Waters AutoPurification System Standard Operation Protocol

Contents

A. User policy (Read before use!)

i. Sample preparation

1. Use 2 ml vials for sample injection.
2. Use 15 mL tubes with diameter of 16 mm for sample collection.
3. Label all your vials and tubes.
4. Dilute samples with starting mobile phase. (No absolute DMSO is allowed.)
5. Filter samples with 0.4 µm or 0.22 µm filter to remove precipitates.
6. The level of sample should reach the bottom line of vials (0.5 mL level).

7. Only flat-bottom and conical-bottom inserts with **NO FEET** are allowed.

ii. Mobile phase and columns

8. Only **reverse phase** chromatography is allowed.
9. Standard mobile phase (provided and refilled by CPOS)

   Polar (A1): H_2O + 0.1% formic acid

   Non-Polar (B1): ACN + 0.1% formic acid
10. **NO running on 100% of A1 phase** is allowed.
11. **Analytical** column provided by CPOS: C18 5 µm, 4.6 mm I.D. x 50 mm length (located at analytic column 2)
12. **Preparative** column provided by CPOS: C18 OBD™, 19 mm I.D. x 50 mm length (located at preparative column 1)
13. Bring your own guard, analytical and preparative columns for any deviations from our standard solvent composition and columns.

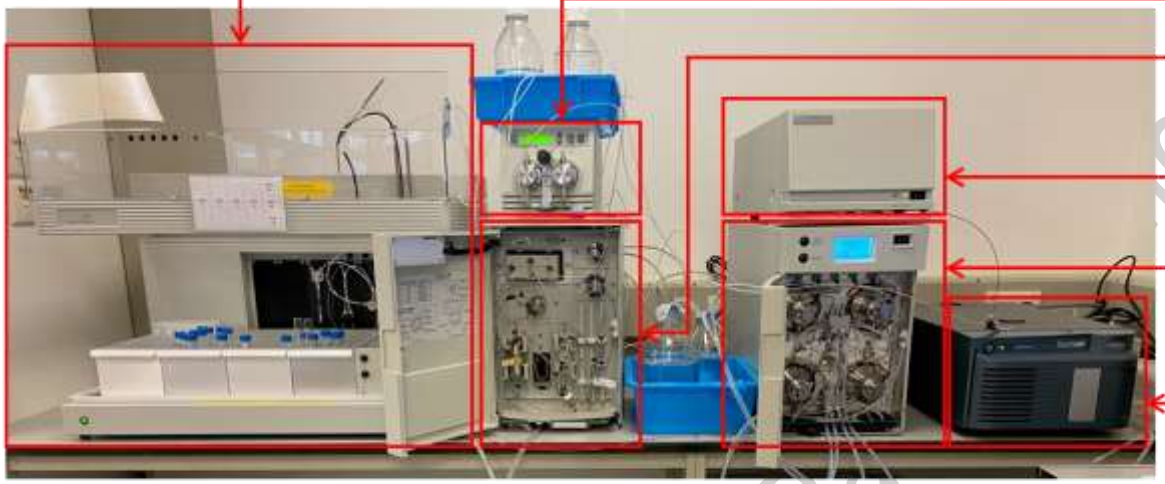
iii. During use

14. Log in PPMS tracker.
15. Create your own projects and save at D:\User Data\Department\PI. Do NOT modify others' projects or methods.
16. Make sure the run time of pump, PDA detector and QDa mass detector are consistent in their corresponding methods.
17. Stopping a run ( / ) is NOT recommended. After you stop the run, the next sample in the queue will NOT be injected but the LC will still keep flowing. Please manually stop the flow by selecting  →  to save solvent.
18. Stop the flow ( → ) when you are still preparing samples or setting up methods to save solvent.

iv. After use

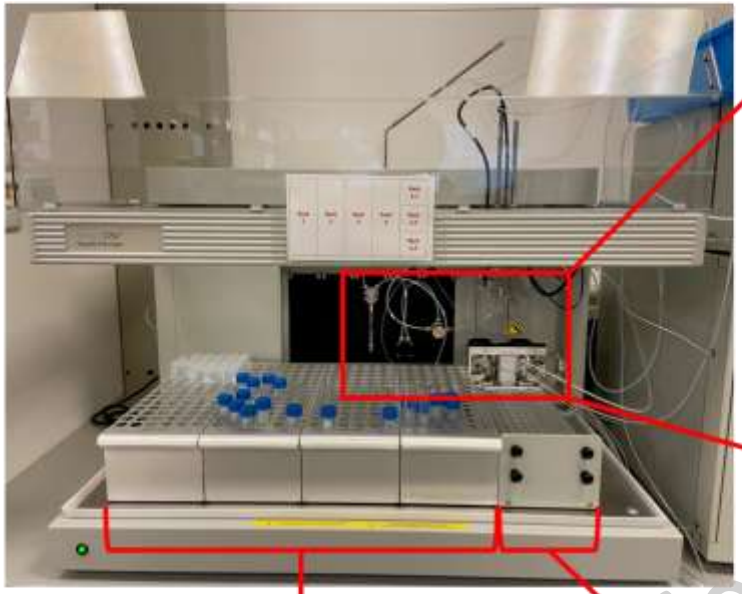
19. You MUST run **<u>at least two blanks</u>** (methanol) with "CPOS_analytical_wash" as the LC method and "CPOS_analytical_wash" as MS method (to monitor any contaminates left) to clean the column. (Part F)
20. You MUST **<u>enable shutdown procedure</u>** so that the flow, PDA detector and QDa mass detector will be automatically shut down after running all samples in the queue. (Part F)
21. Do NOT insert your own USB thumb drive to the PC connecting the instrument. Transfer data with CPOS USB drive and perform analysis on data transfer station (Part G)
22. Remove all your vials and tubes after use.
23. Log out PPMS tracker.

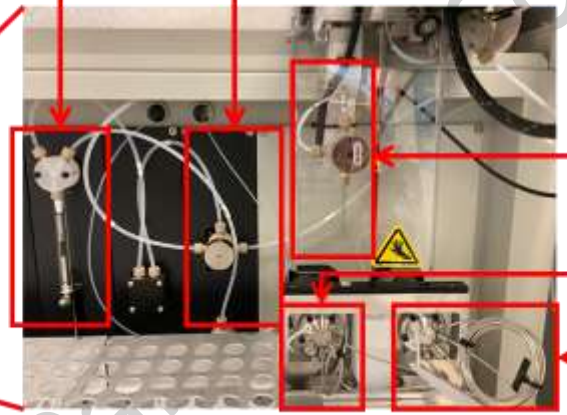
B. System overview



Number in brackets() represent its model no.

Autosampler and collector





Sample syringe

Wash pump

Sample probe and diverter valve

Analytical injection port (20 µl loop)

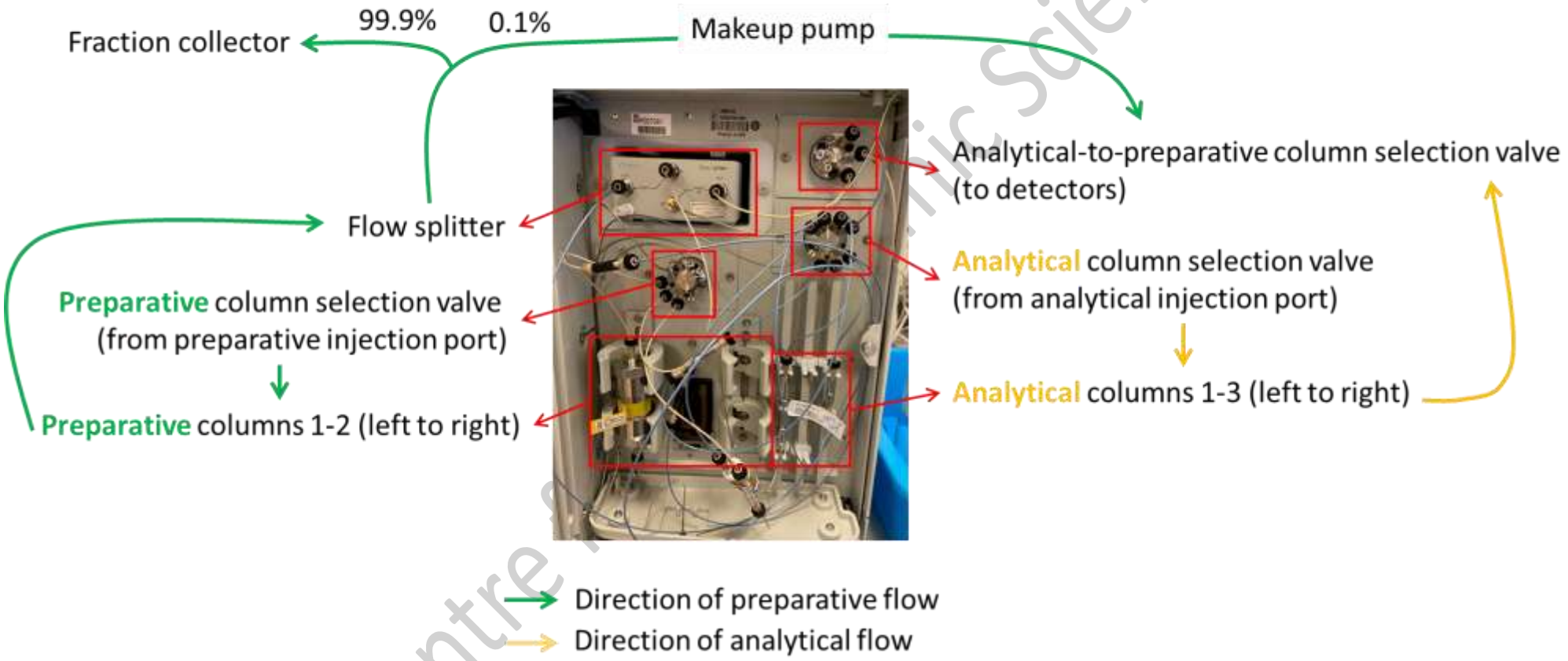
Preparative injection port (1 ml loop)

Rack for 15 ml tubes
for collecting fractions*

Racks for 2 ml vial
for sample injection*

*Do NOT change the rack configuration as it has been calibrated

System fluidic organizer



C. Initialization

1. Open the “MassLynx” software  on desktop.

> ⚠ Keep “MassLynx” software open; otherwise QDa mass detector will be disconnected.

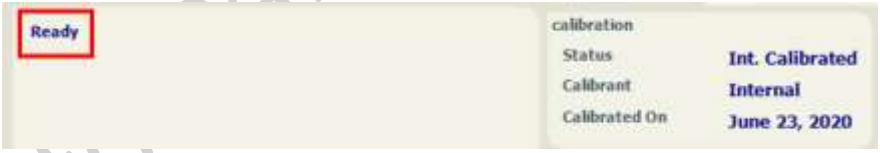
2. Open the “acquity console” software by s electing 

on “MassLynx” software.
3. Initialize QDa mass detector.
   a. Select “QDa Detector” in console.

   b. Select 

to operate QDa mass detector. Green light on the top left of icon represents the detector is on.
   c. Wait ~1 minute for the green status light on QDa mass detector to change from flashing to solid.

   d. Make sure its status is “Ready” in console.



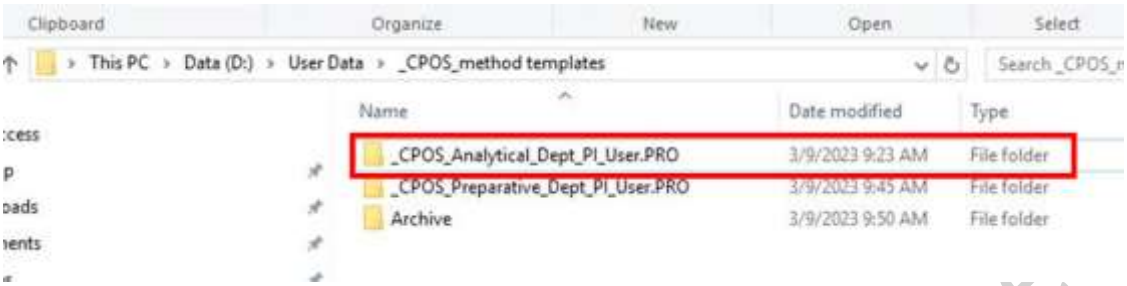
4. Initialize PDA detector.
   a. Select “2998 PDA Detector” in console.

