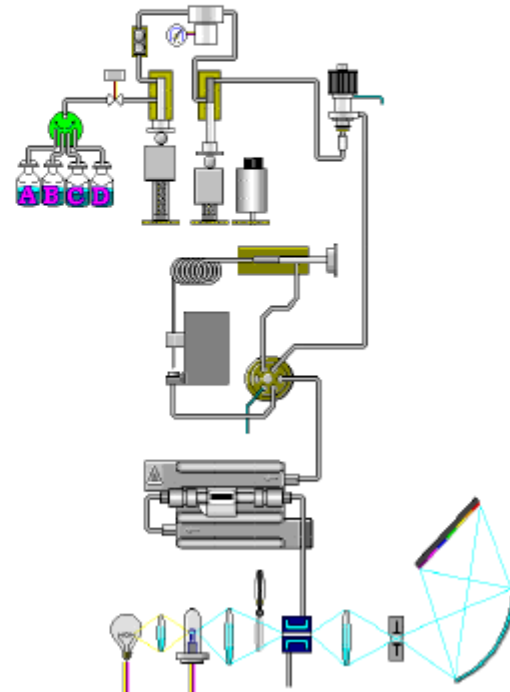
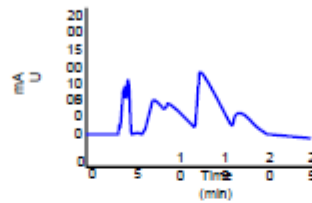
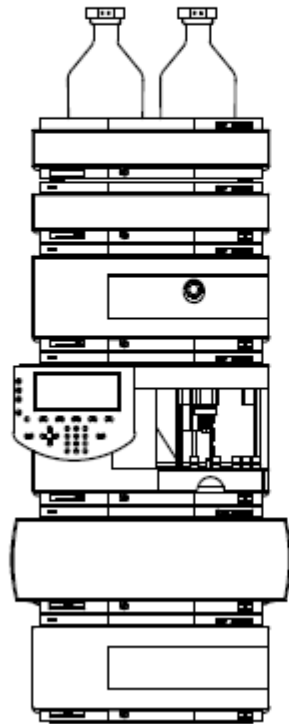


Care, Maintenance, and Troubleshooting of HPLC Columns

Indranath Chakraborty

22/12/12

Troubleshooting in HPLC



Major Areas of Column Problems - Dramatic Changes in 3 Key Areas:

- 1. HPLC System Pressure**
- 2. Chromatogram - Peak Shape**
- 3. Chromatogram - Peak Retention/Selectivity**



1. Pressure Issues

Column Observations

Large pressure change

Potential Problems

Plugged inlet frit

Column contamination

Plugged packing

Determining the Cause and Correcting High Back Pressure

Check pressure with/without column – many pressure problems are due to blockages elsewhere in the system.

If Column pressure remains high:

- Rinse column (remove detector from flow path!)

- Eliminate column contamination and plugged packing
- high molecular weight/adsorbed compounds
- precipitate from sample or buffer

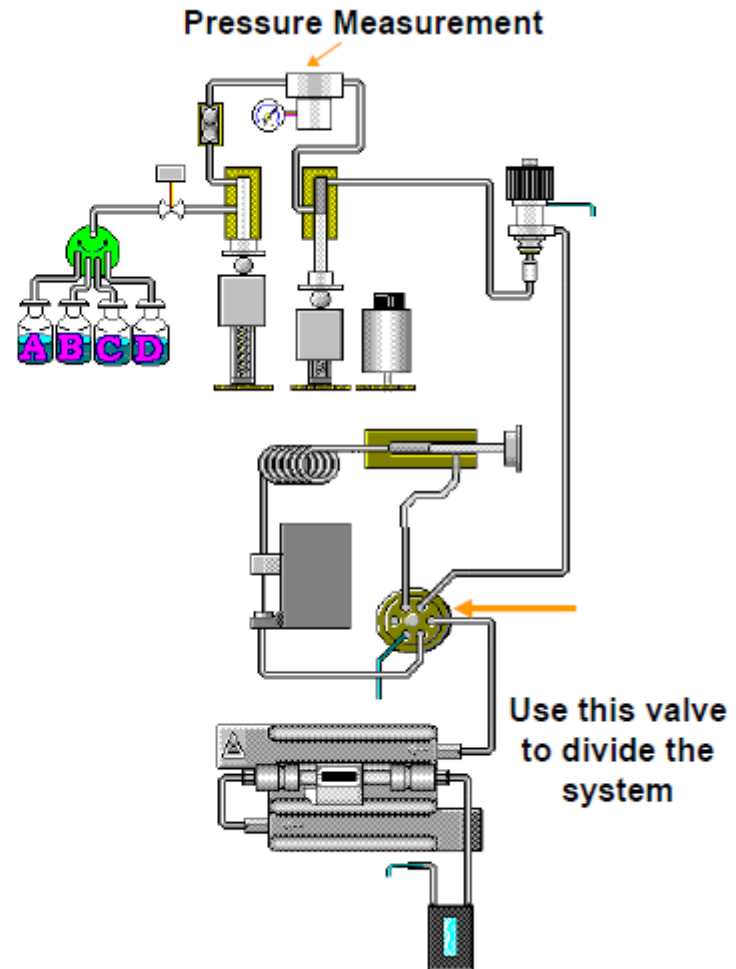
- Back flush column – may clear plugged column inlet frit
- Change column inlet frit (... or discard column)

