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Alliance 2690/5

Jade C. Byrd
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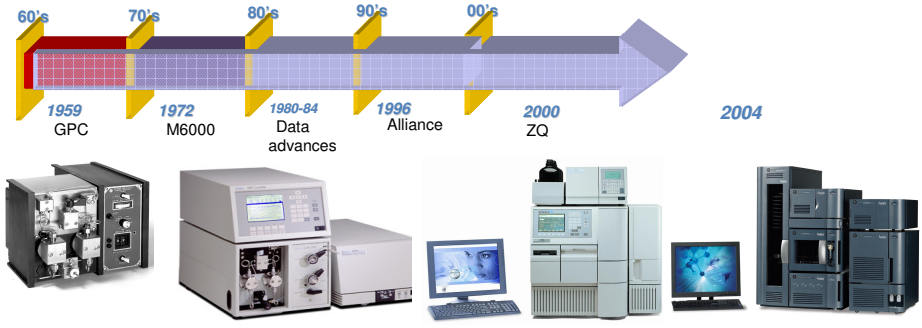

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Novel Technology

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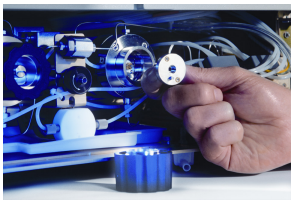
- Solvent Management
  - Quaternary solvent low pressure mixing
  - Vacuum degassing
  - Serial flow path configuration
  - Two inlet check valves with First-in, First-out solvent delivery
  - Programmable piston volume delivering optimum performance without changing pump heads
  - Plunger seal wash
  - Smooth, pulse-free solvent flow



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Solvent Degasser Tray

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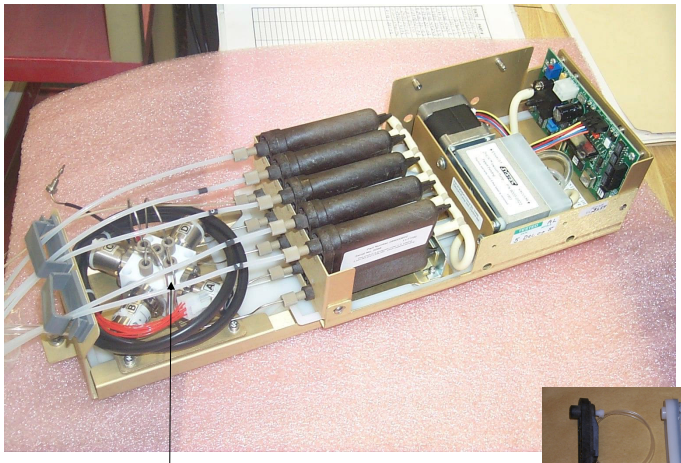

Gradient Proportioning Valve (GPV)

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Low Pressure Mixing

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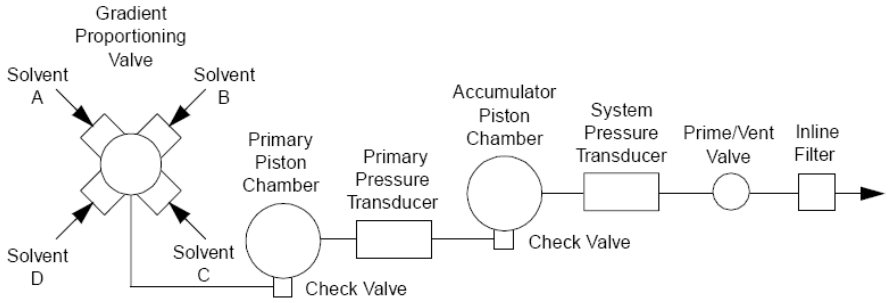

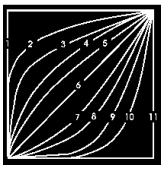

All solvent lines should be primed… even when not in use.

