E. G. Tse, 2019

G25 Preparative LCMS Operation Guide

The operation of the preparative LCMS is identical to the analytical LCMS, with the addition of a fraction collector. Everything in this guide is identical to the analytical LCMS guide with the addition of the section called Fraction Collector Settings. Any sample run through the prep LCMS should first be first run on the analytical LCMS.

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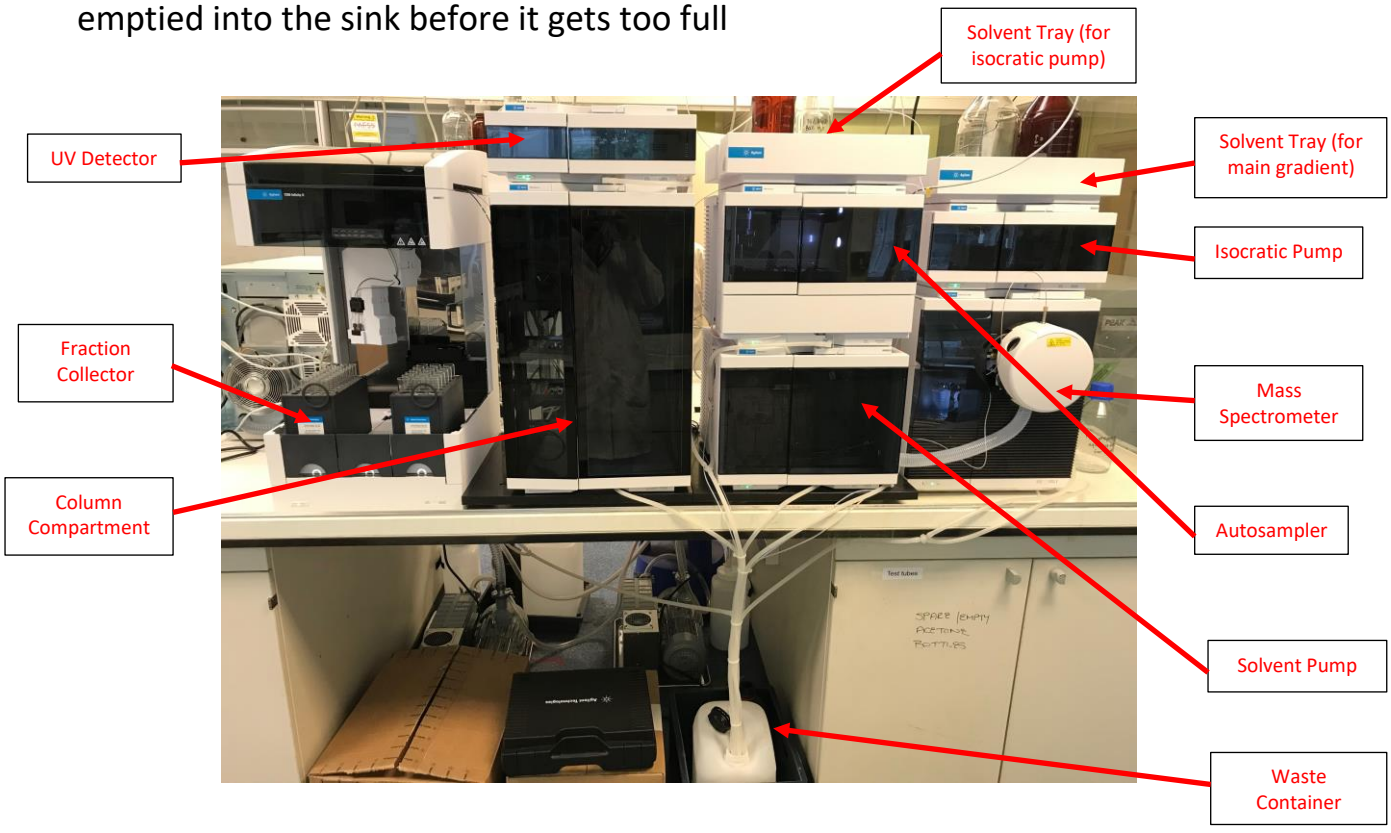
The waste container between the nitrogen generator and the argon cylinder collects the water condensate from the nitrogen generation. Make sure this is emptied into the sink before it gets too full

