Waters

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Calvin Ng
West Region
Principle System Specialist

System Maintenance and Troubleshooting

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- Basic Assumptions…

1. The HPLC is plugged and turned on.
2. The HPLC is plumbed and wired correctly.
3. Solvent is in the reservoir.
4. The pumps are primed and in good working order.
5. Your solvent bottle doesn't have a vacuum on it.
6. You're not using acetone for solvent at 195nm.
7. You're proportioning valve is working properly.
8. You're not mixing MEOH and water without degassing.
9. You're not doing a water to hexane gradient.
10. You're not running a 1M NaCl to 100% ACN gradient
11. No buffer residue in your pump heads or needle.
12. Know your method's normal backpressure profile.
13. Your sample is filtered.
14. You're not trying to make a partial loop injection of 40ul in a 50ul fixed loop injector.
15. The auto-sampler is well purged and in good working order.
16. You're using a shut-down method that works.
17. You're sure you have plenty of sample in the vial.
18. You're not doing gradients with an RI detector.
19. Your detector has a good lamp and is warm-up.
20. You're not running water through a silica column.
21. Solvent pH is not 13 on a silica base column.
22. You're allowing enough time to equilibrate your column.
23. You're RI is not under the air conditioner vent.
24. How's system delta psi and C/D ratio today?

System Maintenance and Troubleshooting

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- Most problems noticed in chromatography
- No Peaks
  - Pump doesn't pump – check flow
  - Injector isn't injecting – test injector
  - Detector isn't detecting – check lamp and flow cell
- Hardware – solvent manager, pressure trace
- Chemistry – compatibility, elution order
- Suggested Connections for System Cleaning Cycles

System Maintenance and Troubleshooting

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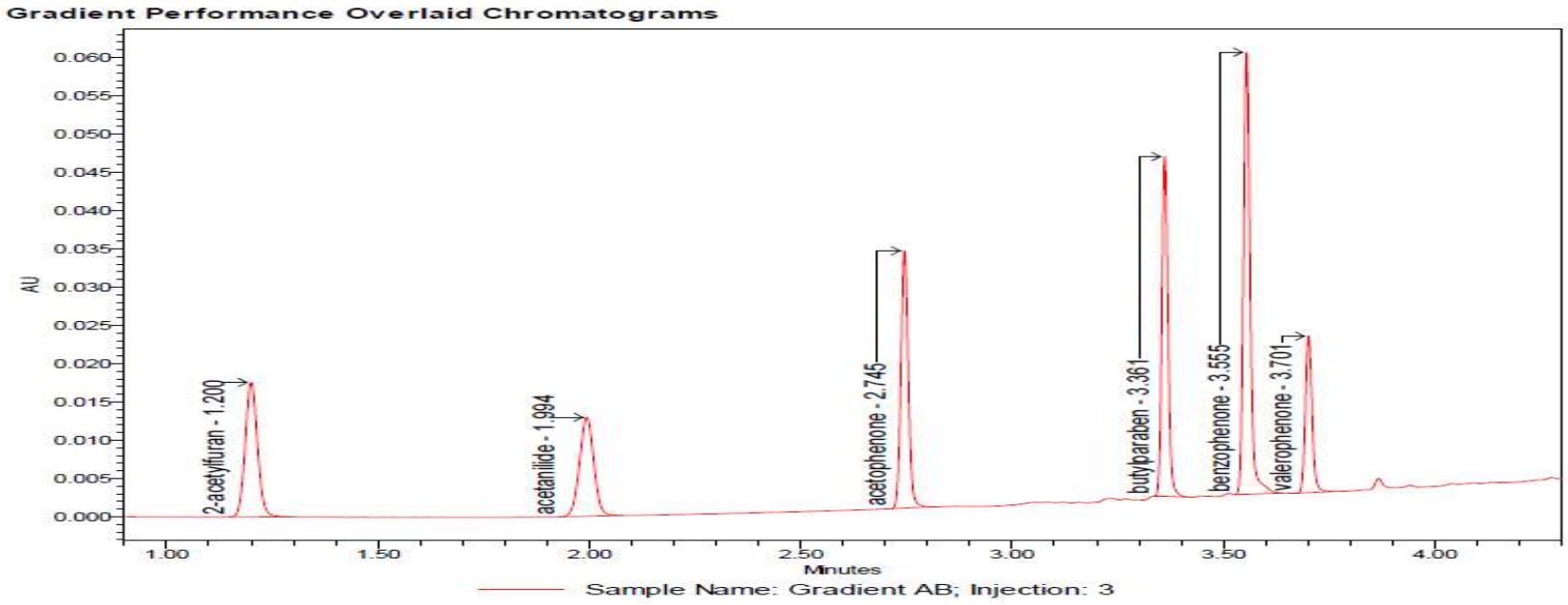
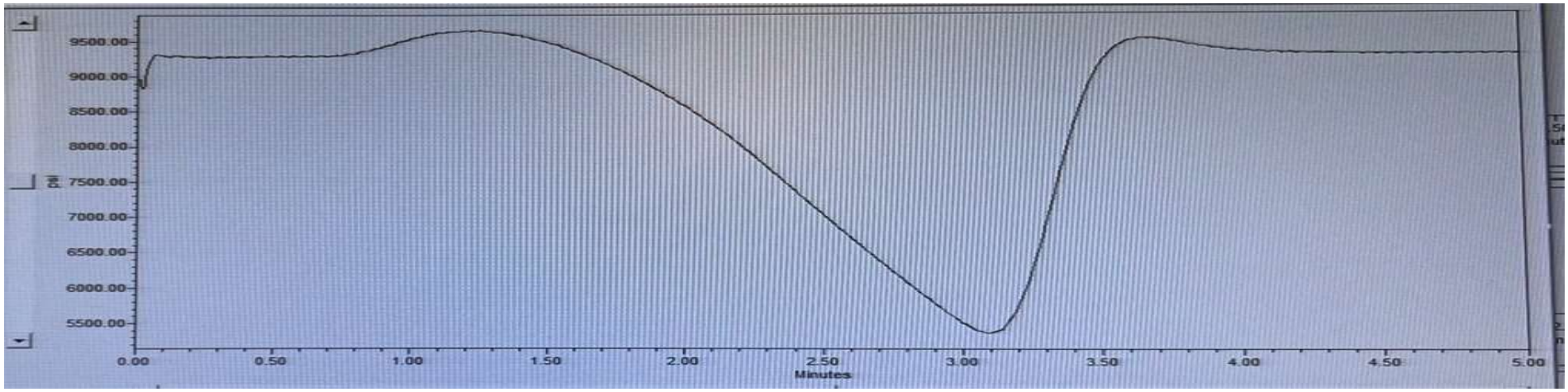
- Most problems noticed in chromatography





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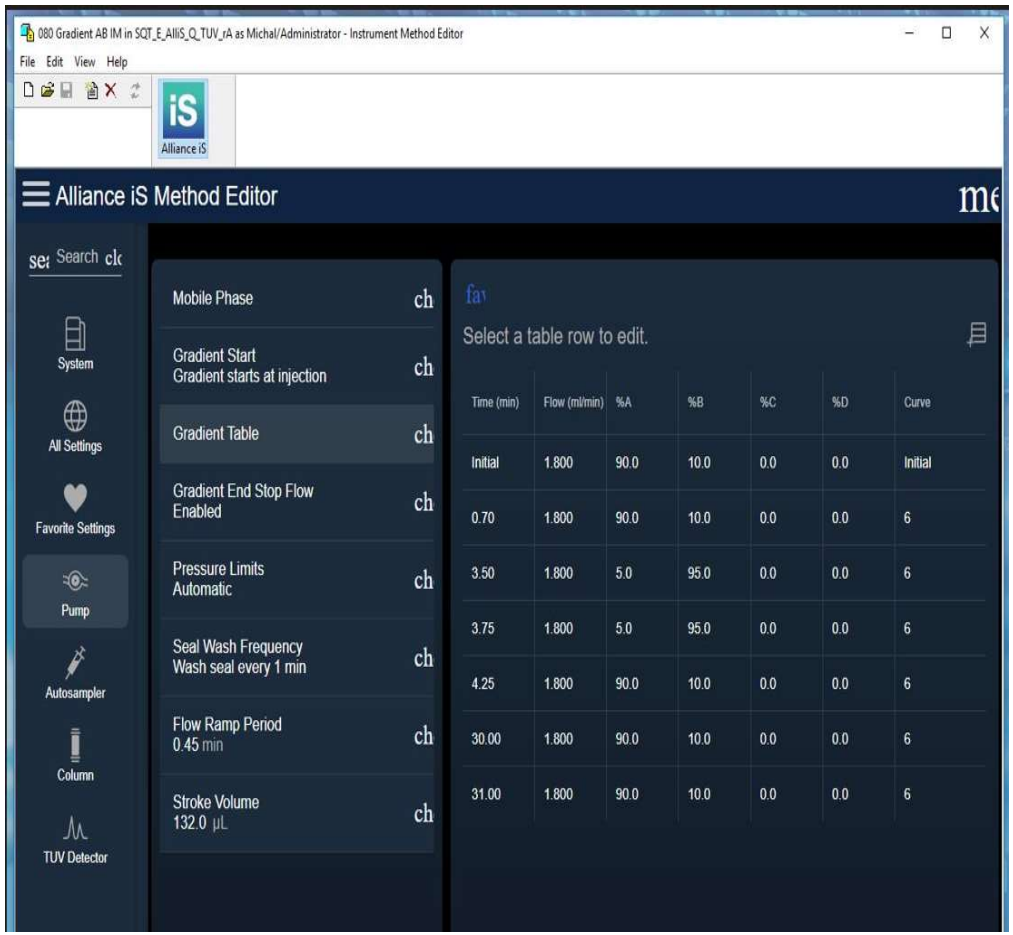