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Solvent Management: Low Pressure Mixing

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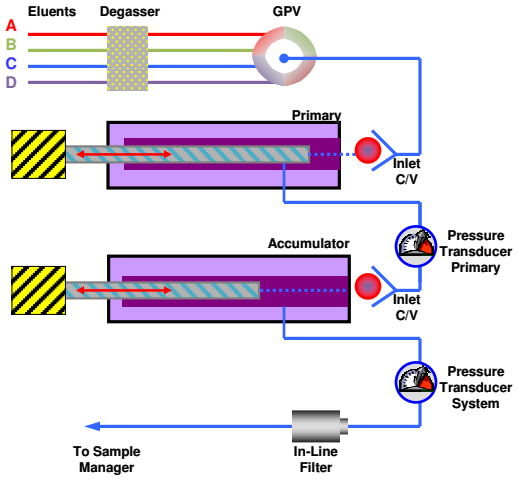

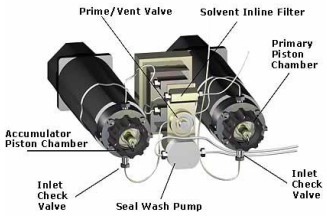

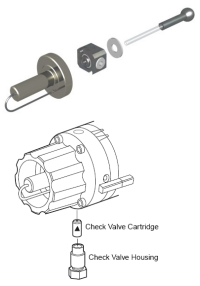

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Solvent Management vs. Pump

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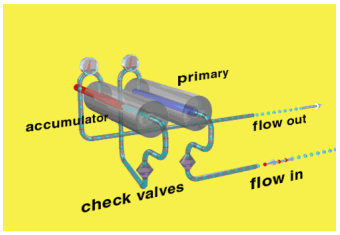
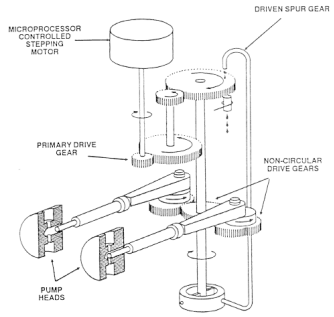
- Solvent Management



- Pump



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Serial Pump Design

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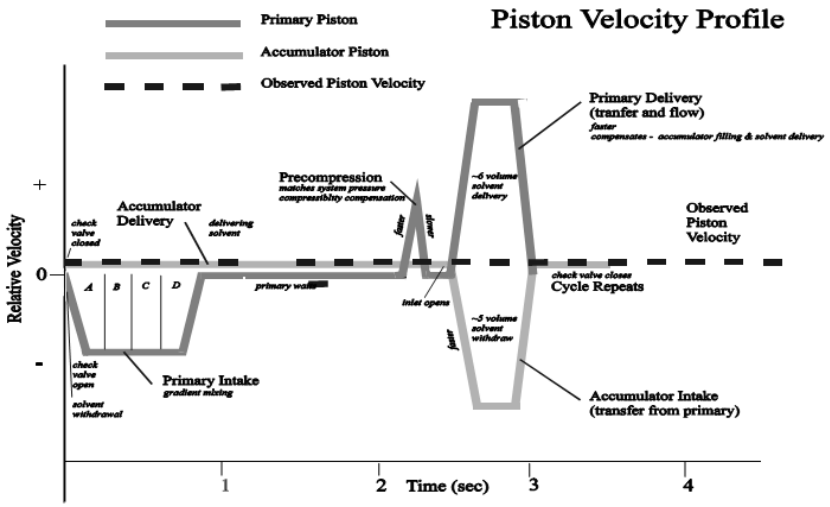

2690/5 Solvent Management Delivery Theory

Proof of Performance

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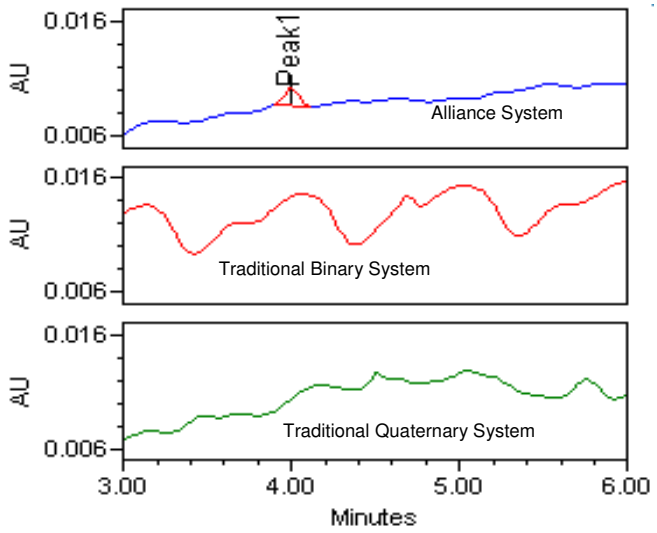