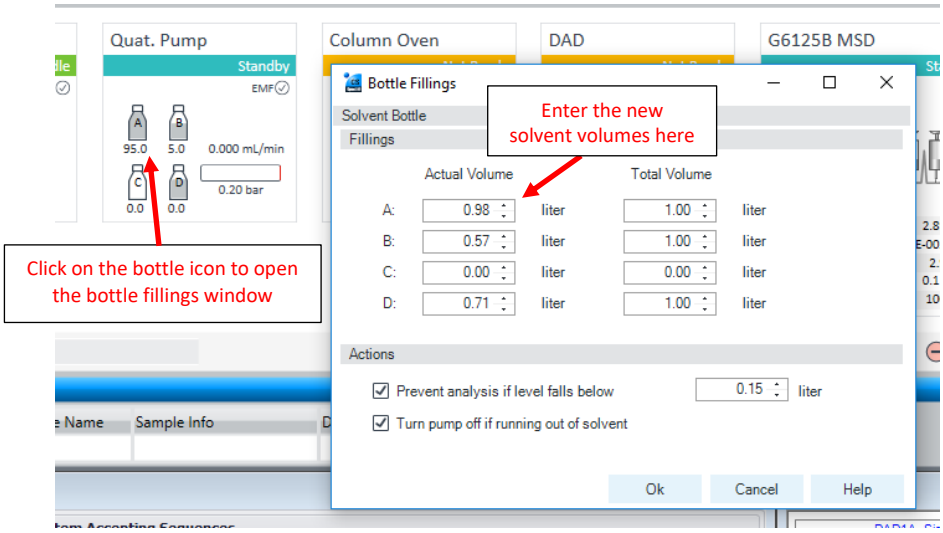


LCMS Supplies from MyFinance

- 2.5 L LCMS grade water (search for Cat no. 10777404)
- 2.5 L LCMS grade acetonitrile (search for Cat no. 10616653)
- Syringe filters (search for Cat no. ANN1322)
- 2 mL LCMS vials (search for Cat no. 5182-0716)
- LCMS vial caps (search for Cat no. 5190-7024)

Prechecks Before Running a Sample

- Check the solvent volumes in the bottles above the LCMS
- If the solvent volume is low, remake the solution and top up the bottle
- ONLY use LCMS grade solvents
    - Line A is water w/ 0.1% formic acid
    - Line B is acetonitrile w/ 0.1% formic acid
- Once refilled, click the bottle icon and enter the new solvent volume



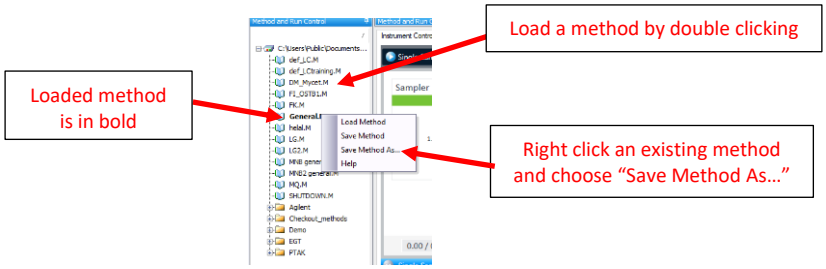
- Check the fraction volumes of the tubes in the rack (see below for how to reset)
- Check the volume in the waste container under the prep LCMS
    - Replace with an empty waste container if full

Sample Preparation

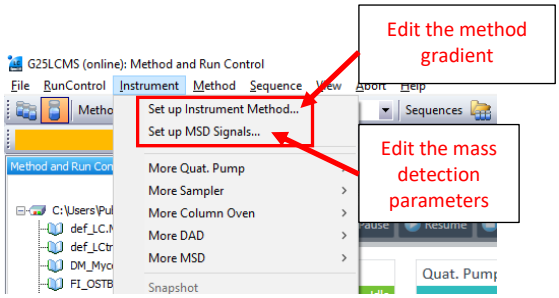
- For preparative LCMS, sample concentration can be higher than for analytical LCMS
- Keep in mind that injection of a more concentrated sample can affect peak shape and separation
- Ideally you want to prepare your sample in a solvent mixture that has less organic than the starting gradient percentage (i.e. if your gradient starts at 5% MeCN, dissolve your sample in this mixture)
- This may not always work so more MeCN can be used
- Filter this sample solution through a syringe filter into an LCMS vial
- Further dilute if necessary

