



H-Class / I-Class Course Workbook



Chris Proehl
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Global Training
& Development

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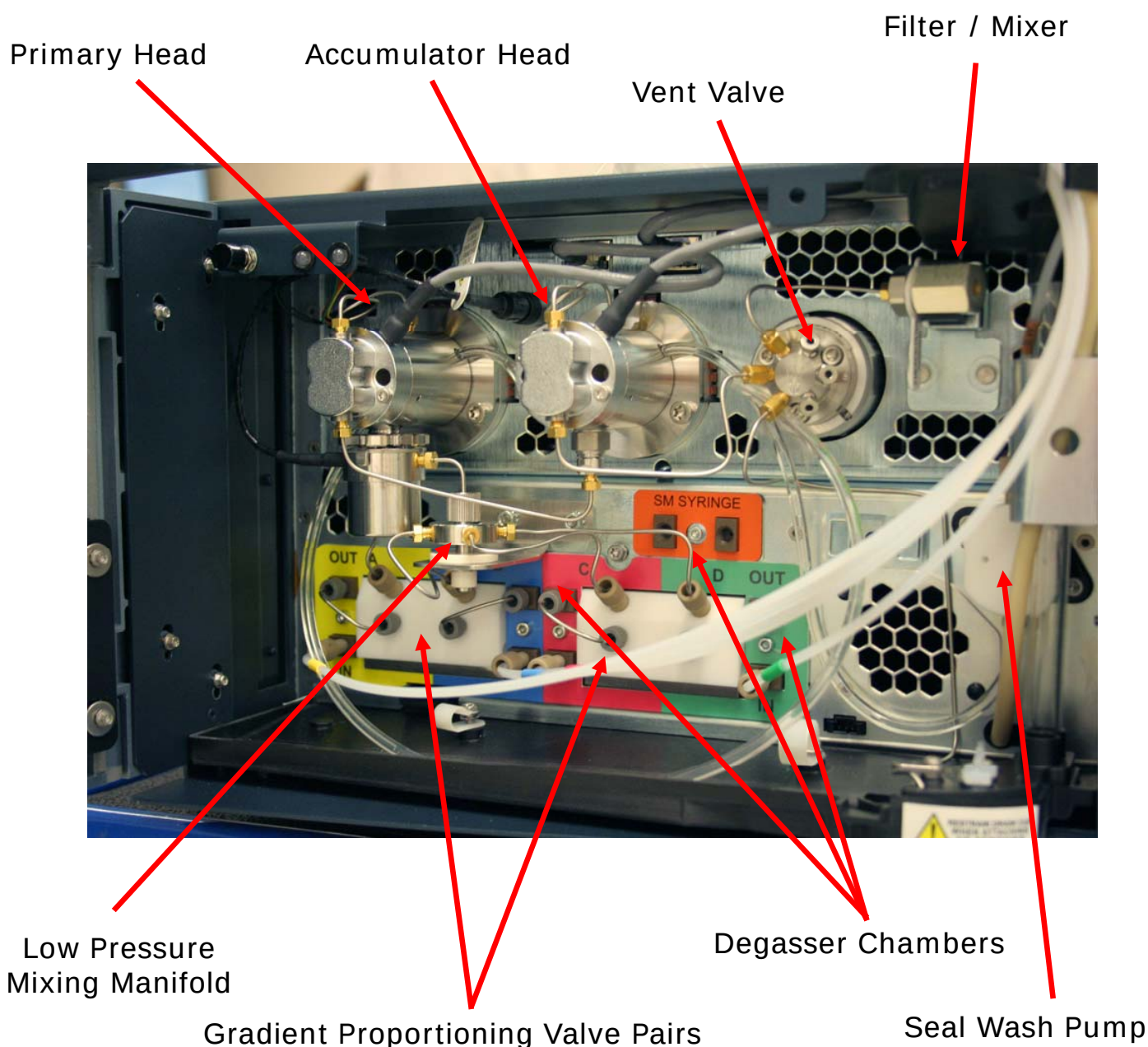
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Section 1 H-Class System Overview



- Open the front panel of the QSM and observe the major components as detailed in the diagram below.



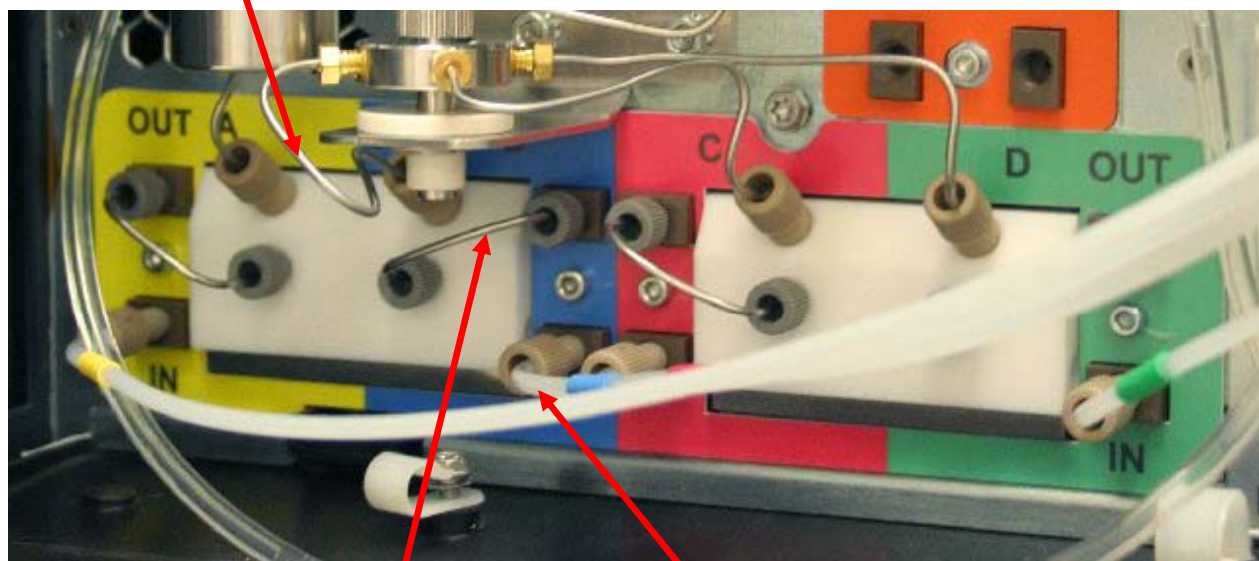
QSM Degasser H-Class Flow Path



- Follow the flow path from the Solvent Degasser through the Gradient Proportioning Valve to the LP Manifold as detailed in the diagram below.

3- GPV Outlet to LP Manifold inlet

Sample Manager Purge Solvent Degasser Chamber



2- Degasser Chamber outlet to GPV inlet

1- Solvent Bottle to Degasser Chamber inlet

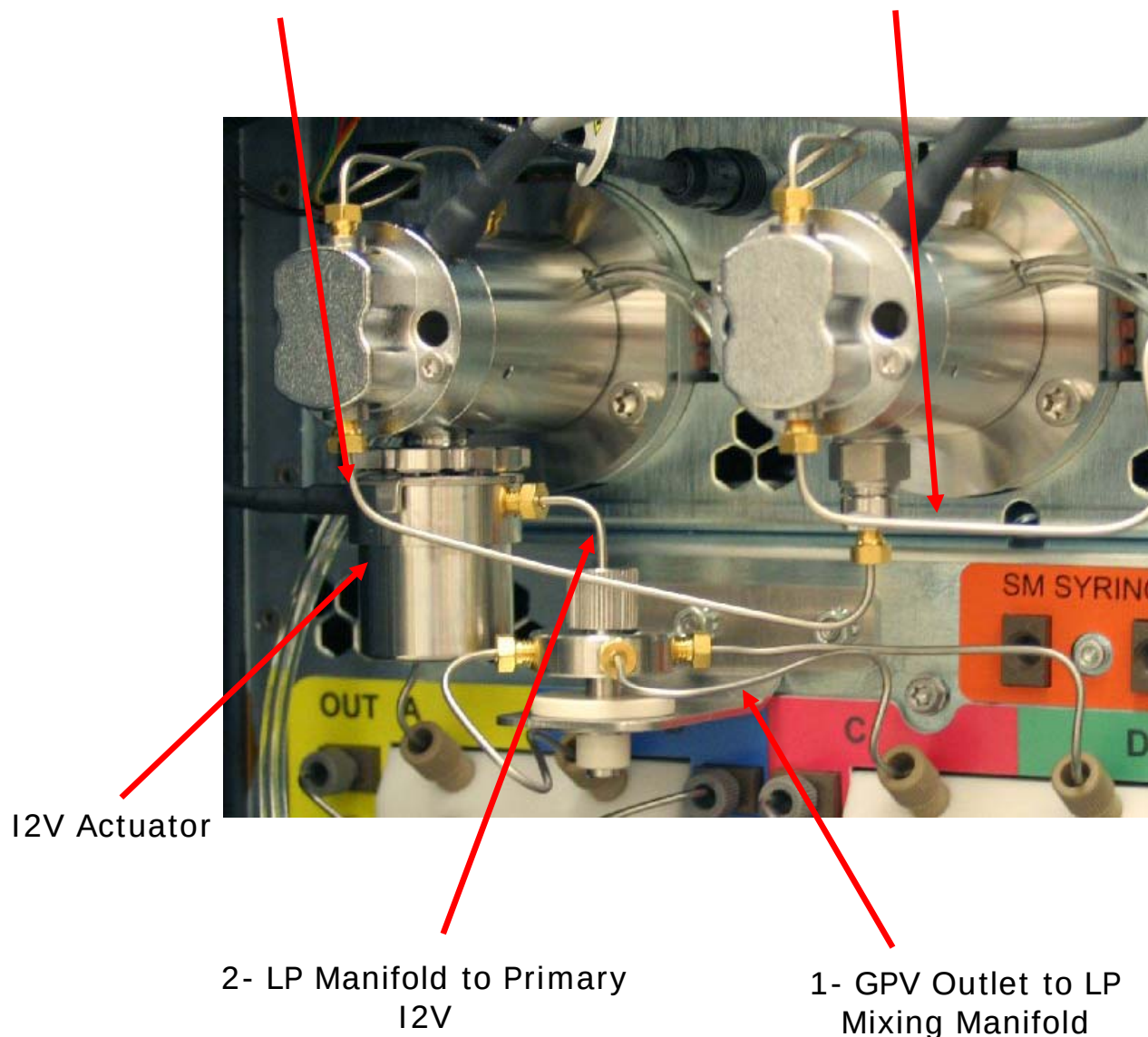
- Follow the flow path from the LP Manifold through the pump heads as detailed in the diagram below.

The solvent is in the low pressure state until it passes the I2V and is compressed by the Primary head.

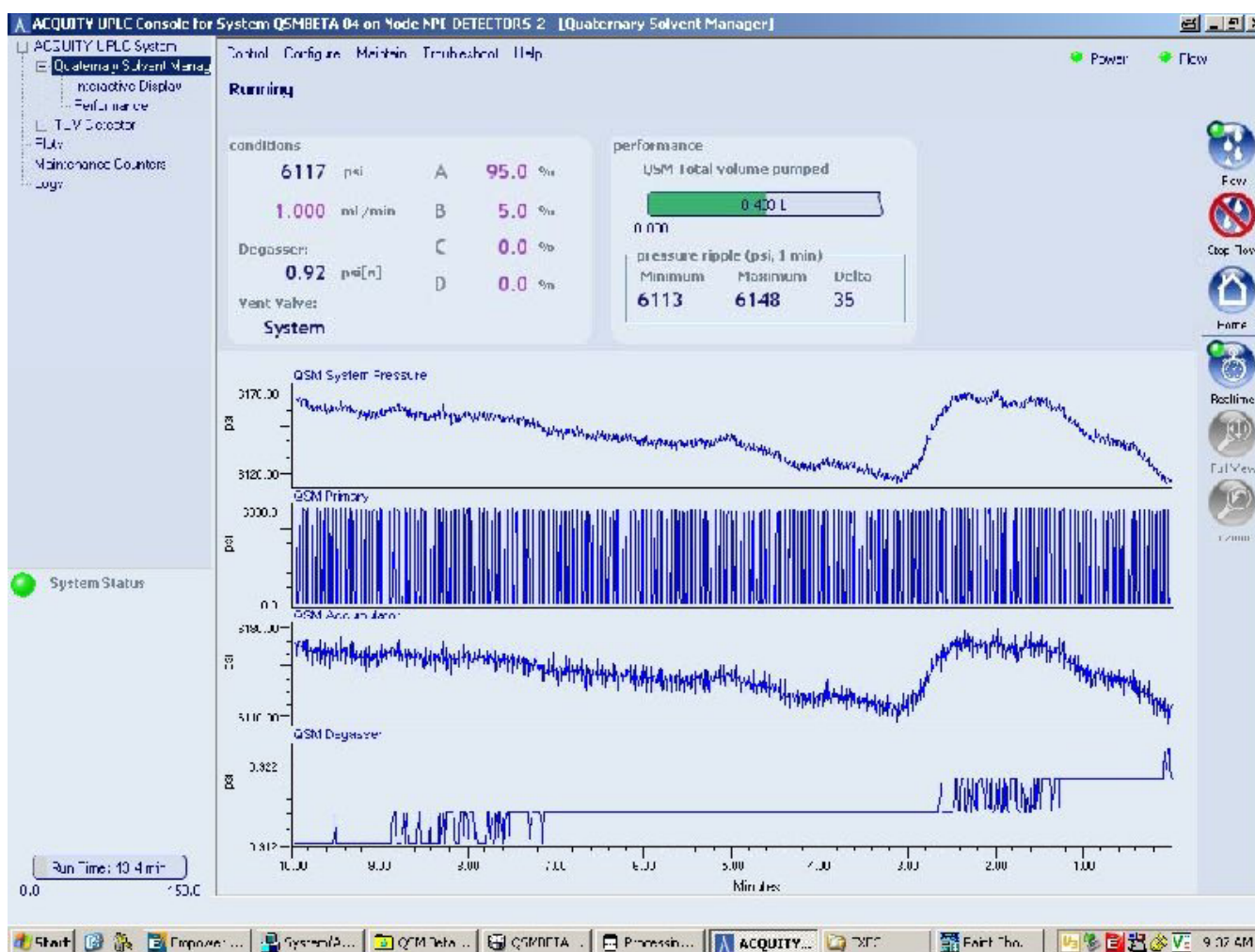
The point at which the 4 solvents meet in the LP Manifold is considered the starting point of a gradient. It is also where the gradient delay volume is measured from. The end point of the gradient delay volume is considered the head of the column.

3- Primary Head Transducer to Accumulator Check Valve

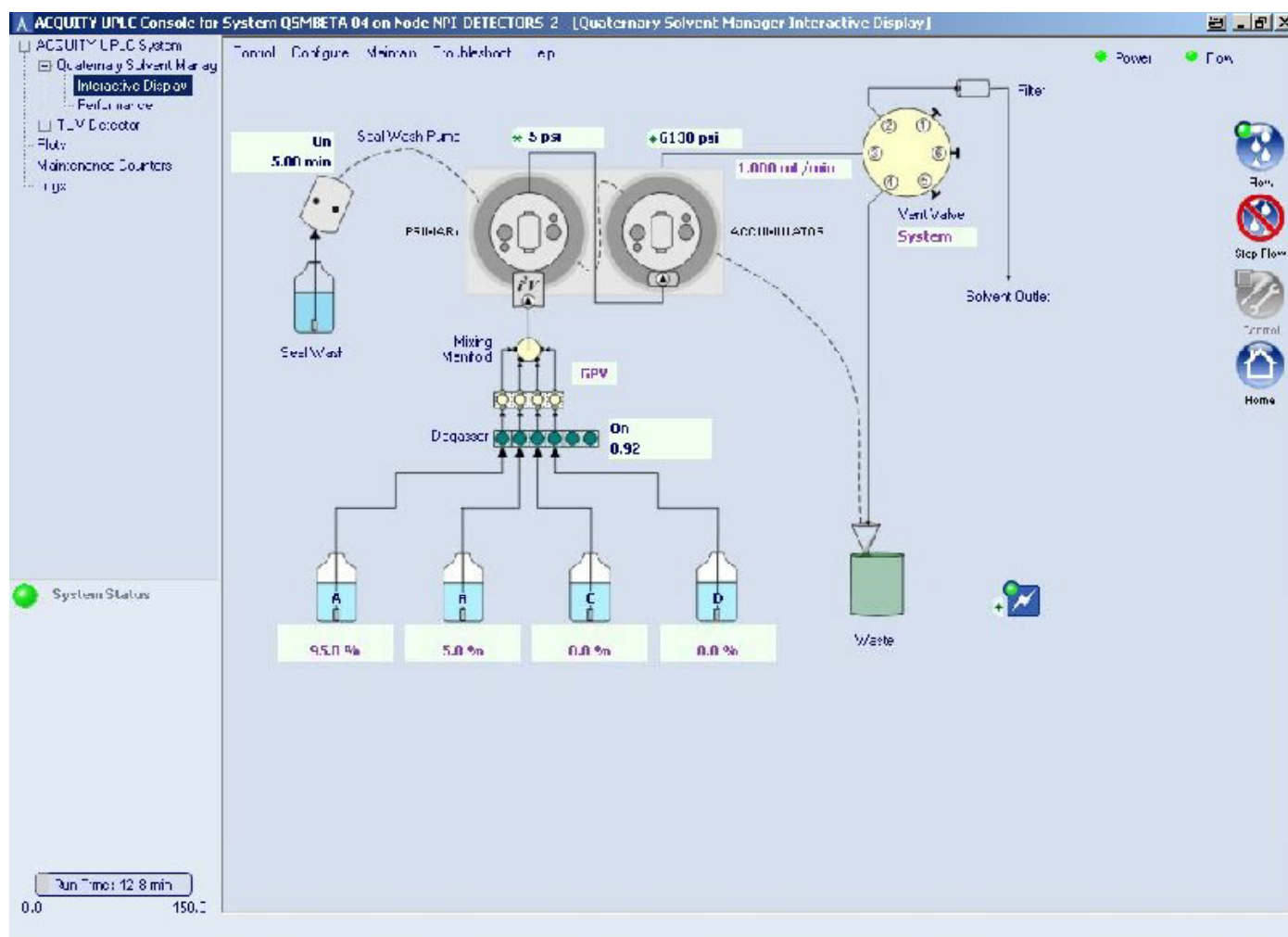
4- Accumulator Head Transducer to Vent Valve



- ❑ Log in to Empower or MassLynx and open the ACQUITY Console menu from the appropriate control panel button.
- ❑ Go to the QSM Top Level menu. Your menu should look similar to the screen below.
- ❑ List two changes in the QSM Console menu compared to the BSM menu.
 1. _____
 2. _____



- ☐ Go to the QSM Interactive menu. Your menu should look similar to the screen below.
 - ☐ Compare the flow path detailed in the Interactive menu to your actual QSM components.
- Is the diagram accurate as compared to the actual front panel components_____?
- ☐ Click on the Control button to turn on interactive control.



- ❑ Click on the GPV link. The Set GPV menu will appear (see screen below).
- ❑ Click on each valve in order. A click should be heard as 24v is applied to actuate the normally closed valve.

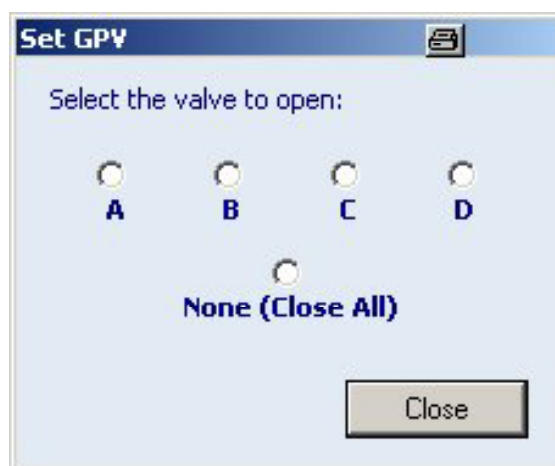
This is an excellent diagnostic tool to test the flow coming from each individual valve and solvent filter. You can troubleshoot a blocked or non-functional valve by observing the gravity fed flow from each valve from the disconnected mixer tube. This will be done in a later section.

- ❑ Check the voltage being read from the personality card by clicking on the  icon.

Enter the voltage readouts for your QSM in the table below.

Voltage	Reading
VCC	
VCC Ground	
+15V	
-15V	
+24V	
+24V Ground	

What is the Degasser pressure? _____psi(a)



- ☐ Click on the Control button to turn off interactive control.
- ☐ Obtain some new filtered water for your system from the appropriate labeled bottle in the lab.
- ☐ Make sure that the solvent lines are inserted to the bottles as follows:

Water	Lines A,C and Seal Wash
Acetonitrile	Lines B,D
10% ACN 90% H ₂ O	SM Wash and Purge Solvent

- ☐ Prime each solvent line for 3 minutes.

What is the total time it takes to prime the QSM lines_____?

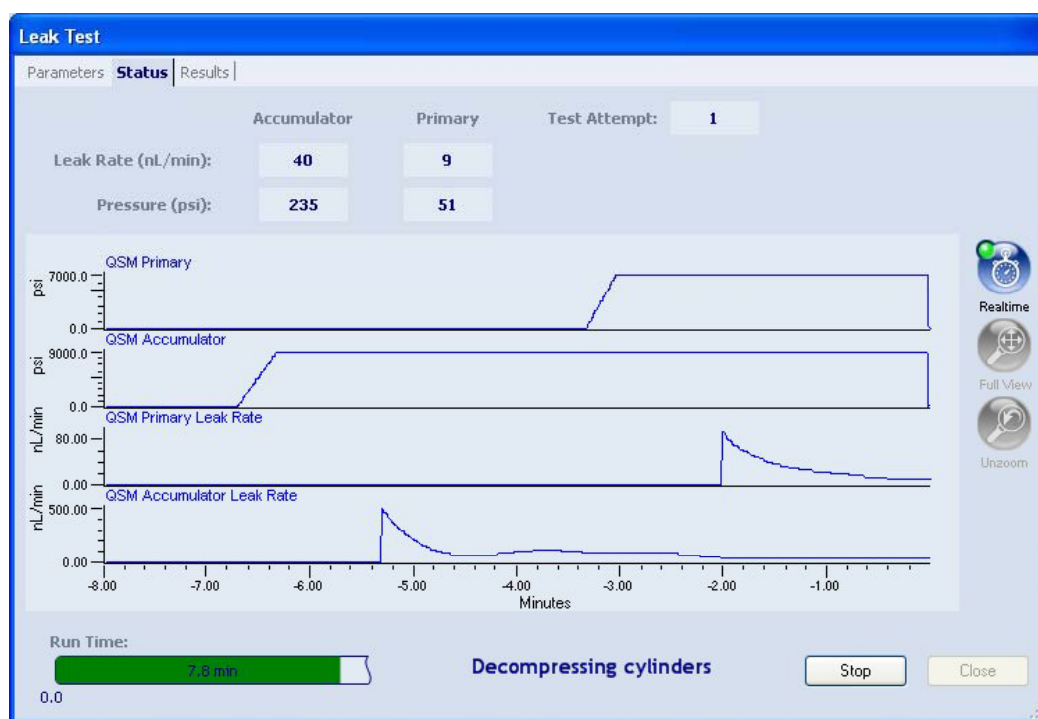
Is the total time longer, shorter or the same as priming four solvents for the BSM_____

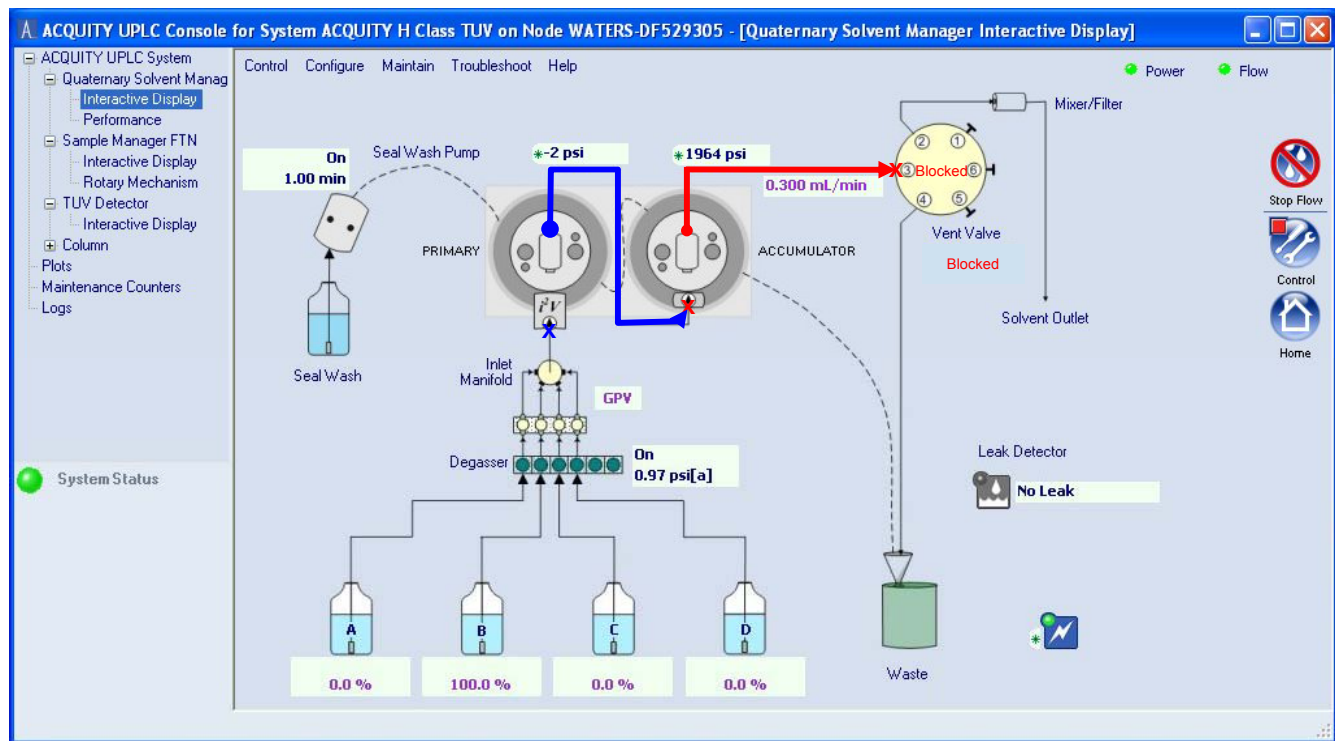
_____?

- ☐ Start the QSM pumping with 100% Water at a flow rate of 0.5ml/min.

-
- Qualcomm Flow Manager Performance
- | In-Use | | pressure ripple (psi, 1 min) | |
|--|------|------------------------------|------|
| Compression - Decompression (0.02 Ratio) | 1.32 | Minimum | 6112 |
| Compressibility | 0.60 | Maximum | 6146 |
| | | Delta | 34 |

- ❑ Shut off the flow using Set Flow from the Control menu.
- ❑ Prime the Seal Wash for about 2 minutes.
- ❑ Trace the path of the Seal Wash line and remove the line outlet line from the waste management system.
- ❑ Observe that there is a steady flow of solvent coming out indicating a good prime then stop the prime.
- ❑ Perform the Wash Plunger function from the Maintain menu. Observe the steps involved in washing the plungers.
- ❑ Switch the console menu to the top level QSM menu so you can see the pressure plots.
- ❑ Run the Leak test using solvent A at 14000 PSI with the Vent Valve as the end point.
- ❑ On the status page, you can right-click the plots and show the Accumulator and Primary pressure plots.





In the QSM interactive menu above diagram, the **red line** indicates what is tested during the Accumulator leak test and the **blue line** indicates what is tested during the Primary leak test.

The criteria for passing the leak test is a leak rate less than 150nl/min when testing to the QSM Vent Valve.

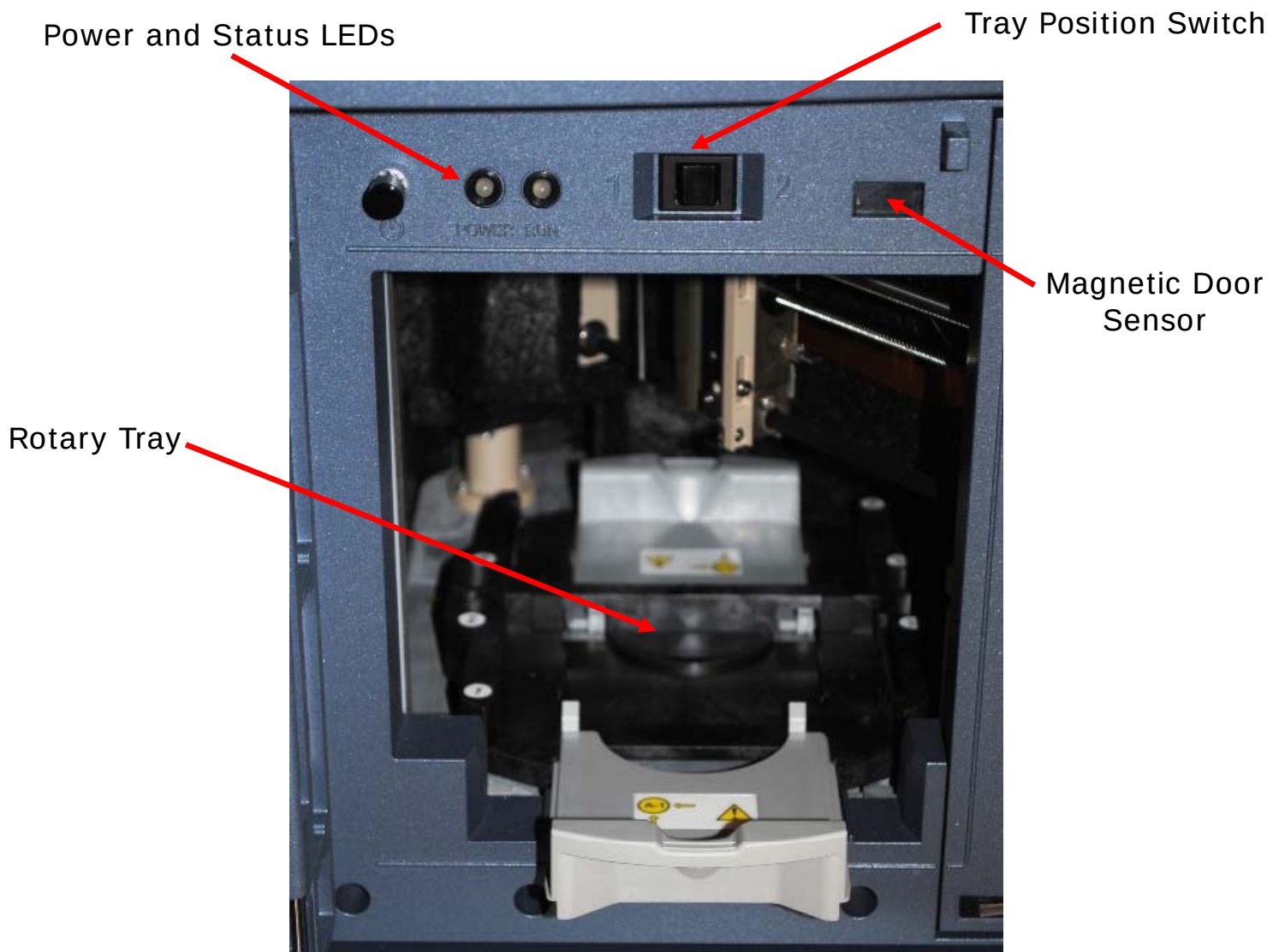
When testing to the column, the criteria for passing the leak test is a leak rate less than 300nl/min.

- Record your results in the appropriate fields:

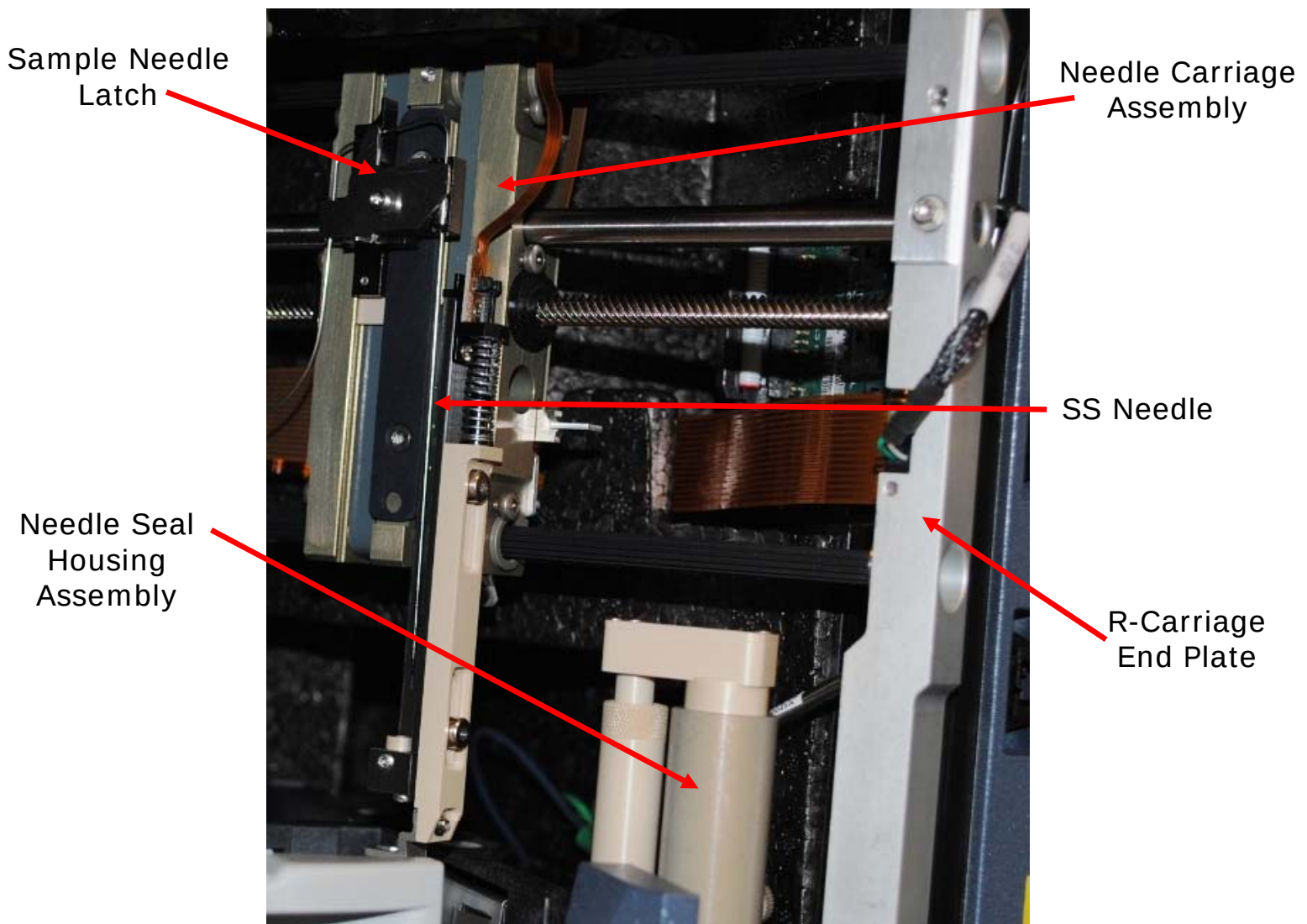
Leak Test	
Parameters Status Results	
	Accumulator Primary
Result:	
Leak Rate:	nL/min nL/min
Maximum Pressure:	psi psi
Percent of Stroke:	% %
Compressed Volume:	uL uL
Compression Attempts:	
Test Attempts:	
Print	
Start Close	

- ❑ Open the door to the SM Sample Compartment.
- ❑ Observe the main components of the Sample Compartment.
- ❑ Click on the Tray Position Switch to change bring the plate holder to position 1 and 2.
- ❑ Practice removing and inserting the plates.

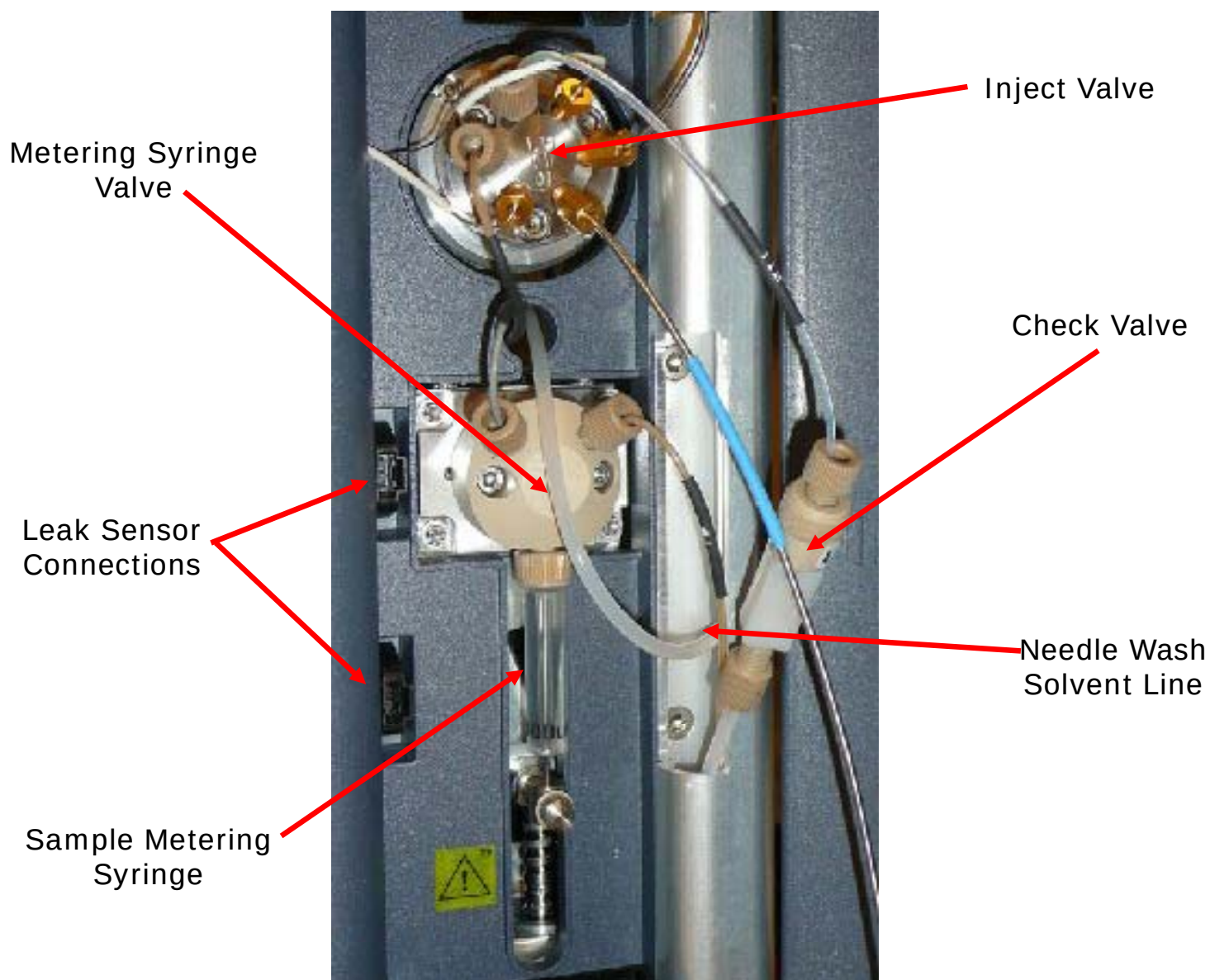
What is the mechanism holding the plate in place _____?



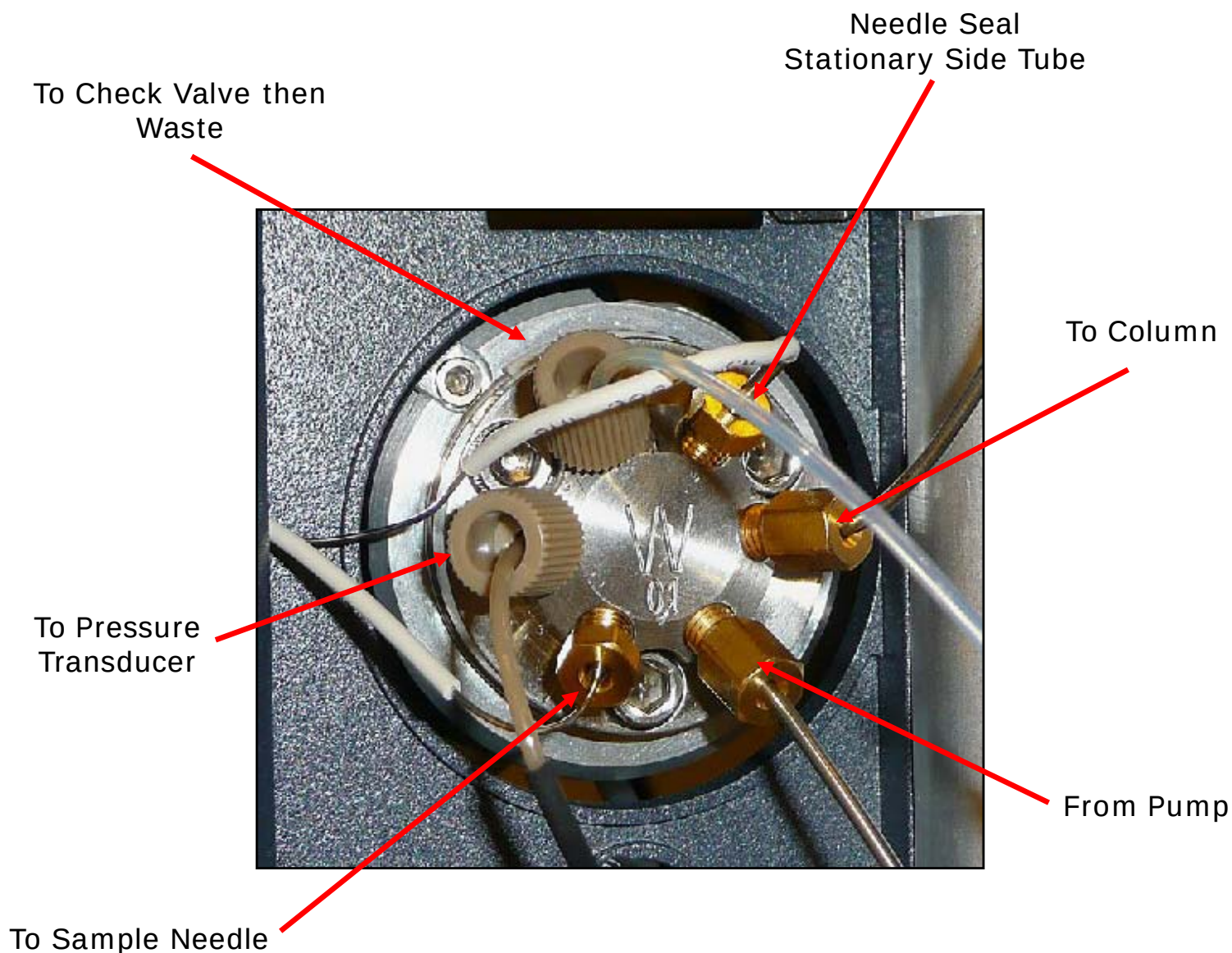
- ❑ Remove the 2 T20 screws securing the access panel in front of the Inject Block and remove the panel.
- ❑ Observe the main components of the Needle Carriage Assembly and the Needle Seal Housing Assembly as seen below.
- ❑ Where possible, trace the solvent lines and SS tubing line back to their origins.
- ❑ You will find it quite useful to compare the actual fluidics layout to the fluidics diagrams listed on the coming pages.



- Observe the main components of the Inject Valve and Sample Metering Syringe as seen below.



- Observe the main connections to the Inject Valve and as seen below and trace each line to its end point. You will need to open the right side panel of the SM to see some of the connection terminations. The internal fluidic components are detailed on the next page.



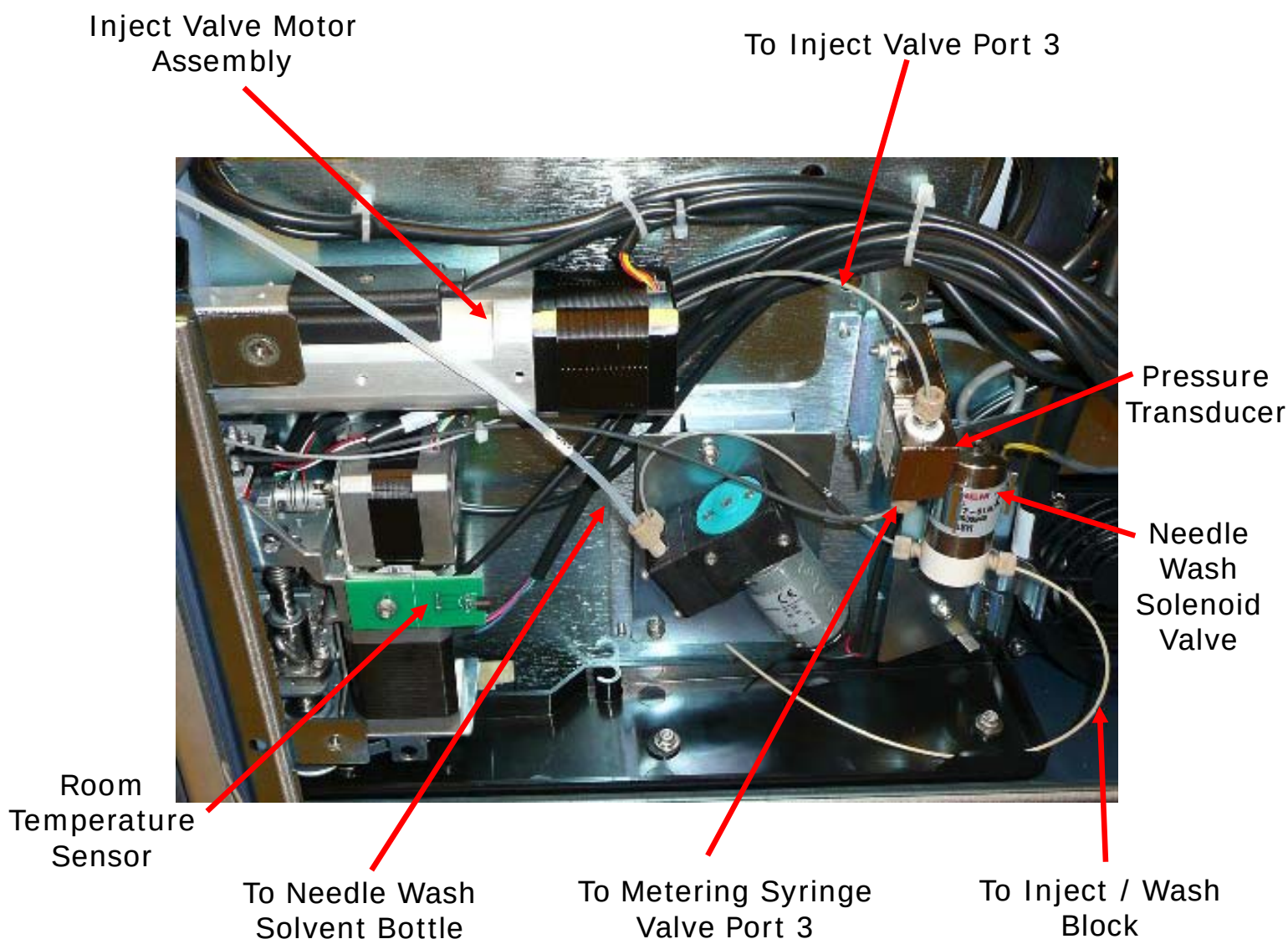
- ❑ Remove the 2 T20 screws securing the access panel in front of the Inject Block and remove the panel.



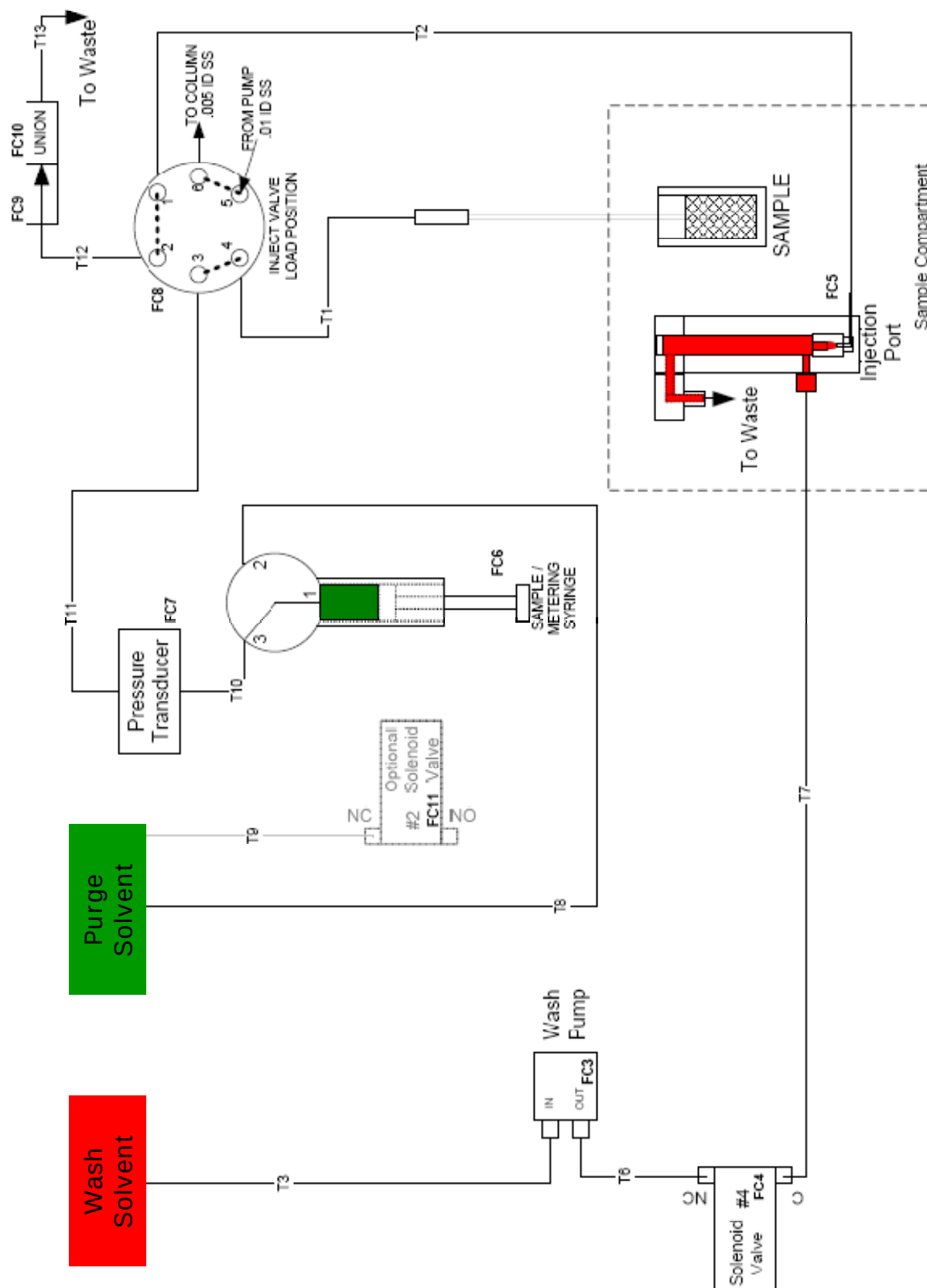
Warning- Beware of live AC and DC voltages when troubleshooting and servicing with side panels removed.

Observe the internal fluidic components as detailed in the picture below.

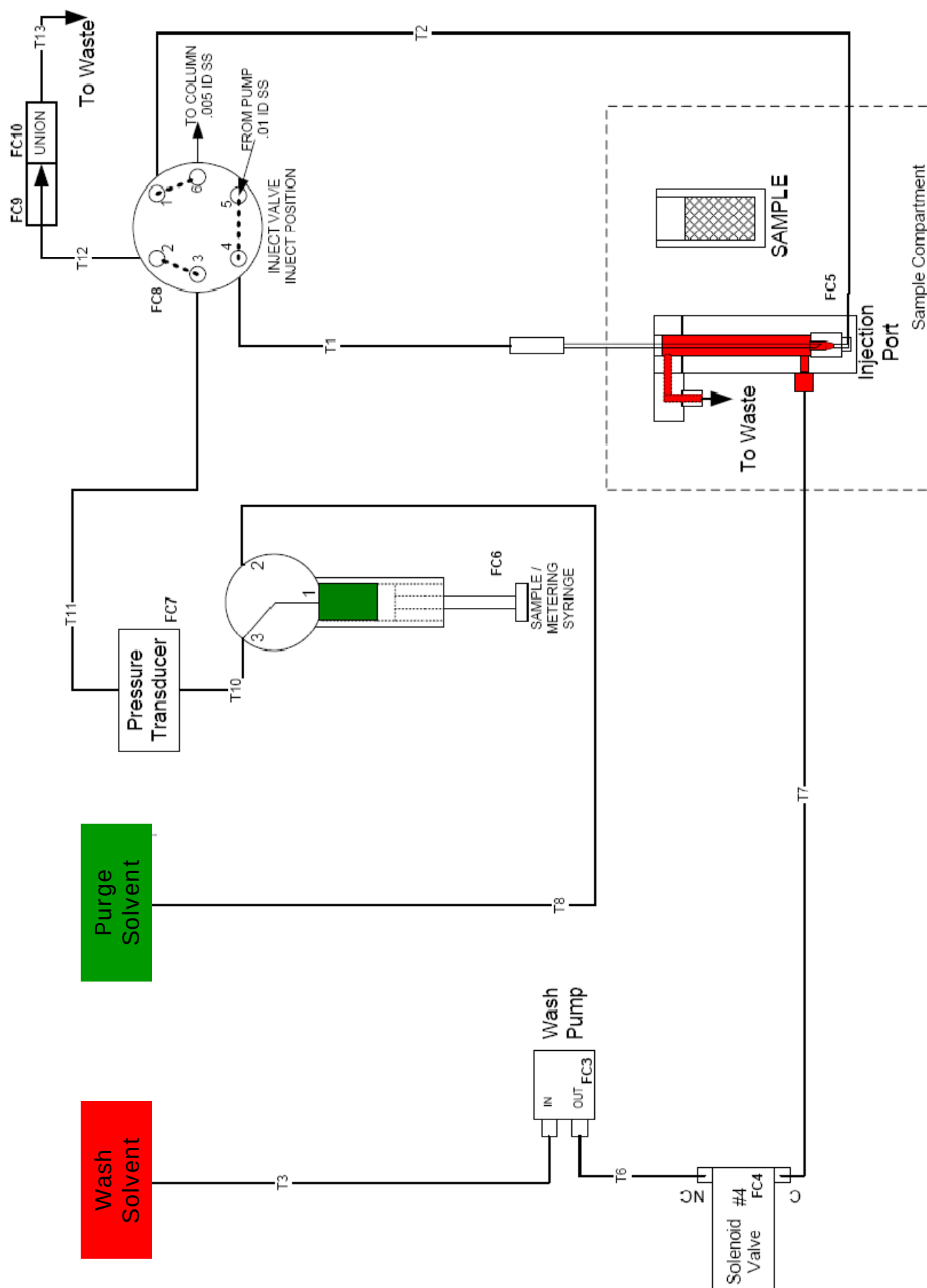
- ❑ As mentioned on the previous page, trace each line to its end point.
- ❑ When you have finished tracing the lines, reinstall the front and side panels.



