LTQ Ion trap and Waters Acquity LC Standard Operating Procedure Guide

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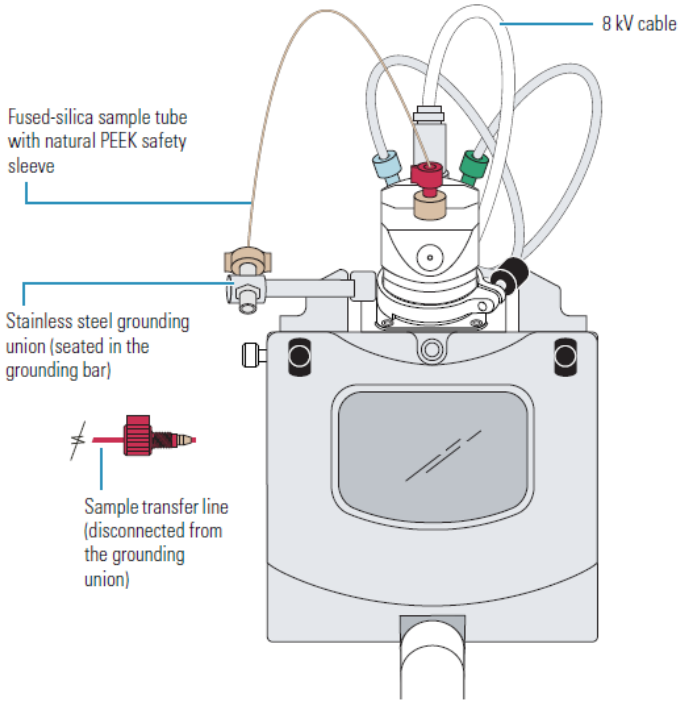
Helpful links:

Hardware manual: https://assets.thermofisher.com/TFS-Assets/CMD/manuals/Man-97055-97072-LTQ-Series-Hardware-Man9705597072-EN.pdf

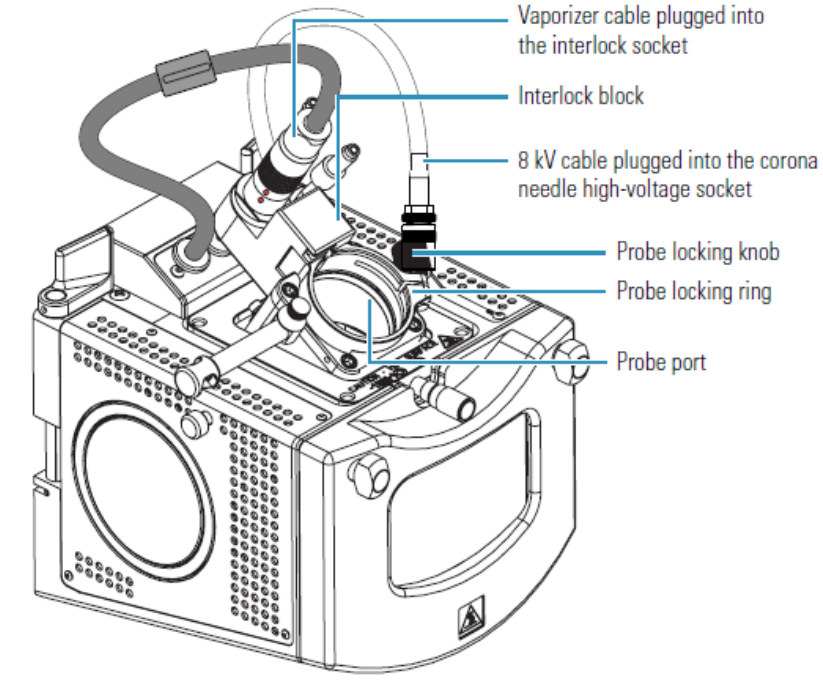
Getting started Guide: https://assets.thermofisher.com/TFS-Assets/CMD/manuals/Man-97055-97073-LTQ-Series-Start-Man9705597073-EN.pdf

LC System Guide: https://www.waters.com/webassets/cms/support/docs/acquity_uplc_h-class_system_guide.pdf