5 ng of the five peptide test mixture on column can be easily distinguished from the baseline in the chromatograms generated on a Waters Alliance HPLC.

At 5 ng, the peak is lost in the baseline ripple present in the chromatograms generated on a competitive "traditional design" Binary pump and Quaternary gradient system.

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2695 Sample Management

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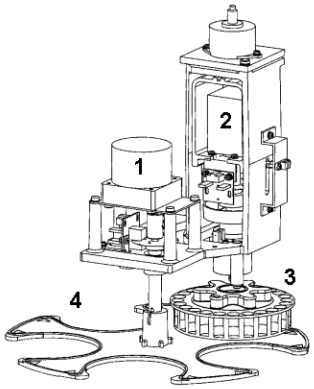
120 x 2mL vials in 5 x 24 vial carousels



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Needle Option

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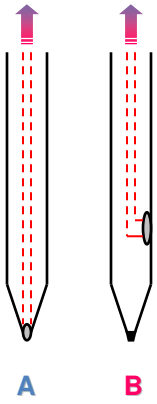
- 2695 utilizes side port needle (B)
  - Stainless steel
  - Standard on 2695
- PerformancePLUS needle
  - Nickel/Cobalt alloy
  - Part Number 700001247



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Injector

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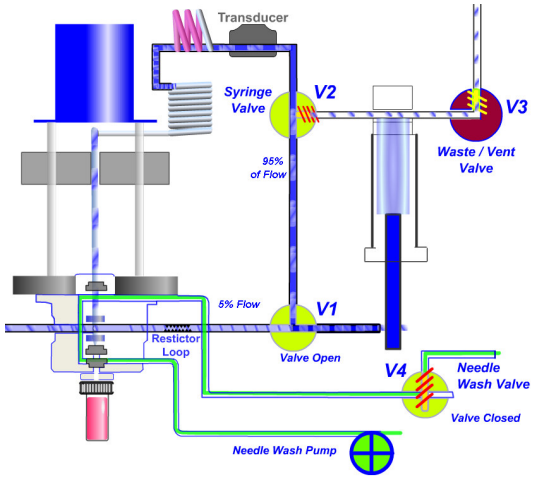

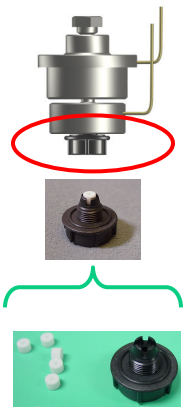

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Syringe Options

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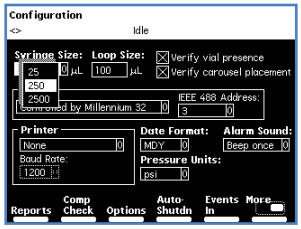

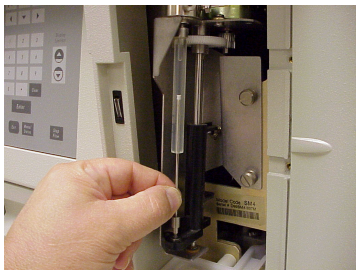

Part Number	Syringe (µL)	Steps per µL	Minimum Sample size (µL)	Minimum draw rate µL/min	Maximum Draw rate µL/min
WAT077343	25	270	0.1 µL	0.1	0.5
WAT044886	250	27	1.0 µL	1.0	5.0
WAT077342	2500	2.8	10.0 µL	10.0	50.0

