

IMSERC User Manual for Amazon SL

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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 the course of 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.

The Amazon SL 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 run samples free from buffer and salts in order to obtain a quick analysis of sample composition.

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

1. Be free from salts and buffers
 - a. Salts and buffers may precipitate and dirty instrument.
 - b. Salts and buffers may inhibit your signal.
2. **NOT** exceed a concentration of $\leq 100 \mu\text{g/mL}$
 - a. Concentrations $\geq 100 \mu\text{g/mL}$ have the potential to contaminate the instrument.
 - b. Concentrations $\geq 100 \mu\text{g/mL}$ 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 3×10^6 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 $10 \mu\text{L}$ 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. 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.

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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	BG75 – Amazon SL	Eye Protection, Gloves
Methanol, Acetonitrile, Dichloromethane, Formic Acid	BG75 - 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.

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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 are able to 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 backup 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 SL 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.

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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.

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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, Dichloromethane	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
Concentration / Purity	$\leq 100 \mu\text{g}/\text{ML}$	Sample(s) may be diluted with modifiers to increase signal/ionization efficiency such as 0.1% Formic or Acetic acid, $\leq 10 \text{ mM}$ ammonium formate or acetate Sample(s) must be free from salts, buffers and strong acids. If sample cleanup is needed it is recommended to seek training on the Amazon X
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

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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. Allow 3-5 minutes for method solvent change
7. Check solvent lines
 - a. Allow time to pass in order to remove bubbles from the solvent lines
8. Check signal. Is background $\leq 3 \times 10^6$
 - 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
9. Click Start
10. Select run preference
11. Monitor background signal, does background return to $\leq 3 \times 10^6$
 - a. Yes > proceed to step 12
 - b. No > run a wash vial
 - i. After several washes and no change Fill out a Bug Report
12. Instrument will default to standby mode after the last sample run
13. Analyze data using sample analysis software, Compass
14. Save processed data and close file
15. End Reservation in NUCore

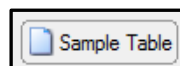
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DATA ACQUISITION

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



2. Open Hystar. You can select the Hystar icon located on the top right screen or on the lower tool bar.

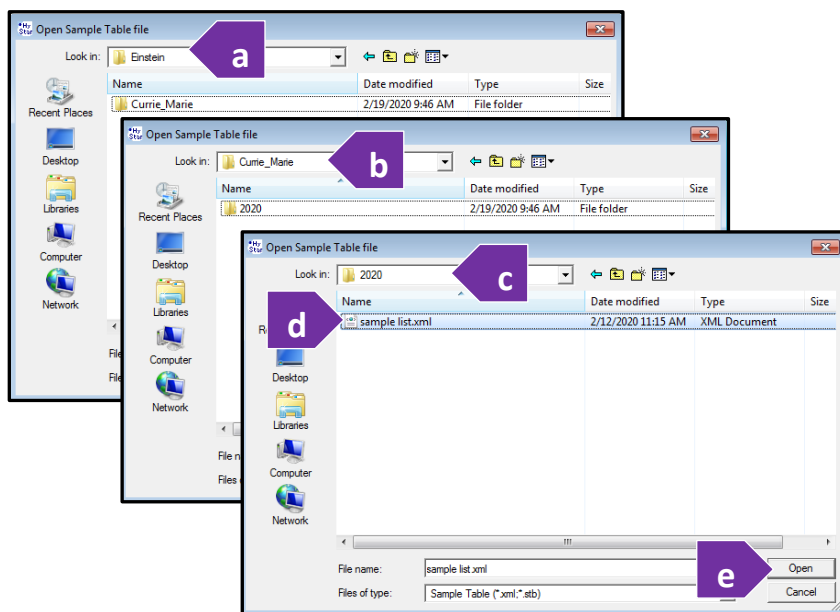


3. To locate your sample table, click the sample table button near the top left of the window. Then click file open.

4. 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.

