

## Waters Acquity Autopurification LCMS system

### NOTE:

- Users should bring in their own supplies, such as sample vials, solvents, test tubes, etc., for their experiments. After the experiment, please take away your samples and supplies. The facility will not be responsible to keep those materials.
- Users may bring in their own columns (analytical columns) for their experiment. The column position 3 is the port to install the user's column. Please notify the facility manager, Chithra Asokan at [casokan@lbl.gov](mailto:casokan@lbl.gov) if you plan to bring in and install your own column to schedule a time to do so.

### Procedure:

1. Check solvent level and positions. The default solvents in A1 and B1 are Water and Acetonitrile respectively. A2 and B2 are used for solvent with additives like 0.1% TFA in Water and 0.1% TFA in Acetonitrile respectively.

If changing solvents, system must be primed. If changing solvents, it is best to reserve the equipment for at least a full day

#### More solvent tips:

- Use fresh solvents for best results (no expired solvents, low Total Organic Carbon)
  - Try to prevent atmospheric exposure by capping solvents
  - Use HPLC grade solvents
  - Use Borosilicate glass bottles
  - Use Amber bottles or wrap bottles in foil to help avoid microbial growth.
  - Solvent should not contain any visible precipitates. Do not filter precipitates, only use fresh solvents.
  - Clean bottles by rinsing with organic solvent and then with water several times. (never use detergent or soap)
2. Place samples in autosampler. Samples should be completely dissolved and particle free.

**Tip:** Sample caps should have single layer septa with no plastics, adhesives, or cap mats.

**Important:** Make sure vials are lined up with holes in metal plate place holder so that injection needle does not hit the metal plate.

3. Check that the nitrogen gas is set at 80 psi at the tank
4. Open Masslynx and select directory specific to your samples (upper left)

The screenshot shows the Masslynx software interface. At the top, the title bar reads "Masslynx - Training\_August2015 - YHQ\_Hartwig.SPL". Below the title bar is a menu bar with "File", "View", "Run", and "Help". A toolbar contains icons for file operations and a "Status" button. The main window displays a table titled "Queue Is Empty" with columns for "File Name", "File Text", "MS File", "MS Tune File", "Inlet Prerun", "Inlet File", "Inlet Postrun", "Bottle", "Inject Volume", "Fraction File", "Fraction Trigg.", and "Fraction Trigg.". The table contains 23 rows of data. On the left side, there is a vertical sidebar with icons for "Instrument", "Inlet Method", "Solvent Monitor", "MS Method", "MS Tune", "MS Console", "Edit Shutdown or Startup", "Shutdown", "Startup", and "Options". Arrows point from these icons to the corresponding columns in the table: "Instrument" points to "File Name", "Inlet Method" points to "Inlet Prerun", "MS Method" points to "MS File", "MS Tune" points to "MS Tune File", "MS Console" points to "Inlet File", "Edit Shutdown or Startup" points to "Inlet Postrun", "Shutdown" points to "Bottle", and "Startup" points to "Inject Volume".

	File Name	File Text	MS File	MS Tune File	Inlet Prerun	Inlet File	Inlet Postrun	Bottle	Inject Volume	Fraction File	Fraction Trigg.	Fraction Trigg.
1	YHQ03184_2_5uL	analytical	20_500_ESI...	Training_Aug...	YHQ_prerun...	YHQ_anal_no...		5.3.1.A	5.000			
2									0.000			
3	YHQ03184_2_15uL	analytical	20_500_ESI...	Training_Aug...	YHQ_prerun...	YHQ_anal_no...		5.3.1.A	5.000			
4	dye test_20210812		dye_test_MS...	Training_Aug...		dye_analyticl...		5.1.8.B	3.000			
5									0.000			
6	YHQ03184_2_15uL	analytical	20_500_ESI...	Training_Aug...	YHQ_prerun...	YHQ_anal_no...		5.1.1.A	10.000			
7	YHQ03184_2_15uL	analytical	20_500_ESI...	Training_Aug...	YHQ_prerun...	YHQ_anal_no...		5.1.1.A	15.000			
8	YHQ03188_5_10uL	analytical	20_500_ESI...	Training_Aug...	YHQ_prerun...	YHQ_anal_no...		5.1.8.B	10.000			
9	YHQ03188_1_10uL	analytical	20_500_ESI...	Training_Aug...	YHQ_prerun...	YHQ_anal_no...		5.1.8.C	10.000			
10	YHQ03184_2_17uL	analytical	20_500_ESI...	Training_Aug...	YHQ_prerun...	YHQ_anal_no...		5.2.8.B	15.000			
11	YHQ03188_1_prep	prep	200_2000_E...	Training_Aug...	YHQ_prerun...	YHQ_prep_n...		5.1.8.B	400.000	YHQ03184_2...		
12									0.000			
13								5.1.8.B	0.000			
14	YHQ03188_1_10uL	analytical	20_500_ESI...	Training_Aug...	YHQ_prerun...	YHQ_anal_no...		5.1.8.B	10.000			
15	YHQ03188_1_5uL	analytical	20_500_ESI...	Training_Aug...	YHQ_prerun...	YHQ_anal_no...		5.1.8.B	5.000			
16									0.000			
17	YHQ03188_1_prep	prep	200_2000_E...	Training_Aug...	YHQ_prerun...	YHQ_prep_n...		5.1.8.B	400.000	YHQ03184_2...		
18									0.000			
19									0.000			
20									0.000			
21									0.000			
22									0.000			
23									0.000			

