Troubleshooting the Agilent 1100 HPLC

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In This Section, You Will Learn:

- General troubleshooting strategy
- Common Problems:
  - Pressure
  - Reproducibility
    - *Retention time (Rt)*
    - *Area*
  - Linearity
  - Baseline noise
  - Sensitivity
  - Peak shape

Important HPLC Parameters

During the course of your work, monitor the following parameters:

- Pressure
- Baseline Noise
- Retention time precision
- Area precision
- Peak height precision
- Peak shape
- Sensitivity

These parameters can tell you if your HPLC is experiencing problems.

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Why Do You Care if Your System Is Functioning Properly?

Quantitative Analysis

- Changes in flow rate caused by pumping problems result in incorrect area counts.
- Noisy baselines will interfere with integration and decrease signal-to-noise ratio.
- Poor autosampler maintenance will result in poor injection volume reproducibility.

Qualitative Analysis

- Retention time fluctuations resulting in incorrect identifications.
- Spectral analysis difficulties.

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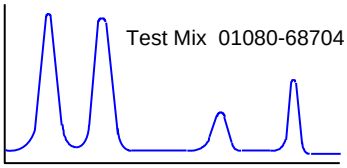
Troubleshooting Key Concept
Divide and Conquer

Question

Is the problem with the application method or the instrument?

Action

Inject a standard test mix of known behavior under known conditions



.....HPLC Test Sample – Keep it simple:

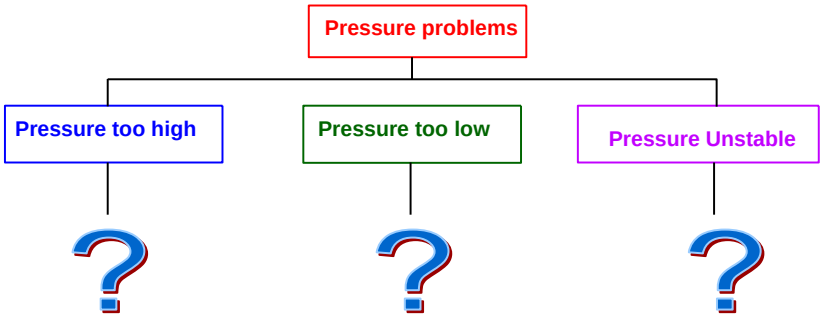
Test sample: **2- 3 Analytes (chemically stable) in sufficient concentration**

Test column: **Record length, internal diameter, stationary phase, particle diameter**

Separation method: **Solvent , flow rate, temperature, pressure, detection parameters**

Results**: t_R, peak width, resolution, peak area, peak height, baseline noise, signal/noise ratio of analytes**

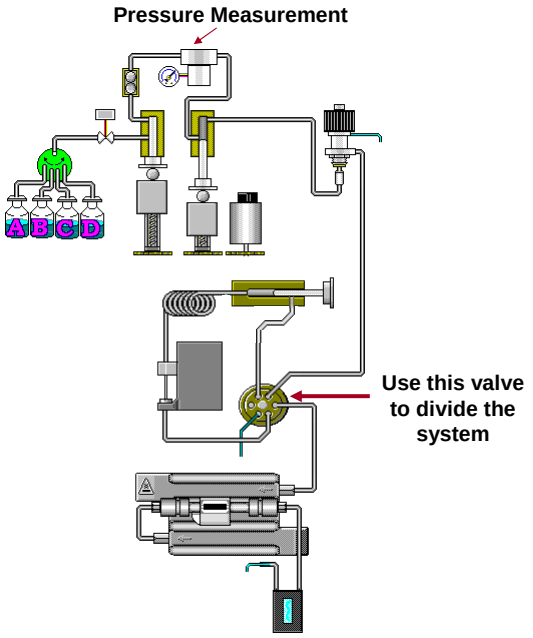
Problems with the System Pressure



Pressure Problem I

Pressure Too High

- Column inlet frit contaminated
- Frit in purge valve contaminated
- Column contaminated
- Blockage in a capillary, particularly needle seat capillary
- Rotor in injection valve plugged
- Injection needle or needle seat plugged