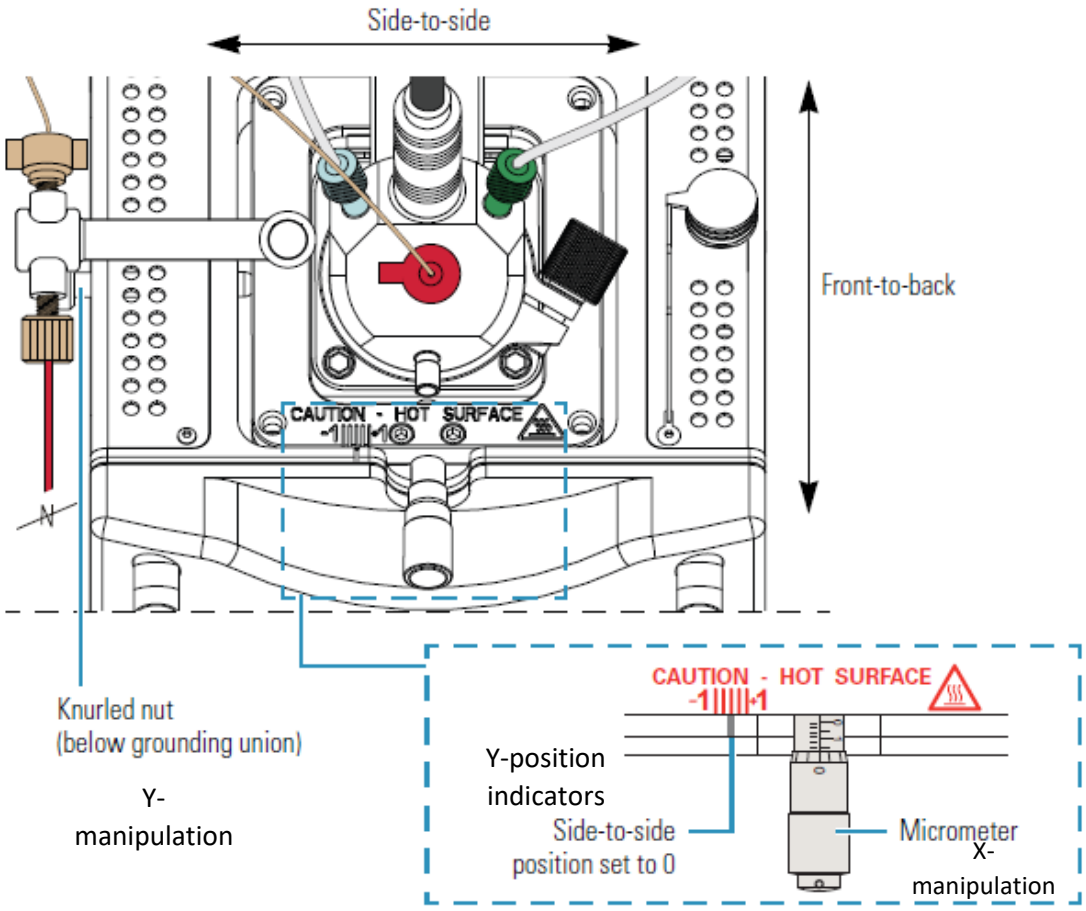
Source Overview:



Front on view of the Ion Source



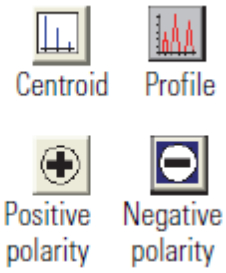
Side view of Ion Source



X & Y manipulation of the ESI probe. Z-axis can be manipulated by adjusting the A-D indicators on the probe.

Other icons of note:

Click on each sign to toggle between its counter-part



Getting Started:

This is a brief introduction about the instrument and some proper practices when using a mass spectrometer.

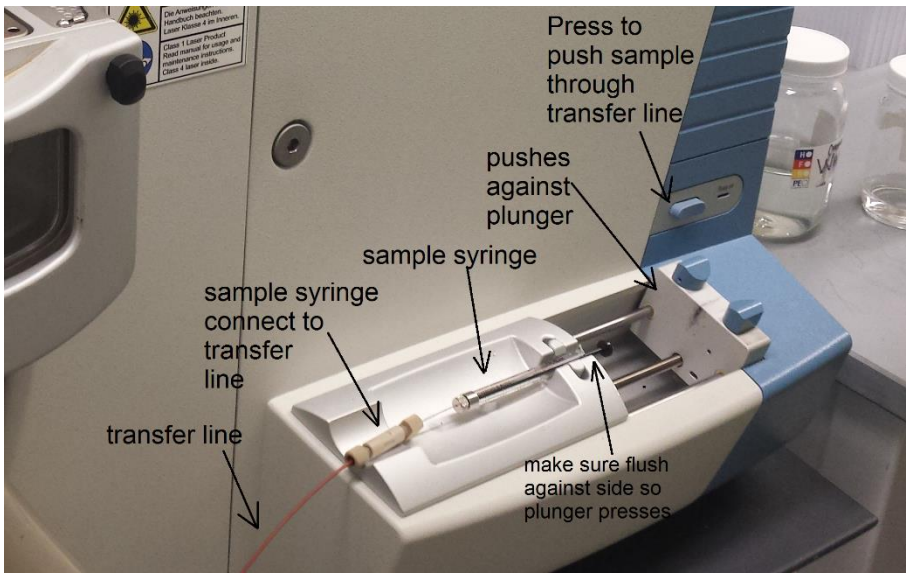
Proper Practices:

