IMSERC User Manual for Amazon X

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INTRODUCTION

Use of this instrument is allowed only by qualified users after receiving training by a staff member. Do not run this instrument without approval from IMSERC staff. Failure to do so may cause damage to the instrument, produce invalid data, and result in additional fees and/or removal of all IMSERC privileges.

This short set of instructions is meant to serve as a guide for 'routine' data collection on the instrument. Please read this standard operating procedure and acquaint yourself with the instrument. If during using the system, something happens that you do not understand, please ***stop,*** and ***get help***. In any event, be completely prepared to justify your actions. The cost of even minor repairs is considerable. Also note, ***you must have had prior training on the Amazon SL before proceeding to train on the Amazon X.***

The Amazon X is a general use instrument capable of analyzing small molecules, proteins, and oligonucleotides. To keep the system simple and useful for a group of diverse users, the default methods are designed to cover about 75% of LCMS requirements. For example, a UV detector is not normally used and specific masses for MS/MS are not user programmable. For specific needs not covered by the method in this guide, please contact IMSERC-MS staff.

All methods use will divert the eluent to waste for the first 0.5 – 1 minute, so buffers may be used. Default methods have a cycle time of about 10 minutes.

injection of your sample and do NOT clean up or separate sample components. Therefore prior to injection your sample must:

1. **NOT** exceed a concentration of ≤ 100 µg/mL
    a. Concentrations ≥ 100 µg/mL have the potential to contaminate the instrument.
    b. Concentrations ≥ 100 µg/mL may produce carryover which will affect the data quality of subsequent users.

You are responsible for maintaining instrument quality prior to and post sample analysis. Always check the background signal is less than 2x10^6 before starting your sample run. If the background signal remains high after your sample run, you are required to inject a methanol wash at a volume of 10 µL to eliminate carryover. An IMSERC methanol wash vial is always kept in vial position 91.

You have the option to inject your own wash vial, but it must be compatible with the chosen method solvents. As good lab practice, it is recommended to always run a wash prior to and following your run. The injection of the wash should be calculated in your time on the instrument. You may use a blank of your choice, i.e. water, methanol, your solvent. If you wish to compare your samples to a blank, then two blanks should be run at the beginning. Do NOT leave the instrument and end your reservation while the instrument is running as it affects other users' reservations. If you are unable to reduce contamination, file a bug report.

SAFETY

All users of IMSERC must review the general safety policies at http://imserc.northwestern.edu/about-policies.html.

Familiarize yourself with the location of standard safety stations like eye wash and shower stations found in just outside of BG76. Protective eyewear is required in this room, and gloves should be removed when using the computer.

Hazard	Location	PPE Required/Hazard Mitigation
Samples	BG70 – Amazon X	Eye Protection, Gloves
Methanol, Acetonitrile, Formic Acid	BG70 - Sample prep area Mobile phase lines	Eye Protection, Gloves

To become an independent user of this instrument, you must have the following safety training and certificates which are offered at https://learn.northwestern.edu:

- Laboratory Safety
- Personal Protective Equipment

Upon completion of the certificate, it will take an overnight to filter through the different systems and get into the files that NUCore uses.

DATA MANAGEMENT

Your personal data folder is created during training which must be located under your supervisor's group folder. See a staff member if you do not have a personal folder on this instrument yet. Your personal file folder must contain your Lastname_Firstname. Inside your personal folder you will create new folder which references the year the data was collected. Inside this folder you can access your sample table contained your history of samples run, as well as the individual acquisition and processed data files from that calendar year.

Every new year, you are responsible to create a new data folder and copy over your sample table. Be sure to also change your subdirectory to the new year, so that the data is collected in the new annual folder.

Over time, files from previous years will be archived to a larger file server to save space on the local drive. If you do not use the designated file structure as outlined in this manual, it may result in a failure to back up your data. See IMSERC staff for clarification if you have any questions.

Once inside the 'annual' folder, you may label/identify your sample data according to your needs.

Example: PI name > Your Lastname_Firstname > YYYY> sampleinfo

Specific Example: Einstein > Currie_Marie > 2020 > polonium_test

Data on this instrument are copied on 'imsercdata.northwestern.edu' under 'MS/Amazon X every 1 hour. Please follow instructions at http://imserc.northwestern.edu/about-general-faq.html#data for details about data access. Please remember to use the northwestern VPN when accessing data from a personal computer.

SOFTWARE

Data acquisition can be performed with Hystar data reduction and analysis are performed using Compass. Both icons to access each software are located on the instrument in the upper right corner of the computer screen.

For offline analysis after your instrument reservation is complete, please use the following resources:

- Analysis Software is also installed on a communal computer located in the area outside room BG51
- Spectrum data is observable through MNOVA and is available through the online communal server 'imsercterm.northwestern.edu'. You must be a registered IMSERC user and have permission to login to the server. Please email IMSERC-ofc@northwestern.edu for details

You have the option to use the instrument computer for analyses, but you must reserve instrument time through NUCore.

SAMPLE PREPARATION

The table below outlines recommendations to ensure proper instrument care and sample analysis.

Detail	Recommendation	Consideration
Vials	Agilent Vial 5182-0714, Agilent cap 5185-5865	The Autosampler picks up the vial. With the wrong vial dimensions, the sample will drop during transport.
Solvent	HPLC grade water, Methanol, Acetonitrile,	Listed solvents include the mobile phase solvents located on instruments. Your sample must be soluble in the solvent you choose and compatible with a combination of the mobile phases listed. Avoid DMSO and DMF. They are not column friendly. If possible, avoid additive like Phosphate Buffered Saline.
Concentration / Purity	≤100 µg/ML	Sample should be prepared in 1-100 µg/mL concentration range. Fully dissolved and filtered through a 0.2 µm filter. Sample(s) may be diluted with modifiers to increase signal/ionization efficiency such as 0.1% Formic or Acetic acid, ≤ 10 mM ammonium formate or acetate. Strong acids (i.e. HCL, H2SO4, HNO3) and bases must be neutralized of avoided. If you have acids or bases, consult IMSERC-MS staff, and disclose your sample/prep information before proceeding.
Volume	400 µL	If volume available is too low, use Agilent vial spring loaded insert 5182-8872
Labeling	Name and solvent	Sample identification & safety / proper disposal

PRE-RUN CHECKLIST

1. Install your column.
2. Change out solvents, if necessary.
3. Purge/ prime solvent lines.
4. Reminder to utilize default method if clean standards are not used.
5. If method optimization is needed, infusion must be performed by staff. Contact MS Staff.
    a. 1 mL of 500 ng/mL of your compound is required.
        i. If more than one compound is to be optimized, prepare mixture to include each component at 200 ng/mL.
    b. Staff will use the provided solution to optimize source conditions and MS or MSn setup.

QUICK START ACQUISITION CHECKLIST

1. Begin reservation in NUCore.
2. Open sample table.
3. Add sample(s)
    a. General tab enter sample name, vial position, injection volume.
    b. Methods tab select method (solvent/mode of detection)
4. Click Acquisition and accept Save.
5. Turn on Mass Spec (right click>status, select “operate”)
6. Check solvent lines.
    a. Allow time to pass to remove bubbles from the solvent lines
7. Check signal. Is background ≤ 2 x 10^6.
    a. Yes > proceed to step 9.
    b. No > run a wash vial.
        i. After several washes and no change, notify staff or fill out a Bug Report.
8. Click Start.
9. Select run preference.
10. Monitor background signal, does background return to ≤ 3 x 10^6
    a. Yes > proceed to step 12
    b. No > run a wash vial
        i. After several washes and no change Fill out a Bug Report
11. Instrument will default to standby mode after the last sample run
12. Analyze data using sample analysis software, Compass
13. Save processed data and close file
14. End Reservation in NUCore

SOLVENTS

Solvent Line	Mobile phase
A1	0.1% Formic Acid in water
A2	0.1% Formic Acid in water
B1	0.1% Formic Acid in acetonitrile
B2	0.1% Formic Acid in methanol

The binary pump allows you to choose, a single mobile phase from line A and one from line B, with default solvents at approximately pH 3.0. You may swap the defaults solvents with your own reverse phase solvent system, but please consult with MS-staff before use. Use line A for high aqueous phase and line B for high organic phase when interchanging with your own solvents. See ***appendix A*** for mobile phase preparations with different pH.

Default or new mobile phases need to be flushed through to the purge valve on the pump for 3 minutes at a flow rate of 3 mL/min before use. Make sure to open the purge valve prior to increasing the flow rate.

It is important to follow these guidelines and notices when preparing and using solvents on the Amazon X:

1. Use only HPLC-grade solvents, including HPLC grade water.
2. Use clean bottle only. Preferably rinse the bottle with the desired solvent before filling.
3. Use borosilicate glass bottles only.
4. Be aware bottles can get contaminated with detergents from the dishwasher.
5. Exchange water-based solvents daily.

a. Algae growth may block the degasser or filters.

