Liquid Chromatography- Mass Spectrometer Manual

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Abstract
This manual will explain the LC/MS, its features, start-up and shutdown, and making methods and sequences.

1 Features

Purge Valve The purge valve, located at the top section of the LC set-up, directs solvent to the waste or to the LC system. To send solvent to the system, turn valve RIGHT. To send solvent to the waste, turn valve LEFT.

Autosampler This system is set up with a 100µL autosampler. This means that 100µL is the maximum sample size. Please note that the door (face) to the autosample section of the LC must be closed for the system to operate.

LC Column The column section of the LC has the capability to run the column from 10°C below ambient temperature to 80°C. Note that the temperature the system is measuring is the heating unit of the LC. You should have the column section at your specific temperature for 30min to ensure the column is at the set point temperature. To install a new column:

1. Remove old column or union.

2. While applying pressure to the capillary, tighten the connection. Repeat for the other side of the connection.

Solvents Solvents must be a minimum HPLC grade. Preferably, the solvents should be LC/MS grade. If you are leaving the system unused for more than three consecutive days:

1. Remove column and replace column with union. Make sure to apply pressure to capillary the whole time when tightening.

2. Flush system with 100% isopropyl alcohol (IPA) and leave IPA as the mobile phase.
UV detector  The Dialray detector of the MS has a 190-650nm detection range.

Lighting on LC/MS  Yellow lights mean not ready. Green lights for all parts of system means run. Red lights means something is wrong.

2  Start-up

This section will explain the proper method to start-up the LC/MS system. The LC/MS should be on for a minimum one day prior to using for experiments. This is to allow the system to clean out.

1. Turn on nitrogen (N\textsubscript{2}) flow behind unit. Nitrogen pressure should be 80-100psi. Make sure to check the pressure holds steady.

2. Plug in the computer and LC/MS power cords. Make sure to be careful plugging in the MS power cord (it is 200V outlet).

3. Turn on computer.

4. Turn on MS. When you push the MS power button, the rough pump (vacuum pump below LC/MS) should turn on first. Keep door to pump open if the ventilation has not been installed. After the rough pump goes on, the turbo pump (pump inside MS) should turn on. If this does not happen contact Agilent.

5. Turn on the LC sections.

6. Once everything is not red, you can open chemstations

3  Shutdown

This section will explain the proper method to shut down the LC/MS.

1. Replace column with union (see instructions in features section. Flush system with 100\% IPA.

2. Within online chemstations, turn off MS on screen.

3. Go to diagnosis tab.

4. Select Maintenance tab, then MSD vent, then More, then Start, and then OK.

5. The MS is now venting. WAIT until the blue timeline is finished. Once the blue timeline is filled, turn off all buttons on LC/MS.

6. Shut off N\textsubscript{2} line.

7. Find and open “CSTools.exe” and hit Kill chemstation to close chemstations program. This needs to be done because Chemstations will not close otherwise.
4 Method Creation

Method creation is quite simple because Chemstations walks you through it. Below are the steps you will follow. You should always start with the default method when making a new method. Once you are done making your new method, use SAVE AS to save your new method.

1. Open either Chemstations (online) or Chemstations (offline). Note that online means you also can control the LC/MS.

2. Open default method.


4. Making sure everything in the pop-up is checked, select OK. See figure 2.

![Figure 1: Red outlined box shows Edit entire method.](image)

![Figure 2:](image)
5. Fill out comments about this method.

6. Always choose Als, which is the autosampler. See figure 3.

7. Quat. Pump section. The pump has a maximum 600bar. However, you should go by the maximum of your column. Stop time is the length of your run. Post time is the amount of wait time. Use the time table to create gradients in solvent. To make an instantaneous switch of mobile phase (solvent), set time in time table to $x + .01$, where $x$ is the time of the previous step. See figure 4.
Figure 4: Red outlined box shows flow rate, green box shows where you can name and select solvents (mobile phases) and blue box shows where you can set up the mobile phase for all the runs

8. Sampler: Set the injection volume (100µL maximum).

9. Column Comp: you will set the temperature you will like for your experiment. See figure 5.
Figure 5: Red outlined box shows where you set column temperature.

10. DAD: This is the step where you set the signals you would like to measure. To measure a signal, make sure it is checked. *Peak width* is how fast you want to collect data points. Selecting *ALL* will collect all peaks under spectrum. See figure 6.
Figure 6: Red outlined box shows where you set the signals to measure and blue outlined box shows where you set peak width.

