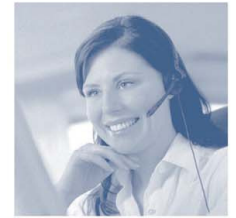

HPLC Troubleshooting

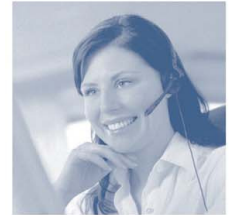


VWR 
Supplier Partnerships for Customer Solutions



Course Outline

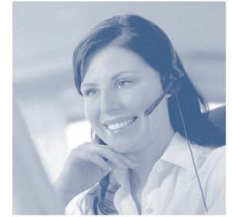
- Potential Sources
- Where to Begin?
- Baseline Troubleshooting
- Column Troubleshooting
- Other Issues



Potential Sources of Chromatographic Problems

- Mobile Phase
- Injector
- In-Line Filter
- Column
- Detector
- Pump
- Guard Column
- Connecting Tubing and Fittings
- Integrator/Recorder

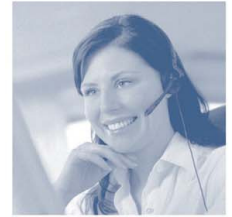
The Scientist/Analyst



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



BASELINE TROUBLESHOOTING



Noisy baseline



Cyclic



Synchronous noise



Spikes



Asynchronous noise



No peaks



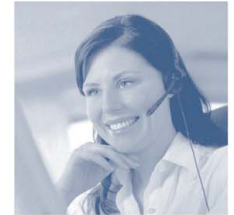
Drift



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

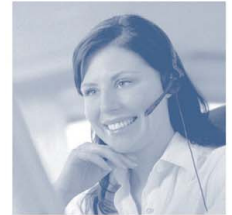


Baseline Troubleshooting

Synchronous Noise

ALMOST ALWAYS CAUSED BY THE PUMP

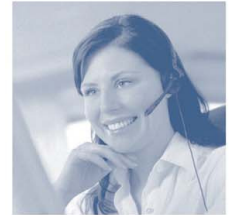
- Air in the pump head
 - Prime pump and degas solvent
- Check valve problem
 - Clean or replace
- Broken plunger
 - Replace
- Mixing Problem
 - Increase system volume
- Electrical noise
 - Remove source



Baseline Troubleshooting

Asynchronous Noise

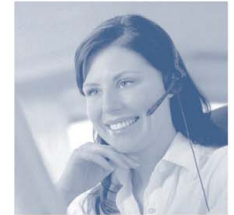
- Bubbles
 - Degas mobile phase
- Gas caught in detector
 - Degas mobile phase. Put backpressure on cell
- Leaks
 - Find leak and repair
- Mixing problems
 - Increase system volume
- Plugged lines
 - Remove plug – flush system
- Electrical problems
 - Remove source



Baseline Troubleshooting

Baseline Drift

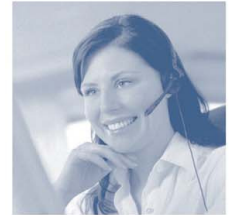
- Gradient – Solvent B absorbs more than Solvent A
 - Try new mobile phase. Use baseline subtraction
- Compounds eluting off column
 - Run strong solvent until baseline is stable
- Solvent composition change (e.g. Evaporation)
 - Ensure solvents are enclosed
- Solvent leaks
 - Tighten, replace fittings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



Baseline Troubleshooting

Cyclic Baseline

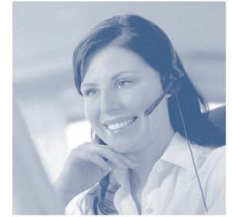
- Temperature fluctuations
 - Thermally insulate. Move away from ventilation. Increase cell temperature
- Mixing problems
 - Increase system volume
- Gas in mobile phase
 - Degas solvents
- Electrical problems
 - Remove source
- Erratic pump
 - Repair
- Plug
 - Remove obstruction – flush system



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



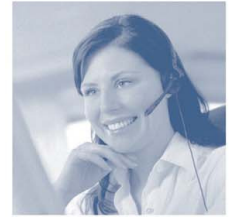
Baseline Troubleshooting

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

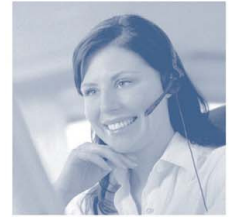
VWR
Supplier Partnerships for Customer Solutions