   b. Select 

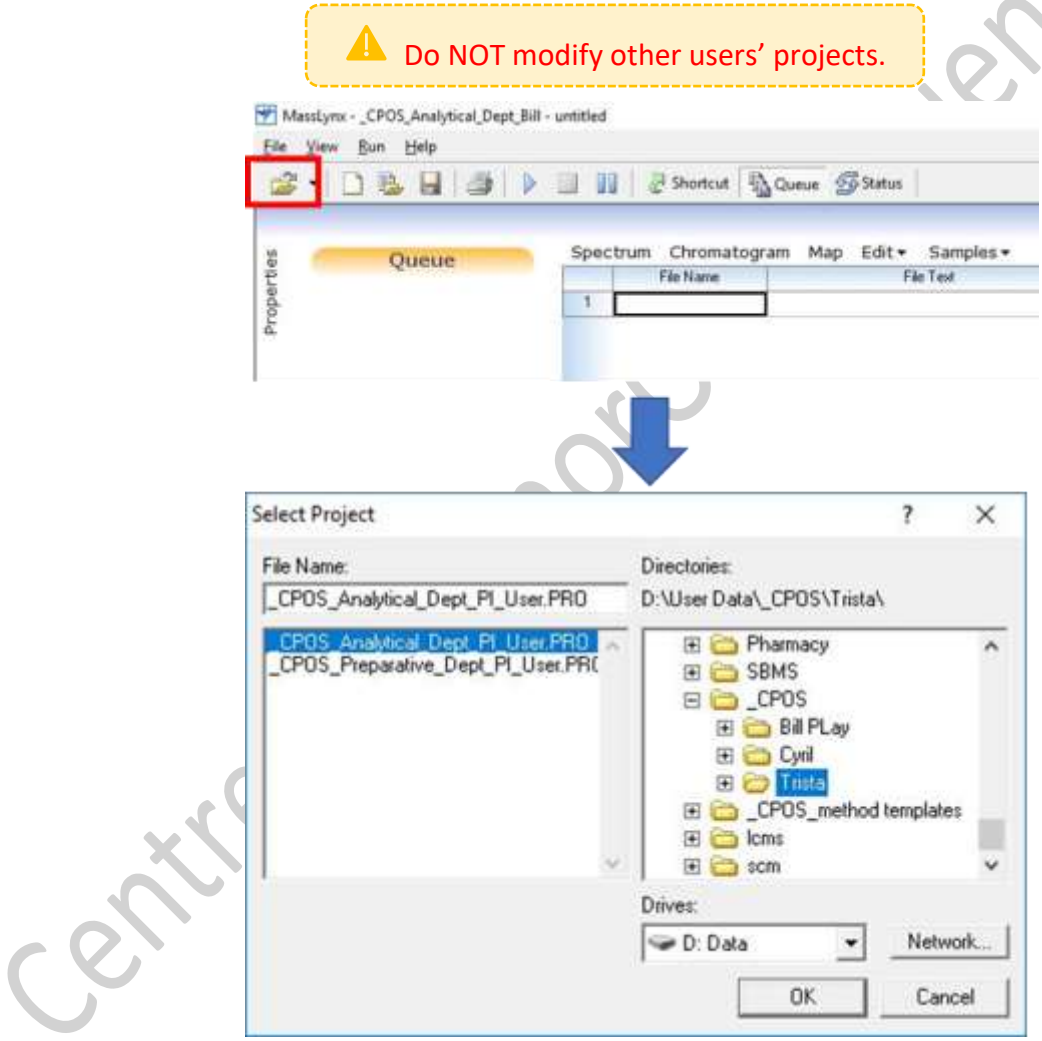
to turn on the lamp of PDA detector. Green light on the top left of icon represents the detector is on.
   c. Make sure the light of lamp on PDA detector turns to green.

D. Analytical analysis

1. Copy the folder "**D:\User Data_CPOS_Analytical_Dept_PI_User.PRO**" to D:\User Data\Department\PI and change the name of the folder.



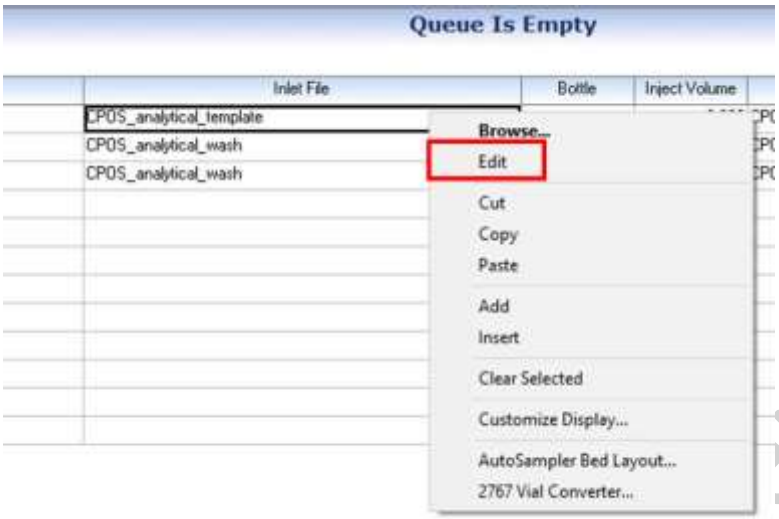
2. Open your PRO file in MassLynx.



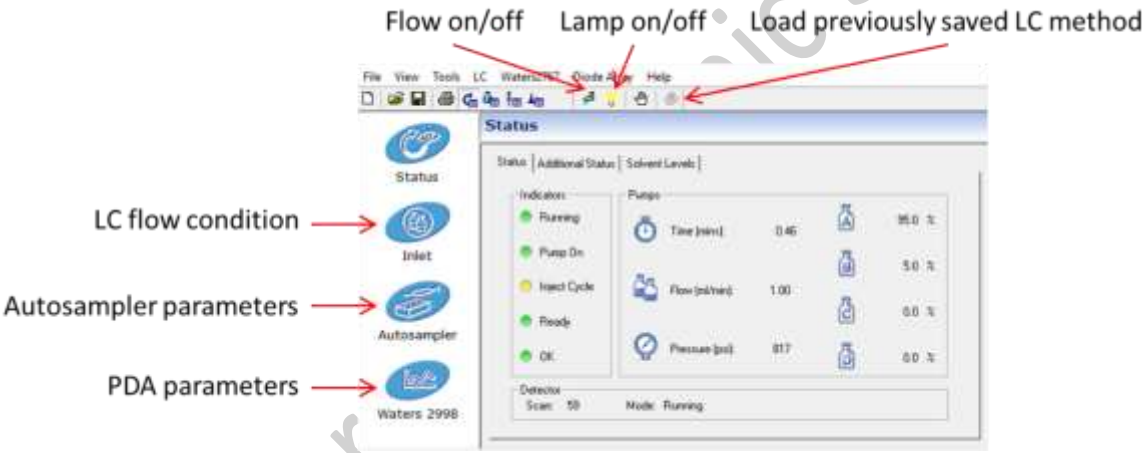
3. Edit File name, File text, bottle position and inject volume.

i. Set up LC and PDA method

4. In the sample list, right click the inlet file name and select "Edit".



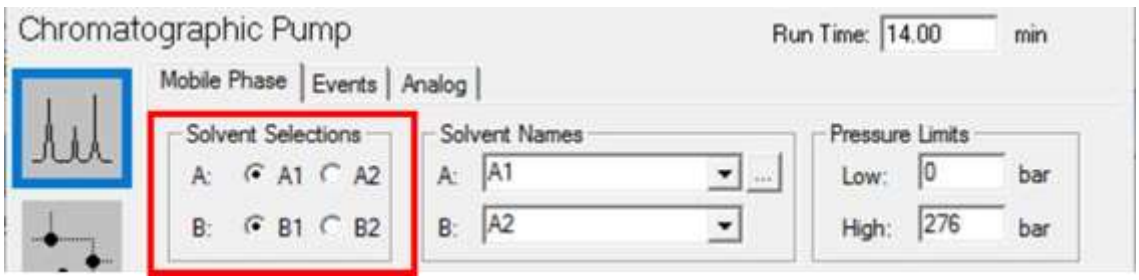
5. Inlet method will be shown in the window below.



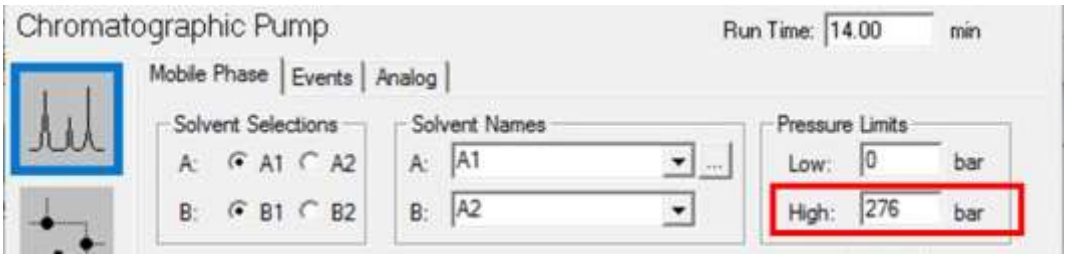
6. Set up LC flow condition by selecting 

.
7. Set the "Solvent Selections" as A1 and B1 if you use the solvents provided by CPOS.
   A1: Water + 0.1% formic acid
   B1: ACN + 0.1% formic acid

⚠ Please inform our staff in advance if you use your own solvents.



8. Set the maximum system pressure at 276 bar or 4,000 psi.

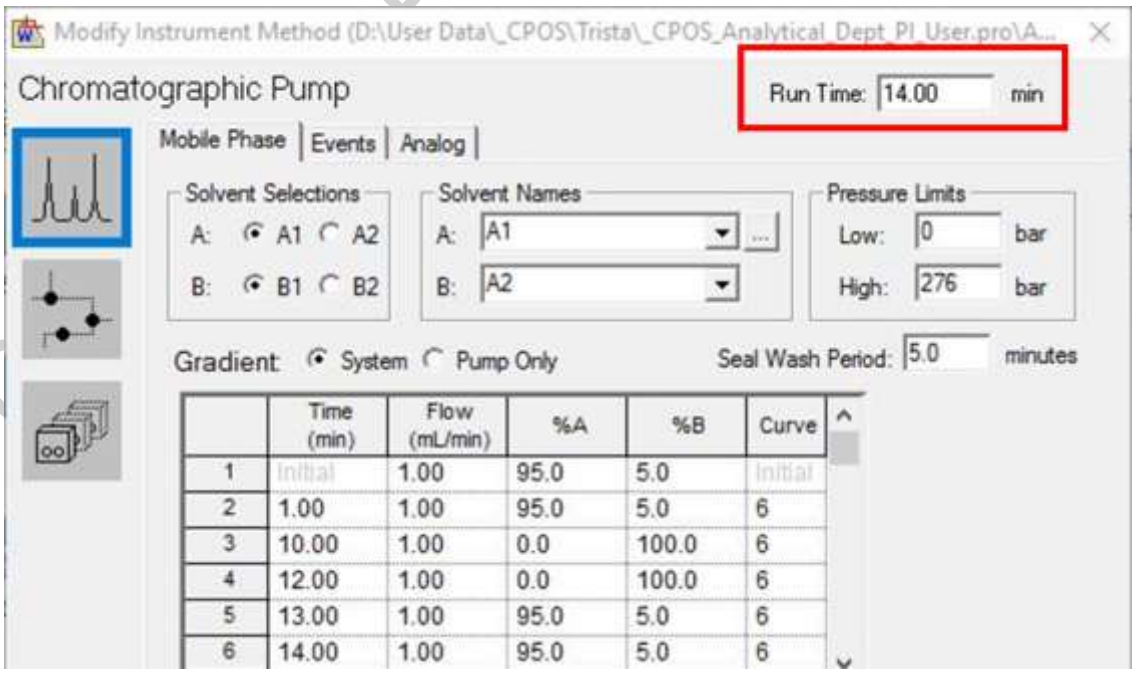


9. Set the gradient table with the following as an example for C18 column (50 mm long). Set flow rate at 1-2 mL/min for analytical run.

⚠ If the length of your own column is > 100 mm, reduce the flow rate to 0.5 - 0.8 mL/min to reduce the pressure.

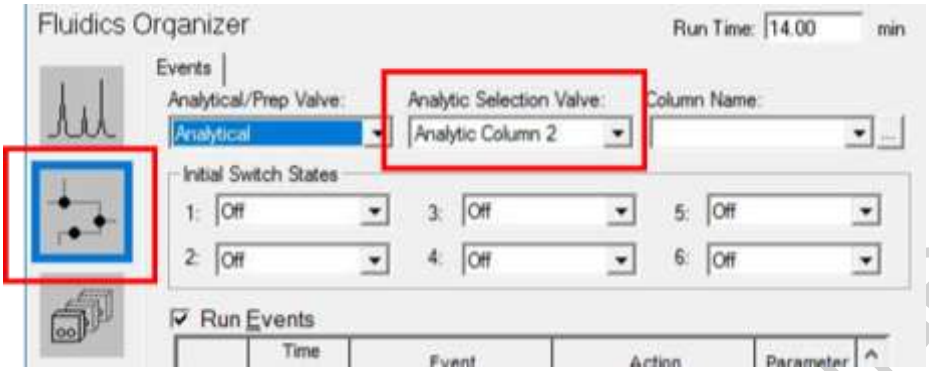
	Time (min)	%A	%B
Sample injection	0th	95	5
Gradient elution	1st	95	5
Column washing	10th	0	100
Column regeneration	12th	0	100
	13th	95	5
	14th	95	5

10. Make sure the "Run Time" of pump (at top right-hand corner) is consistent with that in the gradient table. If they do not match, the "Run Time" of pump will be prioritized.



	Time (min)	Flow (mL/min)	%A	%B	Curve
1	Initial	1.00	95.0	5.0	Initial
2	1.00	1.00	95.0	5.0	6
3	10.00	1.00	0.0	100.0	6
4	12.00	1.00	0.0	100.0	6
5	13.00	1.00	95.0	5.0	6
6	14.00	1.00	95.0	5.0	6

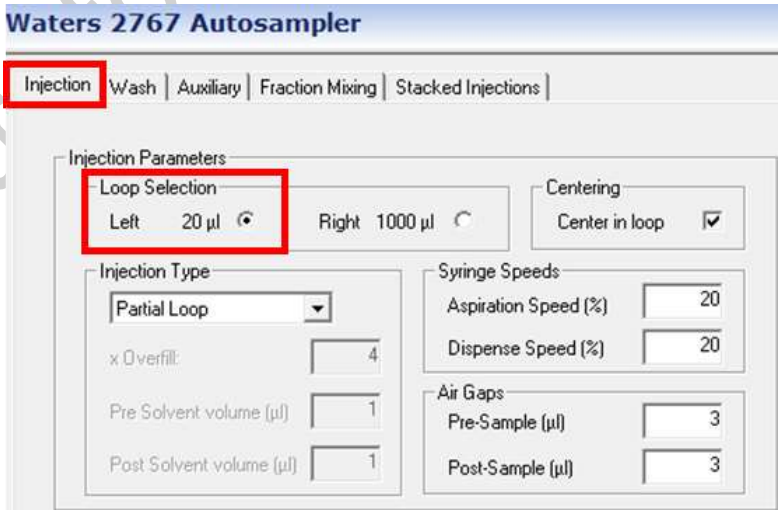
11. Select “Analytical” in “Analytical/Prep Valve” and the appropriate analytic column number (1-3, from left to right) in “Analytic Selection Valve”. The analytical column provided by CPOS (C18 5 µm, 4.6 mm I.D. x 50 mm length) is located at analytic column 2.