Sample Withdrawal Diagram

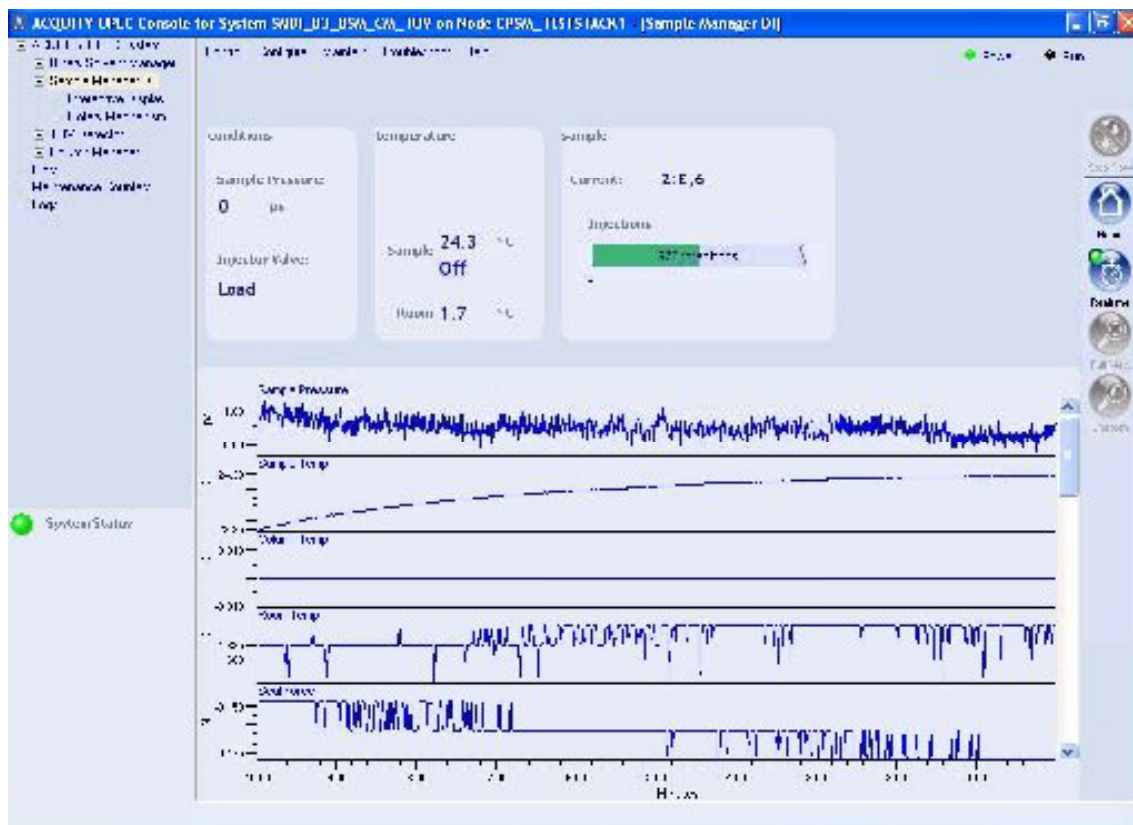


Sample Injection Diagram



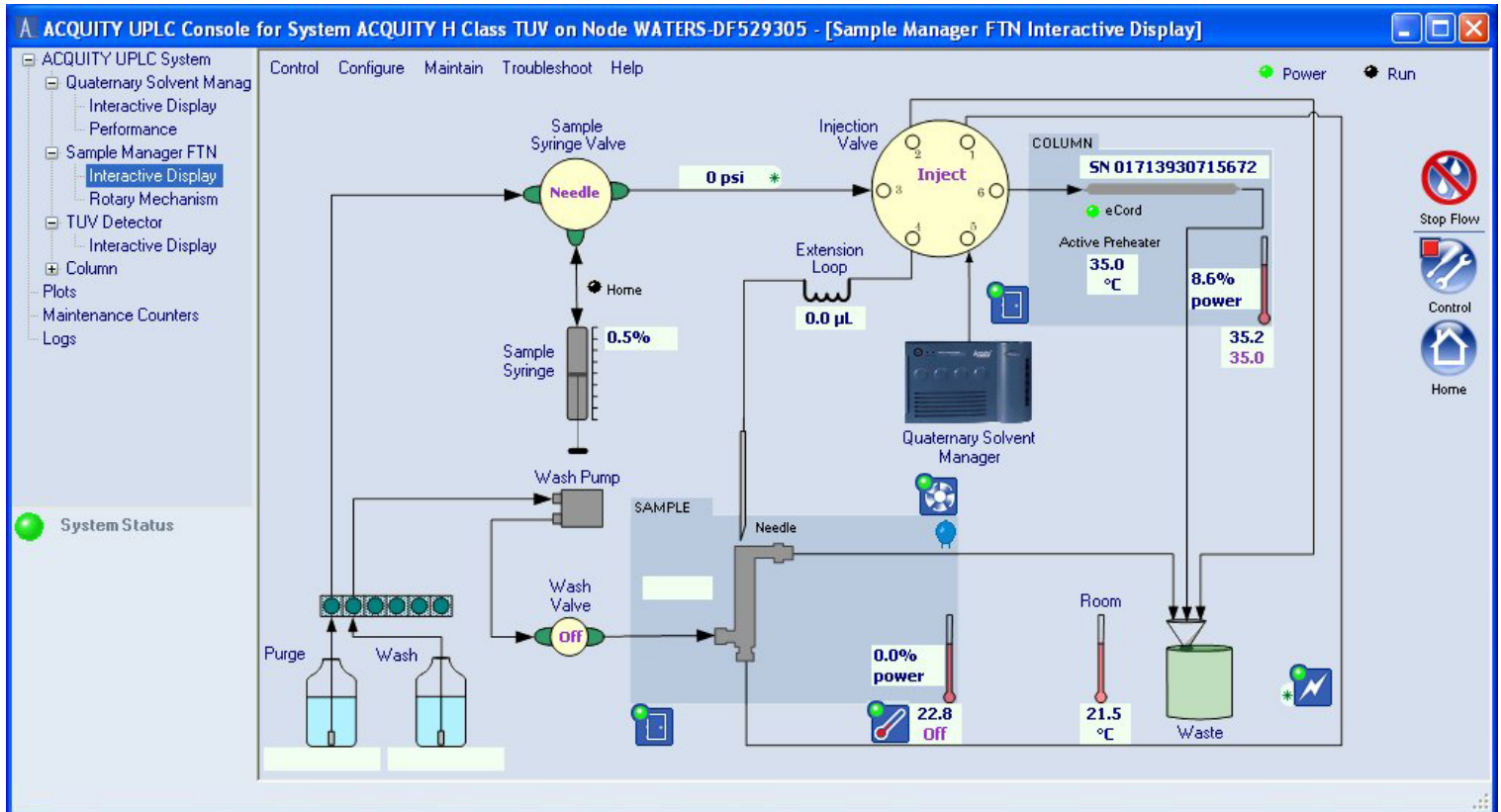
- ☐ Go to the SM Top Level menu. Your menu should look similar to the screen below.
- ☐ Are there any changes in the SM-FTN Console menu compared to the ACQUITY SM menu?

-
-
- ☐ Right-click on the SM plot display and choose properties. List the new plot channels you find compared to the SM menu.
-
-



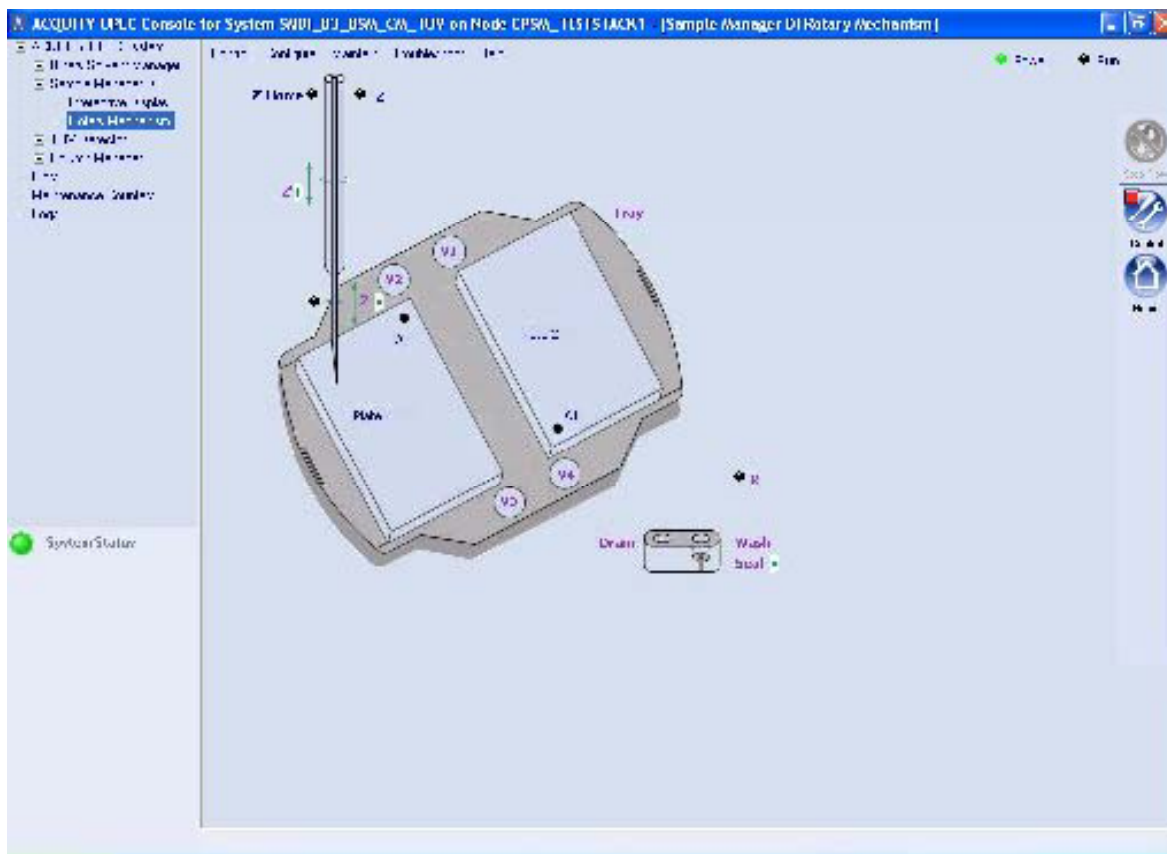
- ☐ Go to the SM Interactive menu. Your menu should look similar to the screen below.
- ☐ Compare the diagram in the Interactive menu to the SM-FTN fluidics diagram. Does the diagram accurately represent the flow path?

- ☐ Click on the Control button to turn on interactive control. What fields are enabled for control?



- ❑ Click on the Control button to turn off interactive control.
- ❑ Switch to the Rotary Mechanism menu. Your menu should look similar to the screen below.
- ❑ Click on the Control button to turn on interactive control.
- ❑ Move the needle to the V1 position while observing the movements in the Sample Compartment.
- ❑ Move the needle to the Injector Wash position then the Injector Seal position.

Caution: Keeps your hands out of the Sample Compartment.



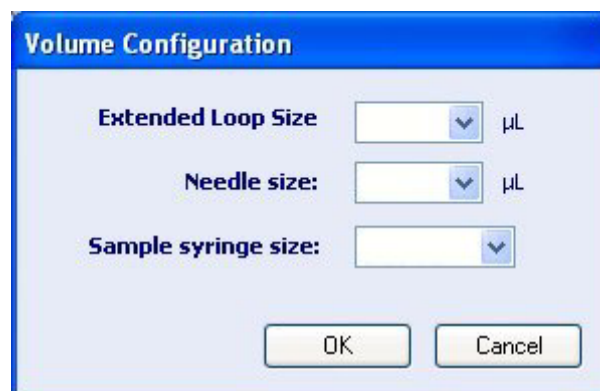
- ❑ Click on the Control button to turn off interactive control. Switch back to the SM-FTN Top Level menu.
- ❑ Click on Preferences from the Configure menu. The Configure menu will appear.



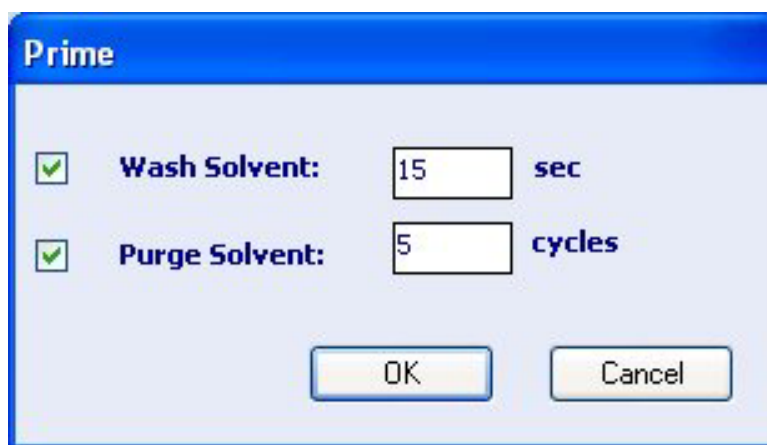
- ❑ Close the Sample Compartment door.
- ❑ Try each setting for “When the door is opened” dialog box and check for the proper result when you open the Sample Compartment door.

Does the light come on when it should_____?

- ❑ Click on Volumes from the Configure menu. The Volumes menu will appear.
- ❑ Fill in the values displayed in the fields below.



- ☐ Switch the SM plots to show only sample pressure.
- ☐ Click on Prime from the Control menu. The Prime menu will appear.



- ☐ Choose the default prime settings and click OK.
- ☐ Observe the sample pressure trace during the prime function.

Is there any pressure change during the Wash Solvent prime (if not then why)_____?

_____.

Is there any pressure change during the Purge solvent prime (if not then why)_____?

- ☐ Click on Wash Needle from the Control menu. The Wash Needle menu will appear. Choose the default setting and click OK.

Is there any SM pressure change_____?



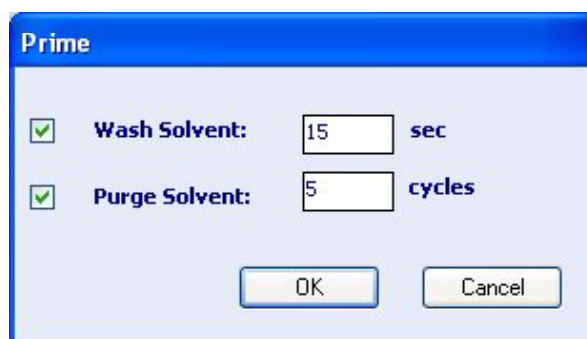
Section 2

Quick-Start Best Practices



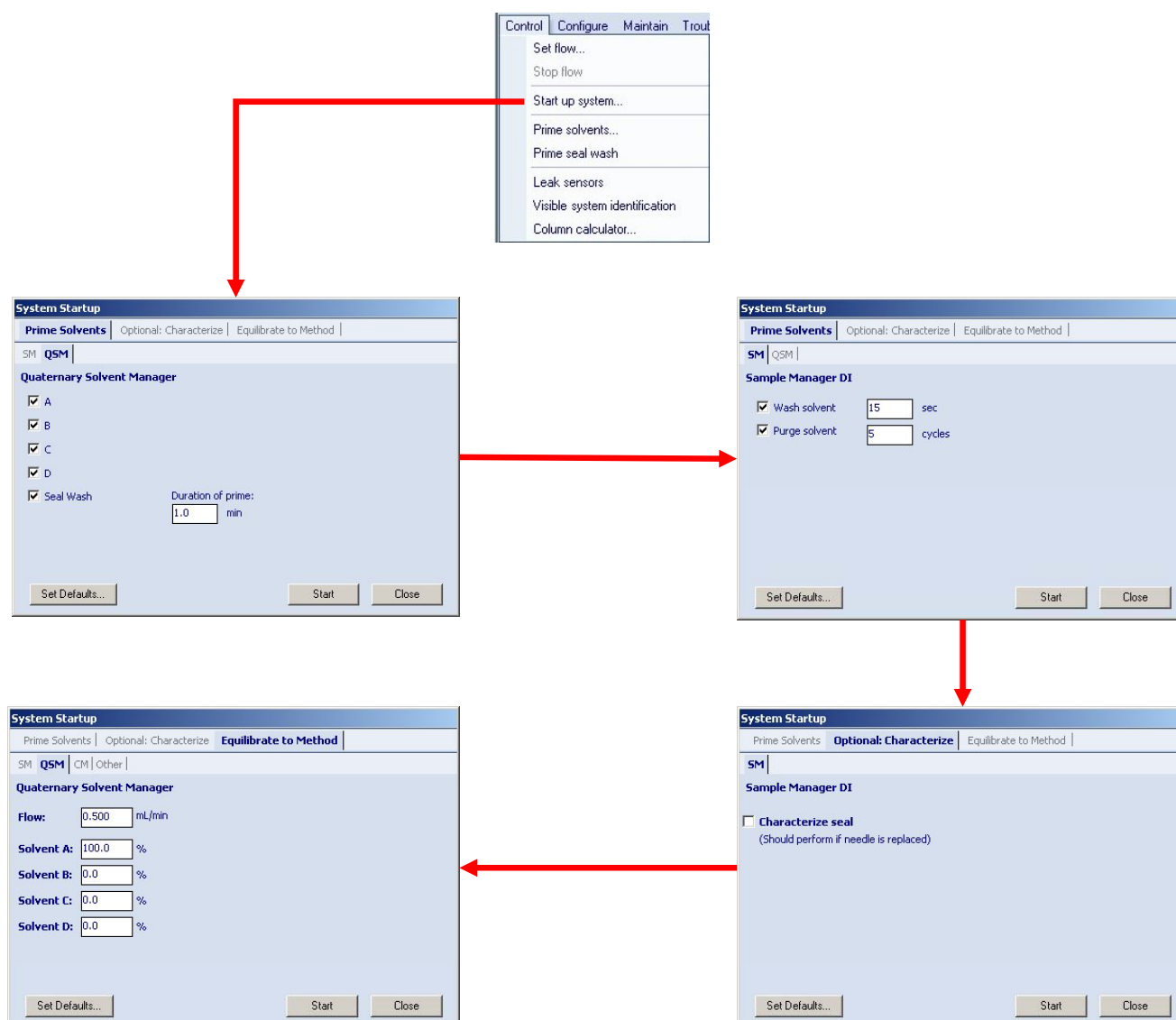
- Objective- to learn the current best practices for restarting the H-Class system after long and short duration shutdown
- Startup prime of the system after a long and short-term shutdown
 - QSM Solvent Lines
 - QSM Seal Wash
 - Solvent Select Valve
 - SM-FTN Wash Solvent
 - SM-FTN Purge Solvent
 - Flow Rate Ramping
 - Setting the CH-A Temperature

- ☐ Observe the following guidelines when starting up the system after a short-term shutdown:
 - ☐ Prime all QSM solvent lines being used for 2 minutes if the system has been idle for 4 or more hours and you will use the solvents that are already in the system.
 - ☐ Prime all QSM solvent lines being used for 4 minutes if you will use new solvents that are of the same composition of what is already in the system.
 - ☐ Prime the Seal wash for 1 minute.
 - ☐ Prime the SM-FTN Wash Solvent and Purge Solvent using the default values of 15 seconds and 5 cycles respectively.
- ☐ Observe the following guidelines when starting up the system after a long-term (greater than 72 hours) shutdown or when completely changing the solvents:
 - ☐ Prime all QSM solvent lines being used for 5 minutes
 - ☐ Prime all QSM solvent lines being used for 7 minutes if changing solvents completely
 - ☐ Prime the SM-FTN Wash Solvent for 60 seconds and the Purge Solvent for 10 cycles.
 - ☐ When priming a dry system, prime the wash solvent for 60 seconds and the purge solvent for 30 cycles.
- ☐ Consult the ACQUITY UPLC H-Class System Quick Reference Card for full details.



The System Startup Menu

- ❑ Go to the Console Top Level menu.
- ❑ Click on Startup System from the Control menu. The System Startup menu will appear.
- ❑ Perform the system startup routine using the default values as shown in the screens below. You do not need to equilibrate the system to a flow rate or temperature at this time.

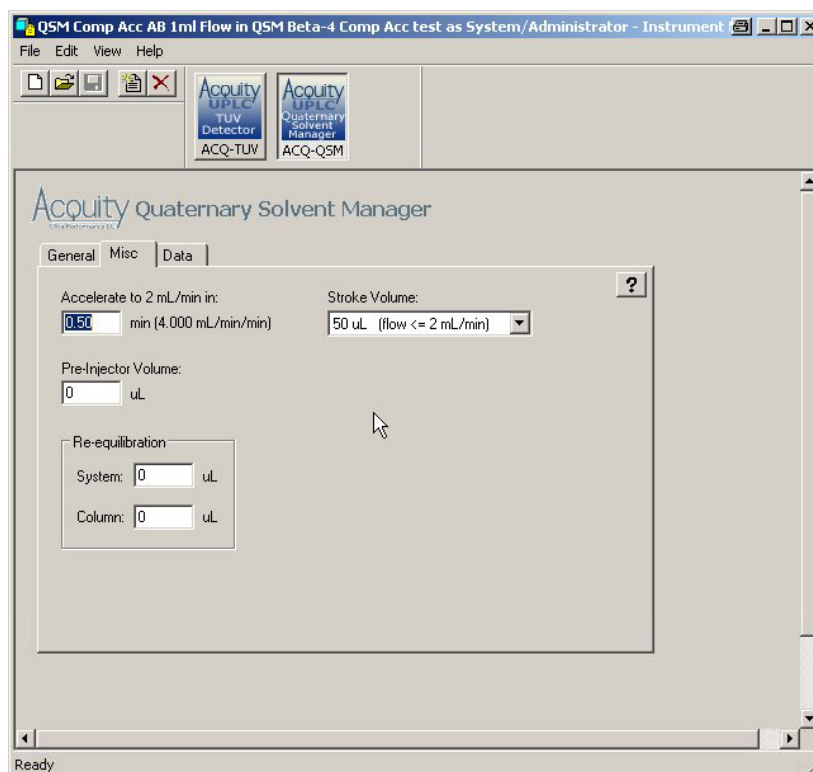


The rate at which the flow rate is ramped after a set flow command is given can be controlled by the Accelerate to 2ml/min field in the QSM instrument method. In order for the entered value to be used, the instrument method must be downloaded to the ACQUITY system.

- ❑ Try the default value of 0.5 and set the flow to 0.5 ml/min. Stop the flow rate after 1 minute by switching the flow to 0ml/min.
- ❑ Set the Accelerate to 2ml/min rate to 1.0.
- ❑ Download the method and set the flow to 0.5 ml/min. Stop the flow rate after 1 minute.

Do you notice a difference in the slopes of the system pressure trace?_____.

- ❑ In preparation for running the Performance Verification tests, set the flow to 0.6 ml/min 90% A 10%B.



Section 3 Monitoring System Health

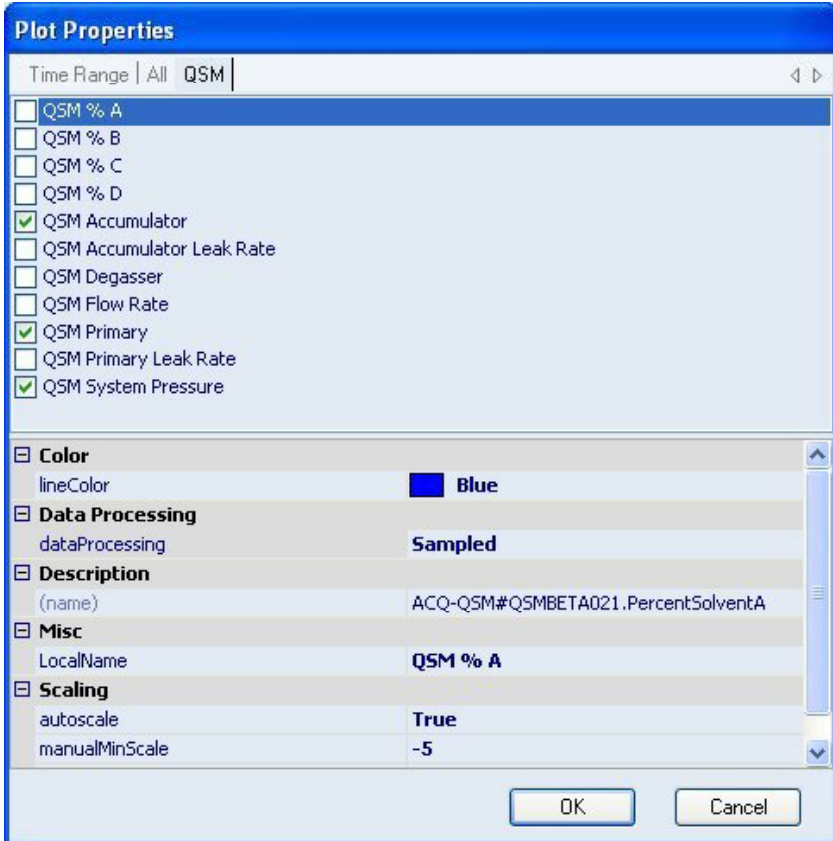


Most Typical QSM Plots

The most useful plots to monitor the health of the QSM for a typical situation are the System pressure, QSM Accumulator and the QSM Primary traces. The other QSM traces can be added at any time if a particular situation warrants it.

To set up the QSM plots:

- ☐ Go to the QSM Top Level menu.
- ☐ Right-click on the QSM plot menu and choose Plot Properties. The Plot Properties menu will appear.
- ☐ Choose the Plots checked below for routine monitoring of QSM health along with monitoring the Delta Pressure Ripple.



Plot Properties

Time Range | All QSM

- ☐ QSM % A
- ☐ QSM % B
- ☐ QSM % C
- ☐ QSM % D
- ☒ QSM Accumulator
- ☐ QSM Accumulator Leak Rate
- ☐ QSM Degasser
- ☐ QSM Flow Rate
- ☒ QSM Primary
- ☐ QSM Primary Leak Rate
- ☒ QSM System Pressure

Color

lineColor ■ Blue

Data Processing

dataProcessing Sampled

Description

(name) ACQ-QSM#Q5MBETA021.PercentSolventA

Misc

LocalName QSM % A

Scaling

autoscale True

manualMinScale -5

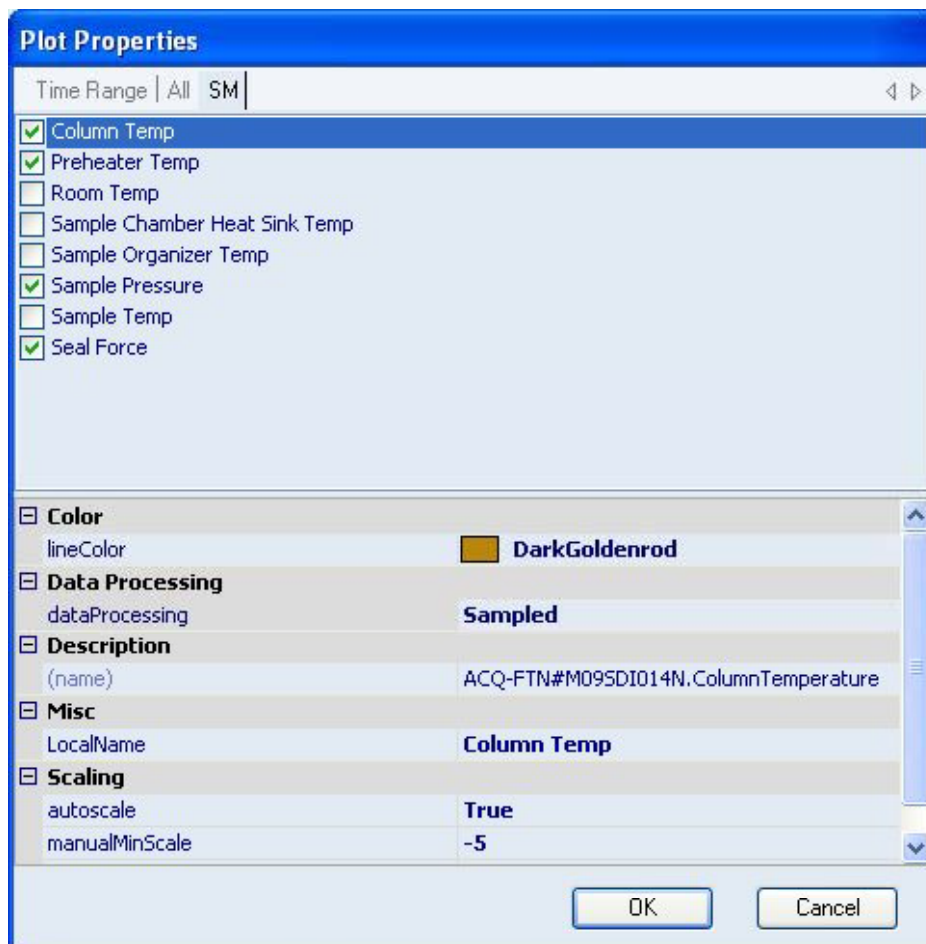
OK Cancel

Most Typical SM-FTN and CHA Plots

The most useful plots to monitor the health of the SM-FTN for a typical situation are the Sample Pressure, Column Temperature, Sample Temperature, Seal Force and Room Temperature traces. The other QSM traces can be added at any time if a particular situation warrants it.

To set up the QSM plots:

- ☐ Go to the SM-FTN Top Level menu.
- ☐ Right-click on the SM-FTN plot menu and choose Plot Properties. The Plot Properties menu will appear.
- ☐ Choose the Plots checked below for routine monitoring of SM-FTN health.



Plot Properties

Time Range | All SM

- ☒ Column Temp
- ☒ Preheater Temp
- ☐ Room Temp
- ☐ Sample Chamber Heat Sink Temp
- ☐ Sample Organizer Temp
- ☒ Sample Pressure
- ☐ Sample Temp
- ☒ Seal Force

Color

lineColor: DarkGoldenrod

Data Processing

dataProcessing: Sampled

Description

(name): ACQ-FTN#M09SDI014N.ColumnTemperature

Misc

LocalName: Column Temp

Scaling

autoscale: True

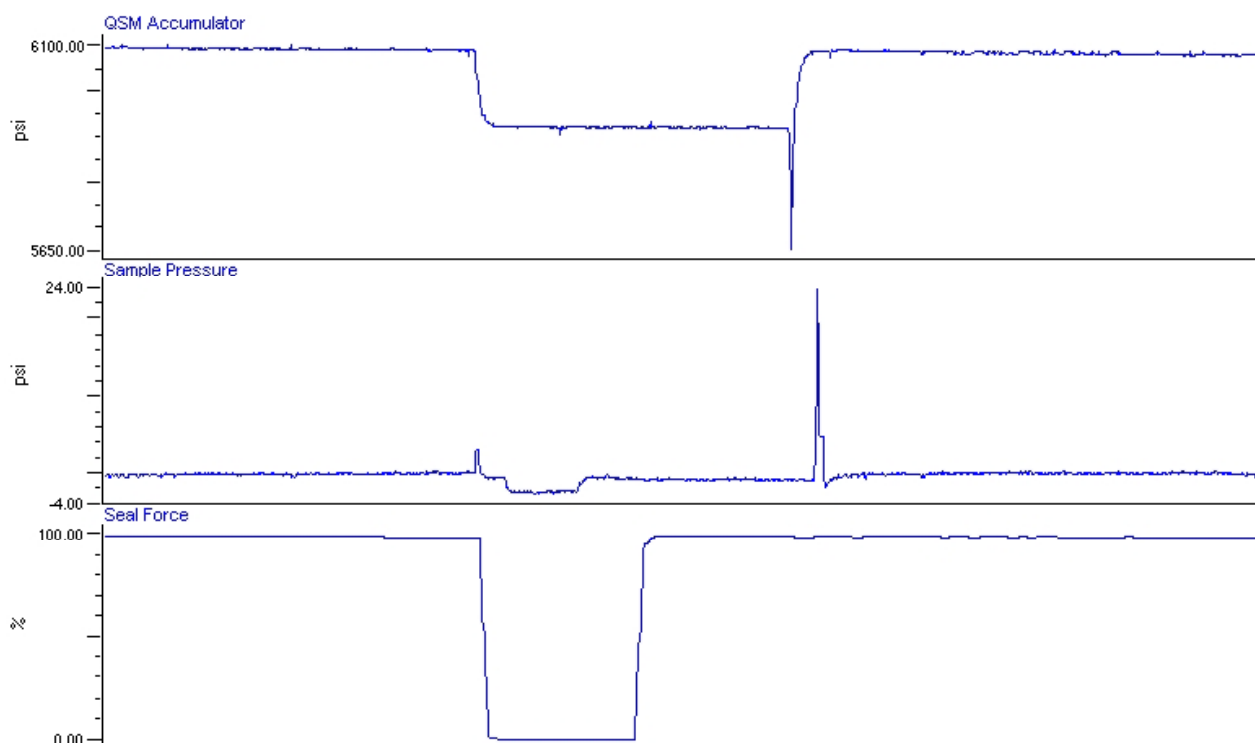
manualMinScale: -5

OK Cancel

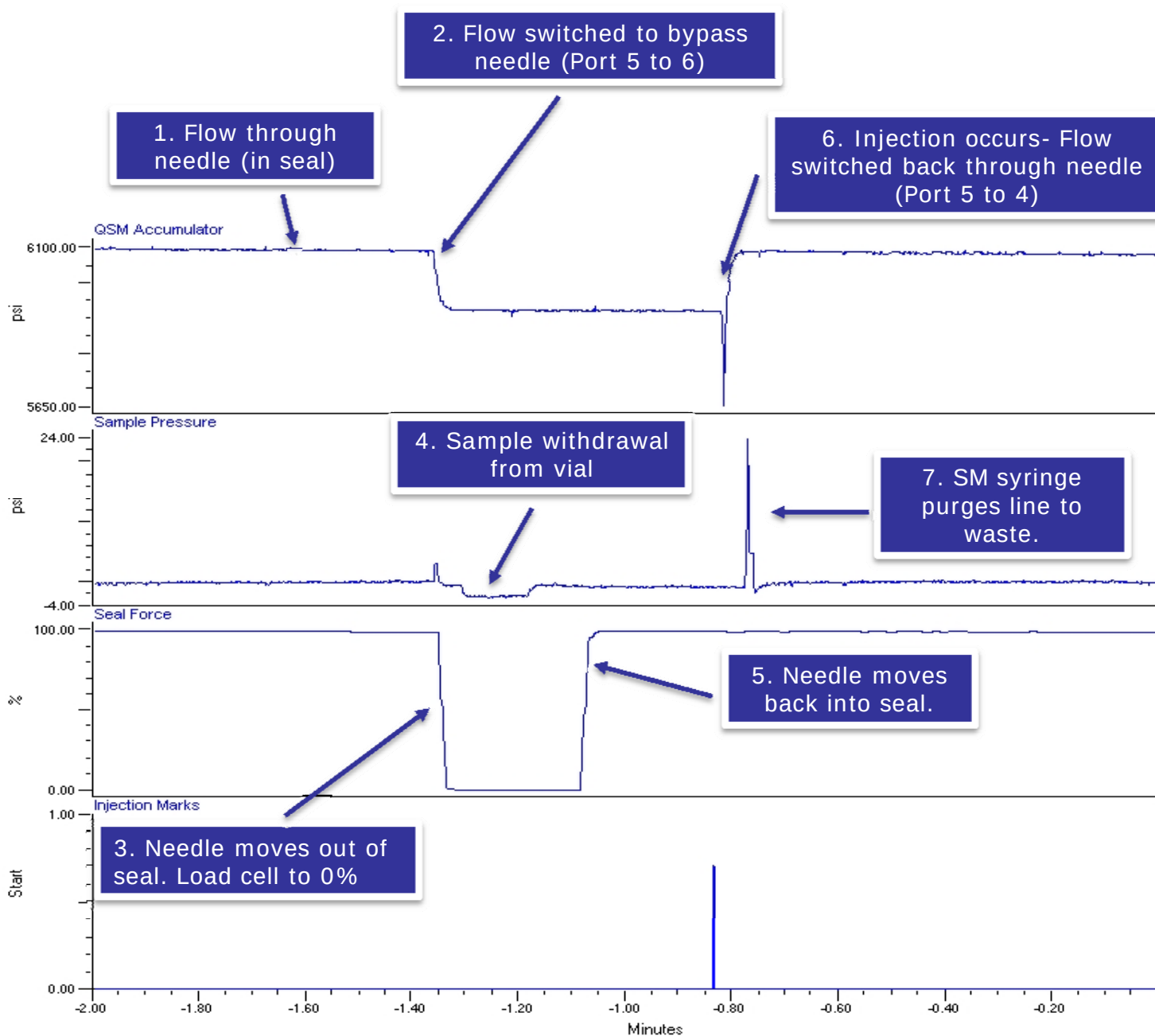
The most useful plots to monitor the health of the system for a typical situation are the System Pressure, Sample Pressure and Seal Force. Injection Marks can be turned on as an option . There are many other cross-module plots that can be set up as well for different purposes, such as Room Temperature versus Column Temperature or Sample Temperature. All traces are recorded for 96 hours whether they are selected or not.

To set up Cross-Module System Health Plot:

- ☐ Go to the Plots Top Level menu.
- ☐ Right-click on the plots menu and choose Plot Properties. The Plot Properties menu will appear.
- ☐ Choose the Plots below for routine monitoring of system health.



Injection Sequence with Plot

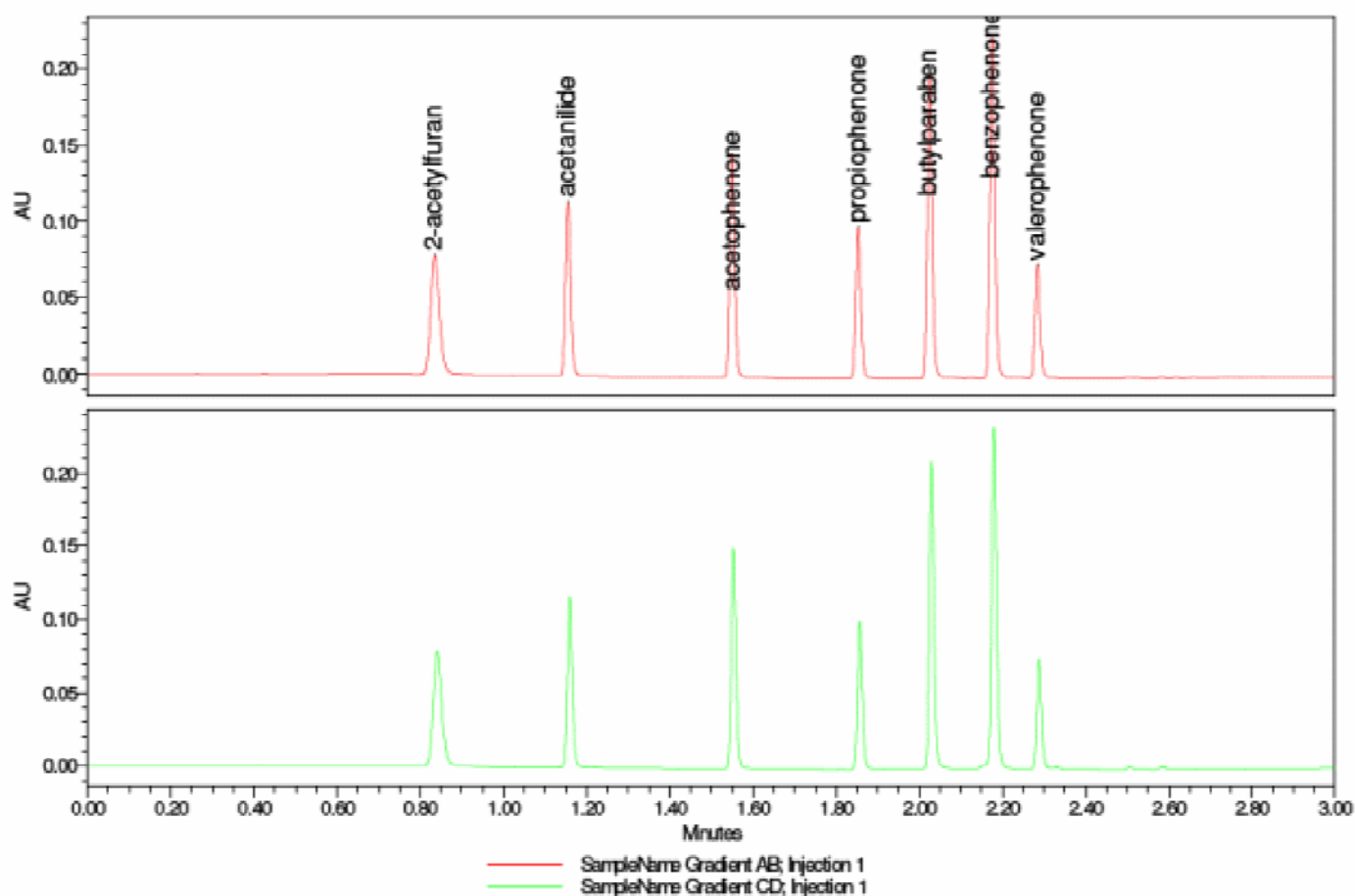




Section 4

Performance Verification Tests

Gradient Performance Chromatogram Overlay



- ❑ The ACQUITY H-Class System Performance Test is designed to test the system in the same fashion as the ACQUITY UPLC System. The following tests are performed to determine that the system is performing to specification:
 - ❑ System Precision- Performs six replicate injections of a Caffeine Standard. The performance of the QSM is checked by looking at the Standard Deviation of the retention times for the Caffeine peak. The performance of the SM-FTN is determined by looking at the area and height precision (%RSD) of the six replicate injections.
 - ❑ Gradient Test- Performs three replicate injections of a 7 component gradient test mixture. The performance of the QSM is checked by looking at the Standard Deviation of the retention times for all peaks. Separate tests will be run for solvent lines AB and CD in order to test the function of all Gradient Proportioning Valves.
 - ❑ *The results are reported in the appropriate fields in the Installation Checklist and shown to the customer for approval.*
 - ❑ *The verification tests can be repeated any time the performance of the system is in doubt. It is an excellent tool to use to separate a system issue from a potential application or chemistry problem at a customer site.*
- ❑ *When combined with a full set of diagnostics test results from each ACQUITY H-Class module, they give a high degree of confidence that the system is running according to the design specifications.*

- ☐ Refer to the ACQUITY H-Class System Performance Test Procedure to run the System Precision test.
You may also use the Sample Set from the Empower SQT template project on your system.
- ☐ Run a blank injection consisting of 90% Water and 10% Acetonitrile first followed by a couple of test injections to check that the system is well equilibrated.
- ☐ Run the six replicate injections of the Caffeine Standard.
- ☐ Record the RT precision (STD Dev), area and height precision (%RSD) of the six replicate injections in the table below and fill out the pass / fail fields
- ☐ An example chromatogram and results table are provided on the next page.

Caffeine Area and Height Results Table

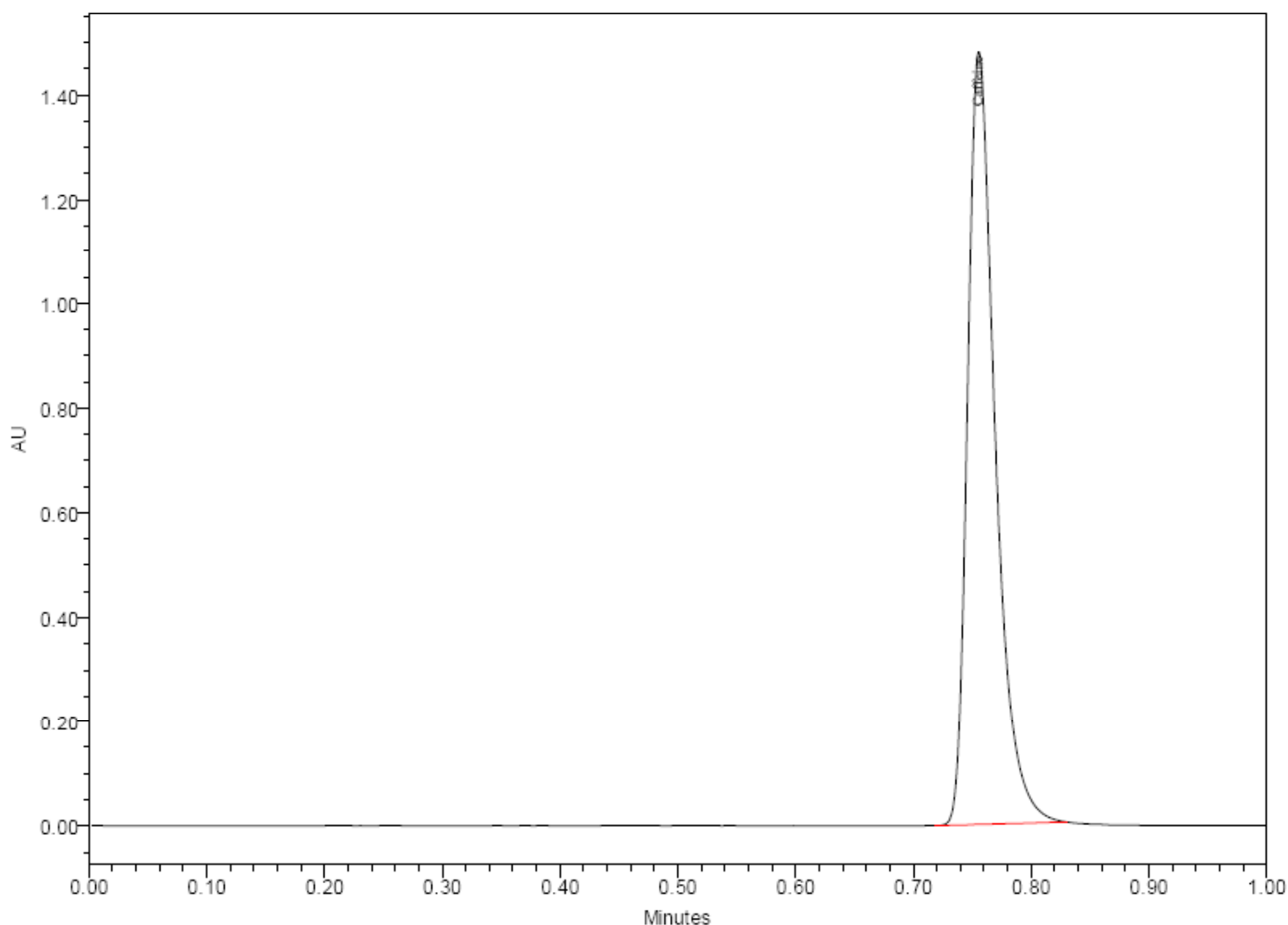
	Area %RSD Specification	Area %RSD	Area %RSD Pass Fail	Height %RSD Specification	Height %RSD	Height %RSD Pass Fail
1	%RSD < or = 0.5			%RSD < or = 1.1		

Caffeine Retention Time (RTsec) Results Table

	Retention time (sec) Standard Deviation Specification	Retention time (sec) Standard Deviation	Retention time (sec) Standard Deviation Pass Fail
1	SD RT < or = 1.0sec		

Precision Test Example Results

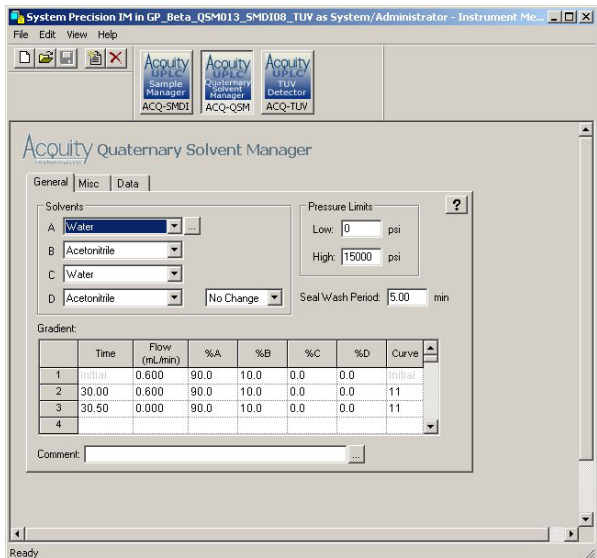
System Precision Chromatograms



System Precision Peak Results

Name: Caffeine

	Vial	Injection	Inj Vol (ul)	Name	RT (min)	Retention time (sec)	Area	Height (μV)	Points Across Peak	Result Id
1	2:E,6	1	5	Caffeine	0.756	45.34	2422500.55	1480032.88	134	3686
2	2:E,6	2	5	Caffeine	0.756	45.33	2421324.73	1491582.87	134	3687
3	2:E,6	3	5	Caffeine	0.756	45.36	2420474.52	1489221.33	133	3688
4	2:E,6	4	5	Caffeine	0.756	45.36	2419922.04	1492776.06	132	3689
5	2:E,6	5	5	Caffeine	0.758	45.47	2421420.72	1488516.45	133	3690
6	2:E,6	6	5	Caffeine	0.759	45.53	2421762.26	1486888.21	133	3691
Mean						45.40	2421234.14	1488169.63		
Std. Dev.						0.08	919.17	4515.41		
% RSD							0.04	0.30		



Acquity Quaternary Solvent Manager

General Misc Data

Solvents:

A: Water
B: Acetonitrile
C: Water
D: Acetonitrile

Pressure Limits:
Low: 0 psi
High: 15000 psi

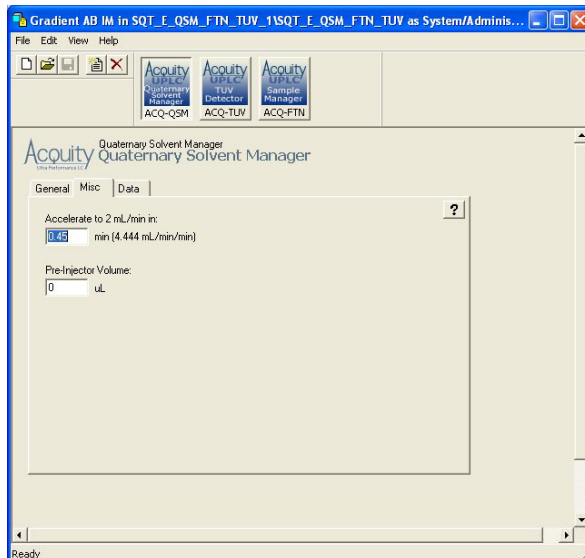
Seal Wash Period: 5.00 min

Gradient:

	Time	Flow (mL/min)	%A	%B	%C	%D	Curve
1	Initial	0.600	90.0	10.0	0.0	0.0	Initial
2	30.00	0.600	90.0	10.0	0.0	0.0	11
3	30.50	0.000	90.0	10.0	0.0	0.0	11
4							

Comment:

QSM Instrument Method General



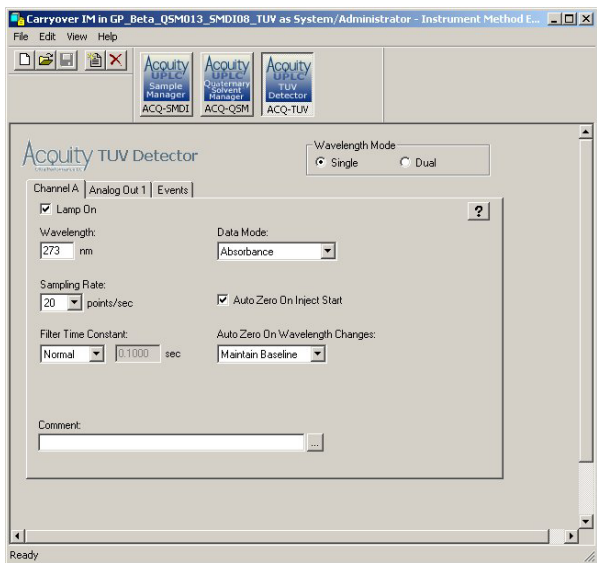
Acquity Quaternary Solvent Manager

General Misc Data

Accelerate to 2 mL/min in:
0.45 min (4.44 mL/min/min)

Pre-Injector Volume:
0 µL

QSM Instrument Method Misc Tab



Acquity TUV Detector

Channel A Analog Out 1 Events

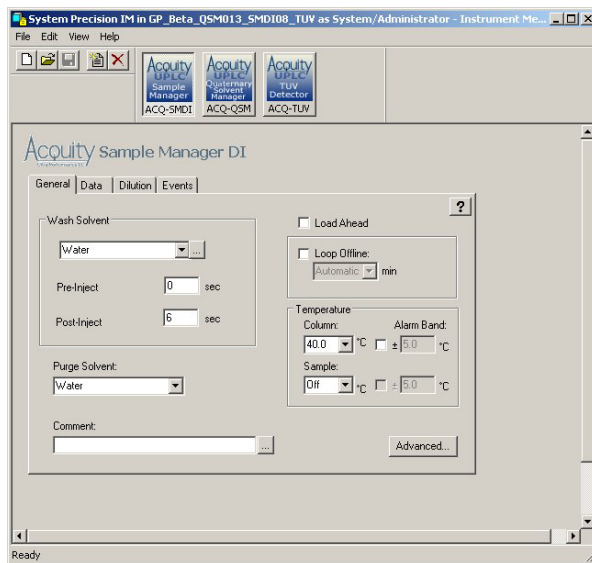
Wavelength: 273 nm
Data Mode: Absorbance

Sampling Rate: 20 points/sec
Filter Time Constant: Normal 0.1000 sec

Auto Zero On Inject Start: ☒
Auto Zero On Wavelength Changes: Maintain Baseline

Comment:

TUV Instrument Method



Acquity Sample Manager DI

General Data Dilution Events

Wash Solvent: Water
Pre-Inject: 0 sec
Post-Inject: 6 sec

Purge Solvent: Water

Temperature:
Column: 40.0 °C
Sample: Off °C

Comment:

SM-FTN Instrument Method

- ❑ Refer to the ACQUITY H-Class System Performance Test Procedure to develop and run the Gradient test.
- ❑ Equilibrate the system for 5 minutes
Important: Make sure to include a next inject delay of 3.0 minutes for Empower or a 6 min. run time for MassLynx.
- ❑ Run the 3 replicate injections of the 7-Component Gradient test mixture.
- ❑ Record the RT precision (STD Dev) of the 3 replicate injections of the 7 Components in the fields provided below.
- ❑ Modify your QSM instrument method to use solvents CD and repeat the above test.

