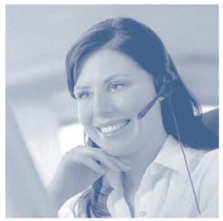
HPLC Troubleshooting



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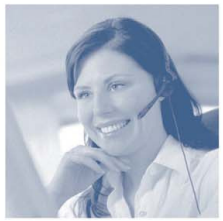
Course Outline



- Potential Sources
- Where to Begin?
- Baseline Troubleshooting
- Column Troubleshooting
- Other Issues

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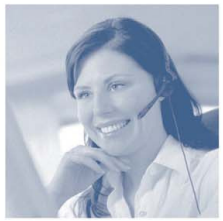
Potential Sources of Chromatographic Problems



- Mobile Phase
- Injector
- In -Line Filter
- Column
- Detector

- Pump
- Guard Column
- Connecting Tubing and Fittings
- Integrator/Recorder

The Scientist/Analyst

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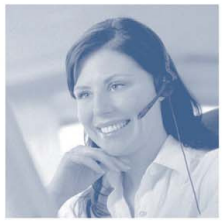
Where to Begin?



System flush (no column in-line)

- Check for little or no back pressure
- Inject blank – No baseline problems
- Compare chromatograms
- Inspect baseline

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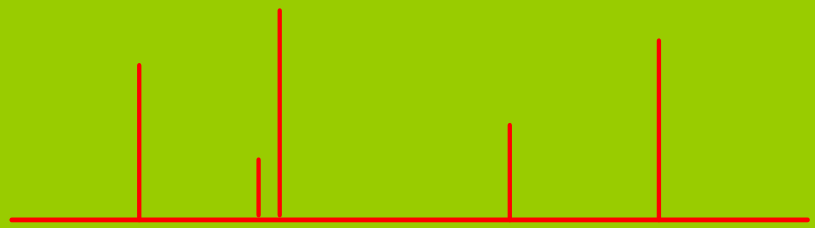
BASELINE TROUBLESHOOTING

Noisy baseline

Cyclic

Synchronous noise



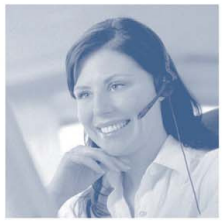
Spikes

Asynchronous noise

No peaks

Drift

Baseline Troubleshooting





Noisy baseline

- Gas in mobile phase
  - Degas mobile phase
- Leaks
  - Find leak and repair
- Electronic noise
  - Remove source. Shield cables
- Weak detector lamp
  - Replace lamp
- Sensitivity too high
  - Lower sensitivity
- Detector cell dirty
  - Flush with 6N nitric acid

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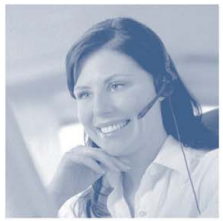
Baseline Troubleshooting





Synchronous Noise

ALMOST ALWAYS CAUSED BY THE PUMP

- Air in the pump head
  - Prime pump and degas solvent
- Check valve problem
  - Clean or replace
- Broken plunger
  - Replace
- Mixing Problem
  - Increase system volume
- Electrical noise
  - Remove source

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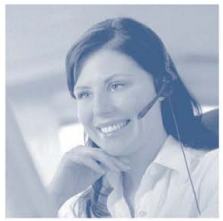
Baseline Troubleshooting





Asynchronous Noise

- Bubbles
  - Degas mobile phase
- Gas caught in detector
  - Degas mobile phase. Put backpressure on cell
- Leaks
  - Find leak and repair
- Mixing problems
  - Increase system volume
- Plugged lines
  - Remove plug – flush system
- Electrical problems
  - Remove source

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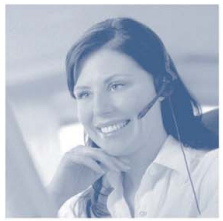
Baseline Troubleshooting





Baseline Drift

- Gradient – Solvent B absorbs more than Solvent A
  - Try new mobile phase. Use baseline subtraction
- Compounds eluting off column
  - Run strong solvent until baseline is stable
- Solvent compostion change (e.g. Evaporation)
  - Ensure solvents are enclosed
- Solvent leaks
  - Tighten, replace fittings
- Backpressure changes
  - Filter solvents and samples. Samples may be too viscous
- Mixing problems
- Increase system volume

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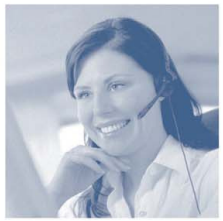
Baseline Troubleshooting





Cyclic Baseline

- Temperature fluctuations
  - Thermally insulate. Move away from ventilation. Increase cell temperature
- Mixing problems
  - Increase system volume
- Gas in mobile phase
  - Degas solvents
- Electrical problems
  - Remove source
- Erratic pump
  - Repair
- Plug
  - Remove obstruction – flush system

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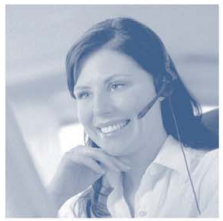
Baseline Troubleshooting