12. Set the “Initial Flow Rate" of 515 pump (makeup pump) to 0.00 mL/min because it will not be used in analytical run.

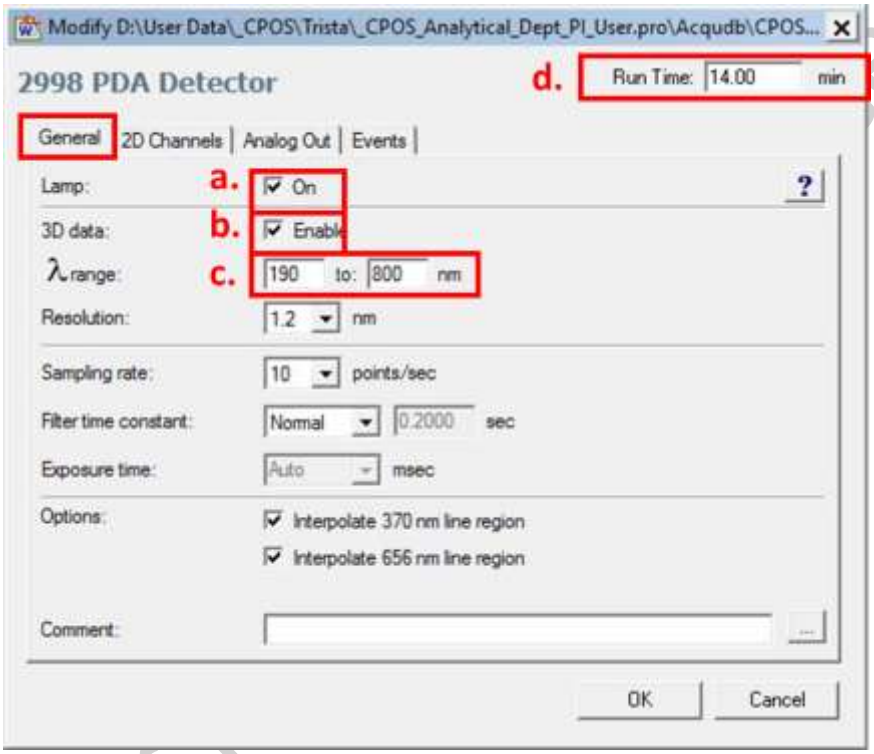
13. Select 

to set up autosampler parameters.
14. Select “Left loop 20 µl” for analytical run. Keep other parameters as default as the graph below.



15. Select 

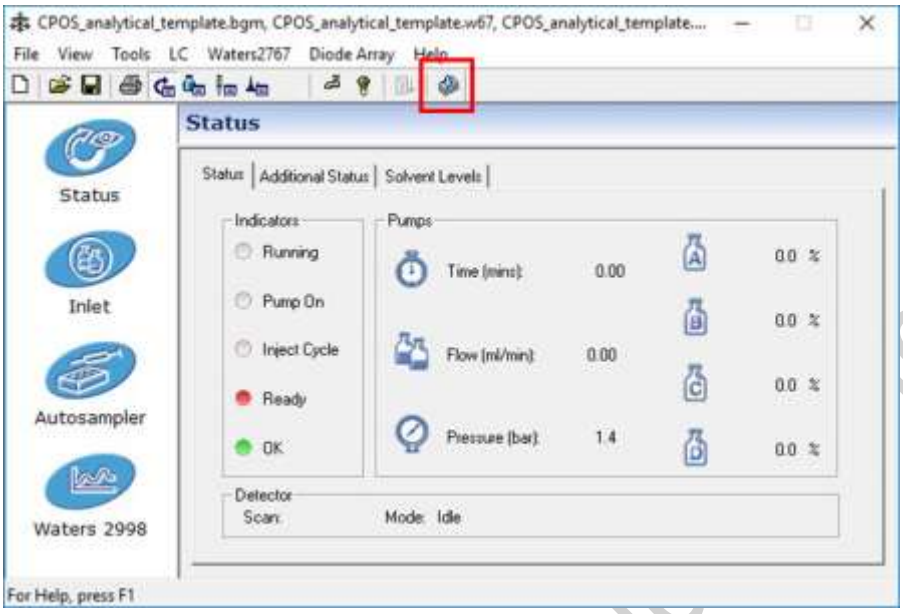
to set up the PDA parameters.
    a. **Lamp**: make sure the lamp is ON.
    b. **3D data**: Enable 3D data for a full scan.
    c. **λ range**: The range of full can be 190 - 800 nm. Signal out of this range cannot be detected.
    d. **Run Time**: Make sure it is consistent with that of the gradient table (step 7). The PDA detector will NOT detect or record any signal if it stops before the gradient stops.
    e. Keep other parameters as default as the graph below.



16. (Optional) If you know the wavelength of UV absorbance of your target(s), set a specific channel for it (maximum 8 channels) for quantitative analysis.

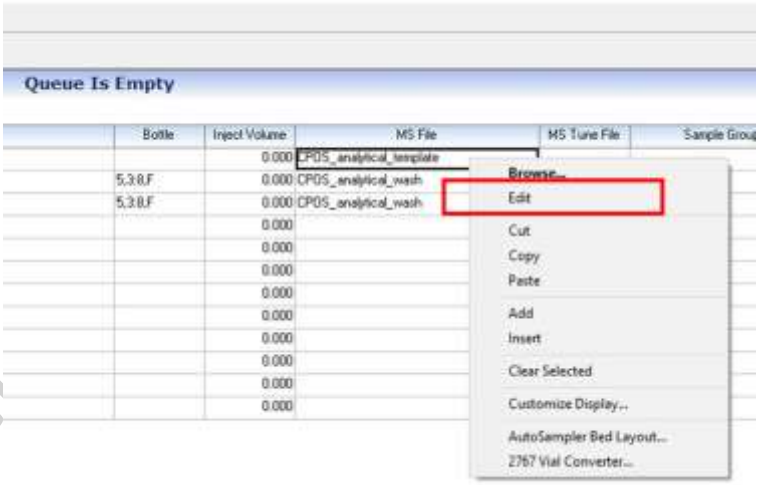
17. Select  to save your LC method.

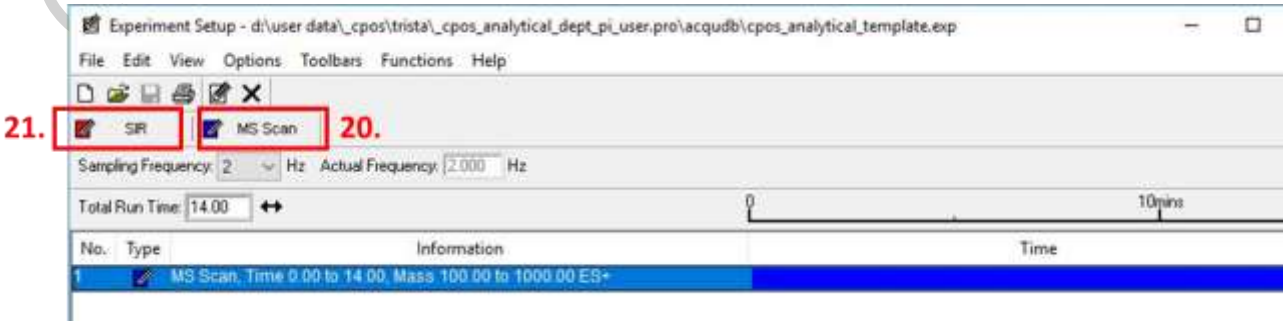
18. Equilibrate LC method by selecting “Load Method”. It will start to flow the initial step of the LC method. Monitor the pressure of the pump until it becomes stable (fluctuating +/-50 psi), which takes 5-10 min. Meanwhile, you can move to the next step.

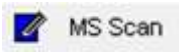


ii. Set up MS method

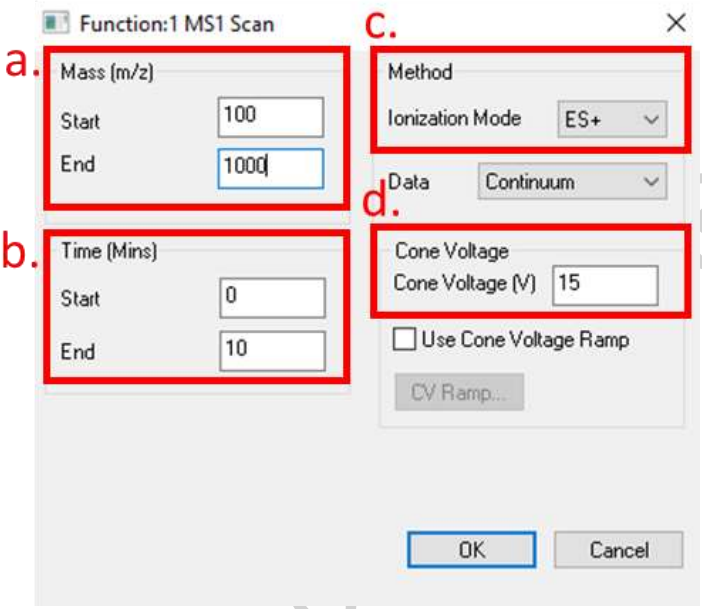
19. Edit MS method by right clicking the MS file in sample list.





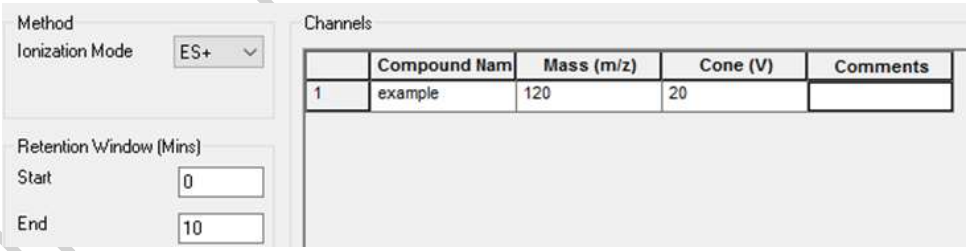
20. Select 

to set MS scan parameters.
   a. **Mass (m/z)**: The maximum MS scan range is 100 - 1000 m/z. Mass < 100 may have much noise while that > 1000 may give no signal.
   b. Time (Mins): Normally set consistent with gradient table but can also specify a shorter timeslot.
   c. **Method**: Select whether ES+/ES- mode you will use.
   d. **Cone Voltage**: the cone voltage needs to be optimized. The higher the mass to charge ratio, the high the cone voltage required. Recommended to start from 15 for ES +ve and 30 for ES -ve.



21. (Optional) Select 

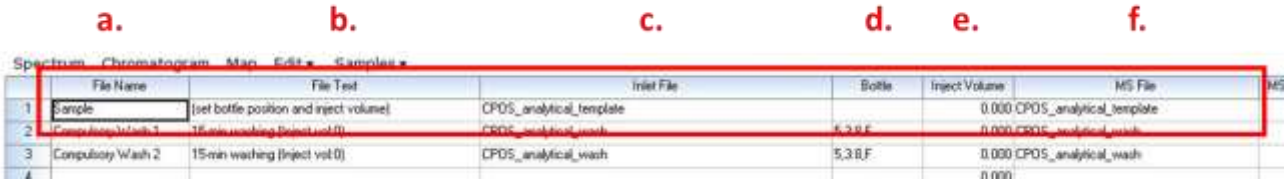
(Selected Ion Recording) to specify target mass to obtain a chromatograph with higher sensitivity for quantitative analysis (maximum 32 SIR channels). You can set specific time slot to increase sensitivity.



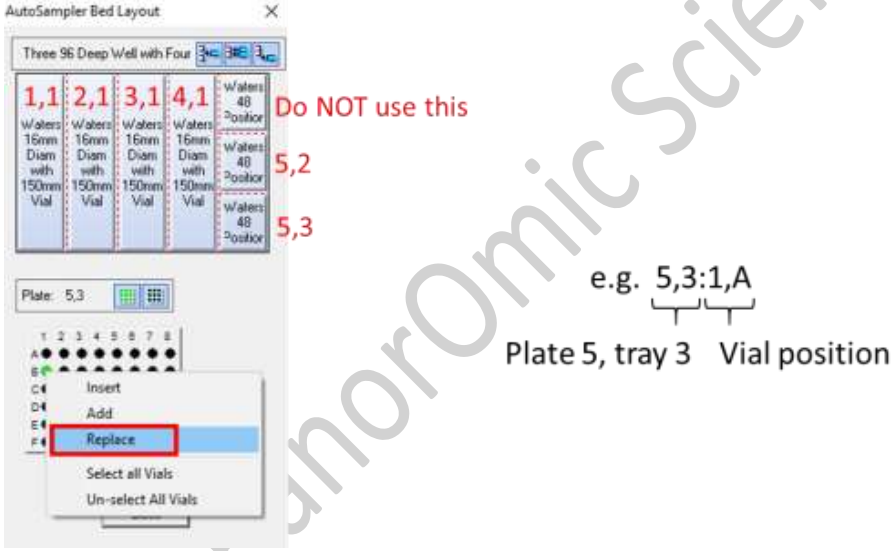
22. Select  to save your MS method.

iii. Create sample list

1. In sample list:



a. **File Name**: sample name. Only include alphabets and "_". Maximum 20-30 characters, otherwise may generate errors.
b. **File Text:** fill in any note related to the sample or setting.
c. **Inlet File**: load your established Inlet method file.
d. **Bottle**: the position of sample vial. Right click → "AutoSampler Bed Layout"



e. **Inject Volume**: maximum 20 µl for analytical run, but recommend to start with 5-10 µl to prevent contamination of the system.
f. **MS File**: load your established MS method file.
g. Other columns can be left blank.