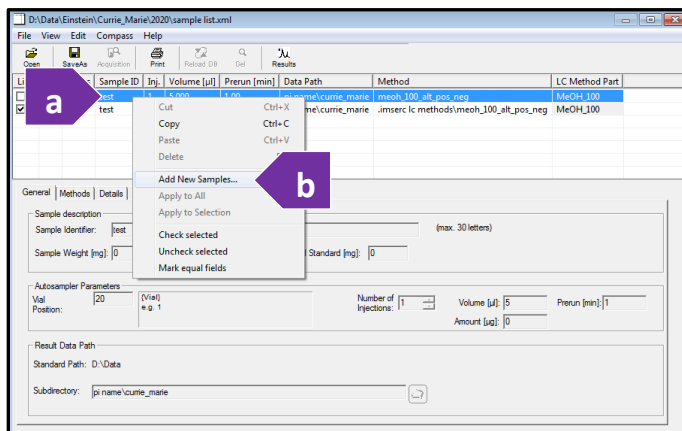
a. In the example below, the PI's name is (a) Einstein, the user's name is (b) Marie Currie and the user is collecting data in the year (c) 2020.

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."

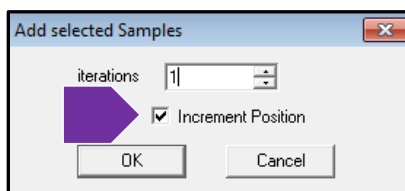


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- To add new samples to your sample list, (a) right click the line of a previous sample which you would like to duplicate and/or amend, then click (b) “Add new samples.”



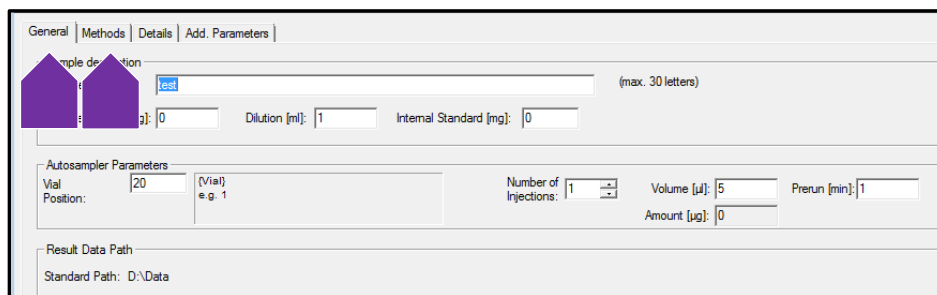
- Enter the number of samples you would like to add. You can check the “Increment Position” box if you would like the program to place the samples in sequential vial positions.



- 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.

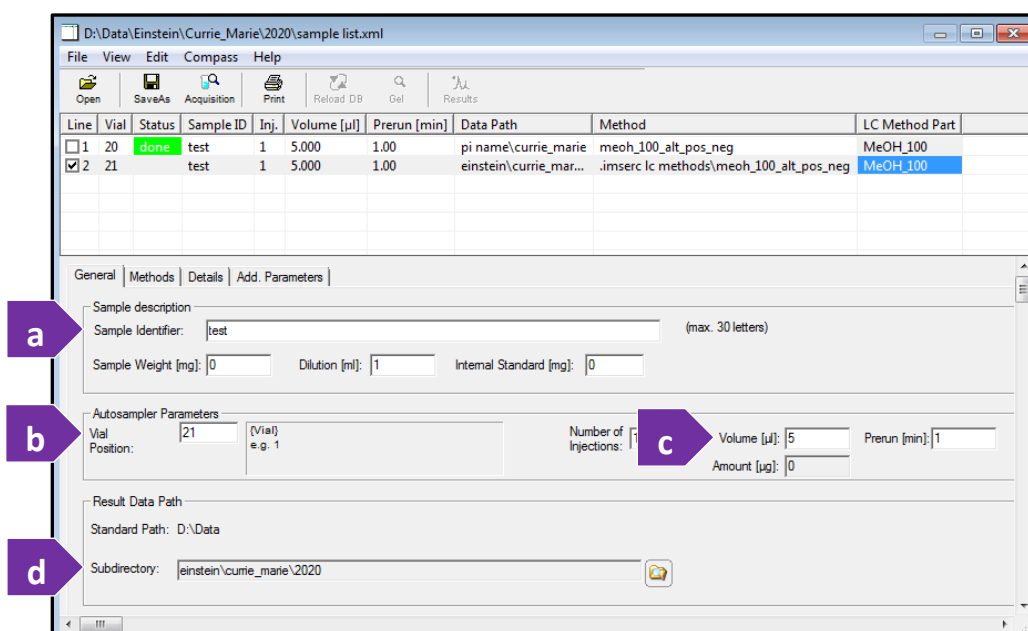
Line	Vial	Status	Sample ID	Inj.	Volume [µl]	Prerun [min]	Data Path	Method	LC Method Part
1	20	test	test	1	5.000	1.00	pi name\currie_marie	.imserc lc methods\meoh_100_alt_pos_neg	MeOH_100
2	21	test	test	1	5.000	1.00	pi name\currie_marie	.imserc lc methods\meoh_100_alt_pos_neg	MeOH_100

- 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.