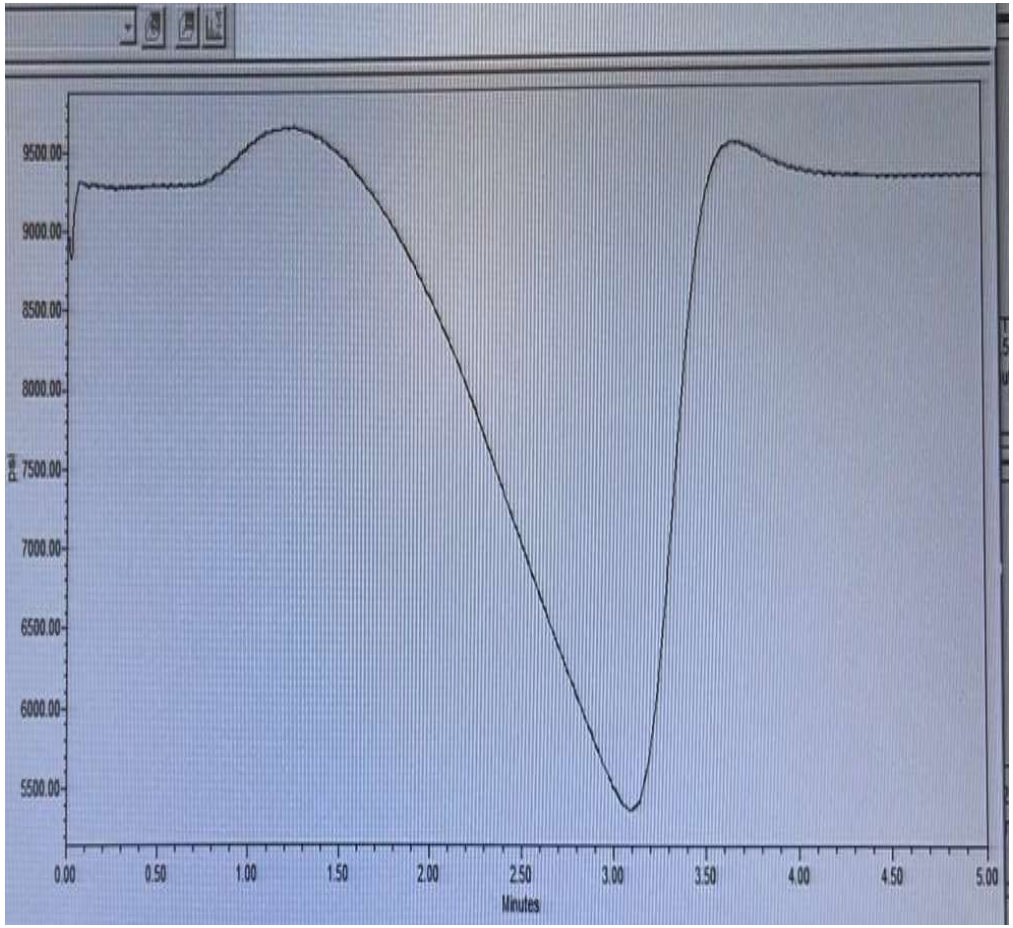

System Maintenance and Troubleshooting
UPLC H Class QSM

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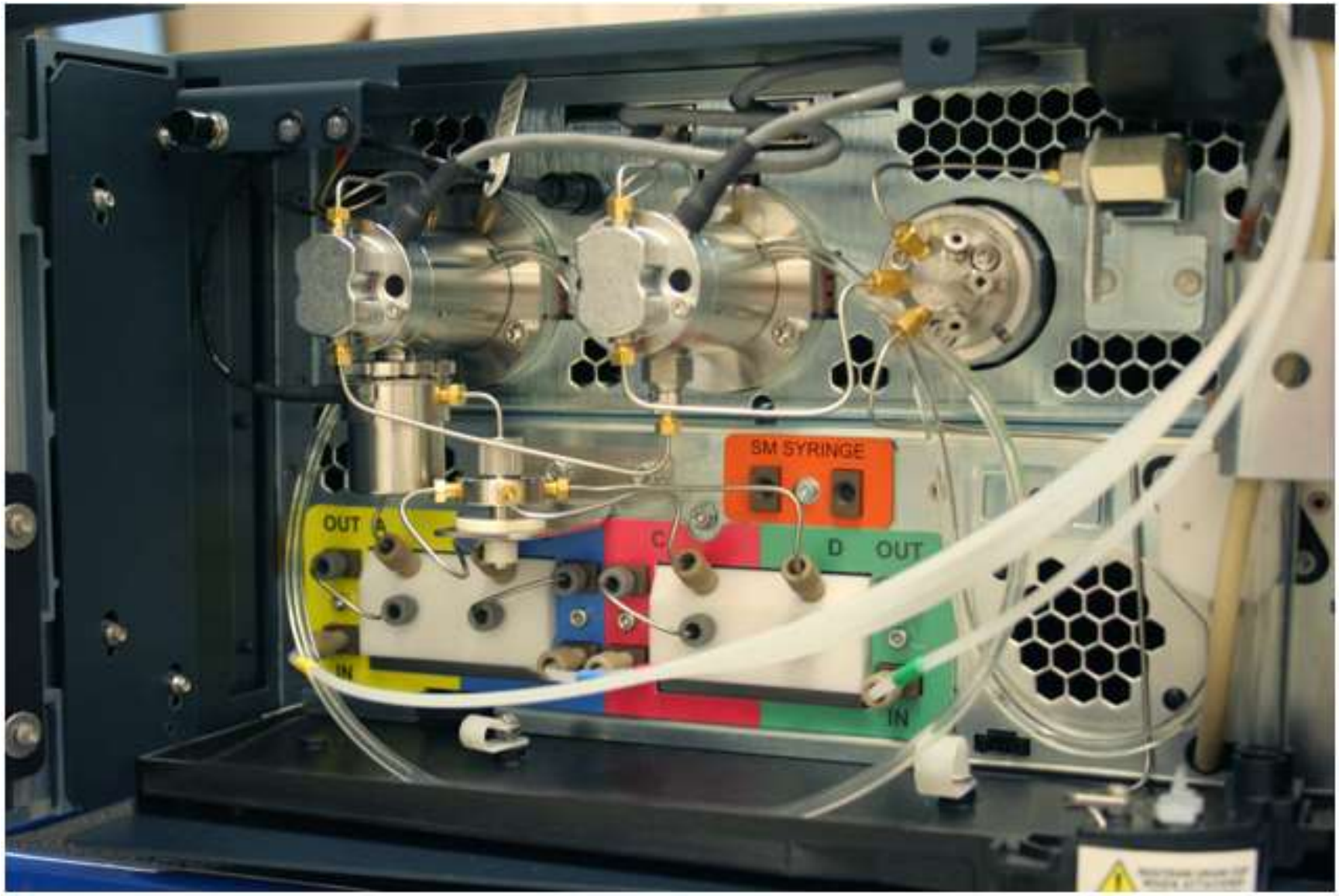