Spikes

- Bubbles
  - Degas solvent
- Poor electrical connection, loose wiring
  - Clean and tighten detector leads, check wiring
- Lamp relay trying to fire a dead lamp
  - Replace lamp
- Electrical noise
  - Remove source. Common sources – Switching valves, compressors, muffle furnaces, fraction collectors, power conditioners, lighting

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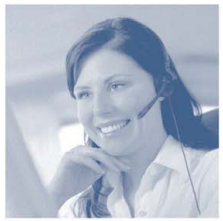
Baseline Troubleshooting





No Peaks

- Injector not injecting
- Pump not pumping
- Dead detector
- Integrator/recorder/PC not connected correctly
- Gain setting too low
- Leaks
- Column retaining all compounds
- Bad or incorrect mobile phase
- Bad or incorrect standard or sample
- Incorrect guard column

INJECT ACETONE SOLUTION TO MAKE A PEAK

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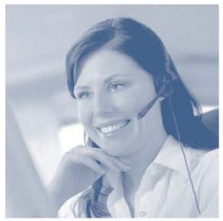
Baseline Troubleshooting





Negative & Positive Peaks

- Some eluting compounds absorb less than solvent
  - Use a different or cleaner solvent
- Air bubbles passing through cell
  - Degas mobile phase
- All peaks are negative
  - Change detector polarity
- Negative peaks with RI detector
  - May be normal (peak direction is function of RI differential from mobile phase

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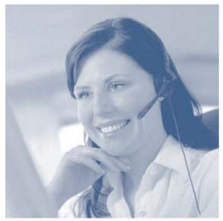
Baseline Troubleshooting





Poor Peak Shape

- Contaminated in-line filter
  - Replace frit
- Column dying
  - Replace column
- System void volume
  - Check system tubing
- Contaminated guard column
  - Replace
- Incorrect or wrong solvent
  - Make new mobile phase. Consider ion pairing/suppression
- Column destroyed
  - pH <2 washes off functional group
  - pH >8 dissolves silica base

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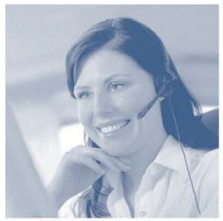
Column Troubleshooting



Common Problems

- Peak shape
- Retention time
- Other

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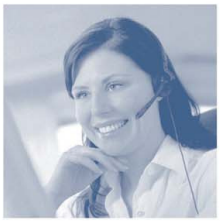
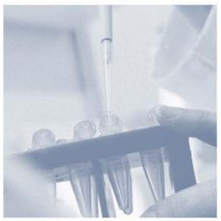
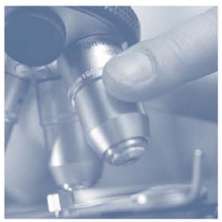
Column Troubleshooting




Chromatographic problems may be related to:

- Instrument
- Sample
- Column

Two Major Problems are:

- Peak Shape/Width
- Retention Time Changes

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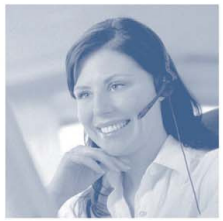
Column Troubleshooting





Peak Shape Problems

Most Common Problem in HPLC:

Distorted peaks will cause integration or resolution problem

Indication that optimal column performance is not being attained

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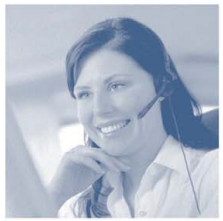
Column Troubleshooting





Peak Shape Problems

- Column Destroyed
- Secondary Interactions
- Incorrect Sample Solvent
- Column Overload
  - Mass Overload
  - Volume Overload
- Other Extra-Column Effects
  - Sampling Rate
  - Time Constant

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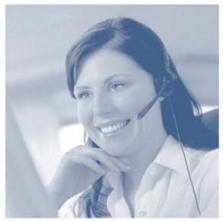
Column Troubleshooting





All Peaks Affected

- COLUMN
  - Connection
  - Replace frit
  - Regenerate or replace column
- COLUMN DESTROYED
  - pH <2 washes off functional group
  - pH >8 dissolves silica base

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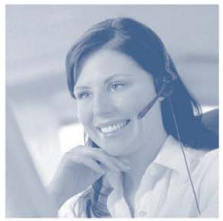
Column Troubleshooting