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5. 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 ‘Subdirectory’ 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.



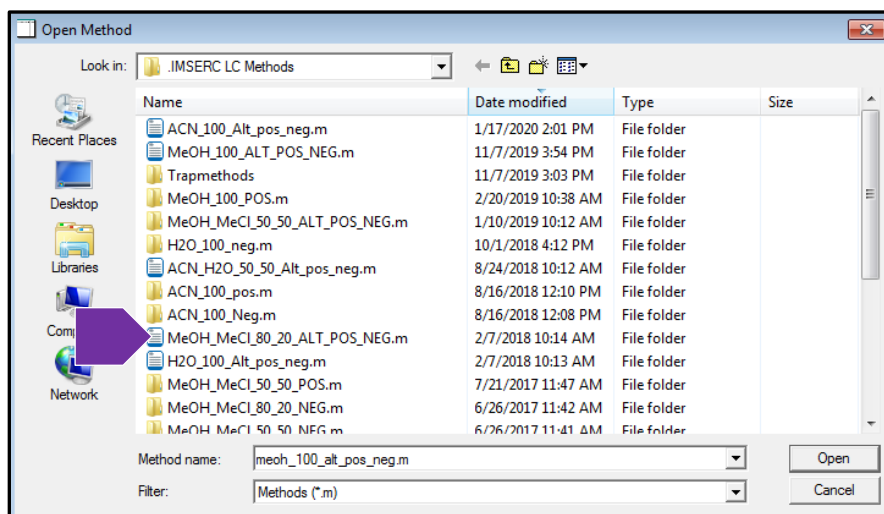
6. Click the “Methods” tab. Here you will select the appropriate mobile phase and mode of detection. Click

on the folder icon . The folder appears listing all method options. Find the best fit method compatible with your injection solvent.

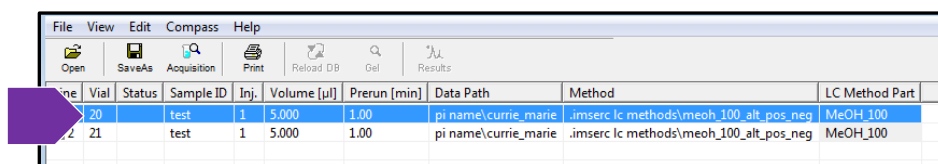
- a. Note: Methods shall not be changed, nor new methods added. If you have an issue with compatibility, please see IMSERC staff for assistance. They may redirect you to another instrument to suit your needs.


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- 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.
 - 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 **POSitive** and **NEGative** spectra.



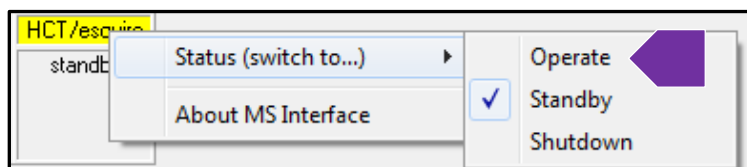
- Once you are ready to submit your sample. Make sure you **highlight** the sample you would like start the run. This tells the instrument to start data acquisition from this sample and move down the sample list.



