HPLC Troubleshooting
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

Synchronous noise

Asynchronous noise

Drift

Cyclic

Spikes

No peaks
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

VWR
Supplier Partnerships for Customer Solutions
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
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

Voided column
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
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

HPLC Analysis:
Effect of Sample Solvent

Sample in MeOH

Sample in HPLC Mobile Phase
(0.1% TFA, 4%ACN and 5%MeOH in Water)
Other Issues

Column/Volume Overload

500 μL  300 μL  100 μL  10 μL
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 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.
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