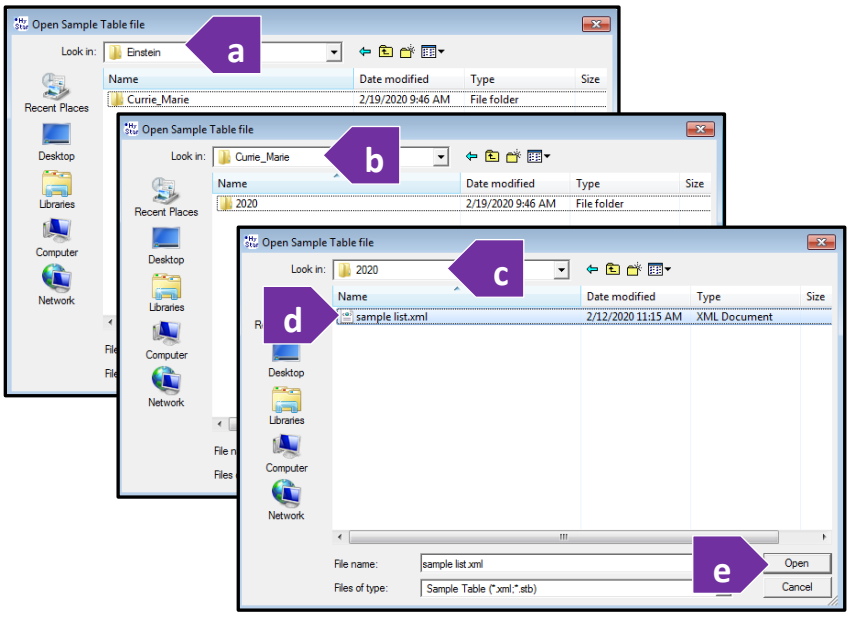
6. Precipitation of insoluble salts may block filters or capillaries.
7. Select solvent volume to be used up within 1-2 days.
8. Residues or contaminations may block filters or capillaries.
9. Label bottles correctly with the bottle contents. Fill out solution name, prep date, expiration date, and your name to identify the owner and contents.

PRIMING SOLVENT LINES

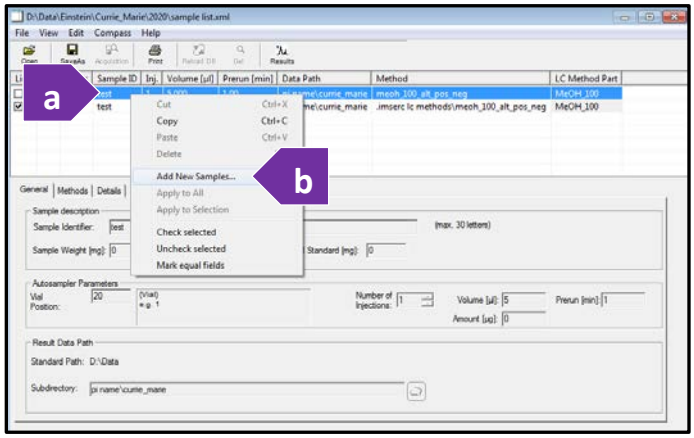
1. Open the purge valve on the pump. Thurn it counterclockwise.

2. Open your sample table, by clicking the sample table button 

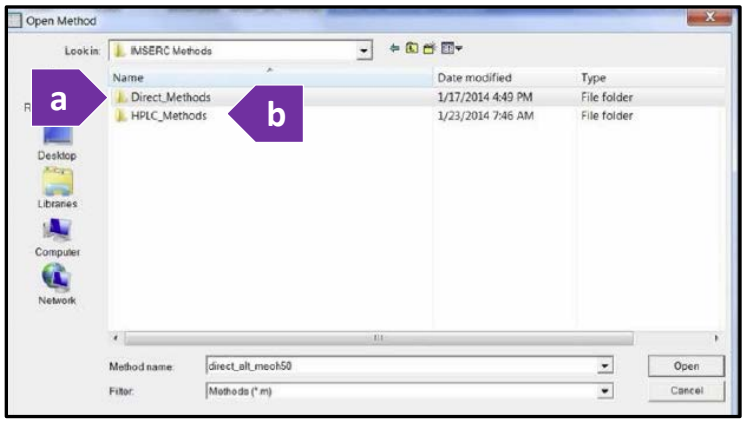
near the top left of the window. Then click file open.
    a. Navigate to your annual data folder, select your sample list, and then click open. It should always be located under your PI Name> Lastname_Firstname > YYYY.
    b. In the example below, the PI's name is *(a)* Einstein, the user's name is *(b)* Marie Currie and the user is collecting data in the year *(c)* 2020.
    c. Your sample list and all data raw and processed will be stored in the folder for that year. Select your *(d)* sample table and click *(e)* "Open."



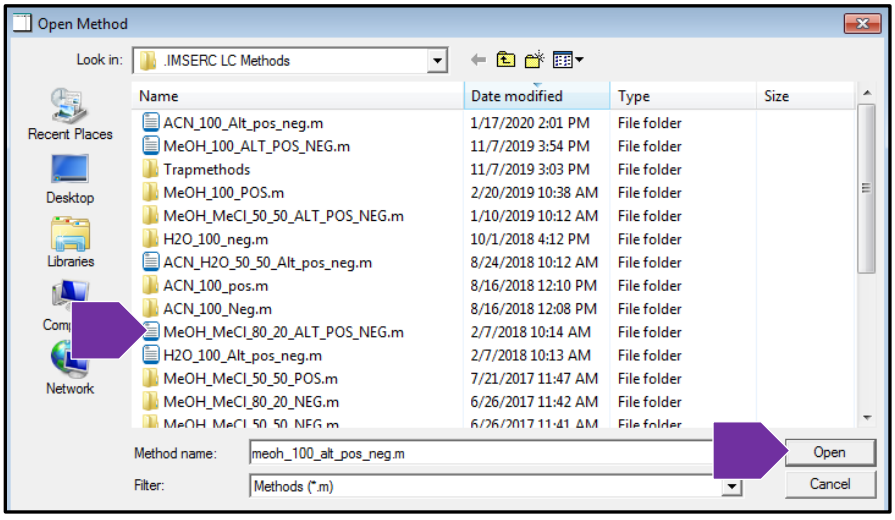
3. Add a new sample to your sample list, *(a)* right click the line of a previous sample which you would like to duplicate and/or amend, then click *(b)* "Add new samples."



4. Click the "Methods" tab. Here you will select the appropriate mobile phase and mode of detection. Click on the folder icon  . The folder appears listing all method options. Find the best fit method compatible with your injection solvent.
    a. For direct methods, you will select and choose from the (a) "Direct_Methods" folder. HPLC methods will be found in the "HPLC_Methods" folder.



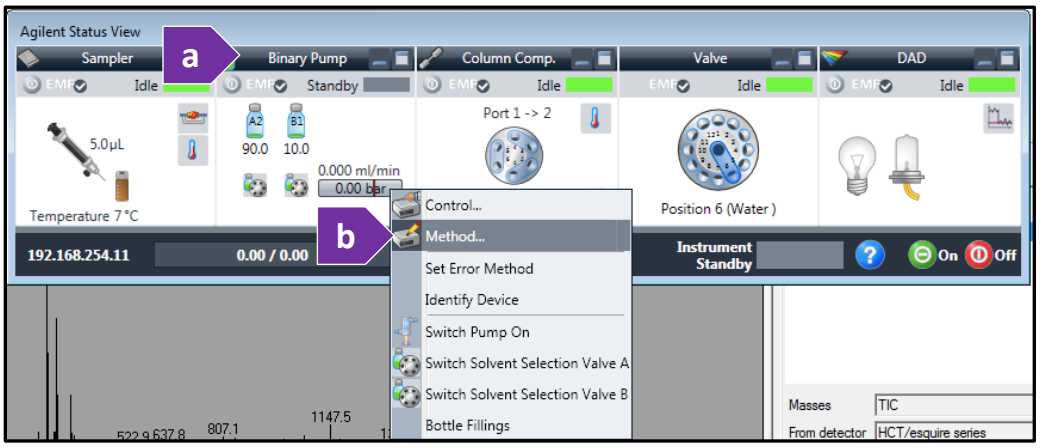
5. Select the method you will be using and click "Open." The first half of each method tells you the mobile phase composition. The second half of the method names tells you the mode of detection. If you are unsure which detection mode your compound will ionize, it is best to select a dual pos/neg mode option which will provide you with both positive and negative ion spectra.
    a. Example: **MeOH_MeCL_80_20_Alt_Pos_Neg** tells you the method will have a mobile phase composition of 80% methanol and 20% dichloromethane and collect both **POS**itive and **NEGative** spectra.



6. Click the “Acquisition” button 

located near the top left and click yes when the box prompts you to save your changes.

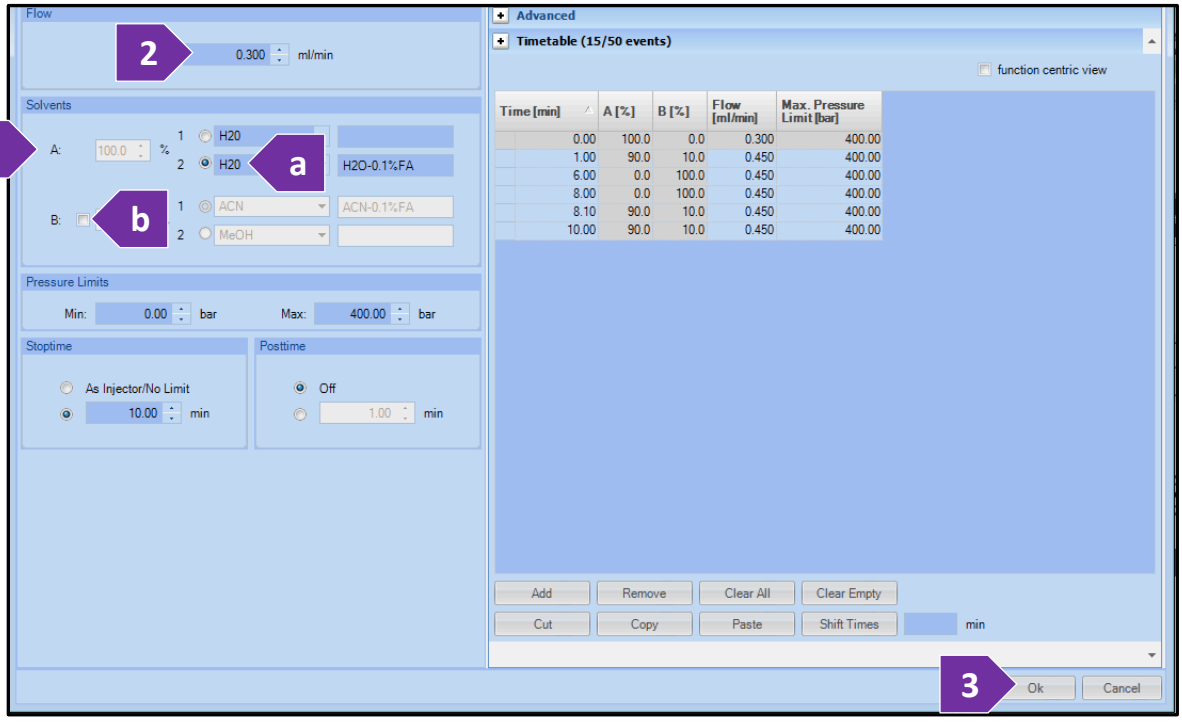
7. On the status view panel point on ‘Binary Pump’ and (a) right click on the mouse. The pump menu will pop up. (b) Click on ‘Method’ to open pump parameters.