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Automate Priming

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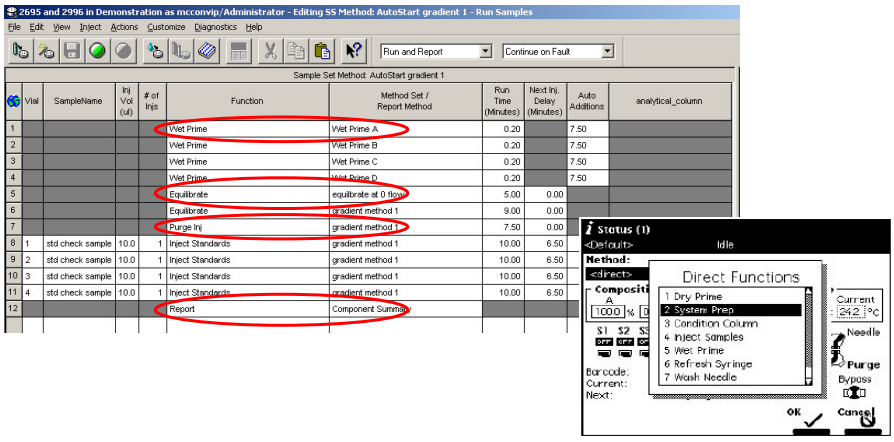
- Ease of Use - Automation benefits
  - — build unattended daily startup routines into sample methods with their Alliance systems – frees up valuable lab time



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Good Practices

Keeping your LC (and chrmotography) working well

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Solvent Preparation

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- Mobile phase difficulties are estimated to account for 70% or more of all liquid chromatographic problems
- Use only clean, HPLC grade solvents
- All buffers should be added, pH adjustments completed, then the solvents should be filtered using a 0.45 micron filter
- Use water only from a high quality water purification system, or HPLC grade water
- Do not "top off" bottles, replace bottles when empty
- Pure water tends to grow bacteria which will clog the system and foul the degasser
  - If the system is idle, replace pure water with water that has 10% organic solvent added to inhibit bacterial growth

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Degassing

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- Regardless of the grade of solvent purchased, bottled reagents contain some particulate matter and dissolved gasses
- Most solvent manufacturers sparge their solvents with nitrogen just prior to bottling
- A freshly opened bottle of solvent is often supersaturated with nitrogen
  - Freshly opened solvents **must** be degassed before use
- Vacuum filtration of all solvents through a .45 micron filter will remove particulates, and degas the solvent

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Performing Quality Separations

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Instrument A --
less **Band spreading --**
(70µL)
MORE PLATES (12,000)

Instrument B --
more **Band spreading --**
(130µL)
LESS PLATES (7,000)

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What contributes to loss of N?

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- Column
  - Void
  - Plugging
- Extra-Column (Non – Column) Band spreading
  - Injection Volume
  - Injector (seal problem)
  - Detector Volume
  - Connecting Tubing
    - Injector to Column
    - Column to Detector
  - End-fittings and Frits

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Extra column bandspreading

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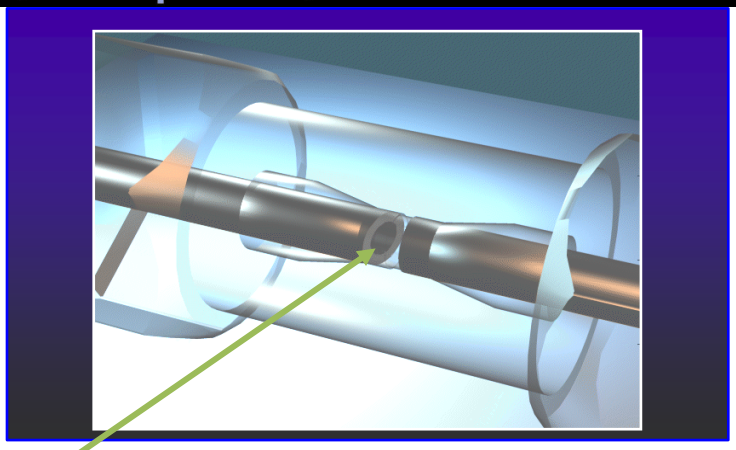

Tubing was cut poorly at an angle rather than smooth and flush
Potential for system band spreading due to mixing chamber effect
- Use tubing cutter

