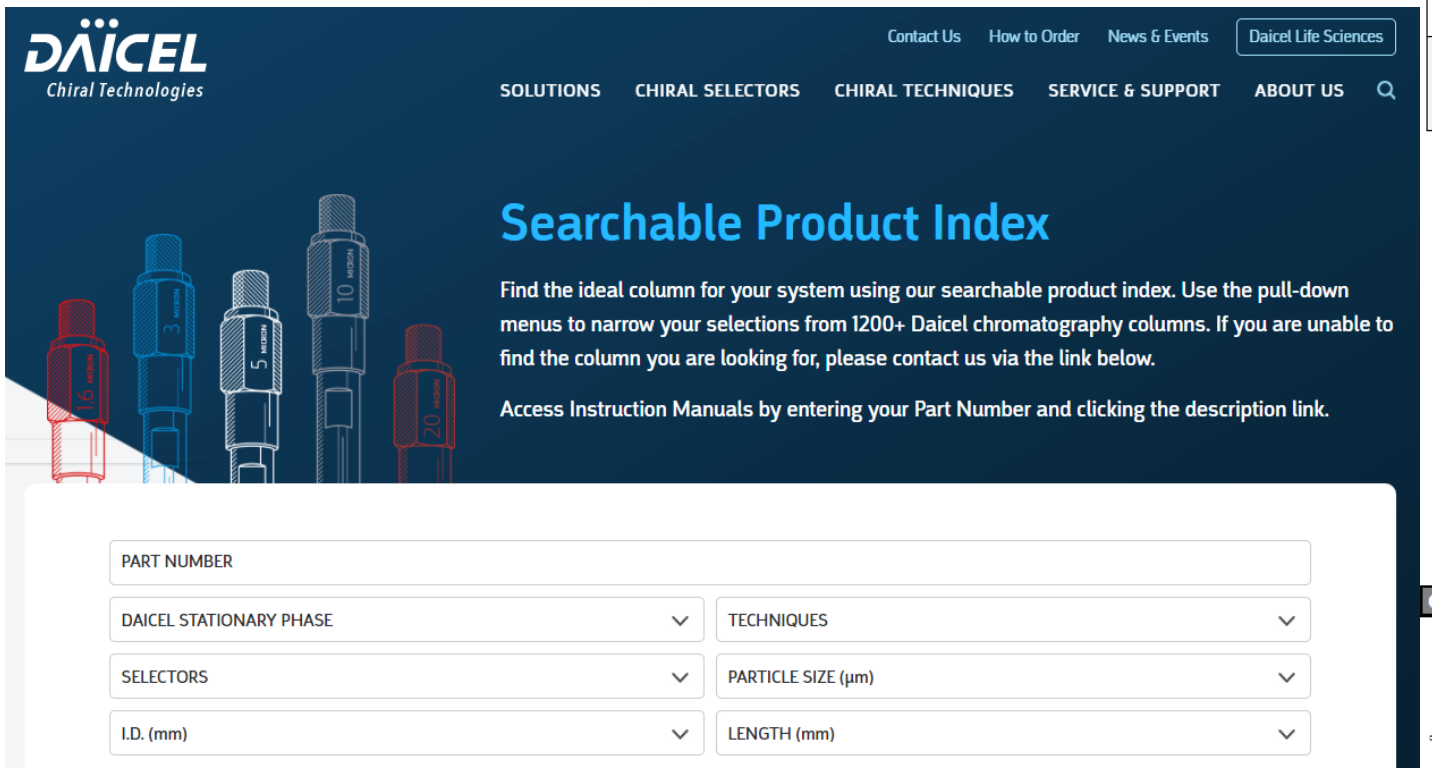


Table 1. Immobilized Primary Screening Solvents

Primary solvent mixtures	Alkane❶/2-PrOH	Alkane❶/EtOH	Alkane❶/MtBE/EtOH❷	Alkane❶/THF❸	Alkane/DCM❹/EtOH
Typical starting conditions	80:20	80:20	0:98:2	70:30	50:50:2
Advised optimisation range	99:1 to 50:50	99:1 to 50:50	80:20:0 to 0:40:60	95:5 to 0:100	85:15:0 to 0:80:20

❶ Alkane = n-Hexane, iso-Hexane or n-Heptane. Some small selectivity differences may sometimes be found.
❷ In absence of alkane, methanol is more efficient than ethanol when combined with MtBE.
❸ In the case of no environmental restrictions, **use of DCM is preferred to THF** in terms of better enantioselectivity that the former may induce.
❹ For excessively retained samples, addition of ethanol up to 20% in pure DCM would be helpful.

D – Additives

For basic or acidic samples, it is necessary to incorporate an additive into the mobile phase in order to optimise the chiral separation.

❶ It has been found that certain amines, such as EDA and AE induce much better behaviour for certain basic compounds than the most commonly used DEA.