Column Collapse

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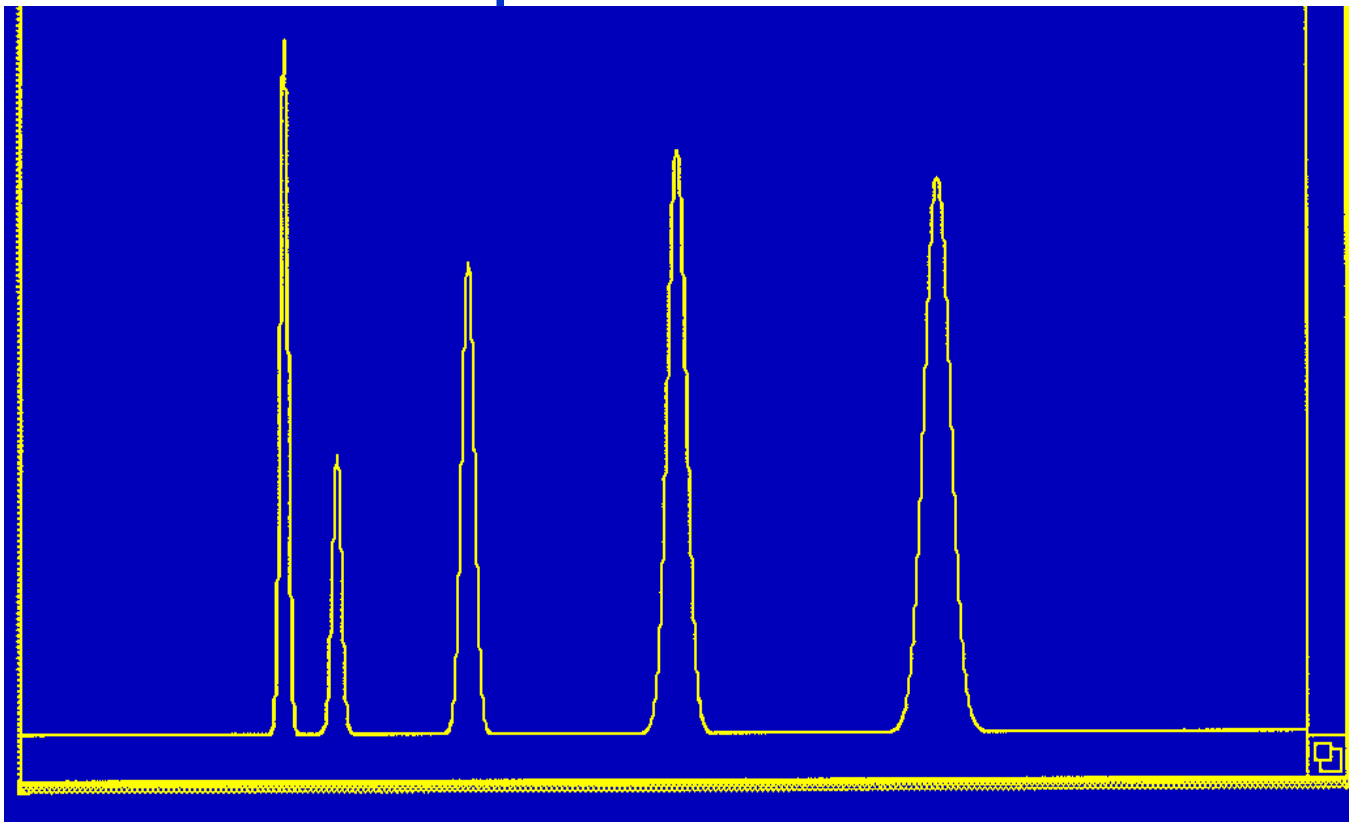
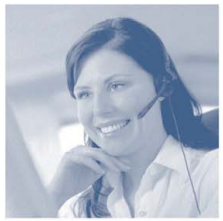
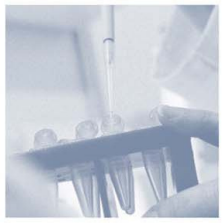
Column Troubleshooting

Well packed column







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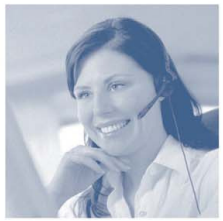
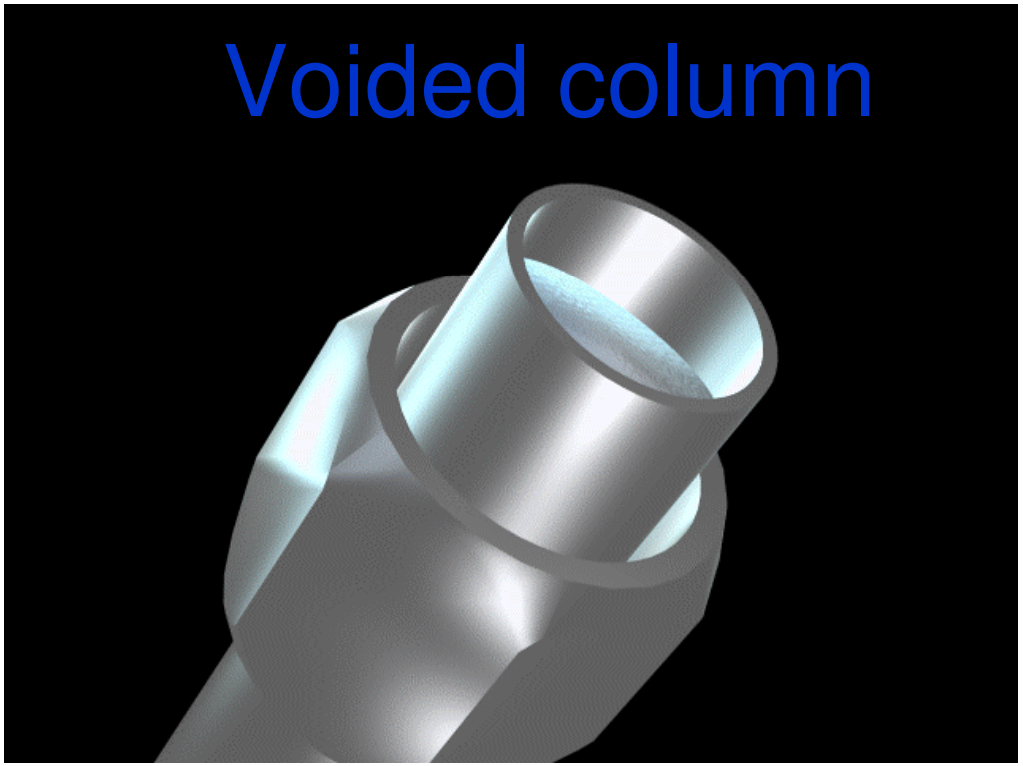
Column Troubleshooting