11. Instrument Curves: This section is where you select the data other than the signals you will like to collect. Always collect pressure data. See figure 7.
12. MSD Spray Chamber: DO NOT CHANGE

13. MSD Signals: To collect MSD signals, you must have this section checked. To collect a signal, check active signal. SIM measures a target mass. SIM ION is the signal mass. Select SIM on Sample Target Masses. Scan will look at a range of signal masses. See figure 8.
Figure 8: Red outlined box is where you select active signals to measure and blue outlined box is where to select scan versus a SIM.

14. No FIA nor Purity.

15. Leave integration on default. You will want to collect data and change data integration of the methods section then.

16. Specify Report: allows you set up how the report from a experiment is printed out.

17. Data aquisition/ data analysis: Set this up in the data analysis tab after the method has been used.

18. You have finished setting up your method. Now you will save the method. Select File tab → Save as → Method

5 Sequence Creation

With a method created, you will want to create a sequence to allow you to run a series of samples fully automated. Again, you start with the default sequence, edit it and save as a new sequence:
1. Select *Sequence* tab → *Sequence table*.

![Sequence Table](image)

Figure 9: Red outlined box shows *Sequence Table*

2. *Column Chooser* lets you select the options you wish to set for your sequence. The options you wish to set must be checked. You will want to have the following *always* selected:

   - *Sample Location*: location of the sample
   - *Sample name*: name of sample
   - *Method*: method to test sample with. Make sure to make and save method prior to making sequence.
   - *Injection volume*: volume of sample to inject into system. This LC/MS has a 100µL maximum.
   - *Inj/Loc*: number of times an injection will be taken from this sample
   - *Sample type*:
   - *Target mass*: appends the signal in the method to measure without creation of a new method.
   - *Data File*: Name of file you wish to have it saved as. Please use the following nomenclature [Group Initials]_[Sample Type]

Hit OK.
3. Fill out the options for each sample

![Figure 10: Red outlined box shows all the options to fill out](image)

4. Sequence parameters (under sequence): Use Auto. Check Post-Sequence command/macro. Select STANDBY for Post-Sequence command/macro. See figure 11.
5. Your sequence is created. Now you will save it: Select File tab → Save as → Sequence. Please name sequence with the following nomenclature [Group Initials]_[Date].

Check List BEFORE running Sequence:

- Solvent Bottles Full in control panel
- Waste bottle ready (look before computer)
- Purge valve closed (Turned to right).
- Rinse Bottle is full and in position 21.

6 Data Analysis

To perform data analysis on data:
1. Select *Data Analysis* tab on left side of software window. See figure 12.

![Image of software window with Data Analysis tab highlighted]

Figure 12: **Red** outlined box highlights *Data Analysis* tab, **blue** outlined box highlights linear integration tool and **green** outlined box highlights print icon.

2. Once the *Data Analysis* is open you can integrate and measure peaks from both the LC (UV detector) and the MS. Use the linear integration tool (see figure 12). To use this tool click the left side, hold and drag to the right side of the peak.

3. Click the print icon to get a print out of data (peak location, peak area, peak width, etc.) (see figure 12).

### 6.1 Mass Spectrum

To visualize the **Mass spectra**, use the tool (shown in figure 13) and just click the peak you wish to see a mass spectra for.
6.2 EIC: Extracted Ion Content

To get a EIC (extracted ion content), the chromatograph for a specific mass, go to file and then select Extract ions... (see figure 14). You will then fill out the pop for the specific range you want for your species.

Figure 13: Red the tool to have selected under the spectrum tab to get a mass spectrum for a specific peak.

Figure 14: Red outlined box highlights the path to getting a EIC chromatograph.
6.3 Extract of Data

**Extract Chromatogram**  Click File→Export File→CSV File.... Then select Signal, name your file and then hit OK. Select the signal you want: DAD is the UV detector, MSD TIC is the total ion content and MSD... EIC is the extracted ion content. You will need all three for all six samples. The file will output two columns: the first is time in minutes and the second is counts.

**Extract UV and Mass Spectra**  First use the Select spectrum at peak apex position tool to show the spectra you want. Next click File→Export File→CSV File.... Then select Active Graphic Window, name your file and then hit OK. You must extract every UV and Mass spectra of interest! Make sure to name the file properly to know the difference between them. The file will output two columns: the first is m/z and the second is content.

**Extract peak area for EIC Peak**  First make and EIC for the specific mass of interest (see above). Next Then select Integration Results, name your file and then hit OK. Select MSD1 xxx, EIC=yyy:zzz. Have the following boxes checked:

- RetTime
- Area
- Height
- Width

Your output file will have four columns: RetTime, Area, Height and Width. You NEED the EIC integration for the glucose peak for all samples. Make sure to note HOW you performed your EIC so you know which standard curve to use to get your glucose concentration.