System Maintenance and Troubleshooting
UPLC H Class QSM

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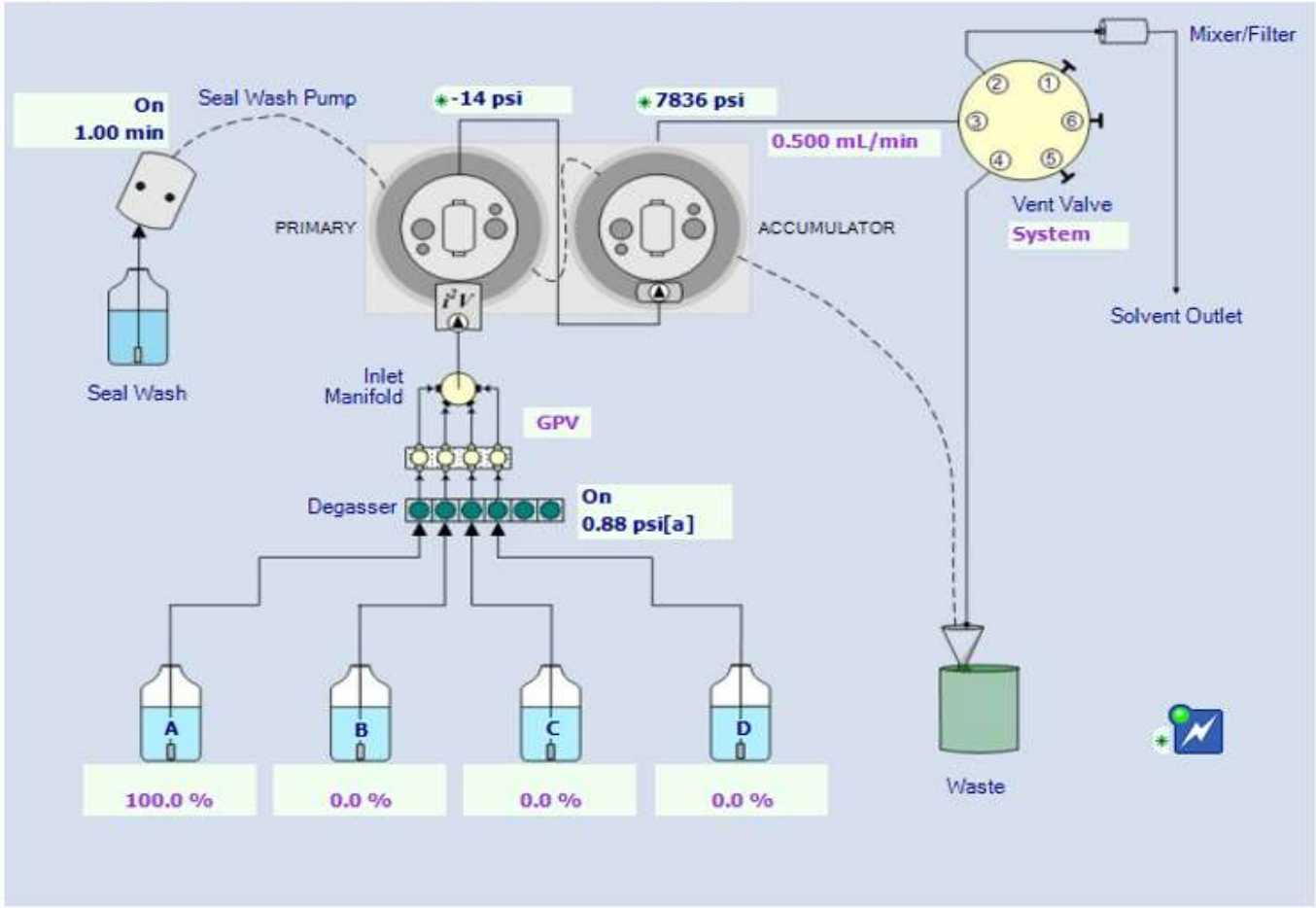
QSM Flow Diagram from Acquity Console



System Maintenance and Troubleshooting UPLC H Class QSM

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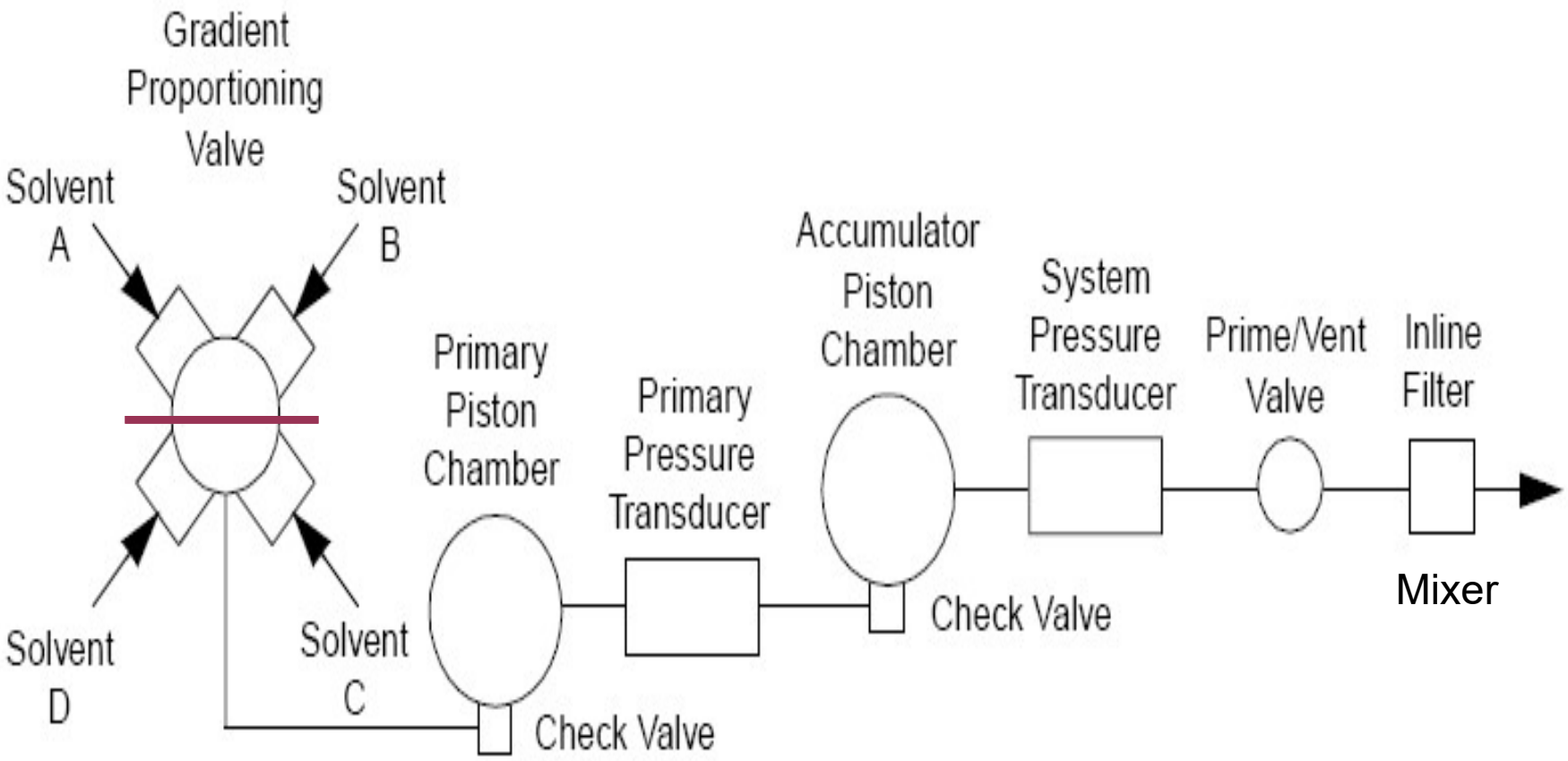
- Low Pressure Gradient Mixing



System Maintenance and Troubleshooting
System Status Check

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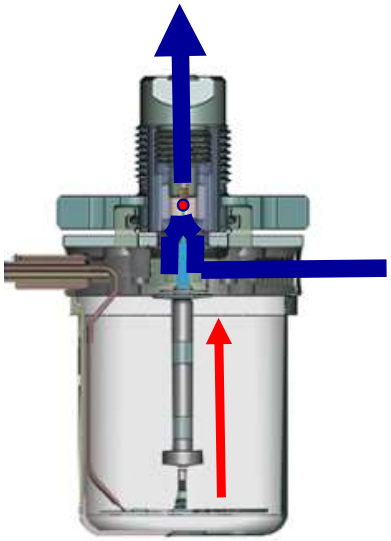
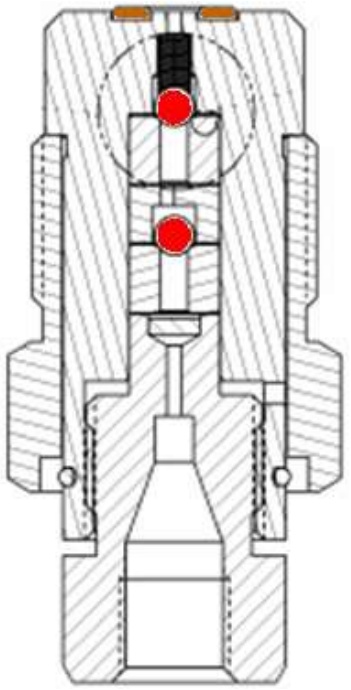
- Pump/system pressure trace

- Pump performance

System Maintenance and Troubleshooting
QSM Check Valves

Note: Check Valve gaskets have an orientation---chamfered side up





Primary

Accumulator

(Double Ball/Double Seat)