Baseline Troubleshooting

Negative & Positive Peaks

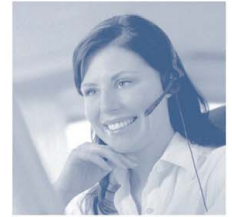
- Some eluting compounds absorb less than solvent
 - Use a different or cleaner solvent
- Air bubbles passing through cell
 - Degas mobile phase
- All peaks are negative
 - Change detector polarity
- Negative peaks with RI detector
 - May be normal (peak direction is function of RI differential from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



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



Column Troubleshooting

Common Problems

- Peak shape
- Retention time
- Other



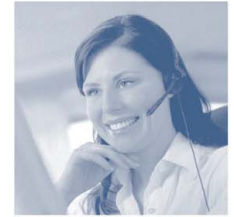
Column Troubleshooting

Chromatographic problems may be related to:

- Instrument
- Sample
- Column

Two Major Problems are:

- Peak Shape/Width
- Retention Time Changes



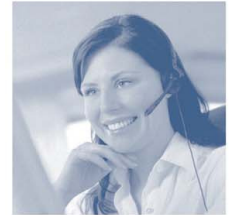
Column Troubleshooting

Peak Shape Problems

Most Common Problem in HPLC:

Distorted peaks will cause integration or resolution problem

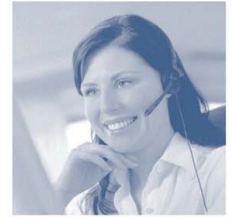
Indication that optimal column performance is not being attained



Column Troubleshooting

Peak Shape Problems

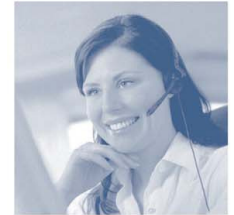
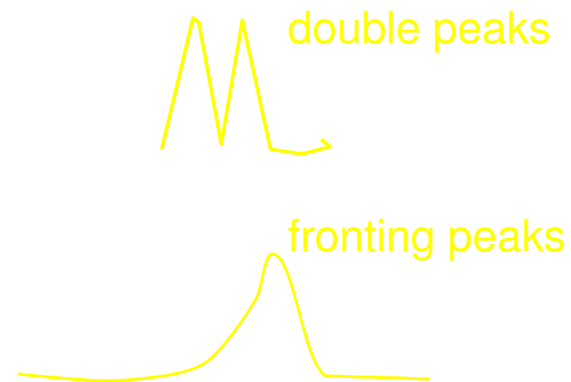
- Column Destroyed
- Secondary Interactions
- Incorrect Sample Solvent
- Column Overload
 - Mass Overload
 - Volume Overload
- Other Extra-Column Effects
 - Sampling Rate
 - Time Constant



Column Troubleshooting

All Peaks Affected

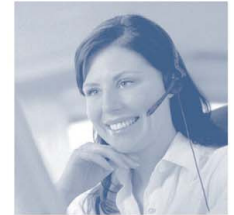
- COLUMN
 - Connection
 - Replace frit
 - Regenerate or replace column
- COLUMN DESTROYED
 - pH <2 washes off functional group
 - pH >8 dissolves silica base