2. Right click sample list to add more samples.

3. Select to save your sample list.

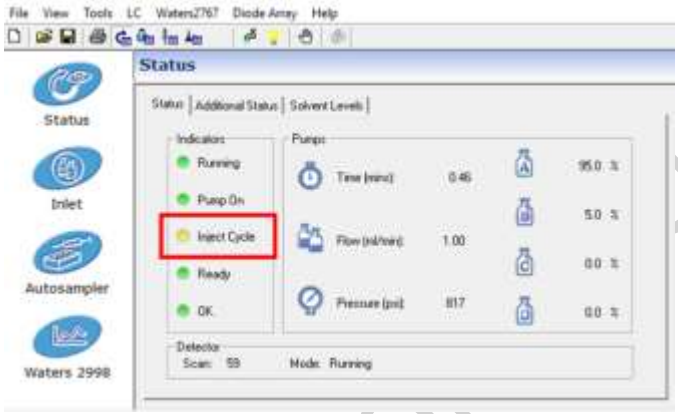
⚠ Before running your sample, check the following:

- The pressure of the pump is less than 2000 psi. If it is >2000 psi, call our staff.
- The pressure of the pump is stable (fluctuating within +/-50 psi).
- No leakage of solvent from the two extremes of column(s)
- The sample vials are in the right position as defined in your sample list.

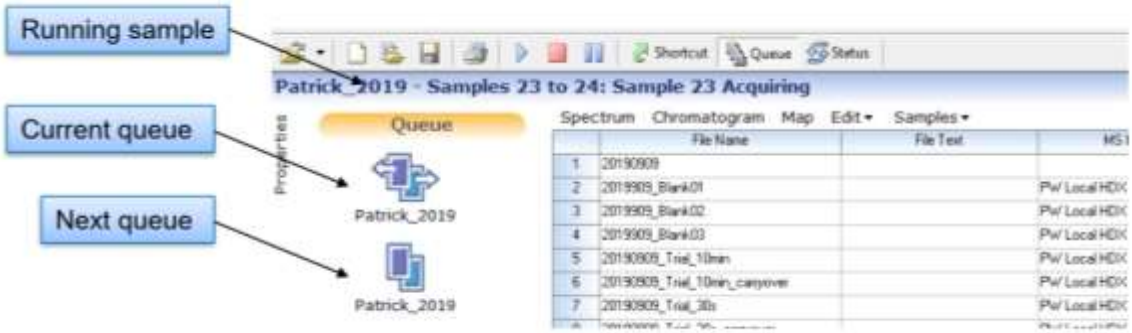
4. Highlight the samples to be run and select  to start the run.
5. Monitor the status of instrument.

   b. The status (at the bottom right of Masslynx software) should have changed from "Instrument Present" or "Not scanning" → "waiting for injection" → "acquiring"

   c. The "inject cycle" should turn to yellow during injection.



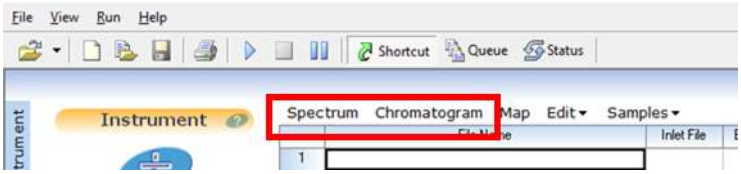
6. You can view the sample list going to be run in "Queue". You cannot edit the running queue but can delete or add a queue or allow a queue to be run first.



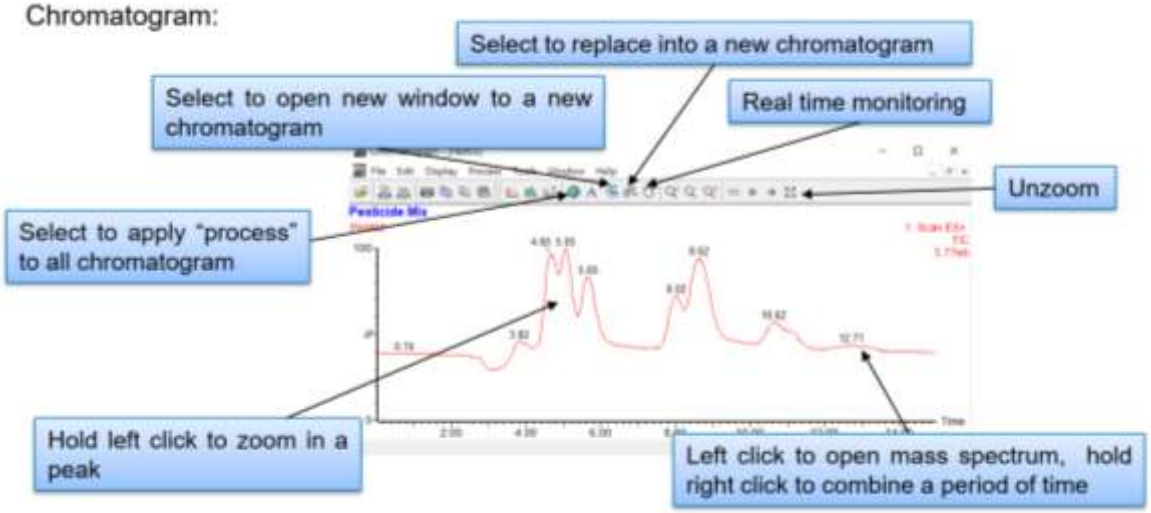
7. If you want to stop run immediately, you can click the red square on the top of Mass Lynx.

iv. View analytical data

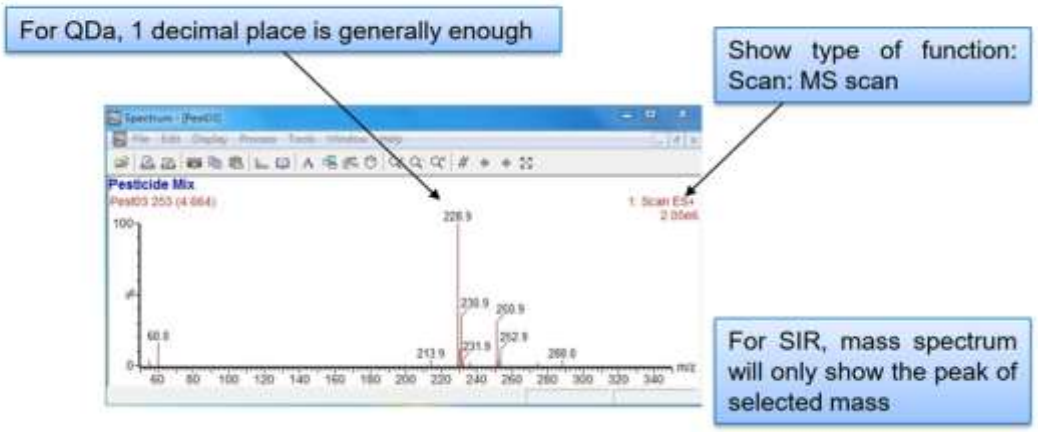
8. Select the sample of interest and then select “Spectrum” or “Chromatrogram” to view data.



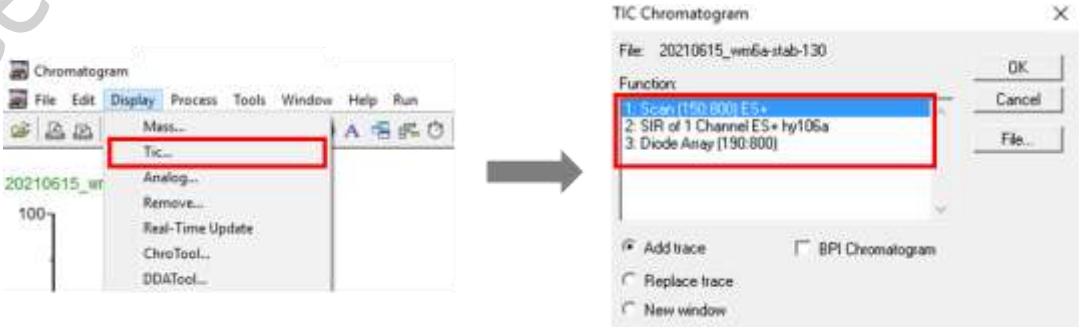
Chromatogram:



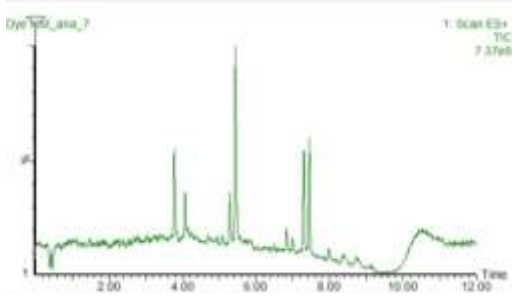
Mass spectrum:



9. In the window of chromatogram, select “Display” → “Tic”



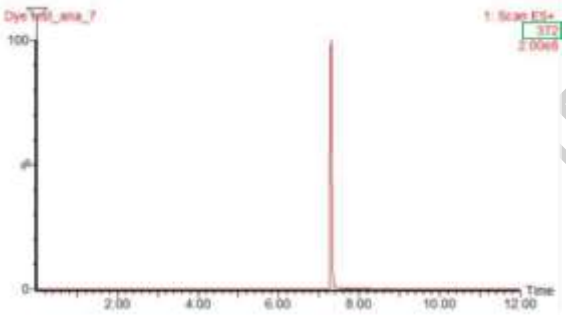
10. Select “1: Scan (xxx:xxx) ES+/-” for the chromatograph of whole mass scan



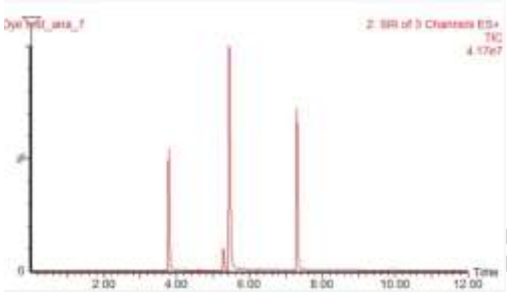
11. To extract target mass, select “Display” → “mass” (less sensitive than SIR)

12. Input target mass

↓

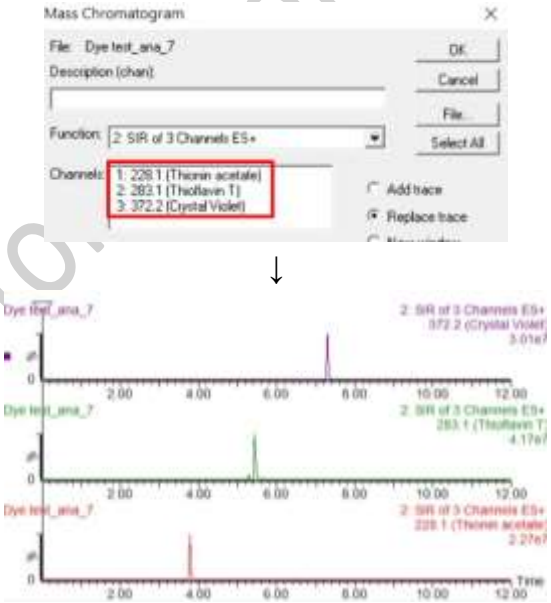


32. Select “2: SIR of x Channel ES+/-” for the chromatograph of SIR of all channels (step 26)

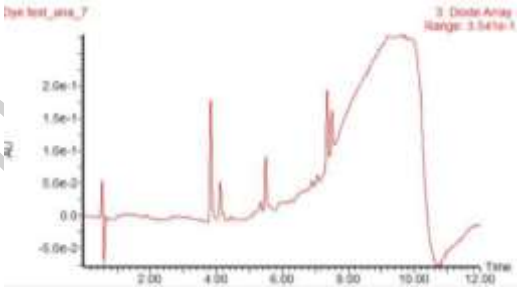


33. To extract specific channel(s), select “Display” → “Mass”

34. DOUBLE click specific channel(s) of interest



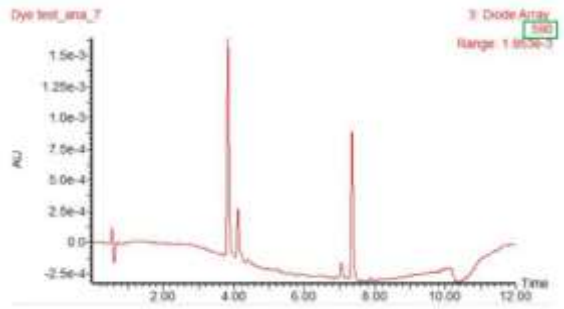
35. Select “3: Diode Array” for the chromatograph of whole PDA scan (step 26)



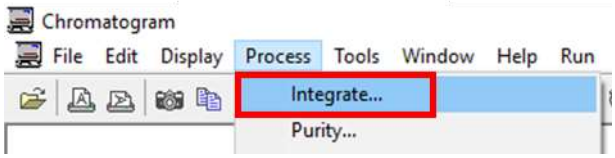
36. To extract specific wavelength, select “Display” → “wavelength”