System Maintenance and Troubleshooting
GPV Valve Pair and Filter

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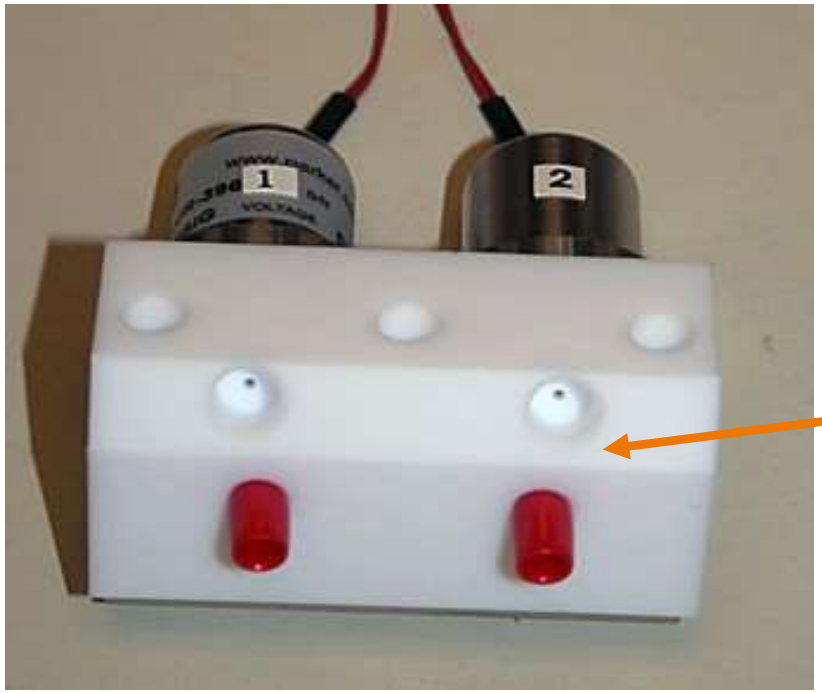

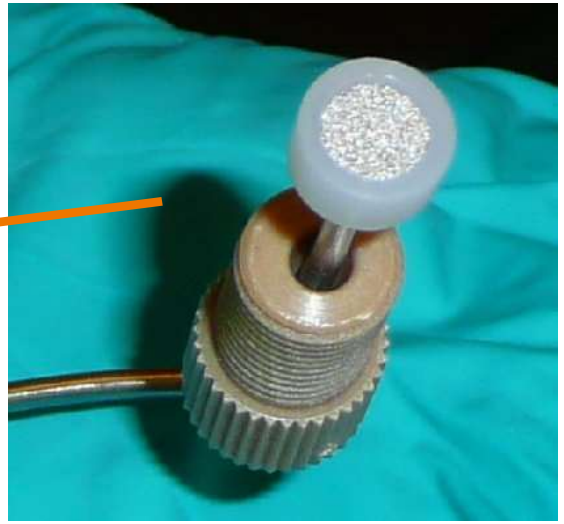

- Similar to Alliance GPV valve
- Located closer to the head to keep dwell volume down
- Can be replaced as a pair

GPV FILTER
(20 MICRON FRIT)
All 4 are changed at PM
p/n 700005173 (4pk)

System Maintenance and Troubleshooting
Degasser Path

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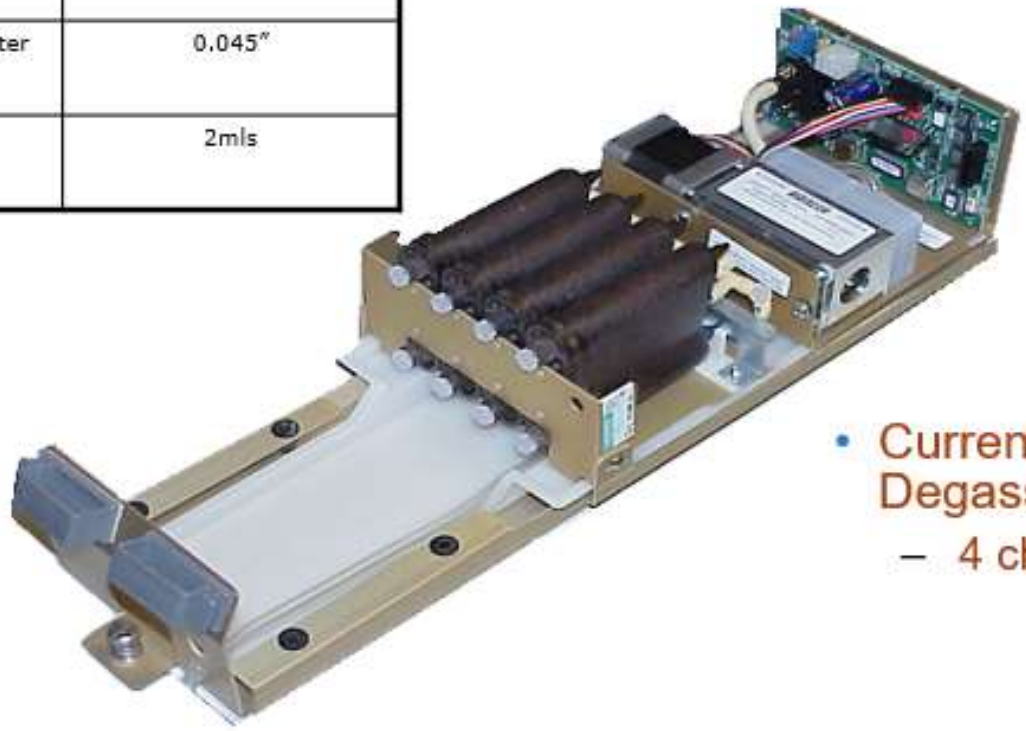
- Mobile phase travels from reservoirs to degasser chambers via gravity.

Performance Plus Degasser Chamber	
Recommended Max Flow rate	5mls/min
Internal Volume	500uls
Internal Tubing Diameter	0.045"
Flush Volume (4x)	2mls

Best Practices

- Have solvent in all 4 Solvent lines
- Prime all solvent chambers
- Flush salts from chambers when not in use



- Current Style Degasser Tray
  - 4 channel only

System Maintenance and Troubleshooting
Gradient Proportioning Valve (GPV)

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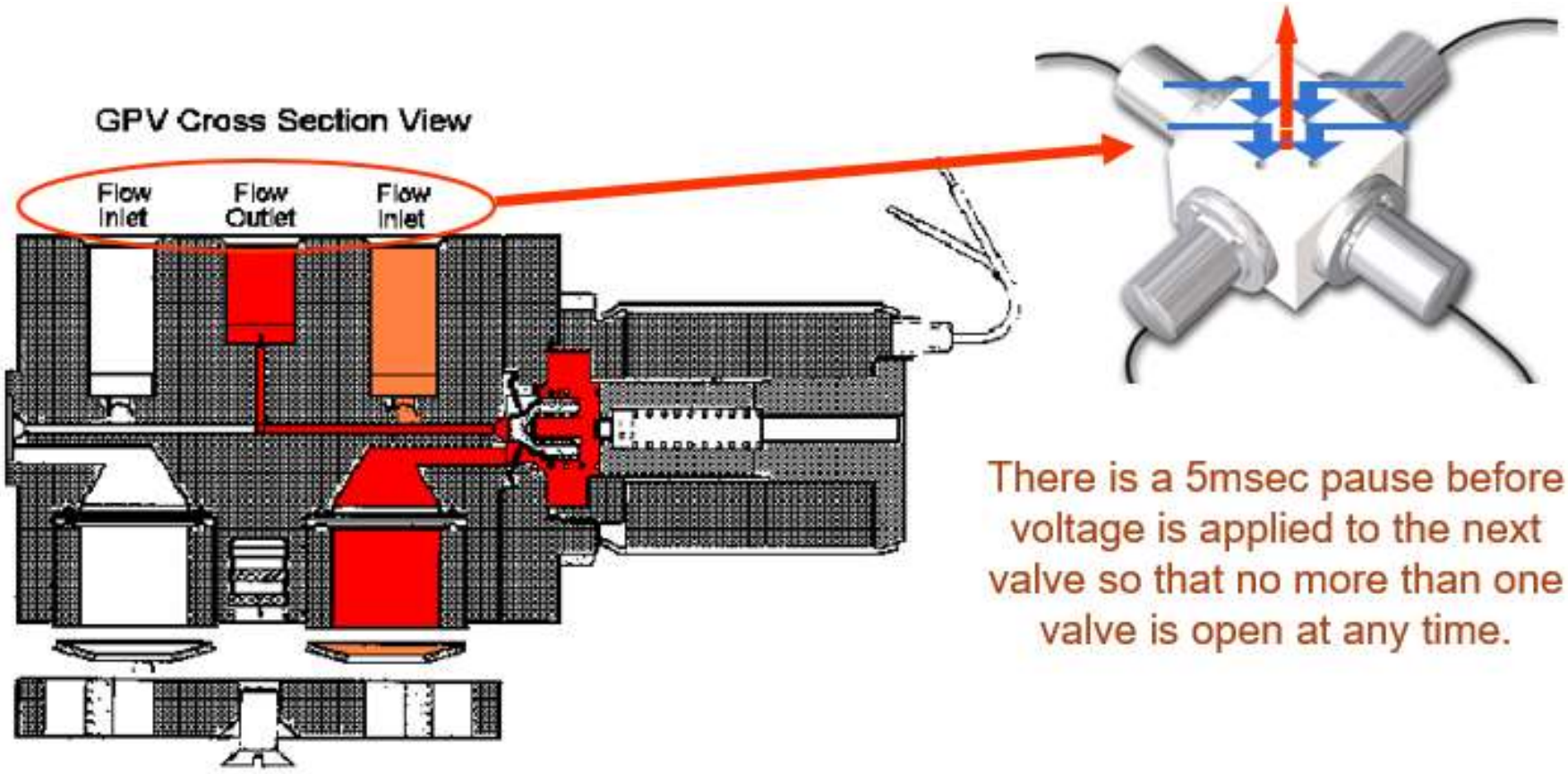
- Mobile phase flows out at degasser chambers into GPV.
- Uses a diaphragm style accumulator to minimize pressure noise.
- Easily damaged if a direct pressure of more than 5 psi.