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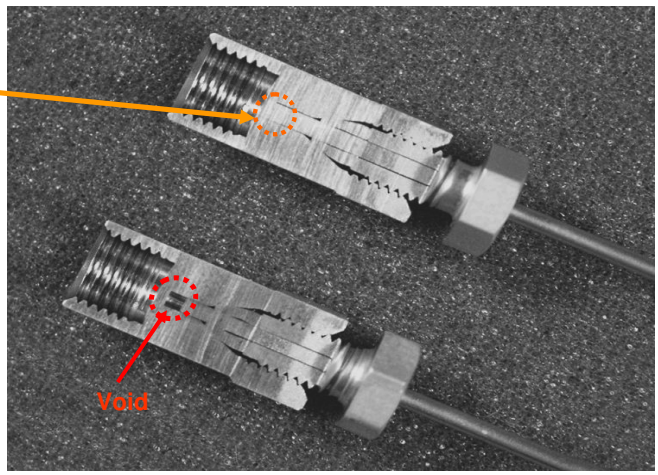
Proper Connection – Tubing Flush with End-fitting and Good Seal

Column tubing not seated properly -- creates a void
(Parker ferrule position into a Waters' 0.130" End-fitting)



(PEEK Slip Connectors Easier to Use)

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Column Installation and Connections

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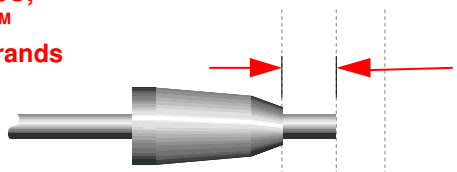
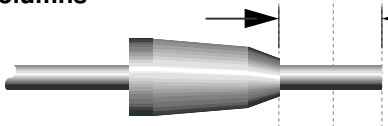
Waters Spherisorb®, ACQUITY UPLC™ and many other brands



0.090" Parker Style

Other Waters® Columns



0.130" Waters Style

If you are using a stainless steel ferrule on stainless tubing, once it is set, it will NOT move

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Effect of Connecting Tubing Volume (ID and Length) on System Band Spreading

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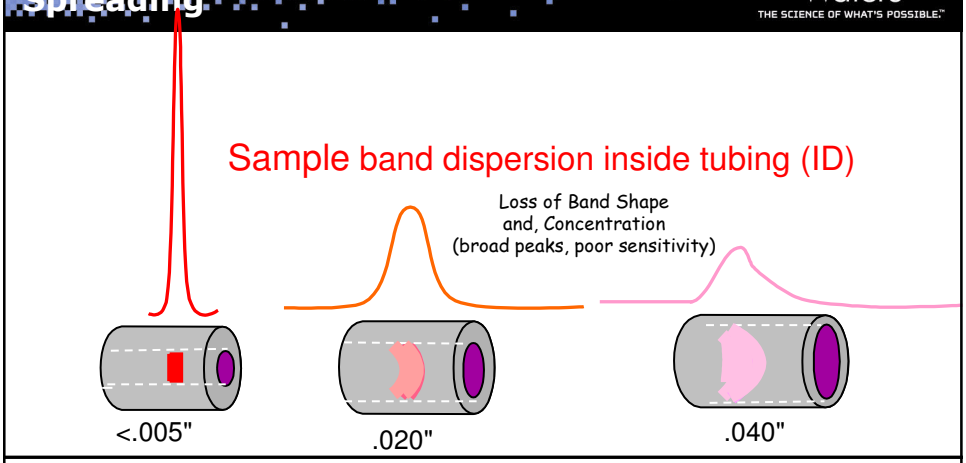
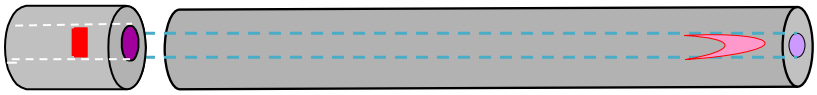


Longer tubing lengths also create more Band spreading



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Tubing Considerations

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- Stainless Steel
  - High pressures (w/ SS fittings)
  - Hydrophobic compounds
    - Can "passivate"—acid wash
- PEEK Tubing