1. Please never analyze samples with compositions of salt, buffer, and DMSO/THF/DMF. Even if the concentration is minute it will cause ion suppression (making it hard for the compounds in your sample to ionize) and stick around in the instrument. If you cannot filter your samples please talk to the Core staff for other options.
2. Always dilute your samples with mass spectrometry safe solvents: acetonitrile, water, methanol, dichloromethane, toluene, and acetone are the most common. Please use HPLC or Optima grade. We have solvents available in both Caudill 040 and 009 that can be used.
3. If you are incorporating LC, please use LC and MS safe solvents. We try to avoid super harsh organic solvents when using LC because it will strip the lining of the tubing. We primarily use acetonitrile, methanol, or water. If your sample is in a specific solvent we may be able to work around it by diluting it with a compatible solvent or injecting very low amounts of the sample.
4. When performing infusions please make sure you are **rinsing the line/source** with the **same solvent your sample is comprised of.** You want to make sure your sample is de-solvated properly from the system. Using a different solvent will not be as efficient and can lead to clogging of the source.
5. Make sure to write in the logbook. When doing infusions write how many samples you infused, what solvent you rinsed with, and what probe you were using (ESI, APPI, or APCI). For LCMS make sure to log which column you are using, the pressure of the column at 0.3 mL/min 100% B1, the pressure of the column at your starting conditions, if you filled the solvent reservoirs/how much solvent is in each, and how many samples you are analyzing.

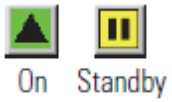
Direct Infusion Instrument Setup

Instrument Set Up:

1. Look in the lab notebook and **see what solvent the last person used**. Put ~500 µL of that solvent in the syringe. Follow the steps below then wash the system a **second** time with the **same solvent your samples is dissolved in**. This will help eliminate clogging up the system. There are solvents in the chemical cabinets and containers in the lab if needed.
2. Connect syringe to red transfer line tubing and place in syringe pump
    a. Be sure syringed pressed against base so depresses plunger properly (note in picture below). To move the plunger squeeze the two blue knobs.



3. In LTQ Tune, press yellow pause button in top right corner of main window so that it changes to green triangle play button to turn on detector



4. Press and hold “Pump On” button just above syringe resting location until blinks. This primes the solvent through.
    a. Priming can only be done if pump is off first
5. In order to check the background on the instrument:
    a. Select your desired Tune File and check signal-to-noise under those settings:
    b. Introduce solvent/blank manually or *via* syringe pump.
    c. Click on “*Bubbles Button*” and be sure analyzer set to “Ion Trap”. Close.

Make sure settings are as follows:

1. *Mass Range:* Normal
2. *Scan Type:* Full
3. *Microscans:* 3
4. *Max. Inject Time:* 10 ms

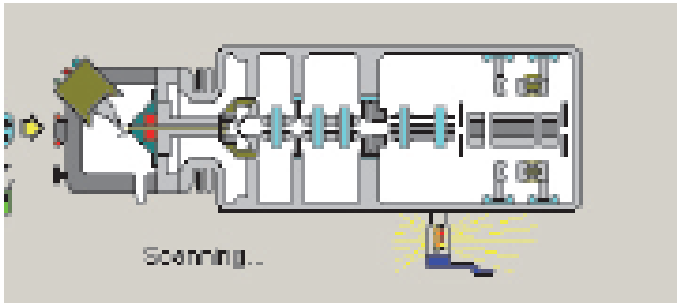
ii. Signal-to-noise check: NL value should be E3 to E4

iii. Note any residual ions from prior runs which are easily observed (in case sample has low signal-to-noise)

Prepare samples:

1. Dissolve and dilute samples so that the concentration is 50 µg/mL for **pure samples** (please remove any salts, buffers, or reagents before analysis)
    a. Samples with **mixtures of products** can be more concentrated, (500 µg/mL)
2. Aim to prepare at least 500 µL of solutionPull up diluted sample solution into syringe (need at least 200 µL for sample analysis time)
3. Connect syringe to transfer line tubing and place in syringe pump holder location properly
    a. Be sure that base of glass syringe barrel is flush with sides of holder
4. Press and hold "Pump On" button to prime sample through transfer line. Be sure green light remains lit indicating pump continuing to push sample through (for approx. 5-10 sec).
5. If not already on, turn on ion trap
    a. In program press *"Bubbles Button"* in top right corner, if command window not already open