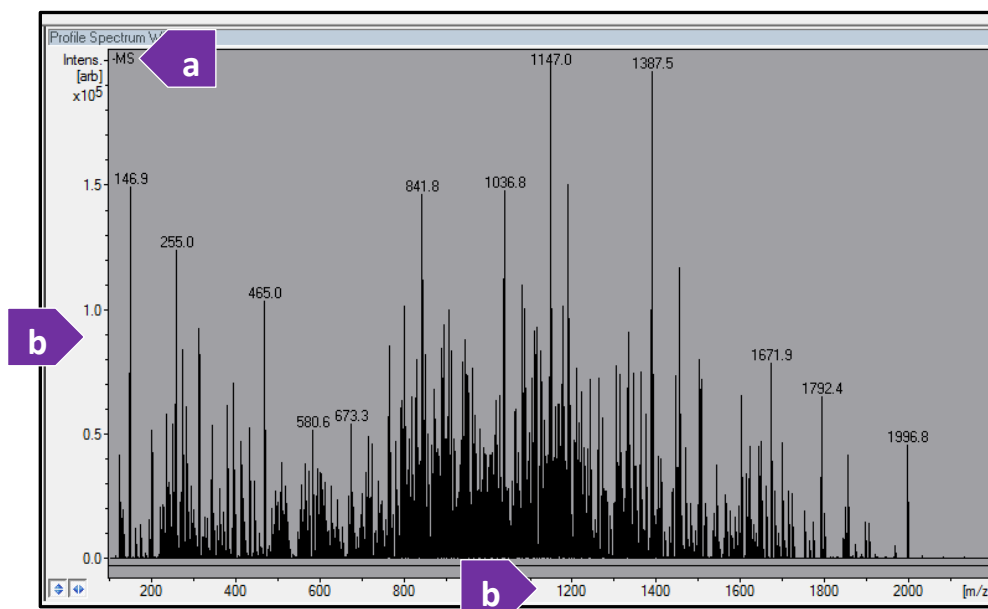
- Click the "Acquisition" button  located near the top left and click yes when the box prompts you to save your changes.

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- 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 **HCT/esquire** and hover over to “Status (switch to...)” and click on “Operate”.




- Note that if the pump ready light stays yellow, you may also have to right click and turn the pump on as well.
- Allow 3-5 minutes for the HPLC to change the mobile phase in the lines from the previous user. During this time you will monitor that background signal is acceptable before officially starting your sample run.
- 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.
 - 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.
 - 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.

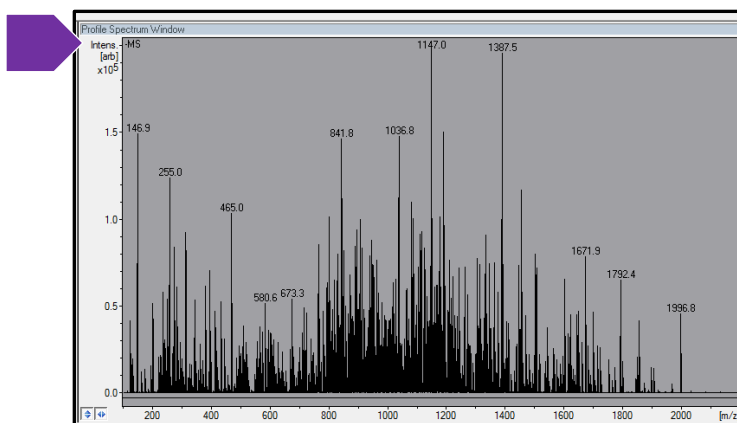


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14. Check that the background signal intensity is $\leq 3 \times 10^6$.

- 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.

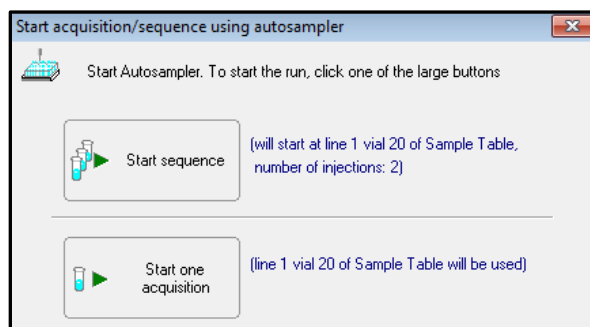


15. Once the instrument is ready and all status lights in the upper right screen are green, the start button will

turn light blue in color. Located under the sample table button is the "Start" button. Click  to start your sample list.

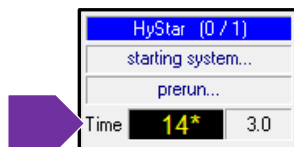
16. Another box will appear asking how you would like to run your sample table.

- 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."



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17. 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.