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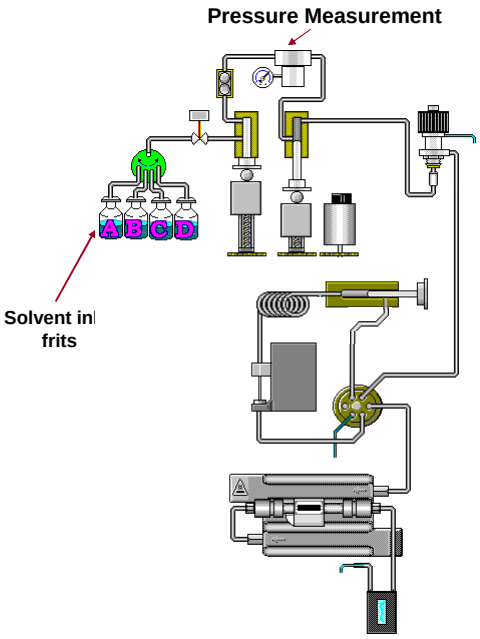
Pressure Problem II

Pressure Too Low

- Solvent inlet frit plugged
- Leak in a capillary connection or other part (pump seals)
- Wrong solvent or flow rate
- AIV (Active inlet valve) defective
- Multichannel Gradient valve incorrectly proportioning
- Ball valve defective
- Column defective (stationaryphase)



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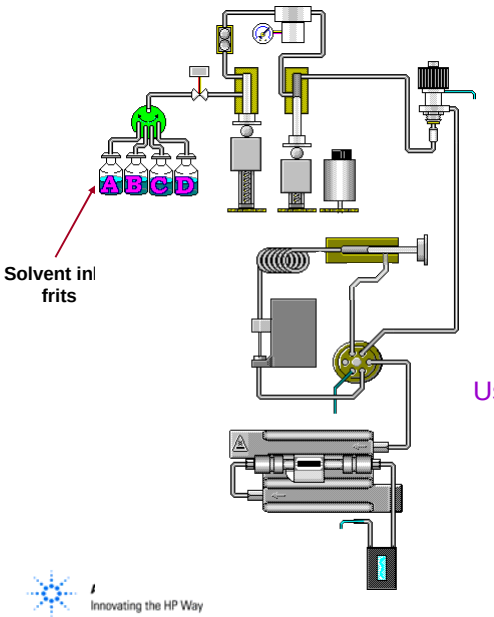
Pressure Problem III



Pressure Fluctuation

- Solvent inlet frits plugged
- Solvent not degassed
- Seals of the pump defective
- Ball valve defective
- AIV defective

Usually an indication there is air in the pump

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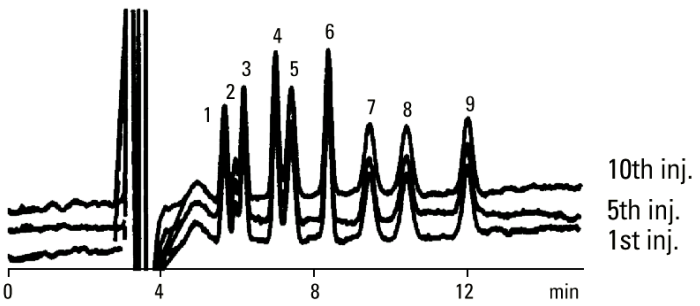
Reproducibility

Peak retention time precision:
⇒ with oven: < 0.3%
⇒ without oven: < 0.7%
Peak area precision: ≤1.5%

Typically,

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



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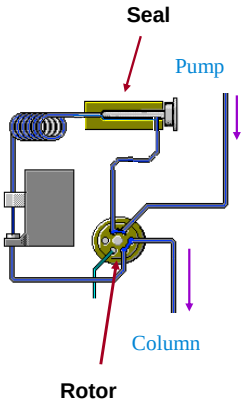
Problems with Reproducibility – Peak Areas

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



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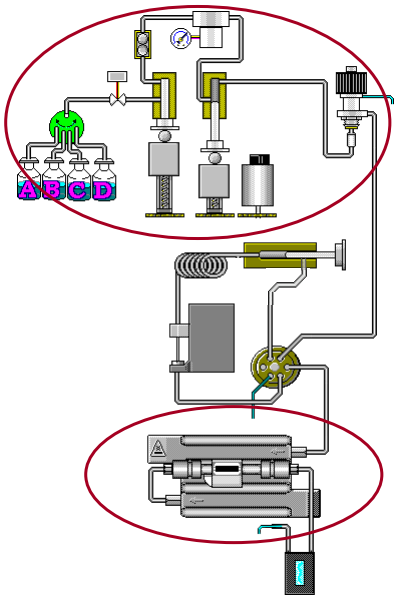
Problems with Reproducibiliy – Retention Time

Retention Times not Reproducible

- **• Pump Problems**
  - **–Mobile phase composition problems**
  - **–Valves AIV, ball valve defective**
  - **–Flow rate problems**
- **•Column Oven Problems**
  - **–Temperature fluctuations**
- **•Other**
  - **–Column equilibration**
  - **–Column deterioration**