37. Input target wavelength

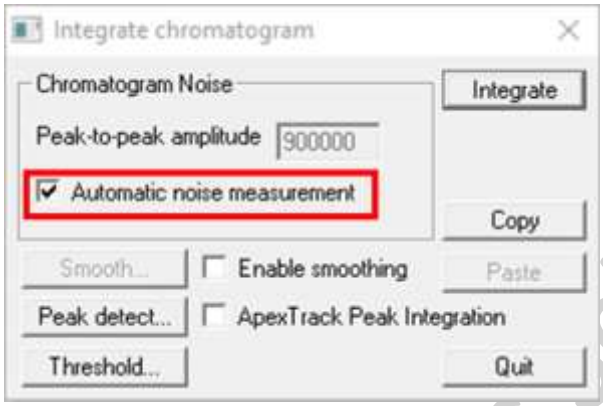
↓



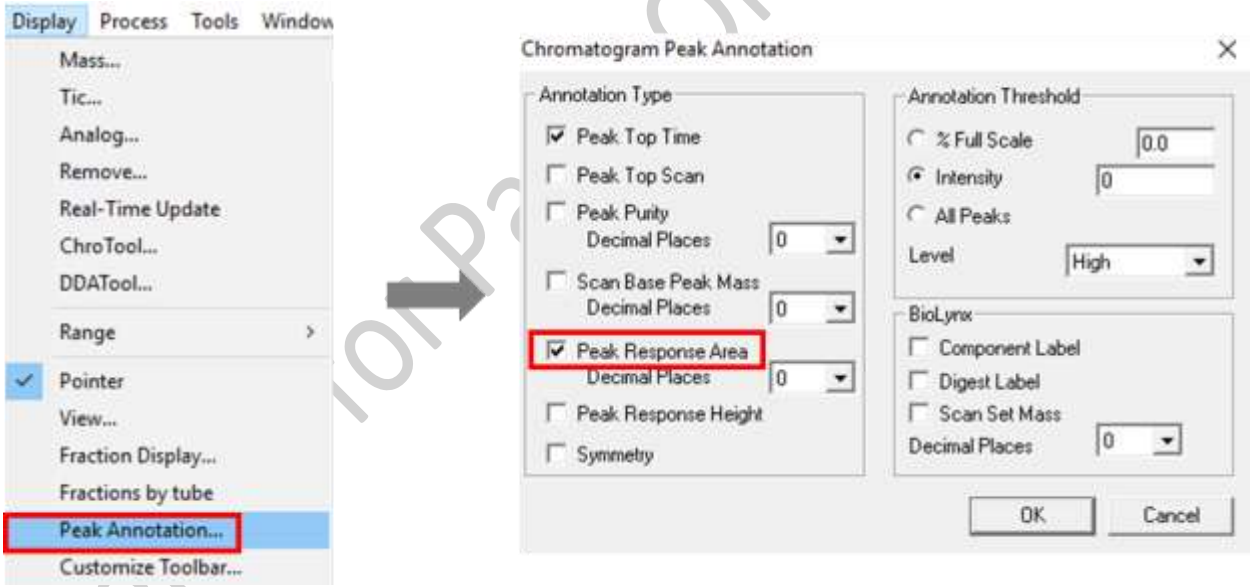
38. To find the peak area, select “Process” → “Integrate”.



39. You are recommended to select “Automatic noise measurement”.



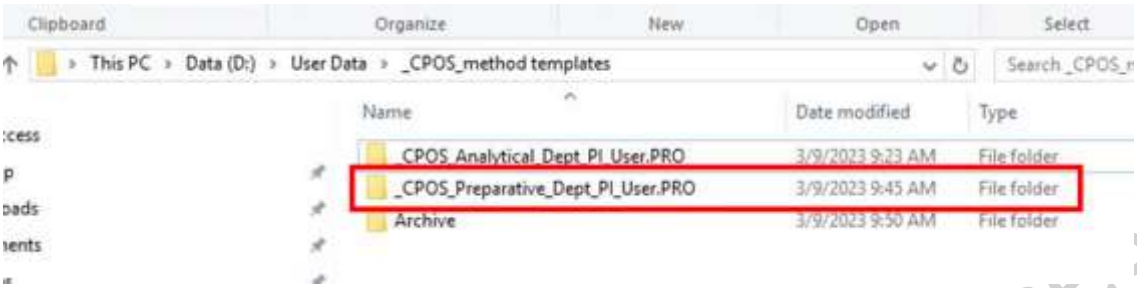
40. If the peak area did not appear after integration, select “Display” → “Peak Annotation” and select “peak response area”.



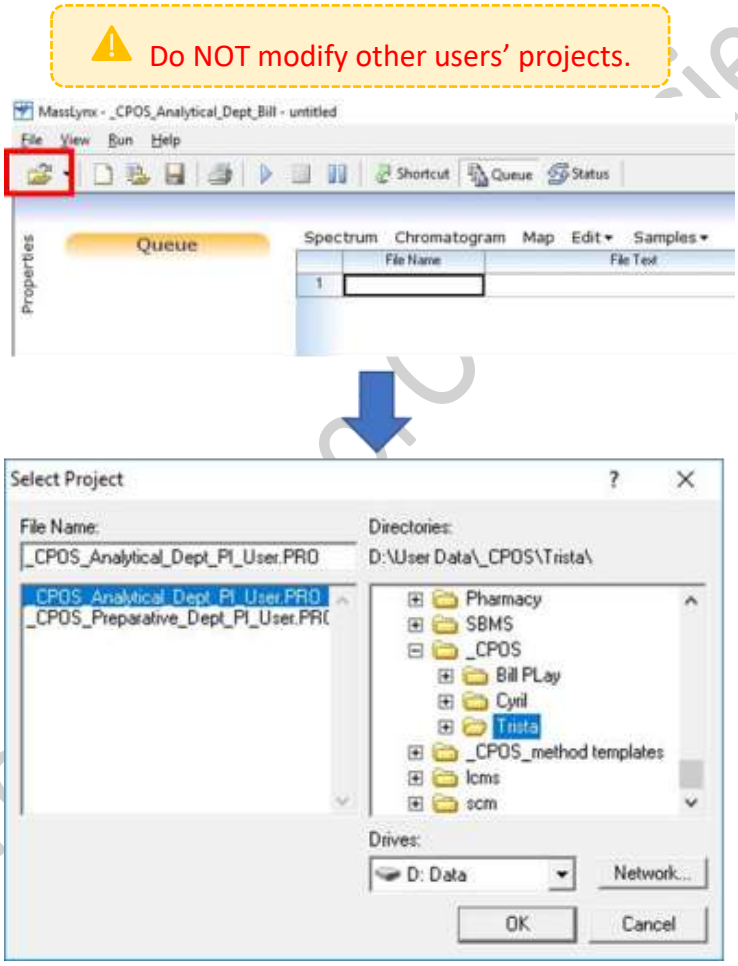
41. You must wash the column and system and enable shutdown after use, please refer to part F. Washing and shutdown (P.32) for details.

E. Preparative analysis

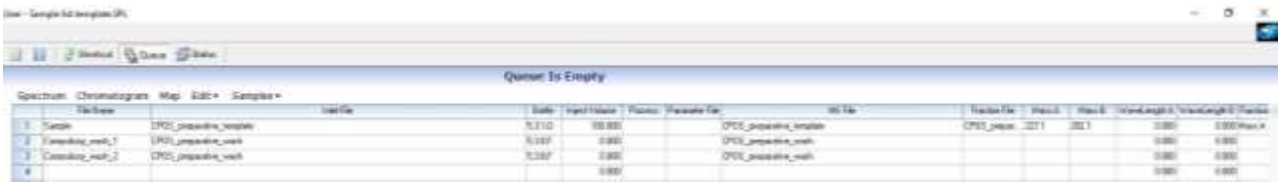
1. Copy the folder "D:\User Data_CPOS_Preparative_Dept_PI_User.PRO" to D:\User Data\Department\PI and change the name of the folder.



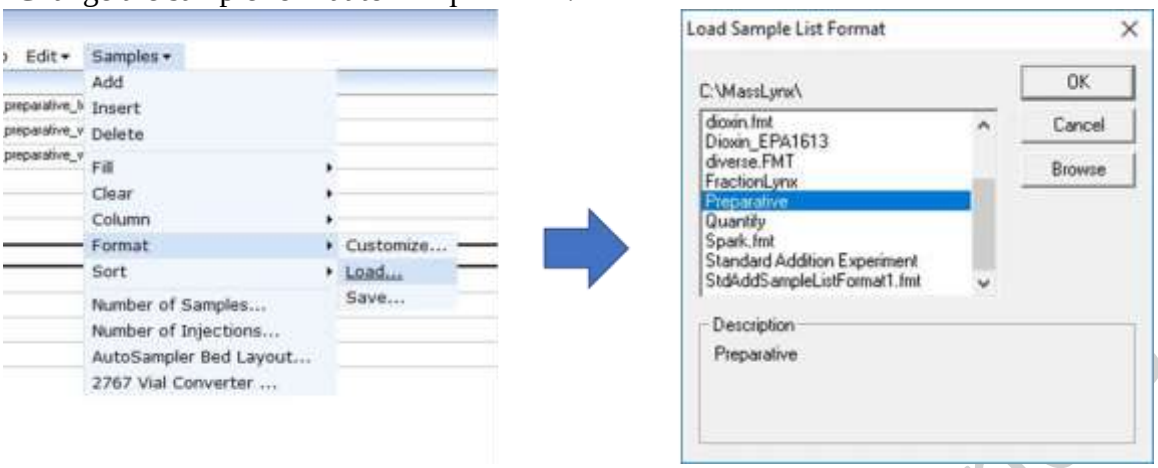
2. Open your PRO file in MassLynx.



3. Edit File name, File text, bottle position and inject volume.

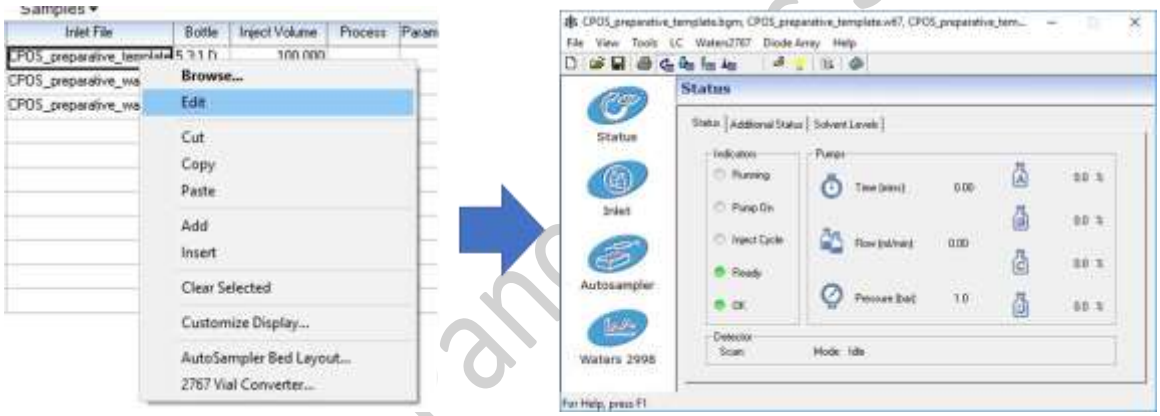


4. Change the sample format to “Preparative”.



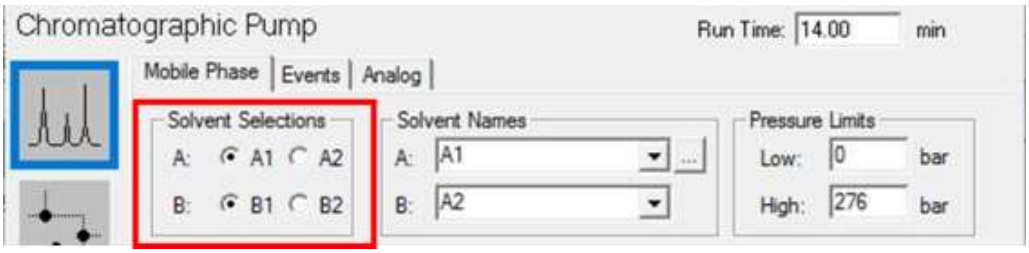
i. Set up LC method

1. In the sample list, right click the inlet file name and select “Edit”. Inlet method will be shown in the window below.

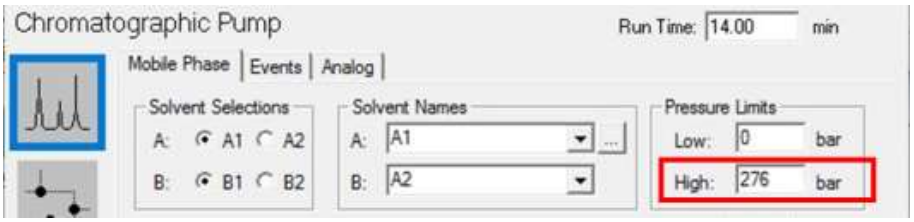


2. Select Inlet to set up LC flow condition.
3. Set the “Solvent Selections” as A1 and B1 if you use the solvents provided by CPOS.
   A1: Water + 0.1% formic acid
   B1: ACN + 0.1% formic acid

⚠ Please inform our staff in advance if you use your own solvents.