Eliminate pressure issues – add a disposable 0.5 or 2 um in-line filter to system.

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



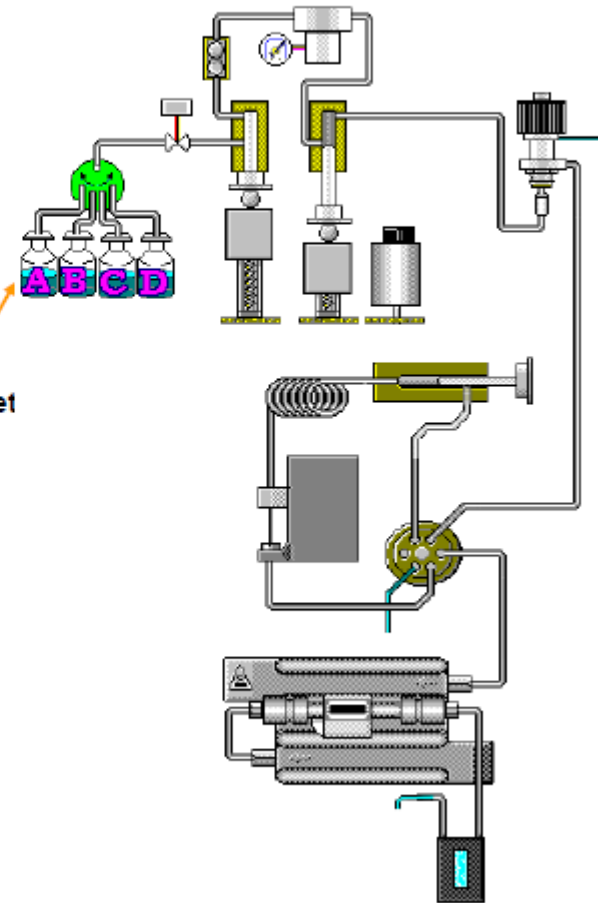
Pressure Problem II

Pressure Too Low

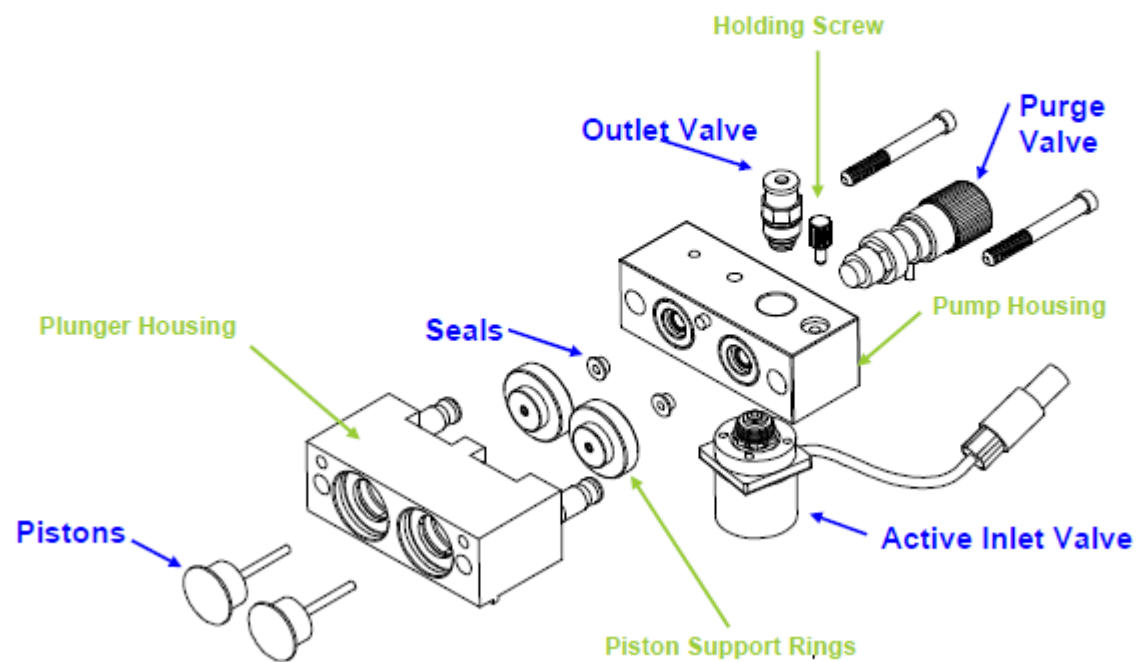
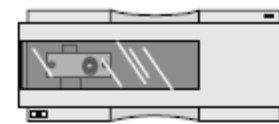
- Solvent inlet frit plugged
- Leak in a capillary connection or other part (pump seals)
- Wrong solvent or flow rate
- AIV (Active inlet valve) defective
- Multichannel Gradient valve incorrectly proportioning
- Ball valve defective
- Column defective (stationary phase)