☞ *The addition of a low percentage of an alcohol (e.g. 2% EtOH or MeOH) in the mobile phase may be helpful to ensure the miscibility of EDA and AE with the low polarity mobile phases.*

Basic Samples require Basic additives	Acidic Samples require Acidic additives
Diethylamine (DEA) 2-Aminoethanol (AE)❶ Ethylenediamine (EDA)❶ Butyl amine (BA)	Trifluoroacetic acid (TFA) Acetic acid Formic acid
< 0.5% Typically 0.1%	< 0.5% Typically 0.1%

⇨ *STRONGLY BASIC solvent additives or sample solutions MUST BE AVOIDED, because they are likely to damage the silica gel used in this column.*

Column Care / Maintenance

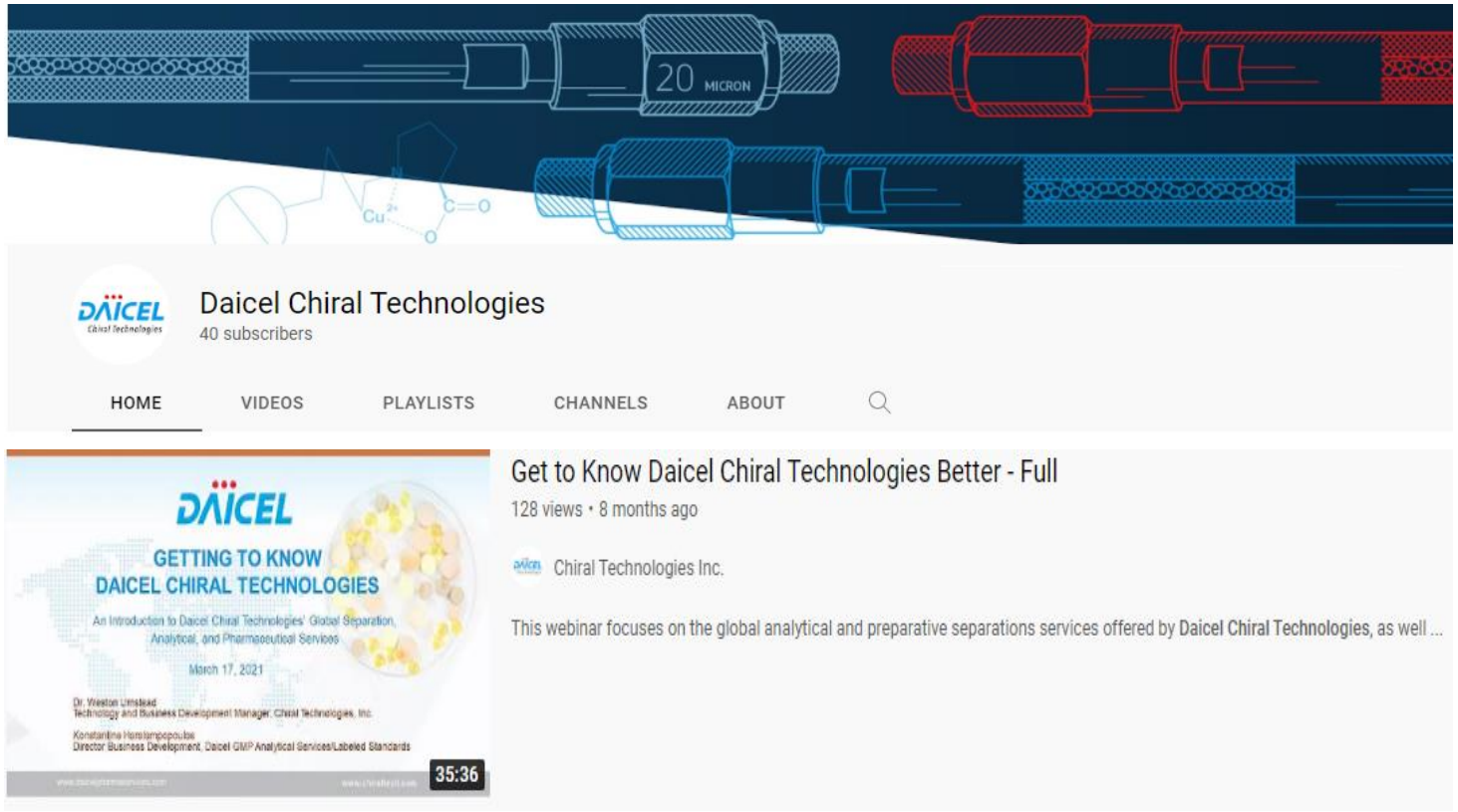
- **The use of a guard cartridge is highly recommended for maximum column life.**
- Samples should be dissolved in the mobile phase. The mobile phase and the sample solution should be filtered through a membrane filter of approximately 0.5µm porosity to ensure that there is no precipitate before using.