4. Set the maximum system pressure at 276 bar or 4,000 psi.



5. Set the gradient table with the following as example. Set flow rate at maximum 10 mL/min for preparative run.

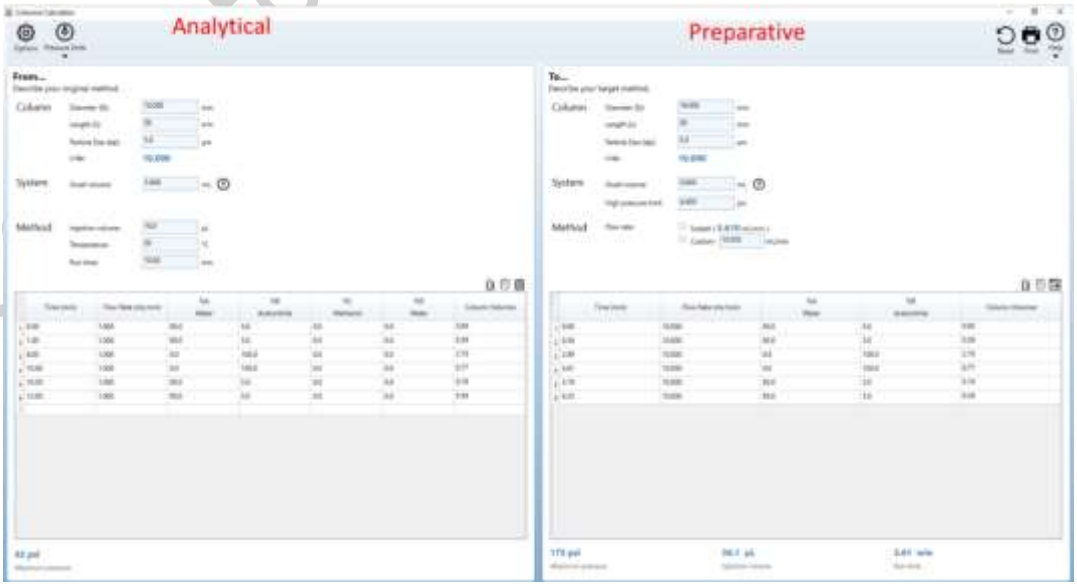
⚠ if you wish to run at >10 mL/min, call our staff to change the loop first.

	Time (min)	%A	%B
Sample injection	0th	95	5
Gradient elution	1st	95	5
Column washing	7.5th	0	100
Column regeneration	8.5th	0	100
	9th	95	5
	10th	95	5

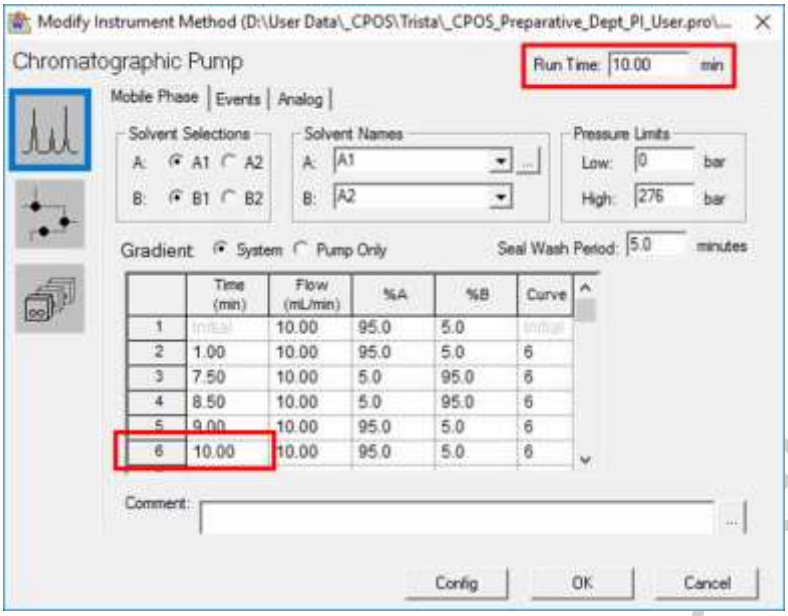
If you wish to translate the **analytical** LC gradient table to **preparative**,

Open "Columns Calculator" 

on desktop.
Type in the **analytical** LC gradient table on the left, then a **preparative** LC gradient table will be automatically generated on the right.

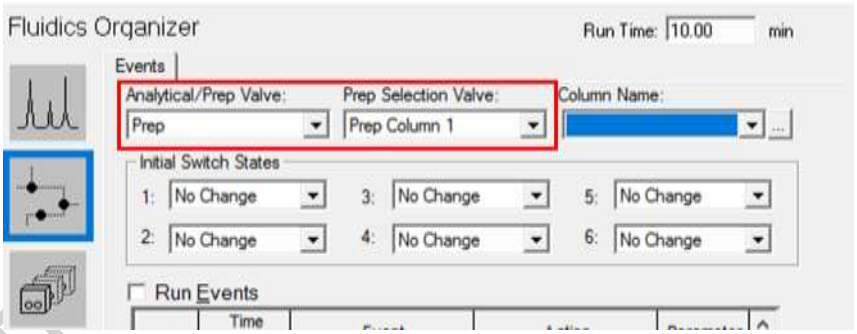


6. Make sure the “Run Time’ is consistent with the gradient table.

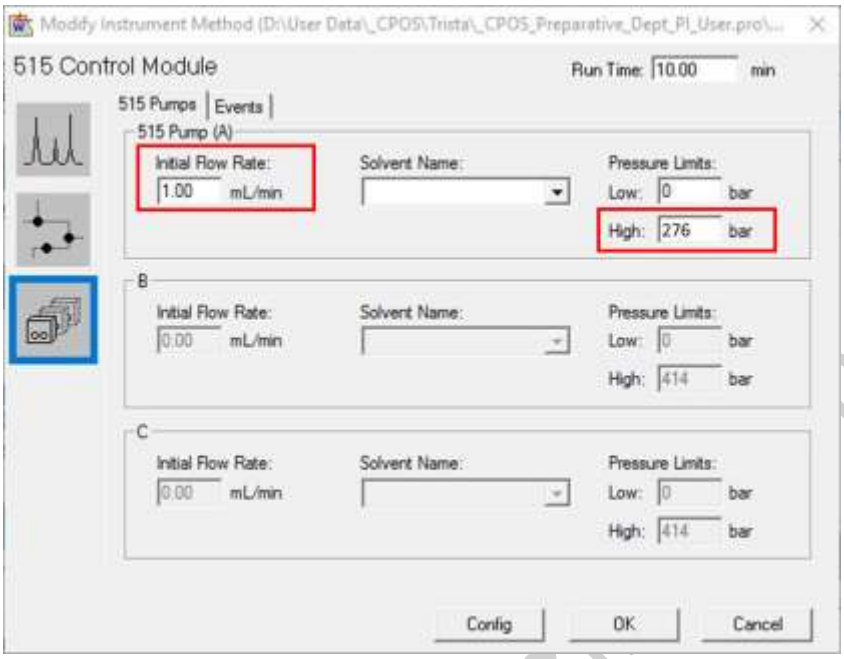


7. Select “Prep” in “Analytical/Prep Valve” and the appropriate preparative column number (1-2, from left to right) in “Prep Selection Valve”. The preparative column provided by CPOS (C18 OBDTM, 19 mm I.D. x 50 mm length) is located at preparative column 1.

⚠ If it is **wrongly** set as “**analytical** column”, the analytical column may break due to unaffordable high pressure!

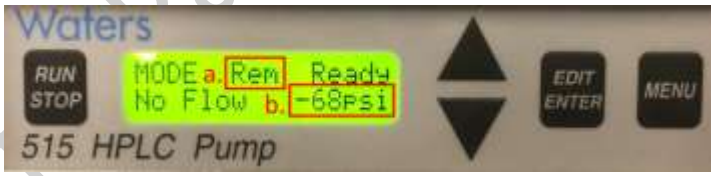


8. Make sure "Initial Flow Rate" of 515 pump (makeup pump) is set to 1 mL/min.
   The high-pressure limit should be set at 276 bar or 4000 psi.
   Make sure the setting is configured.



9. Check the status of makeup pump.
   a. Make sure it is at "Rem" (Remote) mode to be controlled by Masslynx software
   b. The normal pressure should be around 200 psi.

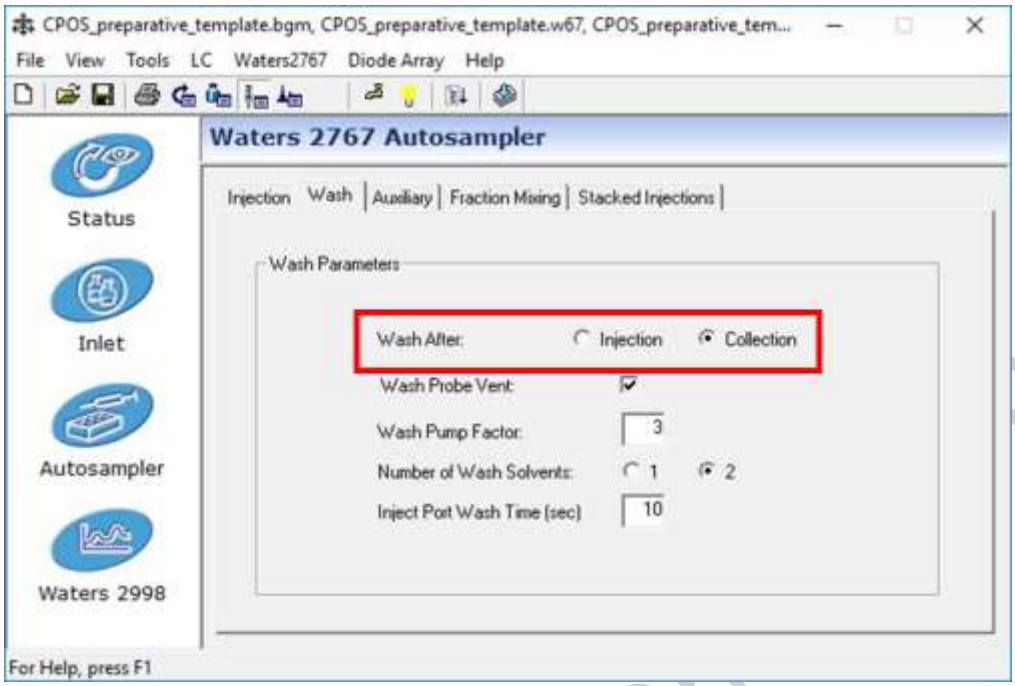
⚠ If the pressure is < 0, call our staff to remove the air bubbles inside before injection.



10. Select 

and select right loop 1000 µl.

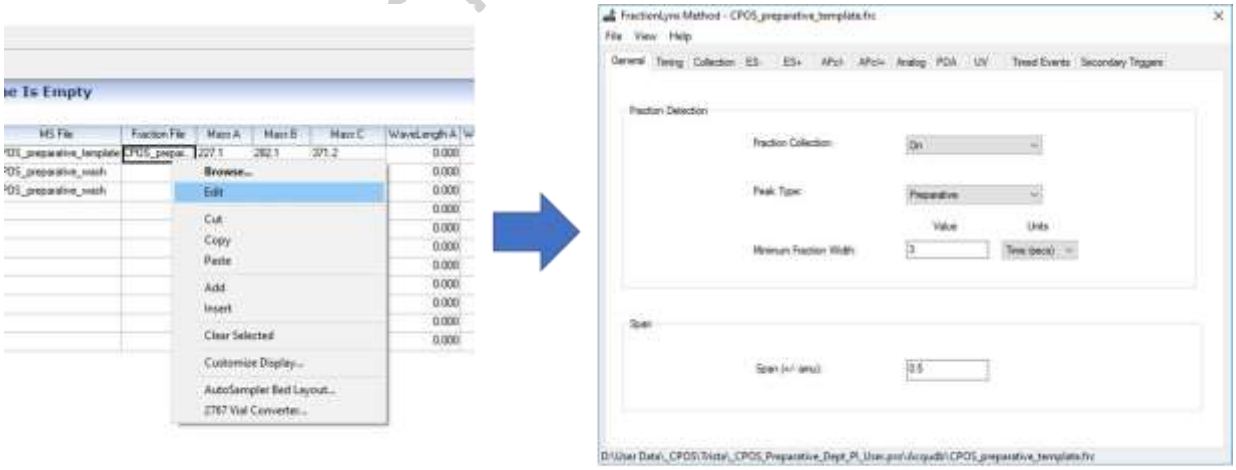
11. Determine the washing procedure of the autosampler.
    a. Wash after injection: suitable for sample that has long retention time.
    b. Wash after collection: suitable for short experiment.