5. Create prerun (right click prerun to edit and save prerun file or select new prerun file) to pump solvent through the system. Prerun can be similar to run file depending on how long the system needs to be purged between samples.
6. Create run (right click run to edit and save run file or select new run file) to inject sample while solvent gradient runs and sample is analyzed.
7. To set up parameters in prerun and run double click the method:
  - 7a. The status screen of your method (indicated in the top ledger) will pop up. Click on Inlet

The screenshot shows the "Status" dialog box in Masslynx. The title bar reads "yHQ\_prerun\_no\_acid\_80h2o-20acn\_5min.bgm, yHQ\_prerun\_no\_acid\_80h2o-20acn\_5min.w67, yHQ...". The dialog has a sidebar with icons for "Status", "Inlet", "Autosampler", and "Waters 2489". The main area is divided into "Status", "Additional Status", and "Solvent Levels" tabs. The "Status" tab is active, showing "Indicators" (Running, Pump On, Inject Cycle, Ready, OK) and "Pumps" (Time (min), Flow (mL/min), Pressure (psi)). The "Pressure (psi)" is set to 9. The "Detector" is "Scan" and the "Mode" is "Idle".

7b. In the next screen that pops up you will specify (Consult literature for best conditions or experiment with gradients and rates to find best separation of peaks):

- Your solvents (Either A1/B1 or A2/B2 depending on where you placed the physical solvent tubing) You can name your solvents for your own reference in the procedure.
- Your gradients: Step 1 will be your initial solvent mixture following step will be the amount of time it will take to switch from one mixture to the next. For example, if you start at a flow rate at 100% A1 to 80% A1 and the 2<sup>nd</sup> step time is 5 minutes, the gradient will change 4% per minute. To create a hold in the gradient, label the next step the amount of time you want the hold and the % you previous changed to that you wish to hold at (for example if the next step from 5 minutes is 8 minutes, the hold is 3 minutes at and type in 80%/20%)
- **Flow rate for analytical samples should be 1 or lower**
- Flow rate for Prep Sample should be 30 or lower.

Chromatographic Pump

Run Time: 15.00 min

Mobile Phase | Events | Analog

Solvent Selections: A:  A1  A2; B:  B1  B2

Solvent Names: A: water; B: Acetonitrile

Pressure Limits: Low: 0 psi; High: 6000 psi

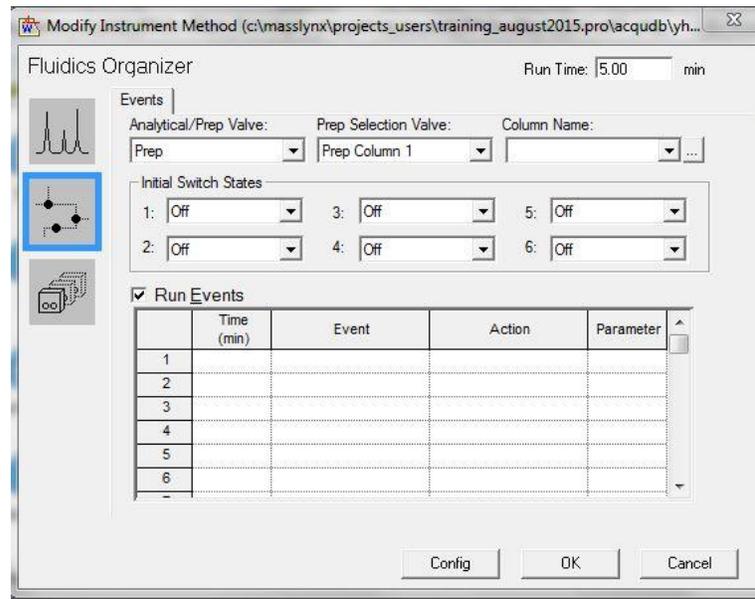
Gradient:  System  Pump Only; Seal Wash Period: 5.0 minutes