Column Collapse



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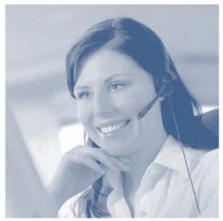
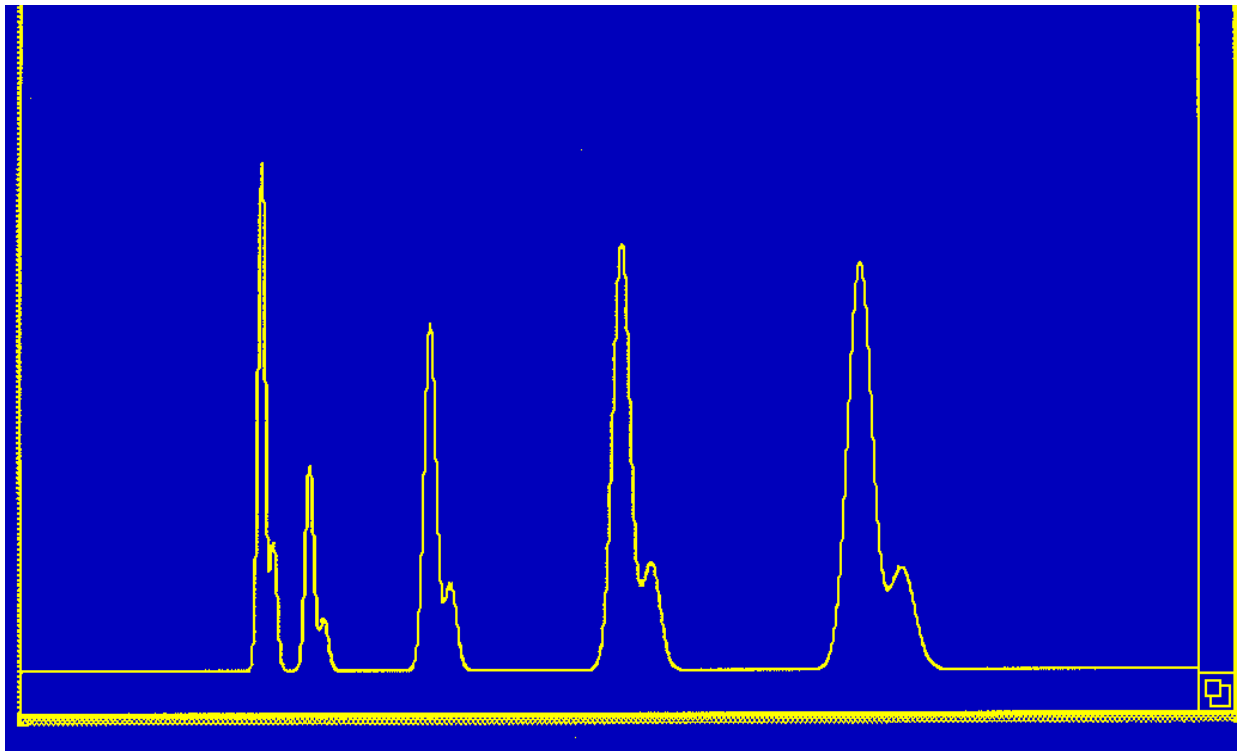
Column Troubleshooting



Voided column



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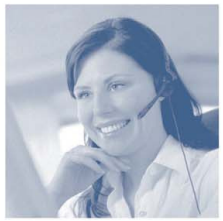
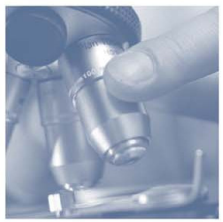
Column Troubleshooting