Retention Time Reproducibility AB

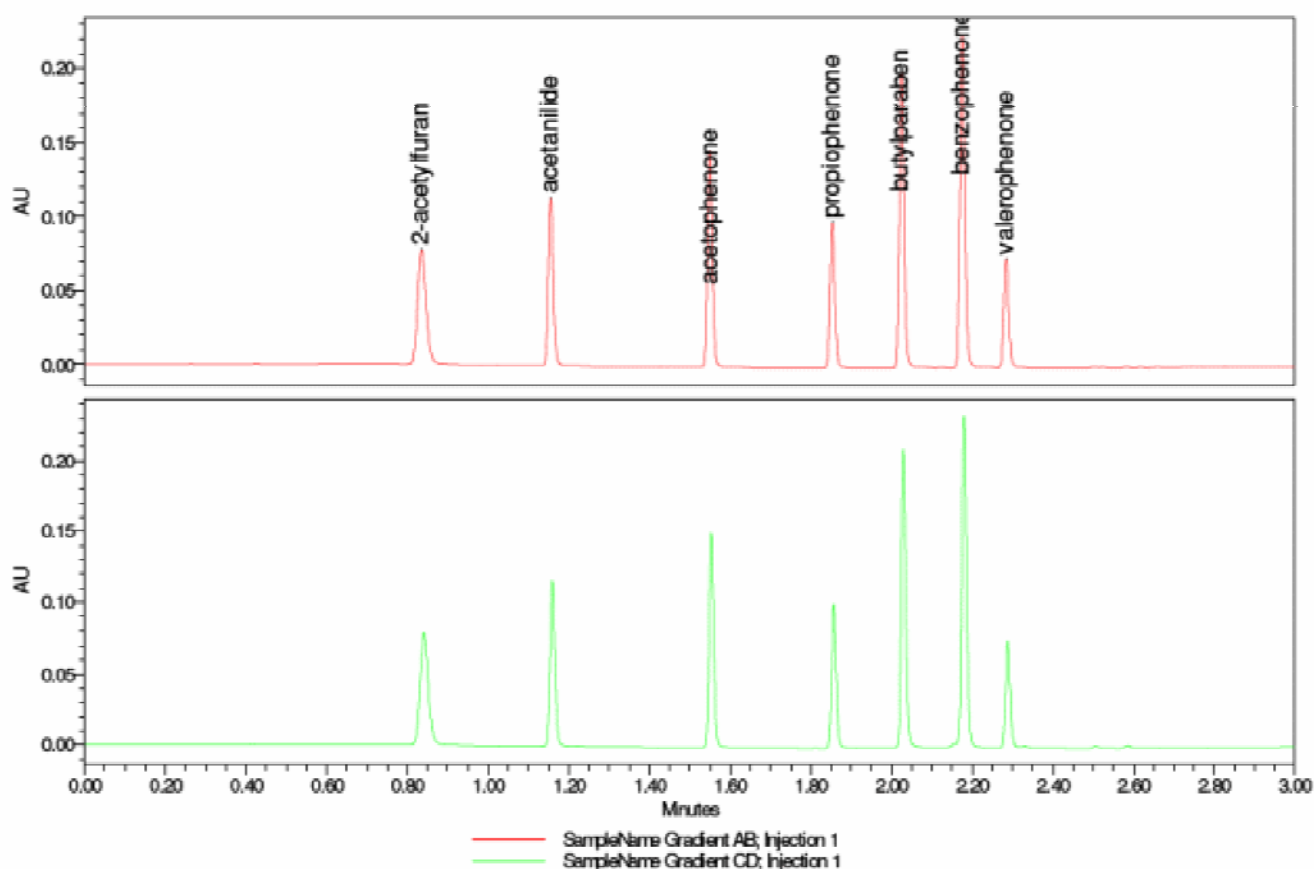
	Name	Retention Time (Seconds) Standard Deviation Specification	Retention Time (Seconds) Standard Deviation	Retention Time (Seconds) Standard Deviation Pass Fail
1	2-acetylfuran	SD RT < or = 1.0sec		
2	acetanilide	SD RT < or = 1.0sec		
3	acetophenone	SD RT < or = 1.0sec		
4	propiofenone	SD RT < or = 1.0sec		
5	butylparaben	SD RT < or = 1.0sec		
6	benzophenone	SD RT < or = 1.0sec		
7	valerophenone	SD RT < or = 1.0sec		

Retention Time Reproducibility CD

	Name	Retention Time (Seconds) Standard Deviation Specification	Retention Time (Seconds) Standard Deviation	Retention Time (Seconds) Standard Deviation Pass Fail
1	2-acetylfuran	SD RT < or = 1.0sec		
2	acetanilide	SD RT < or = 1.0sec		
3	acetophenone	SD RT < or = 1.0sec		
4	propiofenone	SD RT < or = 1.0sec		
5	butylparaben	SD RT < or = 1.0sec		
6	benzophenone	SD RT < or = 1.0sec		
7	valerophenone	SD RT < or = 1.0sec		

- ❑ Modify your QSM instrument method to use a Pre-Inject volume of 350ul and run 1 more injection of the gradient mixture.
- ❑ Overlay this injection with one of your previous CD gradient test injections to see the effect of the using a Pre-Inject volume on the chromatography.

Gradient Performance Chromatogram Overlay



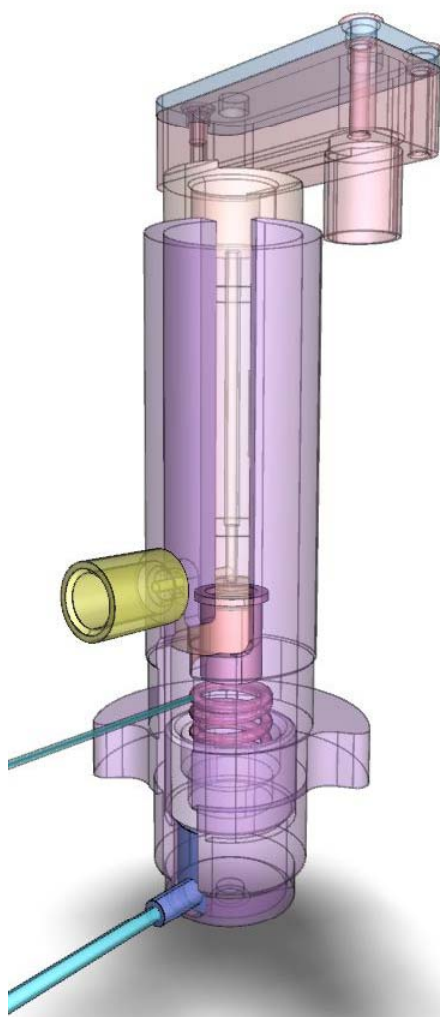
Gradient Performance Summary Results

Component Summary For A RTsec

	SampleName	Inj	Vial	Result Id	2-acetylfuran	acetanilide	acetophenone	propiophenone	butylparaben	valerophenone	benzophenone
1	Gradient AB	1	2:B,8	3511	52.70	68.58	91.43	109.06	118.46	127.65	134.51
2	Gradient AB	2	2:B,8	3512	52.19	68.21	91.14	108.84	118.29	127.50	134.37
3	Gradient AB	3	2:B,8	3513	52.00	68.13	91.10	108.87	118.34	127.53	134.40
Mean					52	68	91	109	118	128	134
Std. Dev.					0.36	0.24	0.18	0.12	0.09	0.08	0.07



Section 5 PM Procedures



Replace/rebuild procedures/diagnostic tests

- ☐ Familiarize yourself with PM procedures as detailed in the “ACQUITY UPLC® H-Class and H-Class Bio Quaternary Solvent Manager Performance Maintenance Technical Reference” document (715002451) available on the portal.

The following performance maintenance procedures and tests for the ACQUITY UPLC® Quaternary Solvent Manager can be found in the ACQUITY UPLC H-Class Quaternary Solvent Manager Operator's Overview and Maintenance Information, in the ACQUITY UPLC Console online help, or in the performance maintenance protocol.

Note: only items in bold will be performed in this training as the others are the same as the SM.

The procedures include:

- ☐ *Checking firmware version*
- ☐ *Inspecting the fan*
- ☐ *Inspecting the waste management*
- ☐ *Reviewing error logs*
- ☐ **Replacing the door air filter**
- ☐ *Replacing the solvent filters*
- ☐ *Replacing the plungers*
- ☐ *Replacing the plunger seals, wash seals and O-rings*
- ☐ *Replacing the accumulator head check valve*
- ☐ **Priming the primary head check valve**
- ☐ **Replacing the low pressure GPV filters**
- ☐ *Replacing the 100 µL mixer/filter assembly*
- ☐ **Performing the dynamic leak test**
- ☐ *Create log entries*

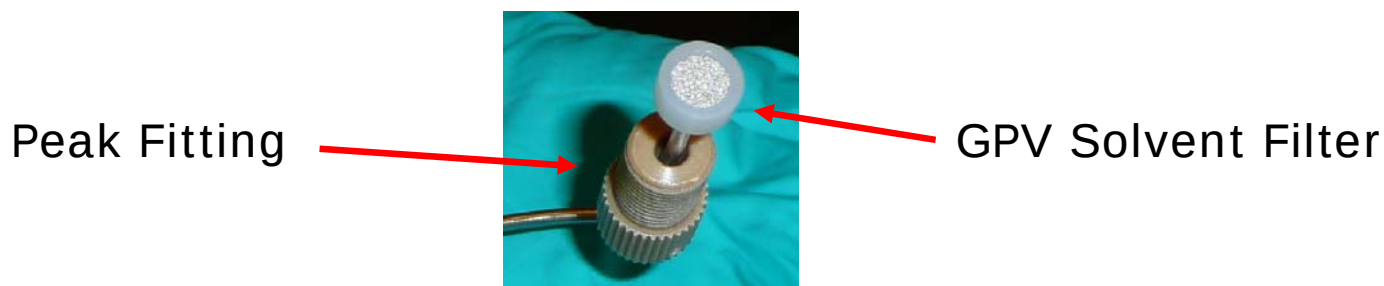
Replacing the GPV Solvent Filters

Important: Wear particulate-free, powder-free non-latex gloves when handling critical clean parts.

Following the procedure in the operators guide, remove the H-Class Solvent Filters from the H-Class valve outlet. It is only necessary to remove and reinstall one filter.

- ❑ Before removing the fitting, go to the QSM interactive menu of the console and click on the Control button.
- ❑ Click on the GPV hyperlink and make sure the valves are turned off. This will prevent any solvent flow during the replacement procedure.
- ❑ Each member of the group should practice this procedure a couple of times to become comfortable and proficient at it.

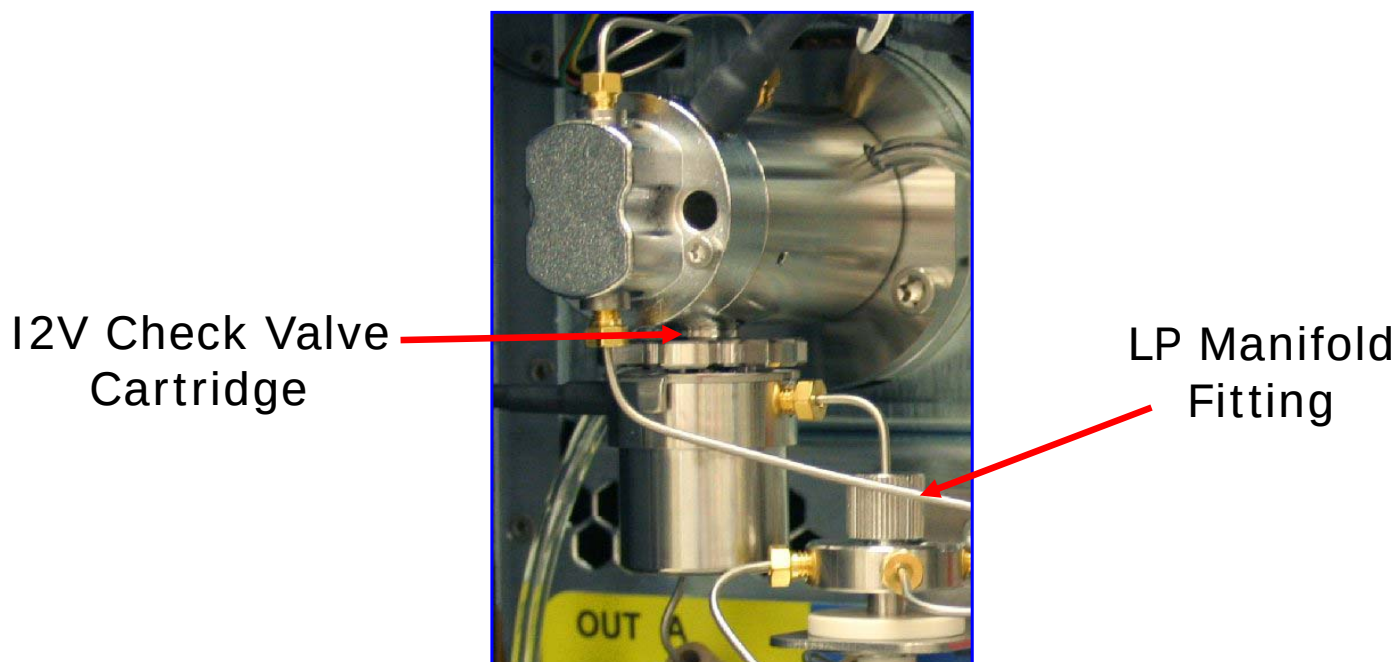
Caution- When reinstalling the outlet tubing ferrule, be careful not to cross-thread the knurled knob fitting. The H-Class valve block threads are easily damaged.



Replacing the Primary Check Valve

Both the Primary and Accumulator Check Valves are normally changed during the PM of the QSM. In our case we will only focus on changing the Primary Check Valve as the Accumulator Check Valve replacement procedure is the same as the BSM. We will perform a virtual PM in this case by removing and replacing the same check valve cartridge.

- ❑ Following the online procedure, remove the Primary and Accumulator Check Valve. Remove only the fittings specified in the procedure.
- ❑ As in the previous procedure, Click on the GPV hyperlink and make sure the valves are turned off. This will prevent any solvent flow during the replacement procedure.
- ❑ Each member of the group should practice this procedure to become comfortable and proficient at it.
- ❑ Reinstall and tighten the I2V when the procedure is completed.



- ❑ Familiarize yourself with PM procedures as detailed in the “ACQUITY UPLC® H-Class and H-Class Bio System Sample Manager - Flow Through Needle Performance Maintenance Technical Reference” document (715002449) available on the portal (see figure on next slide).

The following performance maintenance procedures and tests for the SM_FTN can be found in the ACQUITY UPLC H-Class Sample Manager-FTN Operator's Overview and Maintenance Information, in the ACQUITY UPLC Console online help, or in the performance maintenance protocol.

Note: only items in bold will be performed in this training as the others are the same as the SM.

- ❑ The procedures include:
 - ❑ **Checking firmware version**
 - ❑ Inspecting the fan
 - ❑ **Inspecting the waste management**
 - ❑ Reviewing error logs
 - ❑ **Inspecting the column heater-active**
 - ❑ Replacing the pump outlet tubing
 - ❑ Replacing the sample syringe
 - ❑ **Replacing the sample needle and needle guide**
 - ❑ **Replacing the Vespel seat**
 - ❑ **Replacing the injection valve cartridge**
 - ❑ **Priming the Sample Manager-FTN**
 - ❑ **Calibrating the needle Z-axis**
 - ❑ **Characterizing the needle seal**
 - ❑ **Performing the needle seal readiness test**
 - ❑ Performing the sample syringe leak test
 - ❑ Performing the leak test
 - ❑ Reset the injection counter
 - ❑ Create log entries

Procedures for replacing parts, and diagnostic tests that are not included in the Operator's Guide or performance maintenance protocol will appear in the "ACQUITY UPLC® H-Class and H-Class Bio System Sample Manager - Flow Through Needle Performance Maintenance Technical Reference" document.

☐ The Procedures are:

- ☐ Upgrading the firmware using an Ethernet connection
- ☐ Cleaning the inject/wash station
- ☐ Replacing the side panel filter
- ☐ Replacing the seal extension tube

Replacing the Vespel Seal and seal extension tube

Important: Wear particulate-free, powder-free, non-latex gloves while handling the Stationary Side Seal, tubing and other critical clean parts.

- ☐ Following the procedure, remove and replace the Vespel Seal. The seal can be removed and replaced without damage.
- ☐ While performing this procedure, take the time to disassemble and reassemble all the components of the inject block assembly. A detailed diagram of the assembly is provided on the next page.

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ACQUITY UPLC H-Class

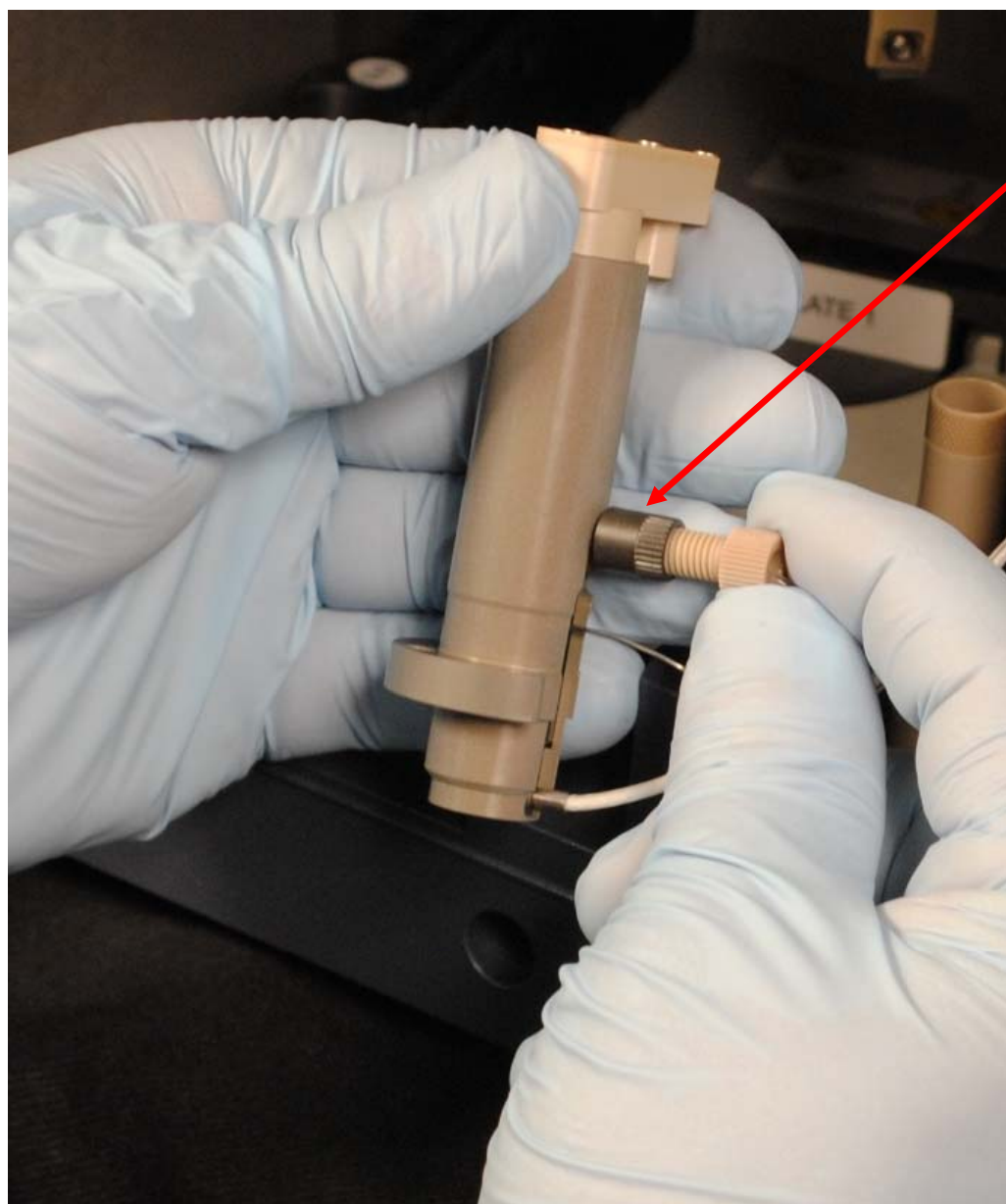
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 - [Sensor Interface PCB](#)
 - [CCPU PCB](#)
 - [Motor/HC Driver PCB](#)
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- [Sample Organizer](#)

Primary Window

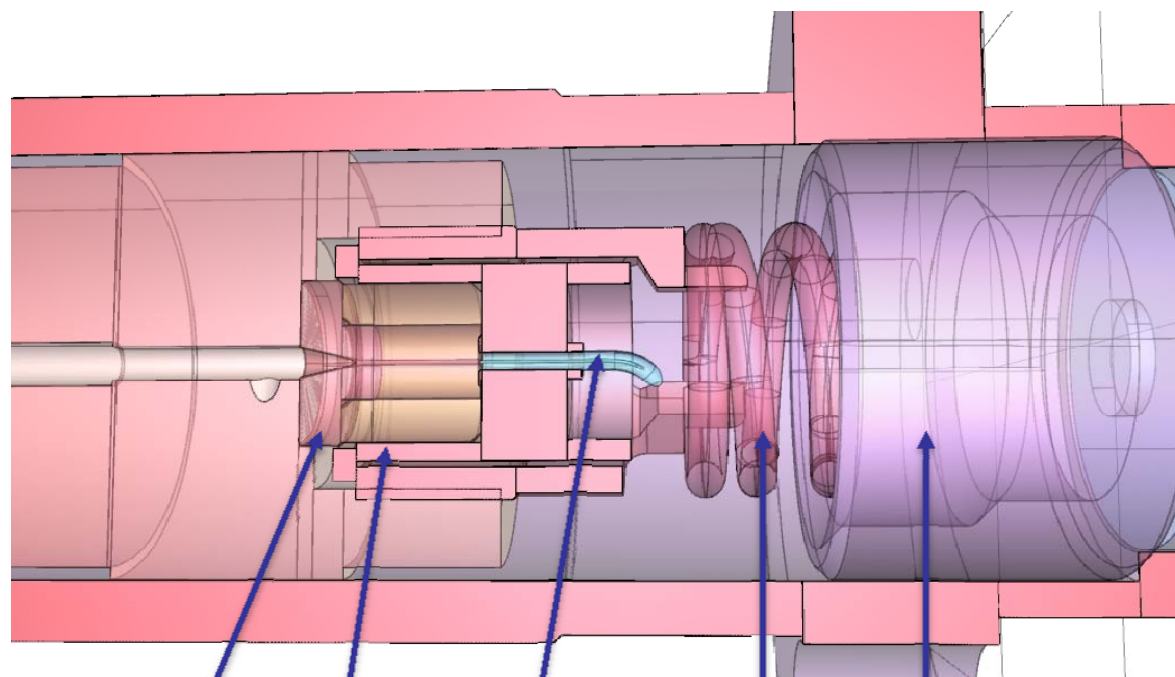
Location of SM-FTN PM Protocol on portal.

*Important: When reattaching the black peak wash line fitting to the needle wash housing, **do not over-tighten the fitting.** This is a low pressure fitting. Finger tight is sufficient. Over-tightening can result in breakage of the peak fitting.*



Peak Wash
Line Fitting

Needle Seal Housing Assembly



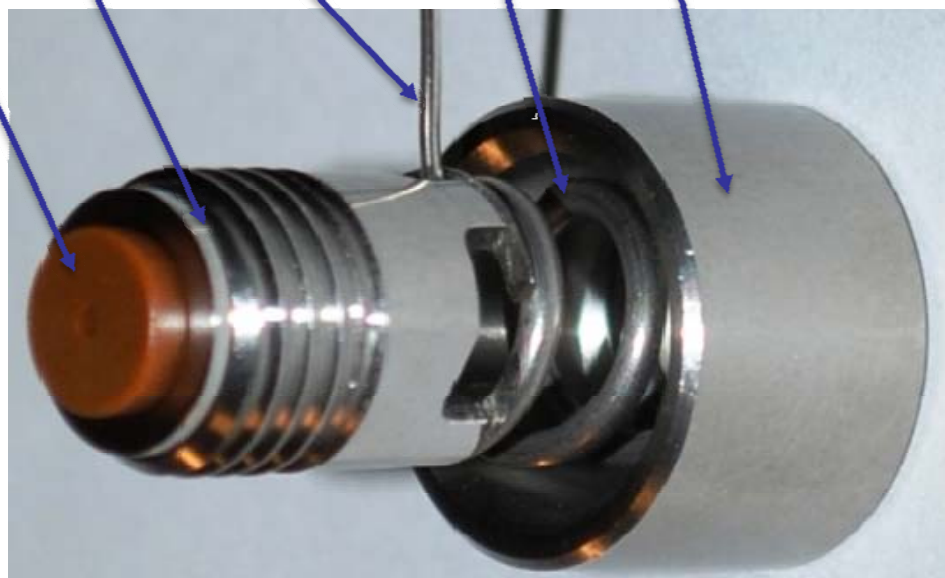
Vespel Seal

Stationary Side
Cup Assembly

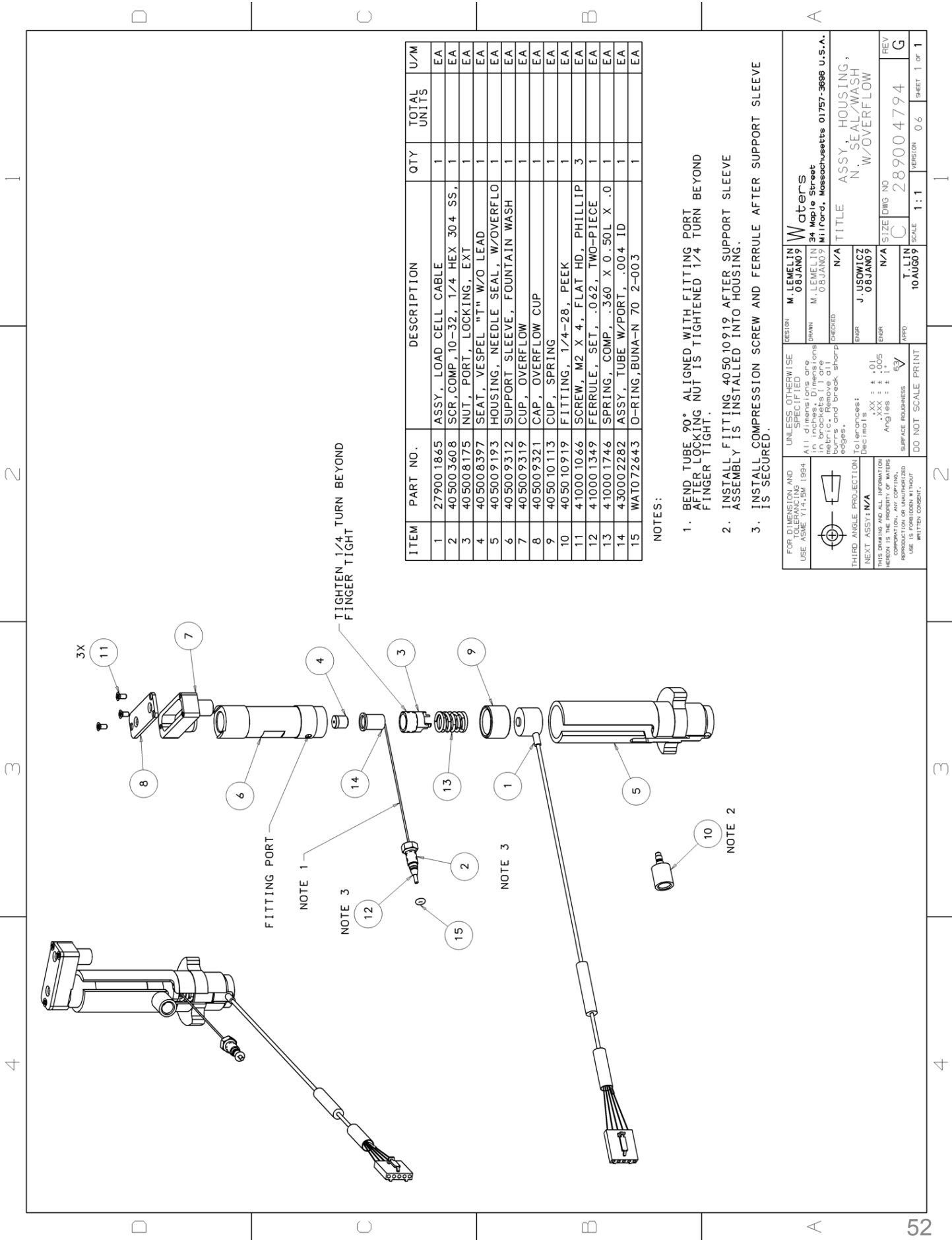
Stationary Side
Tubing (to inject
valve)

Compression
Spring

Spring Cup



Needle Seal Wash Housing Assembly



ITEM	PART NO.	DESCRIPTION	QTY	TOTAL UNITS	U/M
1	279001865	ASSY, LOAD CELL CABLE	1		EA
2	405003608	SCR, COMP, 10-32, 1/4 HEX 30.4 SS,	1		EA
3	405008175	NUT, PORT, LOCKING, EXT	1		EA
4	405008397	SEAT, VESPEL "T" W/O LEAD	1		EA
5	405009193	HOUSING, NEEDLE SEAL, W/OVERFLOW	1		EA
6	405009312	SUPPORT SLEEVE, FOUNTAIN WASH	1		EA
7	405009319	CUP, OVERFLOW	1		EA
8	405009321	CAP, OVERFLOW CUP	1		EA
9	405010113	CUP, SPRING	1		EA
10	405010919	FITTING, 1/4-28, PEEK	1		EA
11	410001066	SCREW, M2 X 4, FLAT HD, PHILLIP	3		EA
12	410001349	FERRULE, SET, .062, TWO-PIECE	1		EA
13	410001746	SPRING, COMP, .360 X 0.50L X .0	1		EA
14	430002282	ASSY, TUBE W/PORT, .004 ID	1		EA
15	WAT072643	O-RING, BUNA-N 70 2-003	1		EA

NOTES:

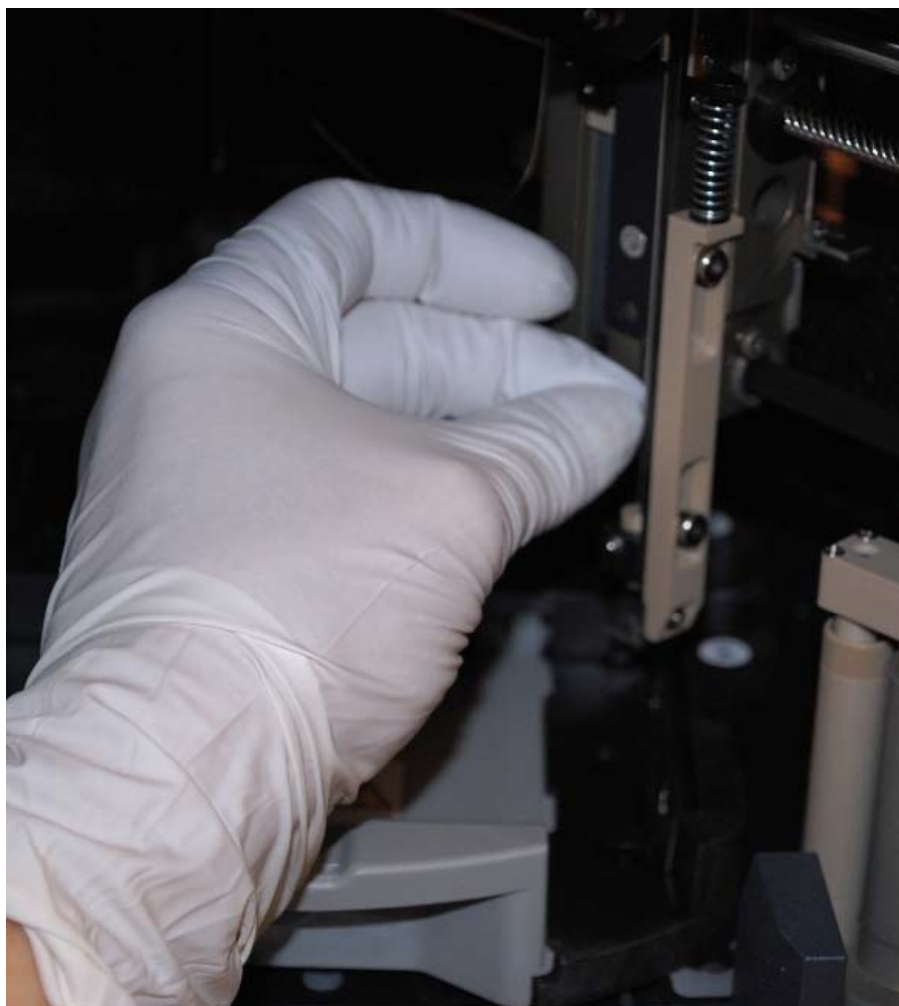
1. BEND TUBE 90° ALIGNED WITH FITTING PORT AFTER LOCKING NUT IS TIGHTENED 1/4 TURN BEYOND FINGER TIGHT.
2. INSTALL FITTING 405010919 AFTER SUPPORT SLEEVE ASSEMBLY IS INSTALLED INTO HOUSING.
3. INSTALL COMPRESSION SCREW AND FERRULE AFTER SUPPORT SLEEVE IS SECURED.

FOR DIMENSIONING AND TOLERANCING USE ASME Y14.5M 1994	UNLESS OTHERWISE SPECIFIED All dimensions are in inches and fractions of inches in brackets () are metric. Remove all leading zeros and break sharp edges.	DESIGN M. LEVELIN 08JAN09	Waters 34 Maple Street Milford, Massachusetts 01757-3686 U.S.A.
THIRD ANGLE PROJECTION	Tolerances: Decimals .XX : ± .01 .XXX : ± .005 Angles : ± .63°	ENGR J. USOWICZ 08JAN09	TITLE ASSY, HOUSING, N. SEAL/WASH W/OVERFLOW
NEXT ASSY: N/A	DO NOT SCALE PRINT	ENGR T. LIN 10AUG09	SIZE C
THIS DRAWING AND ALL INFORMATION HEREON ARE THE PROPERTY OF WATERS CORPORATION. ANY COPYING, REPRODUCTION OR UNAUTHORIZED USE IS FORBIDDEN WITHOUT WRITTEN CONSENT.	SCALE 1:1	VERSION 0.6	REV G
			SHEET 1 OF 1

Replacing the Sample Needle

Important: Wear particulate-free, powder-free, non-latex gloves while handling the Sample Needle and other critical clean parts.

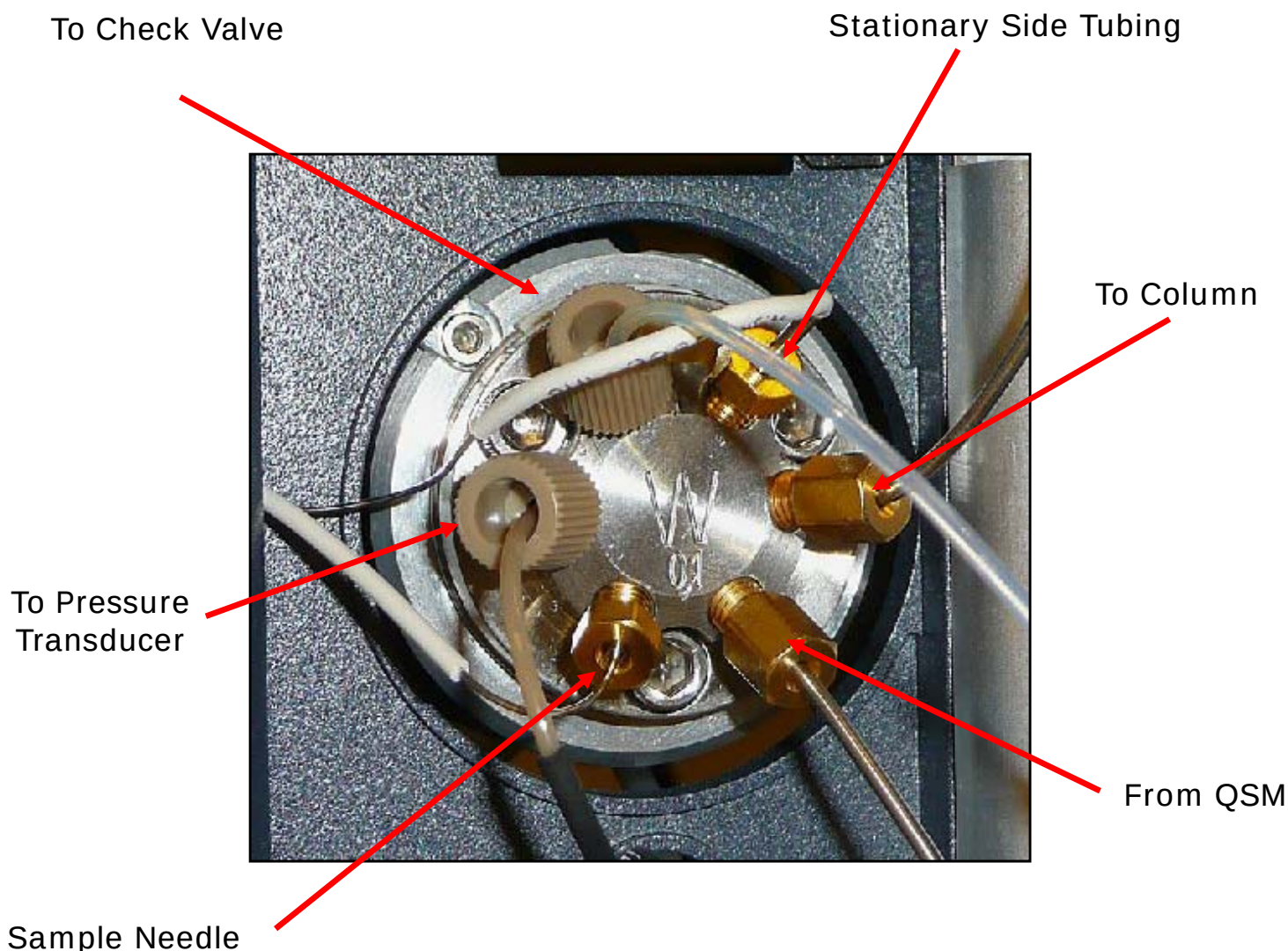
- ❑ Following the procedure in the operators guide, remove and replace the Sample Needle. The same Sample Needle can be removed and replaced without the need for a new one in our case.
- ❑ Practice this procedure several times to get proficient at it. Before reattaching the fitting to the inject valve, perform the next procedure: “Replacing Injector Pod Assembly”.



Replacing the Injector Pod Assembly

- ❑ Following the procedure, remove and replace the Injector Pod.
- ❑ The details of the Injector Pod fitting attachments are listed below.

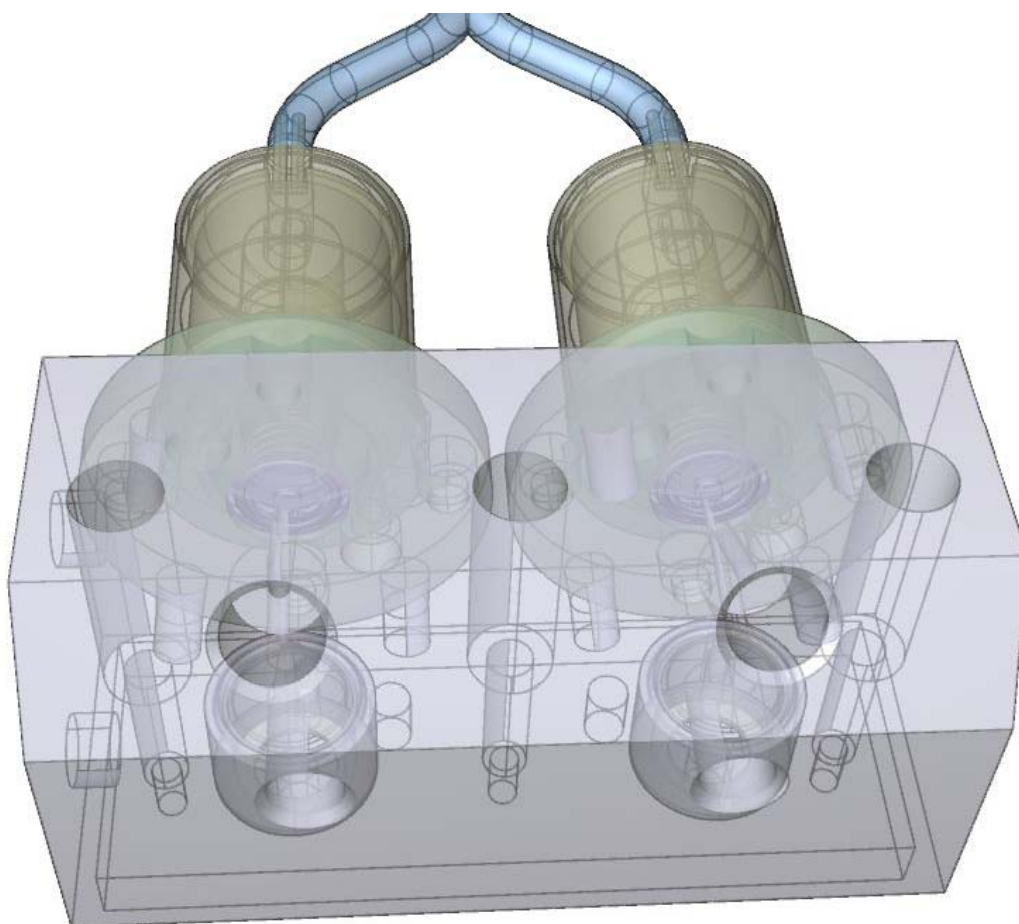
Important: The specification for re-attaching the high pressure fittings is $\frac{1}{4}$ turn past finger tight.





Section 6

QSM Service Procedures



- ❑ Familiarize yourself with all the major service procedures for the QSM located in the GPL section of the portal as detailed in the figure below.
- ❑ Service & Upgrade Procedures
 - ❑ Replacing the CCPU PCB
 - ❑ Performing a Siphon Check
 - ❑ Replacing the CPU/Personality Card
 - ❑ Replacing the Degasser Pump
 - ❑ Replacing a Dual Channel GPV
 - ❑ Replacing the GPV, Inline Filter, and Degasser Chamber
 - ❑ Replacing the GPV Tray Assembly
 - ❑ Replacing the Inline GPV Filters
 - ❑ Replacing the Power Supply
 - ❑ Replacing a Single-channel Degasser Chamber
 - ❑ Installing a Solvent Select Valve
 - ❑ Replacing the Vacuum Transducer PCB

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ACQUITY UPLC H-Class

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 - [Replacing the CPU/Personality Card](#)
 - [Replacing the Degasser Pump](#)
 - [Replacing a Dual Channel GPV](#)
 - [Replacing the GPV, Inline Filter, and Degasser Chamber](#)
 - [Replacing the GPV Tray Assembly](#)
 - [Replacing the Inline GPV Filters](#)
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 - [Replacing the Vacuum Transducer PCB](#)
- [Sample Organizer](#)

Primary Window

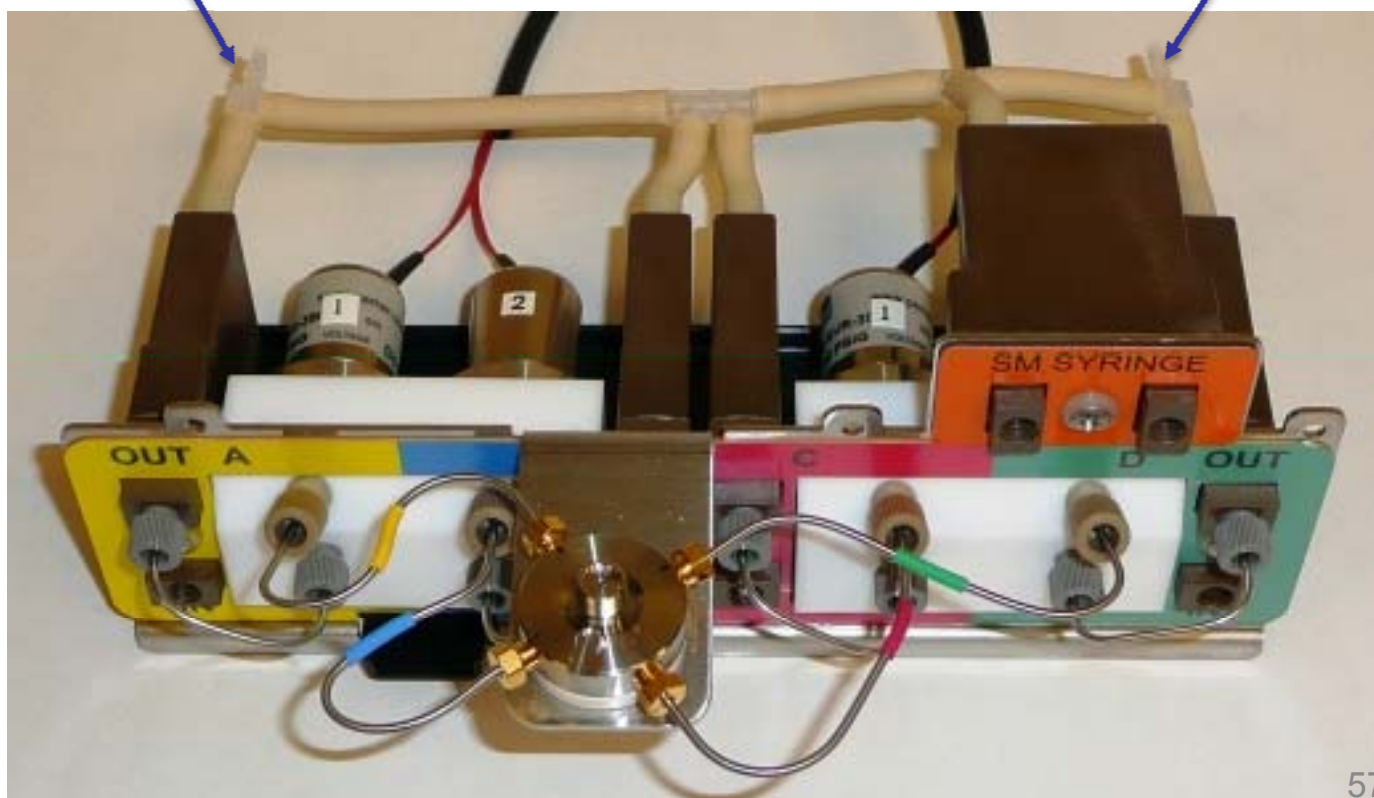
- ❑ Following the Replacing a Dual Channel GPV procedure, remove the GPV Assembly.
- ❑ Once you have the assembly out of the QSM. Remove and replace the C and D GPV pair.
- ❑ Remove and replace the degasser chamber for solvent line A. **Do not take it apart. It is not a serviceable item!**
- ❑ Re-install the degasser chamber and GPV valve pair and re-connect the vacuum and electrical connections.
- ❑ Leave the GPV assembly outside the QSM for now.

Troubleshooting Degasser Issues

- ❑ Remove the vacuum line from the SM degasser chamber.
- ❑ Open up the console and then power up the QSM. You should get a degasser related error message.

From Vacuum Pump

To Vacuum Gauge PCB



Did you receive a degasser related error message?

Error message- _____
_____.

- ☐ Power down the QSM.
- ☐ Open the side panel of the QSM
- ☐ Remove the vacuum pump line from the GPV degasser assembly (see picture on previous page).
- ☐ Remove the vacuum line from degasser gauge.
- ☐ Attach the vacuum pump line directly to the degasser gauge.
- ☐ Power up the QSM again and check the console. Did you get a degasser related error message this time?

This test will determine if the vacuum pump is pumping correctly and that the vacuum inlet line holds sufficient pressure.

You can check the vacuum gauge line by connecting it straight to the vacuum pump.

The vacuum gauge can also be checked separately by connecting the tubing and pulling a vacuum using a priming syringe while watching the Degasser plot in the ACQUITY console.

- ☐ Power down the QSM.
- ☐ Re-attach the vacuum line from the SM degasser chamber.
- ☐ Re-install the GPV assembly back into the QSM.

Removing the Degasser Pump

- ☐ Following the proper procedure, remove the Degasser pump from the rear of the chassis.
- ☐ Re-install and reconnect the vacuum tubing.

Removing the CCPU and Personality PCB

- ❑ Following the proper procedure, remove the CCPU and Personality PCB.

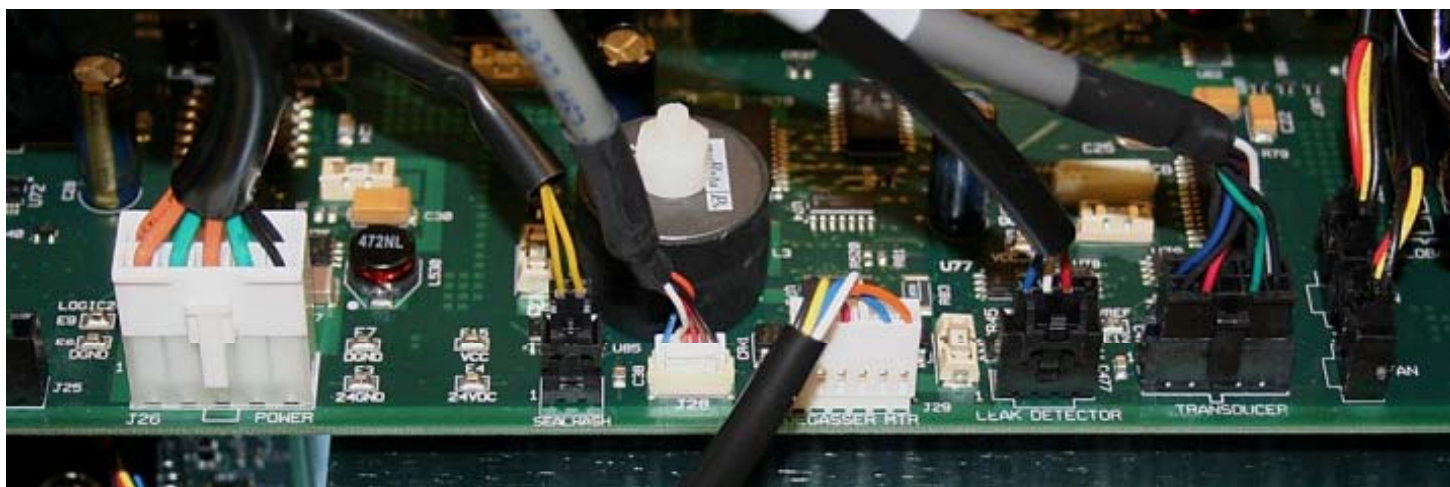


Warning- Beware of live AC and DC voltages when troubleshooting and servicing with side panels removed. Always remove the power cord from the QSM before touching any of the electronics. Make sure you are properly grounded against static discharge.

- ❑ You can try this procedure from the side panel only and alternately with the top panel open as well.
- ❑ Re-install the boards and reconnect all electrical cables except for the power connector (the picture below will show you some of the connection locations).

Removing the Power Supply

- ❑ Following the proper procedure, remove the Power Supply from the back of the QSM.
- ❑ Re-install the Power Supply and reconnect all electrical cables.
- ❑ Plug in the unit and check that the unit is functioning properly in the console. You will re-prime and test the system after all service procedures are completed.

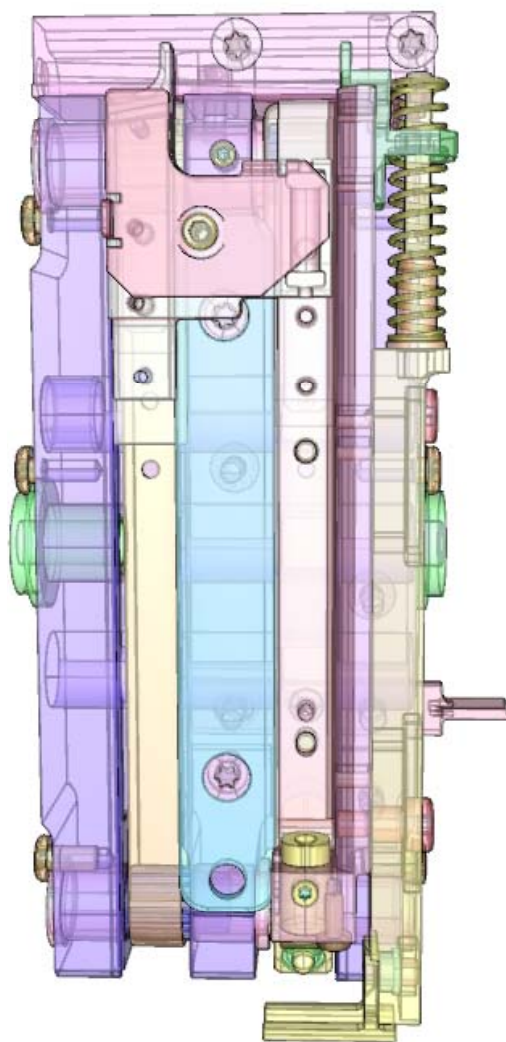


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Section 7 SM-FTN Service Procedures



Removing the RZZ Mechanism Assembly

- ❑ Remove the power cord and both the top and right side covers.

 *Warning- Beware of live AC and DC voltages when troubleshooting and servicing with side panels removed. Always remove the power cord from the SM-FTN before touching any of the electronics.*

- ❑ Remove the top foam insulation covering the Sample Compartment (see photos below for screw locations)
- ❑ Following the proper procedure, remove the RZZ Mechanism Assembly.

Warning- Be very careful handling disconnecting the Needle Carriage flex cable and the Load Cell cable as they are easily damaged.

- ❑ Examine the assembly diagram on the next page and identify all the major parts on the actual assembly.