Column Troubleshooting

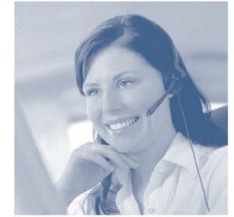
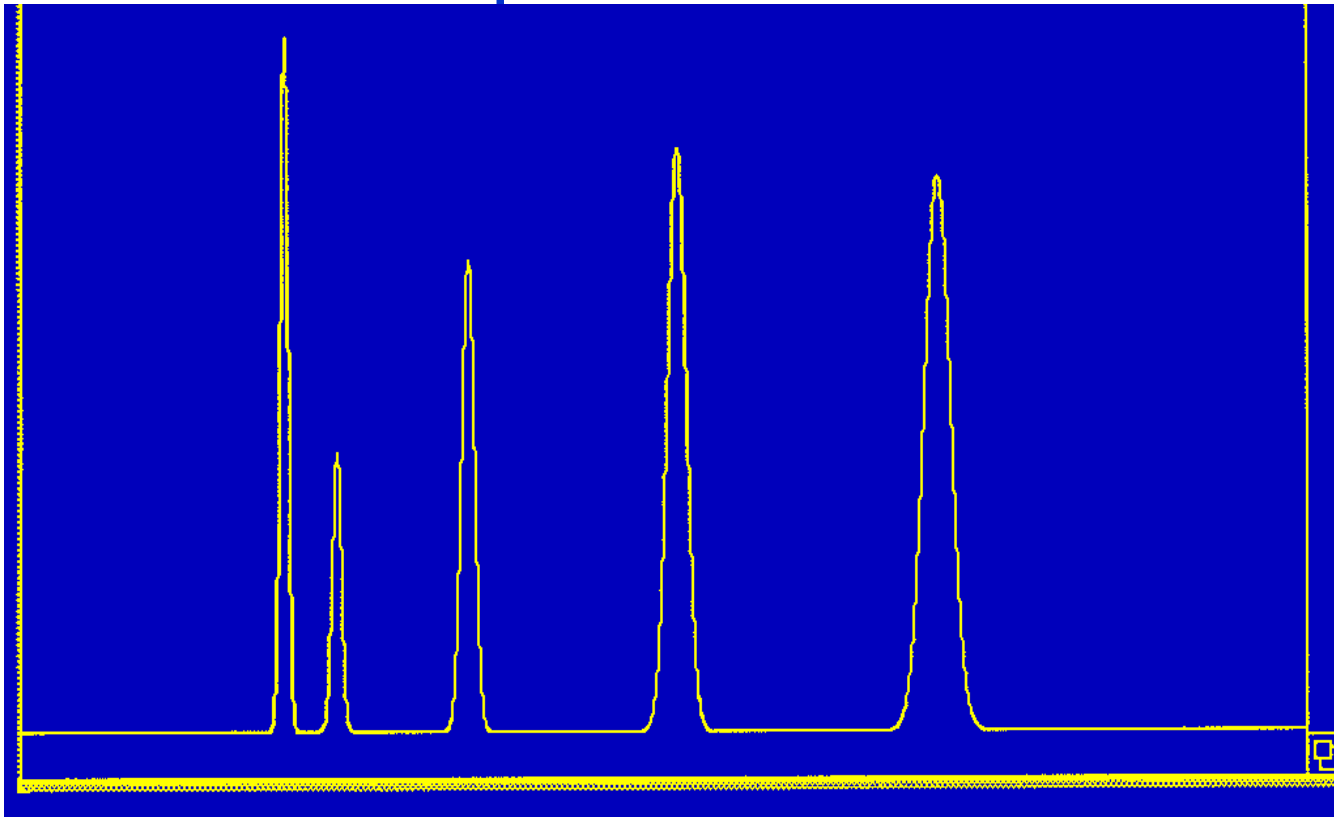
Column Collapse