6. Note signal (NL value) should be E5 or E6 range and IT value should be stable (not bouncing up and down)
7.
    a. If not, click on Source icon



i. Try adjusting Sheath Gas (up and down 1 unit at a time)

ii. Then try adjusting Spray voltage (up and down 0.1 unit at a time)

iii. Then attempt adjusting Sheath Gas again

b. If these don't improve stability consider changing solvent conditions

Acquiring Data:

1. Press Camera Button to begin collecting data

   a. Select the folder you wish to save your data in
   b. Name your file with your sample's identification in the "File Name" field
   c. Define number of scans to collect
      i. Select "Scans" and collect 100-250 scans, depending on signal…good stable signal of E5-E6 need only 100 or so scans, weaker signal/less stable signal needs more.
   d. Press **START** to begin data collection
2. Finished when number on spectrum screen in top left (before NL number) reaches the number of scans you've set
3. Disconnect syringe of sample
4. Clean out syringe with wash MeOH or whatever solvent your sample is dissolved in
5. Flush line manually with wash solvent until your sample peaks are gone and background baseline is restored (NL ≤ E4)
   a. If running another sample in different solvent can flush with new solvent too

Analyze data:

1. To view collected data on desktop or from start menu select program "Xcalibur"
2. Next select "Qual Browser"
3. Open desired data set from C drive in your folder
4. Right click and drag across the top pane or Total Ion Chromatogram (TIC), this will average the scans giving you the most representative mass spectrum

Removing Data from PC:

1. Most only need to report number, can jot down on scrap paper or in lab notebook.
2. You can also select a pane in Xcalibur (make sure the pin is highlighted green) and ctr + C to copy the pane. You can paste this into Wordpad and email the spectra to yourself
3. Will need flash drive to save data files. Files are saved as .RAW files and are only readable by the Xcalibur program. MzMine is an open source software that is able to view .RAW files. Check out our SOP for using MzMine for data visualization.

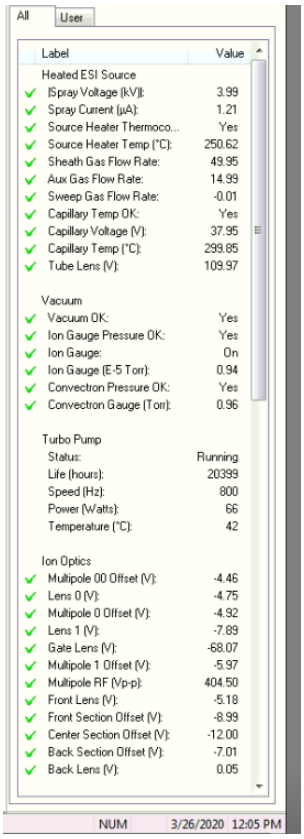
LC-MS Instrument Setup

Waters Acquity UPLC System

1. Open the appropriate tune file in the LTQ tune software

a. The tune file we recommend using is “General-03mL-Flow-LC-Pos” or “General-03mL LC-Neg” based on the polarity you decide to use.

b. Hit the yellow pause button in tune. It should turn into the green play button and the instrument should begin scanning. Make sure all of the instrument parameters have green checks. Below is an example of the parameters for the 03mL-Pos tune file.



2. Check the LC System

a. Check the solvent reservoirs on top of the LC stack. *This is probably one of the most important steps in using the LC system.* The solvents should at least be 1.5 times as full as the solvent you will use in the course of your run. To calculate the amount of solvent you need for your analysis, use the following equation:

 # samples × length of run in minutes × flow rate used = amount of solvent used in mL

Example: 25 samples × 9 minutes × 0.3 mL/min = 67.5 mL of solvent used

The solvent reservoirs contain the following:

A1: H2O optima with 0.1% formic acid

B1: ACN optima with 0.1% formic acid

Seal Wash (SW): 90:10 H2O:ACN optima

Weak Wash (WW): 90:10 H2O:ACN optima (same reservoir as SW)