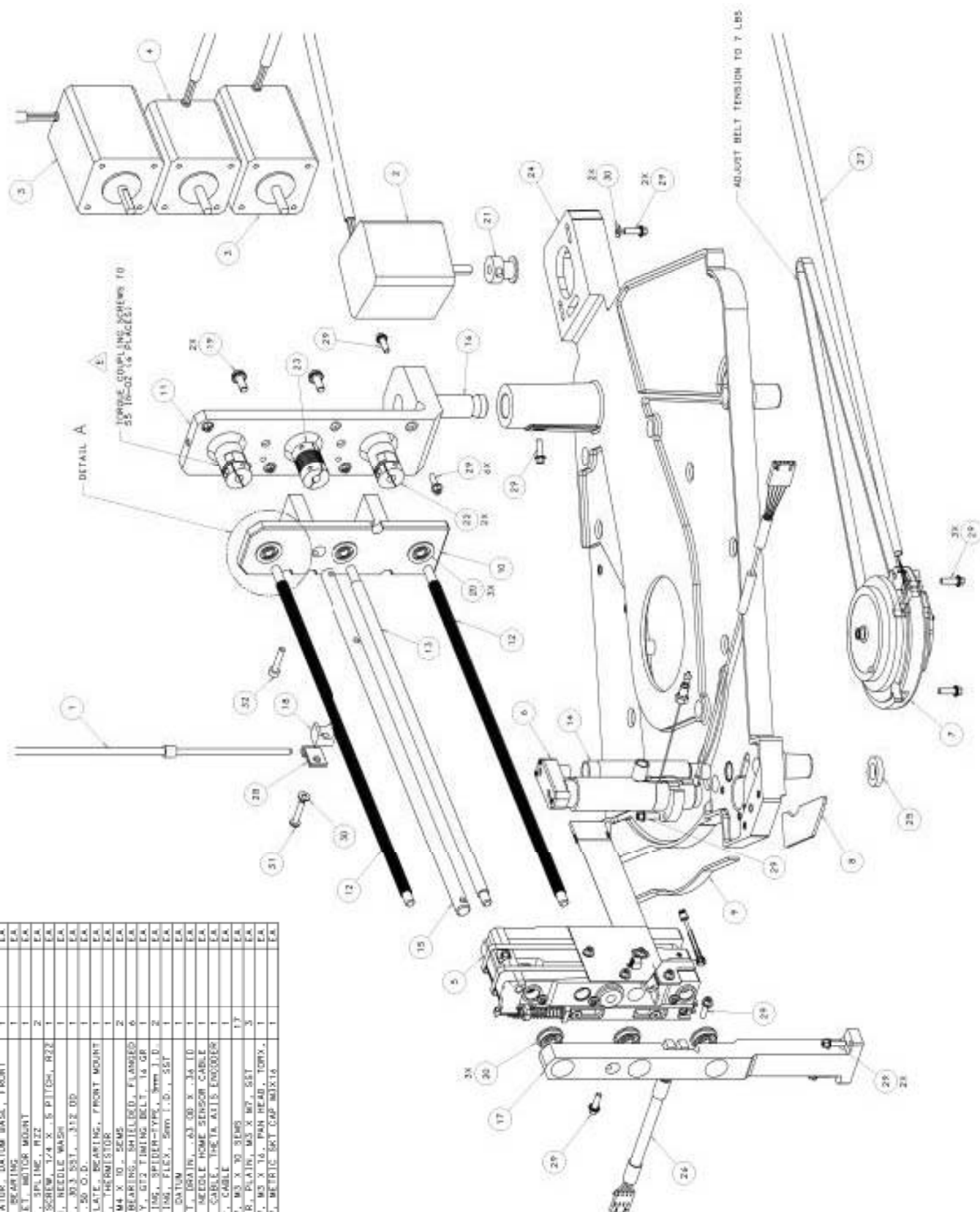


RZZ Mechanism Assembly



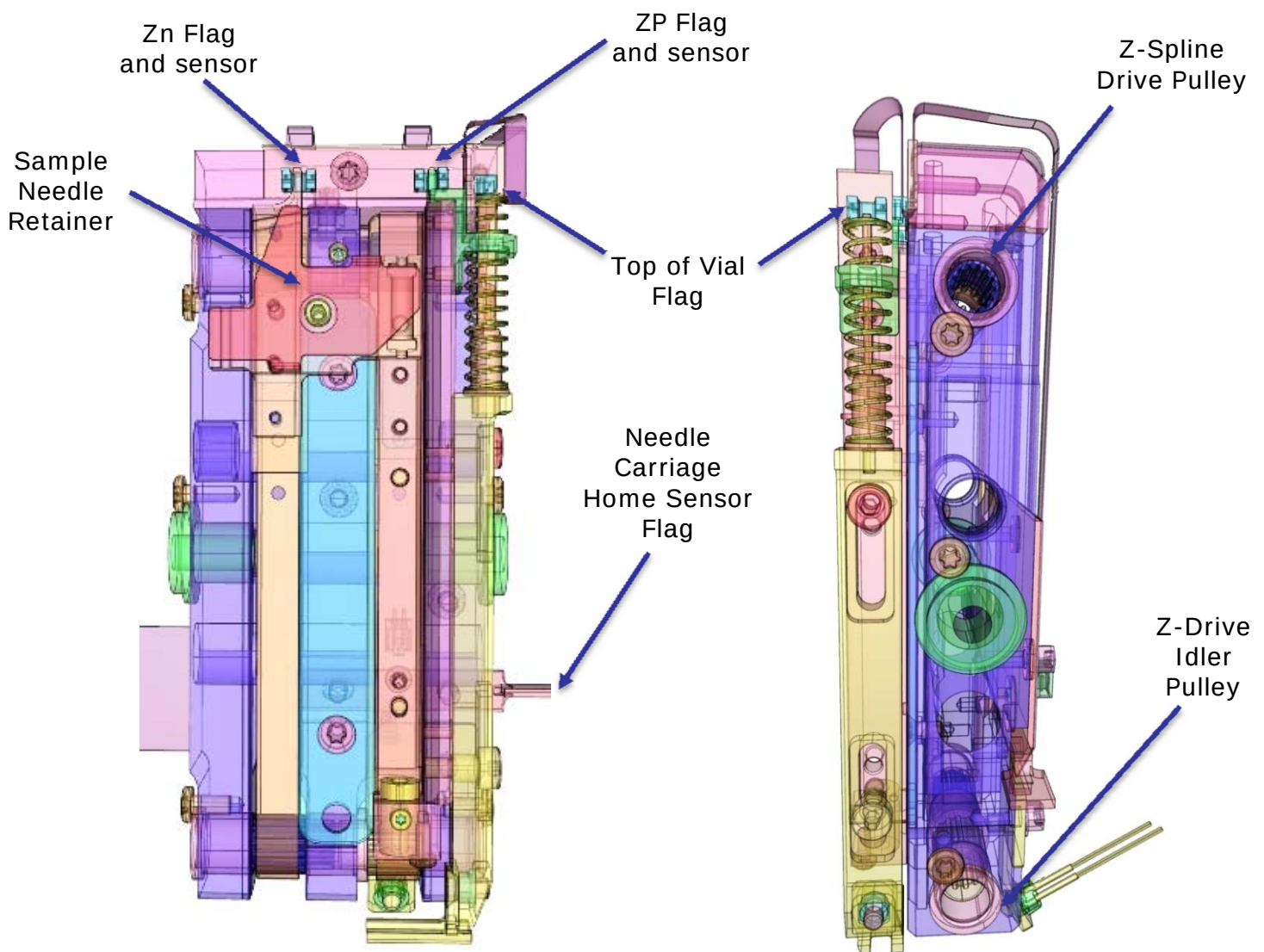
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ITEM	PART NO.	DESCRIPTION	QTY	TOTL UNITS	UOM
1	279005842	THEM MOTOR, AUTOAMPLIFIER, NORM-L	1	EA	EA
2	279001737	ASSY, MOTOR, THETA DRIVE	1	EA	EA
3	279001854	ASSY, MOTOR, Z-PM MECHANISM	2	EA	EA
4	279001854	ASSY, MOTOR, P DRIVE	1	EA	EA
5	289004490	ASSY, NEEDLE CARR, ACID B. INJ.	1	EA	EA
6	289004794	ASSY, HOLDING, N. SEAL/WASH WASH	1	EA	EA
7	289004122	ASSY, THETA DRIVE, SPLIT BEARING	1	EA	EA
8	403005796	INSULATOR, DATUM BASE, LOWER FR	1	EA	EA
9	403005799	INSULATOR, DATUM BASE, FRONT	1	EA	EA
10	403008743	CAGE, BEARING	1	EA	EA
11	403008747	BEARING, MOTOR MOUNT	1	EA	EA
12	403008773	SHAFT, SPLINE, RZZ	2	EA	EA
13	403008814	LEAD SCREW, 1/4 X 5 PITCH, RZZ	1	EA	EA
14	403008963	BASE, NEEDLE WASH	1	EA	EA
15	403009344	SHAFT, 3/32 SBT, .312 OD	1	EA	EA
16	403015031	PIN, 50 O.D.	1	EA	EA
17	403015729	END PLATE, BEARING, FRONT MOUNT	1	EA	EA
18	403015734	WASHER, FRONT MOTOR	1	EA	EA
19	403005419	SCREW, 1/4 X 10, 25MM	2	EA	EA
20	403005834	BEARING, SHIM, 1/8" FLANGED	6	EA	EA
21	403003737	PULLEY, GEAR, THETA BELT, 14 GR	1	EA	EA
22	403003119	COUSING, SPIDER-TYPE, 3mm ID	2	EA	EA
23	403003119	COUSING, FLEX, 5mm I.D., SET	1	EA	EA
24	403001513	BASE, DATUM	1	EA	EA
25	403003795	GEAR, 1/8" X 10, 34.10	1	EA	EA
26	443005814	ASSY, NEEDLE HOME SENSOR CABLE	1	EA	EA
27	443005814	ASSY, CABLE, THETA AXIS ENCODER	1	EA	EA
28	443005814	CLAMP, 1/8 X 10, 34.10	1	EA	EA
29	443005814	CLAMP, 1/8 X 10, 34.10	1	EA	EA
30	443005814	CLAMP, 1/8 X 10, 34.10	1	EA	EA
31	443005814	CLAMP, 1/8 X 10, 34.10	1	EA	EA
32	443005814	CLAMP, 1/8 X 10, 34.10	1	EA	EA
33	443005814	CLAMP, 1/8 X 10, 34.10	1	EA	EA
34	443005814	CLAMP, 1/8 X 10, 34.10	1	EA	EA
35	443005814	CLAMP, 1/8 X 10, 34.10	1	EA	EA
36	443005814	CLAMP, 1/8 X 10, 34.10	1	EA	EA
37	443005814	CLAMP, 1/8 X 10, 34.10	1	EA	EA
38	443005814	CLAMP, 1/8 X 10, 34.10	1	EA	EA
39	443005814	CLAMP, 1/8 X 10, 34.10	1	EA	EA
40	443005814	CLAMP, 1/8 X 10, 34.10	1	EA	EA
41	443005814	CLAMP, 1/8 X 10, 34.10	1	EA	EA
42	443005814	CLAMP, 1/8 X 10, 34.10	1	EA	EA
43	443005814	CLAMP, 1/8 X 10, 34.10	1	EA	EA
44	443005814	CLAMP, 1/8 X 10, 34.10	1	EA	EA
45	443005814	CLAMP, 1/8 X 10, 34.10	1	EA	EA
46	443005814	CLAMP, 1/8 X 10, 34.10	1	EA	EA
47	443005814	CLAMP, 1/8 X 10, 34.10	1	EA	EA
48	443005814	CLAMP, 1/8 X 10, 34.10	1	EA	EA
49	443005814	CLAMP, 1/8 X 10, 34.10	1	EA	EA
50	443005814	CLAMP, 1/8 X 10, 34.10	1	EA	EA
51	443005814	CLAMP, 1/8 X 10, 34.10	1	EA	EA
52	443005814	CLAMP, 1/8 X 10, 34.10	1	EA	EA



Removing the Needle Carriage Assembly

- ❑ Following the proper procedure, carefully remove the Needle Carriage Assembly from the RZZ Mechanism.
- ❑ Compare the major assemblies to the diagram but do not take the assembly apart.
- ❑ Reinstall the Needle Carriage Assembly unto the RZZ Mechanism being careful that it is square to all shafts and that the ribs of the shafts fit into the grooves of the pulleys.
- ❑ Do not reinstall the RZZ Mechanism at this time.



Removing the CCPU and Driver PCB's

- ❑ Following the proper procedure, remove the CCPU and Driver PCB's. Keep as many of the cable connections on as possible.
- ❑ Examine the major components of the board using the assembly diagram on the next page as a reference.
- ❑ Drape the boards over the side of the unit to allow room to get to the Ethernet Switch PCB.
- ❑ Remove the Ethernet Switch PCB from the unit.
- ❑ Remove the last two molded foam covers surrounding the Heater Cooler Engine from the unit.

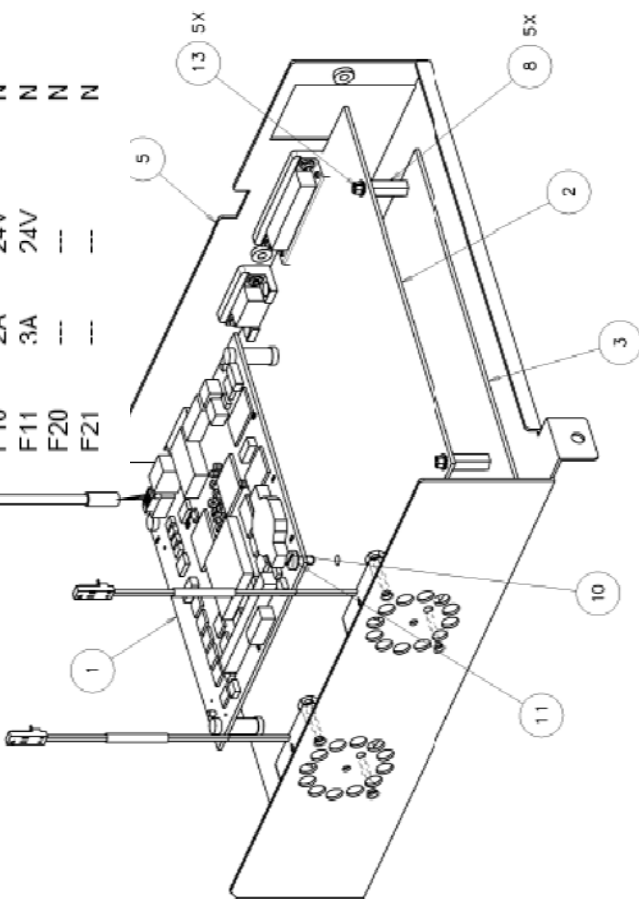
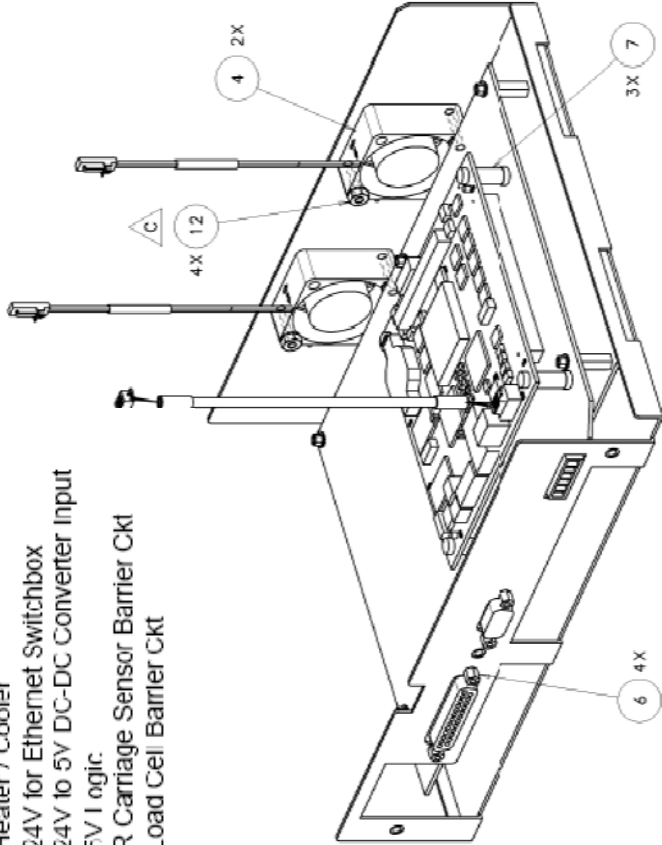


SM-FTN PCB Module Assembly



Control PCB Fuses

REF	Rating	Voltage	Sensed (Y/N)	DESCRIPTION
F4	2A	24V	Y	Fans, Bezel LED's, Pressure X-ducer
F5	---	---	-	
F6	5A	24V	Y	Rear Panel 24V
F7	3A	5V	Y	Rear Panel 5V
F8	7A	24V	Y	Heater / Cooler
F9	3A	24V	N	24V for Ethernet Switchbox
F10	2A	24V	N	24V to 5V DC-DC Converter Input
F11	3A	24V	N	5V I logic
F20	---	---	N	R Carriage Sensor Barrier Ckt
F21	---	---	N	Load Cell Barrier Ckt



ITEM	PART NO.	DESCRIPTION	QTY	TOTAL UNITS	UOM
1	210000273	ASSY, PCB, CPU2K, 3V	1		EA
2	210000367	PCB, CONTROL, CFSM	1		EA
3	210000368	PCB, MOTOR/HC DRIVER, CFSM	1		EA
4	279001786	ASSY, FAN, 24V, 8.1 CFM	2		EA
5	400003513	BRACKET, MOUNTING, PCB	1		EA
6	410000954	SCREWLOCK, FEMALE, 4-40/4-40	4		EA
7	410001195	STANDOFF, PCB, .625 LONG	3		EA
8	410002758	STANDOFF, 6MM HEX X 16MM LG, M3 M	5		EA
9	441000957	ASSY, CABLE, RS232, CPU TO PERS	1		EA
10	WAT022007	SCREW, 6-32X0.5, BINDER HD	1		EA
11	WAT050938	WASHER, LOCK, #6, EXT	1		EA
12	WAT251620	NUT, M4 X 0.7, KEP	4		EA
13	WAT271463	SCREW, M3 X 6 LG, SEMS	5		EA

FOR DIMENSION AND TOLERANCING INFORMATION, SEE ASSEMBLY INSTRUCTION SHEET, 1141-5N 1994

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DATE: 01/11/94

DESIGN: E. DENECKE

DRAWN: E. DENECKE

CHECKED: N/A

DATE: 01/11/94

DESIGN: G. BERTONE

DRAWN: G. BERTONE

CHECKED: N/A

DATE: 01/11/94

DESIGN: N/A

DRAWN: N/A

CHECKED: N/A

Waters

34 Maple Street

Milford, Massachusetts 01757-3000 U.S.A.

TITLE

ASSY, MODULE, PCB

SIZE

27900 1726

SCALE

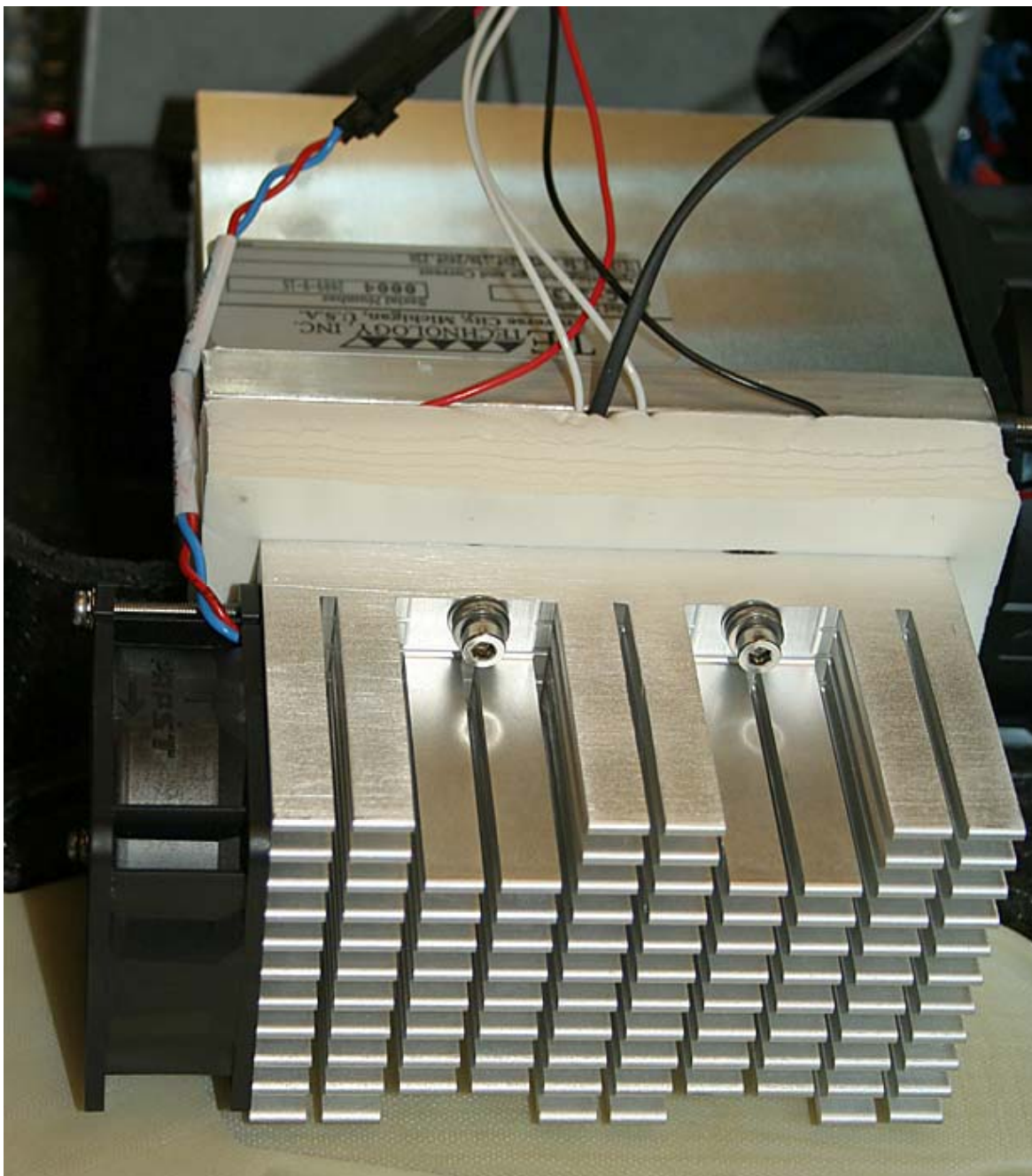
1:1

SHEET

1 OF 1

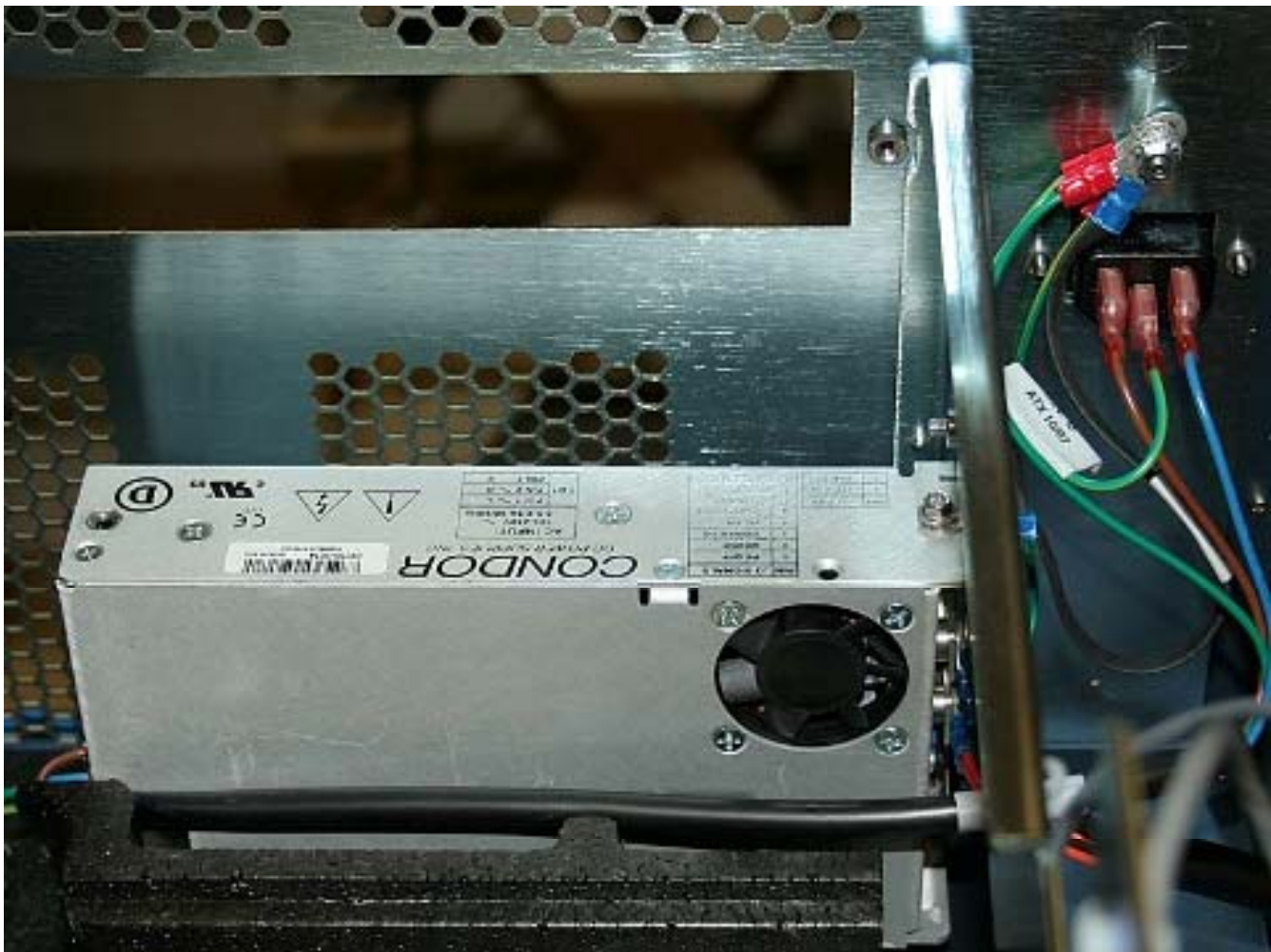
Removing the Heater Cooler Engine

- ❑ Remove the Heater Cooler Engine from the unit.
- ❑ Examine the Heater Cooler Engine and identify all the major parts on the actual assembly without taking it apart. Normally, this unit will be replaced as a whole assembly.



Removing the Power Supply and Datum Base

- ❑ Remove the Power Supply from the unit. This can normally be done without disassembling the unit.
- ❑ Remove the Rotary Tray from the Theta drive bearing.
- ❑ Following the proper procedure, remove the Rotary Tray drive from the unit.
- ❑ Examine the Rotary Tray drive as a unit but do not take it apart. The belt requires a strain gauge to properly set the tension. Refer to the RZZ Mechanism Assembly diagram for details.



Power Supply on Support Foam

- ☐ Reassemble the SM-FTN in the following order:
 - ☐ Power Supply
 - ☐ Heater/Cooler Engine
 - ☐ Heater/Cooler Engine back foam support
 - ☐ Datum Plate and drive assembly
 - ☐ R- Carriage Drive assembly
 - ☐ Heater/Cooler Foam Housing
 - ☐ Ethernet switch
 - ☐ SM-FTN PCB Module Assembly
 - ☐ Sample Compartment Foam Housing
- ☐ Don't hesitate to notify the instructor if you have any issues or questions when reassembling the SM-FTN.
- ☐ Check all cable connections as you go to make sure they are in the proper connector.

Refer to the Electrical Interconnect diagram on the next page or the sticker below the PCB assembly (see picture below) for cable connections.

- ☐ Reconnect the Ethernet cable from the computer and other ACQUITY modules to the SM-FTN Ethernet switch.



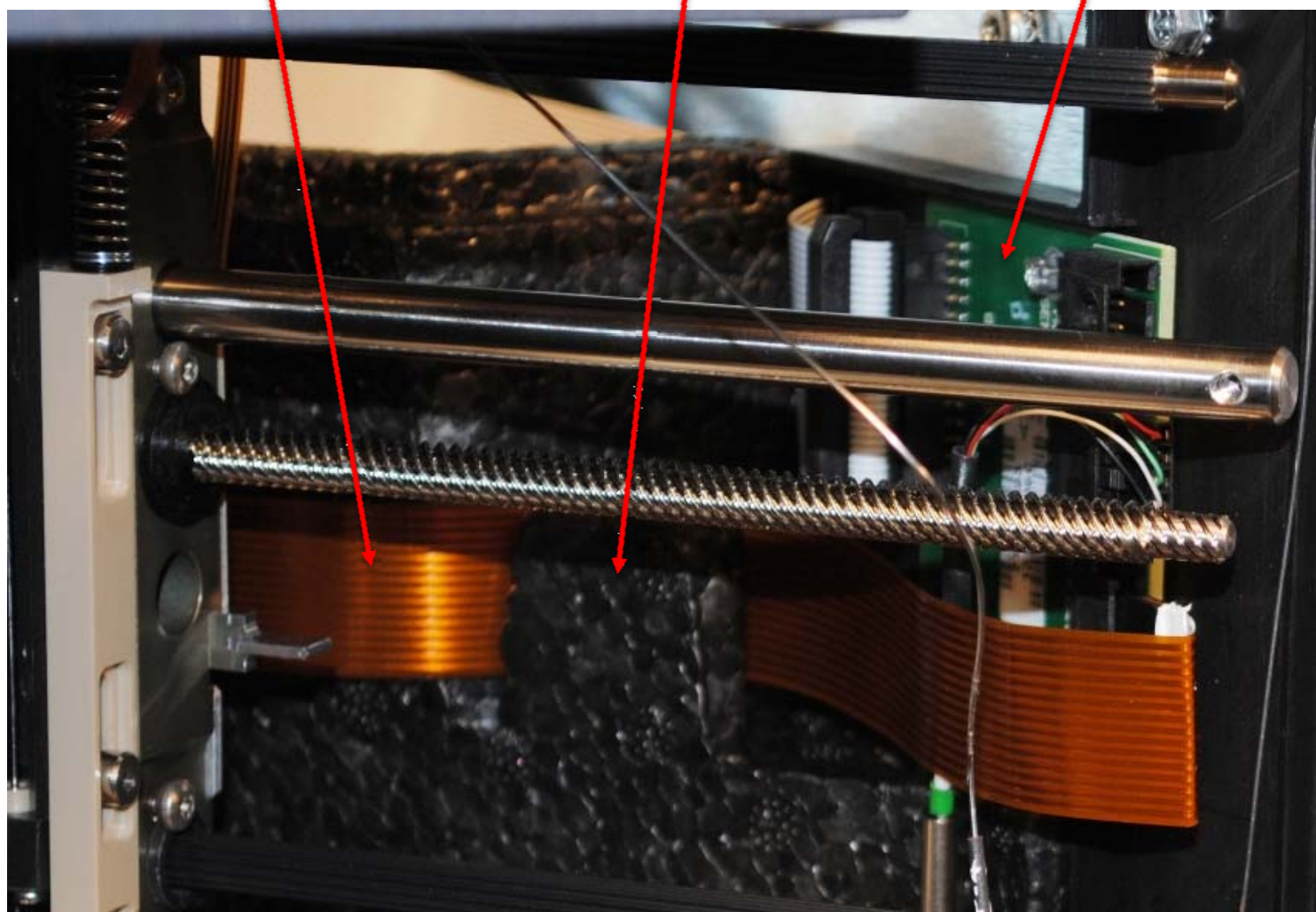
Warning- Make sure to route the needle carriage flex cable through the channel on the foam housing near the Interconnect PCB. Failure to do so could damage the cable on power up! (See Picture on next page).

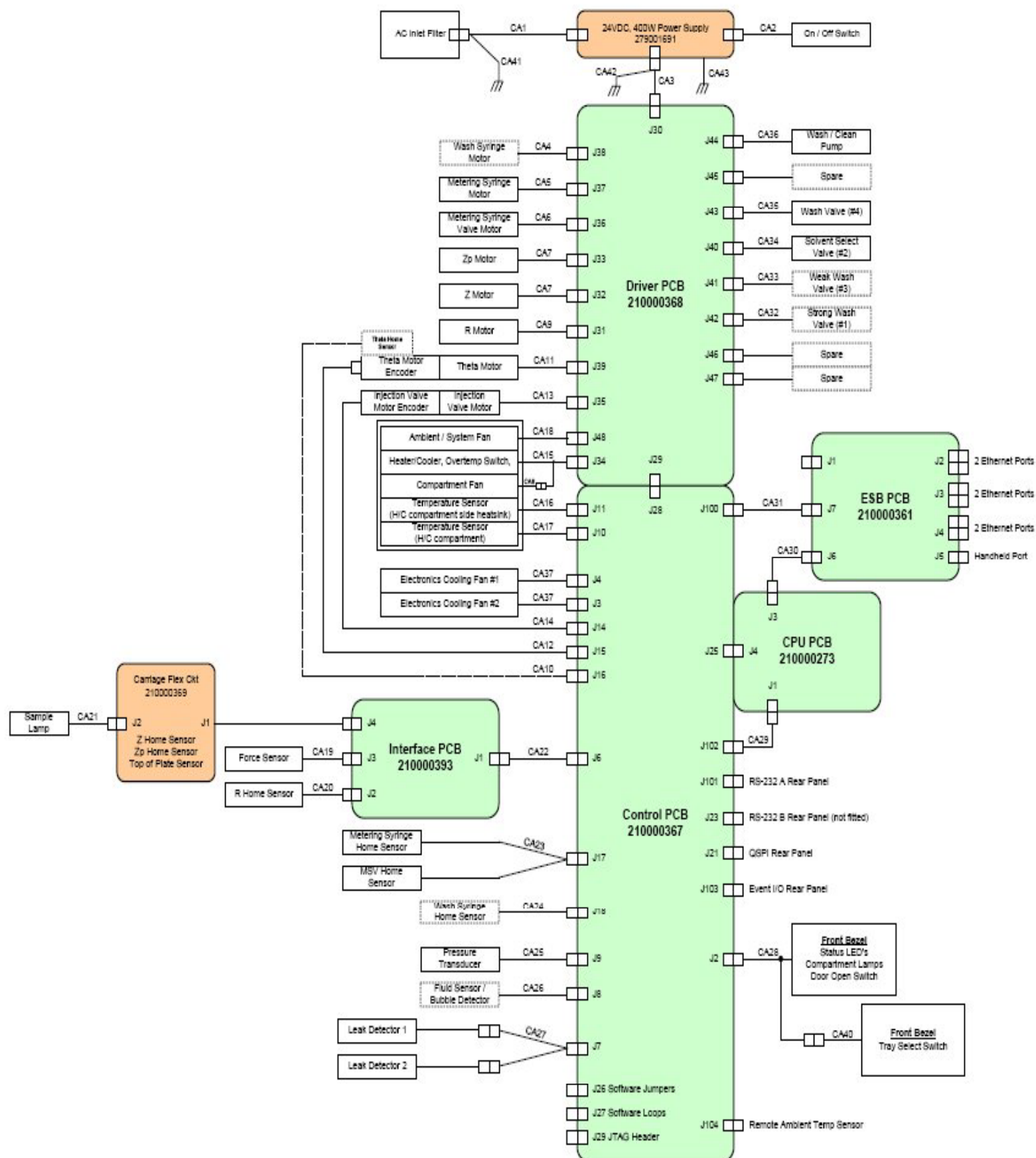
- ❑ Once you are sure all connections are set, connect the power cord and power up the unit.
- ❑ Check the status of the front panel LEDs.
- ❑ Open the console and check the status of the unit.
- ❑ Alert the instructor to any problems you encounter that are not easily fixed.

Needle Carriage
Flex Cable

Flex Cable Guide in
Foam Housing

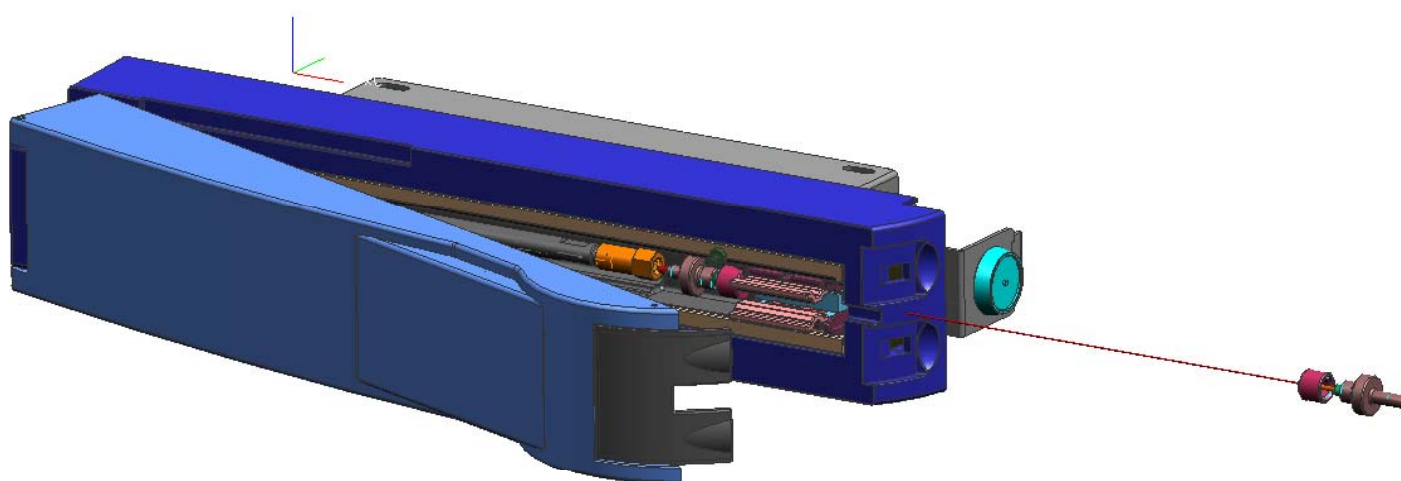
Interface PCB







Section 8 ACQUITY H-Class CH-A



Removing the CH-A Assembly

- ❑ Remove the column and Active Pre-column Heater from the CH-A unit.



Warning- Beware of live AC and DC voltages when troubleshooting and servicing with side panels removed.

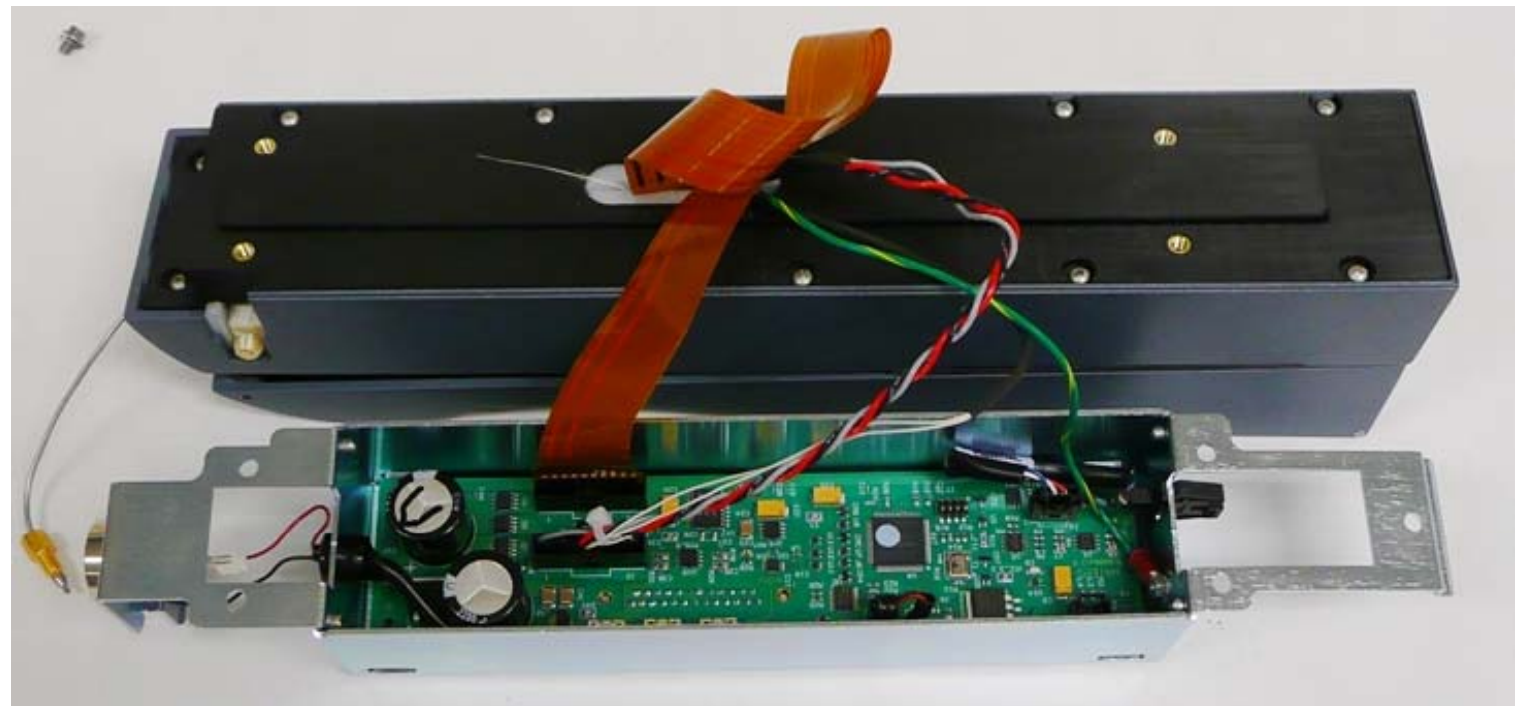
- ❑ Remove the T20 Torx screw from the side of the unit next to the e-Cord reader (see picture below).
- ❑ Following the proper procedure, remove the HTC heater assembly from the front of the chassis.
- ❑ Set the assembly on the lab bench with the column heating compartment facing down.

Remove this T20
Torx screw

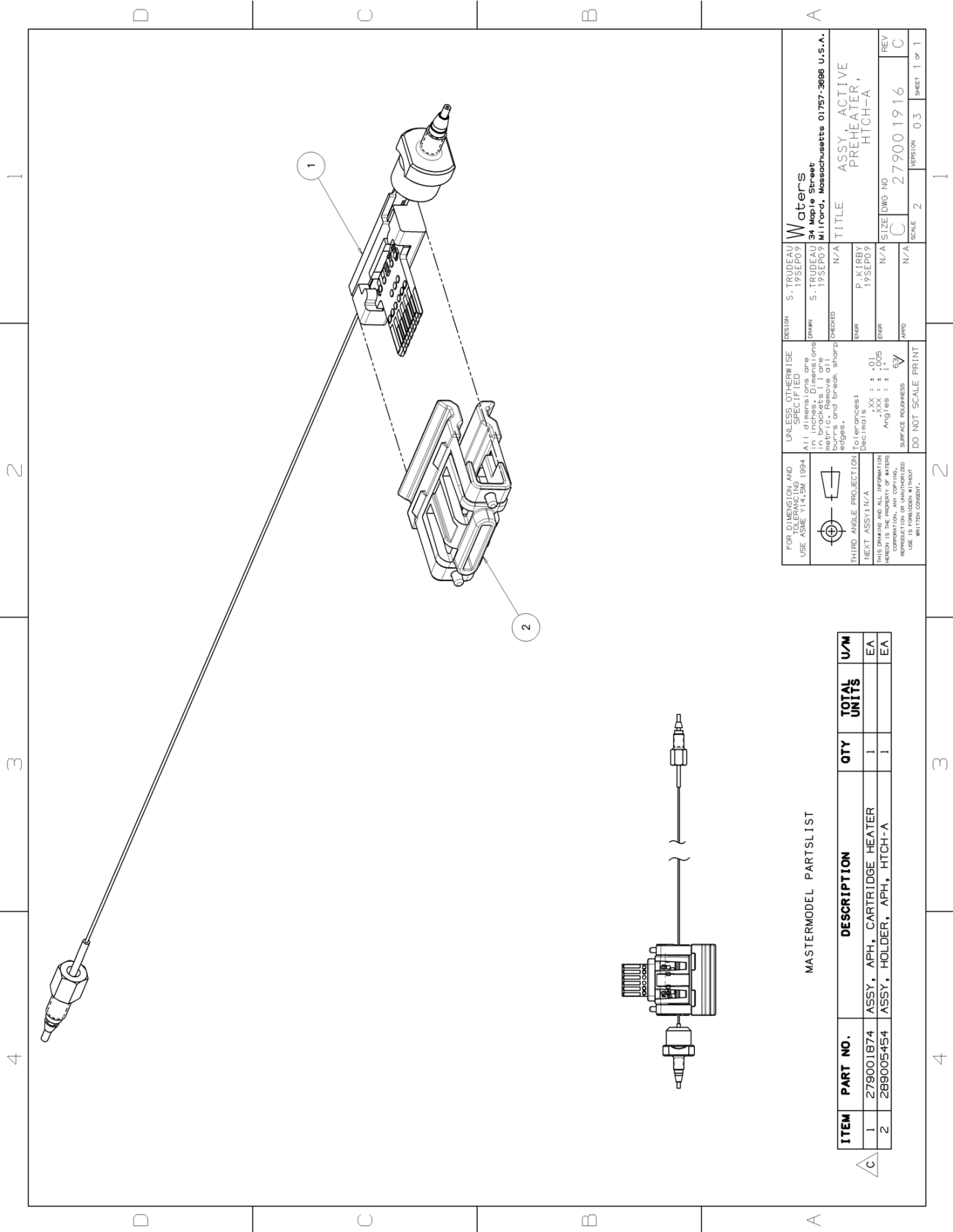


Removing the CH-A Assembly

- ❑ Remove the 5 T20 Torx screws from the back of the heater assembly as shown below.
- ❑ Remove the CH-A PCB assembly from the back of the CH-A. Do not take the PCB assembly apart any further at this time but observe the components and the interconnections.
- ❑ Re-assemble the unit and put it back on heating compartment facing down.



CH-A Active Preheater



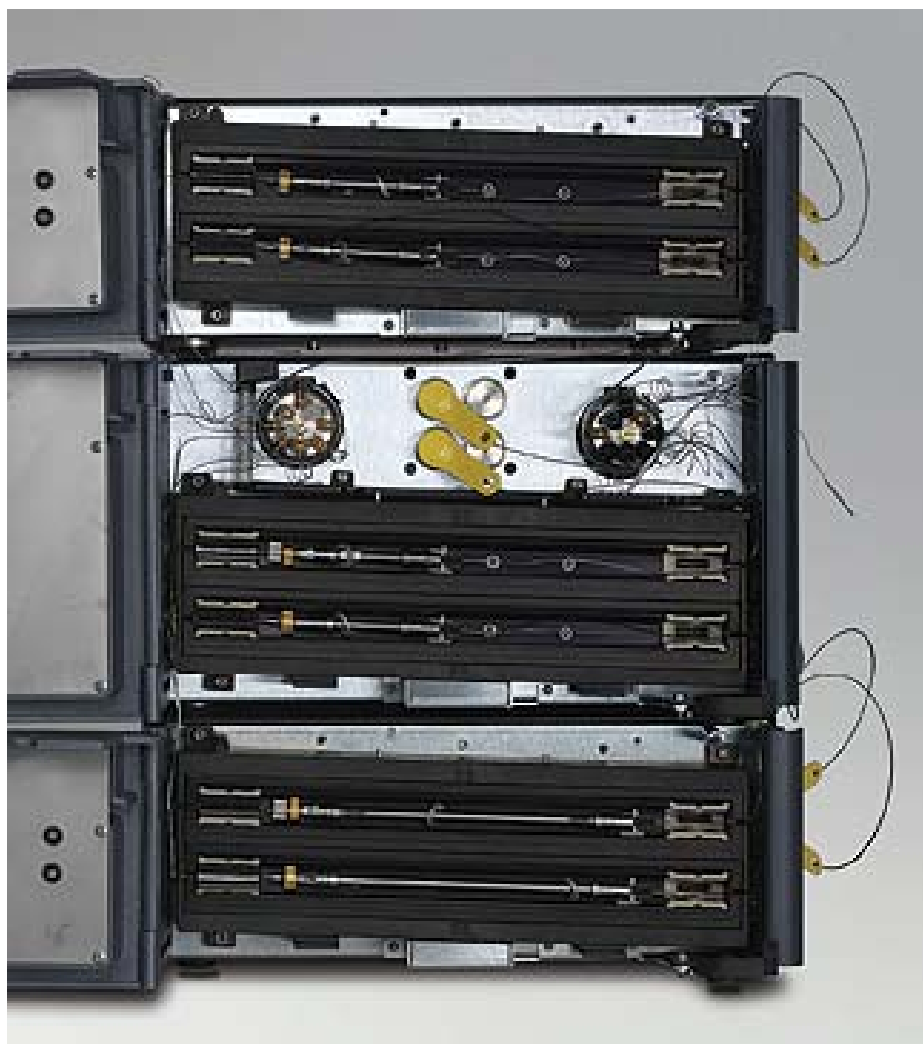
FOR DIMENSION AND TOLERANCE INFORMATION, SEE DRAWING Y14.1M 1994		UNLESS OTHERWISE SPECIFIED, DIMENSIONS ARE IN INCHES, DIMENSIONS IN BRACKETS () ARE IN MILLIMETERS. DIMENSIONS ARE TO BE TAKEN TO THE SURFACE UNLESS OTHERWISE SPECIFIED.		DESIGN S. TRUDEAU 19SEP09		Waters 34 Maple Street Milford, Massachusetts 01757-3698 U.S.A.	
THIRD ANGLE PROJECTION		TOLERANCES: Decimals: .XX : ± .01 Angles : ± 1°		DRAWN S. TRUDEAU 19SEP09		TITLE ASSY. ACTIVE PREHEATER, HITCH-A	
NEXT ASSY: N/A		SURFACE FINISH: Ra .005		CHECKED P. KIRBY 19SEP09		SIZE DWG NO. C 27900 1916	
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				VERSION 03		SHEET 1 of 1	

MASTERMODEL PARTSLIST

ITEM	PART NO.	DESCRIPTION	QTY	TOTAL	U/M
1	279001874	ASSY. APH, CARTRIDGE HEATER	1		EA
2	2890005454	ASSY. HOLDER, APH, HITCH-A	1		EA



Section 9 Column Manager CM-A and CM-Aux



Due to the lower number of Column Managers and Sample Organizers available for this course, your group should alternate this section with the Sample Organizer section following this chapter. Rotate on to the units as they become available.

Reviewing the Sample Organizer Installation Procedure

- While in front of the unit, review the procedure “Installing an H-Class System or H-Class Bio System Column Manager-A / Auxiliary Column Manager (715002684). Thoroughly familiarize yourself with all steps that are in bold below.