System Maintenance and Troubleshooting
HPLC Solvent Manager Compartment

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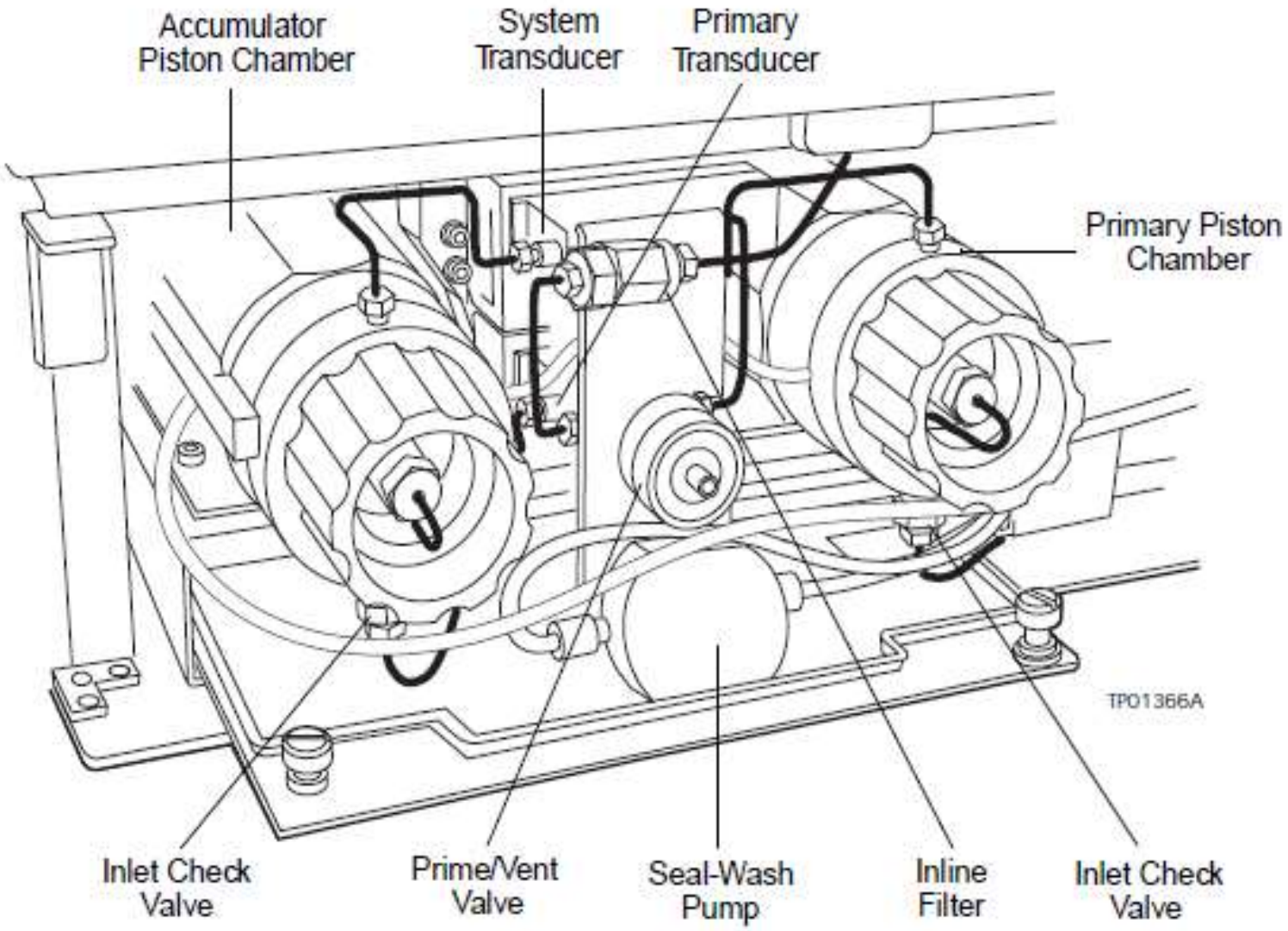

System Maintenance and Troubleshooting
HPLC Performance Plus Check Valve

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- Replacing HPLC Check Valves
  - Wrench 1/2-inch, open-end
  - Wrench 5/16-inch, open-end (optional)
  - New set of check valve cartridges
  - Squirt bottle with 100% alcohol (MeOH or IPA, optional)
  - Use the 1/2-inch wrench to loosen Check Valve Housing from the manifold

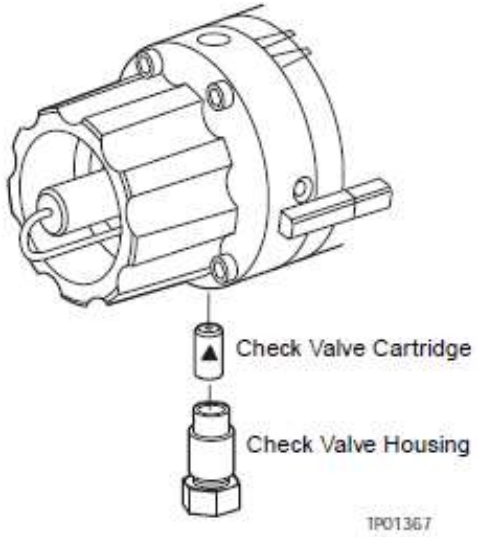

Figure 7-6 Inlet Check Valve

Maintaining the Solvent Management System 212

System Maintenance and Troubleshooting
HPLC Performance Plus Check Valve

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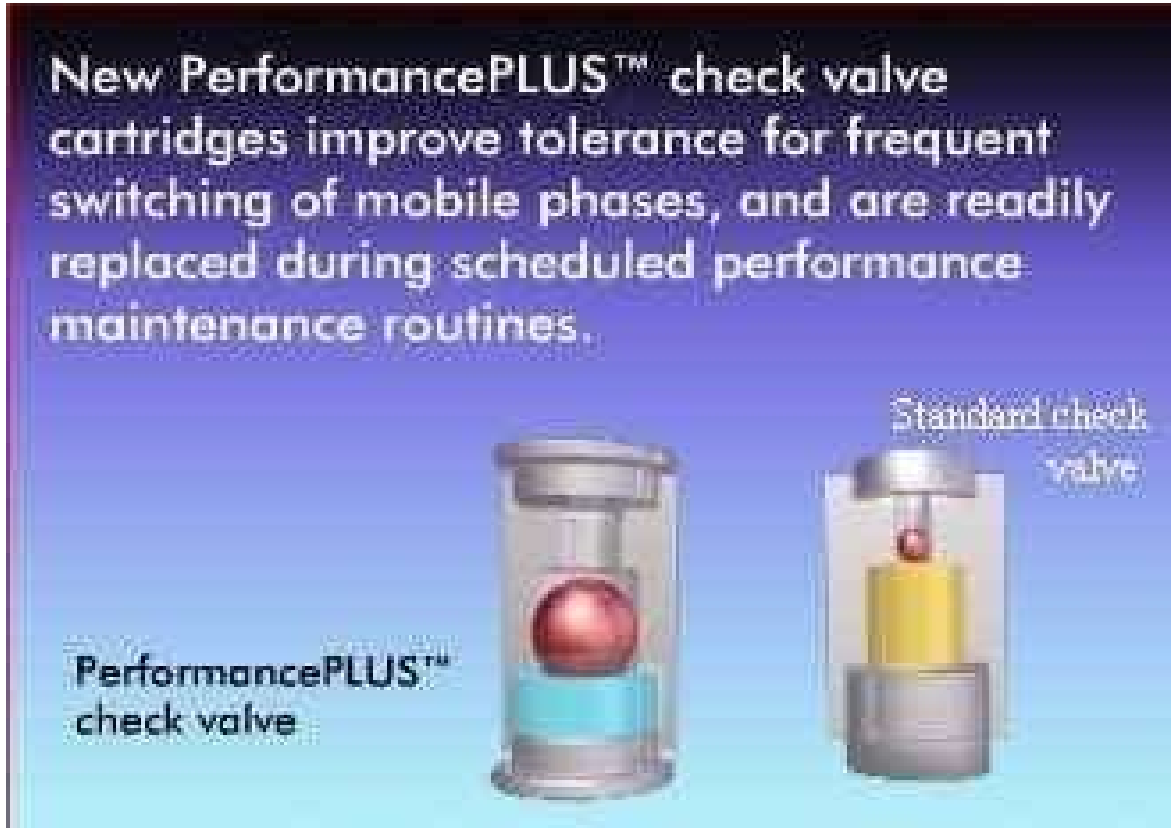
- PN 700000254
- Ruby Sapphire 1/8 in PM kit