Creating a New Method

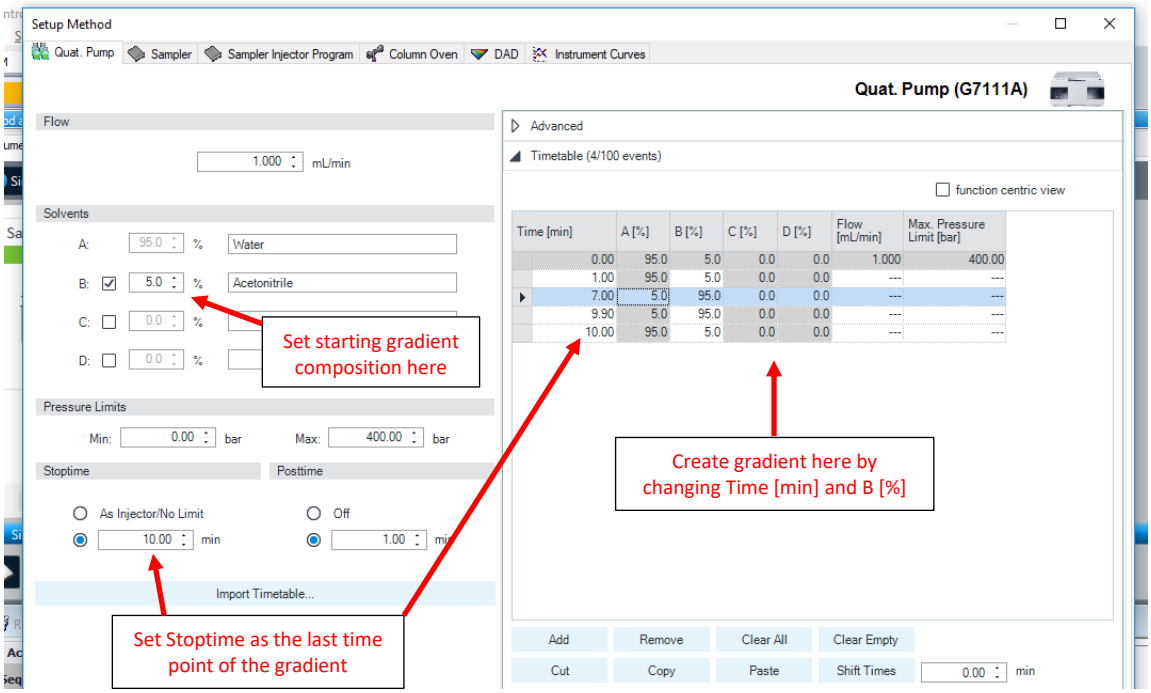
- Only edit your own methods (i.e. DO NOT edit another person's method)
- The loaded method is indicated in bold
- To create a new method (and to save creating a method from scratch), right click on another person's loaded method and choose Save Method As…
- Rename this to your initials



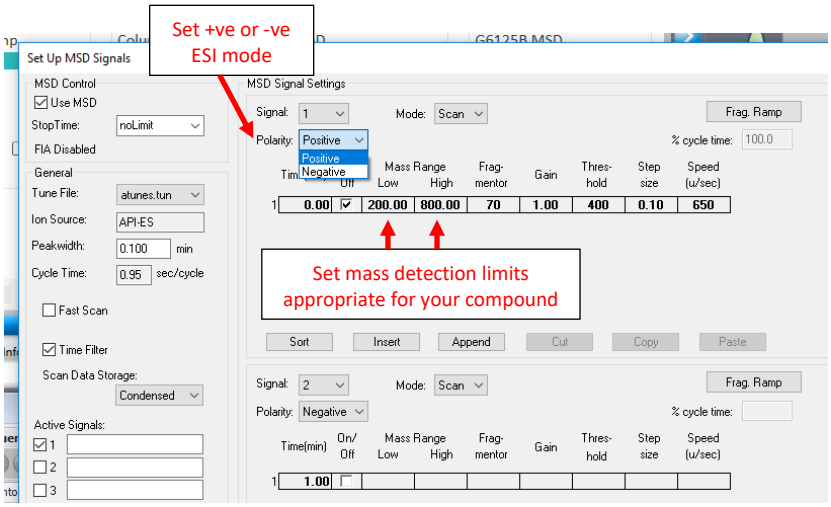
- The method can be edited under Instrument => Set up instrument method…