☞ ***Column cleaning and regeneration procedures***

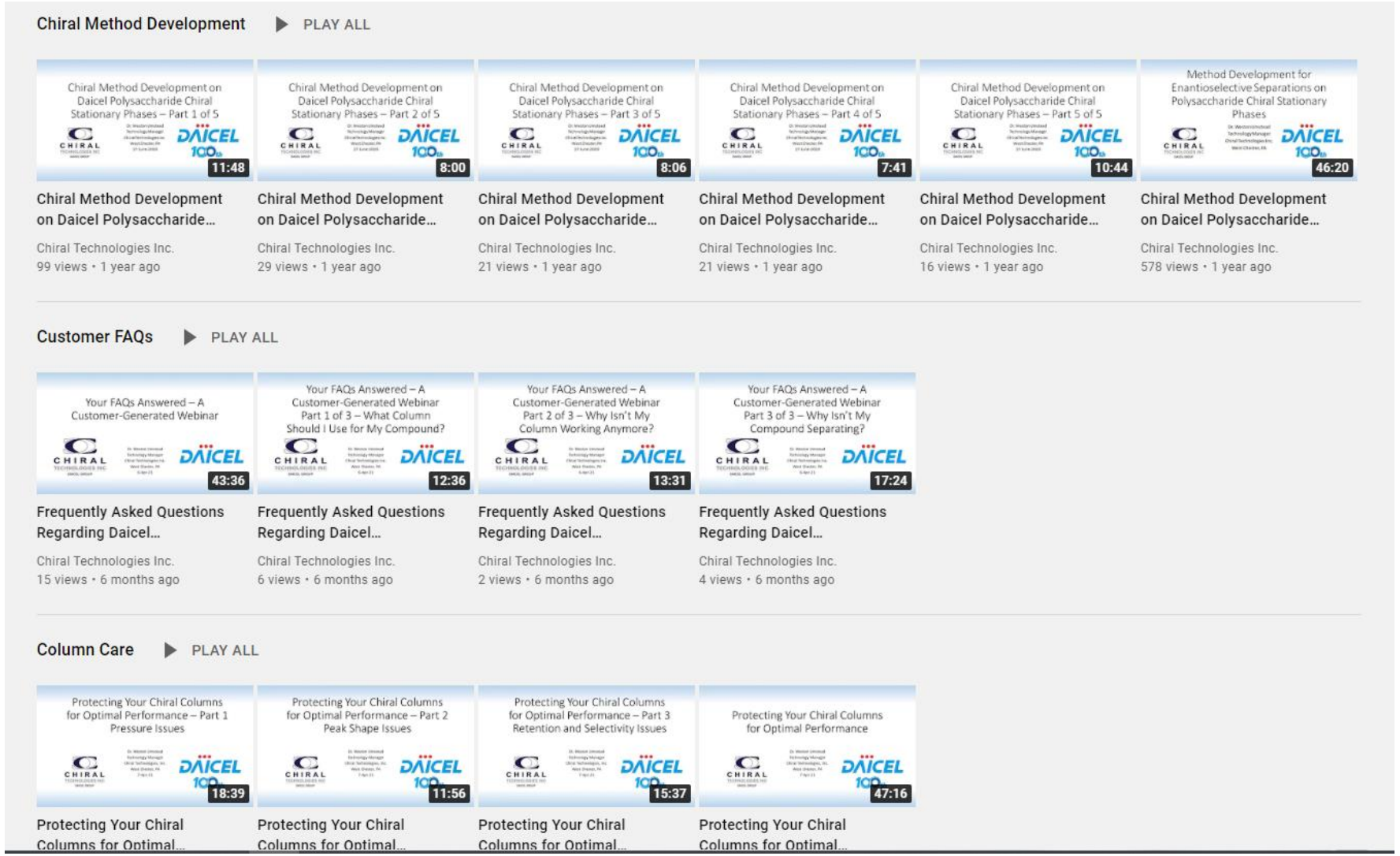
Following extensive use of the column in multiple solvents there may be a change in column reproducibility. In order to ensure consistent performance, a regeneration method may be implemented to eliminate any change in chiral recognition due to the history of the column (mobile phases, additives...).

- Flush with ethanol at 0.5 ml/min for 30 min, followed by 100% THF at 0.5 ml/min for 2 hours.
- Flush with ethanol at 0.05 ml/min(*) for 300 min.

How Can We Help You?



How Can We Help You?



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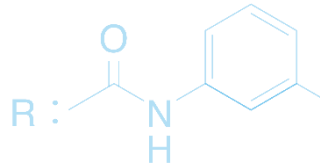
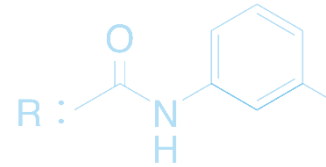
In Summary – Major Causes of Column Failure



- Column or inlet frit fouling
  - Can be avoided with good sample cleanup
  - Filters or guard columns
- Bed compression – void formation
  - Operate at low or reasonable flow rate
  - Avoiding mechanical or thermal “shock”
- Chemical attack or modification
  - Use appropriate pH range mobile phases
  - Avoid restricted solvents for coated phases

Questions



- questions@cti.daicel.com – General column chromatography or method questions

- wumstead@cti.daicel.com – Presentation, chromatography, or column questions