Approach to HPLC Troubleshooting

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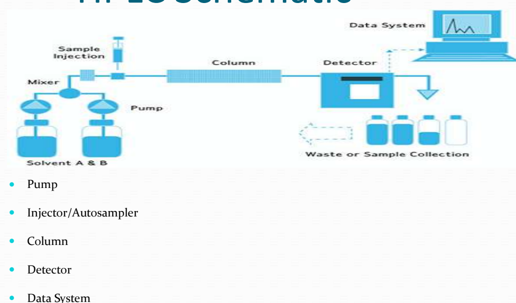
1

Introduction

- Logical approach to the problem: isolate the source
- Check operator's manuals: exploded diagrams
- Seek out technical support from manufacturers. Some offer free seminars, web-based resources on HPLC /UPLC
- Always remember preventative maintenance will reduce frequency of any issues

2

HPLC Schematic



- Pump
- Injector/Autosampler
- Column
- Detector
- Data System

3

Abnormal pressure

- Mobile Phases – grades of solvents, regular preparation, vacuum degassing
- Composition of mobile phases
- Column - ? Correct phase and particle size
- Column temperature
- HPLC Method: check flow rates; gradient programme
- Pressure limit exceeded – check for blockage , check limit settings
- Has anything been changed? Review ALL parameters

4

Low or no pressure

- Verify method settings
- ? Insufficient mobile phase
- Broken piston((sapphire plunger)
- Air trapped in pump head
- Faulty check valves- sonicate 30 seconds
- Major leak: tighten or replace fittings
- Faulty pressure transducer – replace

5

Steady High Pressure

- Flow rate to high
- Column frit blocked: backflush column; replace frit; replace column
- Incorrect mobile phase – replace with correct MP and wash column
- Correct column?
- Injector blockage /replace needle/needle seat assembly
- Column temperature too low
- Blocked guard column
- Blocked in-line filter

6

Pressure fluctuations

- Air in pump : degas solvent
  bleed air from pump
- Faulty check valve: replace
- Pump seal failure
- Insufficient degassing – degas solvent
- Leak in system – locate and degas
- NB use gradient elution; some pressure cycling will be normal due to viscosity changes

7

Injector/Column Leaks

- Loose fitting – tighten
- Overtight fittings- replace DO NOT OVERTIGHTEN!
- Check valves/Pump seals/Purge valves
- Injector leaks rotor seal failure -rebuild or replace
- Column leaks, loose fitting
- How to extend the life of your column: clean sample, high purity reagents, use of solvents compatible with your system, use of in-line filters, dedicated column to one application

8

Chromatogram problems

- Peak tailing- reverse flush column
  replace column frit
  replace column
  wrong mobile phase pH
  replumb: shorter ,narrower tubing
- Peak fronting-possible low temperature
  possible wrong sample solvent
  sample overloading

(decrease volume injected or sample concentration)

9

Chromatogram problems continued

Split peaks

- Possible that column may be fouled with contaminants extra peaks " ghost peaks"
- Change frit or replace column

Broad Peaks

- MP Flow rate too low
- Detector settings?
- Column overloaded –inject a smaller volume; is sample stable?
- Detector response time or cell volume too big
- Tubing between column and detector too long or ID too large
- Poorly made system connections minimum dead volume

10

Other chromatography issues

- Reversed phase mode
  i. add triethylamine (basic samples)
  ii. add acetate (acidic samples)
  iii. add salt or buffer (ionic samples)
  iv. try a different column
- Normal phase
  i. add triethylamine (basic compounds)
  ii. add acetic acid(acidic compounds)

11

Retention time drift

- Poor temperature control
- Mobile phase changing (evaporation/reaction etc)
- Poor column equilibration

12

Detector leaks

- Cracked flow cell window
- Loose fittings
- Blocked waste line – replace
- Ensure waste line is above the surface of waste – so no siphoning
- Blocked flow cell – rebuild or replace

13

Baseline drift

- Column temperature fluctuation
- Non homogenous mobile phase
- Plugged outlet line
- Mobile phase contaminated?
- Mobile phase mixing problem
- Mobile phase recycling?

14

Loss of resolution

- Prepare fresh MP – check pH
- Column fouled with strongly retained contaminants
- Change guard column /and or analytical column

15

Other key problem areas

- Lamp failure – keep a spare
- Bubbles in flow cell (degas MP)
- Wash system between HPLC applications
- Phosphate corrosive/abrasive damage – flush buffer from LC
- Store columns in shipping solvent eg methanol
- Good grade solvents/chemicals required - otherwise irreversible column damage
- Filter samples/ Sample SPE

16

Detectors

- Dirty flow cells : flush warm water 60°C through the flow cell after running buffers or salt solutions
- Always flush system with water-preventing crystallization inside the flow cells
- If LC is not used overnight make sure that flow cell contains MP at least 10% organic
- NB high pressure flow cells required if detectors in series or connected to a fraction collector
- Avoid using MP with alkaline pH > 8 can impair optical performance
- Turn off the UV lamp if no flow
- Lamp failure
- Temperature affects on detectors?

17

Help is out there

- Use your manufacturer's training tools
- Lots of virtual online videos
- Web based tools improve knowledge and skills
- Start with questions , find the answers, answers will lead to solutions
- Keep a Tool box, tubing, fittings, ferrules, needles

18

Thank You

- Any questions?

19