	Time (min)	Flow (mL/min)	%A	%B	Curve
1	initial	1.00	95.0	5.0	initial
2	3.00	1.00	90.0	10.0	6
3	8.00	1.00	0.0	100.0	6
4	15.00	1.00	0.0	100.0	6
5					
6					

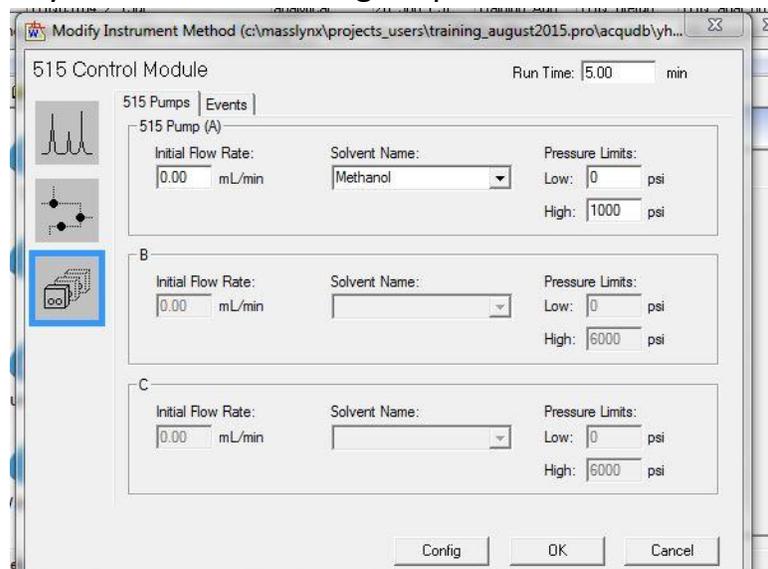
Comment:

Config OK Cancel

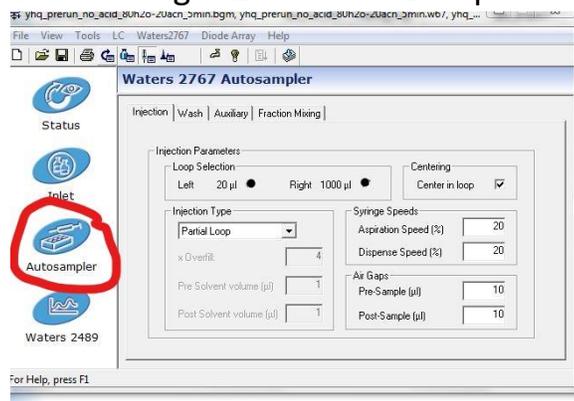
8. **Be sure your run time matches your last step time of your run** (if the last step is 15 min then the total run time is 15 min)
9. In the Selection highlighted below, select if you are running Prep vs Analytical and which column you are using (Choose Analytical 1 or Prep 1 for reverse phase C18 column unless you have installed a different column in a different position)



10. In the next window down below, mark the Initial Flow rate of 5151 Pump A as 0 if running Analytical and 1 if running Prep



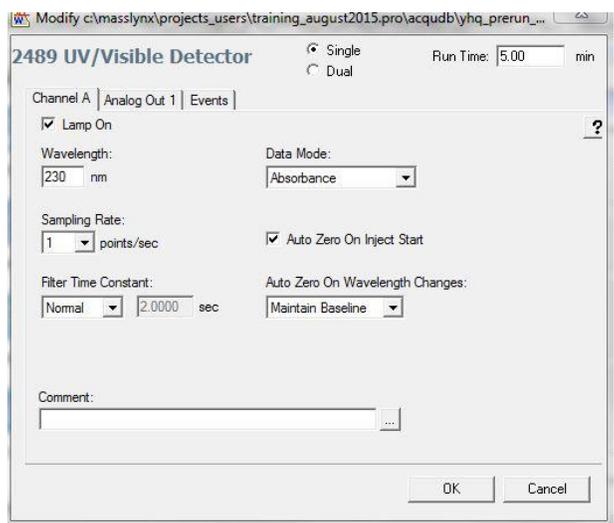
11. Back in the sample file, go to auto sampler: Select the Left 20 microl loop for analytical samples, select the right 1000 microl loop for Prep samples