The CM-A / CM-Aux Installation Process

- **Step1 Determine the stack position(s) – see "Determining the stack position" on page 4.**
- Step 2 Prepare mobile phases and solvents as described in the ACQUITY UPLC® H-Class System and H-Class Bio System with TUV, PDA, ELS, or FLR Detectors Installation Manual (P/N 715002258), or the ACQUITY UPLC® H-Class System and H-Class Bio System with SQD/3100 or TQD Performance Test (P/N 715002380).
- **Step 3 Position all H-Class modules as required – refer to “Installing the ACQUITY UPLC H-Class System modules” in the ACQUITY UPLC® H-Class System and H-Class Bio System with TUV, PDA, ELS, or FLR Detectors Installation Manual (P/N 715002258).**

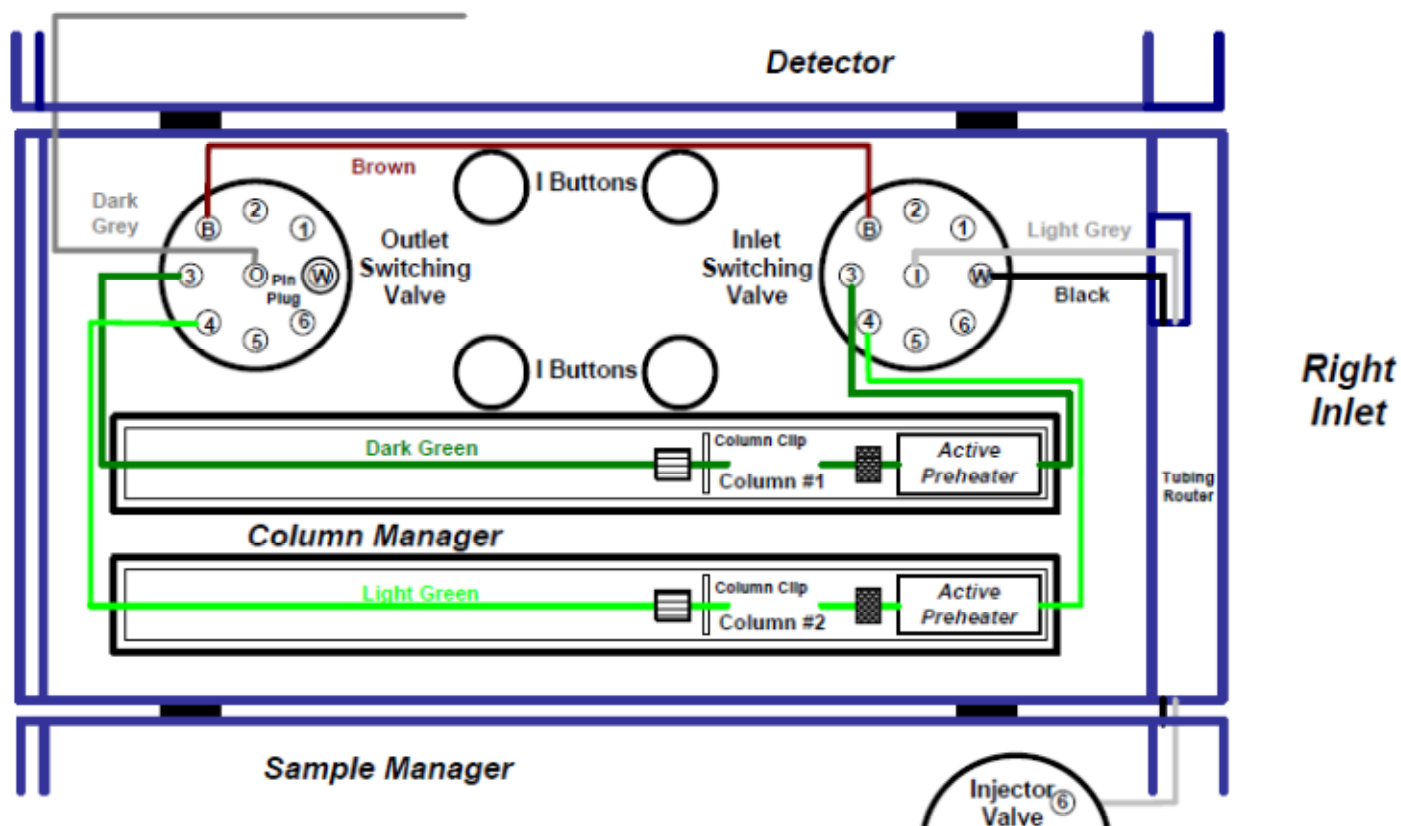
The CM-A / CM-Aux Installation Process

- ❑ Step 4 Install and plumb the QSM, SM-FTN, and any detectors (optical and/or massspectrometer) according to the ACQUITY UPLC® H-Class System and H-Class Bio System with TUV, PDA, ELS, or FLR Detectors Installation Manual (P/N 715002258) and if applicable, the appropriate mass spectrometer installation manual.
- ❑ **Step 5 Install the inlet and outlet switching valve cartridges, as described in "Installing the inlet and outlet switching valve cartridges" on page 7.**
- ❑ Step 6 Make all H-Class module power and electronic connections.
- ❑ **Step 7 Power on all system components, confirm communications, set the desired CM-A / CM-Aux column configuration, confirm CM-A valve positions, and activate the preheaters, as described in "Setting the column configuration, confirming the valve position, and activating the preheaters" on page 8.**
- ❑ **Step 8 Plumb the CM-A / CM-Aux module(s) in preparation for the system performance test, as described in the 2-, 4-, and 6-column installation sub-section(s) in this document.**

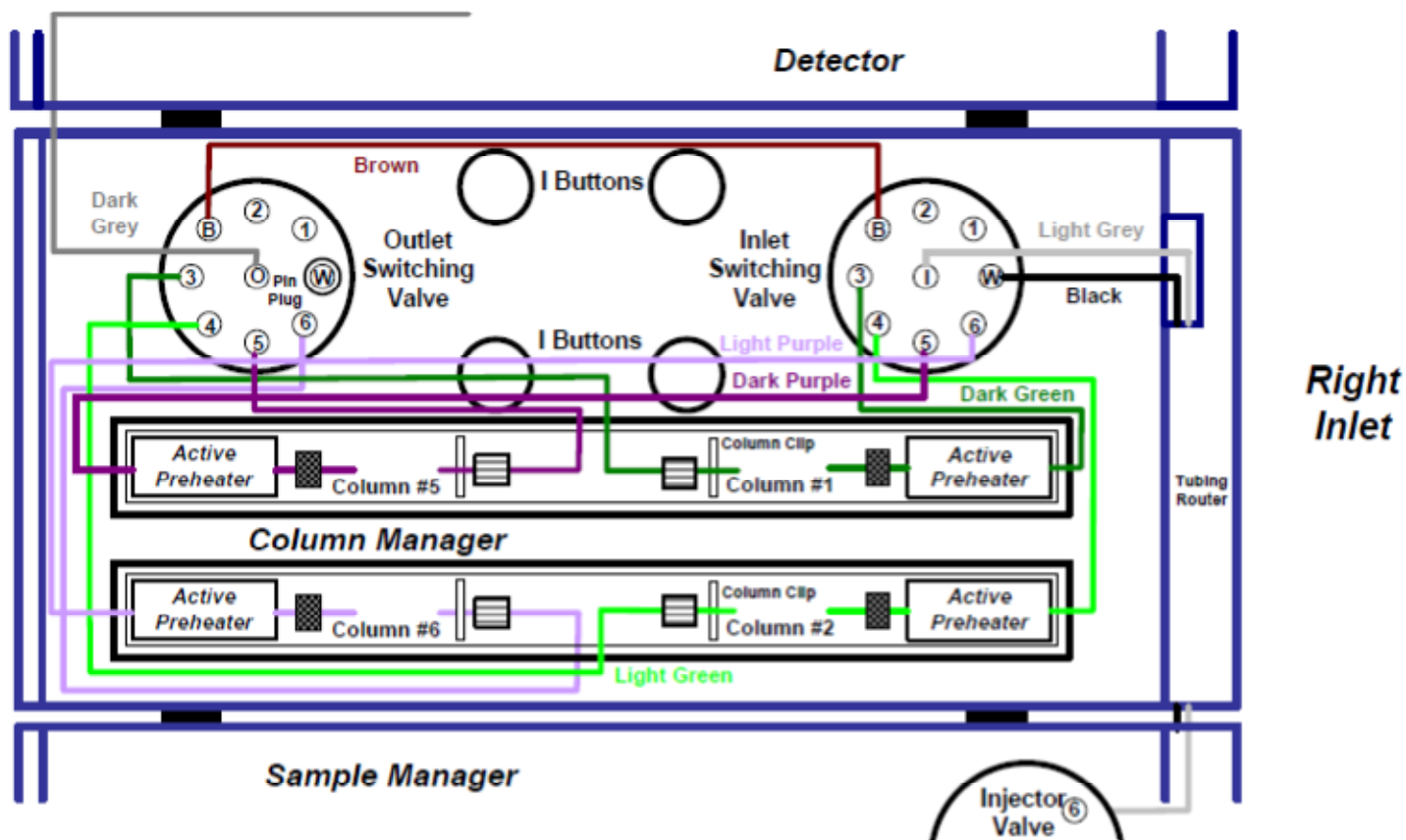
The CM-A / CM-Aux Installation Process

- Familiarize yourself with all plumbing diagrams for the CM-A and CM-Aux combinations as supplied on the next few pages.

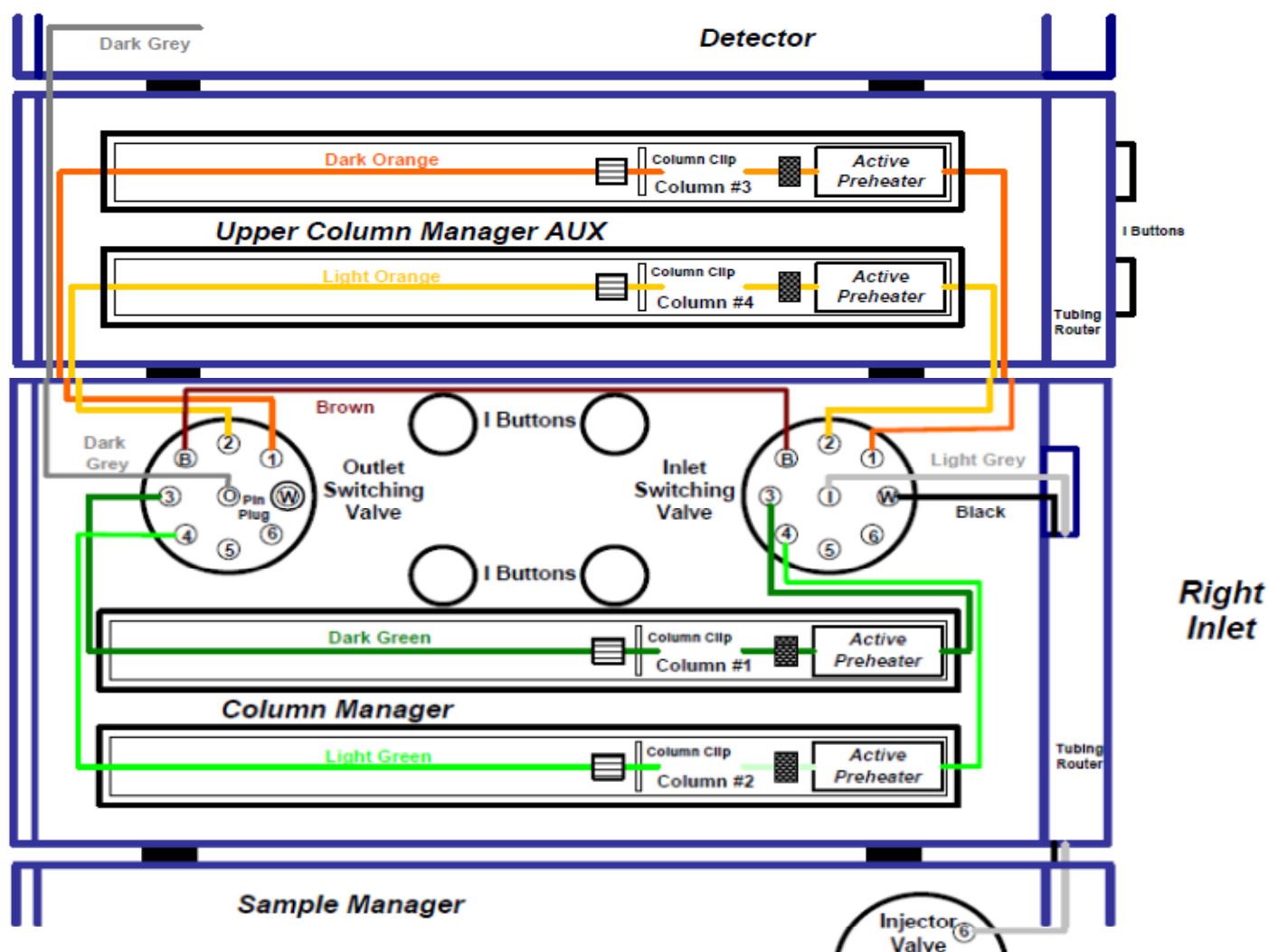
2 x 150mm Configuration



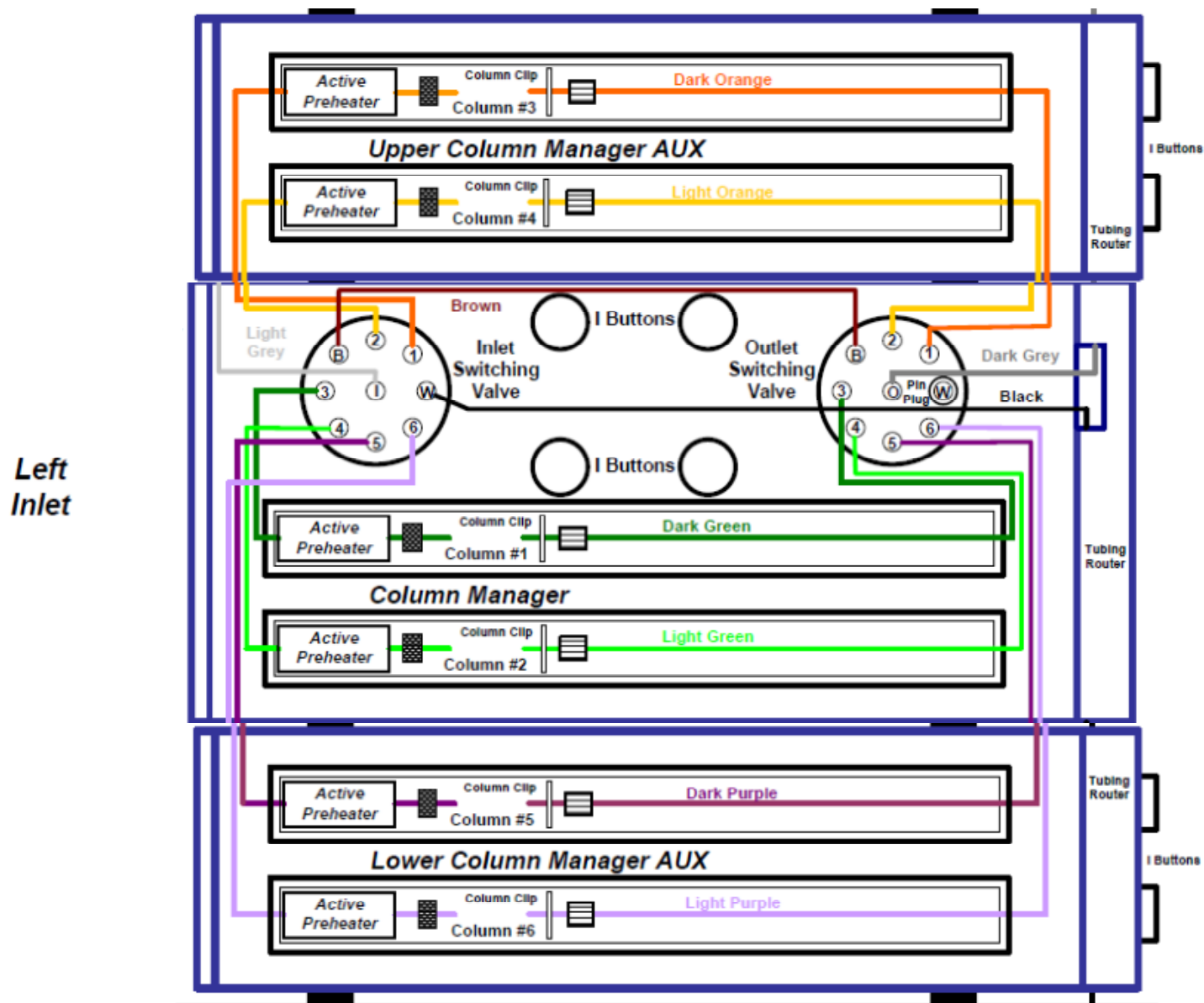
4 x 50mm Configuration



4 x 150mm Configuration



6 x 150mm Configuration



Replacing the Heater / Cooler Engine Assembly

- ❑ While in front of the unit, perform the procedure “Replacing the Heater/Cooler Engine Assembly or the Dual Column Trough Assembly (715002938). Pull the unit apart to the point of Step 14- Separate the trough assembly from the engine assembly as shown in Figure 8 (as seen below).
- ❑ Reassemble the CM-A completely and test for proper function. The unit needs to be left in working order for the next group.



Figure 8 - Separating trough and engine assemblies

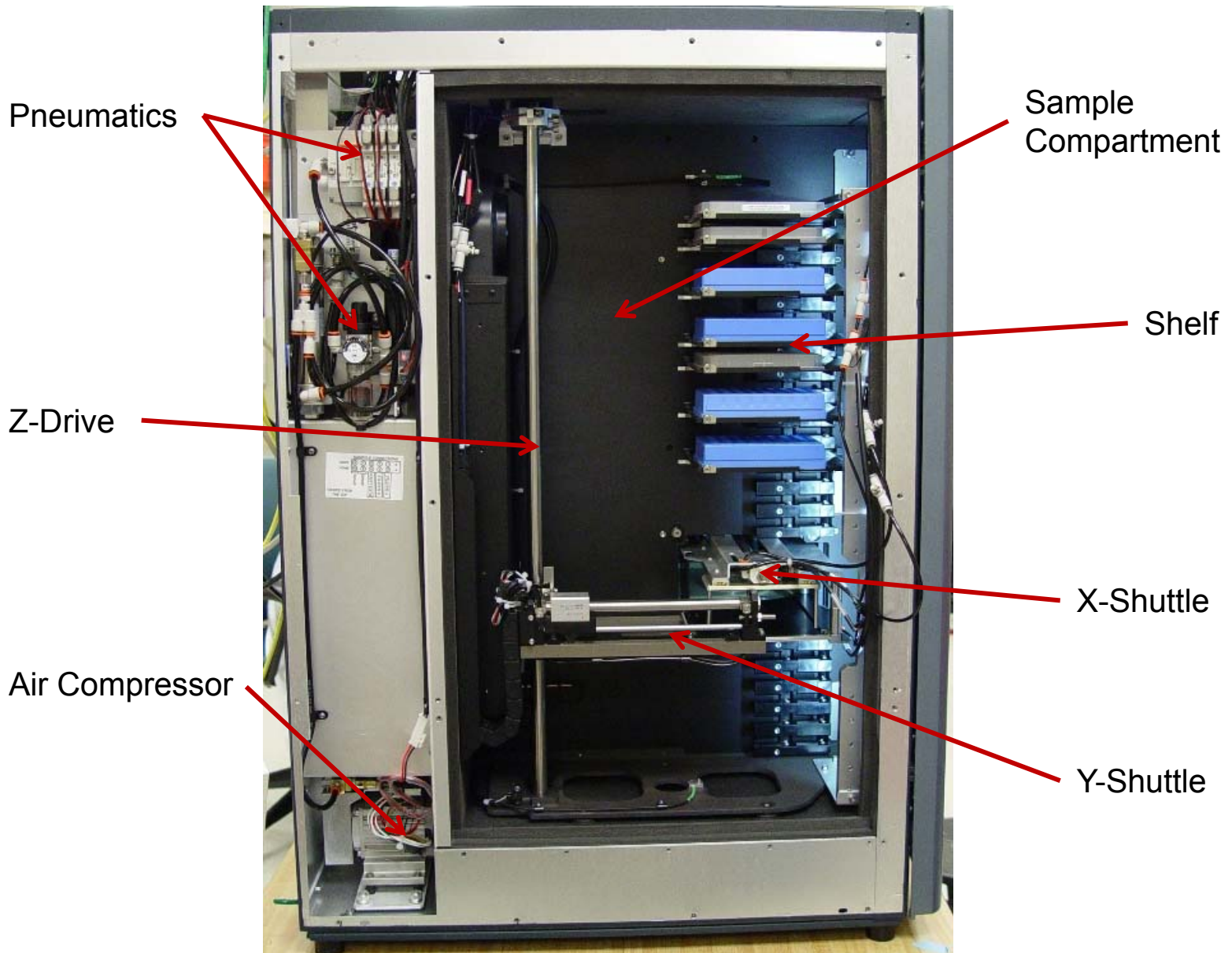


Section 10 Sample Organizer



Reviewing the Sample Organizer Subassemblies

- While in front of the unit, remove the left panel of the and review the major subassemblies of the Sample Organizer as detailed in the diagram below.

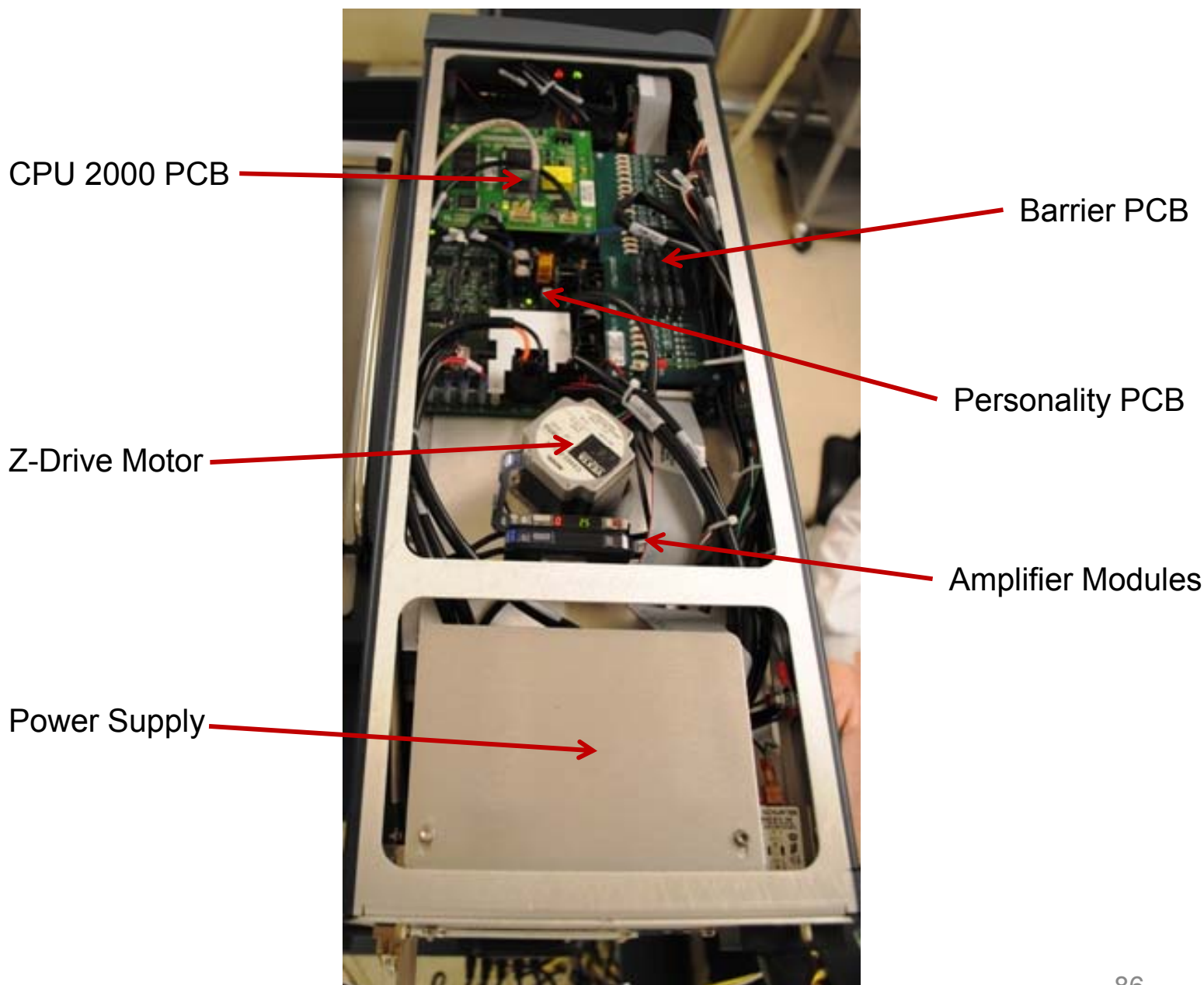


Electronic Subassemblies

- ❑ Remove the top panel of the unit and review the major electrical and control subassemblies of the Sample Organizer as detailed in the diagram below.



Warning- Beware of live AC and DC voltages when troubleshooting and servicing with side or top panels removed. Always remove the power cord from the Sample Organizer before touching any of the electronics.

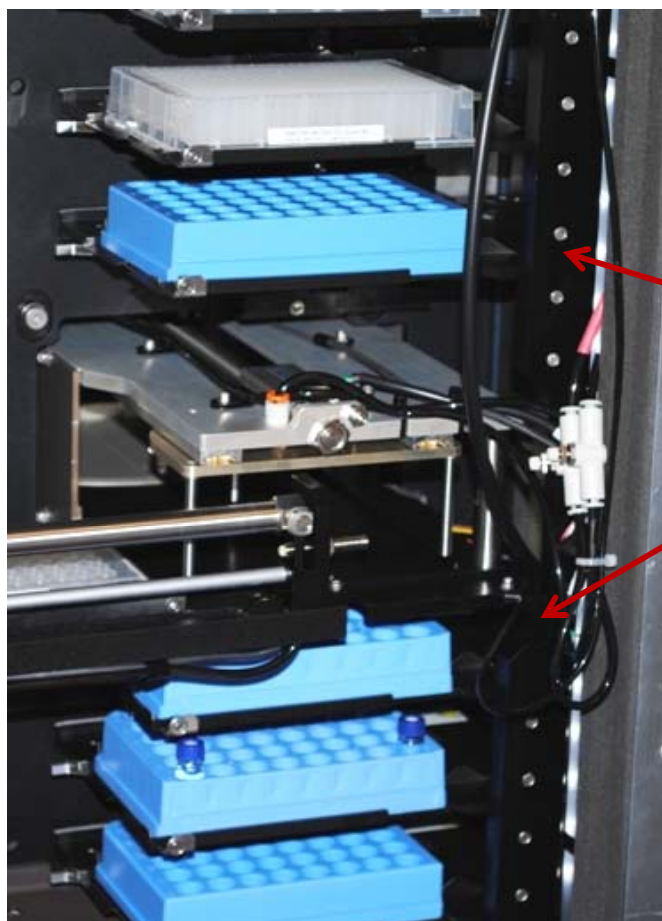


Shelf Stability Bracket and New Wider Shelves

Note the location and details of the Shelf Stability Bracket. It was added, along with the new wider shelves to prevent shelf sag. The new trays are not compatible with the old Sample Organizer.



New wider Shelf



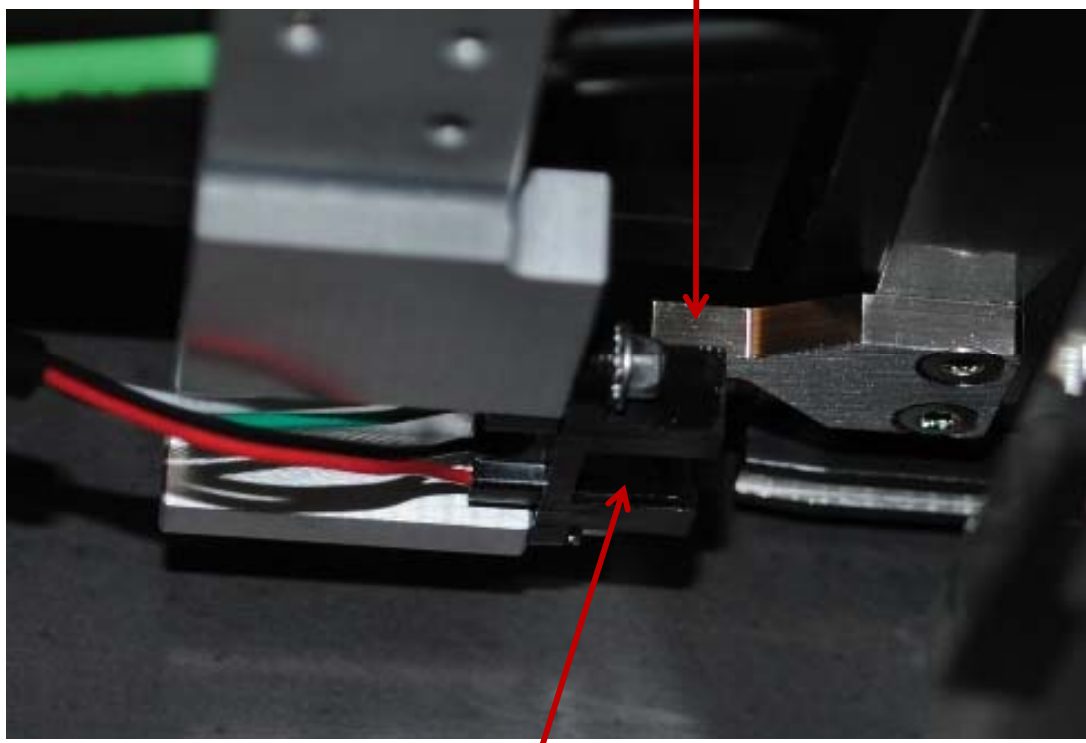
Shelf Stability Bracket

Shelf Sensor and Sensor Flag

The new shelf sensor flag (along with the shelf sensor) greatly improves the detection of shelves in the new Sample Organizer. Find the shelf sensor on the bottom of the YTS.



Shelf Sensor Flag



Shelf Sensor

X-Transfer Shuttle

The X-Transfer Shuttle was modified to accommodate the transfer of plates from the SO to a modified rotary tray in the SM-FTN or SM-FL. This required the transfer pneumatics to be situated on top to facilitate the sliding of the plate into the rotary with the use of 3 prongs.

X-Shuttle Transfer Pneumatics

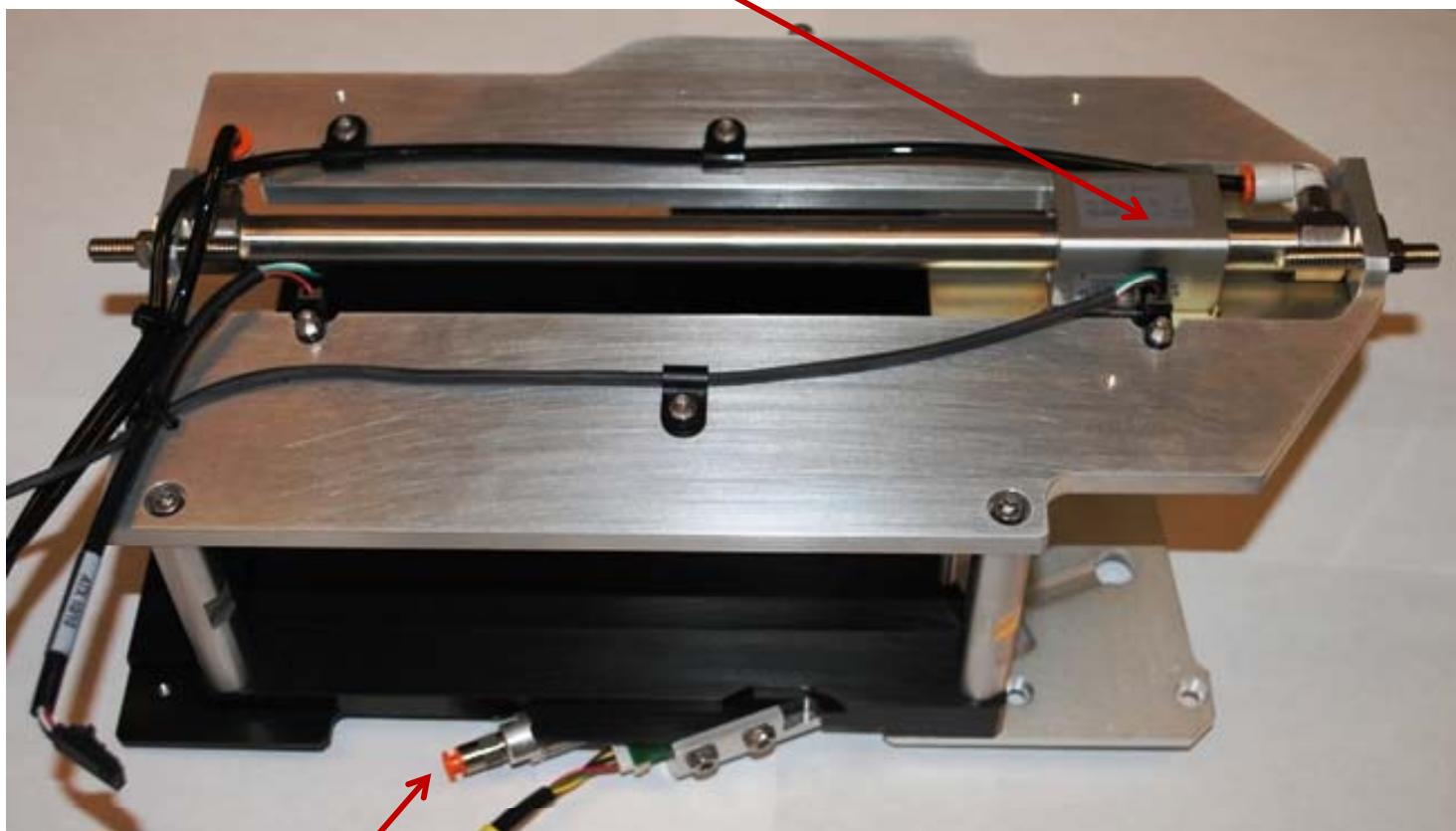
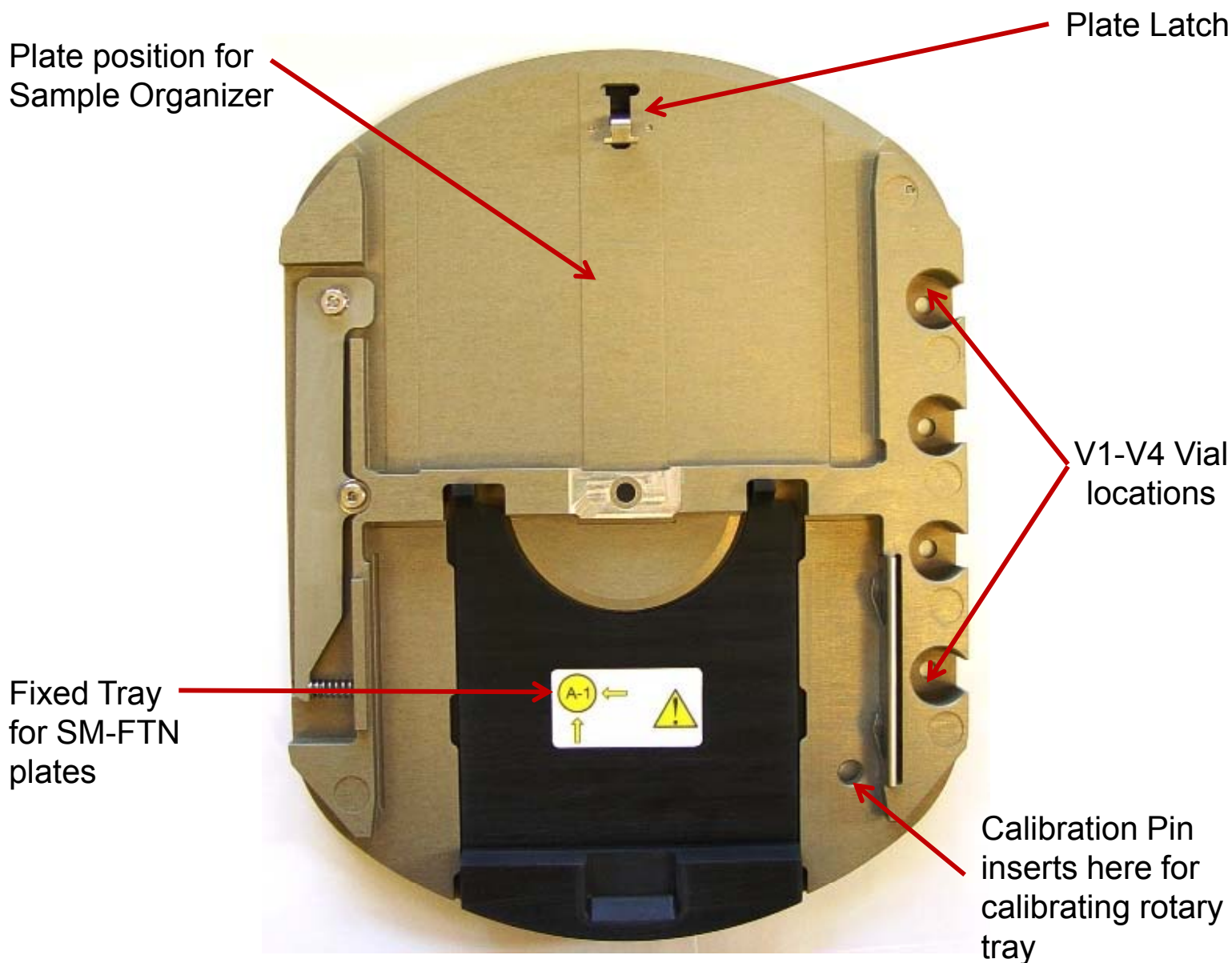


Plate Latch Pneumatic Actuator

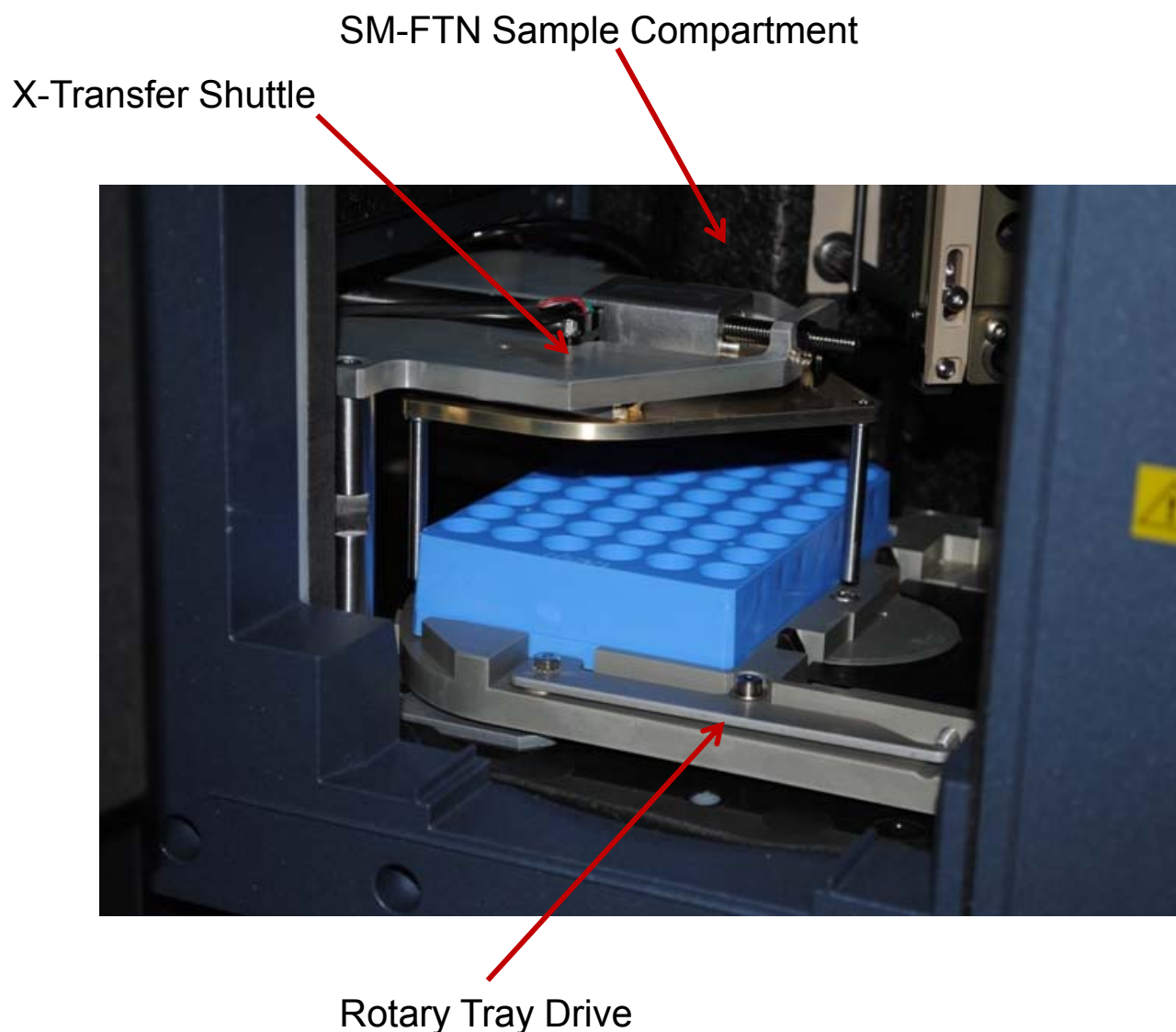
Sample Organizer Rotary Tray

The rotary tray shipped with the SO has been modified to handle the insertion of plates from the SO. This tray is installed into the SM in place of the stock rotary tray. It has a spring-loaded latch to hold the plate from the SO in place. It has a fixed tray location for plates as well as 4 locations for up to 4ml vials (V1-V4).



X-Transfer Shuttle and Rotary Tray Installed

The X-Transfer Shuttle installed in the SM_FTN along with the modified rotary tray. Two of the 3 prongs used in the transfer of the plate can be seen in the below photo.



Reviewing the Sample Organizer Installation Procedure

- ☐ While in front of the unit, review the procedure for assembling the ACQUITY UPLC System Sample Organizer (715002991). Perform all operations that are in bold below. The procedure consists of 13 subtasks:
 - ☐ Disassembling the ACQUITY stack (if necessary)
 - ☐ Unpacking and opening the sample organizer
 - ☐ Attaching the outboard leg and sample manager shelf
 - ☐ Docking the sample manager
 - ☐ **Installing the X-axis transfer shuttle (XTS)**
 - ☐ *Note: For this exercise, remove first then reinstall both the XTS and the rotary tray.*
 - ☐ **XTS Pre-alignment**
 - ☐ **Making electrical and pneumatic connections**
 - ☐ **Configuring the sample organizer**
 - ☐ *Remove the SO from the SM configuration then reconfigure it.*
 - ☐ **Checking the pressure**
 - ☐ **Checking the flow controls**
 - ☐ **Verifying / adjusting the hard stops using the calibration block**
 - ☐ **Installing the sample organizer shelves**
 - ☐ **Testing the sample organizer shelves**

Running the Sample Organizer Diagnostics

- ☐ Once you have the unit operational again, go to the Sample Organizer menu in the console. Perform all operations listed below:
 - ☐ Scan and Store (from the Configure menu).
 - ☐ Put several plates in to check for proper detection.
 - ☐ Troubleshoot> Plate Transfer
 - ☐ Run 3 cycles of the transfer.
 - ☐ Switch the unit to interactive control.
 - ☐ Run the following functions:
 - ☐ X Shuttle- Home and Away
 - ☐ Y Shuttle- Home and Away
 - ☐ Z-Axis- Home and Away
- ☐ Record the shuttle transfer times in the table below.
- ☐ Switch the Sample Organizer back to non- interactive control.
- ☐ Return the unit back to the condition you found it .

Shuttle	Home to Away	Away to Home
X-Shuttle		
Y-Shuttle		
Plate Latch		



Section 11

System Installation Procedure



Post Service Diagnostic Tests

- ☐ Following the “ACQUITY H-Class System Installation Manual” (located online and on the workstation), put the modules back together.
- ☐ Make sure you closely follow the instructions on how to plumb the system together and route the tubing up the side.
- ☐ Make sure all solvent lines back in there proper solvents for the verification tests.
- ☐ Prime all lines for 2 minutes if they still contain solvent otherwise prime for 3 minutes.
- ☐ Start a flow rate of 0.5ml/min with 50% A and 50% B and allow the pressure to stabilize for at least 2 minutes. Record the current pressure and delta pressure ripple in the table below.
- ☐ Repeat the above test with 50% C and 50% D and record the results below.

Solvent Line	System Pressure	Pressure Ripple	Pass / Fail
A-B			
C-D			

The Delta pressure ripple should be below 1% of the system pressure for a passed test result.

Post Service Diagnostic Tests



- ☐ Perform a dynamic leak test using the solvent line in which you replaced the filter to a pressure of 9000 psi.
- ☐ Record the results in the fields below.

Leak Test

Parameters | Status | **Results**

	Accumulator	Primary
Result:		
Leak Rate:	nL/min	nL/min
Maximum Pressure:	psi	psi
Percent of Stroke:	%	%
Compressed Volume:	uL	uL
Compression Attempts:		
Test Attempts:		

QSM Diagnostic Tests Overall Results (Pass/Fail)_____

Initials_____

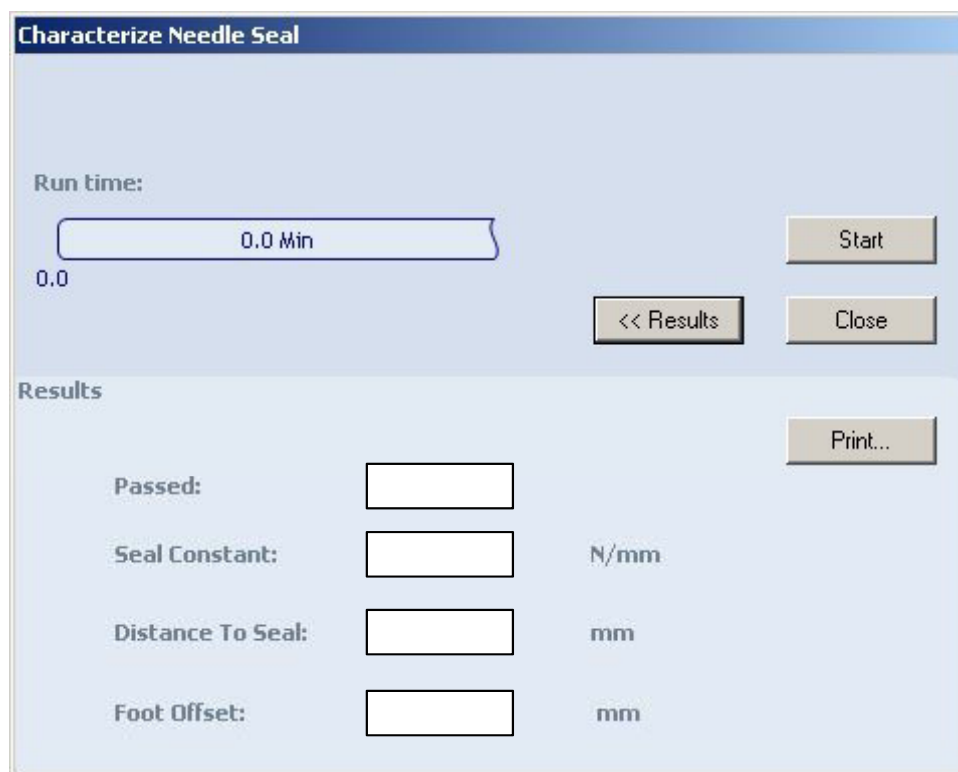
Date_____

Post Service Diagnostic Tests

- ☐ Reset the SM-FTN from the console menu.
- ☐ Prime the Wash Solvent for 1 minute and the Purge Solvent for 10 cycles.
- ☐ Characterize the Needle Z-axis and enter the result below.

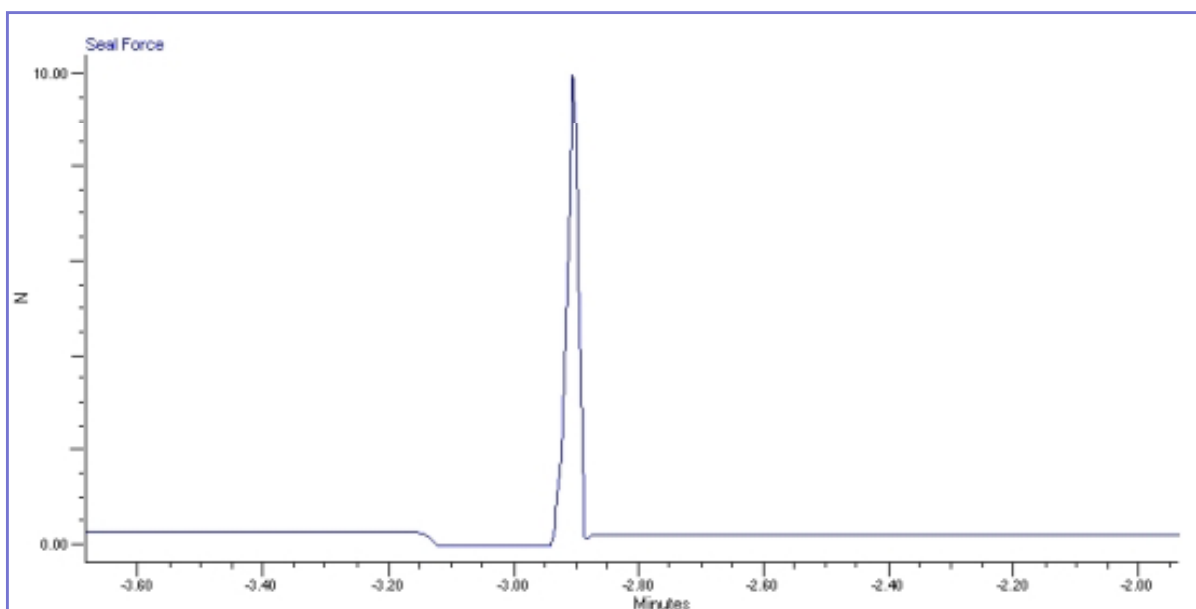
Needle Z-axis Offset: _____ mm.

- ☐ Check the plot properties menu in the SM-FTN top level console and make sure the Seal Force plot is enabled.
- ☐ Characterize the Needle Seal and enter the result below.



The image shows a software dialog box titled "Characterize Needle Seal". It contains a "Run time:" section with a text box showing "0.0 Min" and a "0.0" label below it. To the right are "Start", "<< Results", and "Close" buttons. Below this is a "Results" section with four rows of labels and input fields: "Passed:", "Seal Constant:", "Distance To Seal:", and "Foot Offset:". Each label has a corresponding empty text box. To the right of the "Seal Constant:" field is the unit "N/mm", and to the right of the "Distance To Seal:" and "Foot Offset:" fields is the unit "mm". A "Print..." button is located to the right of the "Results" section.

- Examine the Seal Force plot after the Characterize Needle Seal function is completed. It should look similar to the below plot.



- Start a flow rate of 0.5ml/min on line A and allow the pressure to stabilize.
- Run the Needle Seal Readiness test and enter the result below.
- Observe the QSM system pressure. Does it correlate with the test results _____?

Results		
Passed:		
Pressure Difference:		psi
Flow:		ml/min

- ❑ Run the Sample Syringe static leak test and record the results in the blank fields below.

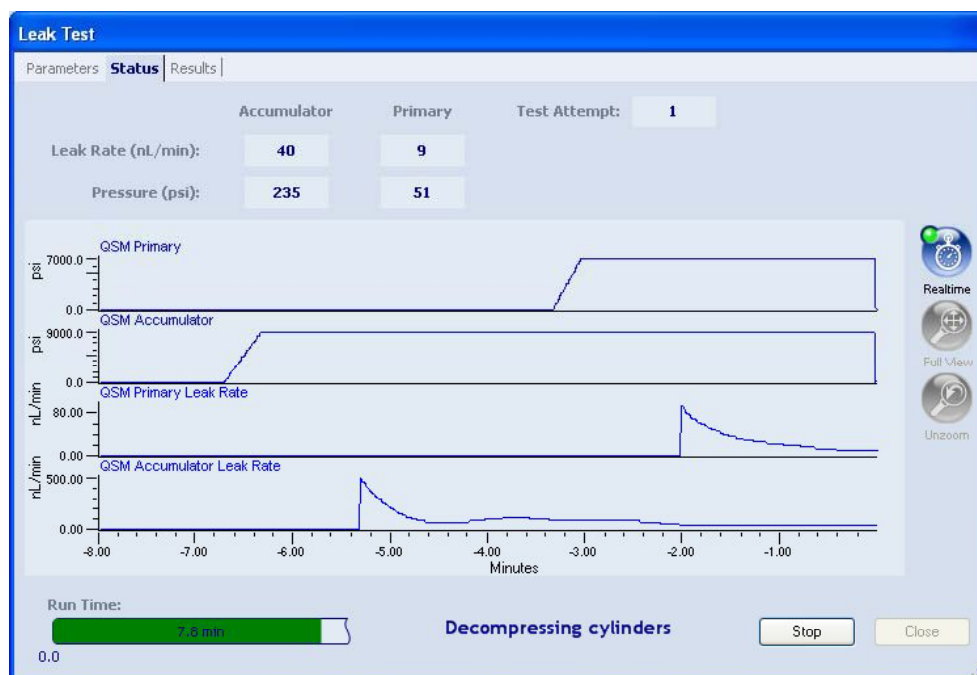
Results	
Leak Test	
Passed:	
Test pressure:	psi
Compressed volume:	μ L
Decompressed volume:	μ L
Start pressure:	psi
End pressure:	psi
Pressure change:	psi
Average pressure leak:	psi/sec

Running the QSM / SM-FTN Dynamic Leak Test

- ❑ Stop the flow and disconnect the column inlet line from the column.
- ❑ Connect the fitting plug (located in the solvent bottle tray front compartment) to the column inlet line to plug the line.
- ❑ Run the Dynamic Leak Test from the QSM menu.
 - ❑ Set the pressure 9000 PSI.
 - ❑ Choose Column as the end point.
 - ❑ Record the results below.

Leak Test	
Parameters Status Results	
Accumulator	
Result:	
Leak Rate:	nL/min
Maximum Pressure:	psi
Percent of Stroke:	%
Compressed Volume:	μ L
Compression Attempts:	
Test Attempts:	
Primary	
Result:	
Leak Rate:	nL/min
Maximum Pressure:	psi
Percent of Stroke:	%
Compressed Volume:	μ L
Compression Attempts:	
Test Attempts:	
Print	
Start Close	

Post Service Diagnostic Tests



Do the results correlate well with the QSM leak tests?

This test should give a good level of confidence that the Stationary Side Seal, tubing and fittings are holding pressure and functioning correctly.

- ☐ If the test failed, recheck all fittings and try the test again. Troubleshoot the problem by changing the location of the pin plug if necessary.
- ☐ Once the test has passed, disconnect the plug and reconnect the column to the inlet line.
- ☐ Fill in the fields below and initial and date it.

SM-FTN Diagnostic Tests Overall Results
(Pass/Fail) _____

Initials _____

Date _____

- ☐ Run the system performance tests to check the system as a whole.
- ☐ Record the results below.

Note- The before and after service results for the system are a key part of the certification exam.

Caffeine Area and Height Results Table

	Area %RSD Specification	Area %RSD	Area %RSD Pass Fail	Height %RSD Specification	Height %RSD	Height %RSD Pass Fail
1	%RSD < or = 0.5			%RSD < or = 1.1		

Caffeine Retention Time (RTsec) Results Table

	Retention time (sec) Standard Deviation Specification	Retention time (sec) Standard Deviation	Retention time (sec) Standard Deviation Pass Fail
1	SD RT < or = 1.0sec		

Precision Tests Overall Results (Pass/Fail)_____

Initials_____

Date_____

Retention Time Reproducibility AB

	Name	Retention Time (Seconds) Standard Deviation Specification	Retention Time (Seconds) Standard Deviation	Retention Time (Seconds) Standard Deviation Pass Fail
1	2-acetylfuran	SD RT < or = 1.0sec		
2	acetanilide	SD RT < or = 1.0sec		
3	acetophenone	SD RT < or = 1.0sec		
4	propiphenone	SD RT < or = 1.0sec		
5	butylparaben	SD RT < or = 1.0sec		
6	benzophenone	SD RT < or = 1.0sec		
7	valerophenone	SD RT < or = 1.0sec		

Retention Time Reproducibility CD

	Name	Retention Time (Seconds) Standard Deviation Specification	Retention Time (Seconds) Standard Deviation	Retention Time (Seconds) Standard Deviation Pass Fail
1	2-acetylfuran	SD RT < or = 1.0sec		
2	acetanilide	SD RT < or = 1.0sec		
3	acetophenone	SD RT < or = 1.0sec		
4	propiphenone	SD RT < or = 1.0sec		
5	butylparaben	SD RT < or = 1.0sec		
6	benzophenone	SD RT < or = 1.0sec		
7	valerophenone	SD RT < or = 1.0sec		

Gradient Tests Overall Results (Pass/Fail)_____

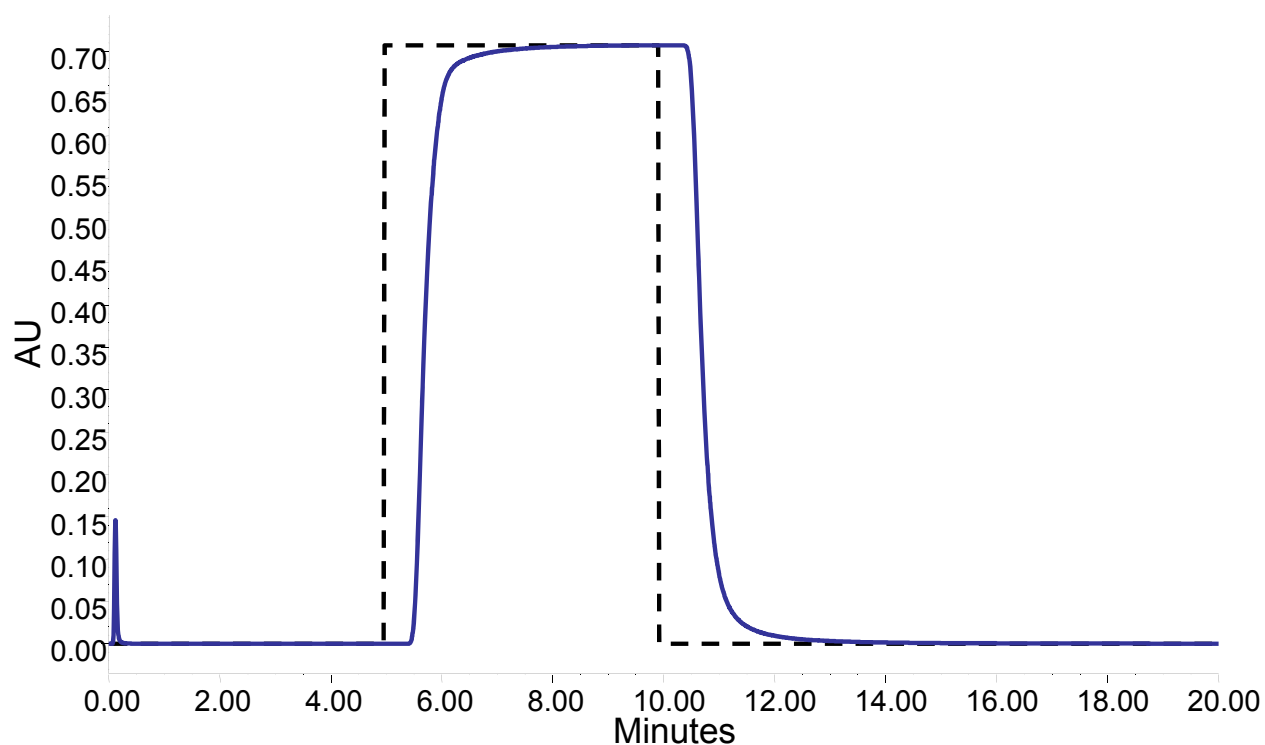
Initials_____

Date_____



Section 12

Determining H-Class System Volumes



The extra-column volume comprises the volume of the system from the point of the injection to the point of detection.