18. Note that you can open your sample table and add additional samples to update your list in realtime. 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.
19. Each method is no longer than approximately 3-5 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 $\leq 3 \times 10^6$.
20. 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 methanol wash vial is refreshed daily. The wash vial is located in position 91 in the autosampler, and should be injected at a 10 μ L volume.
 - 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.
21. After your sample run is complete, the instrument will automatically go into standby mode. The status lights will turn to yellow.
22. 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.
23. 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.

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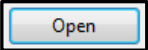
DATA ANALYSIS

1. To analyze your data, click on the “Data Analysis” icon .

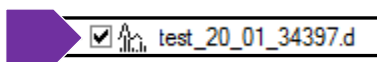
2. Click the open folder button .

3. Navigate to your folder then select your annual data folder. It should be located under

a. Data > PI Name > Lastname_Firstname > YYYY

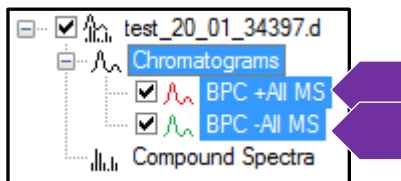
4. Select the sample file you would like to view and click  on the lower left corner.

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



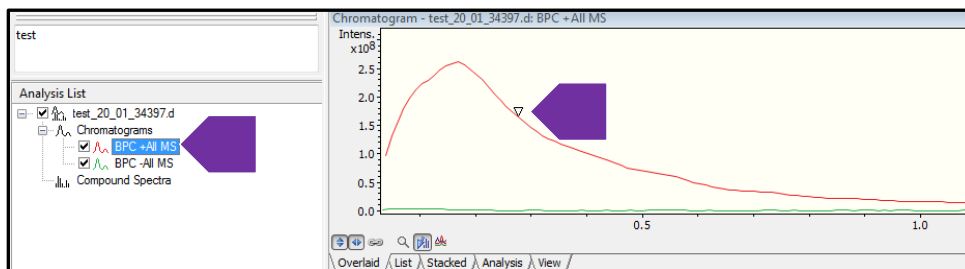
a. Ensure other users' files are unchecked. This will remove their data from the chromatogram and prevent confusion between data files. You will only need to uncheck the far left box which tells the software to deactivate the whole file.

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.

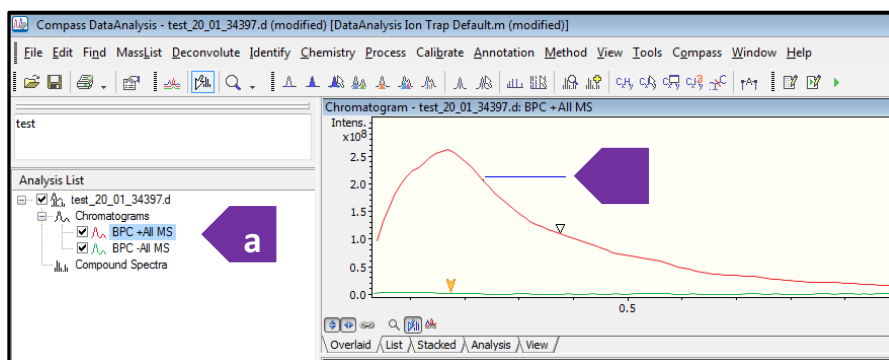


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7. Highlighting the trace in the analysis list will active that specific trace. The software will also color match the trace.