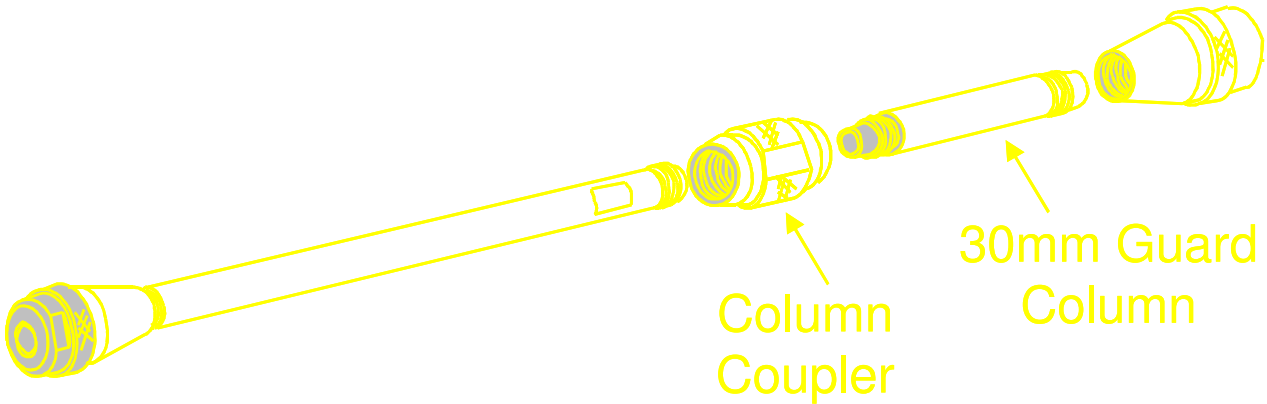



Column Protection

Major cause of column deterioration is contamination.

Use of guard columns may increase column life-time to > 10,000 analyses



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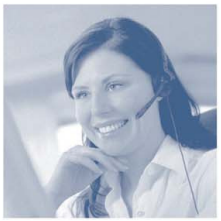
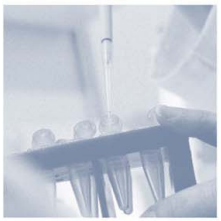
Column Troubleshooting





Column Protection

Guard column should be regarded as a cost-effective sacrifice to extend analytical column life-time

Should contain IDENTICAL packing material as the analytical column

e.g. using a different C18, with different retention properties could actually destroy the separation or impair protection

Well designed, well packed guard columns will actually IMPROVE the analytical separation efficiency

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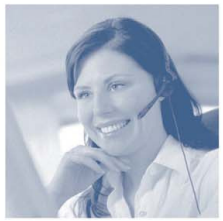
Column Troubleshooting





Column Protection

Other Techniques to Protect the Column:

- In-line Filter between the Injector and Column
- Filtering of the Sample (Doesn't Protect against Seal Shedding)
- Sample Cleanup through Solid Phase Extraction (SPE)

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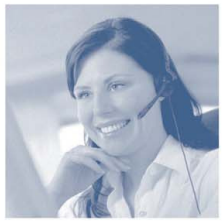
Column Troubleshooting



Column Storage

Store in Mobile Phase for Short Periods of Time (<72hrs.)

Store in Shipping Solvent for Longer Periods of Time

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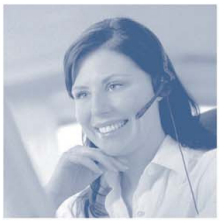
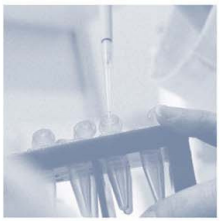
Column Troubleshooting





Column Storage

- Column should be stored in solvent which manufacturer recommends
- For bonded phases, use organic solvent
  - (eg. MeOH or ACN) Using non aqueous solvents minimizes hydrolysis.
- Some bonded phases (CN) become unstable in polar organic mobile phases.
  - Storage in water or buffer is then okay.
- Worst mobile phase for CN column is CH3CN (Acetonitrile)

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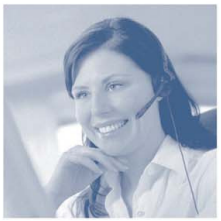
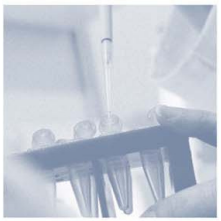
Column Troubleshooting





Column Storage

Columns which may be stored in Water or Buffered Solvents:

- Ion exchangers
- Aqueous SEC packings

<u>However:</u>

Prevent microbial growth by using 0.05% sodium azide in mobile phase

OR

Small quantity of organic solvent (acetonitrile 5% or methanol 10%)

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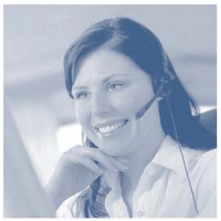
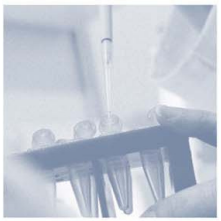
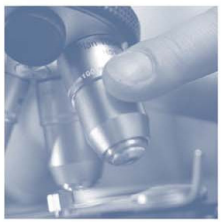
Column Troubleshooting