A more accurate determination would include all parts of the column (the connectors and frits) minus the packing material. However this is quite difficult to measure without sophisticated equipment.

- ☐ Switch the Gradient table to isocratic using 100% A at 0.5 ml/min.
- ☐ Disable the loop offline setting in the SM-FTN instrument method.
- ☐ Switch the TUV method sampling rate to 40 pts/sec and the filter to none.
- ☐ Use the Caffeine sample from the system precision test.
- ☐ Run 3 replicate **1ul** injections of the test with a Run Time of 30 seconds.
- ☐ Calculate the extra-column volume in ul, using the following formula:

$$X = Rt * F * 1000 \text{ (ul / ml)}$$

Rt = Retention time of the peak

F = flow rate in ml/min

Extra-column Volume = _____ul



Bandspreading is the broadening of the peaks as they flow through the extra-column volume of the system. It is an important measure of the system impact on the efficiency of the chromatography.

- ☐ Calculate the system bandspreading volume in ul, using the following formula:

$$B = W * F * 1000 \text{ (ul/ml)}$$

W= Width of the peak at 4.4% height

F= flow rate in ml/min

Bandspreading = _____ul

- ☐ Return the system to its normal configuration and reinstall the ACQUITY column.



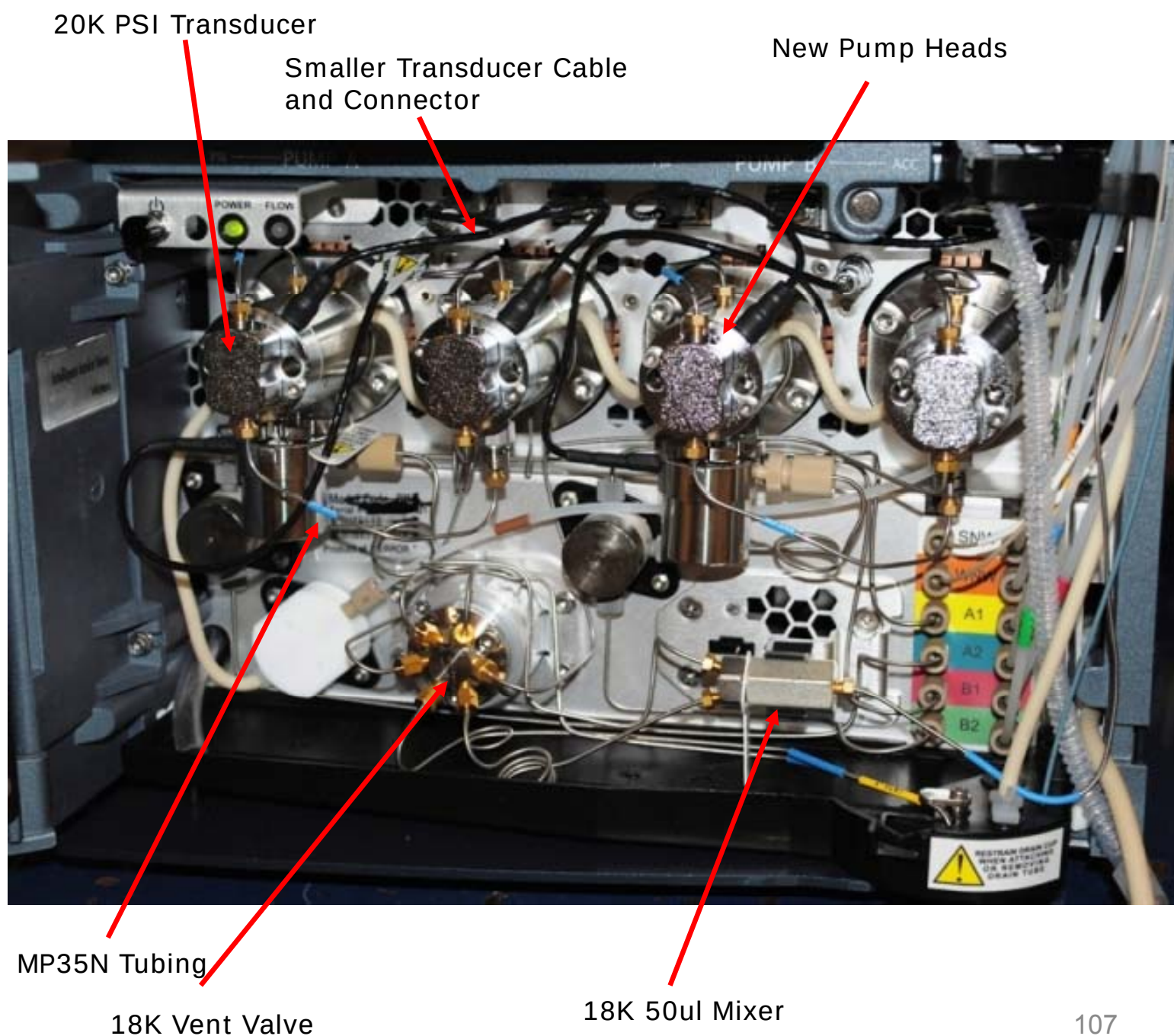
Section 13

I-Class System Overview



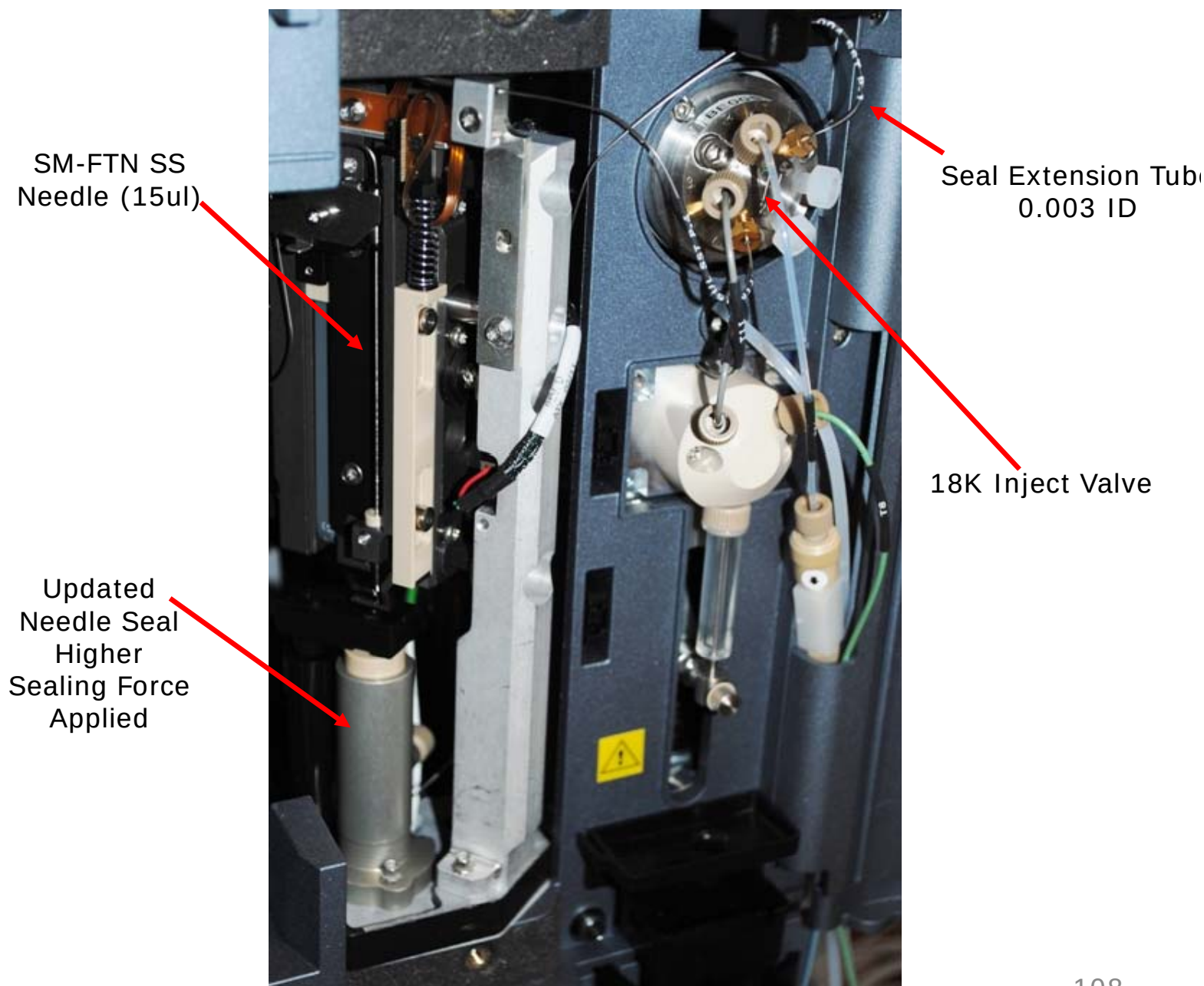
I-Class BSM Component Changes

- Open the front panel of the BSM and observe the major differences from the original BSM as detailed in the diagram below.

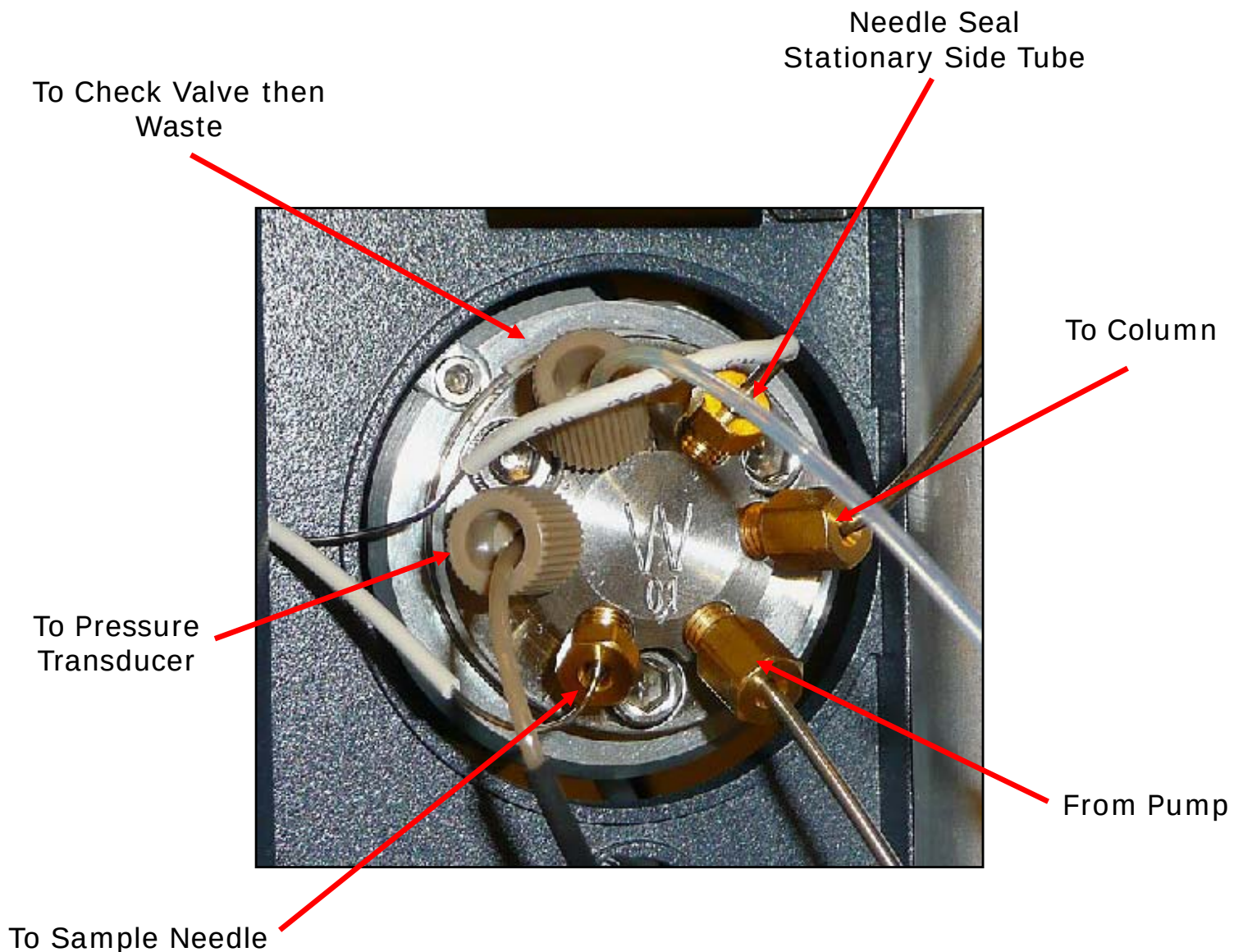


I-Class SM-FTN Changes

- ❑ Remove the 2 T20 screws securing the access panel in front of the Inject Block and remove the panel.
- ❑ Observe the main component changes of the Needle and the Needle Seal Housing Assembly as seen below.
- ❑ Where possible, trace the solvent lines and SS tubing line back to their origins.



- Observe the main connections to the Inject Valve and as seen below and trace each line to its end point. You will need to open the right side panel of the SM to see some of the connection terminations. The internal fluidic components are detailed on the next page.



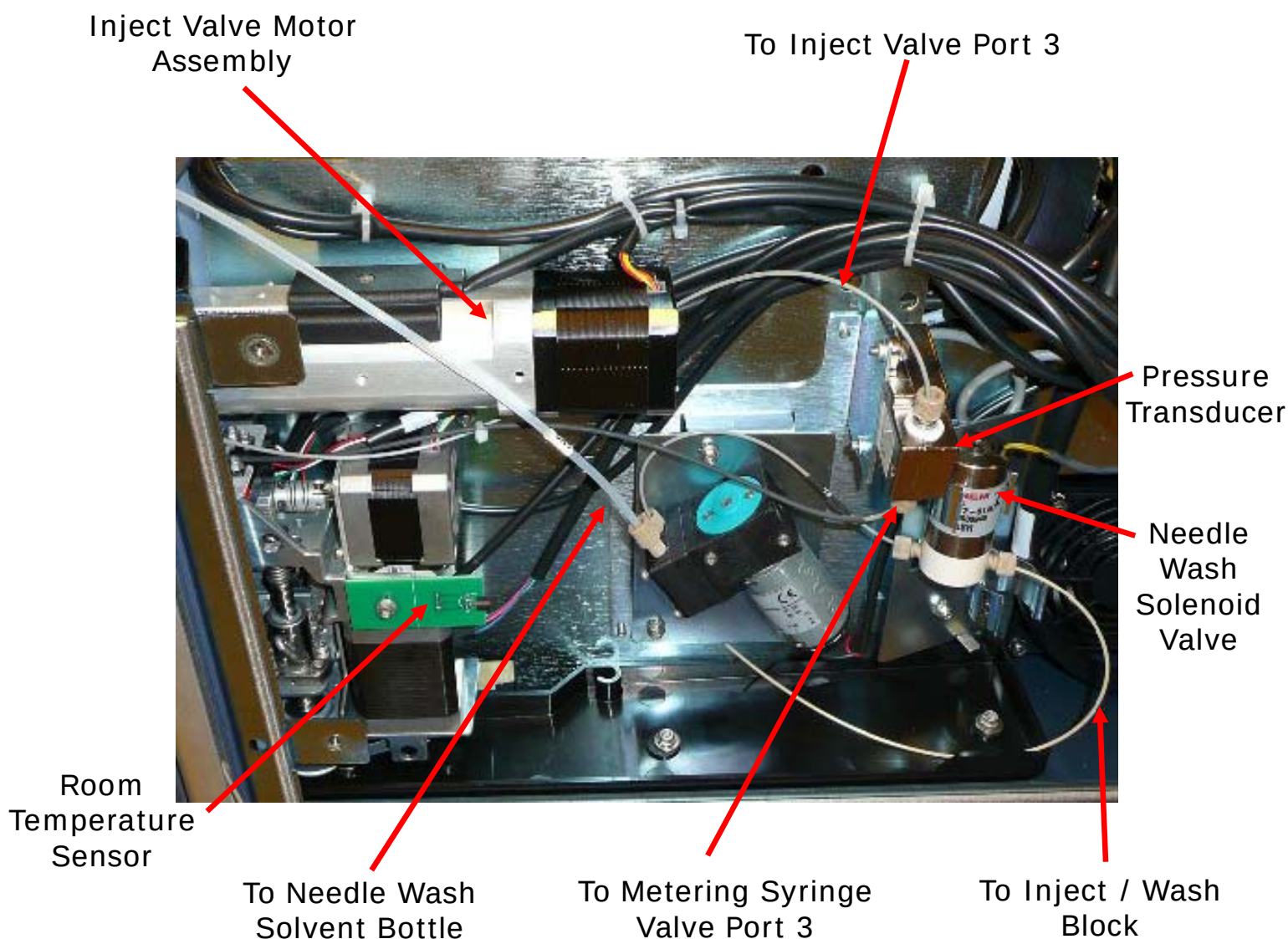
- ❑ Remove the 2 T20 screws securing the access panel in front of the Inject Block and remove the panel.



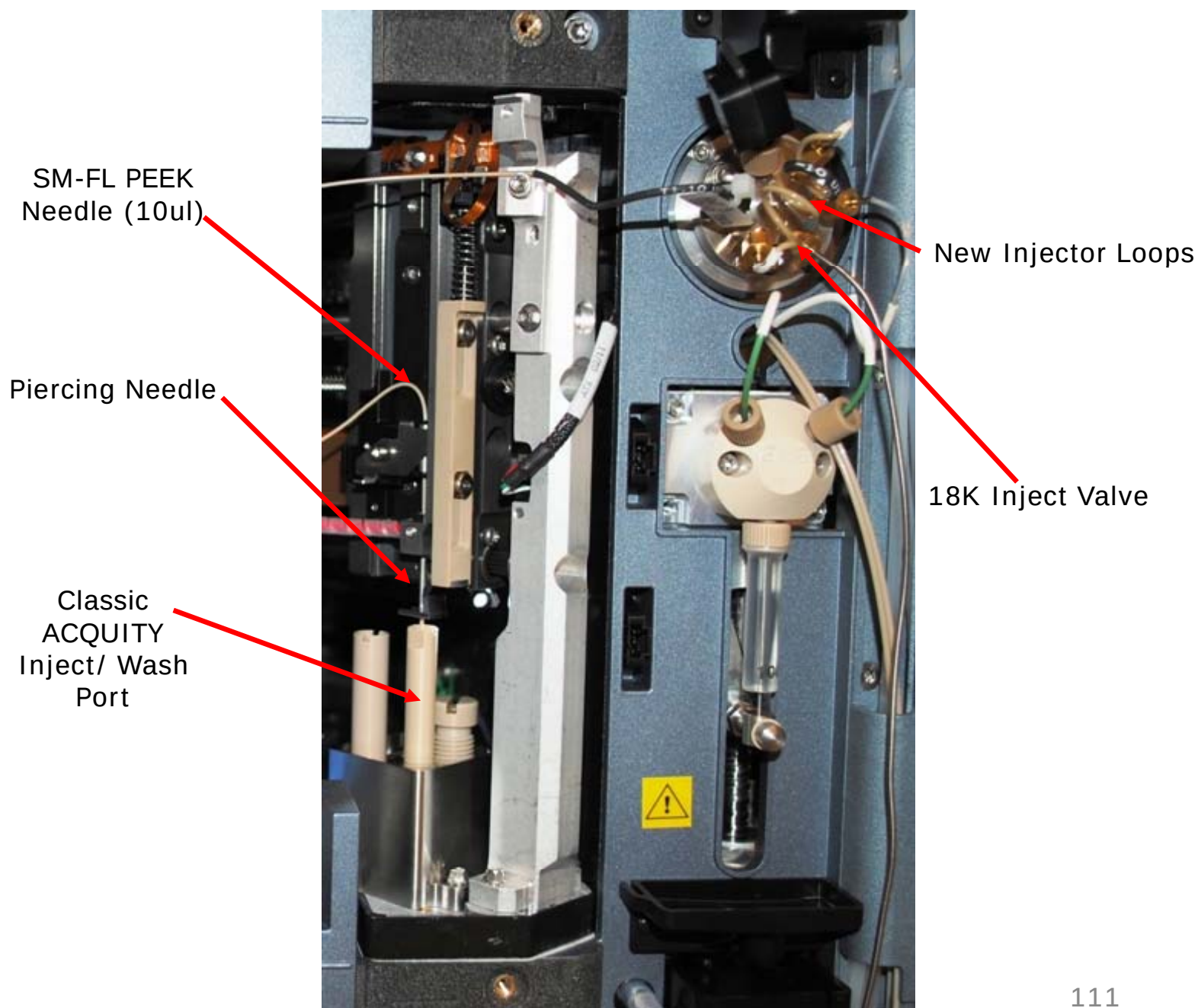
Warning- Beware of live AC and DC voltages when troubleshooting and servicing with side panels removed.

Observe the internal fluidic components as detailed in the picture below.

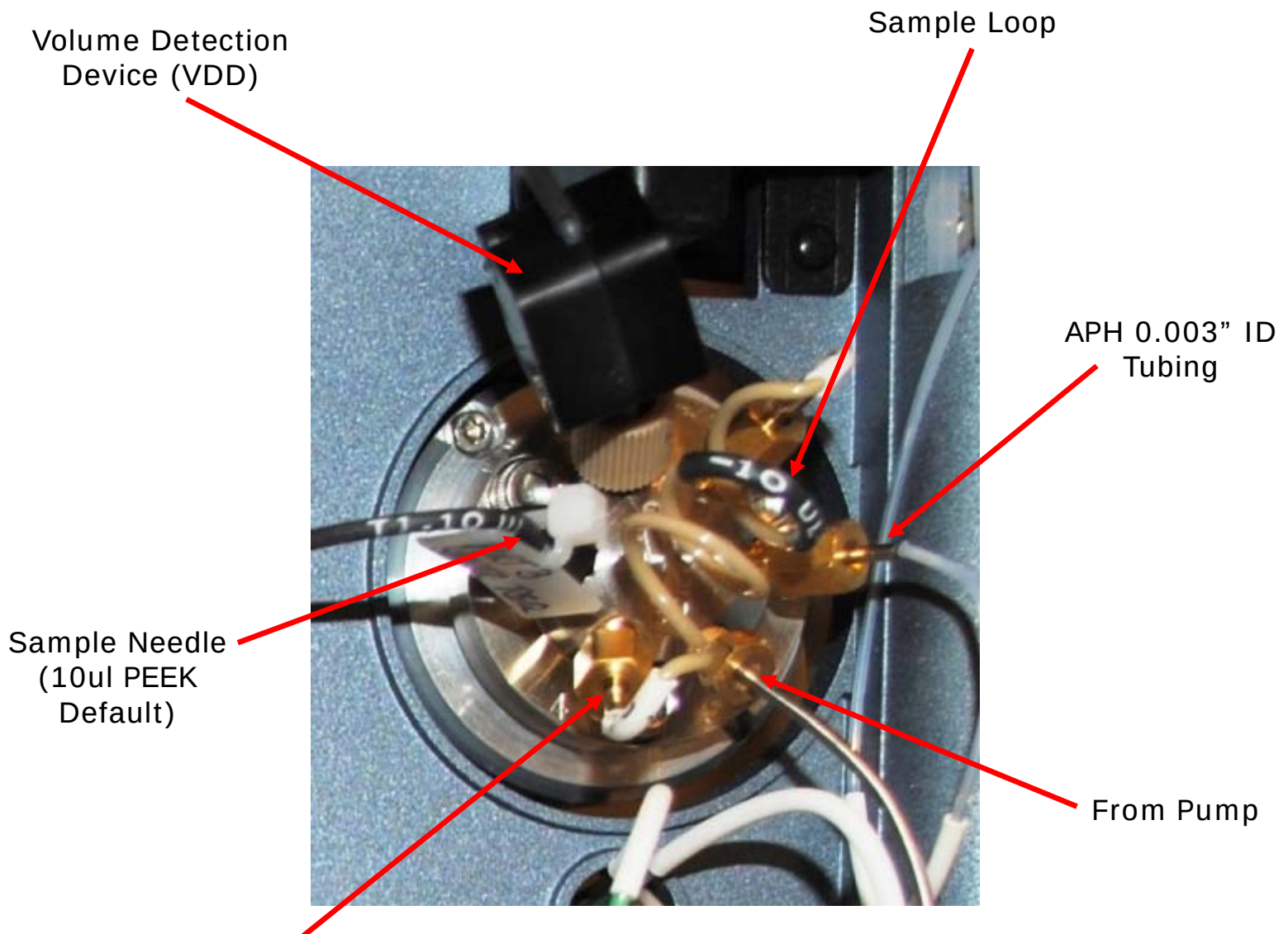
- ❑ As mentioned on the previous page, trace each line to its end point.
- ❑ When you have finished tracing the lines, reinstall the front and side panels.



- ❑ Remove the 2 T20 screws securing the access panel in front of the Inject Block and remove the panel.
- ❑ Observe the main component changes to adapt the fixed loop injection technique of ACQUITY Classic SM to the common platform SM.
- ❑ Where possible, trace the solvent lines and SS tubing line back to their origins.



- Observe the main connections to the SM-FL Inject Valve and as seen below and trace each line to its end point. You will need to open the right side panel of the SM to see some of the connection terminations. The internal fluidic components are detailed on the next page.



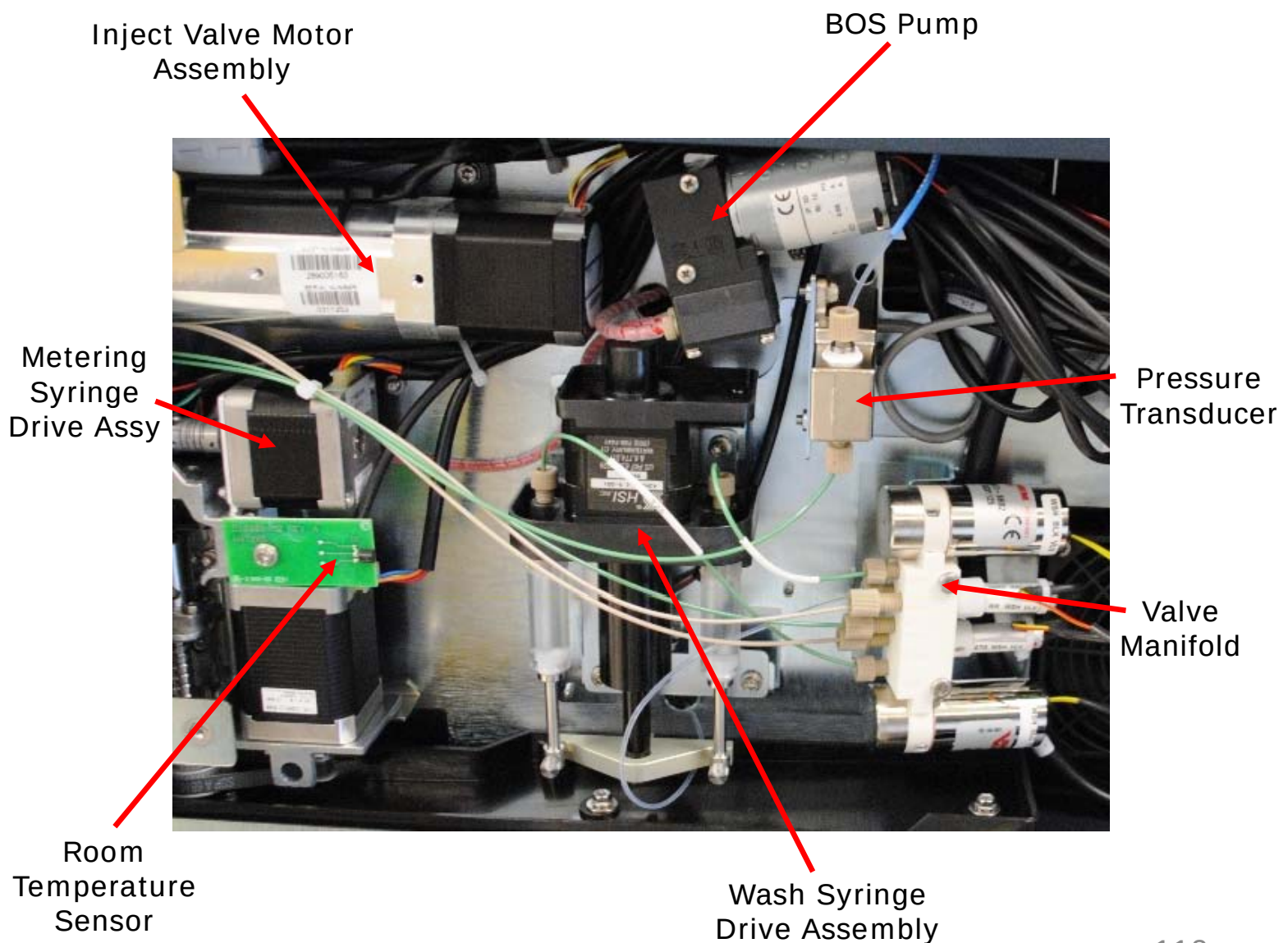
- ❑ Remove the 2 T20 screws securing the access panel in front of the Inject Block and remove the panel.



Warning- Beware of live AC and DC voltages when troubleshooting and servicing with side panels removed.

Observe the internal fluidic components as detailed in the picture below.

- ❑ As mentioned on the previous page, trace each line to its end point.
- ❑ When you have finished tracing the lines, reinstall the front and side panels.



- ☐ Obtain some new filtered water for your system from the appropriate labeled bottle in the lab.
- ☐ Make sure that the solvent lines are inserted to the bottles as follows:

Water	Lines A1, A2
Acetonitrile	Lines B1 and B2
10% ACN 90% H ₂ O	Strong Wash, Weak Wash and Seal Wash

- ☐ Prime each solvent line for 3 minutes.

What is the total time it takes to prime the BSM lines_____?

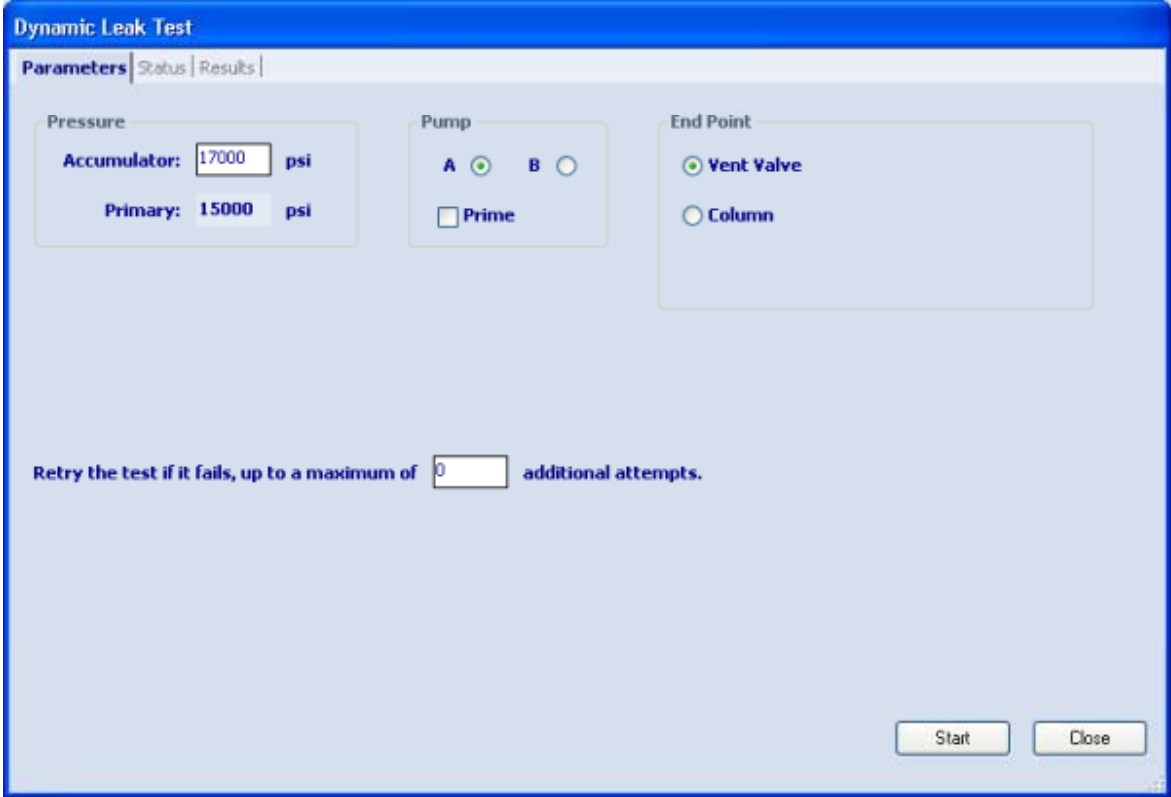
Is the total time longer, shorter or the same as priming four solvents for the BSM_____

_____?

- ☐ Start the BSM pumping with 90% Water and 10% CAN at a flow rate of 0.5ml/min.
- ☐ Switch the channel plots to view the Primary Pressure and Accumulator Pressure for the A and B pumps.
- ☐ Give the pressure at least 3 minutes to settle down and record the Pressure Ripple

Delta Pressure Ripple-_____PSI

- ☐ Prime the Seal Wash for about 1 minutes.
- ☐ Observe that there is a steady flow of solvent coming out indicating a good prime then stop the prime.
- ☐ Switch the console menu to the top level BSM menu so you can see the pressure plots.
- ☐ Run the Dynamic Leak test for pump A using solvent A1 at 17000 PSI with the Vent Valve as the end point.
- ☐ On the status page, you can right-click the plots and show the Accumulator and Primary pressure plots.
- ☐ Run the Leak test using solvent B1 at 17000 PSI with the Vent Valve as the end point.
- ☐ Record the results in the fields on the next page.
- ☐ What is the range of pressures you can test the Accumulator head on each pump _____?



The screenshot shows a software window titled "Dynamic Leak Test" with three tabs: "Parameters", "Status", and "Results". The "Parameters" tab is active and contains the following settings:

- Pressure:** Accumulator: 17000 psi, Primary: 15000 psi
- Pump:** A (selected with a green dot), B (unselected), Prime (checkbox is unchecked)
- End Point:** Vent Valve (selected with a green dot), Column (unselected)

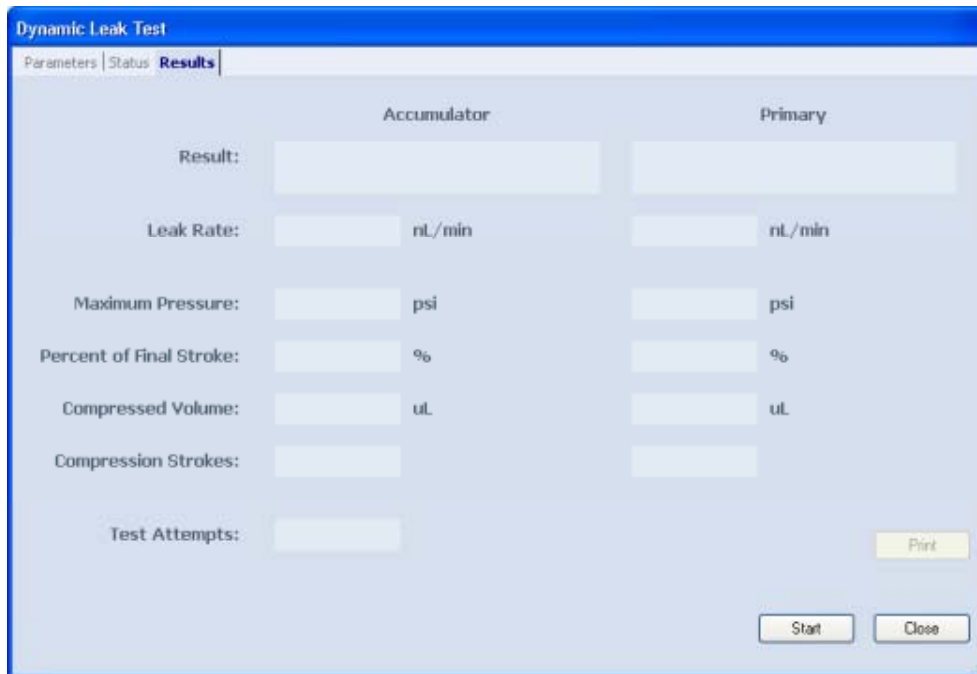
At the bottom, there is a field for "Retry the test if it fails, up to a maximum of 0 additional attempts." and two buttons: "Start" and "Close".

The pass criteria for an individual pump test is a leak rate of less than 200 nL/min. The pass criteria for a system test is a leak rate of less than 600 nL/min.

The new leak test can use multiple strokes to compress fluids to the test pressure condition, which accommodates highly compressible fluids and the additional fluid volume compressed during a system test.

- ❑ Record your results in the appropriate fields:

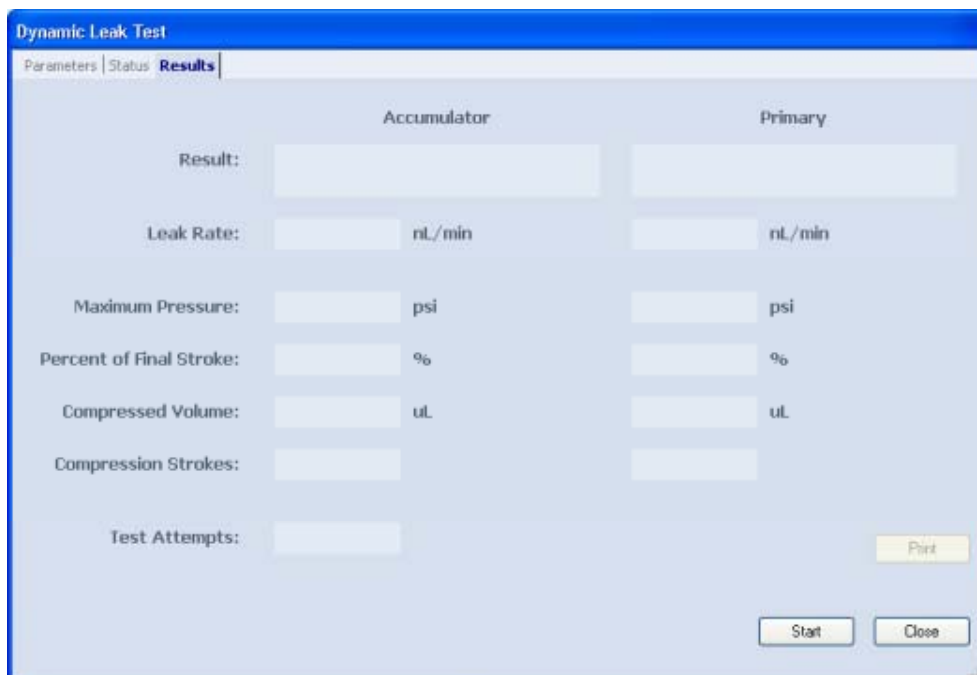
Pump A Results



The form is titled "Dynamic Leak Test" and has three tabs: "Parameters", "Status", and "Results". The "Results" tab is active. It is divided into two columns: "Accumulator" and "Primary". Each column has a "Result:" field, a "Leak Rate:" field with a unit of "nL/min", a "Maximum Pressure:" field with a unit of "psi", a "Percent of Final Stroke:" field with a unit of "%", a "Compressed Volume:" field with a unit of "uL", and a "Compression Strokes:" field. There is also a "Test Attempts:" field at the bottom left. A "Print" button is located at the bottom right, and "Start" and "Close" buttons are at the very bottom.

	Accumulator	Primary
Result:		
Leak Rate:	nL/min	nL/min
Maximum Pressure:	psi	psi
Percent of Final Stroke:	%	%
Compressed Volume:	uL	uL
Compression Strokes:		
Test Attempts:		

Pump B Results

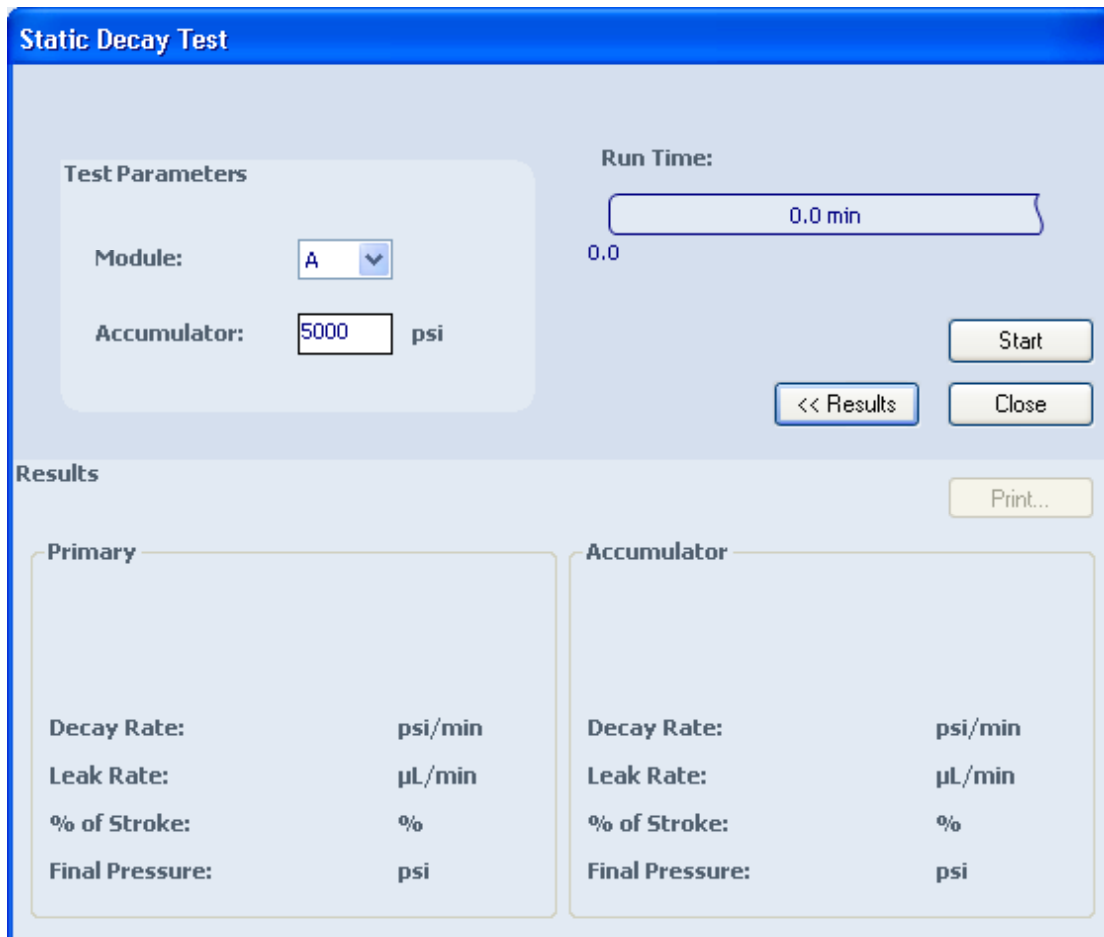


This form is identical to the one for Pump A, titled "Dynamic Leak Test" with "Parameters", "Status", and "Results" tabs. It features the same layout of input fields for "Accumulator" and "Primary" results, including leak rate, maximum pressure, percent of final stroke, compressed volume, compression strokes, and test attempts. It also includes "Print", "Start", and "Close" buttons.

	Accumulator	Primary
Result:		
Leak Rate:	nL/min	nL/min
Maximum Pressure:	psi	psi
Percent of Final Stroke:	%	%
Compressed Volume:	uL	uL
Compression Strokes:		
Test Attempts:		

- ☐ Enter Service mode in the console by entering the normal service password.
- ☐ Set up the BSM console plots to show just the Accumulator A and Primary A plots.
- ☐ Run the Static Leak test for pump A using solvent A1 at 5000 PSI with the Vent Valve as the end point.
- ☐ Record the results in the fields below.
- ☐ Do the plots resemble the normal plots of a static leak test_____?

Note from the release notes: Because of improvements to the dynamic leak test, the static leak test is no longer available. While the menus are still available in the June 2011 DP, the test functions as a dynamic leak test.



The image shows a software dialog box titled "Static Decay Test". It is divided into several sections. The "Test Parameters" section on the left contains a "Module:" dropdown menu set to "A" and an "Accumulator:" text box containing "5000" followed by "psi". To the right of this is a "Run Time:" section with a text box showing "0.0 min" and a "0.0" label below it. Below the "Run Time" section are three buttons: "Start", "<< Results", and "Close". At the bottom right is a "Print..." button. The "Results" section at the bottom is divided into two columns: "Primary" and "Accumulator". Each column contains four rows of labels: "Decay Rate:", "Leak Rate:", "% of Stroke:", and "Final Pressure:", each followed by a unit (psi/min, µL/min, %, and psi respectively).

Test Parameters		Run Time:
Module:	A	0.0 min
Accumulator:	5000 psi	0.0

Buttons: Start, << Results, Close, Print...

Primary		Accumulator	
Decay Rate:	psi/min	Decay Rate:	psi/min
Leak Rate:	µL/min	Leak Rate:	µL/min
% of Stroke:	%	% of Stroke:	%
Final Pressure:	psi	Final Pressure:	psi



Section 14

I-Class BSM PM Procedures



Since the prerequisite for this course is the ACQUITY Systems course, it will be assumed that you already have knowledge of the basic BSM PM procedures. We will therefore only focus on the parts of the I-Class BSM that differ from the original BSM.

- ❑ Parts Replaced (only items in bold will be performed in this training as the others are the same as the BSM)
 - ❑ Solvent Reservoir Filters
 - ❑ Solvent Filters
 - ❑ **Primary and Accumulator Check Valve Cartridges**
 - ❑ **Primary and Accumulator Head Seals (do not remove seals from heads)**
 - ❑ Primary and Accumulator Plungers
 - ❑ High Pressure Filter Assembly
 - ❑ Air Filter

Primary and Accumulator Check Valve Cartridges

Important: Wear particulate-free, powder-free non-latex gloves when handling critical clean parts.

- ❑ Following the proper procedure from the Operators guide, remove then replace both the Primary and Accumulator check valves from the A pump.

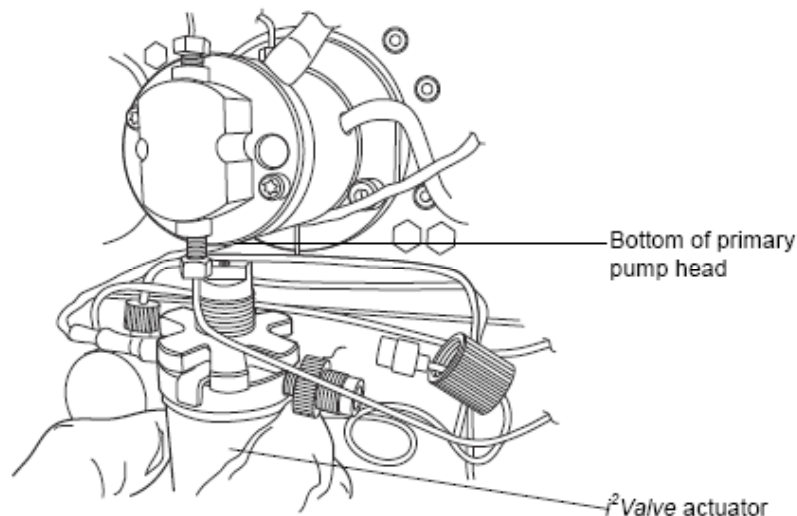
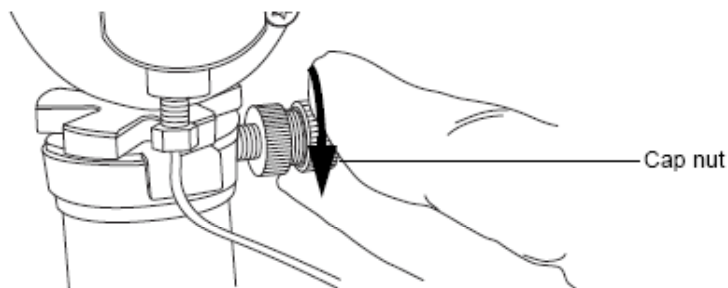
Note: It is necessary to install a new check valve. Just reinstall the old check valve.

Note the presence of the gold gaskets rather than the normal peek gaskets. These help extend the working range of the check valves beyond 15K psi.