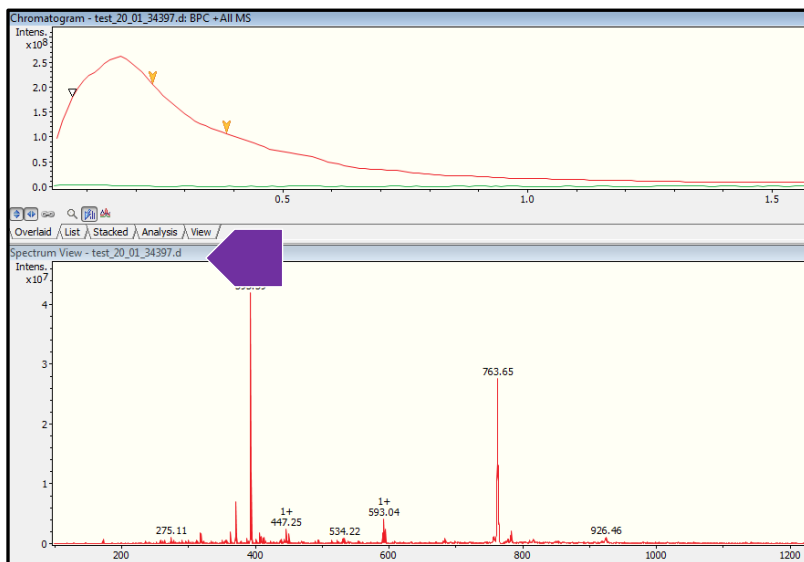


8. Use the left mouse button to view your data file.
 - a. To zoom in, you can choose between several options.
 - i. Hold down the shift key. This creates a zooming tool with the mouse where you can repeatedly select where you would like to zoom in the spectra.
 - ii. Right click, select "zoom." This 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.

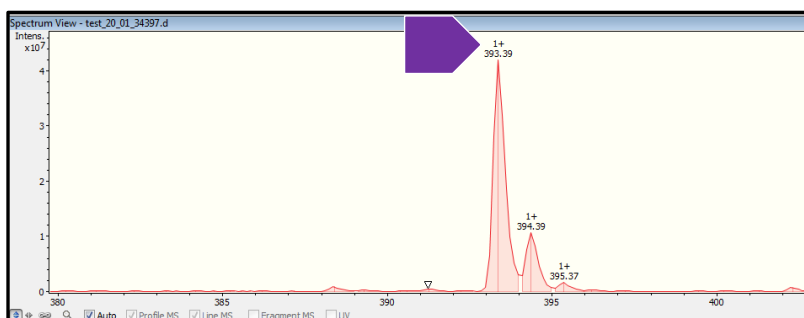


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10. By clicking and dragging over the chromatogram you can view the averaged spectra for that range. And it will appear in the "Spectrum View" window below the chromatogram trace.

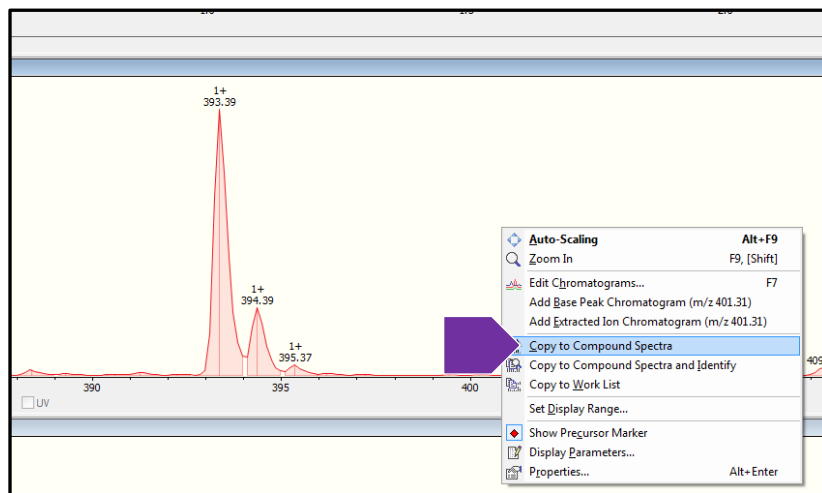


11. You can use the same zoom instructions to look closer at your acquired spectra. In some instances you will see the software assisting you with the charge state by calculating the spacing between isotopes and place the calculated values above the peak.