Well packed column



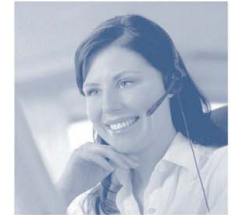
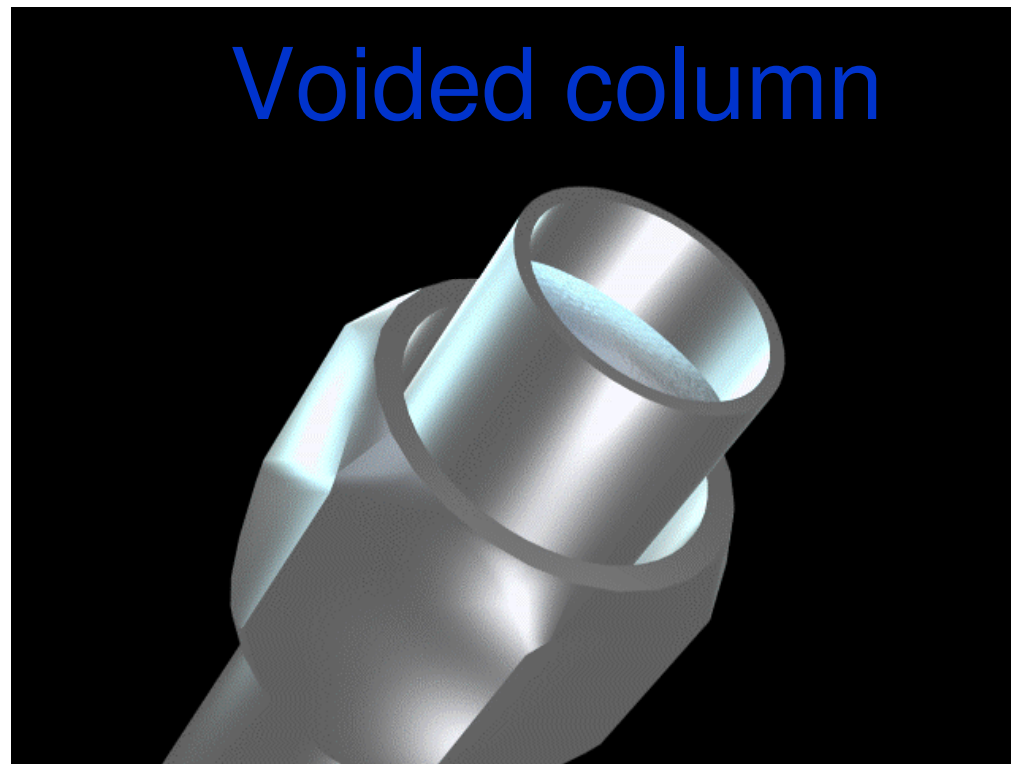
Column Troubleshooting

Well packed column



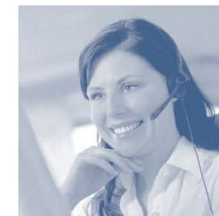
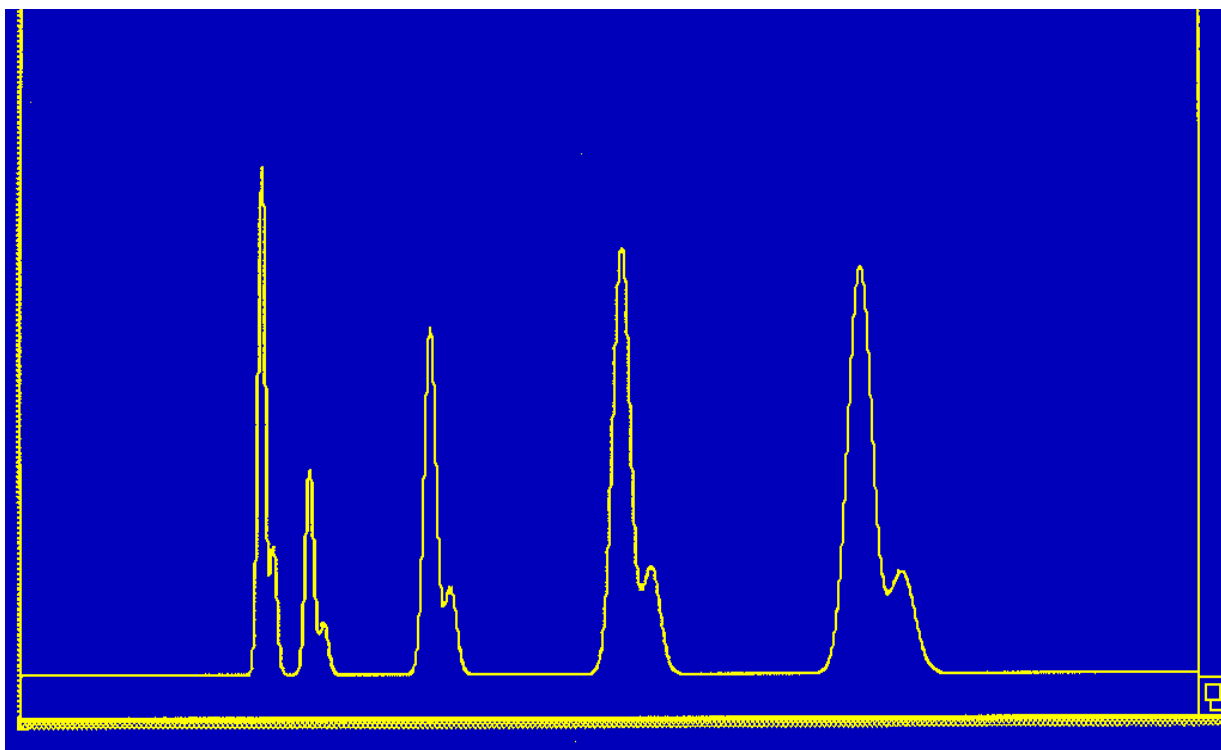
Column Troubleshooting