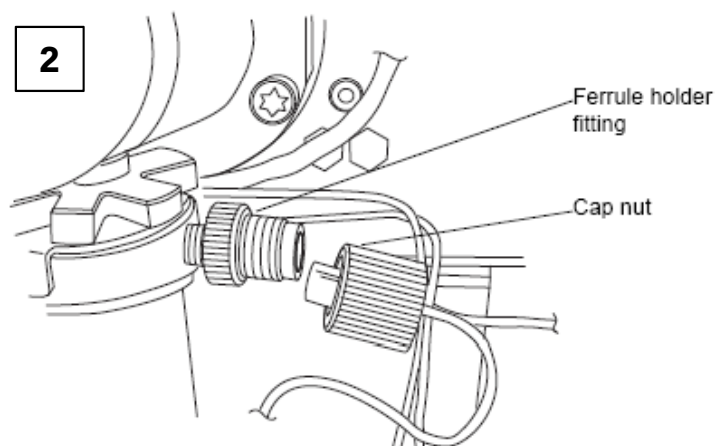
1

From the I-Class Operators Guide

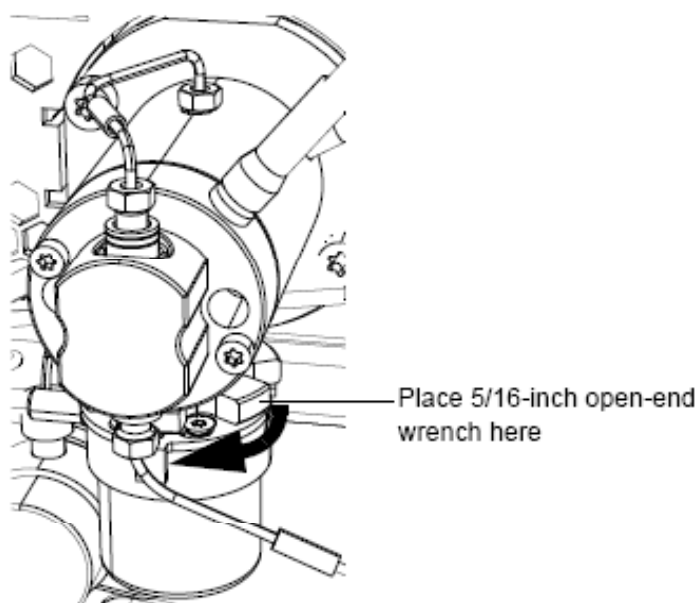
4



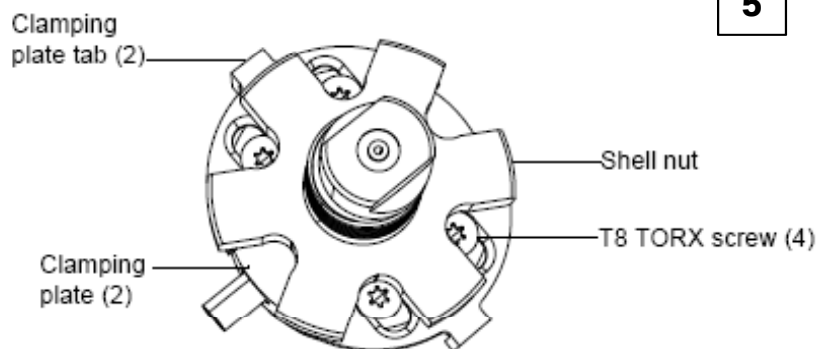
2



3

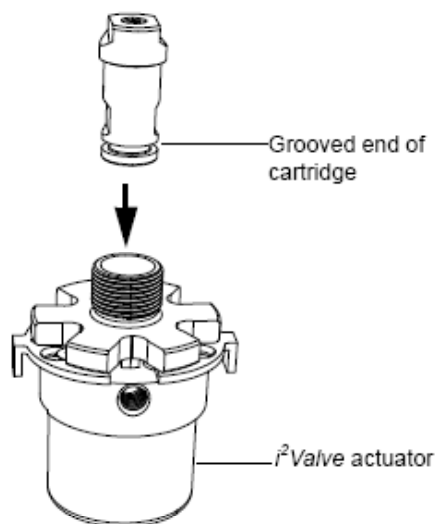


5

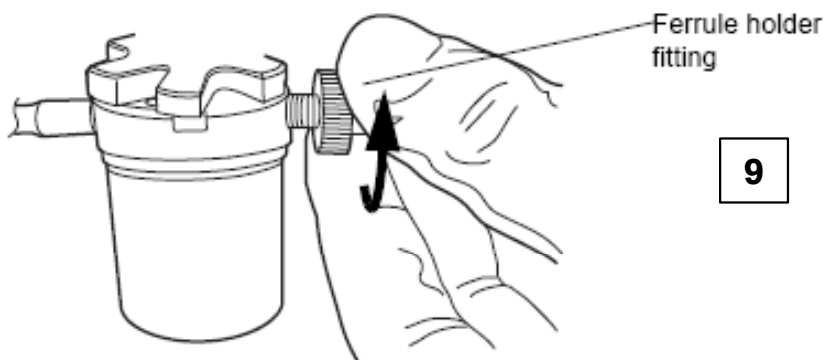


From the I-Class Operators Guide

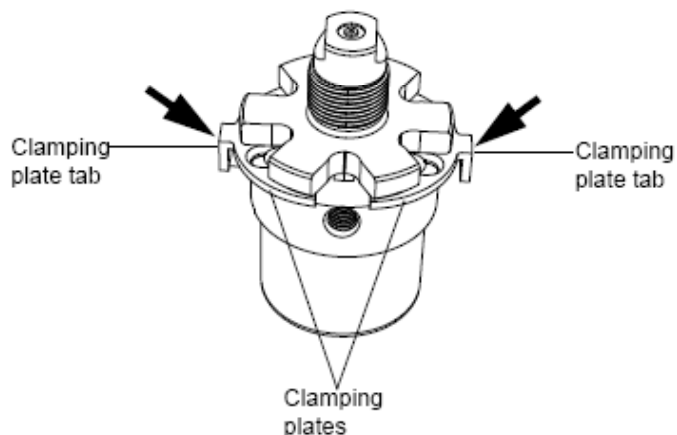
6



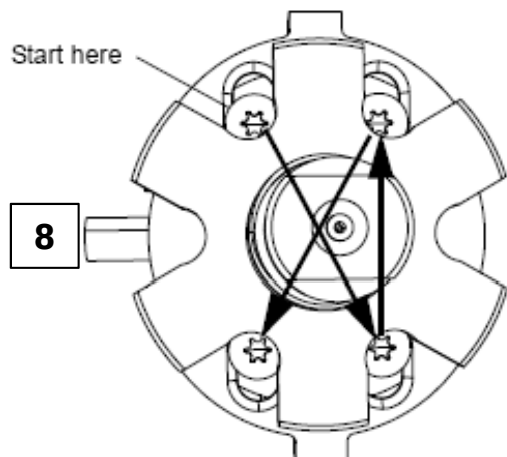
9



7

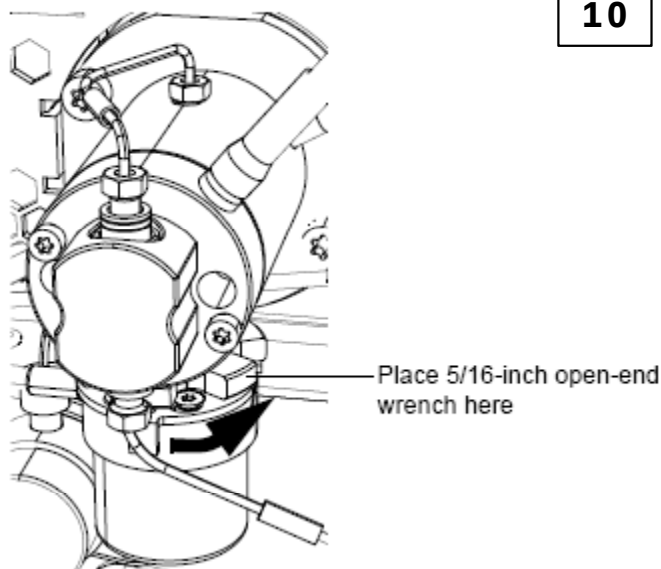


While squeezing the clamping plate tabs, use the T8 TORX driver to tighten the 4 screws that secure the plates, repeating the torquing pattern shown below at least 3 times and gradually increasing the torque until the screws are uniformly tight



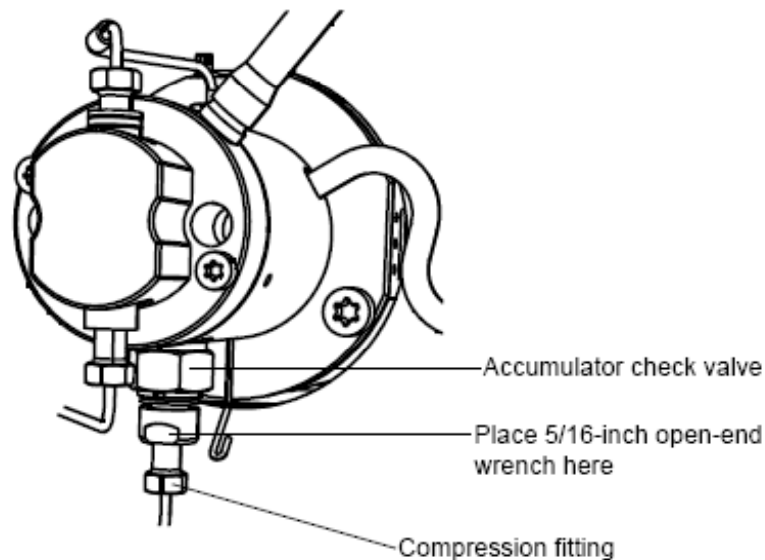
8

10



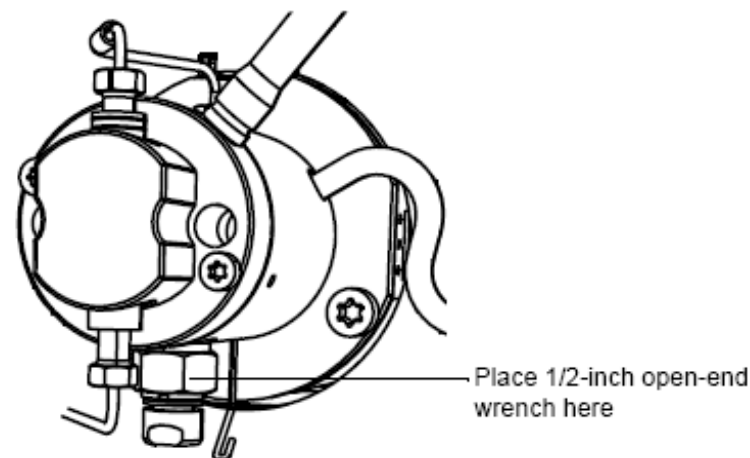
From the I-Class Operators Guide

4. Using the 5/16-inch open-end wrench to hold the check valve in place, disconnect the compression fitting by using the 1/4-inch open-end wrench.



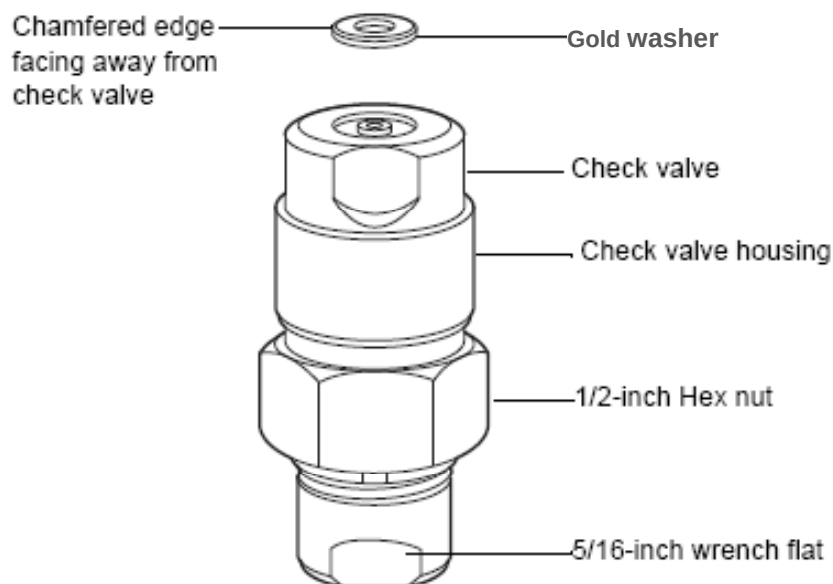
Caution: To avoid leaks, ensure the Gold washer, which is normally on the top face of the accumulator check valve, does not remain in the head when you remove the valve assembly (see [page 71](#)).

5. Use the 1/2-inch open-end wrench to loosen the check valve, and then remove the check-valve assembly from the head.



From the I-Class Operators Guide

- ❑ Ensure the new Gold washer is inserted into the new check valve, its chamfered edge facing away from the check valve.
- ❑ Insert the check-valve assembly into the head, and use the 1/2-inch wrench to tighten the check-valve nut 1/8-turn beyond finger-tight.
- ❑ Finger-tighten the compression screw and ferrule, and then, using the 5/16-inch open-end wrench to hold the check valve in place, use the 1/4-inch wrench to tighten the compression screw up to 1/6-turn beyond finger-tight for an existing stainless steel tubing assembly, or 3/4-turn beyond finger-tight for a new stainless steel tubing assembly.



Pump A Head Removal and Seal Inspection

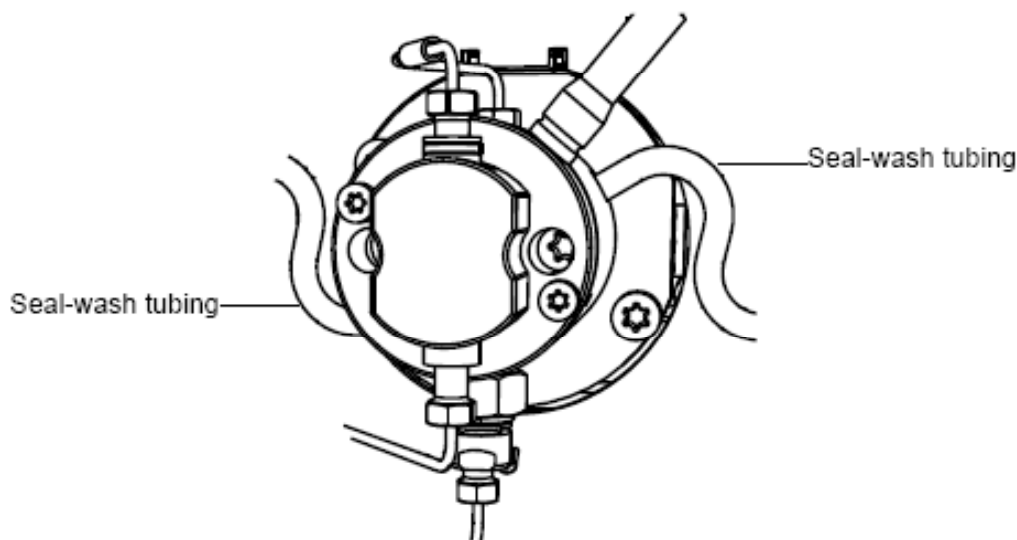
Important: Wear particulate-free, powder-free non-latex gloves when handling critical clean parts.

- Following the proper procedure from the Operators guide, remove the Accumulator Head from the A pump.

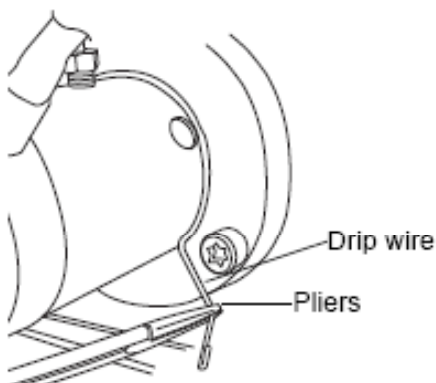
Note: It is necessary to remove the seals. Just observe the positioning of the new plunger seal at this time until spares are available.

From the I-Class Operators Guide

Remove the seal-wash tubing secured to the seal-wash housing by barbed fittings by using a tool or by pulling on the tubing as close to the head as possible.



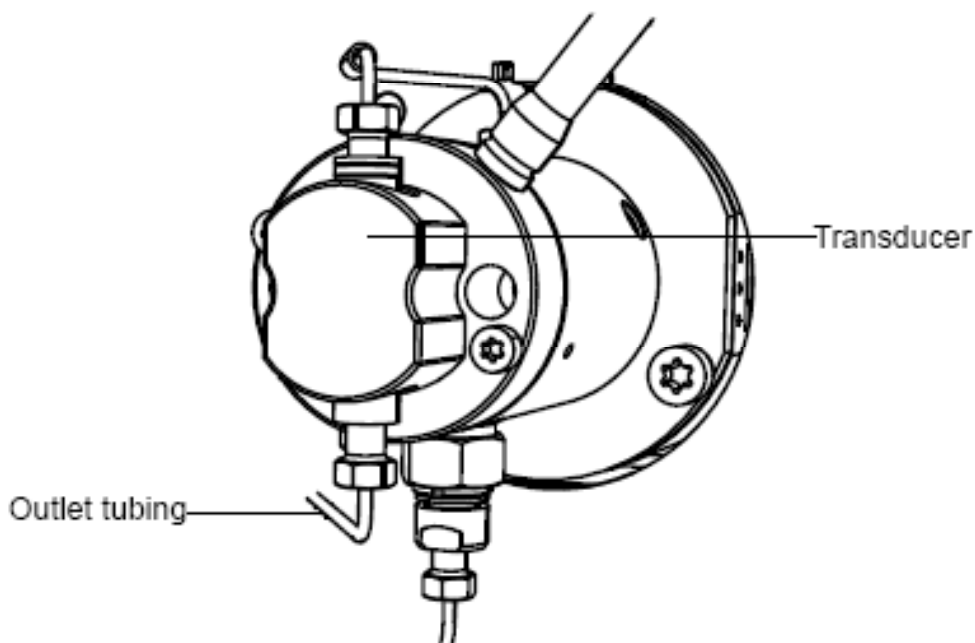
Using a pliers, remove the drip wire from the head assembly.



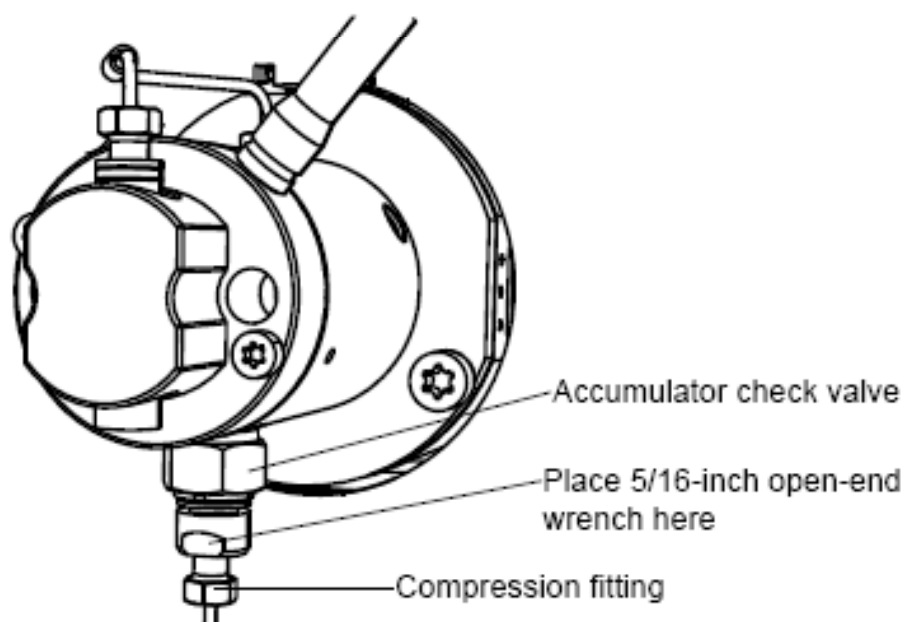
Pump A Head Removal

From the I-Class Operators Guide

Using the 1/4-inch open-end wrench, disconnect the outlet tubing from the transducer.

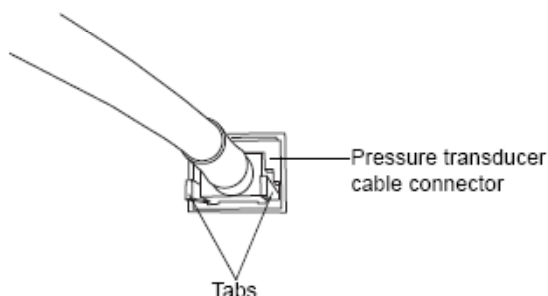


Using the 5/16-inch open-end wrench to hold the check-valve cartridge in place, disconnect the tubing connection from the check valve with the 1/4-inch open-end wrench.



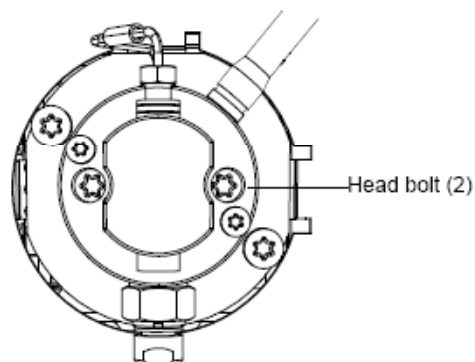
Pump A Head Removal

Disconnect the pressure transducer cable from the bulkhead by squeezing on the tabs and pulling gently.

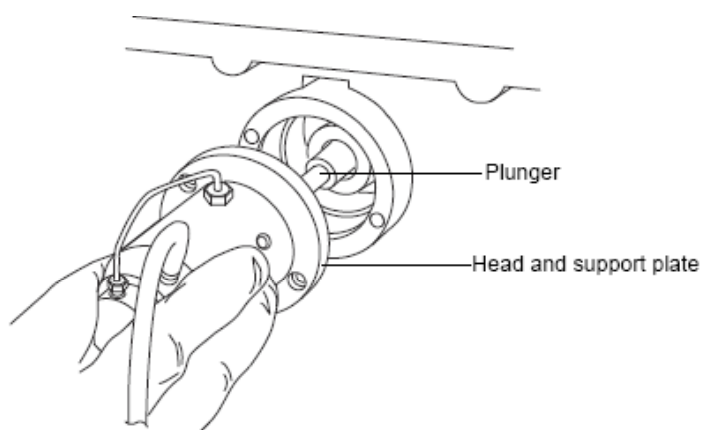
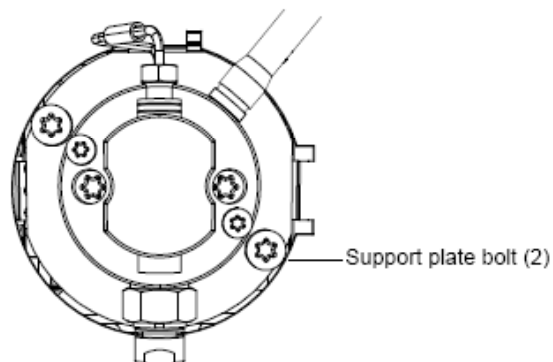


Using the T27 TORX driver, loosen the 2 head bolts 1/2-turn.

Tip: The bolts are accessible from the front of the pressure transducer.



Using the T27 TORX driver, loosen and remove the 2 support plate bolts, and then gently pull the head and support plate off the actuator housing, making sure not to tilt the head during the extraction.

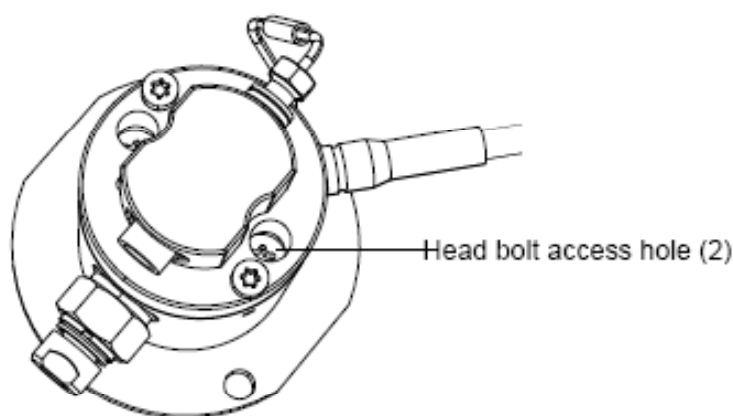


From the I-Class Operators Guide

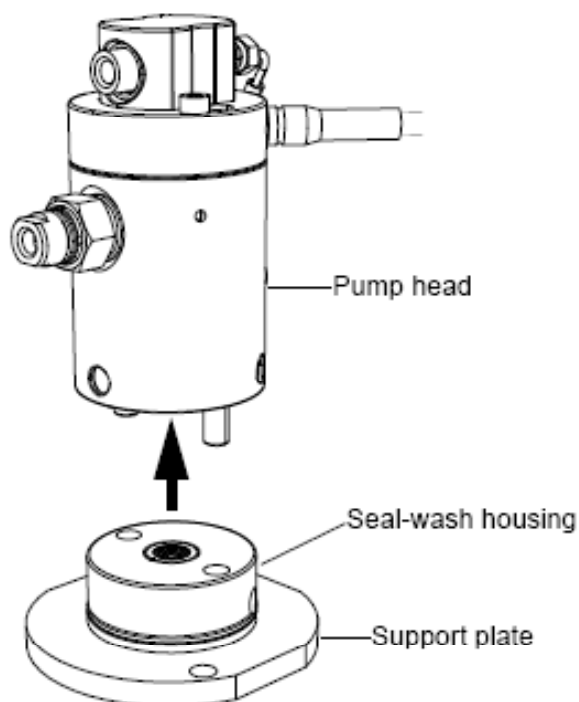
To remove the accumulator plunger seals:

1. Stand the head upright on a clean surface.
2. Using the T27 TORX driver, completely loosen the 2 head bolts to release the support plate from the pump head.

Requirement: If you remove the transducer and head bolts, be sure to reuse the head bolt washers when reassembling the pump head.

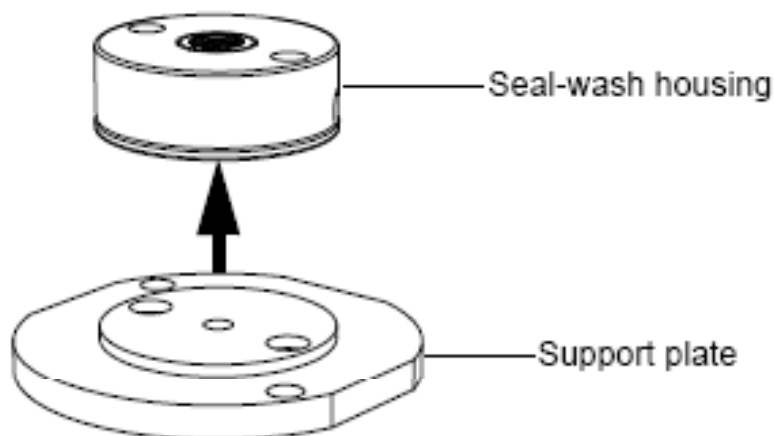


3. Lift the pump head from the support plate.

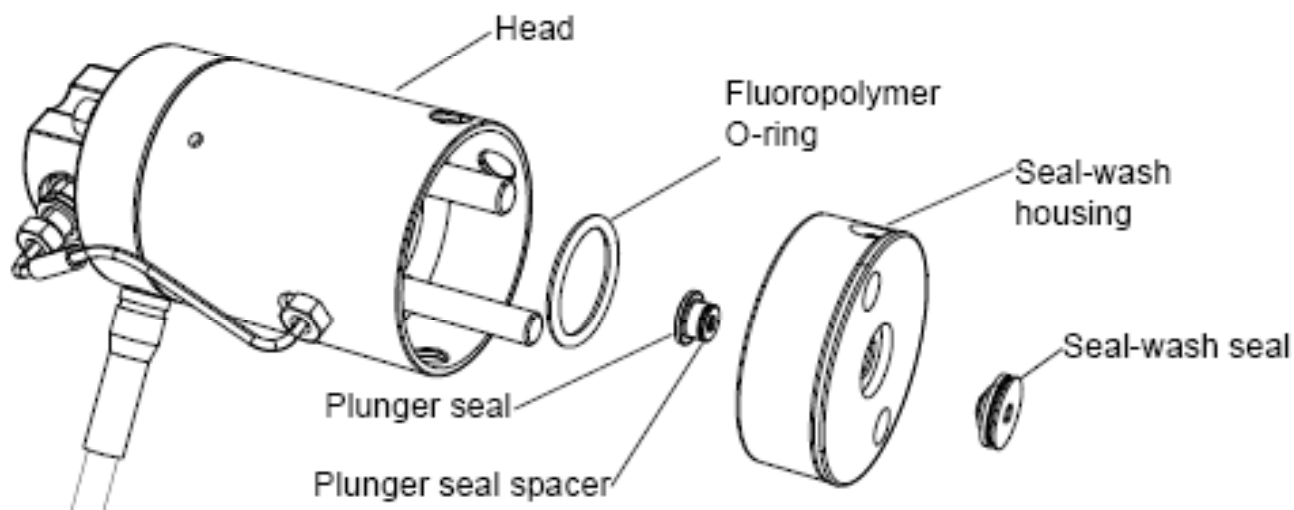


From the I-Class Operators Guide

Lift the seal-wash housing from the support plate.



Plunger seals:

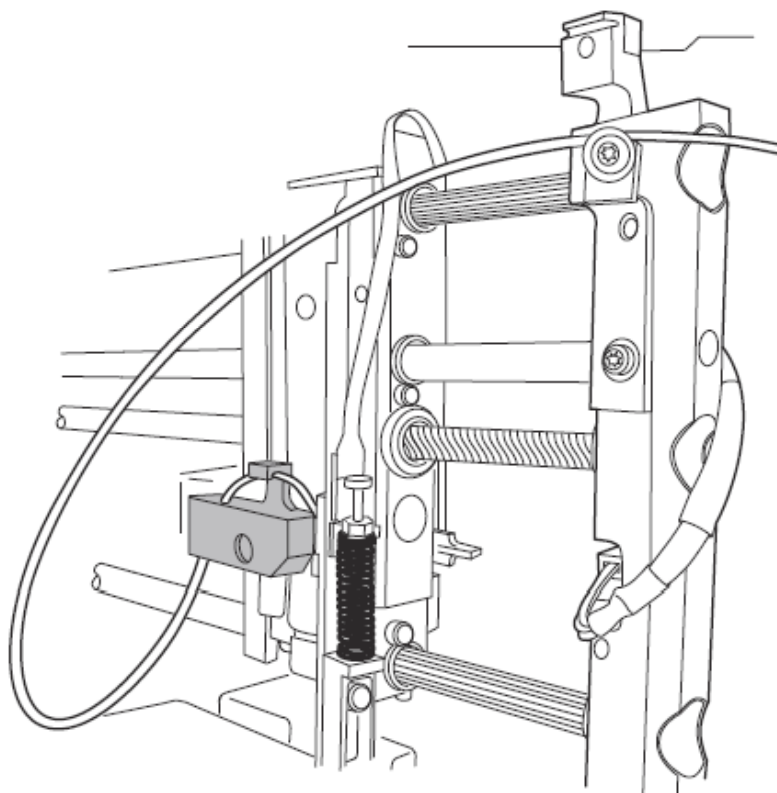


Note: Do not remove the seals at this time unless the instructor has indicated that spare seals are available.



Section 15

I-Class SM-FL PM Procedures



- ☐ Parts Replaced (only items in bold will be performed in this training as the others are the same as the SM)
 - ☐ **PEEK Sample Needle**
 - ☐ Injector Pod Assembly
 - ☐ Sample Metering Syringe
 - ☐ **Strong and Weak Wash Syringes**
 - ☐ **Inject Port O-Ring**
 - ☐ **Injector Loop**
 - ☐ Solvent Reservoir Filters
 - ☐ Air Filter

Replacing the Sample Needle

Important: Wear particulate-free, powder-free, non-latex gloves while handling the Stationary Side Seal, tubing and other critical clean parts.

- ☐ Following the proper procedure, remove and replace the Sample Needle. The same Sample Needle can be removed and replaced without damage.

To replace the sample needle:

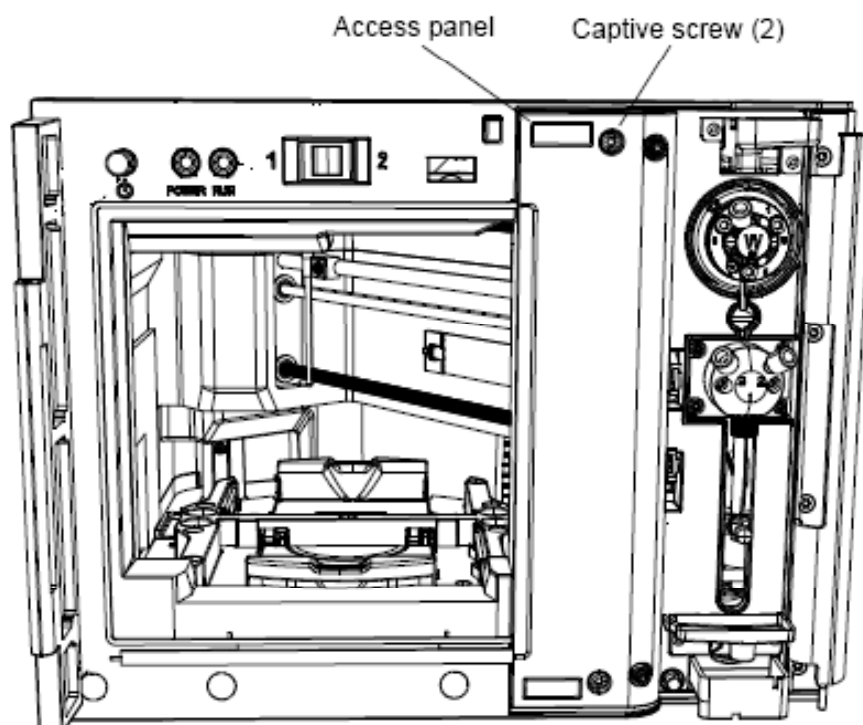
1. In the ACQUITY UPLC Console, select Sample Manager-FL from the system tree.
2. Remove the sample plate, if a plate is loaded.
3. Click Maintain > Replace > Needle.

Result: Doing so invokes a wizard that moves the sample needle assembly to an accessible position.

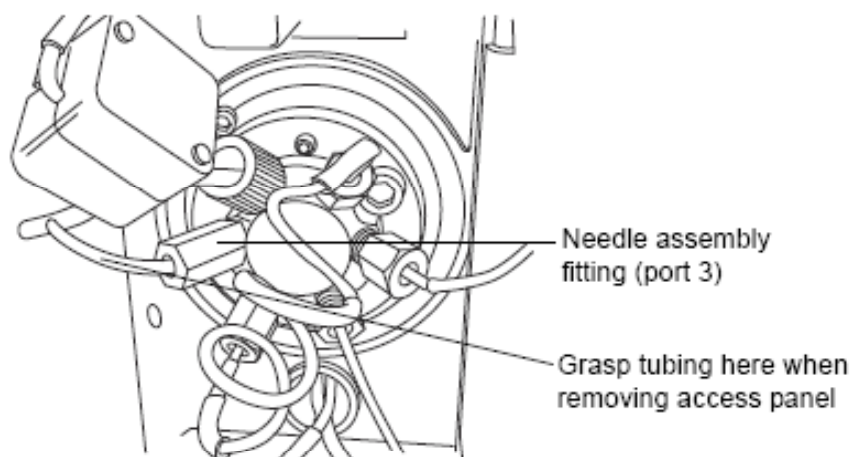
4. Open the sample compartment and fluidics compartment doors.

From the I-Class Operators Guide

5. Using the T20 TORX driver, loosen the two captive screws that secure the access panel.

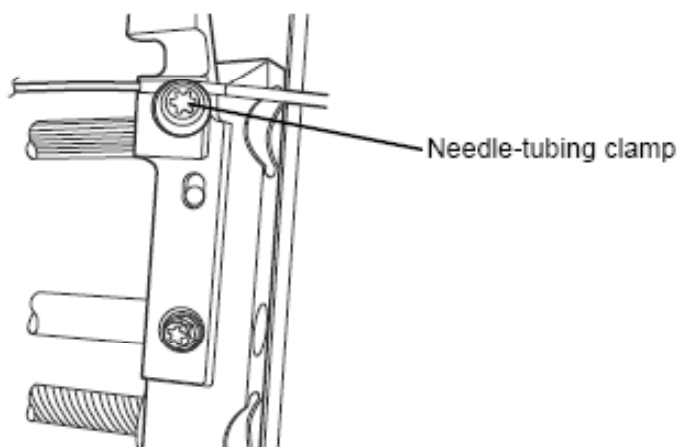


6. Carefully grasp the needle tubing, and hold it securely while you remove the panel.
7. Unscrew the needle assembly's fitting from injector port 3.

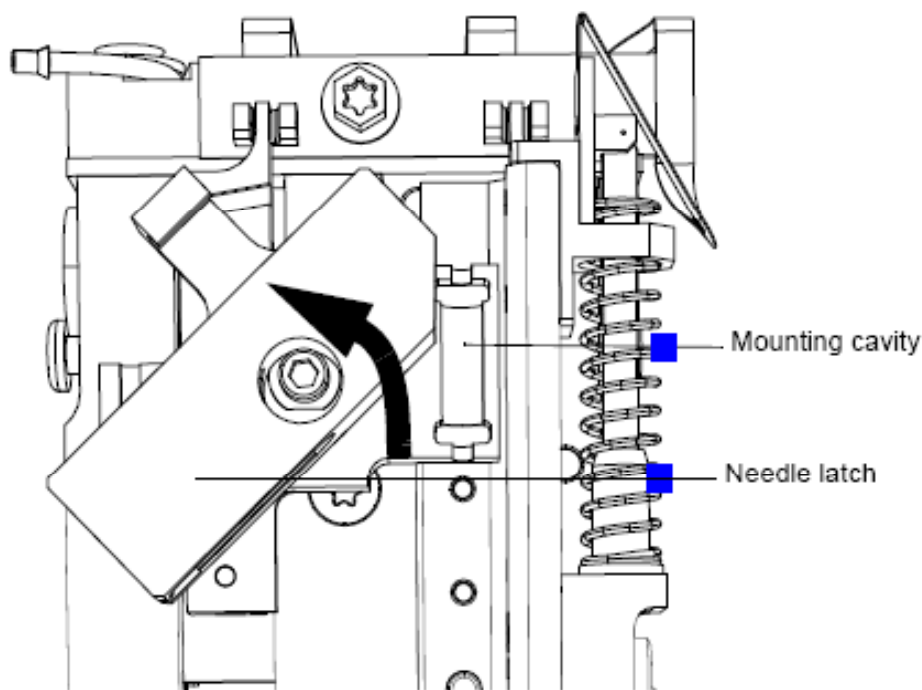


From the I-Class Operators Guide

8. Hold the needle tubing, and use the TORX T10 driver to loosen the screw on the needle-tubing clamp.

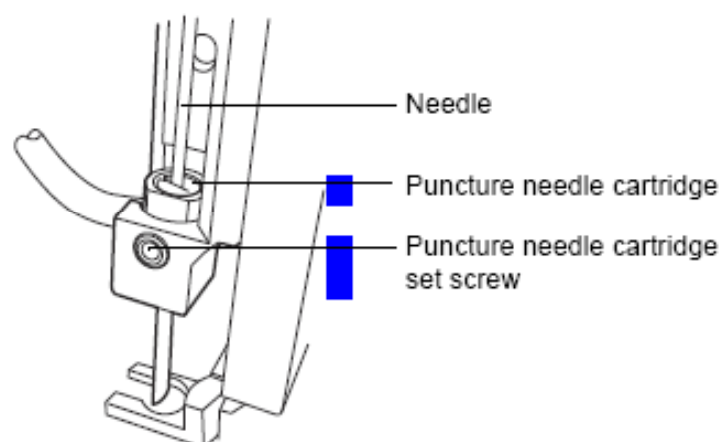


9. Remove the needle tubing from the slot in the clamp assembly.
10. Hold the fitting end of the needle tubing, and push the needle latch back, to release the needle mounting cylinder from its mounting cavity.



From the I-Class Operators Guide

11. Lift the needle tip from the puncture needle cartridge at the bottom of the needle mechanism.



Warning: To avoid puncture wounds, do not press or otherwise touch the needle tip.



Caution: To avoid damaging the needle, do not press or otherwise touch its tip.

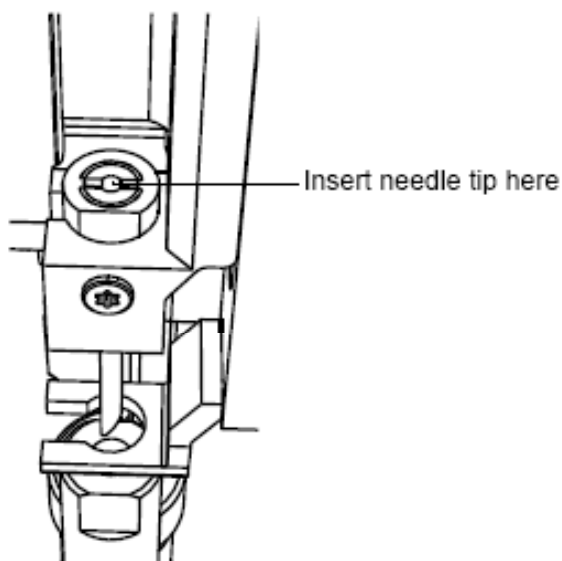


Caution: To avoid damaging the needle tubing, and to ensure the needle fits correctly in the device, do not bend or in any way deform the needle.

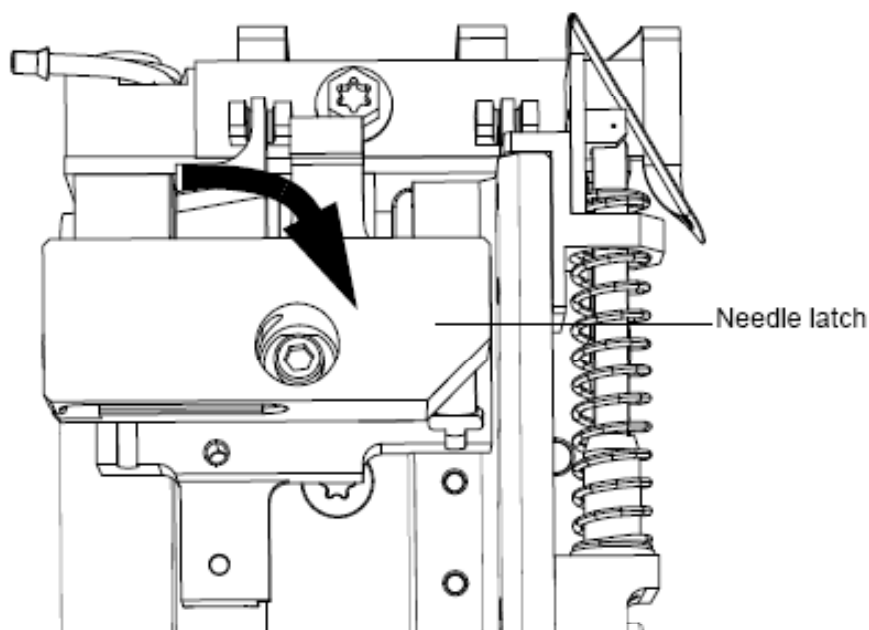
12. Using the T6 TORX driver, loosen the puncture needle cartridge set screw, and then remove the puncture needle cartridge.
13. Install the new puncture needle cartridge, and tighten the set screw.

From the I-Class Operators Guide

14. Holding the new needle assembly with both hands to control its position within the sample compartment, insert the needle's tip into the puncture needle cartridge at the bottom of the needle mechanism.

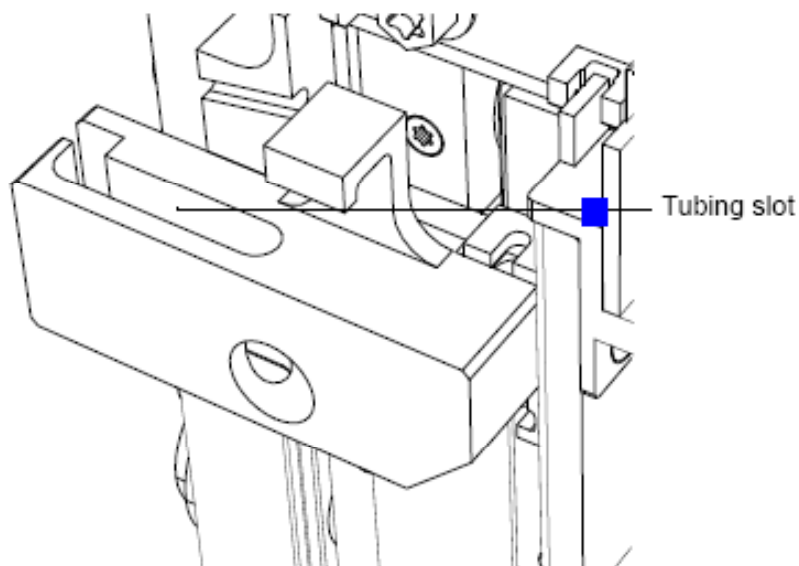


15. Insert the needle mounting cylinder into the mounting cavity on the carriage assembly.
16. Push the needle latch downward, to secure the needle assembly.

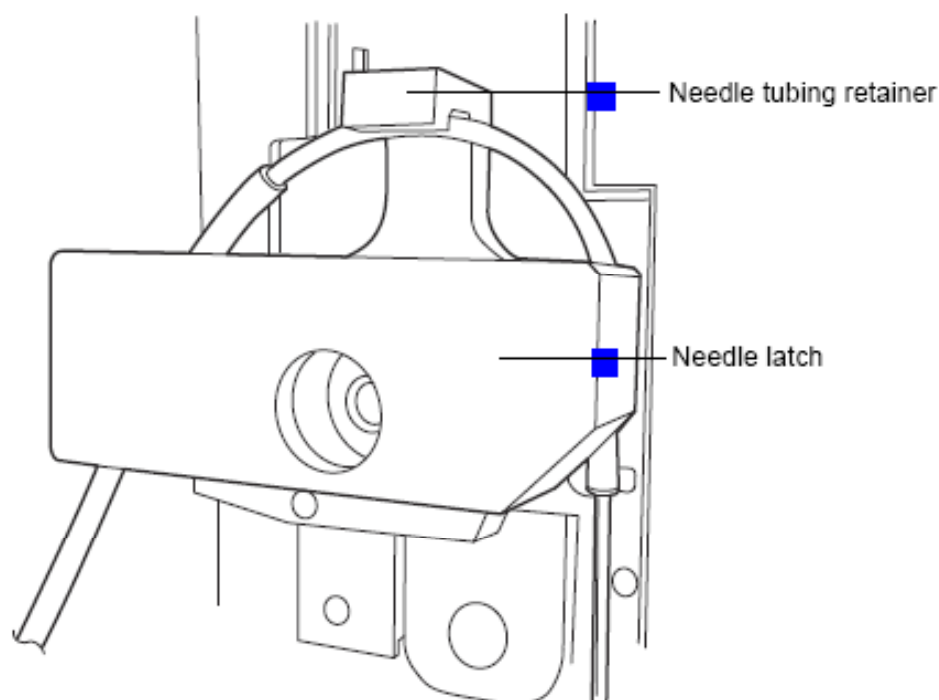


From the I-Class Operators Guide

17. Route the needle tubing into the slot on the backside of the latch.



18. Carefully bend the needle tubing until it snaps into place underneath the retainer on the needle latch.



19. Bring the needle tubing up toward the slot in the clamp assembly.

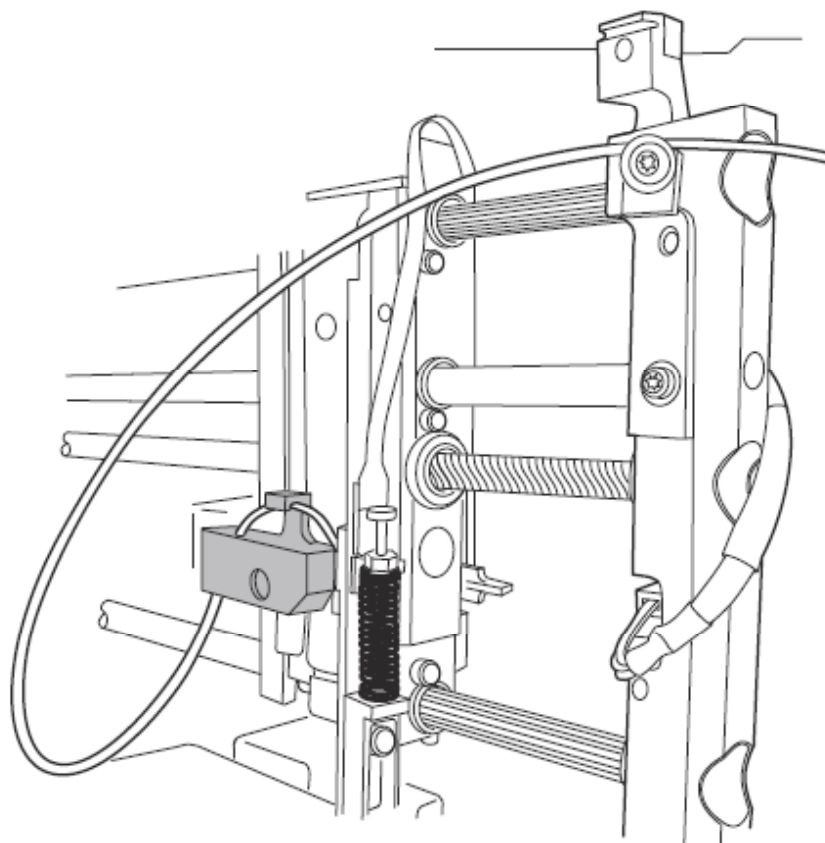
Tip: The tubing bends, to accommodate the routing.

From the I-Class Operators Guide

20. Ensure that the tubing is routed as pictured below.



Caution: To prevent damage to the needle tubing, do not force it into position.

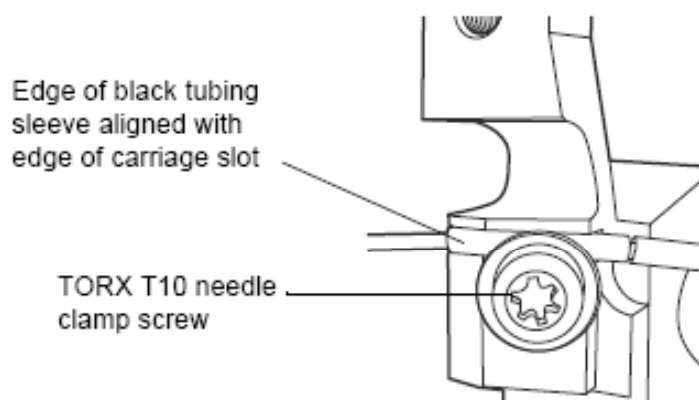


21. Route the needle tubing through the slot in the clamp assembly.



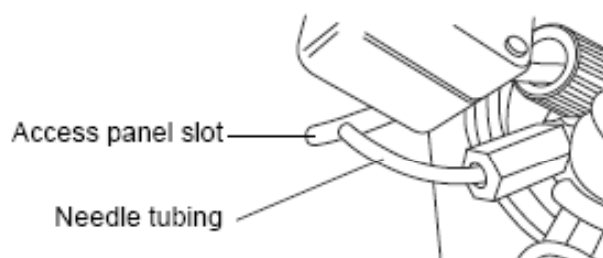


22. Ensure that the edge of the black tubing sleeve aligns with the left edge of the slot, as shown below.



Caution: To avoid damage to the needle during operation, allow the needle to release any residual tension before you tighten the clamp.

23. Using the T10 TORX driver, tighten the screw on the needle-tubing clamp.
24. Insert the needle tubing fully into port 3 on the injection valve, with the two-piece ferrule assembly bottomed in the port, and then thread the fitting into the port.
25. Tightening the fitting by hand, and then add 1/4-turn using the wrench.
26. Reinstall the access panel, ensuring that the needle tubing is routed through the slot on the side of the access panel.
27. Use the T20 TORX driver to tighten the two screws that secure the access panel to the front of the unit.



28. Close the sample compartment and fluidics compartment doors.
29. Calibrate the needle's Z axis (see ["Calibrating the needle's z axis" on page 1-54](#)).
30. Characterize the needle seal (see the ACQUITY UPLC online Help).



Calibrating the needle's z axis

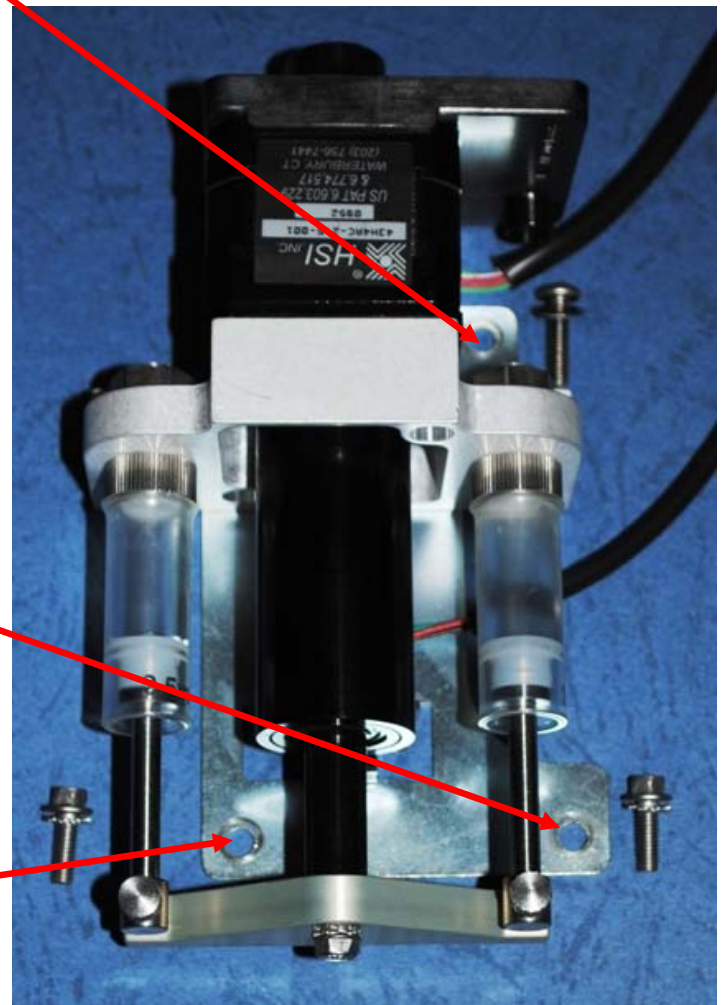
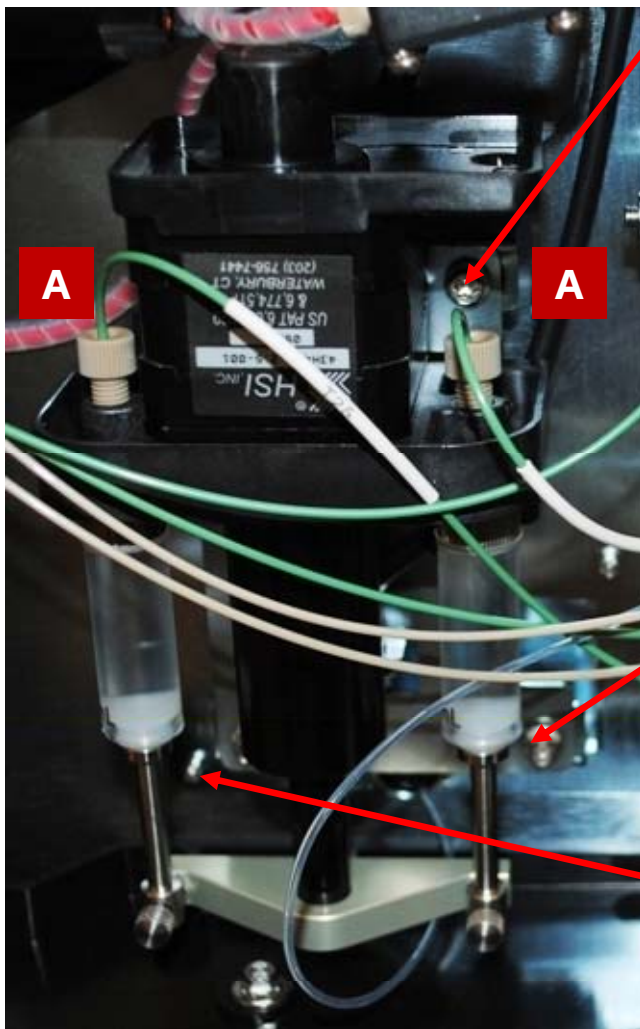
You must calibrate the needle's z axis whenever you replace the sample needle. Failing to calibrate the needle can damage it. The calibration procedure is the same for all needles.

To calibrate the needle's z axis

1. Open the sample manager door.
2. Remove the plates from the trays.
3. In the console, select Sample Manager > Control > Reset SM.
Result: The mechanism moves to the home position, with the needle above the wash station.
4. Click Maintain > Calibrate needle Z axis.
5. Click Start, and then click OK in the confirmation window.
Tip: To easily and efficiently achieve the correct needle setting, slide a business card under the needle.
6. Select 1.0 mm for displacement per keystroke.
7. Use the +Z button (Page Down key) to drive the needle down to within 1 millimeter of the tray surface.
8. Switch the displacement increment to 0.1 millimeter, and lower the needle until it is almost touching the surface of the business card.
9. Click Save > Yes, and then click Close.
10. Remove the card.
11. Specify the sample needle and syringe volumes, if they changed.
12. Characterize the needle seal (see the ACQUITY UPLC online Help).

- ❑ Remove the Wash Syringe drive assembly by disconnecting the peek tubing **A** and removing the 3 T20 torx screws.
- ❑ Reinstall the assembly and then practice replacing the strong and weak wash syringes (detailed on the next 3 pages).

Screw Locations



Replacing the Strong and Weak Wash Syringes

- Using the procedure that follows, remove and replace the Strong and Weak Wash Syringes. The same syringes can be removed and replaced without damage.