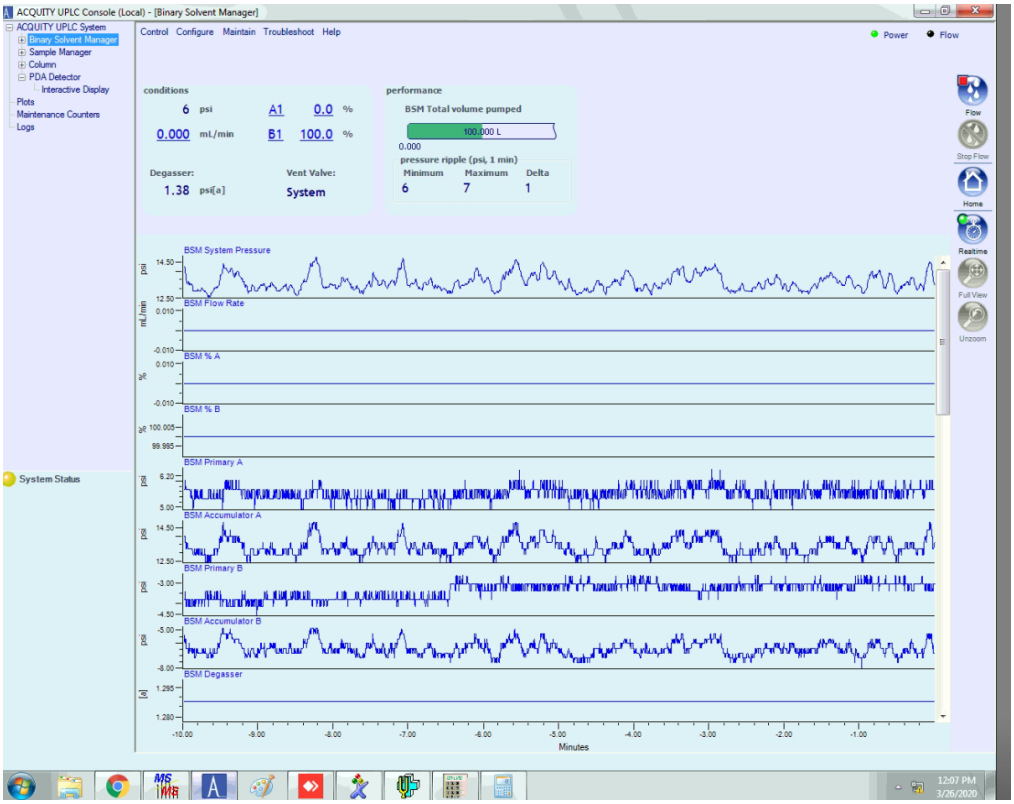
Strong Needle Wash (SNW): 100% CAN or MeOH.

b. Check the column tag to see what column is currently in the system. Our default column is a Waters BEH C18 50 mm x 2.1 mm, 1.7 µm. If you need a different column and we have instructed you on how to do this, change the column now. If you have **not** been instructed on how to do this please go get the Core staff for assistance.

c. Make sure the solvent line from the PDA is going into the source. If not you will not see any signal.

3. Start the LC System

a. Open the Acquity software (denoted by a blue A). Below is an example of what the software looks like.



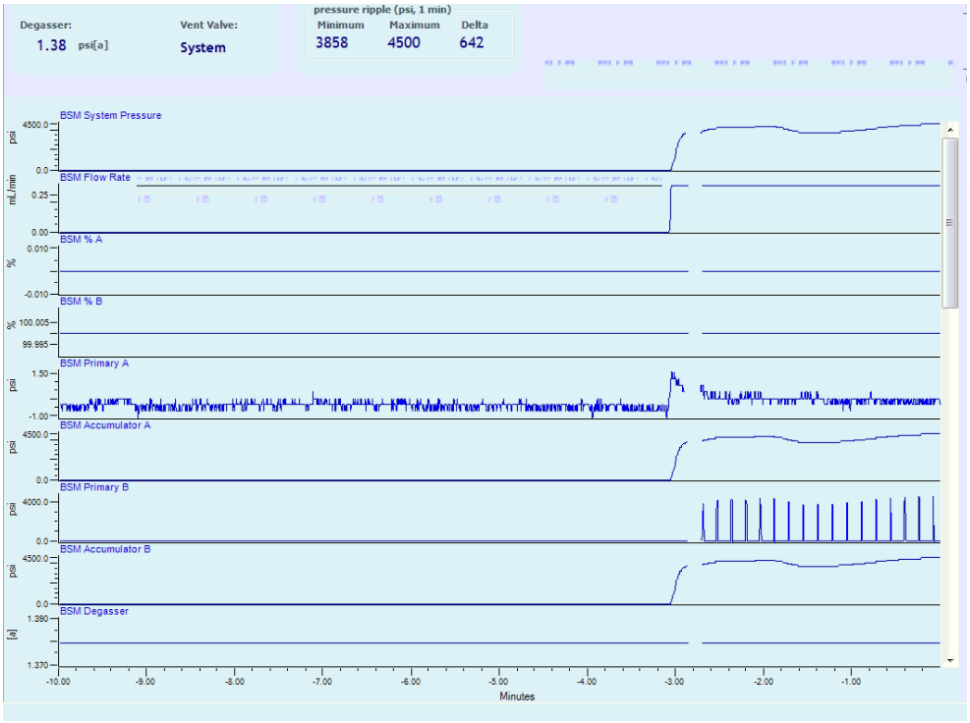
b. Go to the "Binary Solvent Manager" tab. There will be a portion of the screen that says "conditions". This is where you will start/stop the flow and/or change it.

c. Go to the "Sample Manager" tab and set the column temperature at 40 °C (do NOT set the column to temperatures higher than 50 °C). We recommend you set the sample temperature at 10 °C-this cools the autosampler chamber and will prevent evaporation after your samples have been injected.

d. Once the column temperature has reached 40 °C go back to the Binary Solvent Manager tab. Now turn on the flow rate (0.3 mL/min for a standard method) and make sure it is set to 100% B1. This will help wash sticky compounds from the run before off (if any). Let this go for ~7-10 minutes.