Column Collapse



Column Troubleshooting

Voided column

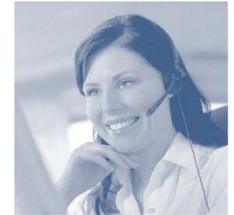
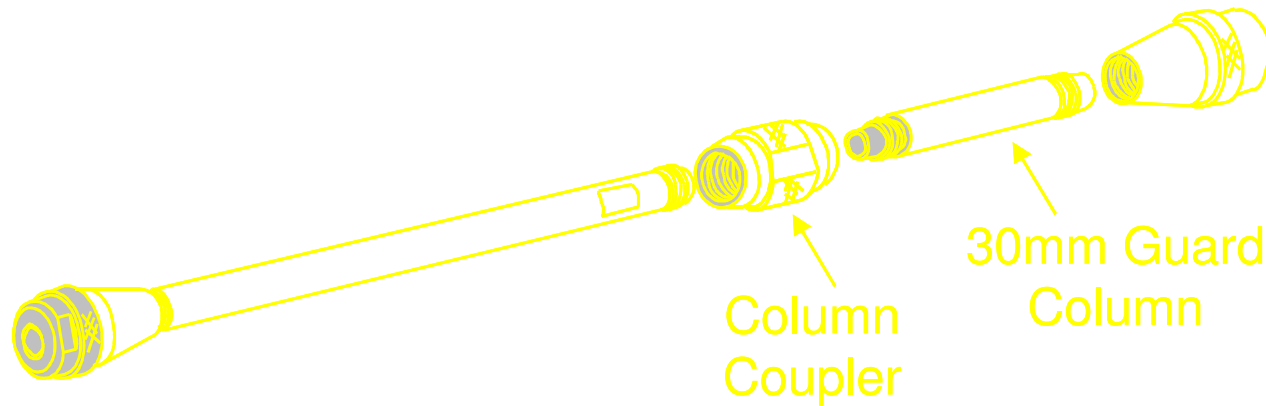


Column Troubleshooting

Column Protection

Major cause of column deterioration is contamination.

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



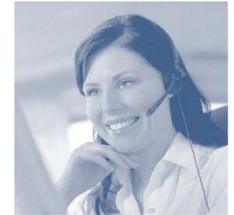
Column Troubleshooting

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

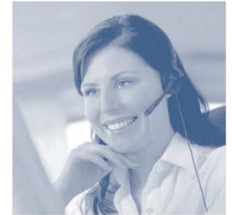


Column Troubleshooting

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)



Column Troubleshooting

Column Storage

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

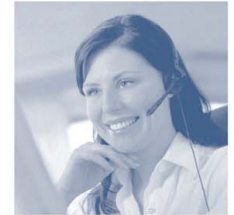
Store in Shipping Solvent for Longer Periods of Time



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)**



Column Troubleshooting

Column Storage

Columns which may be stored in Water or Buffered Solvents:

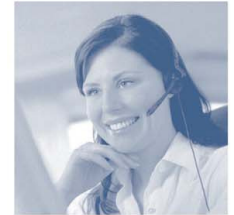
- Ion exchangers
- Aqueous SEC packings

However:

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

OR

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



Column Troubleshooting

Column Storage

Columns which should be stored in Mobile Phase:

- Normal Phase
- Organic SEC (GPC)