System Maintenance and Troubleshooting
Solvent Inline Filter

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- Replacing Inline Filter Cartridge
  - Wrench 5/8-inch (2X), open-end
  - Wrench 5/16-inch, open-end (optional)
  - New inline filter cartridge
  - Squirt bottle with 100% alcohol (MeOH or IPA, optional)

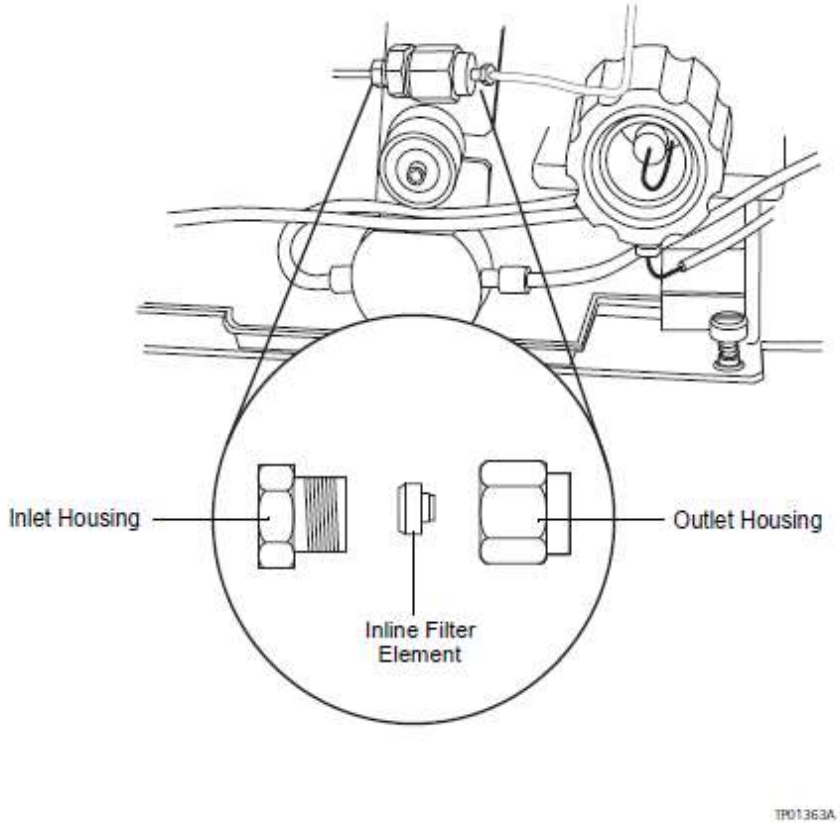

Figure 7-7 Replacing the Inline Filter

Maintaining the Solvent Management System 214

System Maintenance and Troubleshooting Chemistry

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- The different chromatographic analysis are defined by solvent matrices or composition(s)
- Understanding the polarity characteristics of mobile phase(s)
- Comparing normal and reversed-phase chromatography relative to the packing material

System Maintenance and Troubleshooting Chemistry

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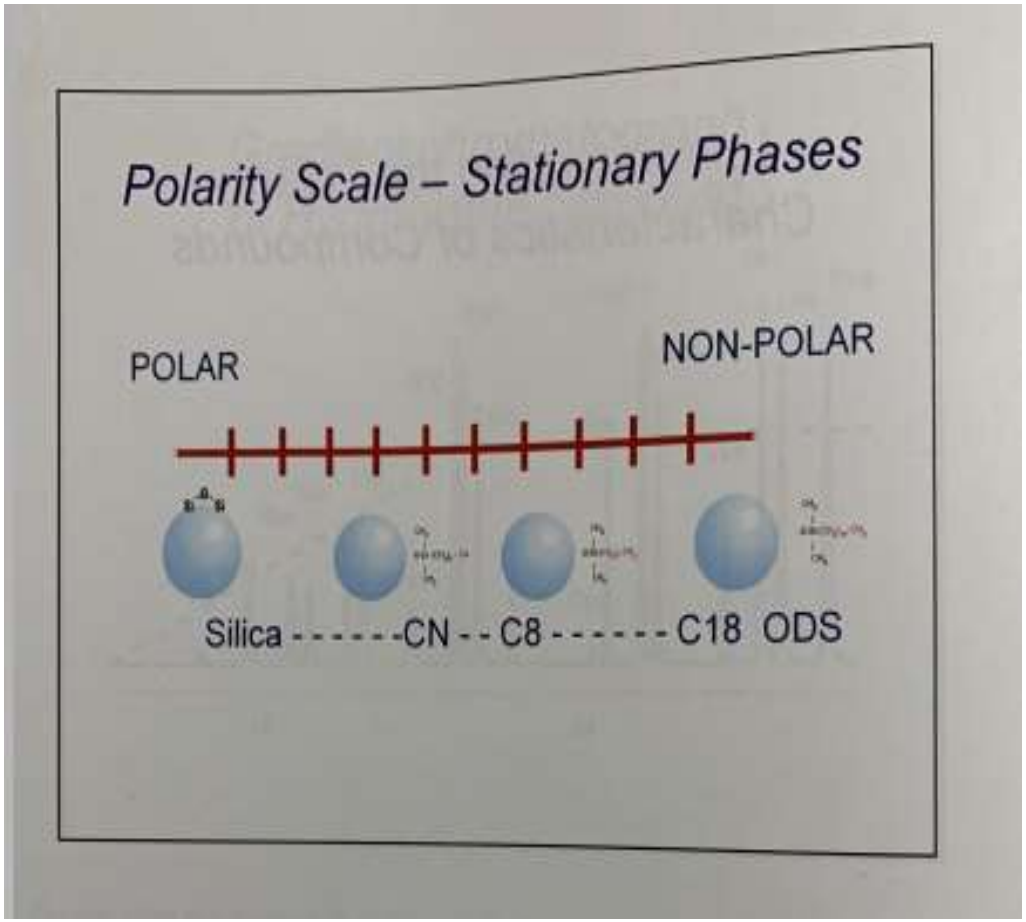

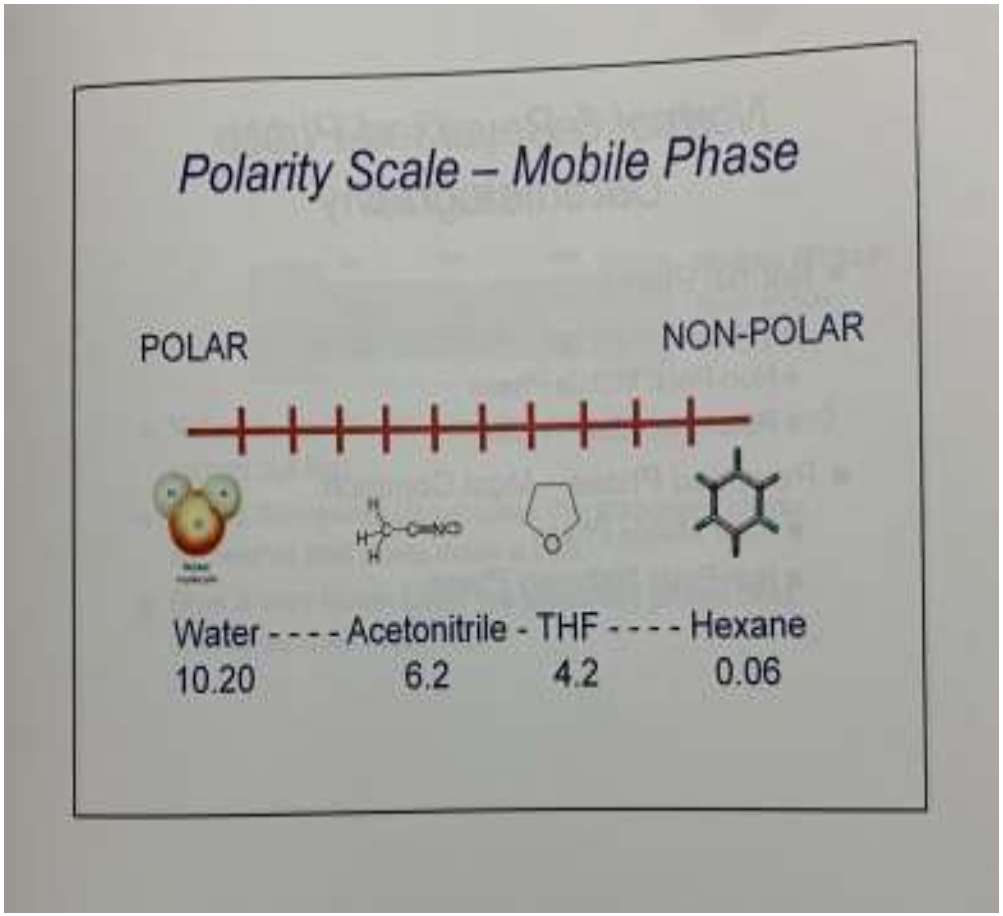

Column

System Maintenance and Troubleshooting Chemistry

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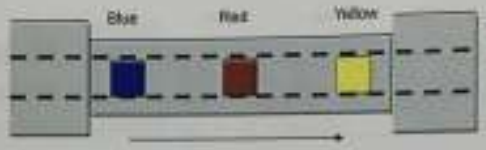
Why Do They Separate?: Reverse Phase

Mobile Phase: Polar (Water)

- Yellow is Non-Polar: Likes the stationary phase best and comes out LAST.
- Red is Somewhat Polar: Likes the stationary phase somewhat and slows down a little.
- Blue is very Polar: Likes the mobile phase best and comes out FIRST.