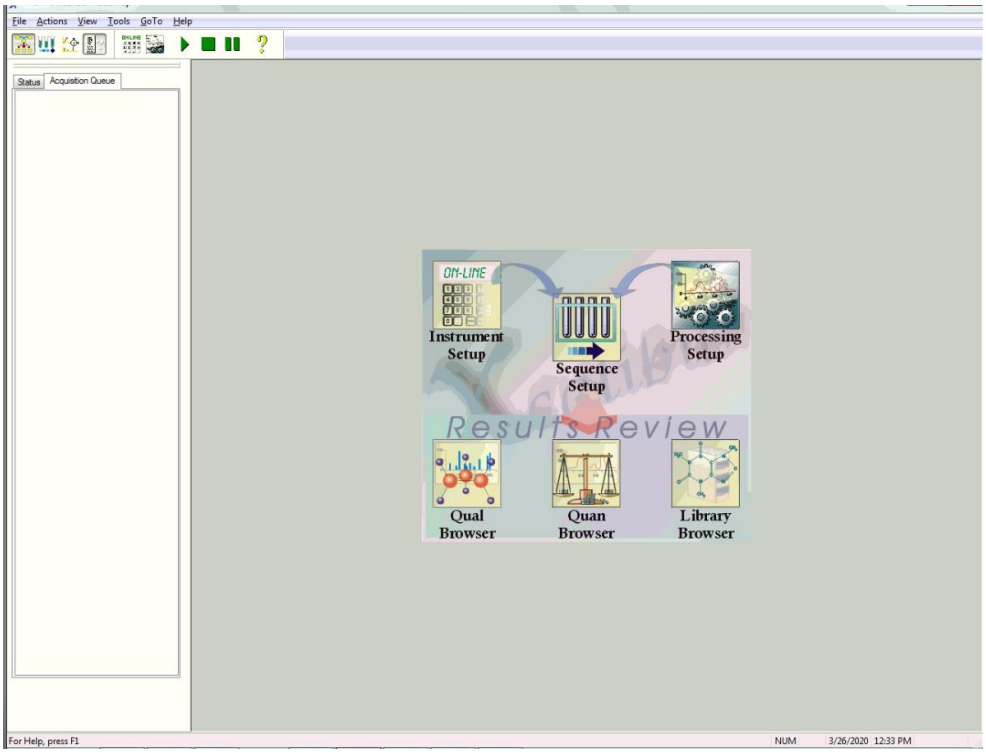
e. While the column is washing you can load your samples in the autosampler chamber. We typically use 48 vial x 2mL holders which are a light blue color. Make sure to note whether you placed the tray in position 1 or 2.

f. Once the column has finished washing you can change the flow composition to your starting conditions. The general method starts at 95% A 5% B with a flow rate of 0.3 mL/min. It usually takes about 20-30 minutes for the column to fully equilibrate. The delta or change in pressure should be 10 psi or lower. There are a bunch of charts that track the pressure. The main one to look at is the top one-BSM System Pressure. This is the pressure measured on the column and it should eventually level out and be very consistent.



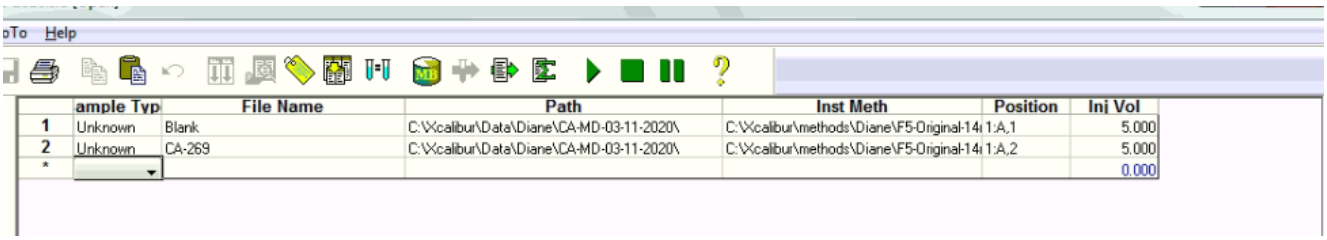
4. Writing a Sequence and Starting a Queue

a. Now that the mass spectrometer is on and scanning and the LC is ready we can write a sequence and then start the queue. To write a sequence go to Xcalibur or the blue X. A roadmap view with several icons will come up (pictured below). Click “Sequence Set Up” to write a sequence.