Other Issues

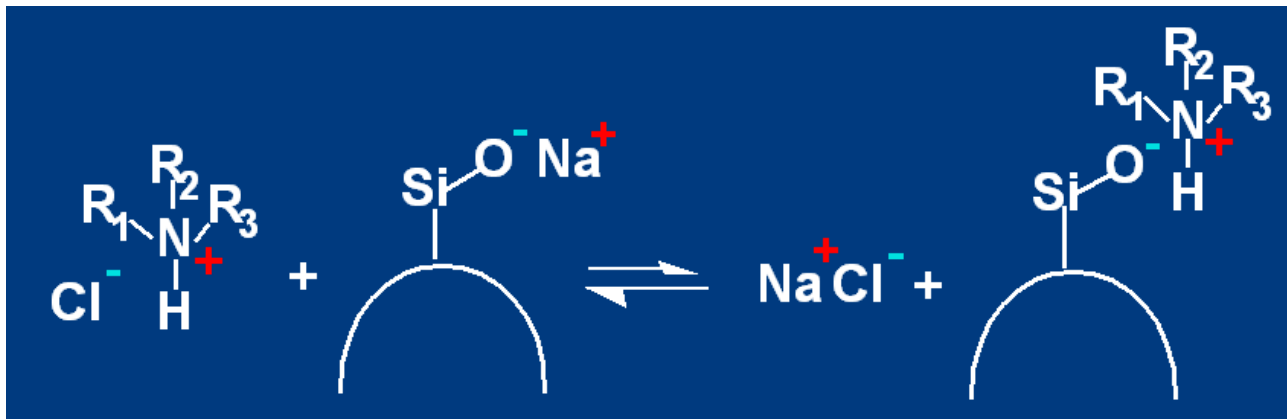
Tailing

What Causes Tailing?

Mixed mode retention

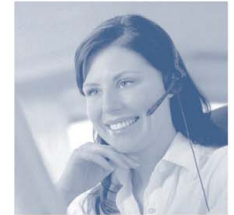
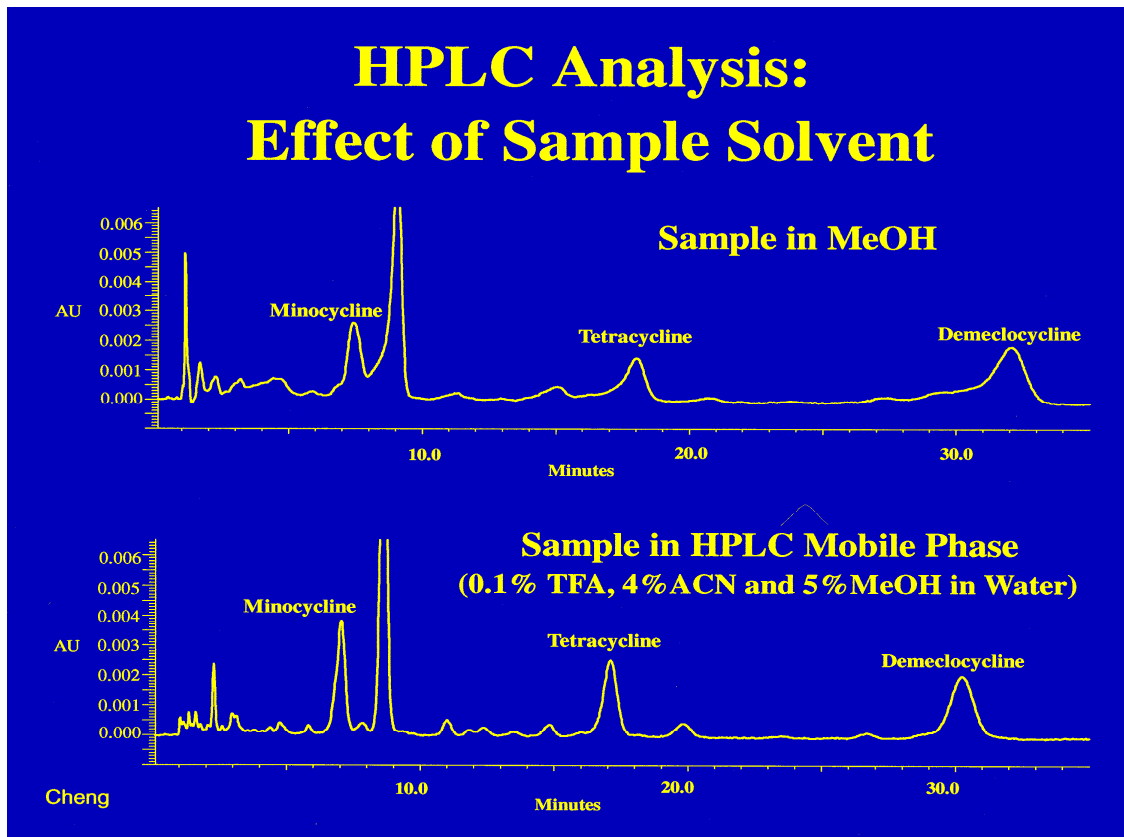
Hydrophobic – interaction with bonded phase