8. (1) Set line A to 100%. (2) Set the flow rate to 3 ml/min. (3) Click “OK.”

   a. Make sure the correct line 1, or 2 is selected, this is set in the method and should be loaded.

   b. You may need to uncheck the “B” to adjust the % of A accordingly.

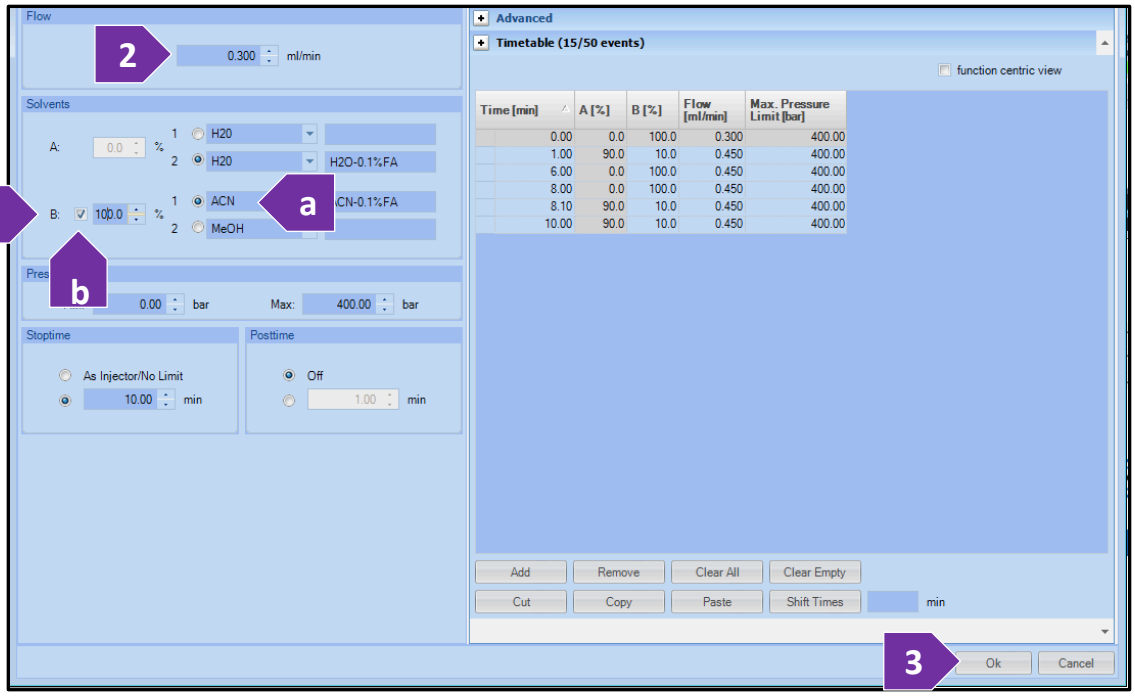


9. Flush line A for 3-5 minutes.

10. Repeat step 7. (1) Set line B to 100%. (2) Set the flow rate to 3 mL/min. (3) Click “OK.

    a. Make sure the correct line 1, or 2 is selected, this is set in the method and should be loaded.

    b. You need to check the “B,” then adjust the % accordingly.



11. Flush line B for 3-5 minutes.

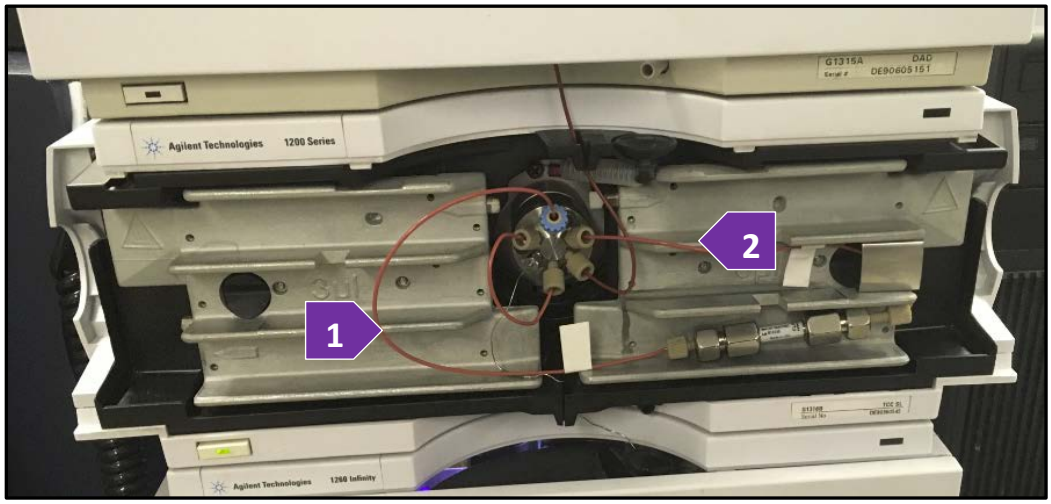
12. Open your sample table, again and click “Acquisition” to re-load your method.

13. Close the purges valve, by rotating the knob clockwise.

14. Ensure no air bubbles are trapped in the system.

COLUMN INSTALLATION

1. Set the flow rate to 0.1 mL/min and remove a plastic union from the solvent line in the column compartment.
2. Connect tubing to the (1) column inlet, ensuring that flow is in the direction indicated by the arrow on the column. Tighten the end fitting to the (2) outlet line, going into the column and increase flow to 0.3 mL/min for 2-3 minutes.
    a. Instrument max flow should not exceed 0.5 mL/min.



3. Stop flow and wipe outlet end of column to remove any particulates prior to connecting to the outlet line.
4. Connect column outlet and pass approximately 10 column volumes through the system at 0.3 mL/min while observing the back pressure.
    a. A steady back pressure indicates constant flow - while fluctuations may indicate air in the system.
    b. Wide fluctuations may shock and damage the column. When a steady pressure has been attained within the common limits, the column is ready for use.

DATA ACQUISITION

1. The computer screen is by default, deactivated. You must start your reservation through NUCore to be able to turn on the computer screen. If screen is already on, start your reservation through NUCore.

15. Open Hystar. You can select the Hystar icon 

located on the top right screen or on the lower tool bar.

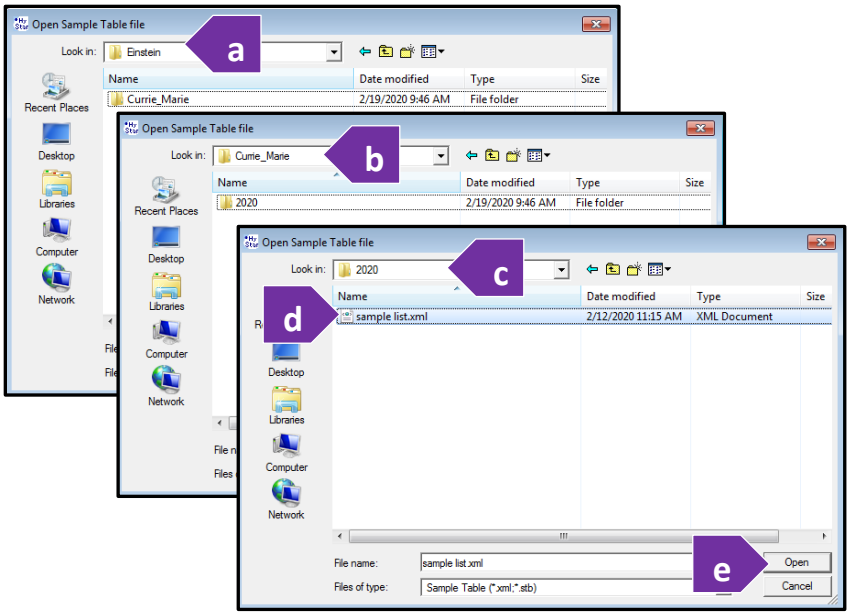
16. To locate your sample table, click the sample table button 

near the top left of the window. Then click file open.