- Edit the gradient as you see fit
- The Stoptime (time at which the data for a run will stop being collected) should be set as the last time point in your gradient
- The Posttime (time after a run has completed/time between sample injection in a sequence) should be set to at least 1 min
- The remaining tabs (sampler/sample injector program/column oven/dad/instrument curves) can be left with the default settings



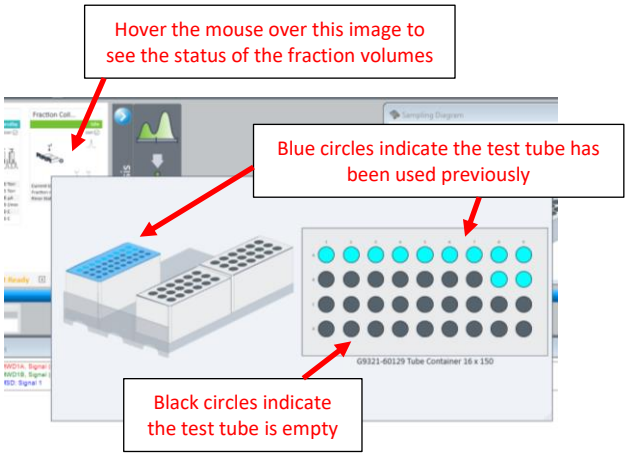
- The mass detection settings can be edited under Instrument => Set up MSD signals…
- Choose the polarity of the detector (+ve ESI or -ve ESI)
- Choose the mass range appropriate for your desired compound (typically from 200 to 800, but expand if masses are higher or lower)
- The remaining settings can be left as default



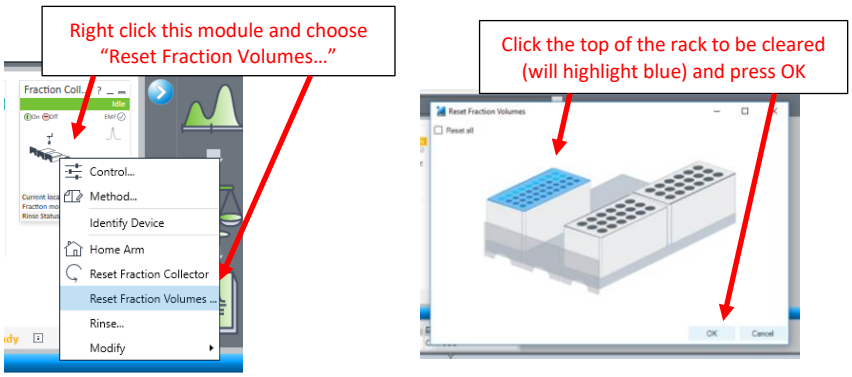
- To ensure that the instrument goes back to standby mode after a run, check under Method => Run Time Checklist… that Post-Run Command/Macro is selected and that it is set to Turn Instrument Standby
- Once done editing the method, right click the method name and choose “Save Method”

Fraction Collector Settings

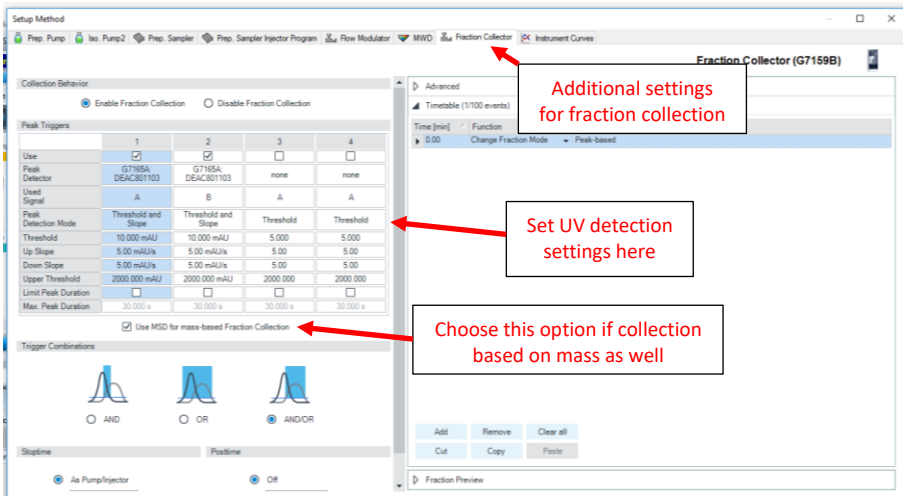
- Before running a sample, refill the test tube rack if empty
    - Hover over the fraction collector module to check the state of the tubes (blue circles indicate a fraction has previously been collected in that tube)