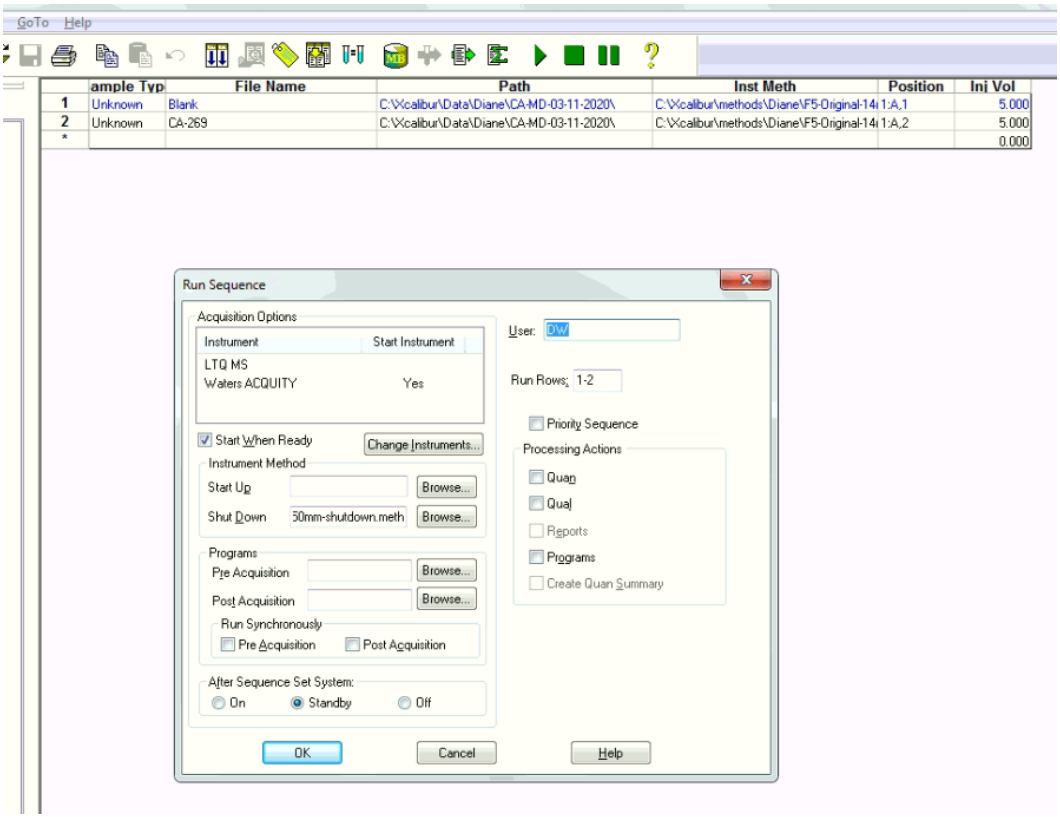
b. Once in sequence setup we can begin filling out the sequence.

- Simply put in the file names for each sample and select a path which is where those files will then be saved.
- Select the correct instrument method for the analysis. We have a couple of general methods that can be used.
- The format for the sample position should be “1:A,1” the first number denoting the tray, the letter denoting the row and the second number denoting the number of vial on that row. Each row has 8 slots. If there is a problem with the position, double check that the “48 Vial 2 mL Holder” is selected in Change → Tray Name.
- The injection volume depends on the sample loop currently in place. Unless otherwise stated the sample loop is 5 µL. That means you can inject a maximum of 5 µL. A typical injection volume is 3-5 µL.



c. After you have saved your sequence and the LC is fully equilibrated, you may start your analysis. Highlight the entire sequence and then click the green arrow with a list. A box will then pop up (pictured below).

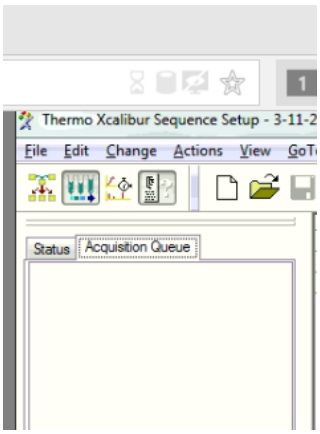
- Notice that under “Shut Down” there is a method added. The general method is simply called “Shutdown”. This will turn the UV/Vis lamp off, wash the LC column, and turn the flow off after the sample queue. If you are not staying for the remainder of your analysis it is **essential** that you have a shutdown method in place so that the solvent reservoirs do **not** run dry.
- Also notice that in the “After Sequence Set System” section standby is checked. This turns the LTQ into standby so it is not scanning after the run has completed.
- Once those are clicked hit “OK” and your run will start.