Ion exchange – interaction with charged sites



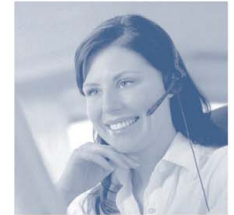
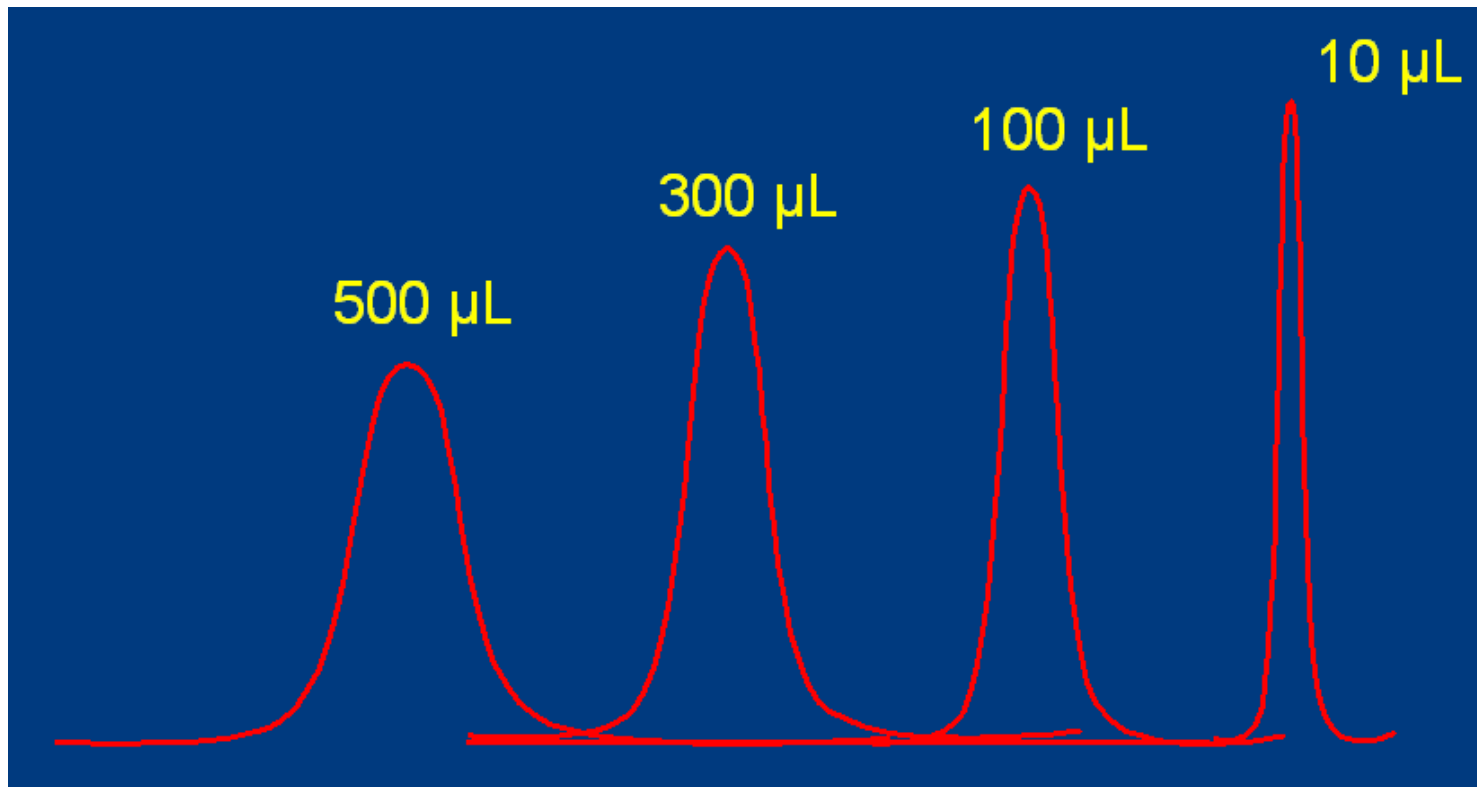
Other Issues