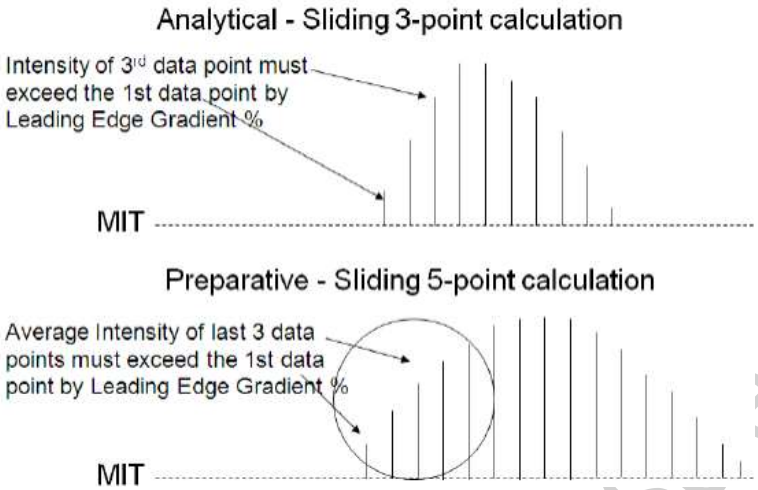
12. For the setting of PDA detector and QDa mass detector, please refer to Part D analytical analysis steps 12-17.

ii. Set up fraction collection method

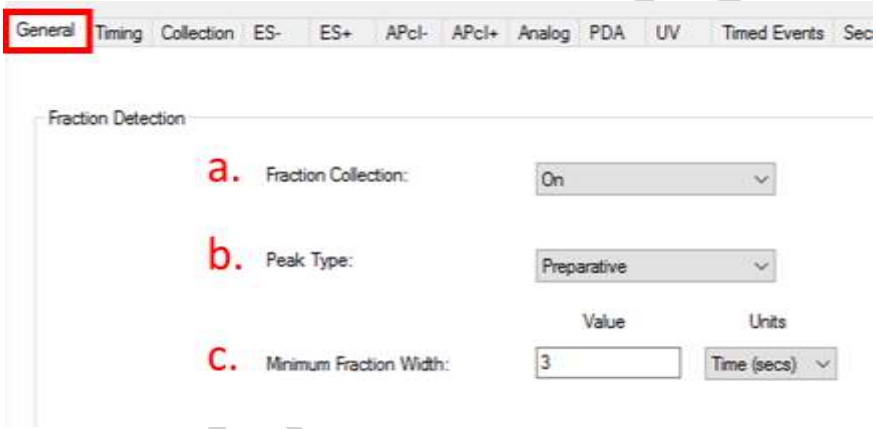
13. Right click the "fraction file" in the sample list and the FractioLynx Method will be shown in the window as shown below.



14. In the tab of "General",
    a. Turn on the fraction collection. You can turn it off during trials before actually collecting fractions.

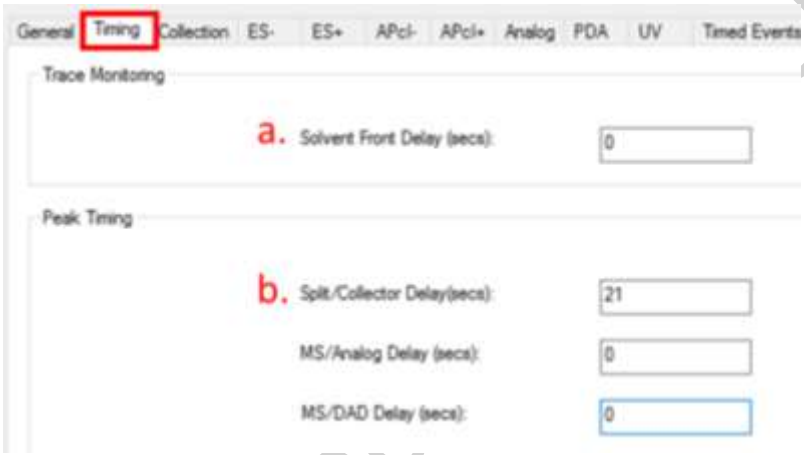


    b. Peak narrower than set value will not be collected.

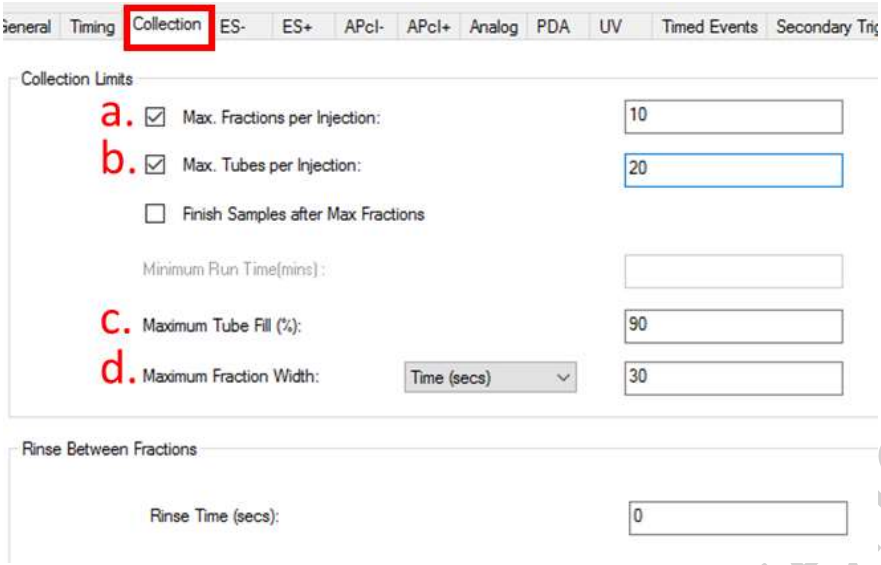


15. In the tab of “Timing”,
    a. Avoid collection of early eluted peak that may contain impurity
    b. There is time delay between flow path to detector and flow path to collector. This delay depends on flow rate. Set according to the table below:

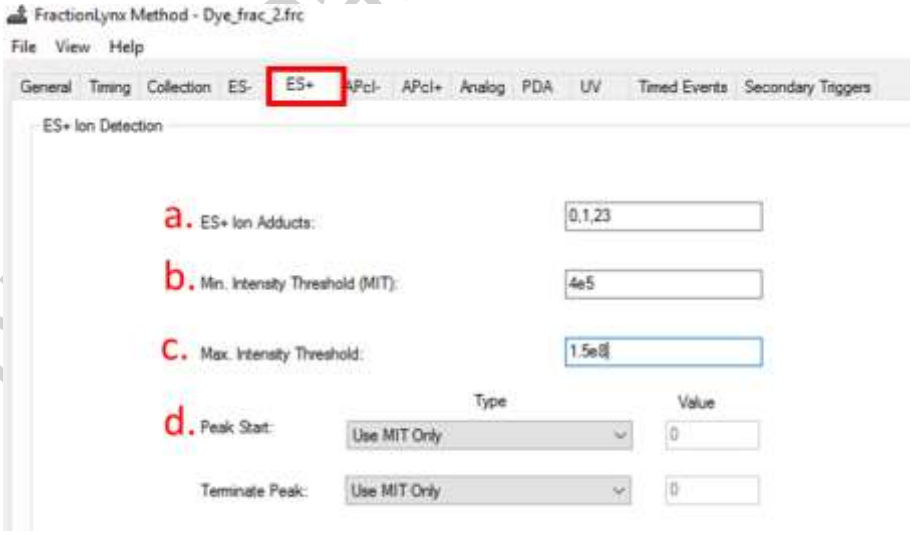
Flow rate (mL/min)	Delay (secs)
20	11
15	16
10	21



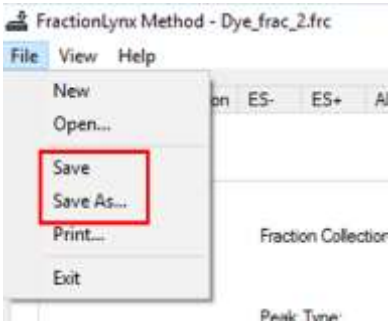
16. In the tab of “Collection”,
    a. set the maximum fraction collected in one injection.
    b. One fraction can be collected to more than one tube, so normally set b = 2a.
    c. To prevent overfill of tube. Normally set as 90% for easy handling, i.e. 90% of a 15 mL tube = 10 mL will be filled.
    d. Collection will terminate after set value. This will override “terminate peak” setting in “ES+” tab.



17. In the tab of "ES+",
    a. 0, 1 (+H), 23 (+Na)
    b. The minimum response of the target required for collection to occur. To avoid collection of undesired fractions, the MIT must be set to a value greater than the intensity of the background of the detector.
    It can be determined by:
        - Previous experience, e.g. QDa mass detector often have background of at least 1e5 level
        - Run a trial
        - Run a solvent blank
        - Auto MIT (run a blank and allow software to calculate MIT)



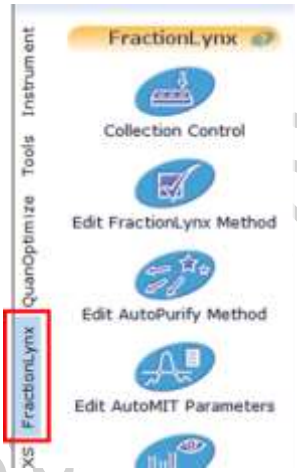
18. Save fraction collection method.



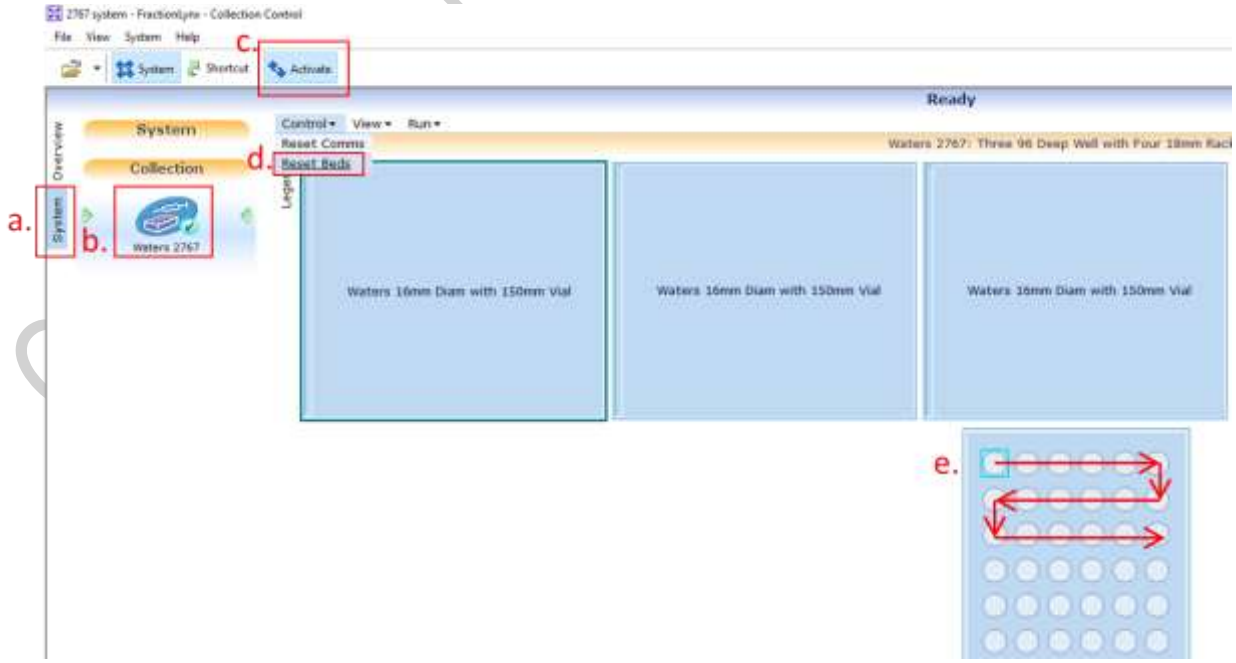
iii. Define fraction collection position

19. Select 

under "FractionLynx" tab.

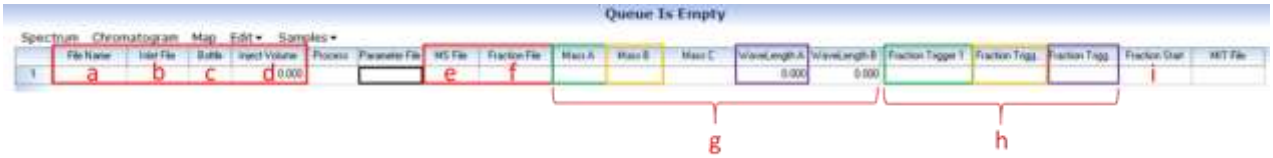


20. Activate the system (c) and reset the collection bed (d).

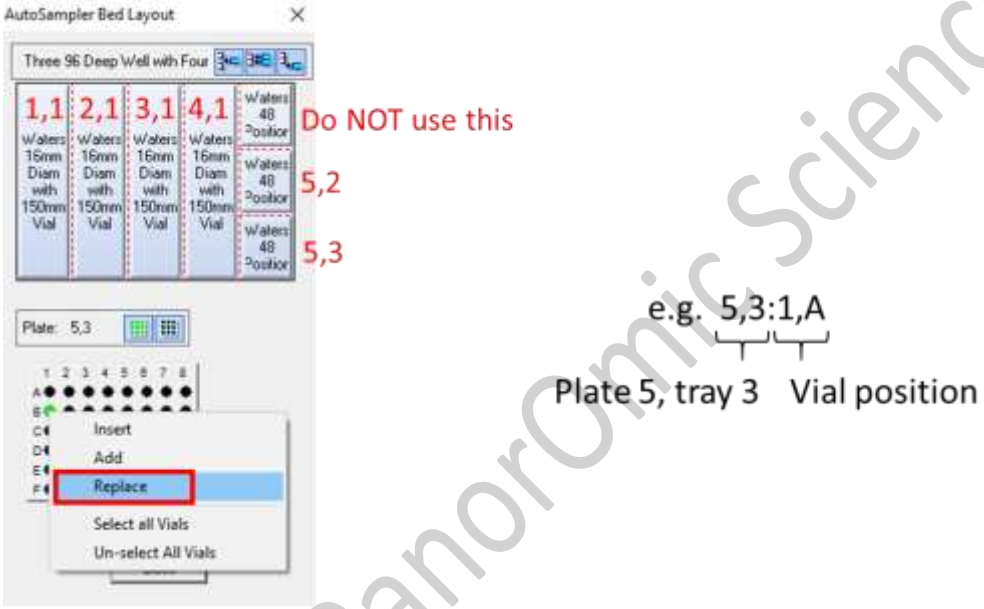


iv. Create sample list

21. In the sample list:



a. **File Name**: sample name. Only include alphabets and "_". Maximum 20-30 characters, otherwise may generate errors.
b. **Inlet File**: load your established Inlet method file.
c. **Bottle**: the position of sample vial. Right click → "AutoSampler Bed Layout"



d. **Inject Volume**: maximum 1000 µl for preparative run but recommend to start with 100 µl to prevent contamination of the system.
e. **MS File**: load your built MS method file.
f. **Fraction File**: load your built fraction collection method file.
g. **Mass A/B/C or Wavelength**: enter the molecular mass or wavelength of your targets.
h. **Fraction trigger 1/2/x**: double click to select the corresponding triggers.
i. **Fraction Start**: define starting collection tube position (optional).
j. Other columns can be left blank.