5. Data Collection and Analysis

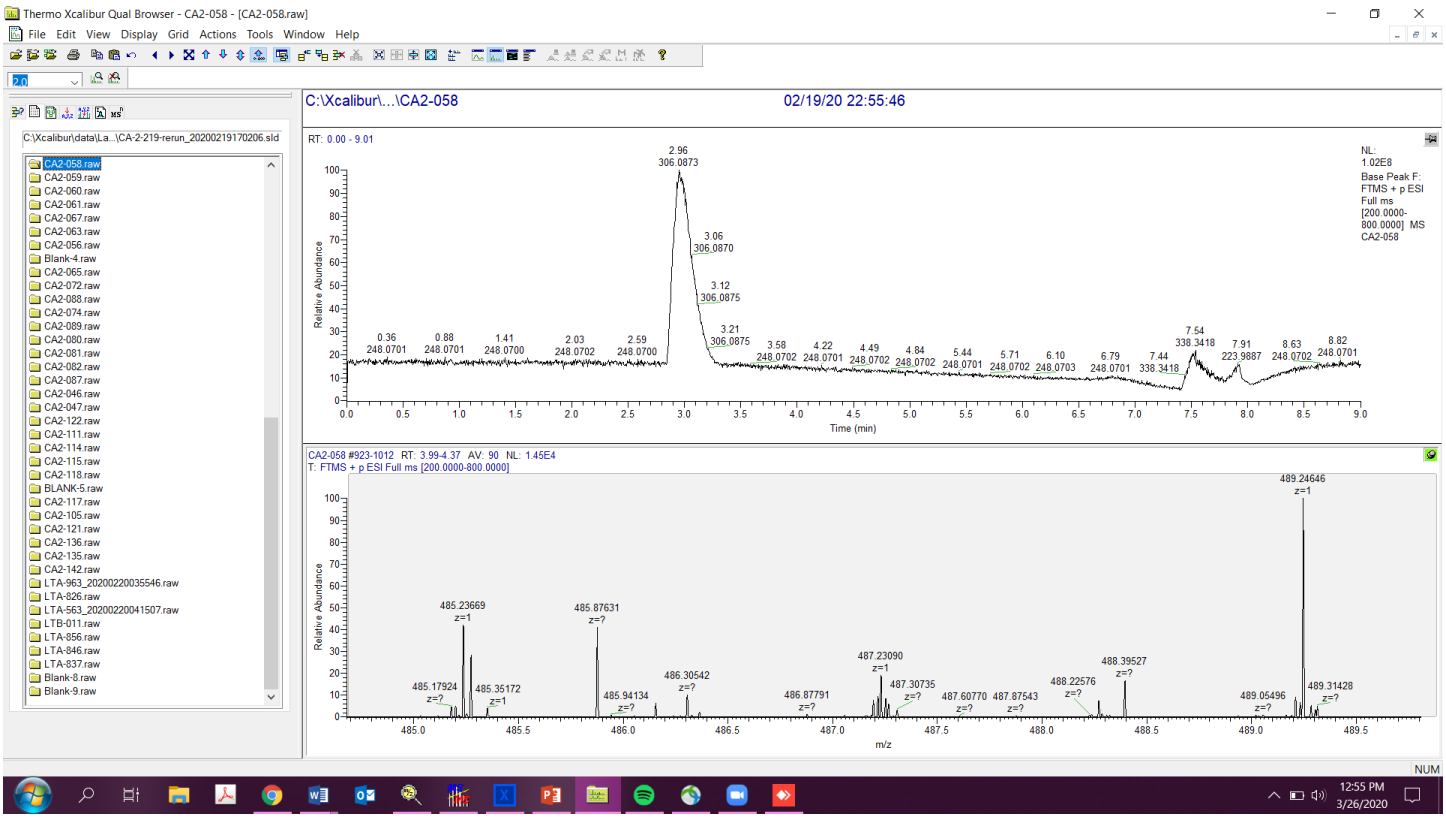
a. Once your run has started it is important to watch the first two injections to make sure there are no issues.

- In sequence set up there is an “Acquisition Queue” section that will list all of the samples in the sequence.
- The sample will be highlighted green when acquiring and will be checked off once acquired. You can keep track of the progress here.
- There is a real time view available as well. Click the clock button under the change tab and it will show you real time spectra as it is being acquired.



b. Once your analysis is completed you can analyze the data in Xcalibur.

- Go back to the roadmap but this time open “Qual Browser”. You can now open your sequence and click on each file name to see a different sample. Single samples can be opened as well.



- You can export your data via flash drive and work it up at your convenience on one of our data processing computers in the low field NMR bay across from Brandie’s office.