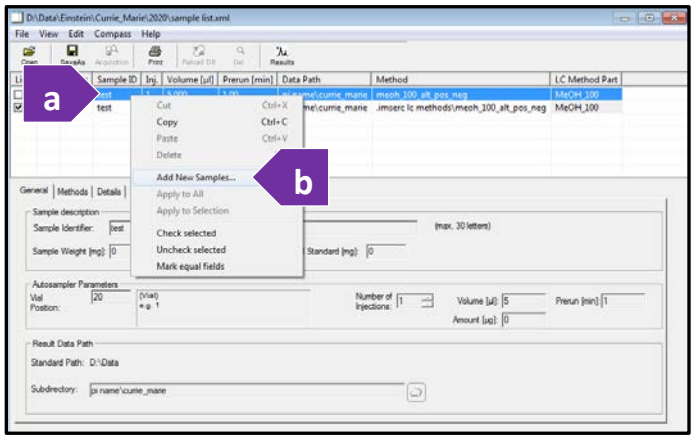
17. Navigate to your annual data folder, select your sample list, and then click open. It should always be located under your PI Name> Lastname_Firstname > YYYY.

    a. In the example below, the PI's name is *(a)* Einstein, the user's name is *(b)* Marie Currie and the user is collecting data in the year *(c)* 2020.

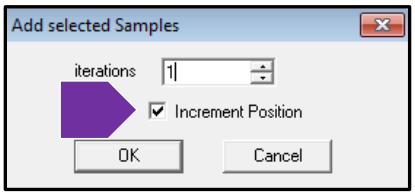
    b. Your sample list and all data raw and processed will be stored in the folder for that year. Select your *(d)* sample table and click *(e)* "Open."



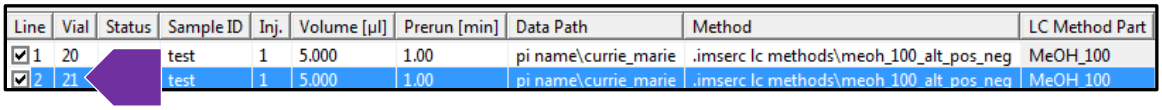
18. To add new samples to your sample list, *(a)* right click the line of a previous sample which you would like to duplicate and/or amend, then click *(b)* "Add new samples."



19. Enter the number of samples you would like to add. You can check the "Increment Position" box if you would like the program to place the samples in sequential vial positions.

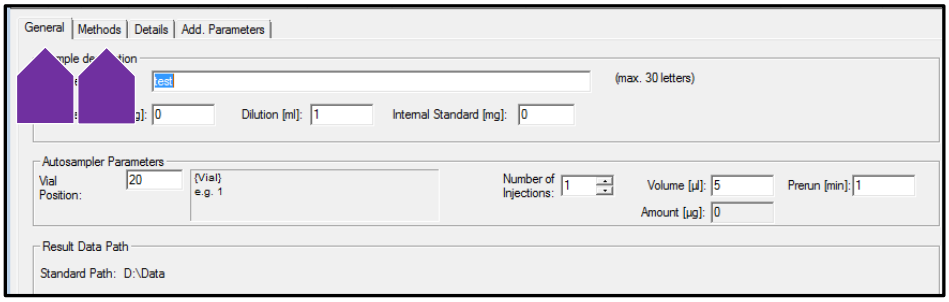


20. The added sample will be an exact copy of the original. The exception to this is if "increment position" was selected in the step above. Then only the vial position will increase by one more than the sample position from which you copied.

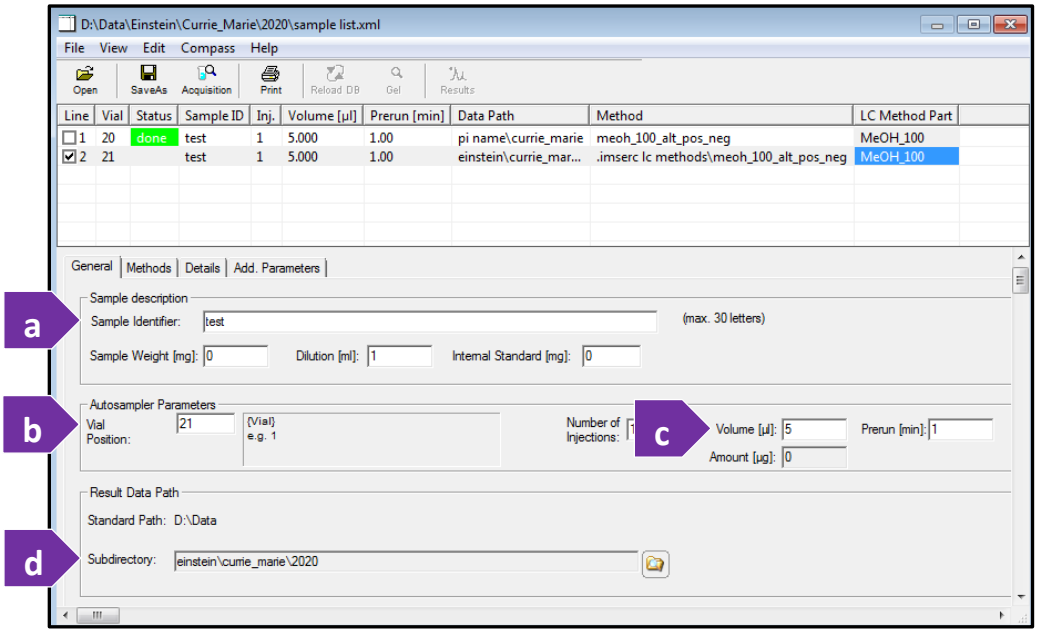


Line	Vial	Status	Sample ID	Inj.	Volume [µl]	Prerun [min]	Data Path	Method	LC Method Part
1	20		test	1	5.000	1.00	pi name\currie_marie	.imserc lc methods\meoh_100_alt_pos_neg	MeOH_100
2	21		test	1	5.000	1.00	pi name\currie_marie	.imserc lc methods\meoh_100_alt_pos_neg	MeOH_100

21. To make changes to your new sample, you will need to utilize the two tabs in the bottom table. The two tabs used for standard operation at IMSERC are the "General" and "Methods" tab.

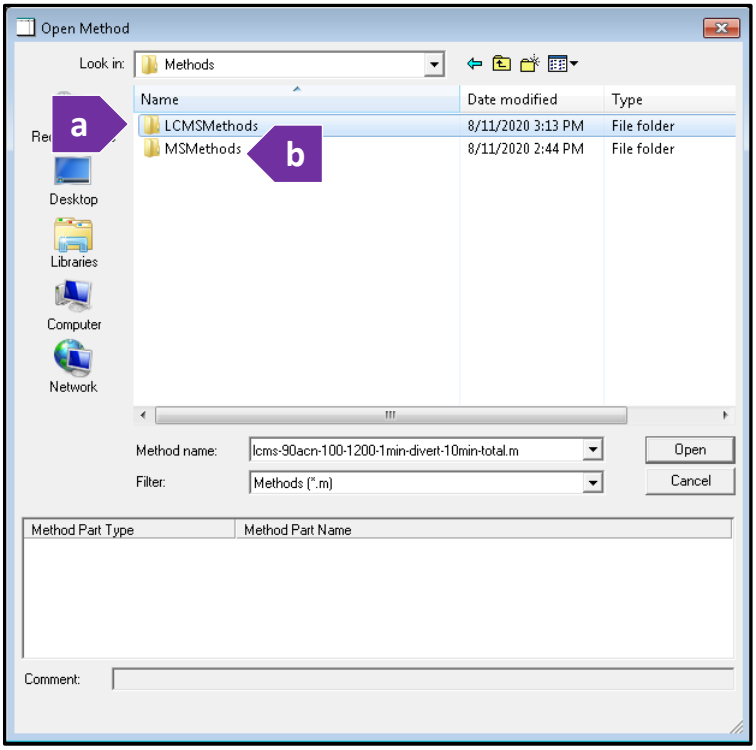


22. Changes made in the bottom two tabs will populate into your list above. Fill out all necessary information on the "General" tab according to your sample information:
    a. Change the sample name/identifier.
    b. Change the vial position.
    c. Change the injection volume.
    d. The 'Subdirectroy' file is where your files are being saved. Ensure that the files are being saved in the same location as your sample table so you can access them remotely. Again, they should be located under your PI > Lastname_Firstname > YYYY.

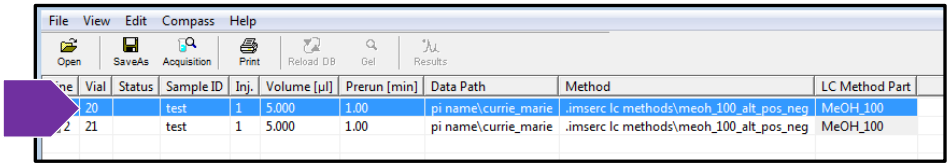


23. Click the "Methods" tab. Here you will select the appropriate LC method and mode of detection. Click on the folder icon  . The folder appears listing all your method options. Find the best fit method compatible with your injection solvent.
    a. Note: Default methods shall not be changed.
    b. To edit your method, see ***appendix B***. *Consult IMSERC staff before editing a method for the first time. This is option is for advanced users only.*

24. For LCMS methods, you will select and choose from the (a) “LCMS_Methods” folder. MS methods will be found in the “MS_Methods” folder.

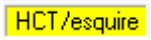


25. Once you are ready to submit your sample. Make sure you ***highlight*** the sample you would like start the run. This tells the instrument to start data acquisition from this sample and move down the sample list.

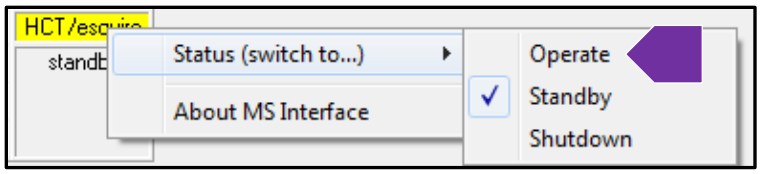


26. Click the “Acquisition” button 

located near the top left and click yes when the box prompts you to save your changes.