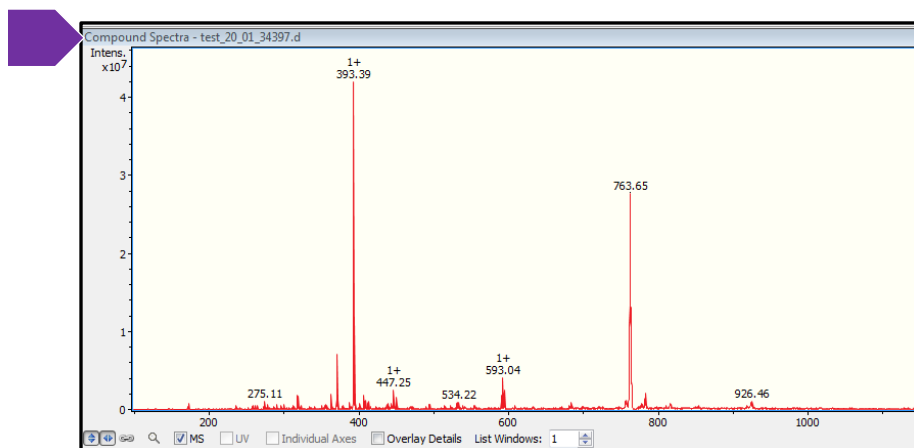


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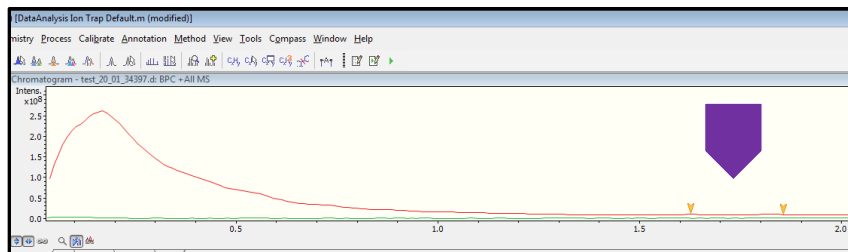
12. To collect your extracted spectra, right click the “Spectrum View” window, and select “Copy to Compound Spectra”