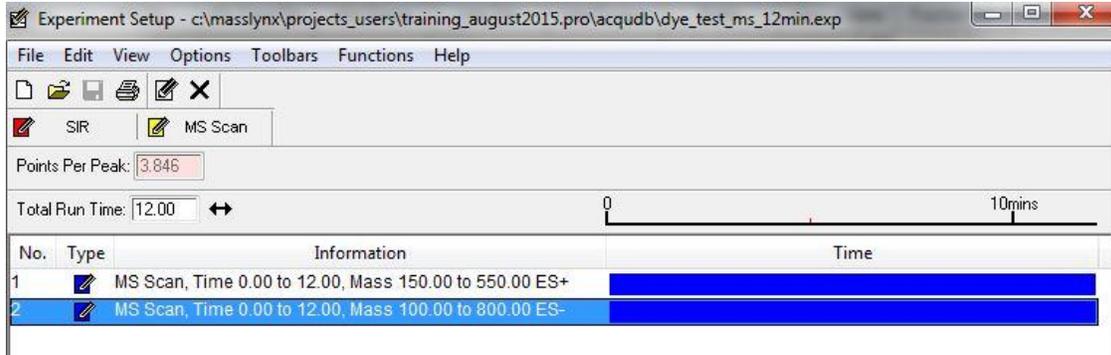
12. Difference between Prep and Analytical Settings:

	Analytical	Prep
Flow rate	0-1 ml/min	20-30 ml/min
Column selection	Choose Analytical 1, 2, or 3	Choose Prep 1, or 2
515 Pump A Methanol flow	0	1
Autosampler Loop	20 microl	1000 microL
Fraction file	(none)	For set up see step 22 and 23

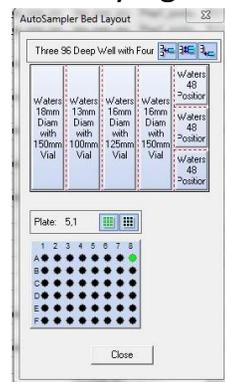
13. Then press Waters 2489 (see photo step 11) to set up UV-vis measurement, choose Single or Dual Wavelengths. **Make sure Run Time is the same Runtime as Mass setting in Inlet settings.**



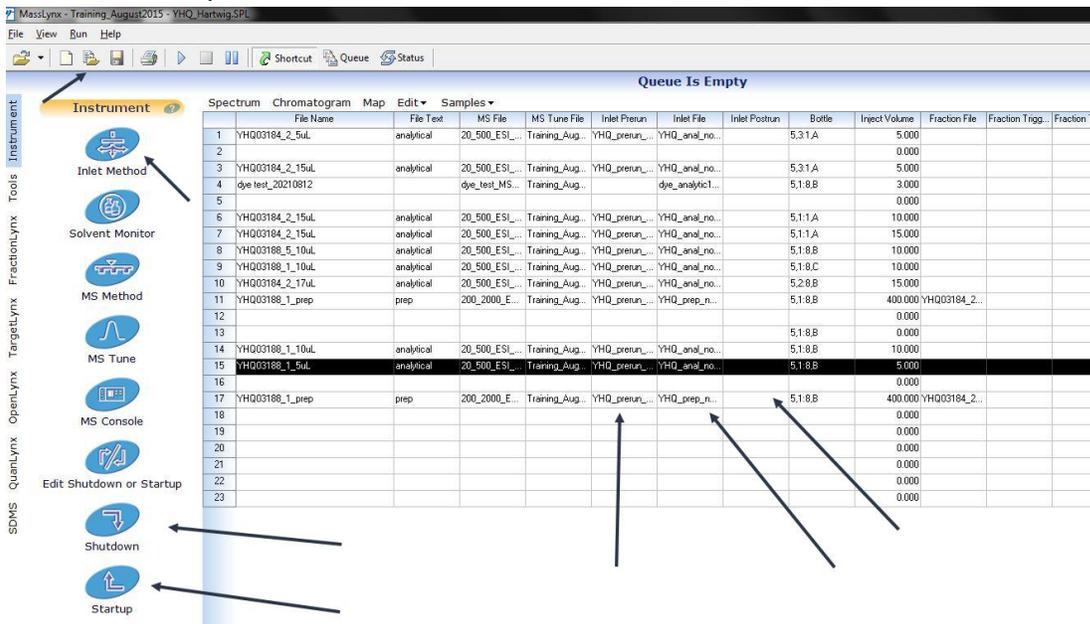
- Copy MS Tune File from template. This is a standard file, do not edit
- Copy or Edit, rename, and save MS file to specify Ion modes (ES+ and ES-), the range of the Mass Spec, and the runtime (the same as the MS and UV Runtime)