Solvent inlet
frits

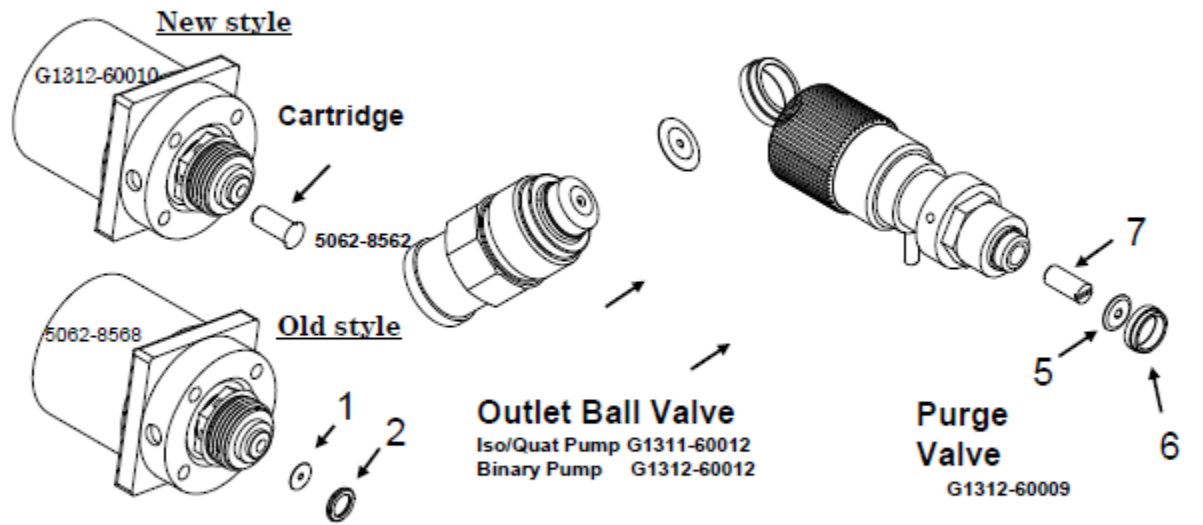
Pressure Measurement



1100 and 1200 Pumps Exploded View



Pump Check Valves



Active Inlet Valve
(common to all)

- | | | | |
|----------------|-------------|---------------|-------------|
| 1. Gold Washer | 5001-3707 | 5. Gold Seal | 5001-3707 |
| 2. Plastic cap | 01018-21207 | 6. Cap(4pk) | 5062-2485 |
| 3. Gold Seal | 5001-3707 | 7. PTFE (5pk) | 01018-22707 |
| 4. Cap(4pk) | 5062-2485 | | |

Column Cleaning

Flush with stronger solvents than your mobile phase.
Make sure detector is taken out of flow path.

Reversed-Phase Solvent Choices in Order of Increasing Strength

1. Mobile phase without buffer salts (water/organic)
2. 100% Organic (MeOH or ACN)
3. Is pressure back in normal range?
4. If not, discard column or consider more drastic conditions:
75% Acetonitrile:25% Isopropanol, then
5. 100% Isopropanol
6. 100% Methylene Chloride*
7. 100% Hexane*

When using either Hexane or Methylene Chloride the column must be flushed with Isopropanol before returning to your reversed-phase mobile phase.