Why Do They Separate?: Normal Phase



Mobile Phase: Non-Polar (Hexane)

- Yellow is Non-Polar: Likes the mobile phase best and comes out first.
- Red is Somewhat Polar: Likes the stationary phase somewhat and slows down a little.
- Blue is very Polar: Likes the stationary phase best and slows down the most.

Like Dissolved Like
Like Carries Like
Like Retained Like

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Comparison of Normal vs Reversed Phase

	Normal Phase	Reversed Phase
Column Polarity	Polar > Moderate Amide, PFP, Z-HILIC	Non-polar > Moderate C18, C8, HSS T3
Solvent Polarity	Non-Polar > Moderate	Polar > Moderate
Elution Order	Most Non-Polar first	Most Polar first
Effect of Increasing Solvent Polarity	Reduces RT	Increases RT

System Maintenance and Troubleshooting Chemistry

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- Size Exclusion – Gel Permeation Chromatography
  - SEC specifically GPC
  - Chromatography achieved by a physical size separation of the molecules as they pass through the packing material
  - Specialty field in liquid chromatography devoted to measuring the Molecular Weight Distribution (MWD) of polymers

System Maintenance and Troubleshooting Chemistry (GPC)

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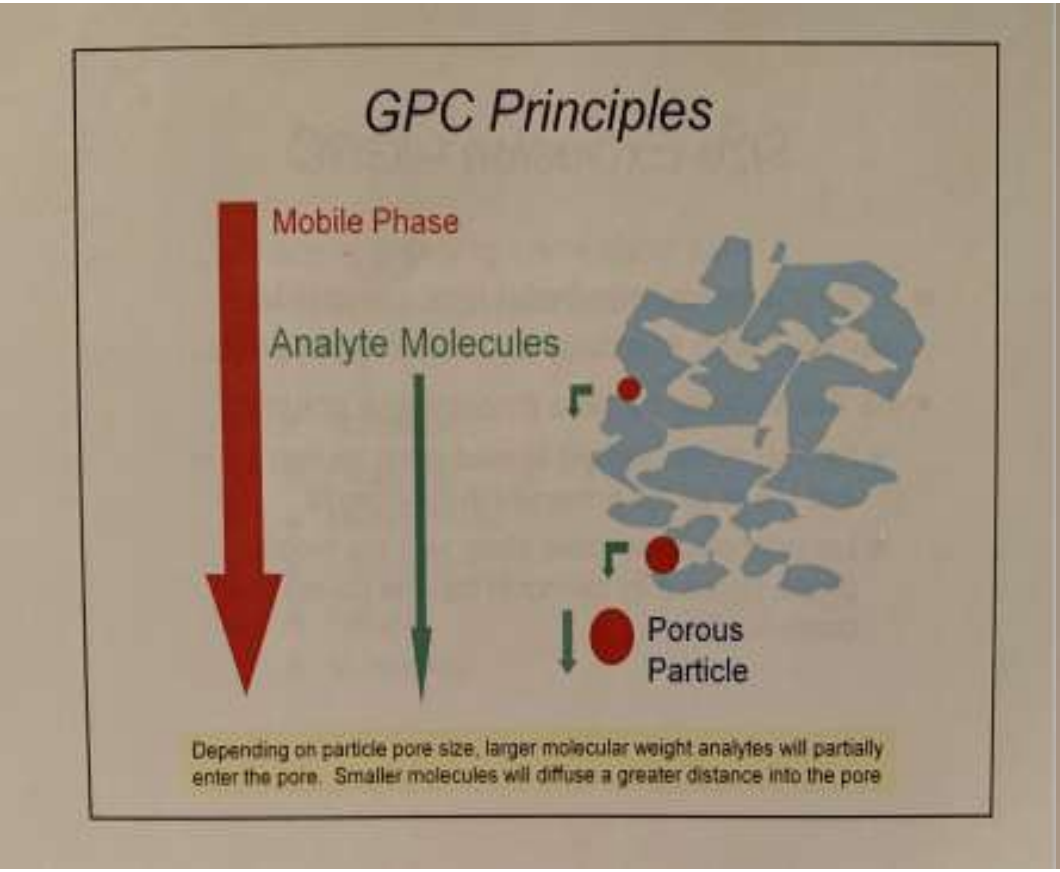

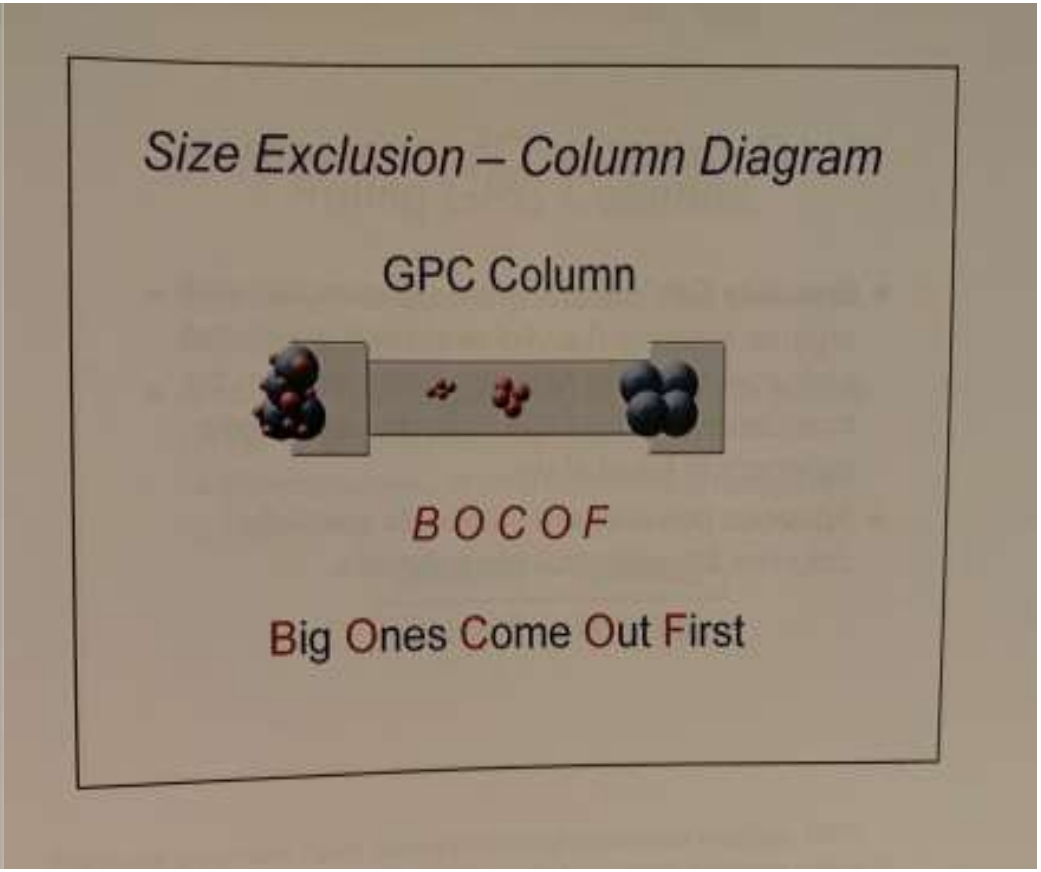

System Maintenance and Troubleshooting Chemistry

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- Size Exclusion Chromatography Tips (aqueous)
  - Remove mobile phase line filter when performing SEC with 100% aqueous mobile phase (PBS)
    - Best source to be contaminated with microbes, thus contaminating freshly prepared solutions of filtered MP and new or existing column
  - Filter prepared mobile phase through compatible 0.22um or smaller sterile disposable filters
    - Avoid or reduce the potential of introducing silicates which could alter column performance

System Maintenance and Troubleshooting Chemistry

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- Size Exclusion Chromatography Tips (aqueous)
  - High Ionic strength mobile phase (>150mM) should be replaced every two months
  - Low Ionic strength mobile phase (<150mM) should be replaced every 2-3 days
  - Prevent potential precipitation of buffer residue in LC system, maintain a low flow rate to sustain 700 -1000 psi after SEC column is removed

System Maintenance and Troubleshooting Chemistry

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- Size Exclusion Chromatography Tips (aqueous)
  - If LC system will be idle for > 2 days, after column is removed, prime each solvent line for 15mins with high purity water
  - Next, flush and store system in 70/30 IPA/water to prevent microbial growth
  - Pro Tip: 100% organic alcohol does not penetrate the cell wall of bacteria.