Column Storage

Columns which should be stored in Mobile Phase:

- Normal Phase
- Organic SEC (GPC)

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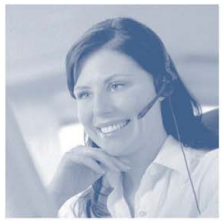
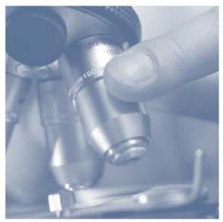
Other Issues



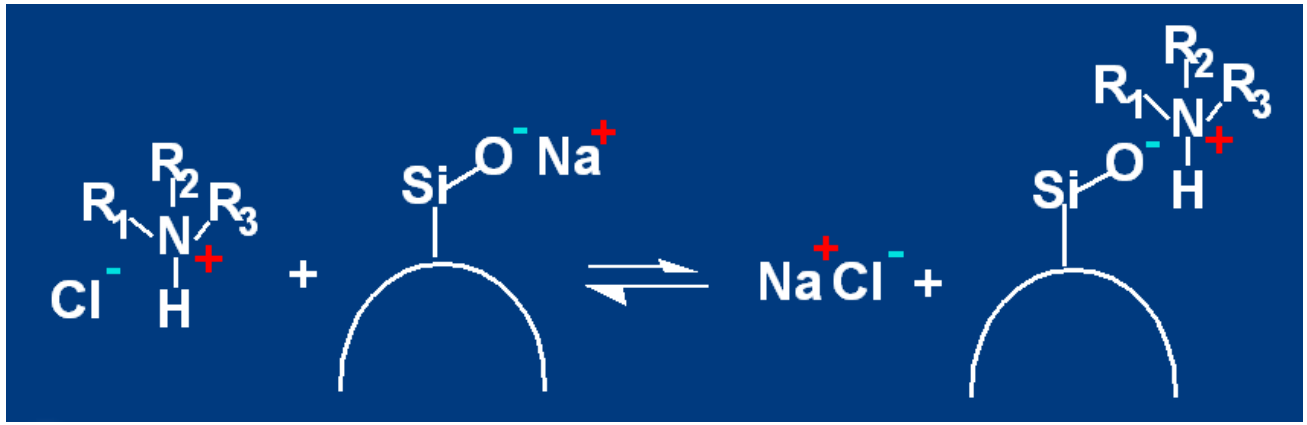

Tailing

What Causes Tailing?

Mixed mode retention

Hydrophobic – interaction with bonded phase

Ion exchange – interaction with charged sites



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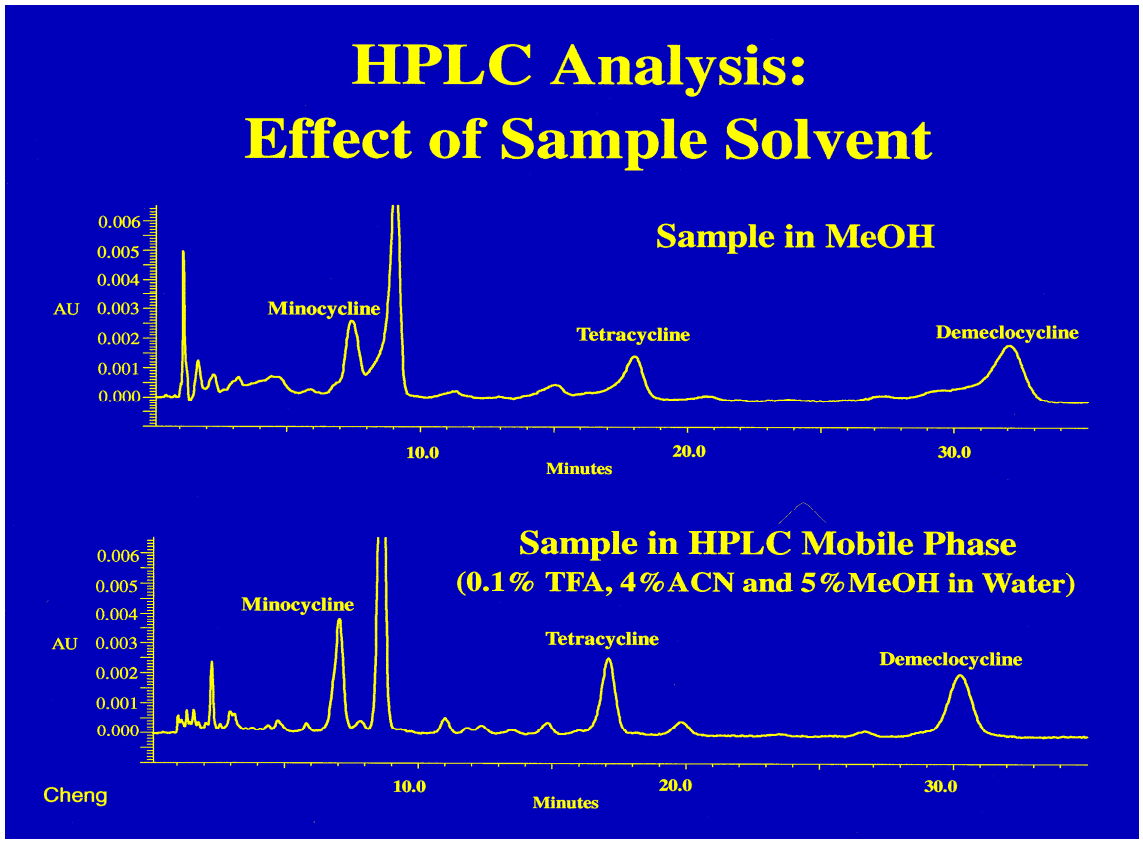
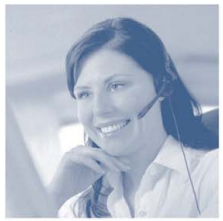
Other Issues