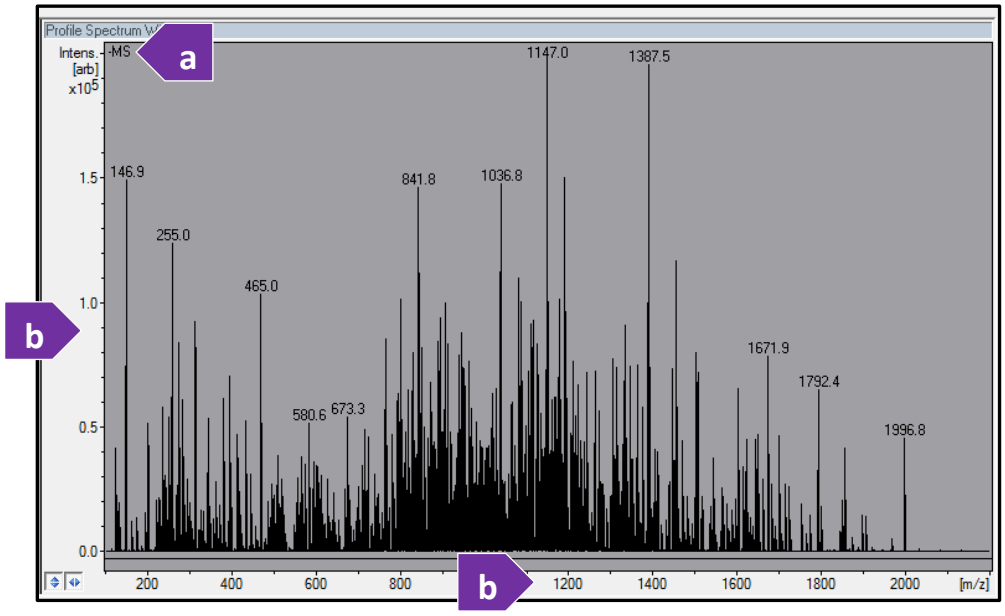
27. The method will load and the autosampler and the pumps will turn on, but the mass spec will need to be manually turned on. Right click on the yellow “HCT/esquire,” button 

and hover over to “Status (switch to…)” and click on “Operate”.



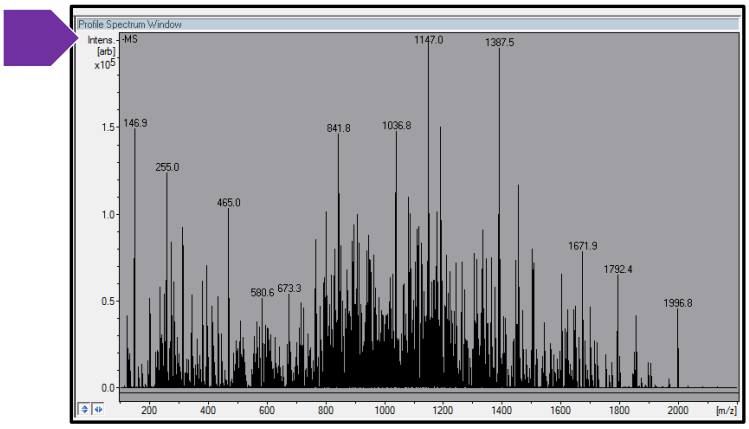
28. Make sure you have primed the pumps/lines prior to starting the run. Monitor that background signal is acceptable before officially starting your sample run.

29. Check the "Profile Spectrum Window," which is the window on the right side of the split screen. Make sure that you have a full view of the detection window.
    a. Note: If you are running in dual positive and negative mode, the detector window will flash simultaneously between negative and positive mode. You will see which mode is showing in the upper left corner. In this example –MS denotes negative mode detection.
    b. You can double click the X and Y axis to bring the window to view the full window ensuring you are viewing the full detection window and intensities.



30. Check that the background signal intensity is ≤ 2 x 10^6.
    a. YES > proceed to step 21.
    b. NO >
        i. Run 1-3 washes to eliminate the noise.
        ii. Notify IMSERC-MS staff is available to fix the issue.
        iii. Place a 'Stop' sign on the keyboard and then click the 'Bug report' icon 

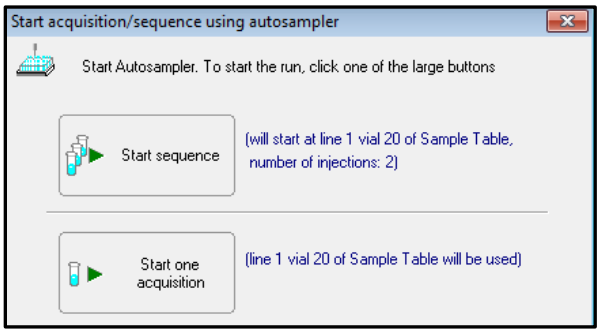
and submit the issue to staff online.



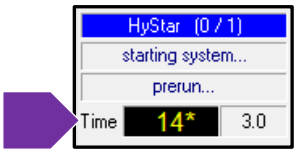
31. Once the instrument is ready and all status lights in the upper right screen are green, the start button will turn light blue in color. Located under the sample table button is the "Start" button. Click 

to start your sample list.

32. Another box will appear asking how you would like to run your sample table.
    a. If you would like to run all samples in your list, select **"**Start Sequence."
    b. If you only want to run one sample, select "Start One Acquisition."



33. A 60-90 second pre-run will start a countdown. This is built into each method to allow more time for the system to switch in the lines. When the pre-run is complete the autosampler will inject your vial.



34. Note that you can open your sample table and add additional samples to update your list in real time. The software will recognize the change and add the sample(s) to the queue. Remember to click acquisition and save changes. If the instrument goes into standby before you complete your changes, you will need to submit your samples and go through the same instructions listed above.

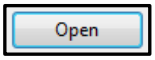
35. Default method is no longer than approximately 10 minutes. Monitor the background signal intensity at the end of your sample run in the same "Profile Spectrum Window" to ensure the signal intensity returns to ≤ 2 x 10^6.

36. If the background signal does not return to acceptable limits, you may need to run additional washes. Washes are to be complete during your reservation time and should be monitored until complete. You must react if carryover is still seen during your wash. If you still see high contamination after several attempts, place a stop sign on the keyboard, and fill out a bug report both online.
    a. A methanol wash vial is refreshed daily. The wash vial is located in position 91 in the autosampler and should be injected at a 10 µL volume.
        i. Note: This is a communal wash vial. It is subject to contamination depending on frequency of use. Users are encouraged to bring their own wash vial to troubleshoot their data more effectively.
37. After your sample run is complete, the instrument will automatically go into standby mode. The status lights will turn to yellow.

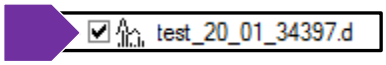
38. Remove your sample from the autosampler. Any samples left in the autosampler vial are subject to immediate disposal. It is your responsibility to take your sample with you after use.

39. Acquisition and quantitation software should remain open. Leave the acquisition software open when you are done with the measurement. And end your reservation to log off the instrument.

DATA ANALYSIS

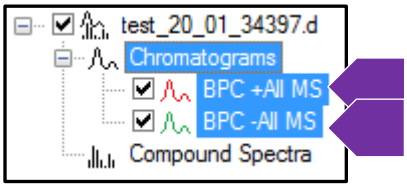
1. To analyze your data, click on the "Data Analysis" icon  .
2. Click the open folder button  .
3. Navigate to your folder then select your annual data folder. It should be located under
    a. Data > PI Name> Lastname_Firstname > YYYY
4. Select the sample file you would like to view and click 

on the lower left corner.
5. Your file will appear with a check inside a box. This indicates that the data will be seen in the chromatogram trace.

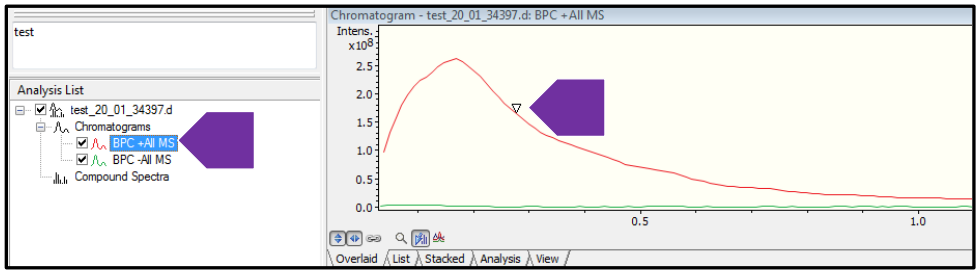


    a. Ensure other users' files are unchecked. This will remove their data from the chromatogram and prevent confusion between data files. You will only need to uncheck the far-left box which tells the software to deactivate the whole file.

6. If you have selected dual mode detection, when you click on the analysis list window, you will see two options to select a positive or negative chromatogram trace to extract your spectra.

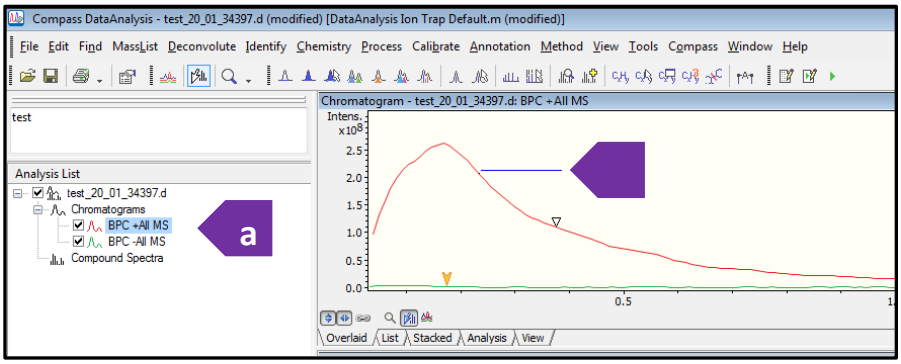


7. Highlighting the trace in the analysis list will active that specific trace. The software will also color match the trace.