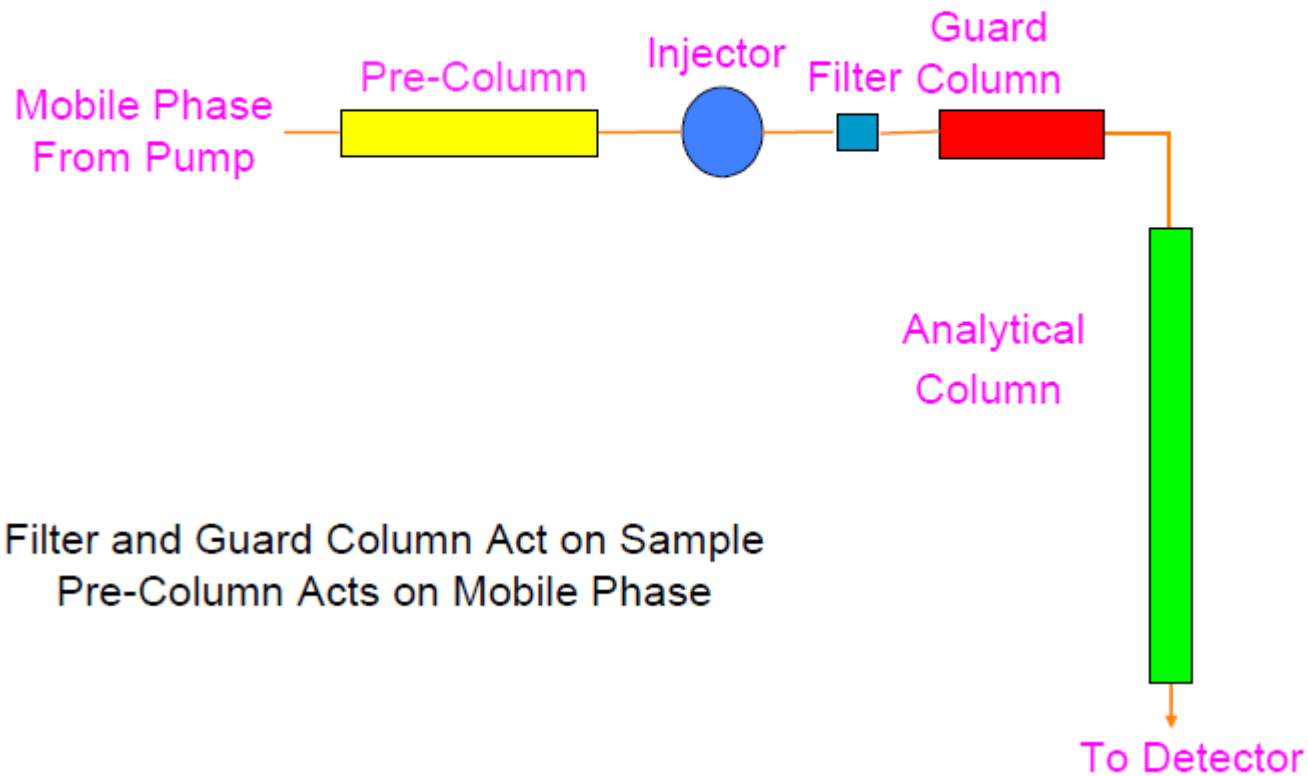
Column Cleaning

Normal Phase Solvent Choices

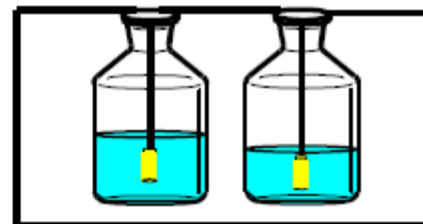
in Order of Increasing Strength

- Use at least 50 mL of each solvent
- 50% Methanol : 50% Chloroform
- 100% Ethyl Acetate

Preventing Back Pressure Problems: In-Line Devices



Preventing Column Back Pressure Problems



1. Filter mobile phase:
 - filter non-HPLC grade solvents
 - filter buffer solutions
 - Install an in-line filter between auto-sampler and column (removes pump seal debris, ALS rotor debris, and sample particulates). Use 2 μm frit for 3.5 μm columns, use 0.5 μm frit for 1.8 μm columns.
2. Filter all samples and standards
3. Perform sample clean-up (i.e. SPE, LLE) on dirty samples.
4. Appropriate column flushing – flush buffers from entire system at end of day with water/organic mobile phase.

Thank you

Will be continued in next presentation.....