    - To reset the fraction volumes, right click the fraction collector module and choose “Reset Fraction Volumes…”
    - In the new window, click the top of the rack and press OK to reset the volumes to zero



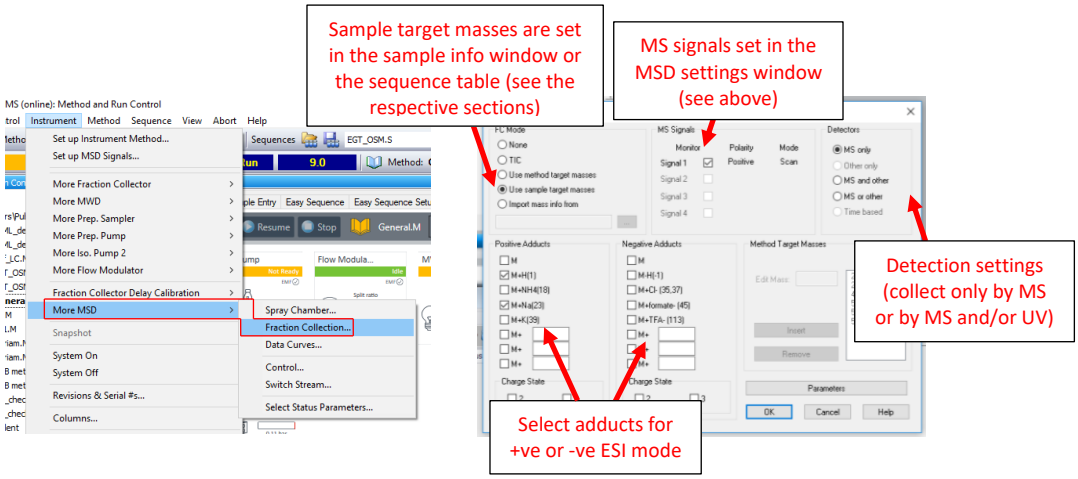
- There is an additional tab in the method editor called “Fraction Collector”
    - Key settings are Peak Detection Mode, Threshold, Up Slope and Down Slope



- Extra settings for collection by mass are under Instrument => More MSD => Fraction Collection…
    - Select “Use sample target masses”

- Choose Signal 1
- Choose the appropriate Adducts (usually M+H and M+Na on +ve ESI mode)
- Choose the detectors
  - MS only: Only collect when sample mass is detected
  - MS and other: Collect when sample mass and UV are detected
  - MS or other: Collect when sample mass or UV are detected

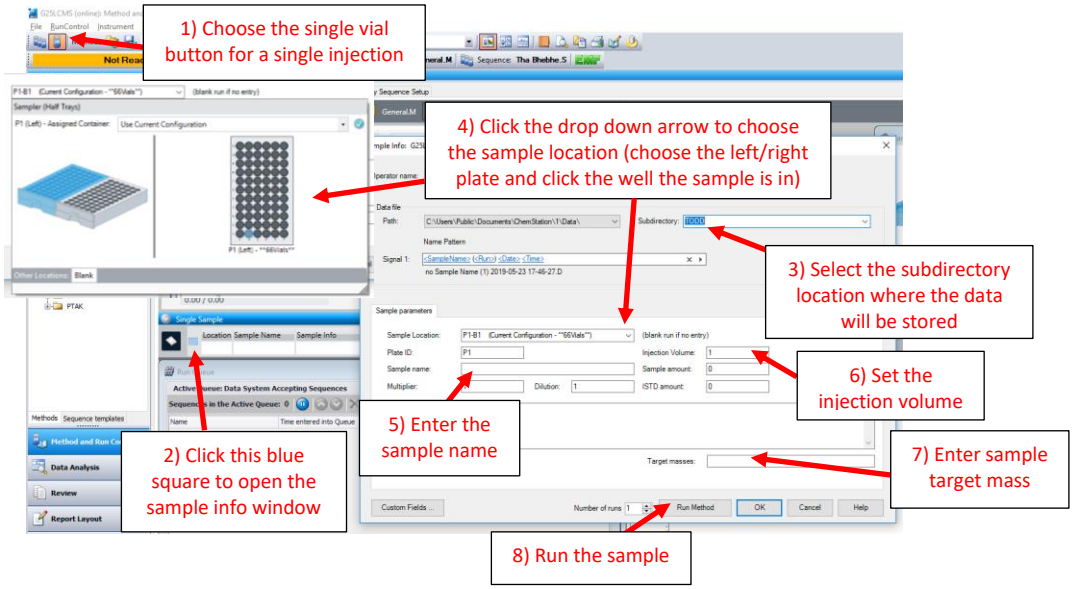
- Sample target masses are set in the sample info window (for single injections) or in the sequence table (for sequence injections)