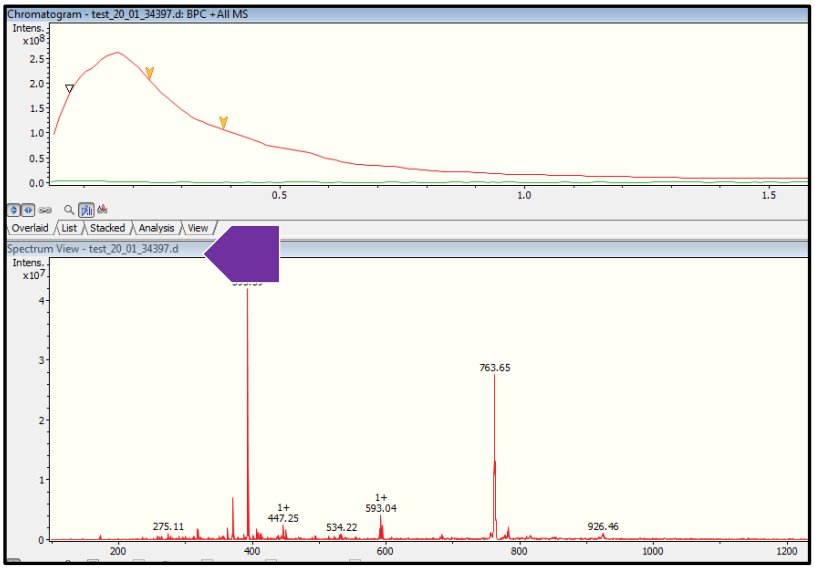


8. Use the left mouse button to view your data file.
    a. To zoom in, you can choose between several options.
        i. Hold down the shift key. This creates a zooming tool with the mouse where you can repeatedly select where you would like to zoom in the spectra.
        ii. Right click, select “zoom.” This creates the same zooming tool. Repeat to zoom again.
        iii. Click on the x or y axis, then use the scroll on the mouse to zoom in and out.
    b. To zoom out:
        i. Double click the x or y axis to send the view back to its original view.

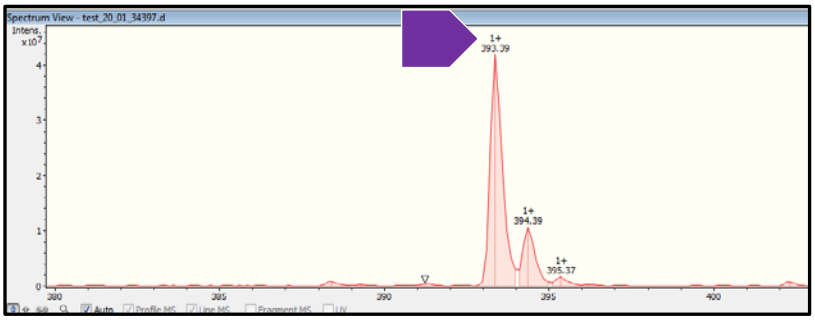
9. Use the right-hand mouse button to extract spectra. Hold down the right mouse button and drag across your selected chromatogram. You will see a line appear showing you the start and stop point of your selection.
    a. Note that the peak is highlighted in the analysis list. This is a reminder that the peak from which you would like to extract must be highlighted/active.



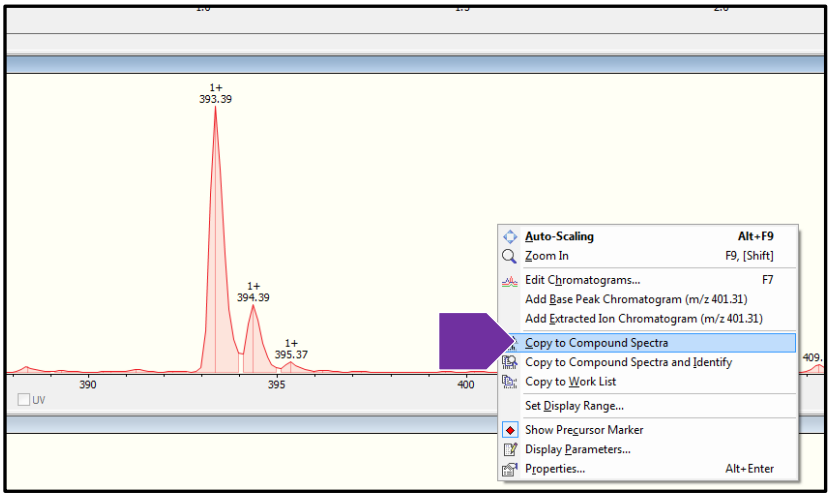
10. By clicking and dragging over the chromatogram you can view the averaged spectra for that range. And it will appear in the “Spectrum View” window below the chromatogram trace.



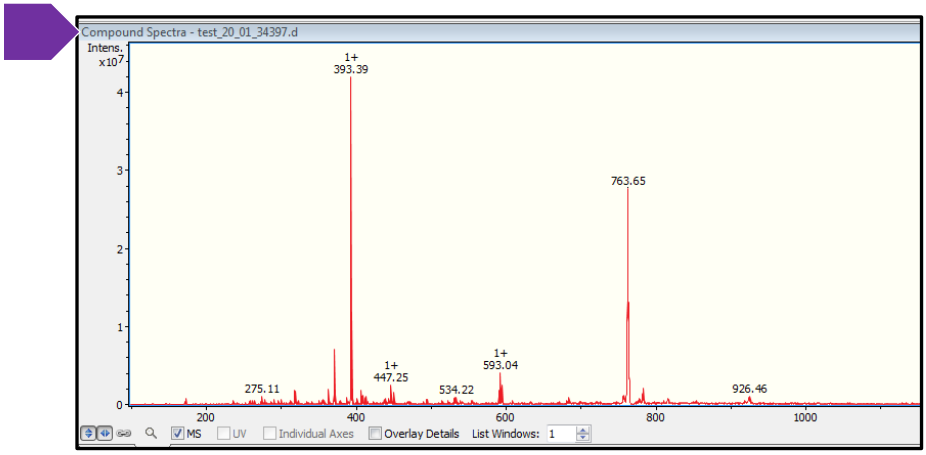
11. You can use the same zoom instructions to look closer at your acquired spectra. In some instances, you will see the software assisting you with the charge state by calculating the spacing between isotopes and place the calculated values above the peak.



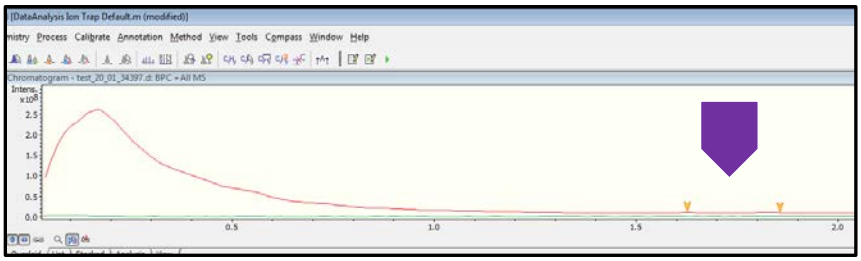
12. To collect your extracted spectra, right click the “Spectrum View” window, and select “Copy to Compound Spectra”



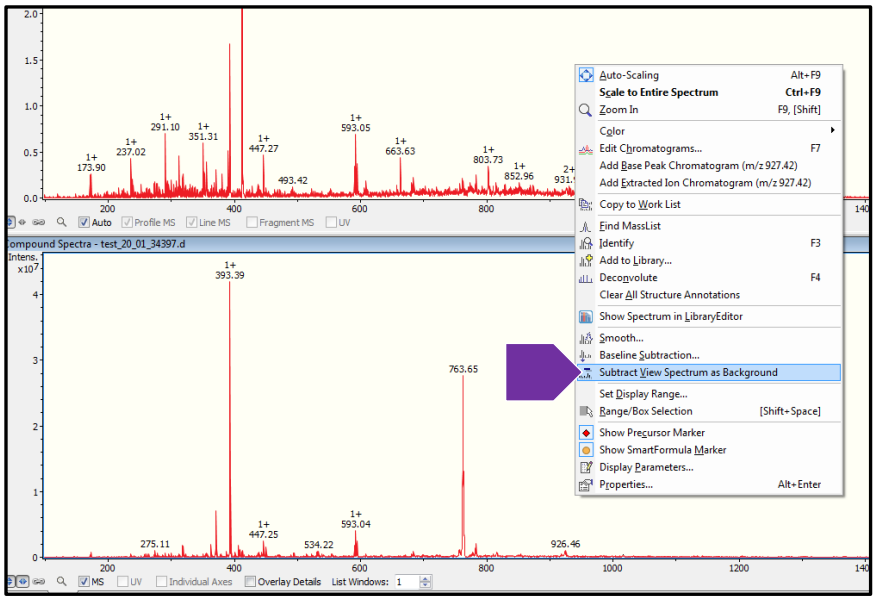
13. The spectra will now appear in the “Compound Spectra” window below.