Incorrect Sample Solvent



Other Issues

Column/Volume Overload



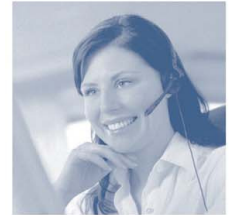
Other Issues

Column/Volume Overload

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

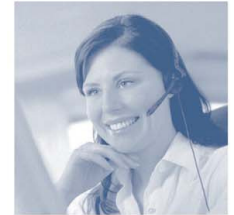
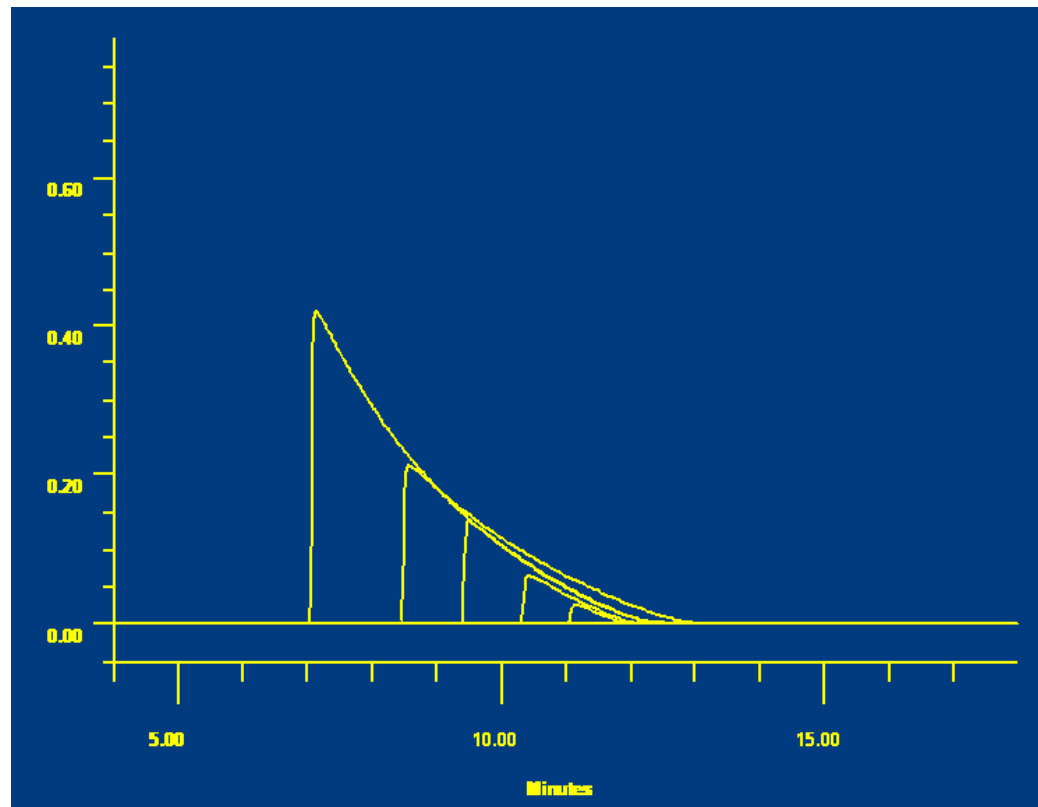
This is fine enough to find the point of maximum load but coarse enough to cover a large concentration quickly

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



Other Issues

Mass Overload



Other Issues

Mass Overload

How to identify?

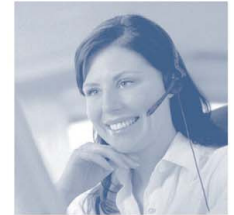
- Indicated by a rapid detector response

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.



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



HPLC Troubleshooting

Thank you for your attention