Running a Single Injection

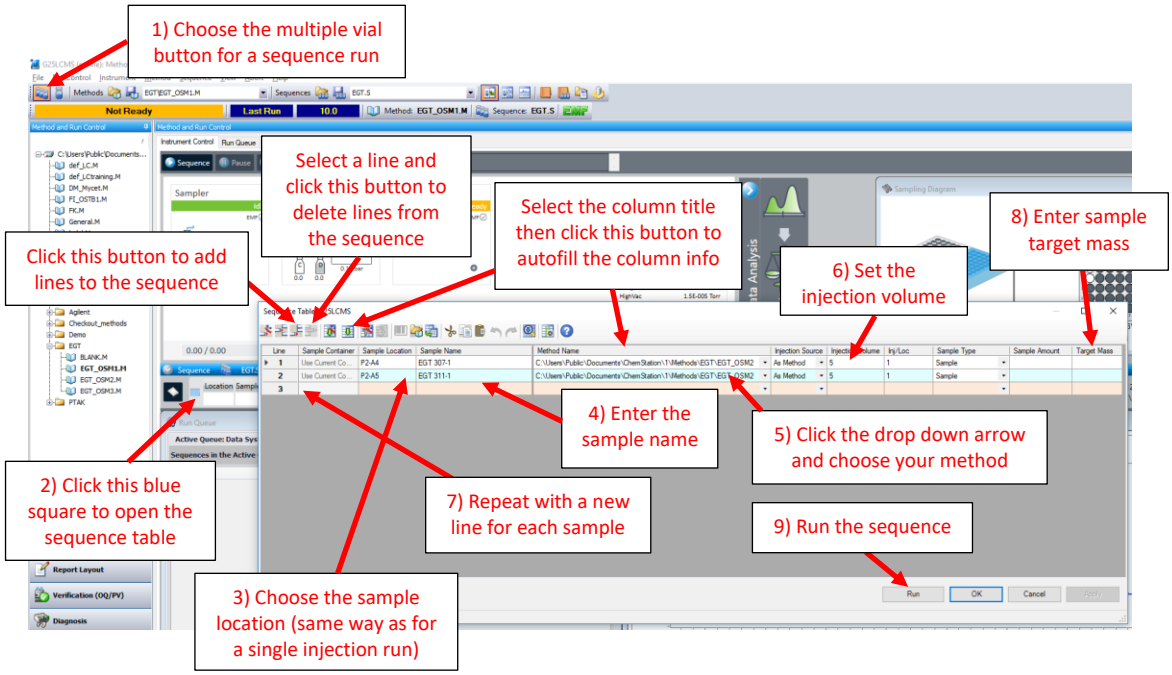
- Load your method then:

1) Select the single injection mode
2) Click the blue square to enter the sample info window
3) Choose the appropriate subdirectory for your group
4) Click the drop-down arrow to choose a sample location (click the left or right plate and click the well location)
5) Enter a sample name
6) Set the injection volume (max 900 uL)
7) Enter the sample target mass
8) Click “Run Method”