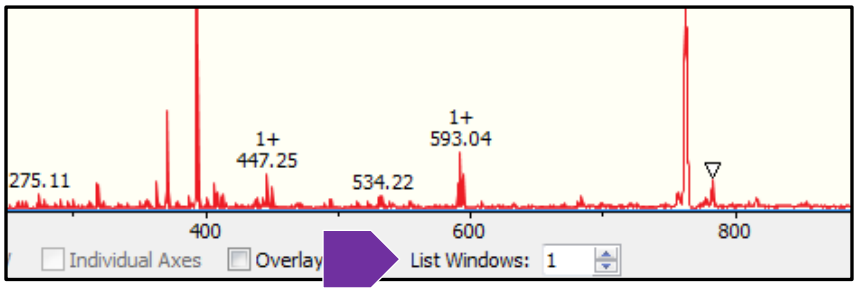
14. To extract background. Select an area of baseline following your sample.



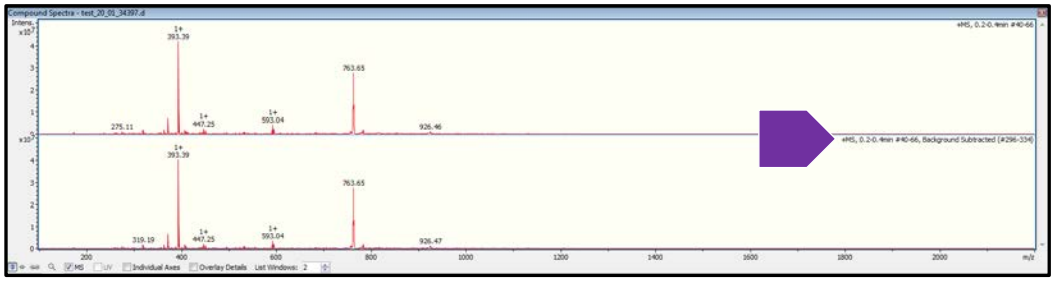
15. Right click the “Compound Spectra” (the spectra that you have just collected) and then select “Subtract View Spectrum as Background.” This will subtract the baseline spectra visible in the “Spectrum View” from your spectra in the “Compound Spectra.”



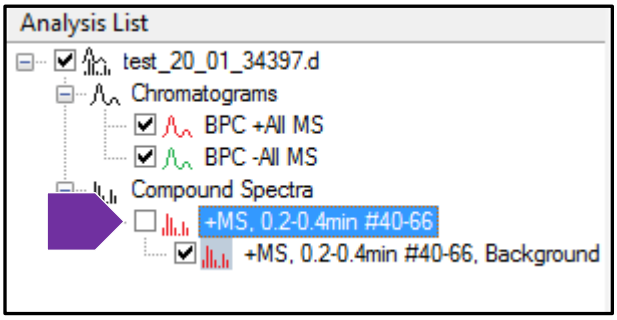
16. If you are unable to view your new spectra with background removed, you need add more windows to view the added spectra. Check the “List Windows” selection, located on the bottom of the “Compound Spectra” window. Change the number of list windows below from 1 to 2.



17. You should now be able to see the new spectra with the label of each on the upper right-hand corner.



18. Remember you can select and deselect which chromatograms you would like to view as well as the spectra you have collected by checking the boxes on the left panel of the analysis list.

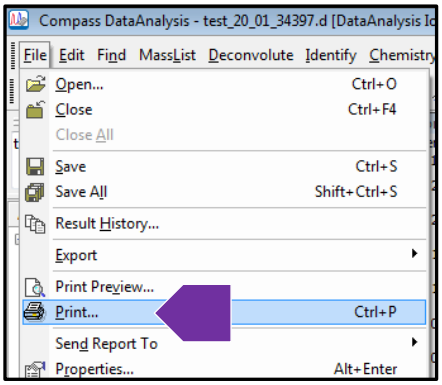


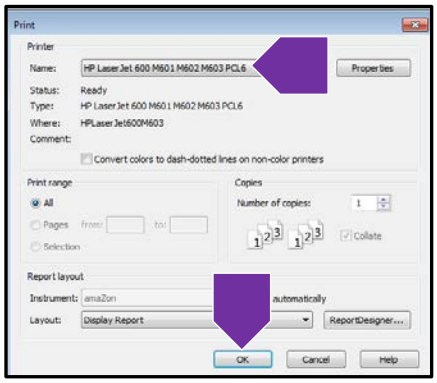
19. You can repeat the procedures with a second chromatogram in the data file if you ran an Alt Pos/Neg method.

20. Save your file by clicking the save icon  .

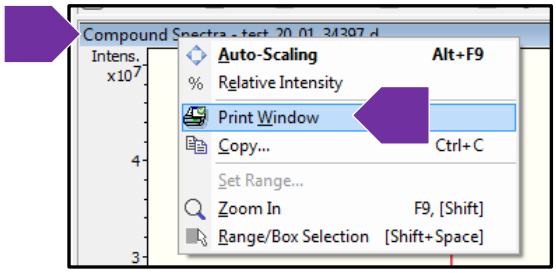
21. You have two options to print.

    a. Go to the top of the window and select file> print. Then select the printer named "HP Laser Jest 600 M601 M602 M603 PCL6." The printer is located in the NMR area outside of BG75. This will print out what is visible in your Chromatogram, the Spectrum View, and the Compound Spectra.





    b. Or right click on top of the "Compound Spectra" window and select Print Window. This will print only the data visible in the compound spectra.



22. Close your file when you are done.

23. If you have made changes to default view settings of the software, return them to the original view for the next user.

PUBLICATION

EXPERIMENTAL SECTION

Mass Spectrum data was collected on the Bruker Amazon X using Hystar Version 3.2 data acquisition software and processed using Compass Version 4.4 for data analysis. The mass spectrometer was configured with an Agilent 1200 Series HPLC module, and the mass spectrometer was configured with an ESI source and 3D ion trap mass analyzer. The sample was run using direct injection of the sample at ***<<insert your injection volume here >>*** µL injection volume. Mobile phase composition was ***<<insert chosen mobile phase composition here >>*** and ***<<insert mode of detection here >>*** detection mode was utilized.

ACKNOWLEDGEMENT

All results gained from the use of this instrument, and used in publication must use the following acknowledgement:

"This work made use of the IMSERC at Northwestern University, which has received support from the Soft and Hybrid Nanotechnology Experimental (SHyNE) Resource (NSF ECCS-1542205), the State of Illinois, and the International Institute for Nanotechnology (IIN)."

TROUBLESHOOTING

NUCORE / RESERVATION

1. The computer Screen will not Turn On?
    a. Begin Your reservation in NUcore to initiate access to the instrument
1. There is an error with my reservation?
    a. If you have already started your reservation using NUCore, please logoff by selecting the error reporting option and a brief description about the issue.
    a. If you have not started your reservation using NUCore, please report problems with the instrument at http://imserc.northwestern.edu/contact-issue.html add place the 'Stop' sign near the instrument computer. 'Stop' signs are located at XXXX and online at the link above.
    b. Email or talk to a staff member.

INSTRUMENT

1. The autosampler shows an error?
    a. This is most likely due to the wrong vial placed and the instrument detected no vial.
        i. Right click the autosampler on the acquisition page and select "Reset communications"
        ii. Close the software completely
        iii. Click on the Compass software and allow time to reload
        iv. Open your sample table make necessary change to vial position, save, and submit your sample

APPENDICES

APPENDIX A: MOBILE PHASE PREPARATION

0.1% methanol in water: In a 1 L volumetric flask add 10 mL methanol; Bring to 1 L mark with water; Mix.

0.1% acetonitrile in water: In a 1 L volumetric flask add 10 mL acetonitrile. Bring to 1 L mark with water: Mix.

0.1% Formic Acid with 2.0% acetonitrile and 12.5 mM ammonium acetate: In a 1 L volumetric flask add 20 mL acetonitrile; Add 0.963 g ammonium acetate; Add 0.72 mL of glacial acetic acid; Bring to the mark with water; Mix. The pH should be approximately 5.

0.1% methanol and 0.2% ammonium hydroxide: In a 1 L volumetric flask add 10 mL methanol; Add 0.200 mL of ammonium hydroxide; Bring to the mark with water; Mix.

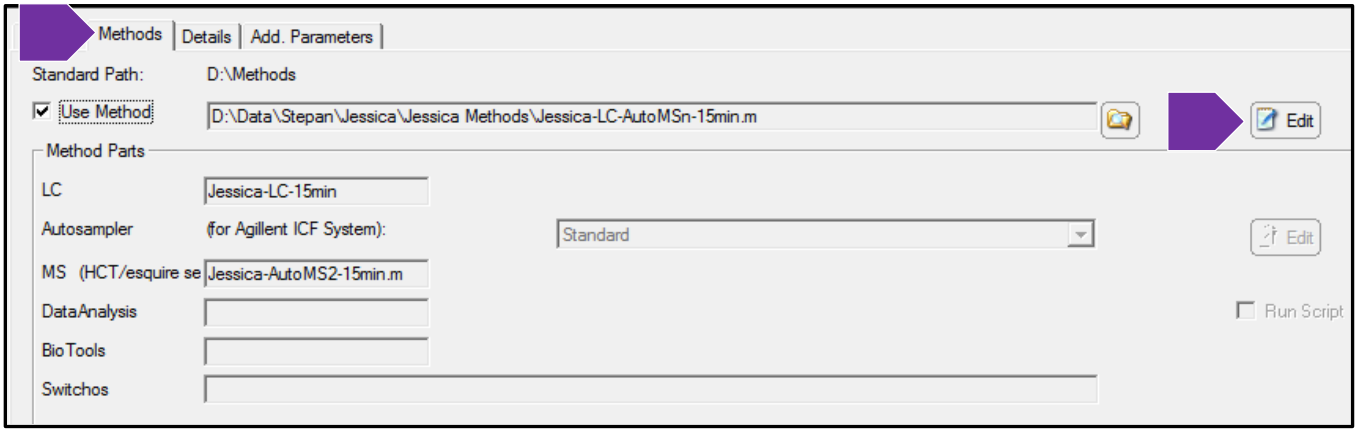
0.1% acetonitrile and 0.1$ formic acid in water: In a 1 L volumetric flask add 10 mL acetonitrile; Add 2 mL of formic acid; Bring to the mark with water; Mix.