System Maintenance and Troubleshooting Chemistry (SEC Cont.)

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- Previously mentioned Like Dissolved Like

- Current understanding, improvement: Light Dissolved Like
  - Biological: Osmosis, Diffusion
  - Chemistry: Dilution
  - Physics: Potential Difference
    - From our own experiences as well as from some of our customers' experiences, for some unique SEC aqueous application the "clean up" process is NOT about a complete system turn-over. Instead, it is about reducing the background signal to not suppress peak of interest(s) sensitivity. Hence, consider a concentration migration approach, such as 10-15% concentration of the mobile phase diluted in water. Then, proceed with pump priming, sample manager priming, and inject this "clean agent" through sample path.

System Maintenance and Troubleshooting Chemistry (Ion Exchange)

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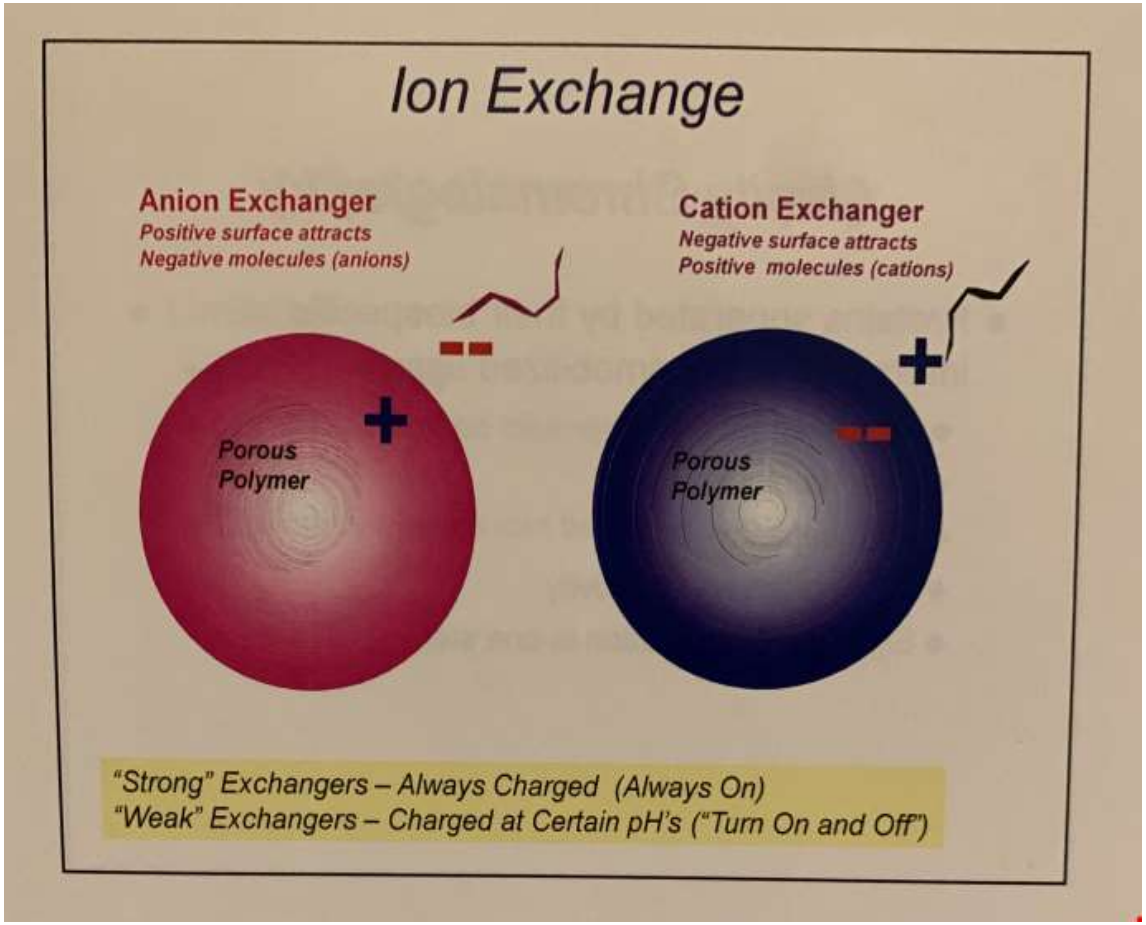
- Retention Mechanism
  - Opposite charge attraction
  - Charged Analyte Molecule(s)
  - Oppositely Charged Stationary Phase
- System "Clean Up"
  - Prepare new freshly filtered mobile phase
  - Install new mobile phase to system
  - Prime and flush using new mobile phase
  - DO NOT STOP flow for long duration, which may damage column packing material



System Maintenance and Troubleshooting
Prepare System

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- Prior to perform DIAG, system MUST be thoroughly prime with appropriate solvents.
  - UPLC pump should be primed for about 4 mins per lines per method usage, at a minimum.
  - UPLC H Class SM FTN should be primed for about 200 seconds on Wash solvent, and 40 cycles on Purge solvent via sample syringe.

System Maintenance and Troubleshooting
Prepare System

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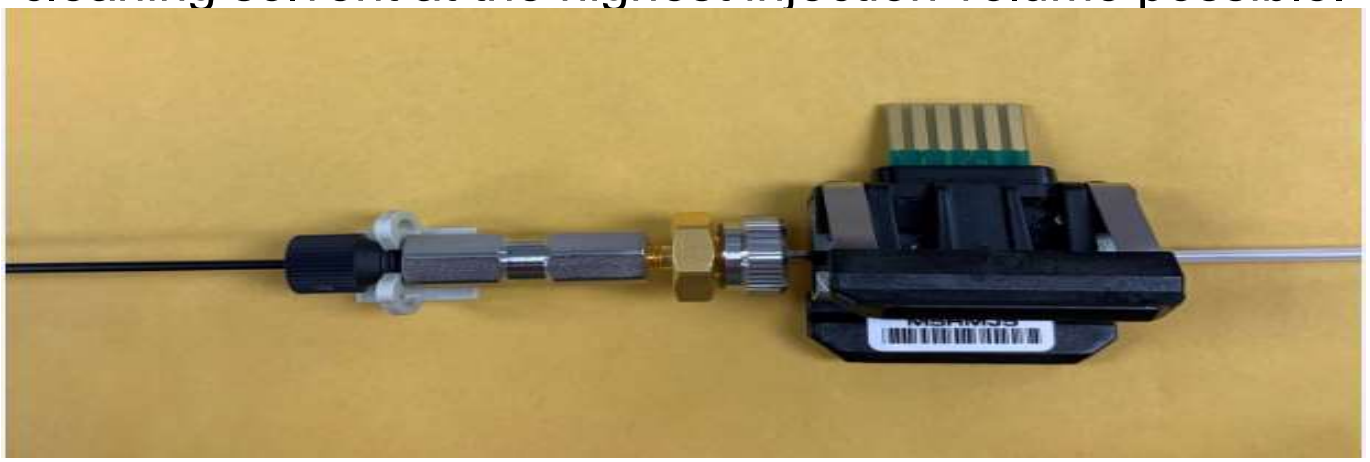
- Typical Reverse phase mobile phase.
  - You can use high aqueous to clean system, such as 80/20 (water / ACN) or (water/MeOH)

- Typical Normal phase mobile phase
  - You can use IPA and a polar organic solvent to clean system, such as 50/50 IPA/MeOH

- Regardless of which kind of chemistry, please setup system to make injections using your cleaning solvent at the highest injection volume possible.



System Cleaning or Storage Condition

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	Plate/Well	Inj Vol (uL)	# of Injs	Label	SampleName	Level	Function	Method Set / Report or Export Method	Label Reference	Processing	Run Time (Minutes)
1							Wet Prime	01 UV System Readiness MS			6.00
2							Purge Inj	01 UV System Readiness MS			
3							Purge Inj	01 UV System Readiness MS			
4							Purge Inj	01 UV System Readiness MS			
5							Purge Inj	01 UV System Readiness MS			
6							Equilibrate	01 UV System Readiness MS			3.00
7	2:F,6	10.0	10		Blank		Inject Samples	01 UV System Readiness MS		Don't Process or Report	1.50
8	2:F,7	10.0	10		Blank		Inject Samples	01 UV System Readiness MS		Don't Process or Report	1.50
9							Wet Prime	01 MS System Readiness MS			6.00
10							Purge Inj	01 UV System Readiness MS			
11							Purge Inj	01 UV System Readiness MS			
12							Purge Inj	01 UV System Readiness MS			
13							Purge Inj	01 UV System Readiness MS			
14							Condition Column	01 UV System Readiness MS			6.00
15	2:F,6	10.0	10		Blank		Inject Samples	01 UV System Readiness MS		Don't Process or Report	1.50
16	2:F,7	10.0	10		Blank		Inject Samples	01 UV System Readiness MS		Don't Process or Report	1.50

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Questions or Concerns ???