22. Right click sample list to add more samples.
23. Select  to save your sample list.

⚠ Before running your sample, check the following:

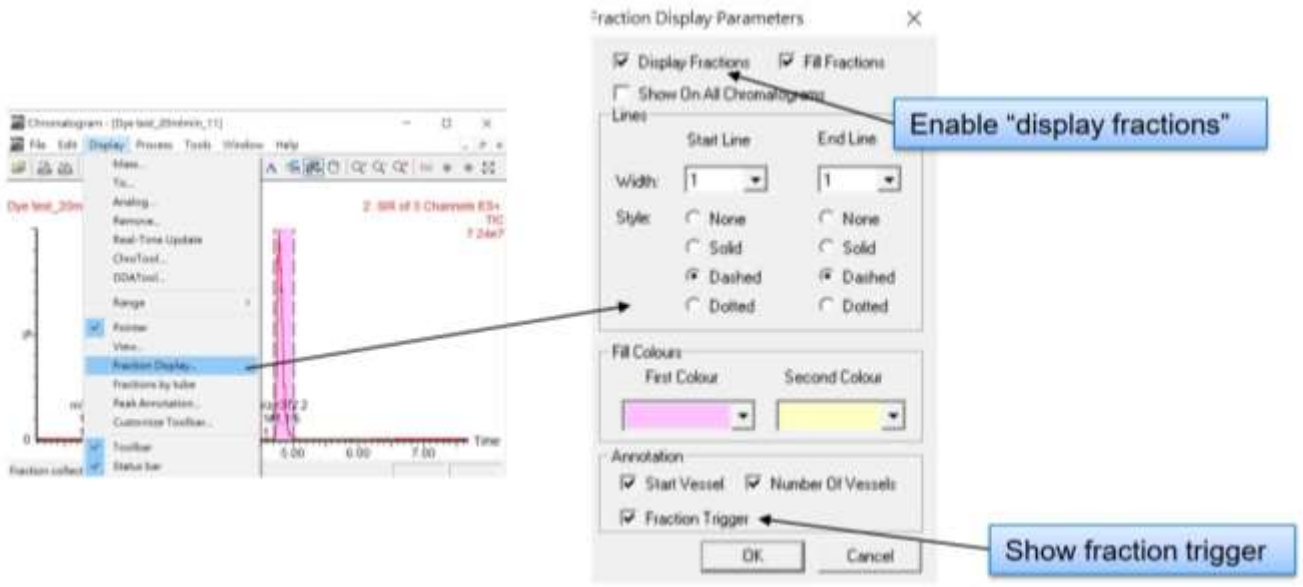
- The pressure of the pump is less than 2000 psi. If it is >2000 psi, call our staff.
- The pressure of the pump is stable (fluctuating within +/-50 psi).
- No leakage of solvent from the two extremes of column(s)
- The sample vials are in the right position as defined in your sample list.
- The fraction collection tubes are in the right position as defined in fraction collection method.

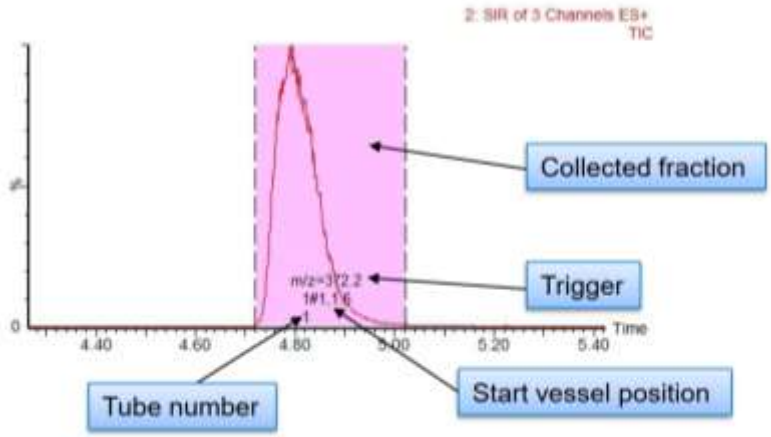
24. Highlight the samples to be run and select  to start the run.

⚠ After experiment, remember to remove waste in the waste bottle.

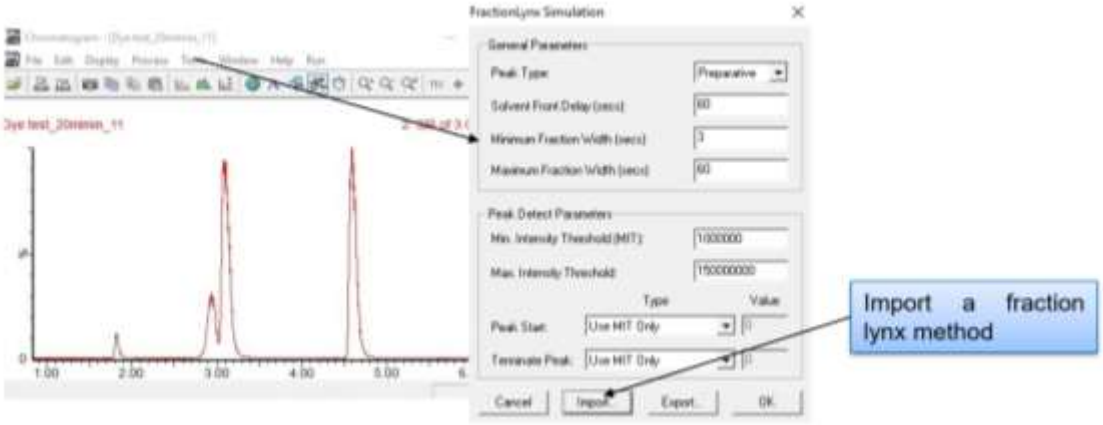
V. View collection results

25. In the window of chromatogram, select “Display” → “Fraction Display” to enable Display fractions and show fraction trigger.

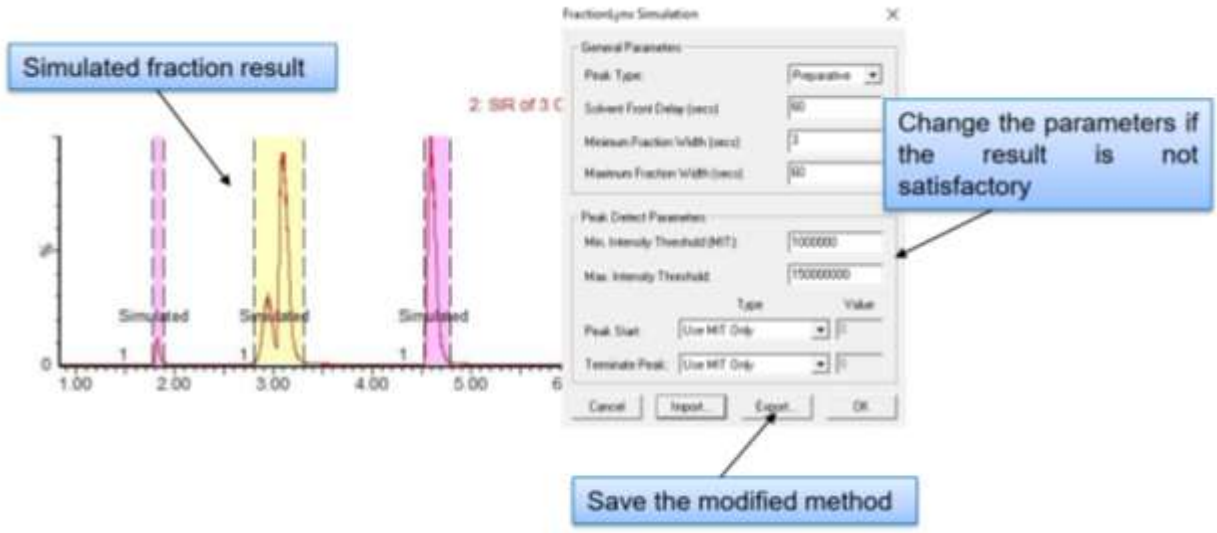




26. If you found the fraction result is not good enough or if you have run a trial and want to modify the fraction collection method, select “Process” → “FractionLynx Simulation”. Import a fraction collection method for modification.



27. Change the parameters and the fraction result will be simulated in the chromatogram. Save the modified fraction collection method.



F. Washing and shutdown

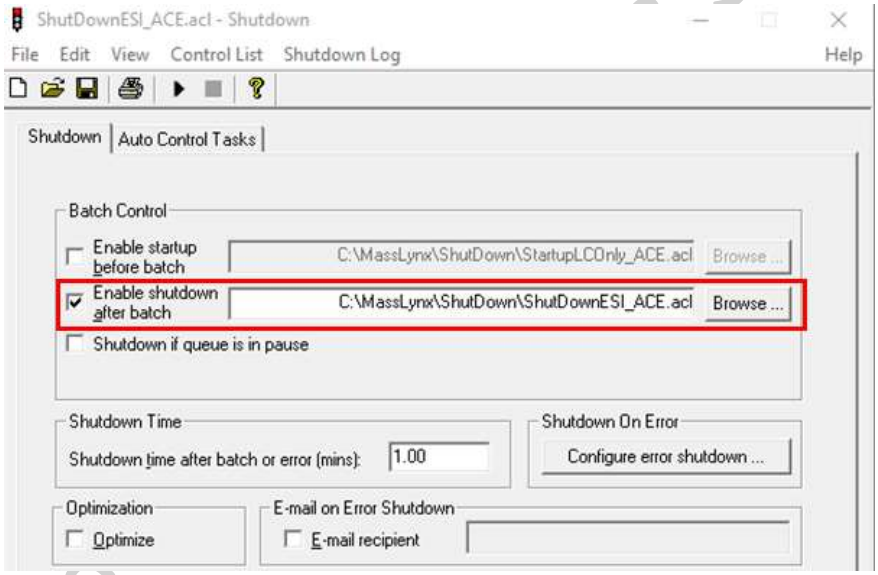
1. Run two blanks (Methanol) with "CPOS_Wash" as inlet method and "CPOS_wash" as MS method (to monitor any contaminates left) to wash the column and system
   For example,

Spectrum Chromatogram Map Edit Samples

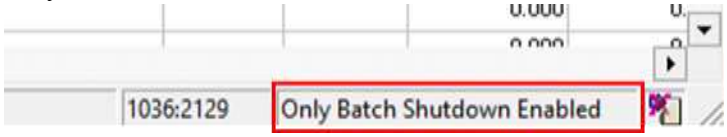
	File Name	Inlet File	Bottle	Inject Volume	Process	Parameter File	MS File
1	wash1	CPOS_wash	5,2:1,C	0.000			CPOS_wash_MS
2	wash2	CPOS_wash	5,2:1,C	0.000			CPOS_wash_MS

2. Enable shutdown after batch (all the samples waiting in queue). This will turn off the flow, PDA detector and Qda mass detector.
   a. Click "Shutdown Disabled' at the bottom right-hand corner.

   b. Select "enable shutdown after batch".



   c. Select  to save the setting.
   d. Make sure "Only Batch Shutdown Enabled" is shown.



3. Keep "MassLynx" software 

open, otherwise QDa mass detector will be disconnected.
4. Keep the PC and other parts of instrument on.

G. Data transfer

- NO personal USB thumb drive to the PC connecting to Waters autopurification system
- Use CPOS USB thumb drive to transfer data to data transfer stations shown below
- On data transfer stations, you can copy data with your own USB thumb drive or through uploading to Imaging and Flow Cytometry Core Server

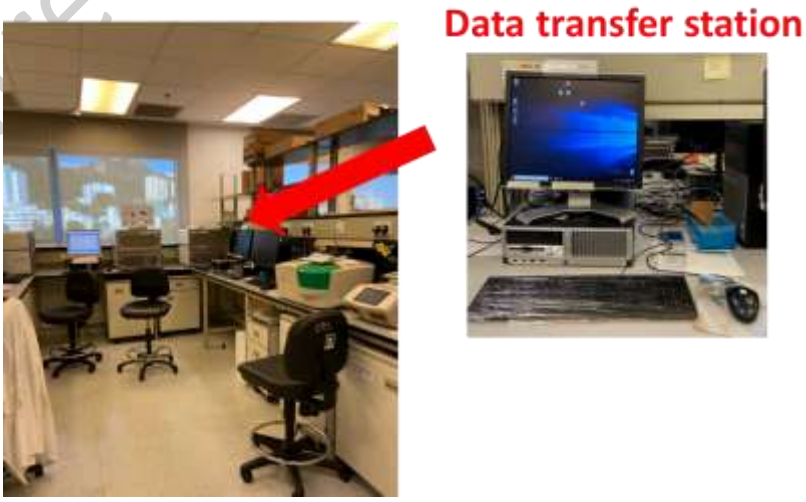
Data Transfer Station for Waters Autopurification System

(WITH Masslynx software for analysis)

Located at the end of the lane of Waters autopurification system

Data Transfer Station

(WITHOUT Masslynx software for analysis)



Located at one lane behind Waters autopurification system