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Linearity Problems

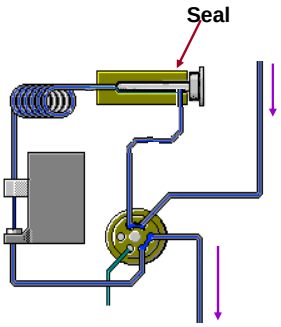
Peak Areas not Linear

Autosampler

- Rotor seal cross-port leak or injection valve not tight
- Piston seal of metering unit defective
- Needle partially blocked

Detector

- Satuaration



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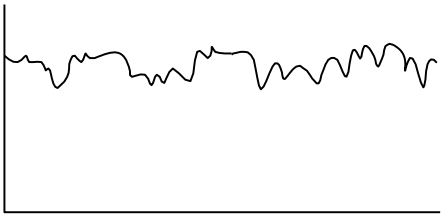
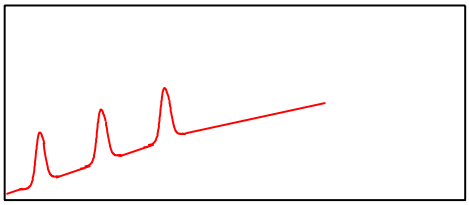
Baseline Fluctuations





Possible Causes:

- Dirty Flow Cell
- Dirty mobile phase
- Detector Lamp Failing
- Pulses from Pump (if Periodic)
- Temperature Effects on Detector (RI)
- Air Bubbles passing through Detector
- Gradient elution
- Immiscible Solvents

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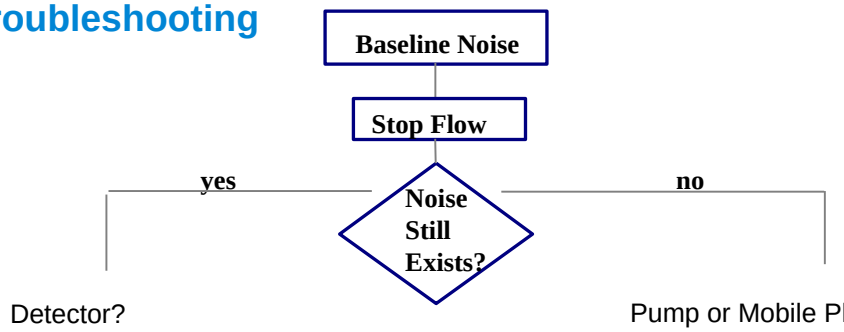
Troubleshooting



Other Questions to Ask

- ➔Have you changed your mobile phase composition?
- ➔Have you changed your acquisition wavelength?
- ➔What mobile phase was last used in your instrument?
- ➔Do you have a miscibility problem?
- ➔Are your solvents dirty?

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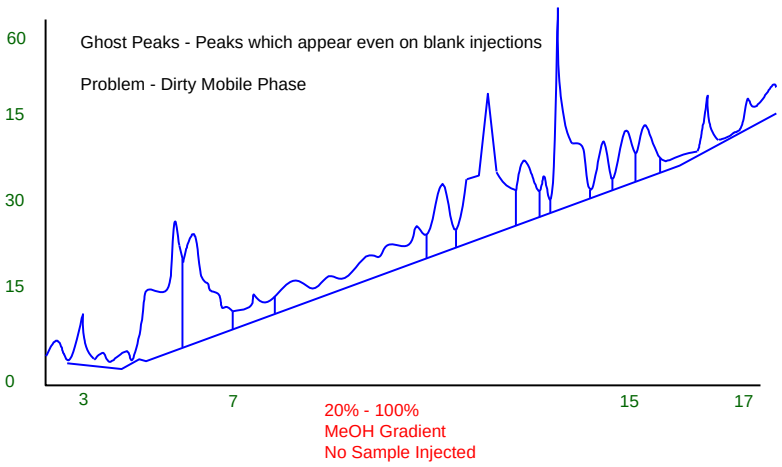
Example - Ghost Peaks



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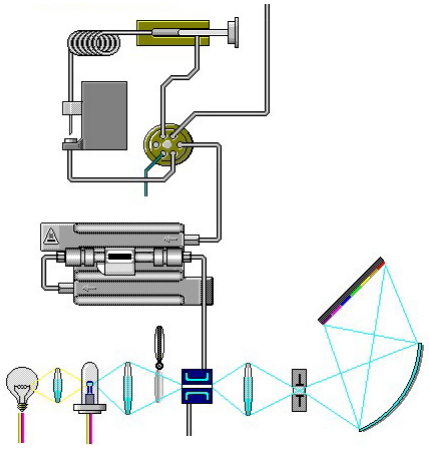
Sensitivity Problems