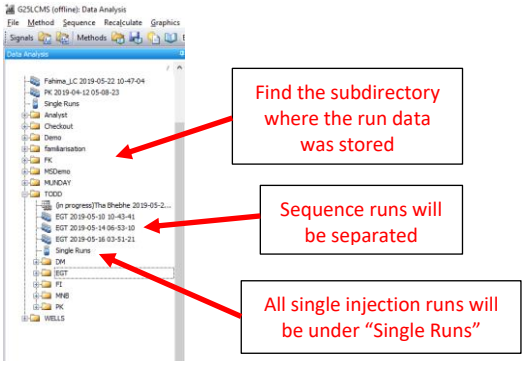
Running Sequence Injections

1) Select the sequence injection mode
2) Click the blue square to enter the sample info
3) Click the drop-down arrow to choose a sample location (click the left or right plate and click the well location)
4) Enter a sample name
5) Click the drop-down arrow to choose your method (if not on the list, click <browse> to find it)
6) Set the injection volume (max 900 uL)
7) Repeat for each sample
    - To add sample lines to the sequence, click the append lines button
    - To delete lines, select the line number and click the delete lines button
    - To automatically fill the info fields for other samples, select the column title and click the filldown button
    - If doing multiple injections of the same sample, change the value in the "Inj/Loc" column
8) Enter the sample target masses for each sample
9) Click "Run"



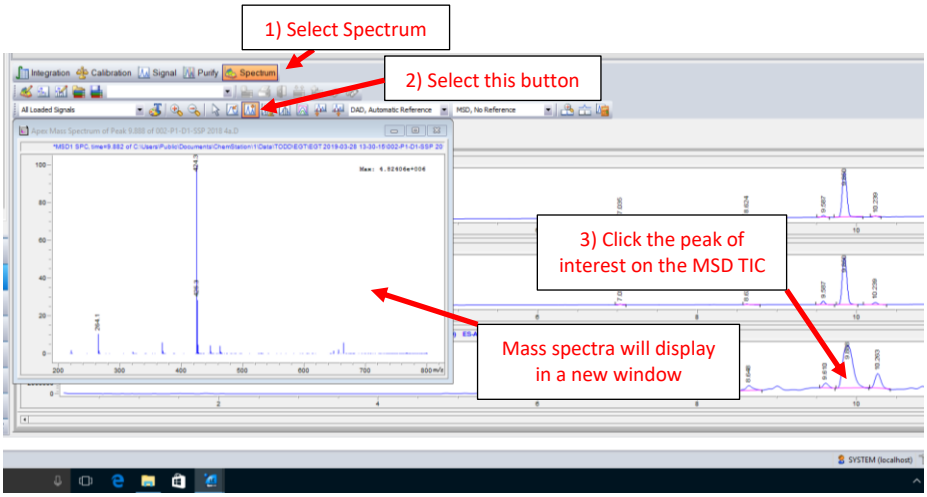
Viewing the Data

- To view the data, switch to offline mode
- Find the chosen subdirectory location
- For single injection runs, the file will be under Single Runs
- For sequence injection runs, the files separated in its own entry

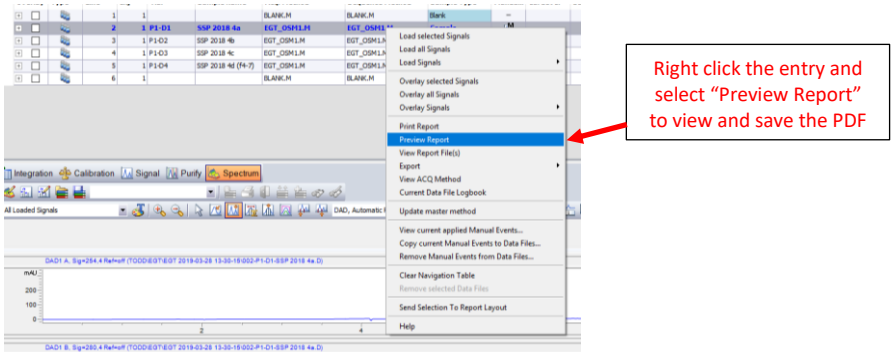


- Double click an entry to load the data

1) To view the mass spectra from the MSD TIC, first select Spectrum mode
2) Click the peak apex position button
3) Click on the peak on the MSD trace to view the mass



- To save the report as a PDF, right click on the entry and choose Preview Report
- Save this PDF



- If you have software such as MestreNova, you can copy the entire raw data folder and view the LCMS data elsewhere
- A shortcut to the raw data files can be found in the quick access menu
    - If no subdirectory was chosen, they will appear here

- If a subdirectory was chosen, they will be in the respective folder

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