- Specify the amount for injection in Inject volume (~5 for analytical)
- Specify location of vials under Bottle by right clicking on the cell



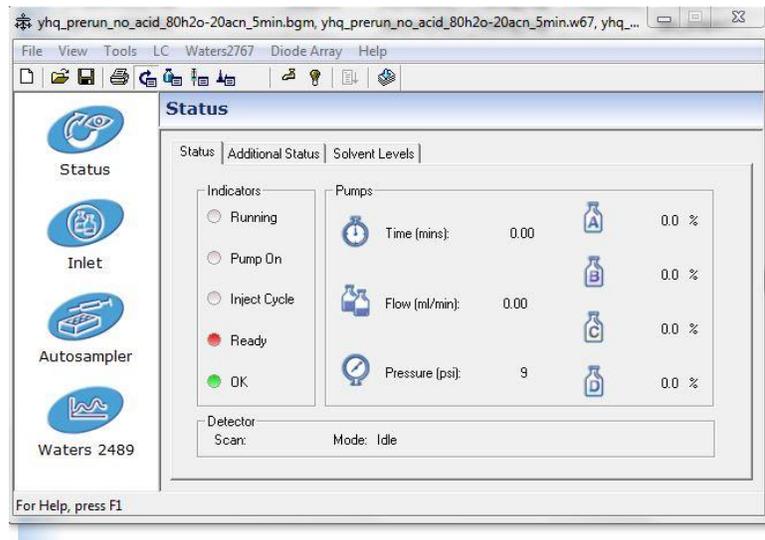
- Press [Start up] in the shortcuts tab.



19. While system is warming up create sample files (as described previously).

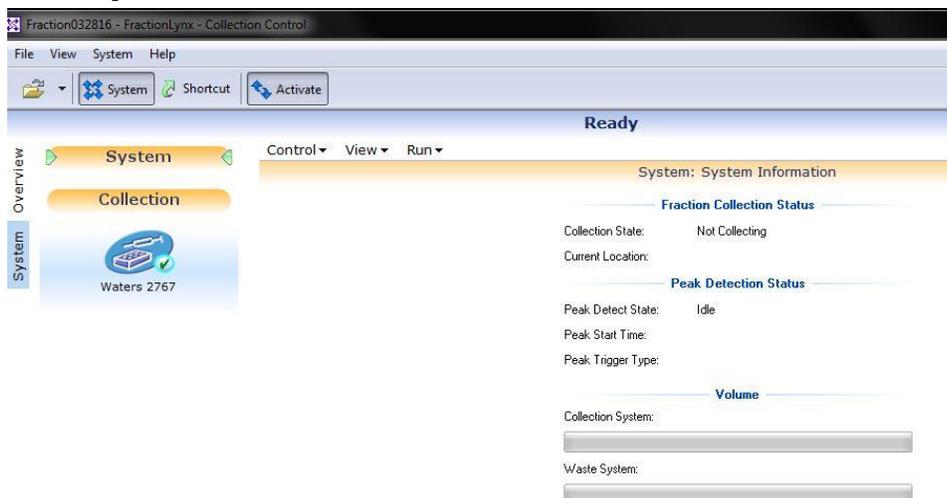
\*If using different solvent press prime on solvent pump. Or create a run with 0 injection volume and flowing new solvent through system for ~20 min at 1 ml/min