Triethylamine / hexafluoroisopropanol in water: Add 16.8 g (or 10.4 mL) of hexafluoroisopropanol (Sigma-Aldrich 105228-25g) to a 500 mL volumetric flask. Add 390 mL of HPLC water. Mix thoroughly; Add 0.6 mL of triethylamine (Sigma 90335 – 100 mL;) Mix again; Bring to the mark with water; Check the pH with pH paper or a meter. The pH should be approximately 8.

APPENDIX B: EDITING A LC METHOD

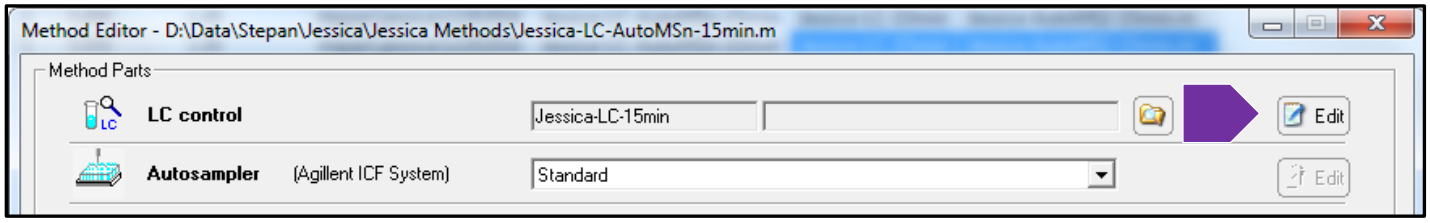
1. Once your sample table is open, after clicking on the 'Methods' tab, click 

.



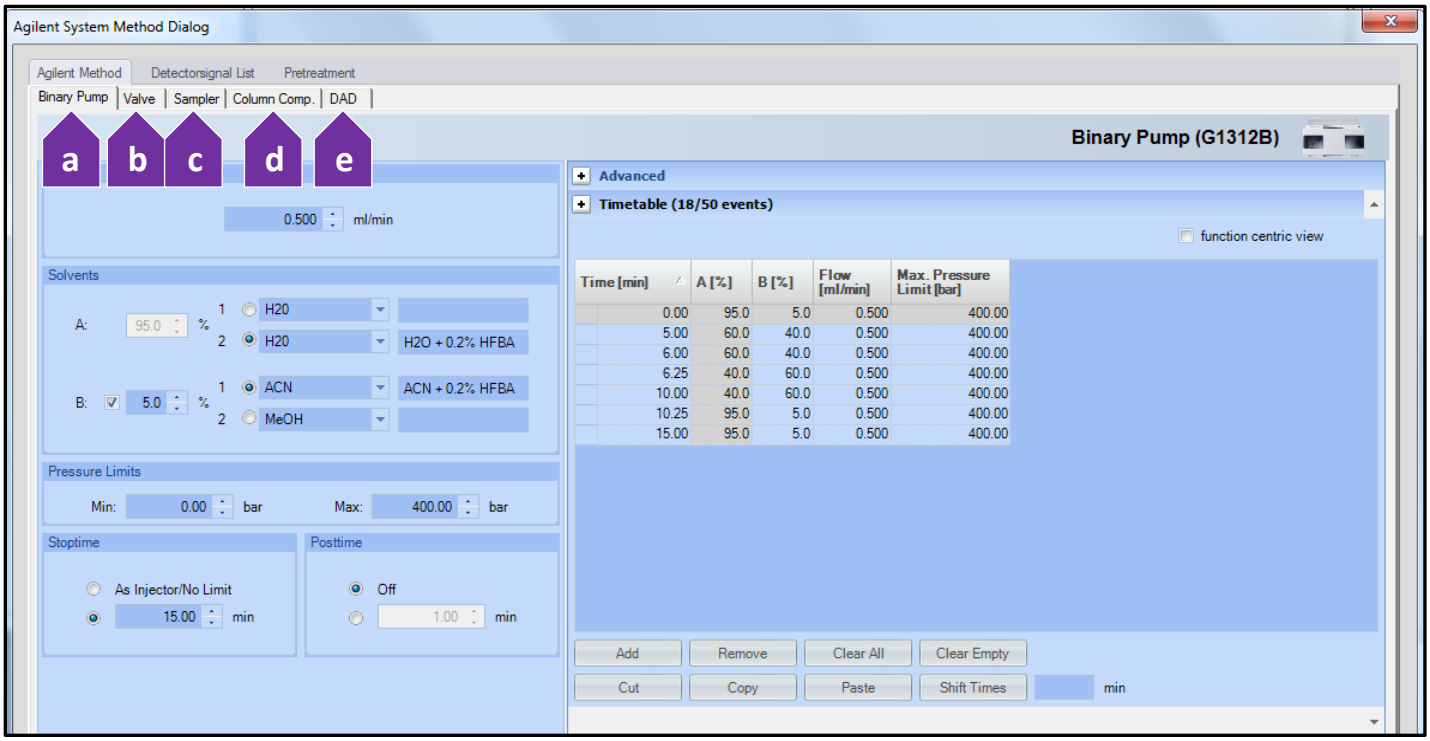
2. When the new window appears, click 'Save As,' and assign a new name to the new LC method you are about to create.
    a. The new method should include follow the format of Last name-LC method length.
        i. Example: *Curie-LC-10min*
    b. Performing this step first, prevents making unintended changes to other methods.
3. Click on the 

button on the top line of 'LC Control.'

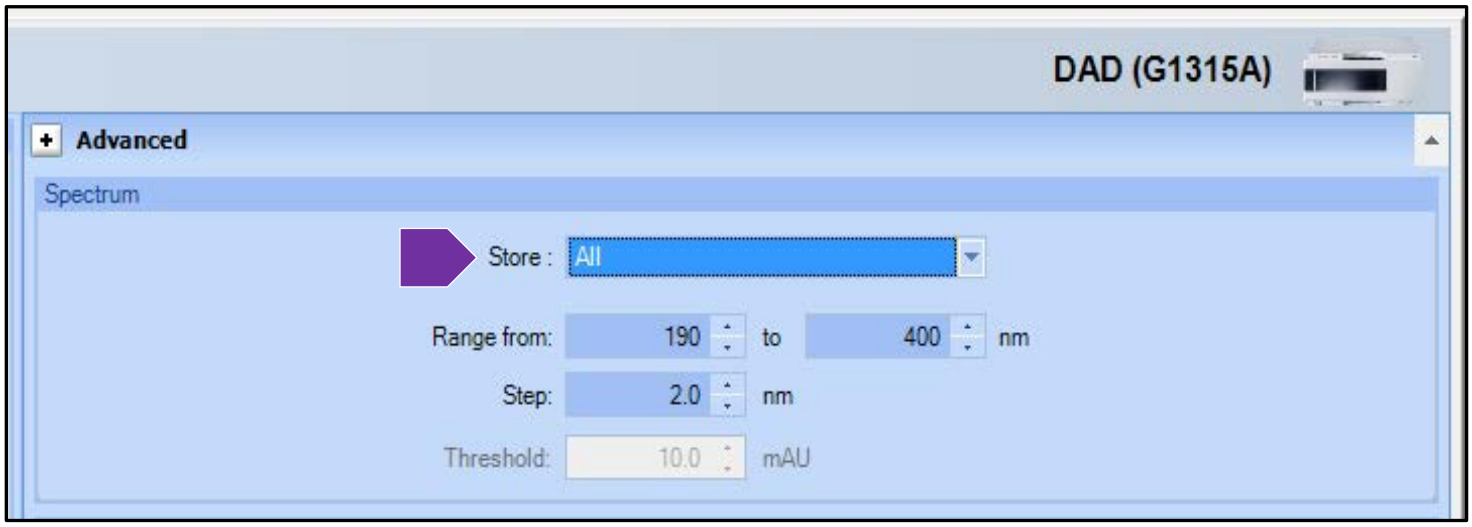


4. Click on the 'Parameters' button to display method specifics.

5. Go through the five tab and set up the parameters for your method.
    a. Binary pump
        i. Define your solutions, flow and gradient.
    b. Valve
    c. Sampler
    d. Column Comp
    e. DAD



6. On the 'DAD' tab, set the 'Store' to 'All.' You cannot enter the wavelength selection on this tab.
    a. You can do it in Hystar by right clicking on DAD on Agilent Status view window.



7. Import the MS or MSn method that you already have in Trap Control. Notice you can only click on the folder icon to brows for a method. You cannot modify MS methods from here.
8. At this point the LCMS or LCMSn method is complete.
9. Click 'Save As' to ensure that all the changes are saved.
10. When you close the editor window, HyStar will ask you if you want to use the new method (in place of what was entered on the line you used to modify the method. Confirm that you would like to use the newly edited method.
    a. If you chose the new method, now any copy and paste of this new line will use the method.
11. Test the method with some blanks and clean standard, before queing up a full run.
12. A time will need to be chosen to "divert to waste." This is found and set up in the Trap Control Method.
    a. This option is used to reduce the amount of uncessary solvent/sample to be introduced to the instrument, isolating the peak of interest.

REVISIONS

V1.0 2020/08/12	• Release of original version of USER MANUAL for Amazon X V1.0