Solvents to Avoid with PEEK Tubing

Attacks PEEK	**Swells PEEK**
Conc. HNO_3	DMSO
Conc. H_2SO_4	THF
	CH_2Cl_2

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Reducing Contamination/Ghost Peaks/Carryover

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- Test pH using an aliquot and then dispose
  - pH probe can contaminate the bottle of mobile phase
- Change your needle wash filter
  - Especially needed if the needle wash has run dry
  - Remember to pre-moisten
- Ensure your needle wash is compatible/good for your sample
  - E.g. salts need some water, maybe acid
- Put a wash step in your gradient
  - Remember the inside of the needle is washed by the gradient
  - Cleans out the column also
- Acid-clean/passivate the HPLC system
  - H_3PO_4, Nitric
- Use certified/pre-cleaned vials

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Vials

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- Unreactive Glass
  - Type I borosilicate
    - Least reactive
  - USP
- Consistent dimensions
  - Don't crash needles
- Caps/Septa
  - Core easily
  - Won't detach
  - Release vacuum
    - Pre-slit
  - Low Contaminants
    - PTFE
    - QC tested

Resources

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- Online Library
  - Technical notes
  - Methods and application-specific solutions
- Call/email Waters Home Office
  - Nature of symptom
  - Instrument SN
  - Method parameters
    - Flow rate, solvents/mobile phases, detector wavelength, type of column
  - Operating Pressure
  - Sample type, matrix
- Service Engineer/Plan
  - Plan cost is 85% cost of parts

www.waters.com/techassist
1.800.252.HPLC

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Questions?

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What is a Compression Check?

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- Automated routine to check for leaks in
  - Sample loop
  - Syringe
  - Needle Seal Pack
  - Valves
- Also detects bubbles in the sample withdrawal path
  - Bubbles will cause problems with system accuracy and precision

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What happens during a Compression Check?

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- Sample Loop is isolated from the mobile phase flow
- Sample Loop is vented to atmosphere
- A closed system is created that includes the Sample Loop, Valves, Syringe, and Needle Seal Pack
- The closed system is pressurized to a set pressure by the syringe
  - The volume of solvent required to pressurize the closed system is recorded
- A leak down test is run for a specified time, and the loss of pressure is recorded

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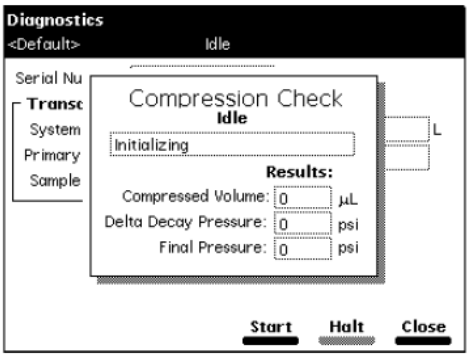
Compression Check Results Page



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How do I Interpret the Results?

- **Compressible Volume**- the volume of solvent required to pressurize the sample loop to a pre set pressure
  - Typically less than 5 ul using a degassed solvent
  - If the value exceeds 5 ul, possible causes are
    - Mobile phase not degassed
    - Bubble in the syringe
    - Leak in the syringe, sample loop, valves, or needle seal pack
  - Repeating the compression check often reduces the compressible volume

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How do I Interpret the Results?

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- **Delta Decay Pressure-**change in sample loop pressure after completing the leak down test
  - Typically less than 30% of the compression check pressure
  - If the value is high, the problem is usually a worn syringe
  - If the value is positive, then there is a leak in one of the valves or in the needle seal pack that is allowing high pressure mobile phase to leak into the sample loop
- **Final Pressure**-sample loop pressure after completing the compression check

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Recommended System Startup Checklist

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1. See that power is turned on for the Alliance and detector.
2. Turn on degasser, column and sample temps.
3. Check to see there are sufficient solvents for test.
4. Run a wet prime for each solvent you will use. Prime the seal wash and needle wash.
5. Turn on flow with the needed composition.

Allow the delta psi to stabilize.

6. Purge the injector.
7. Program Empower for method, and prepare samples.

~ Allow approximately 30 min for equilibration.

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