Peak response too low

Causes:

- **insufficient injection amount**
- **Detector problems**
  - **Lamp aging**
  - **Detector cell contamiated**
  - **Solvent absorption high**



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Peak Problems – Split/Tailing/Fronting
Appendix

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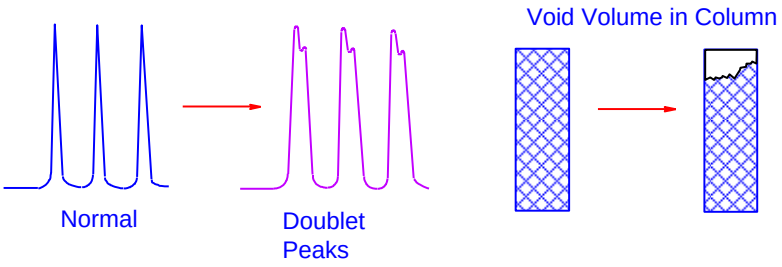
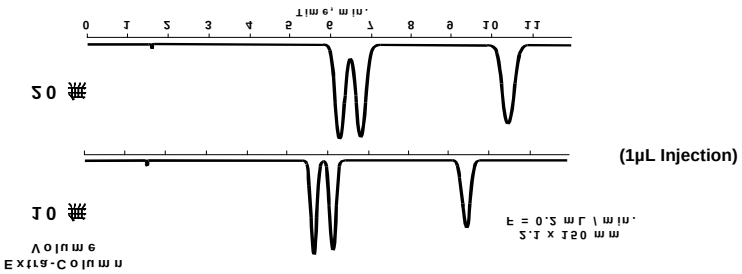
Peak Shape - Doublets



- Void Volume in Column
- Partially Blocked Frit
- Only One-Peak a Doublet- Coeluting Components

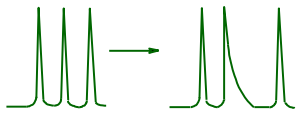
Effect of Extra-Column Volume



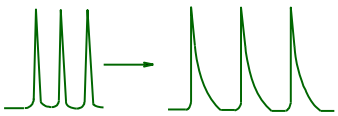
- Use short, small internal diameter tubing between the injector and the column and between the column and the detector.
- Make certain all tubing connections are made with matched fittings.
- Use a low-volume detector cell.
- Inject small sample volumes.

Peak Shape - Tailing

Symmetry > 1.2



Normal Tailing



Normal Tailing

Causes

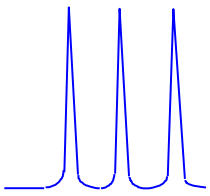
Some Peaks Tail

- Secondary - Retention Effects
  - Residual Silanol Interactions
- Small Peak Eluting on Tail of Larger Peak

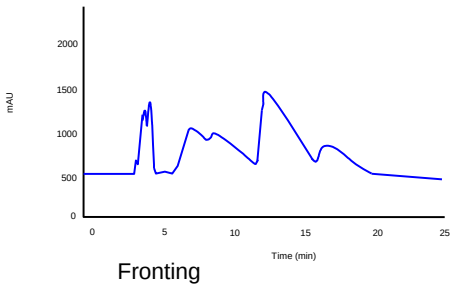
All Peaks Tail

- Extra-Column Effects
- Build up of Contamination on Column Inlet
- Heavy Metals
- Bad Column

Peak Shape - Fronting Peaks



Normal



Fronting

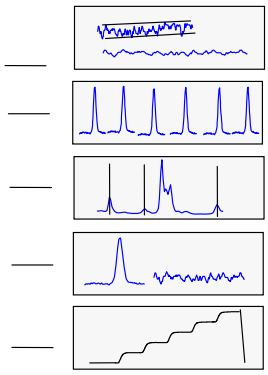
Symmetry < 0.9

Causes:

- ➔Column Overload
- ➔Small Band Eluting Before Large Band

Review of Important Tests

- Leak test
- Temperature stability & accuracy
- Baseline noise/drift
- Precision of peak areas & RT
- Wavelength accuracy
- Detector linearity
- Autosampler carry over
- Autosampler linearity
- Composition accuracy/precision
- Composition ripple



Summary - Troubleshooting

Exclude application errors

Take a methodical and systematic approach

Localize error in a part of the system

Change only one thing at a time

Ask for help where appropriate

Plan course of action

Verify if problem has been eliminated (test injection)

Periodic maintenance of the Agilent 1100 prevents downtime