Incorrect Sample Solvent





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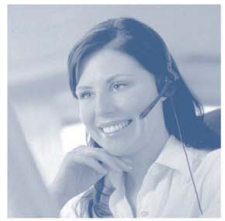
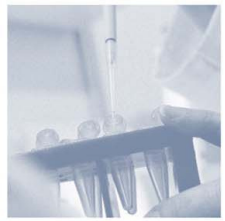
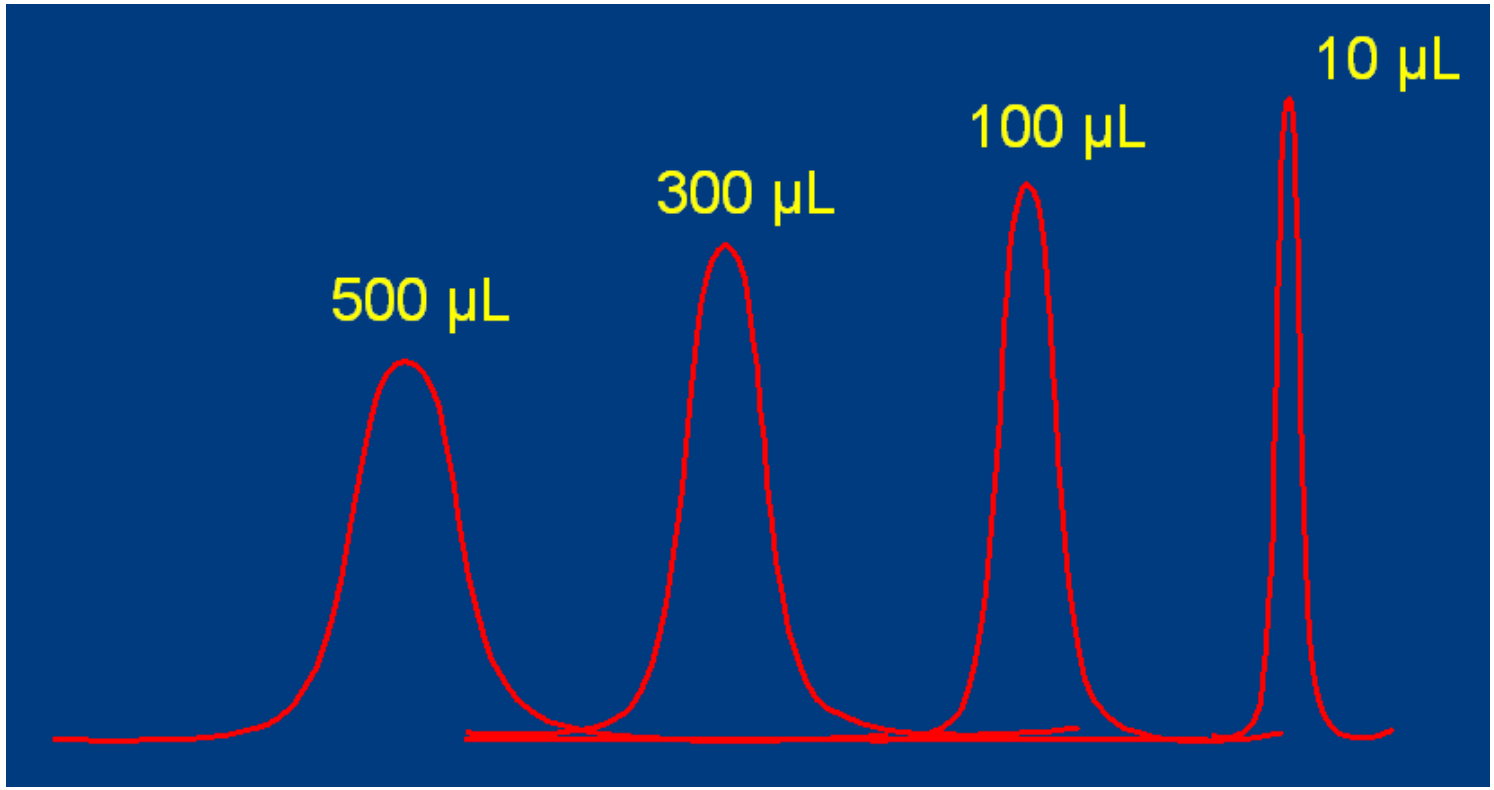