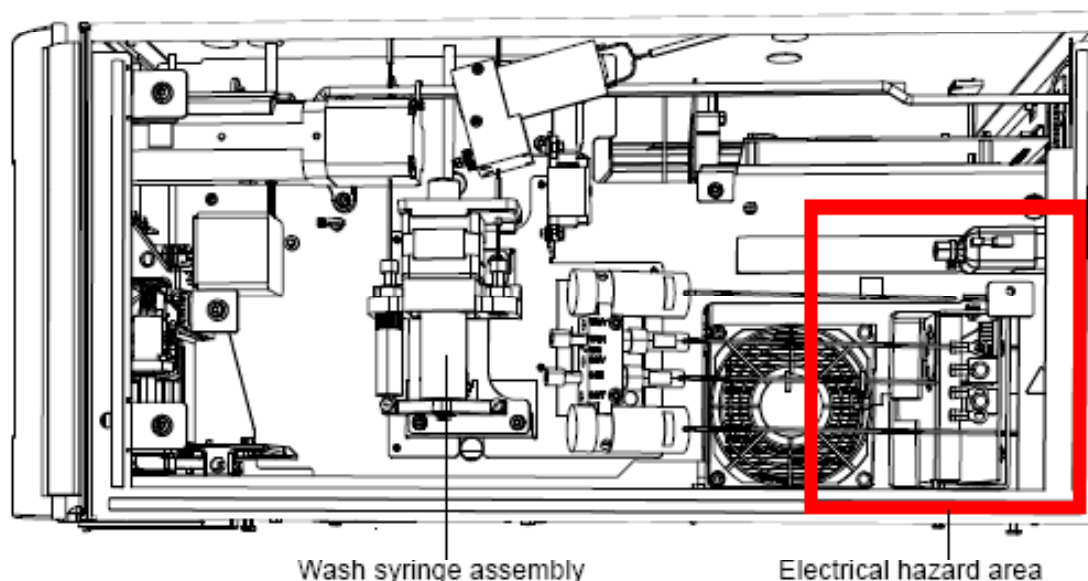
from the I-Class Operators Guide

To replace the wash syringes:

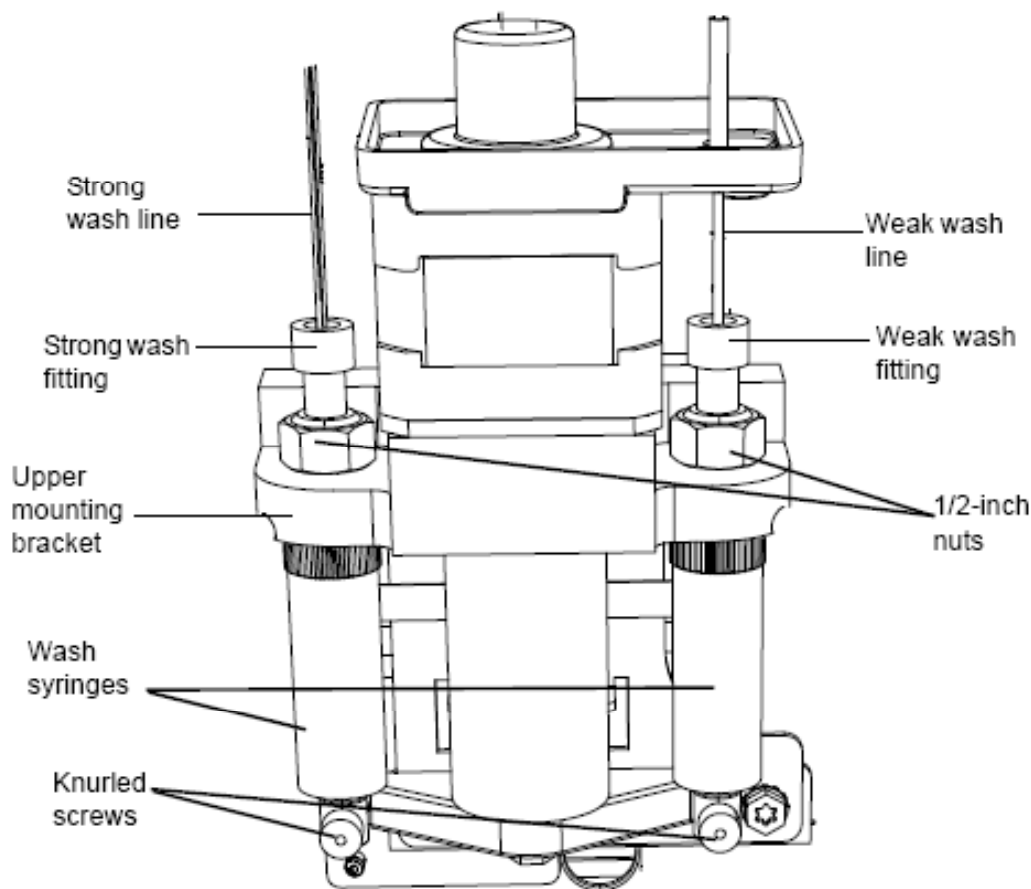
1. Remove the wash solvent lines from the solvent bottles.
2. Remove the two TORX T20 screws that fasten the instrument's side panel.
3. Slide the side panel back about 2.5 cm, tilt the bottom of the panel to disengage the top lip from the instrument chassis, and then remove the panel.



Warning: To avoid injury from electric shock, do not contact any of the components in the electrical hazard area shown below.



4. Unscrew the weak and strong wash fittings, and then move the wash lines away from the seal wash assembly.



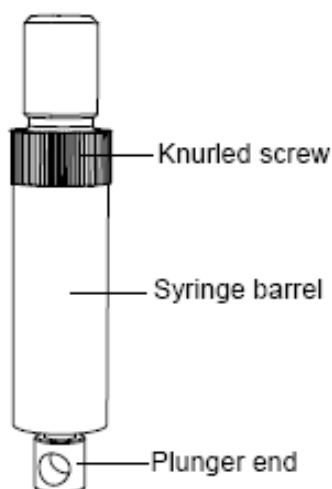
5. Using the 1/2-inch open-end wrench, remove the nuts that secure the wash syringes to the upper mounting bracket.
6. Loosen the knurled screws that secure the wash syringe plungers to the wash syringe mounting bracket.
7. Unscrew each syringe until it separates from the mounting bracket.



Caution: To avoid breaking the syringe, do not hold it by its glass barrel. Instead, hold the syringe by its knurled collar.

8. Pull down the syringe barrel to clear the upper mounting bracket, and remove the syringe.
9. Unpack each replacement wash syringe.

Wash syringe



10. Partially fill each new wash syringe (by hand) with weak or strong wash solvent (to help remove air bubbles and to lubricate the syringe plunger).
11. Slide the plunger end of the new wash syringe over the threaded post on the syringe guide mounting bracket.
12. If necessary, depress the syringe barrel to clear the upper mounting bracket, and then screw each new wash syringe into the mounting bracket.
Tip: You sometimes must pull upward on the syringe barrel as you screw the wash syringe into the mounting bracket.
13. Using the 1/2-inch open-end wrench, install the nut that secures the wash syringe to the upper mounting bracket.
14. Install and finger tighten the knurled screw that holds each wash syringe barrel to the mounting bracket.
15. If plugs are fitted in the wash syringe tips, remove them.
16. Reattach the weak-wash and strong-wash lines, connecting the fittings finger-tight.
17. Prime the system until no bubbles appear in the syringes.
18. Grip the device's side panel by its bottom edges.
19. Position the side panel so that it extends approximately 2.5 cm beyond the rear of the device, with the top edge of the panel beneath the device's top cover.

from the I-Class Operators Guide

20. Tilt the bottom of the side panel inward, and slide the side panel forward until it meets the front of the device.

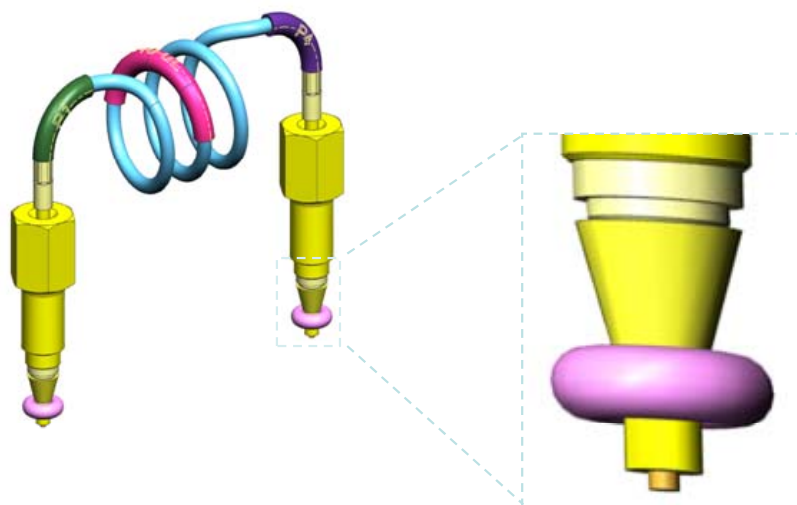
Tip: The holes in the panel align with the screw holes in the chassis.

21. Secure the side panel using the two TORX T20 screws.

I-Class Sample Loops

The Sample Loops for the I-Class have been redesigned to:

- ☐ Improve Volume Accuracy
 - ☐ Hypodermic style tubing
 - ☐ 0.025" OD
- ☐ Improve Dispersion
 - ☐ Protruded end
 - ☐ Positive contact point
- ☐ Coil style is flexible
 - ☐ User friendly easy to install



- ❑ Following the proper procedure, remove and replace the Sample Loop. The same Sample Loop can be removed and replaced without damage.

Replacing the sample loop

Replace the sample loop when it clogs or when its capacity is inappropriate for your chromatographic need.



Caution:

- To prevent bandspreading or excessive carryover, be sure to reinstall a previously installed sample loop in exactly the same way that it was installed before, so that the same end goes into the same port.
- To avoid leaks, remove the O-rings prior to installing the sample loop. The O-rings on the new sample loop hold the seals in place during shipping.

Required materials

- 1/4-inch open-end wrench
- ACQUITY UPLC sample loop

To replace the sample loop

1. Open the fluidics compartment door.

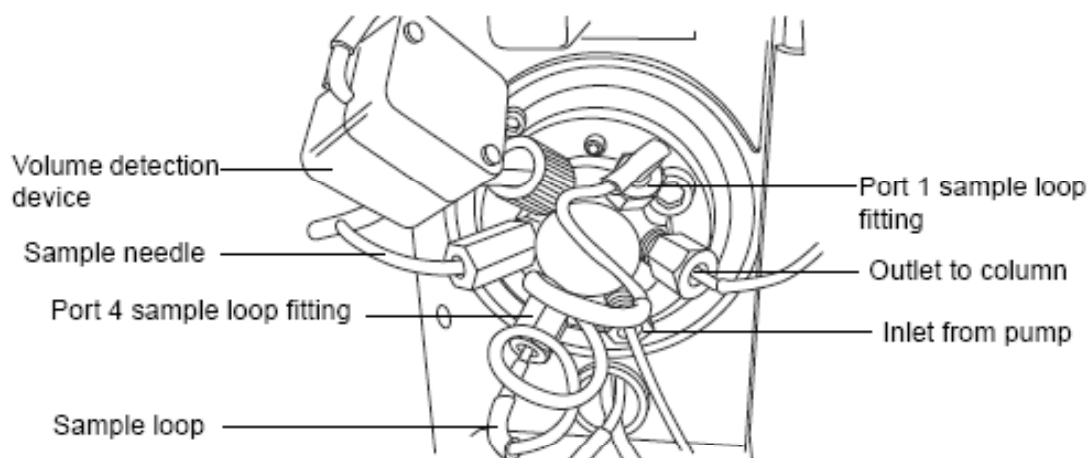


Caution: To avoid kinks, do not pull on the volume detection device (port 2) or sample needle (port 3) tubing.

2. Use the wrench to remove the fittings on ports 1 and 4 of the injection valve.



Sample loop fittings on the injection valve



3. Remove the sample loop and its fittings.
4. If you are going to reuse the sample loop, label each end with the port it was connected to.

Tip: If you replaced the injection valve cartridge, do not install previously used sample loops on the new cartridge.

5. Place the sample loop and its fittings in a contamination-free bag.
6. Unpack the replacement sample loop and fittings.



Caution: To avoid leaks, remove the O-rings prior to installing the sample loop. The O-rings on the new sample loop are used to hold the seals in place during shipping.

7. Remove the O-rings from the sample loop.



Caution:

- To prevent dead volume in the system, ensure that the ends of the loop are fully inserted into the ports of the injection valve before swaging the ferrules.
- Use only Waters approved ACQUITY UPLC loops with ACQUITY UPLC fittings. Each loop must be fitted to its injector.



8. Ensure the 2-piece ferrule is in place on one end of the sample loop. If not, slide a 2-piece ferrule and compression screw onto one end of the sample loop, and then seat the end into injection valve port 1.
9. Finger-tighten the fitting and then add 3/4-turn using the wrench.
See also: ["Installation recommendations for fittings" on page 15.](#)
10. Remove the fitting and inspect the ferrule to ensure it has not moved.
11. Repeat step 8 through step 10 for the other end of the sample loop and for injection valve port 4.
12. Reinstall the sample loop fitting into valve ports 1 and 4.



Caution:

- To avoid kinks, do not push on the volume detection device (port 2) or sample needle (port 3) tubing.
- To prevent bandspreading or excessive carryover, be sure to reinstall the sample loop in exactly the same way that it was installed before, so that the same end goes into the same port.

13. Close the fluidics compartment door.



Caution: Be careful not to crush the loop or volume detection device line.

14. Ensure the sample compartment door is closed.
15. In the ACQUITY UPLC Console, select Sample Manager from the system tree.
16. Click Maintain > Calibrate System Volume to characterize the new system volumes.

Note: You can wait until the Post Service Diagnostic Tests section to Characterize the Needle and Loop volume.

- Following the proper procedure, remove and replace the Inject Valve. Note: You can leave the Sample loop on the Inject Valve while removing and reinstalling it.

Replacing the injection valve cartridge



Warning: To avoid personal contamination with biologically hazardous or toxic materials, wear clean, chemical-resistant, powder-free gloves when performing this procedure.

Requirement: When you replace the injection valve, you must also replace the sample loop.

Required materials

- 2-mm Allen wrench
- Gloves: clean, powder-free, chemical-resistant
- Injection valve cartridge

To replace the injection valve cartridge:

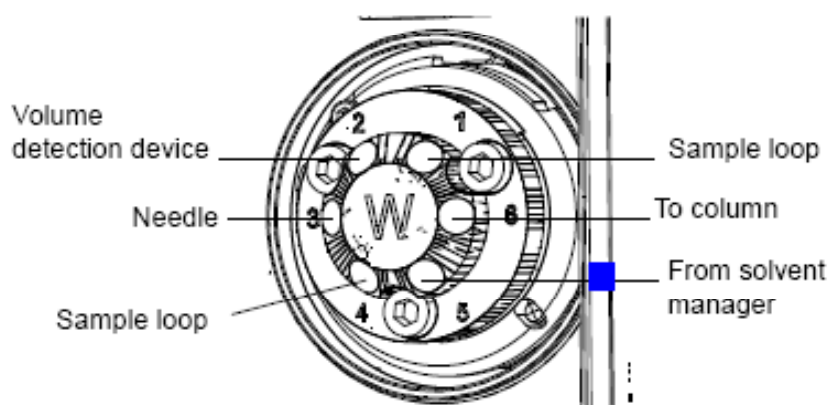
1. In the ACQUITY UPLC Console, select Sample Manager FL from the system tree.
2. In the SM-FL information window, click Maintain > Park needle and inject valve > Yes.



Caution: To avoid damaging electrical parts, never disconnect an electrical assembly while power is applied to an instrument. To completely interrupt power, set the power switch to Off, and then unplug the power cord from the AC source. Wait 10 seconds thereafter before you disconnect an assembly.

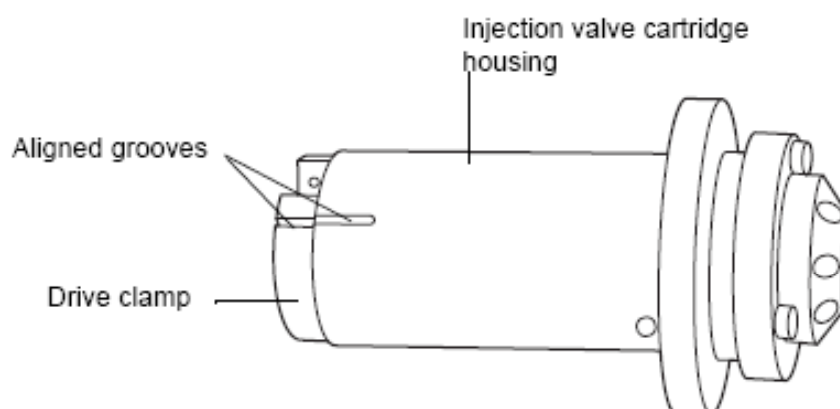
3. Power-off the SM-FL.
4. Open the fluidics compartment door.

5. Remove the fittings attached to the injection valve cartridge.



6. Use the 2-mm Allen wrench to remove the screw at the 10 o'clock position on the injection valve cartridge.
7. Remove the injection valve cartridge from the injection valve assembly.
8. Carefully unpack the replacement injection valve cartridge.
9. Ensure that the groove in the cartridge housing and the groove on the drive clamp align.

Tip: If they do not align, turn the drive clamp until the grooves line up.



10. Insert the new injection valve cartridge into the injection valve assembly.
- Note:** If the injection valve cartridge does not slide fully into the injection valve assembly, contact your Waters service representative.
11. Insert the 2-mm Allen screw at the 10 o'clock position on the injection valve cartridge, and tighten it.
 12. Reattach all fittings, ensuring the tubes bottom in their fitting holes.
 13. Close the fluidics compartment door.
 14. Power-on the SM-FL.



Section 16

I-Class Post Service Diagnostic Tests



Post Service Diagnostic Tests

- ☐ Following the “ACQUITY I-Class System Installation Manual” (located online and on the workstation), put the modules back together.
- ☐ Make sure you closely follow the instructions on how to plumb the system together and route the tubing up the side.
- ☐ Make sure all solvent lines back in there proper solvents for the verification tests.
- ☐ Prime all lines for 2 minutes if they still contain solvent otherwise prime for 3 minutes.
- ☐ Start a flow rate of 0.5ml/min with 50% A and 50% B and allow the pressure to stabilize for at least 2 minutes. Record the current pressure and delta pressure ripple in the table below.

Solvent Line	System Pressure	Pressure Ripple	Pass / Fail
A-B			

The Delta pressure ripple should be below 1% of the system pressure for a passed test result.

Post Service Diagnostic Tests

- ☐ Perform a dynamic leak test using the solvent line in which you replaced the filter to a pressure of 9000 psi.
- ☐ Record the results in the fields below.

Leak Test

Parameters | Status | **Results**

	Accumulator	Primary
Result:		
Leak Rate:	nL/min	nL/min
Maximum Pressure:	psi	psi
Percent of Stroke:	%	%
Compressed Volume:	uL	uL
Compression Attempts:		
Test Attempts:		

BSM Diagnostic Tests Overall Results (Pass/Fail)_____

Initials_____

Date_____

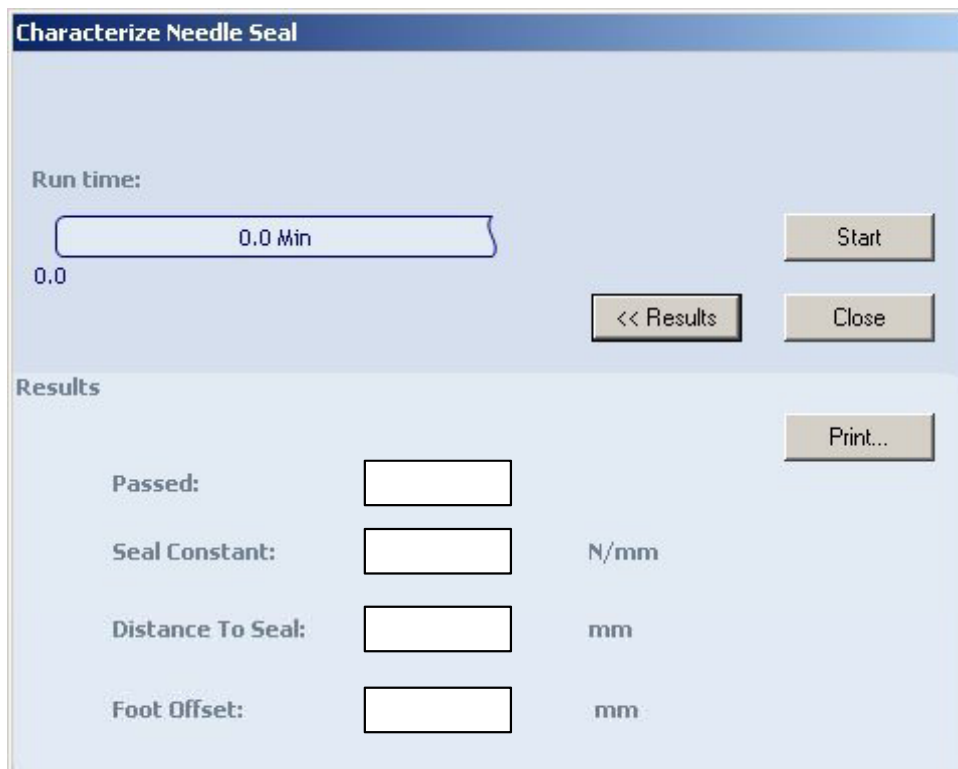
Post Service Diagnostic Tests-SM-FTN

This section deals with the SM-FTN. If your system has an SM-FL then proceed to the Post Service Diagnostic Tests-SM-FL section.

- ☐ Reset the SM-FTN from the console menu.
- ☐ Prime the Wash Solvent for 1 minute and the Purge Solvent for 10 cycles.
- ☐ Characterize the Needle Z-axis and enter the result below.

Needle Z-axis Offset: _____mm.

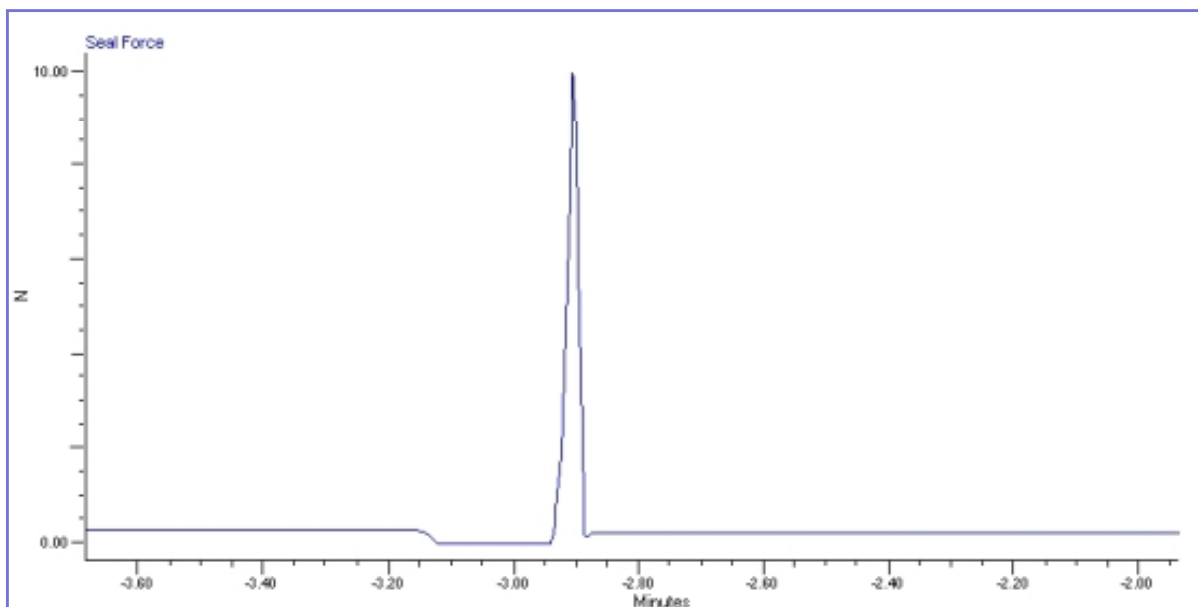
- ☐ Check the plot properties menu in the SM-FTN top level console and make sure the Seal Force plot is enabled.
- ☐ Characterize the Needle Seal and enter the result below.



The image shows a software dialog box titled "Characterize Needle Seal". It has a light blue background. At the top, there is a section for "Run time:" with a text box containing "0.0 Min" and a "Start" button. Below this is a "Results" section. It contains four rows of labels and text boxes: "Passed:", "Seal Constant:", "Distance To Seal:", and "Foot Offset:". To the right of the "Seal Constant:" text box is the unit "N/mm", and to the right of the "Distance To Seal:" and "Foot Offset:" text boxes is the unit "mm". There are also buttons for "<< Results", "Close", and "Print..." in the dialog.

Field	Unit
Passed:	
Seal Constant:	N/mm
Distance To Seal:	mm
Foot Offset:	mm

- Examine the Seal Force plot after the Characterize Needle Seal function is completed. It should look similar to the below plot.

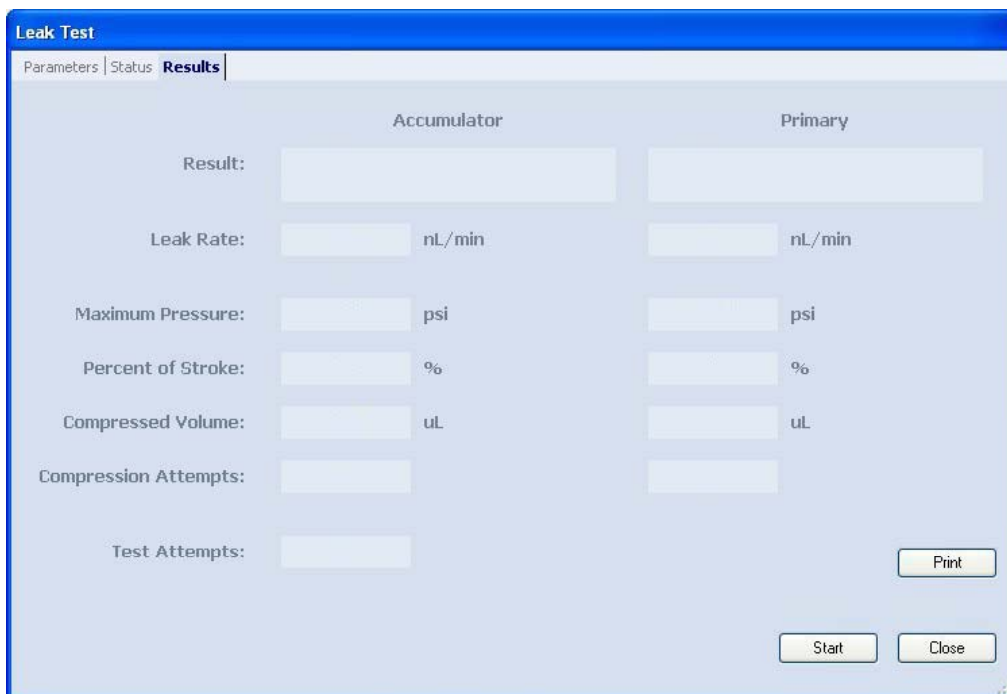


- Start a flow rate of 0.6ml/min with 90% A 10%B and allow the pressure to stabilize.
- Run the Needle Seal Readiness test and enter the result below.

Results		
Passed:		
Pressure Difference:		psi
Flow:		ml/min

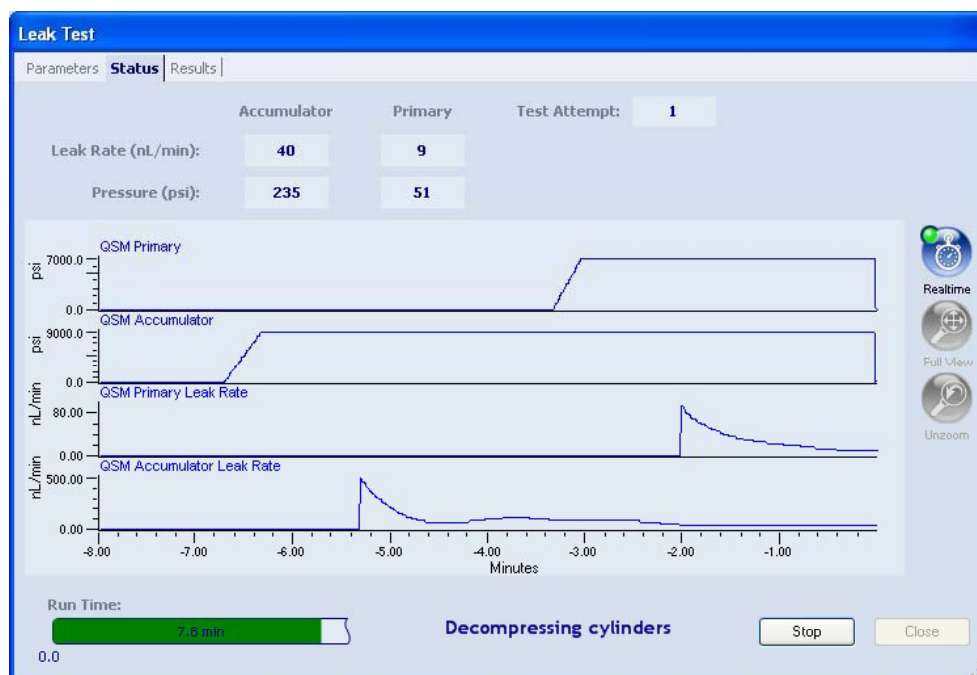
Running the BSM / SM Dynamic Leak Test

- ☐ Stop the flow and disconnect the column inlet line from the column.
- ☐ Connect the fitting plug (or union and plug, located in the solvent bottle tray front compartment) to the column inlet line to plug the line.
- ☐ Run the Dynamic Leak Test from the BSM menu.
 - ☐ Use the A Pump
 - ☐ Set the pressure 17000 PSI.
 - ☐ Choose Column as the end point.
 - ☐ Record the results below.



The screenshot shows the 'Leak Test' software window with the 'Results' tab selected. The window is divided into two columns: 'Accumulator' and 'Primary'. Each column has a 'Result:' field at the top, followed by 'Leak Rate:' (nL/min), 'Maximum Pressure:' (psi), 'Percent of Stroke:' (%), 'Compressed Volume:' (uL), 'Compression Attempts:', and 'Test Attempts:'. There are 'Start' and 'Close' buttons at the bottom right, and a 'Print' button above them.

	Accumulator	Primary
Result:		
Leak Rate:		
Maximum Pressure:		
Percent of Stroke:		
Compressed Volume:		
Compression Attempts:		
Test Attempts:		



Do the results correlate well with the BSM leak tests?

This test should give a good level of confidence that the Stationary Side Seal, tubing and fittings are holding pressure and functioning correctly.

- ☐ If the test failed, recheck all fittings and try the test again. Troubleshoot the problem by changing the location of the pin plug if necessary.
- ☐ Once the test has passed, disconnect the plug and reconnect the column to the inlet line.
- ☐ Fill in the fields below and initial and date it.
- ☐ Proceed to the System Performance Tests Section

SM-FTN Diagnostic Tests Overall Results
(Pass/Fail) _____

Initials _____

Date _____

Post Service Diagnostic Tests-SM-FL

This section deals with the SM-FL If your system has an SM-FTN then go to the previous section; Post Service Diagnostic Tests-SM-FTN.

- ☐ If needed, reset the SM-FTN from the console menu.
- ☐ Prime the Sample and Wash Syringes for 3 cycles.
- ☐ Characterize the Needle Z-axis and enter the result.
Needle Z-axis Offset: _____mm.
- ☐ Characterize the Needle Seal and enter the result.
Needle Seal- (Pass/Fail & Offset): _____mm
- ☐ Calibrate the Air Sensor and enter the result.
Air Sensor - (Pass/Fail & Volume): _____ul
- ☐ Characterize the Needle and Loop Volumes and enter the results below.

Characterize Needle and Loop Volumes

Nominal Configuration

Loop size:	10.0	µL
Needle size:	10	µL
Sample syringe size:	100.0	µL

Run time:
0.0

Results

Measured volume	
System volume with loop:	µL
System volume without loop:	µL
Loop:	µL
Needle:	µL

- Run the Sample Syringe Leak Test and record the results in the blank fields below.

Results		Leak Test	
Passed:			
Test pressure:			psi
Compressed volume:			μL
Decompressed volume:			μL
Start pressure:			psi
End pressure:			psi
Pressure change:			psi
Average pressure leak:			psi/sec

- Run the Wash Syringe Leak Test and record the results in the blank fields below.

Weak Wash Leak Test

Results		Leak Test	
Passed:			
Test pressure:			psi
Compressed volume:			μL
Decompressed volume:			μL
Start pressure:			psi
End pressure:			psi
Pressure change:			psi
Average pressure leak:			psi/sec

Strong Wash Leak Test

Results		Leak Test	
Passed:			
Test pressure:			psi
Compressed volume:			μL
Decompressed volume:			μL
Start pressure:			psi
End pressure:			psi
Pressure change:			psi
Average pressure leak:			psi/sec

- Run the Needle Seal static leak test and record the results in the blank fields below.

Results		Leak Test	
Passed:			
Test pressure:			psi
Compressed volume:			μL
Decompressed volume:			μL
Start pressure:			psi
End pressure:			psi
Pressure change:			psi
Average pressure leak:			psi/sec

- ❑ Run the Back Pressure Regulator test and record the results in the blank fields below.

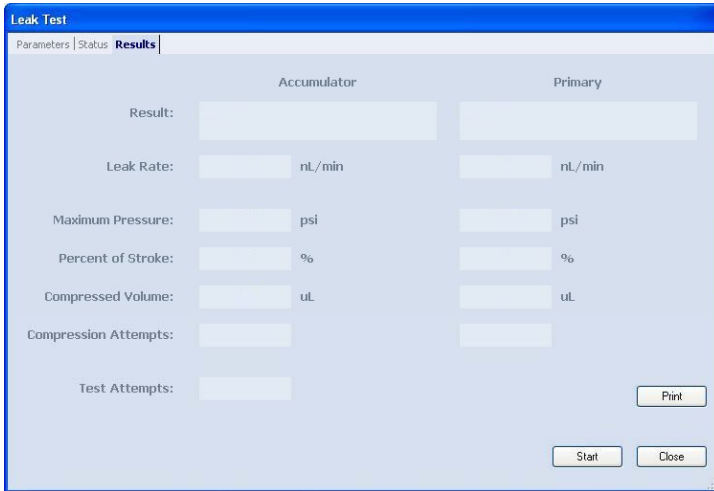


The screenshot shows a software window titled "Back Pressure Regulator Test". It contains a "Run time:" label with a text box showing "0.0 Min" and a "0.0" value below it. To the right are "Start", "<< Results", and "Close" buttons. Below this is a "Test Results" section with a "Print" button. The results are listed as follows:

Test Results	
Passed:	
Start pressure:	psi
End pressure:	psi
Average pressure:	psi
Standard deviation:	psi

Running the BSM / SM Dynamic Leak Test

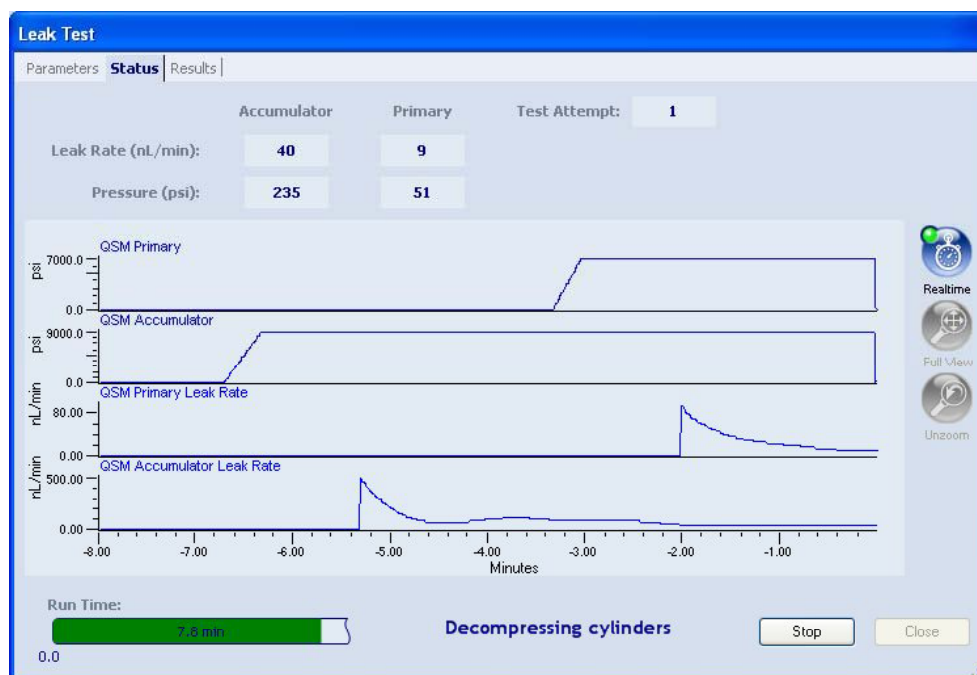
- ❑ Stop the flow and disconnect the column inlet line from the column.
- ❑ Connect the fitting plug (or union and plug, located in the solvent bottle tray front compartment) to the column inlet line to plug the line.
- ❑ Run the Dynamic Leak Test from the BSM menu.
 - ❑ Use the A Pump
 - ❑ Set the pressure 17000 PSI.
 - ❑ Choose Column as the end point.
 - ❑ Record the results below.



The screenshot shows a software window titled "Leak Test" with tabs for "Parameters", "Status", and "Results". The "Results" tab is active, showing two columns: "Accumulator" and "Primary". Each column has a "Result:" label and a text box. Below these are labels for "Leak Rate:", "Maximum Pressure:", "Percent of Stroke:", "Compressed Volume:", "Compression Attempts:", and "Test Attempts:", each followed by a text box and a unit. At the bottom right are "Print", "Start", and "Close" buttons.

	Accumulator	Primary
Result:		
Leak Rate:	nl/min	nl/min
Maximum Pressure:	psi	psi
Percent of Stroke:	%	%
Compressed Volume:	uL	uL
Compression Attempts:		
Test Attempts:		

Post Service Diagnostic Tests



Do the results correlate well with the BSM leak tests?

This test should give a good level of confidence that the Stationary Side Seal, tubing and fittings are holding pressure and functioning correctly.

- ☐ If the test failed, recheck all fittings and try the test again. Troubleshoot the problem by changing the location of the pin plug if necessary.
- ☐ Once the test has passed, disconnect the plug and reconnect the column to the inlet line.
- ☐ Fill in the fields below and initial and date it.

SM-FL Diagnostic Tests Overall Results (Pass/Fail) _____

Initials _____

Date _____

- ☐ Run the system performance tests as detailed in the I-Class Installation procedure to check the system as a whole.
- ☐ Record the precision results below.

Note- The before and after service results for the system are a key part of the certification exam.

Caffeine Area and Height Results Table

	Area %RSD Specification	Area %RSD	Area %RSD Pass Fail	Height %RSD Specification	Height %RSD	Height %RSD Pass Fail
1	%RSD < or = 0.5			%RSD < or = 1.1		

Caffeine Retention Time (RTsec) Results Table

	Retention time (sec) Standard Deviation Specification	Retention time (sec) Standard Deviation	Retention time (sec) Standard Deviation Pass Fail
1	SD RT < or = 1.0sec		

Precision Tests Overall Results (Pass/Fail)_____

Initials_____

Date_____

- ☐ Record the Gradient test results below.

Note- The peak retention times must be converted from minutes to seconds before doing the statistics in order to properly determine the standard deviation in seconds.

Retention Time Reproducibility

	Name	Retention Time (Seconds) Standard Deviation Specification	Retention Time (Seconds) Standard Deviation	Retention Time (Seconds) Standard Deviation Pass Fail
1	2-acetylfuran	SD RT < or = 1.0sec		
2	acetanilide	SD RT < or = 1.0sec		
3	acetophenone	SD RT < or = 1.0sec		
4	propiophenone	SD RT < or = 1.0sec		
5	butylparaben	SD RT < or = 1.0sec		
6	benzophenone	SD RT < or = 1.0sec		
7	valerophenone	SD RT < or = 1.0sec		

Gradient Tests Overall Results (Pass/Fail)_____

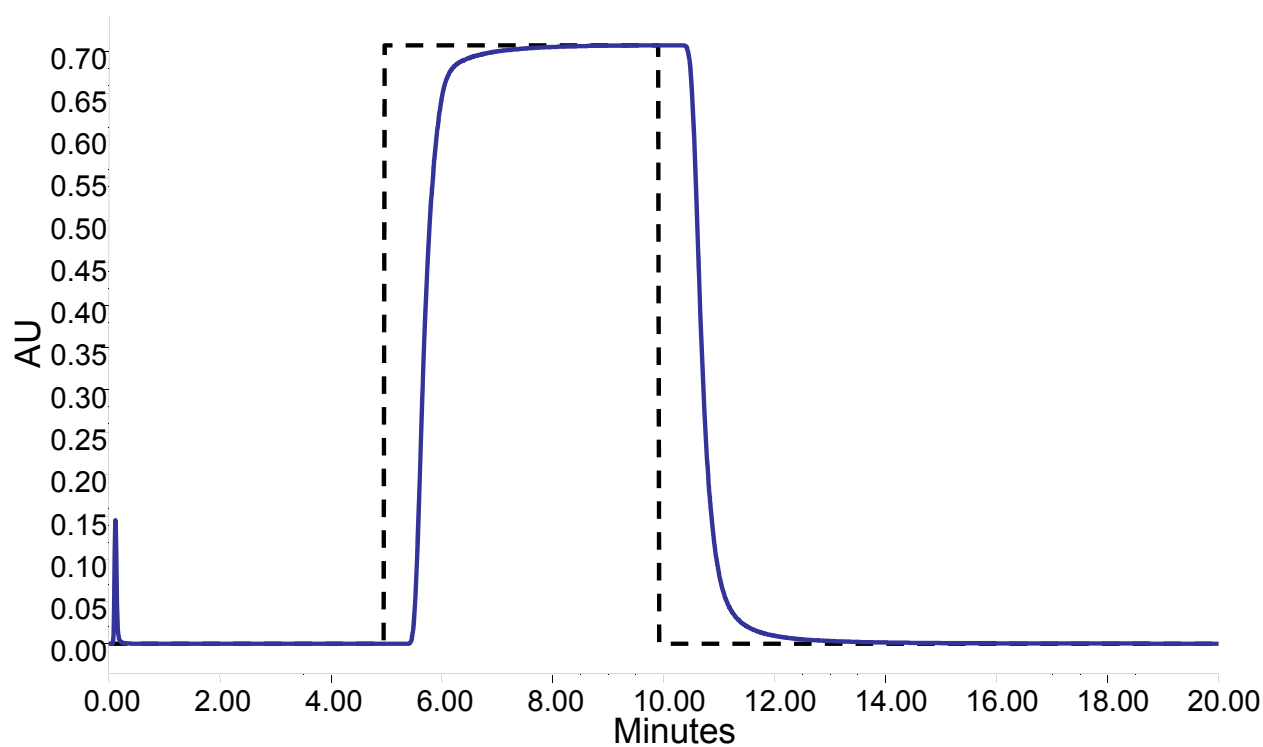
Initials_____

Date_____



Section 17

Determining I-Class System Volumes



The extra-column volume comprises the volume of the system from the point of the injection to the point of detection.

A more accurate determination would include all parts of the column (the connectors and frits) minus the packing material. However this is quite difficult to measure without sophisticated equipment.

- ☐ Modify the Precision (Caffeine) method to the following parameters and then save it as System Bandspreading.
 - ☐ Switch the flow in the Gradient table to isocratic using 90% A 10%B at 0.5 ml/min.
 - ☐ Disable the loop offline setting in the SM-FTN instrument method.
 - ☐ Switch the TUV method sampling rate to 40 pts/sec and the filter to none.
- ☐ Use the Caffeine sample from the system precision test.
- ☐ Run 3 replicate **1ul** injections of the test with a Run Time of 30 seconds.
- ☐ Calculate the extra-column volume in ul, using the following formula:

$$X = Rt * F * 1000 \text{ (ul / ml)}$$

Rt= Retention time of the peak

F= flow rate in ml/min

I-Class Extra-column Volume= _____ ul

Bandspreading is the broadening of the peaks as they flow through the extra-column volume of the system. It is an important measure of the system impact on the efficiency of the chromatography.

- ❑ Calculate the system bandspreading volume in ul, using the following formula:

$$B = W * F * 1000 \text{ (ul/ml)}$$

W= Width of the peak at 4.4% height

F= flow rate in ml/min

- ❑ If you are using a system with the SM-FL, find out the values for the SM-FTN from another group and visa-versa.

I-Class SM-FL Bandspreading = _____ul

I-Class SM-FTN Bandspreading = _____ul

- ❑ Remove the I-Class active pre-heater (APH) and use the APH from your H-Class system.
- ❑ Run the bandspreading test again and record the results below.

SM-FL Bandspreading: H-Class APH= _____ul

SM-FTN Bandspreading: H-Class APH= _____ul

What is the Bandspreading difference using the I-Class and H-Class APH.

Bandspreading Difference: (H-Class APH - I-Class APH) = _____ul

- ❑ Return the system to its normal configuration and reinstall the ACQUITY column.



Section 18 ACQUITY Method Transfer

ACQUITY UPLC Columns Calculator - Untitled *

File Edit Help

Analytical Factors

☐ Isocratic ☒ Gradient

Molecular Weight: 0-100 Da

Column Temperature: 30 °C

Calculate

A,B,C,D

From HPLC → To UPLC

QSM

HPLC

Column Length: 150 mm

Column Diameter: 4.6 mm

Particle Size: 5 µm

Injection Volume: 20 µL

Dwell Volume: 1.00 mL

UPLC

Columns...

50 mm x 2.1 mm
100 mm x 2.1 mm
30 mm x 2.1 mm

Maximum Pressure: 15000 psi

Extra Mixer Volume: 0.00 mL

Pre-Injector Volume: 0.00 mL

HPLC Gradient

	Time (min)	Flow (mL/min)	%A (Aqueous)	%B (Acetonitrile)	%C (Methanol)	%D (Other)	Column Volumes
▶	Initial	1.00	90.0	5.0	5.0	0.0	---
*							

- ❑ Transfer the following method from the Alliance to the ACQUITY H-Class System using the new ACQUITY Calculator.
- ❑ Use the values you derived for Pre-Injector volume and Gradient Delay Volume.

HPLC Method Overview

The test sample is a 7 component mix consisting of some common analgesics along with some degradation products and impurities. The HPLC run time is 25 minutes with a 10 minute re-equilibration time for a total analysis time of 35 minutes. The method requires that all components have a minimum resolution of 2.0.

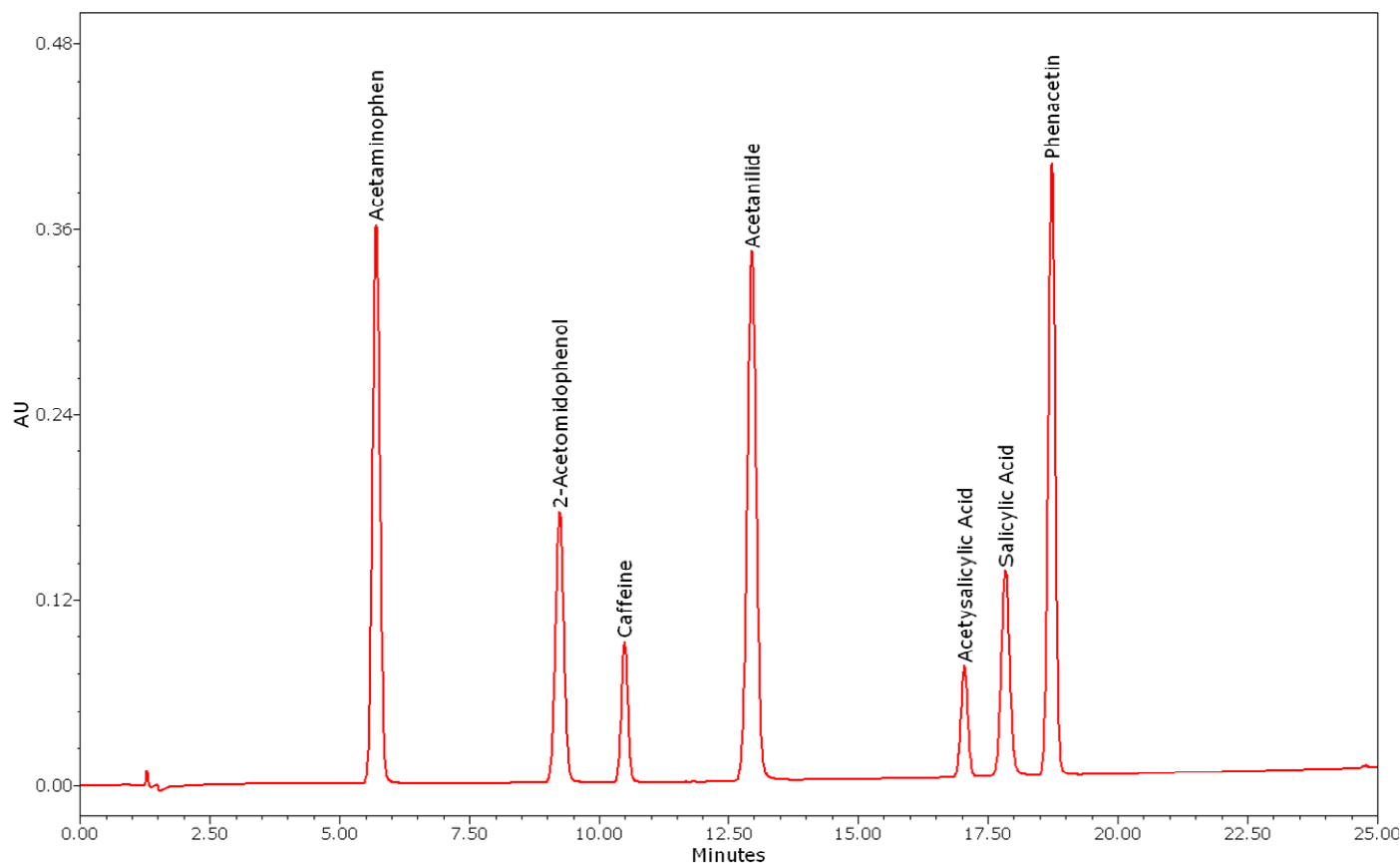
System:	Alliance® XC HPLC System
Column:	4.6 x 100mm XTerra™ MS C ₁₈ 5µm
Sample:	Analgesic Mix @ 50µg/mL in 90/10 Water/Acetonitrile
Injection Volume:	20µL
Column Temperature:	35°C
Flow Rate:	1.0 mL/min
Mobile Phase A:	0.1% Formic Acid in Water
Mobile Phase B:	0.1% Formic Acid in Acetonitrile
Gradient:	See Table
Detection:	245nm; 5Hz; Normal (0.4s)
Dwell Volume	1.04ml

Time (min)	% A	% B
0.0	98	2
10.0	90	10
25.0	65	35
25.01	98	2
35.00	98	2

- ❑ Convert the Binary Gradient to a Ternary Gradient using 1% Formic Acid in Water as the solvent in line C. A fresh supply will be available from the instructor. A quantity of 300-500ml should be all that is necessary.
- ❑ A constant 0.1% Formic acid can be delivered to the column by entering a 10% value for the C line for each row of the gradient table and subtracting 10% from the line A. This will keep the composition of the aqueous mobile phase the same.

Flow Rate: 1.0 mL/min
Mobile Phase A: Water
Mobile Phase B: Acetonitrile
Mobile Phase C: 1% Formic Acid in Water
Gradient: See Table

Time (min)	% A	% B	% C
0.0	88	2	10
10.0	80	10	10
25.0	55	35	10
25.01	88	2	10
35.00	88	2	10



Example Chromatogram- HPLC Method

- Determine the average MW from the table of compound MWs below.

Compound	Resolution	MW
Acetaminophen	--	151.16
2-Acetamidophenol	12.6	151.16
Caffeine	4.7	194.19
Acetanilide	8.9	135.16
Acetylsalicylic Acid	14.4	180.16
Salicylic Acid	2.8	138.12
Phenacetin	3.4	179.22

Average MW _____

Selecting the Column Chemistry

- ☐ Use the Waters® Interactive Reversed-Phase Column Selectivity Chart to select the most closely matched UPLC chemistry.

Selected UPLC Chemistry: _____

Determining the Column Dimensions

- ☐ Calculate the L/dp ratio of the HPLC column to determine the appropriate column dimensions.

Calculations:

Selected UPLC Column Dimensions: _____

	20mm	30mm	50mm	100mm	150mm	250mm
1.7µm UPLC BEH		17,650	29,410	58,820	88,230	
1.8µm UPLC HSS	11,110	16,670	27,770	55,556	83,333	
2.5µm	8,000	12,000	20,000			
3.5µm	5,700	8,600	14,300	28,600	42,900	71,400
5.0µm	4,000	6,000	10,000	20,000	30,000	50,000

- ☐ Use the ACQUITY UPLC Columns Calculator to determine the new UPLC conditions. We want to determine the conditions for the geometrically scaled gradient method (will look the most like the original HPLC separation) and the scaled gradient method for UPLC velocity.
- ☐ Fill the HPLC Linear Velocity values in the fields below.

Optimally Scaled UPLC Gradient Method (disregarding particle size)

Flow Rate: _____

Injection Volume: _____

Gradient Table

Time	% A	% B

Calculated HPLC Peak Capacity: _____

Calculated UPLC Peak Capacity: _____

Data Rate: 20Hz

Time Constant: 0.1 second (Normal)

- ☐ Use the ACQUITY UPLC Columns Calculator to determine the scaled gradient method for UPLC velocity.
- ☐ Fill the values in the fields below.

Geometrically Scaled Gradient Method (accounting for particle size)

Flow Rate: _____

Injection Volume: _____

Gradient Table

Time	% A	% B

Calculated HPLC Peak Capacity: _____

Calculated UPLC Peak Capacity: _____

Data Rate: 20Hz

Time Constant: 0.1 second (Normal)

Recommended Wash Solvent: _____

Recommended Purge Solvent: _____

Inject and Evaluate the Scaled UPLC Method

- ☐ Obtain a sample from the instructor.

Scaled Gradient Method (HPLC Linear Velocity)

- ☐ Setup your system with the proper mobile phase and flow conditions for the Geometrically Scaled Gradient Method (HPLC Linear Velocity) determined on the previous pages.
- ☐ Prime the system and equilibrate at the initial conditions.
- ☐ Make two injections of the sample and evaluate the quality of the separation.

Scaled Gradient Method (UPLC Linear Velocity)

- ☐ Setup your system with the proper mobile phase and flow conditions for the Geometrically Scaled Gradient Method (UPLC Linear Velocity) determined on the previous pages.
- ☐ Equilibrate at the initial conditions.
- ☐ Make several injections of the sample and evaluate the quality of the separation.
- ☐ Optimize the method as time allows.
- ☐ Fill the values in the fields below.

Compound	Resolution		
	HPLC	Geometrically Scaled UPLC	Optimally Scaled UPLC
Acetaminophen	--		
2-Acetamidophenol	12.6		
Caffeine	4.7		
Acetanilide	8.9		
Acetylsalicylic Acid	14.4		
Salicylic Acid	2.8		
Phenacetin	3.4		

- ☐ Using the geometrically scaled gradient method (UPLC Linear Velocity) determined on the previous pages, set up and equilibrate your system.
- ☐ Obtain Sample “B” from the instructor.
- ☐ Make a 5ul injection of your previous sample (Sample “A”) first. This will be considered the reference chromatogram.
- ☐ Make a 5ul injection of Sample “B”.
- ☐ Compare the two samples and give your observations as to the cause of the issues below.