20. Double click on your Inlet file. Load method by pressing button below Help tab

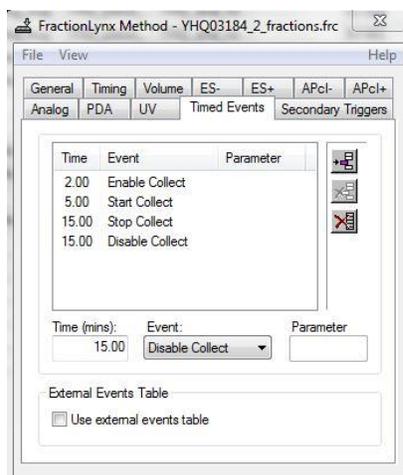


21. Go to Waters 2767 tab and **Prime system**

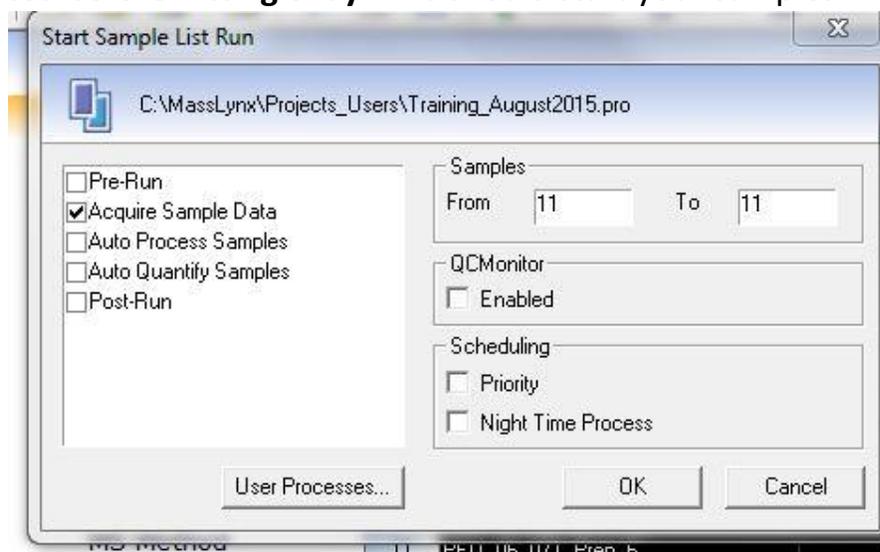
22. FOR PREP ONLY: In the Short Cut tab click the [FractionLynx] tab to the far left. Go to [Control] and [Reset Beds]. Press [Activate]



23. FOR PREP ONLY: Create a Fraction File: Insert and Specify time of enabling Collection (at least 30 seconds before start collect), Start collect, Stop collect, and Disable collect (Stop collect and Disable collect can be the same time)



**24. To Start Run(s):** Highlight sample rows that you wish to run and Press the Play button. **Double check that the only the rows you have highlighted and wish to run are listed before hitting okay.** This should start your samples



**Tips if run is not starting:**

- If Prep run was previous to analytical run: In the Short Cut tab click the [FractionLynx] tab to the far left and **make sure [Activate] is NOT pressed**
- Make sure Method is loaded before pressing run (see step 20)
- Make sure all solvents are filled (end is submerged) including Methanol Wash Solvent
- Restart software including running stop files found on desktop and restarting (See step 26)
- Reset Sample injection under the LC tab in the Method File
- Rest Communication under the LC tab in the Method File

25. After run(s) are finished, press the [Shutdown] and close software.
26. To look at data, highlight data row and press [Chromatogram]. Double click the spot in the chromatogram to have specific Mass spec and UV results. To save results go to edit to either take a screen shot or copy data over to wordpad.
27. Run Stop Acquisition and Stop Mass Lynx files on desktop.



28. Users are required to clean up the system after using the instrument. The standard cleaning procedure includes, 1) Add wash samples (clean solvent) after the running sequence. 2) Use wet KIM wipe to clean the outside of the injection probe (please be very gentle when you wipe the probe). 3) In the “Inlet Method” setup page, under “Waters 2767” menu, choose “prime system” and “wash probe/inlet port”.

Tip: Please check the rinse (Methanol) solvent frequently. The wash procedure will not work if the rinse solvent is used up. And the injector will fail to take sample without the rinse solvent.

**Users who fail to follow the procedure, and cause contamination to other user’s samples, will be removed from the current user list and not be allowed to access the instrument for at least two weeks.**