Column/Volume Overload



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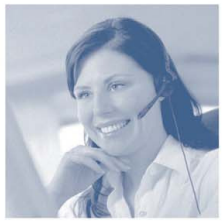



Column/Volume Overload

A good approach to a loading study is to increase the amount injected by a factor of 2

This is fine enough to find the point of maximum load but course enough to cover a large concentration quickly

It is often not necessary to perform measurements on the chromatogram. A visual inspection is often sufficient

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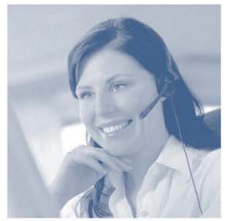
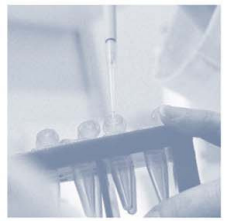
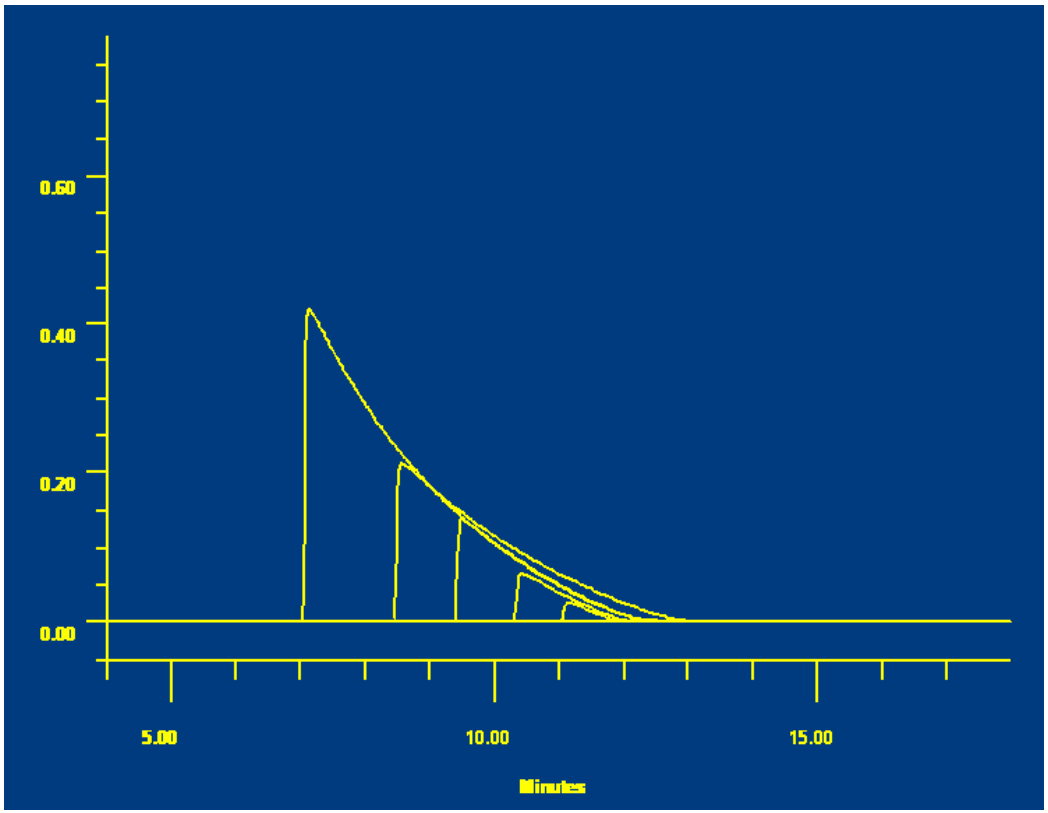




Mass Overload



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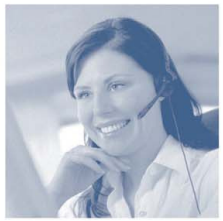
Other Issues





Mass Overload

How to identify?

- Indicated by a rapid detector response

How to remedy the problem?

- Dilute the sample by 50% then re-inject

One can observe better resolution and slightly rounded leading edge of the peak

We then dilute the sample by 50% again until we observe a much more pronounced leading edge of the peak of interest.

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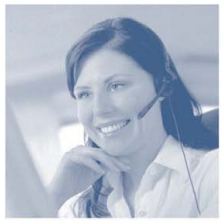
Summary





- Assess all potential sources
- What is the baseline telling you?
- Treat your columns well
- Assess the suitability of your method
  - Mobile phase composition etc.
- Ensure injection volume and sample concentration are suitable

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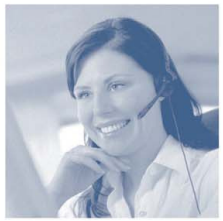
HPLC Troubleshooting



Thank you for your attention

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