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

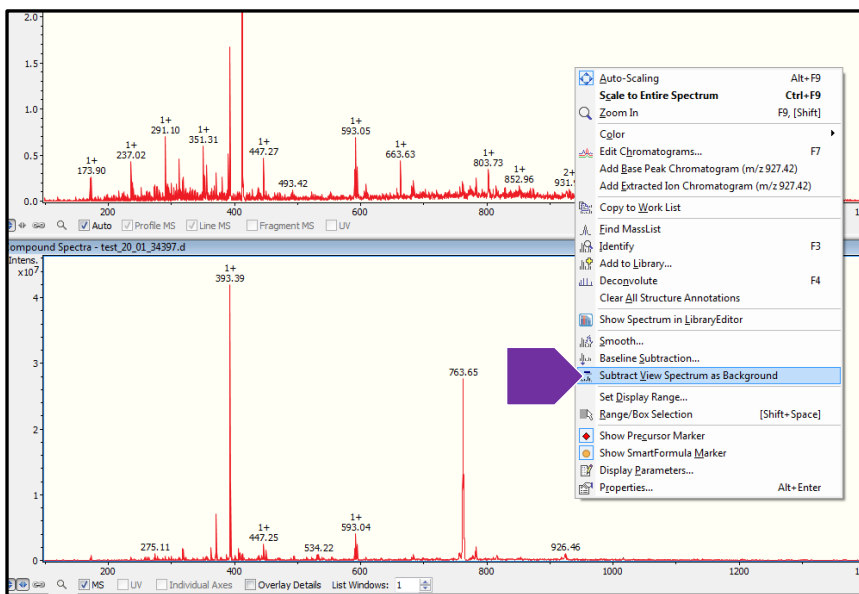


14. To extract background. Select an area of baseline following your sample.

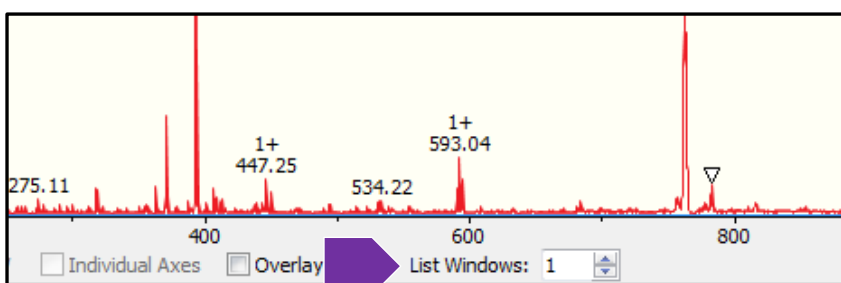


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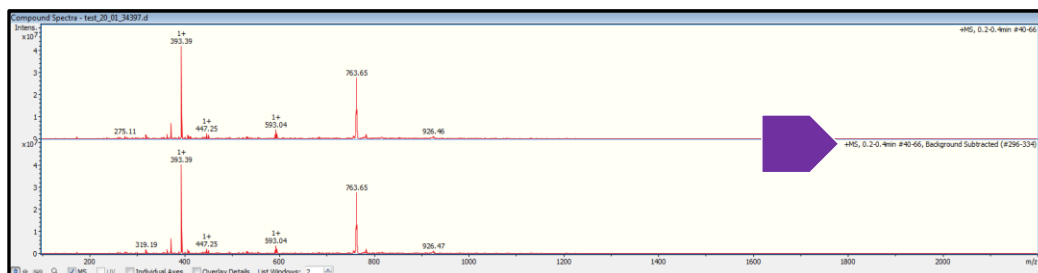
15. Right click the “Compound Spectra” (the spectra that you have just collected) and then select “Subtract View Spectrum as Background.” This will subtract the baseline spectra visible in the “Spectrum View” from your spectra in the “Compound Spectra.”



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.

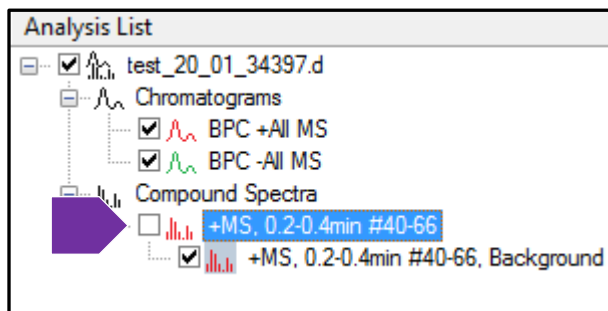


17. You should now be able to see the new spectra with the label of each on the upper right hand corner.



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18. Remember you are able to 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.



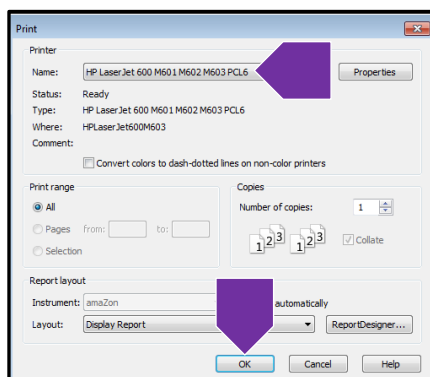
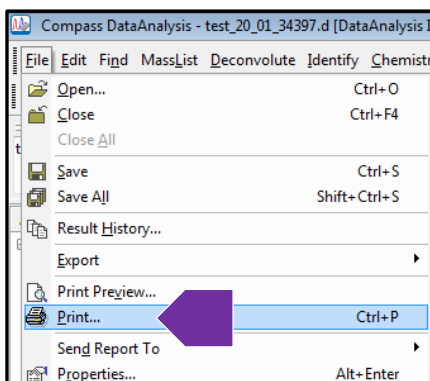
19. You can repeat the procedures with a second chromatogram in the data file if you ran an Alt Pos/Neg method.

20. Save your file by clicking the save icon  .

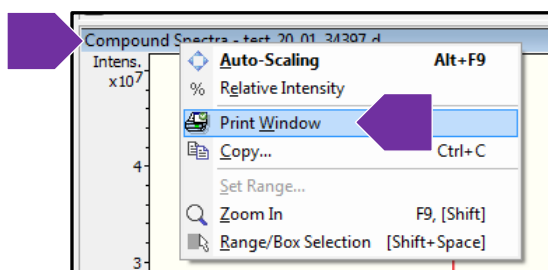
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21. You have two options to print.

- 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.

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PUBLICATION

EXPERIMENTAL SECTION

Mass Spectrum data was collected on the Bruker Amazon SL 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 1100 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:

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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.
 - b. 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.
 - c. 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

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APPENDICES

APPENDIX A: ADVANCED OPERATIONS 1

REVISIONS

V1.0 2020/01/30	<ul style="list-style-type: none">• Release of original version of USER MANUAL for Amazon SL V1.0
V1.1 2020/02/18	<ul style="list-style-type: none">• Release of original version of USER MANUAL for Amazon SL V1.2• Fixed spelling errors. Adjusted image sizes.• Formatted tables and arrows.• Changed wording in publication section.• Updated how data is to be saved on the instrument.