How to properly use the LC/MS.

- **Walk up and use**
- **Data analysis**
- **Restart protocol**
- **How to change the nitrogen dewar**
- **General reminders**

SECTION 1: Walk up

You will be able to walk up to the machine and simply insert your sample into the sequence table (without having to stop any sequences or create ANY methods). If you follow this step by step you will not have a problem with the instrument.

- Prepare your samples so it is roughly 5-10 nanograms / ml in HPLC grade solvent. Preferably CH_3CN, H_2O, etc. Make sure that there are no solid particles in your sample which may clog the lines.
- See if the solvents are full, if not, then fill them with the appropriate solvent in the cabinet labeled "LC/MS and $[\alpha]_d$ solvents". Make sure that you fill reservoir A1 with H_2O/0.1%AcOH, B1 with CH_3CN / 0.1% AcOH, and B2 with pure CH_3CN. If the desired solvent is empty call it to the attention of whoever is in charge of the LC/MS or add 4ml of AcOH to the appropriate 4L solvent bottle.
- <u>If a sequence is running, and you wish to insert a sample:</u> Go to the SEQUENCE drag down option, select "sequence table", and insert your run into the running sequence. Save the modified sequence.
- <u>If a sequence is NOT running and you wish to insert a sample:</u> Go to the SEQUENCE drag down option, select "New Sequence". Go to the SEQUENCE drag down option, select "sequence table", and insert your runs and click OK. Click on the SEQUENCE drag down option, select "sequence parameters", type your subdirectory in the appropriate place. **Click the "Post-Sequence CMD/Macro" and select "standby."** If you fail to do this then the system will run until it is out of solvent. Click OK. Click on the SEQUENCE drag down option, select "Save sequence as" and type the date in numeric form such as MM_DD.
- **Please do not write or modify methods.** We have written a series of methods that should cover the usage requirements for everyone. If you find that you need a special method, ask the person in charge of the LC/MS to help you write one from the default. Modifying methods and resaving them with the same name is possibly the source of our problems with the LC/MS. The method names are in code, which translates as follows.
    - First letter is **P** or **N**. This stands for Positive or Negative ionization.
    - Second and third letters are **ES** or **AP**, these stand for ESI or APCI. PLEASE note whether the machine is configured for ESI or APCI before loading the appropriate method. If the system is configured for ESI and an APCI method is loaded, the machine crashes.
    - Fourth letter is **P**, **N** or **F**. This stands for **P**olar, **N**onpolar or **F**lashed compounds. Flashed compounds (polar or nonpolar) have been purified and do not need LC separation (i.e. one compound). The table below shows the two gradient methods available for **mixtures** of compounds. For the flashed compounds the method is 100% MeCN for 5 min.

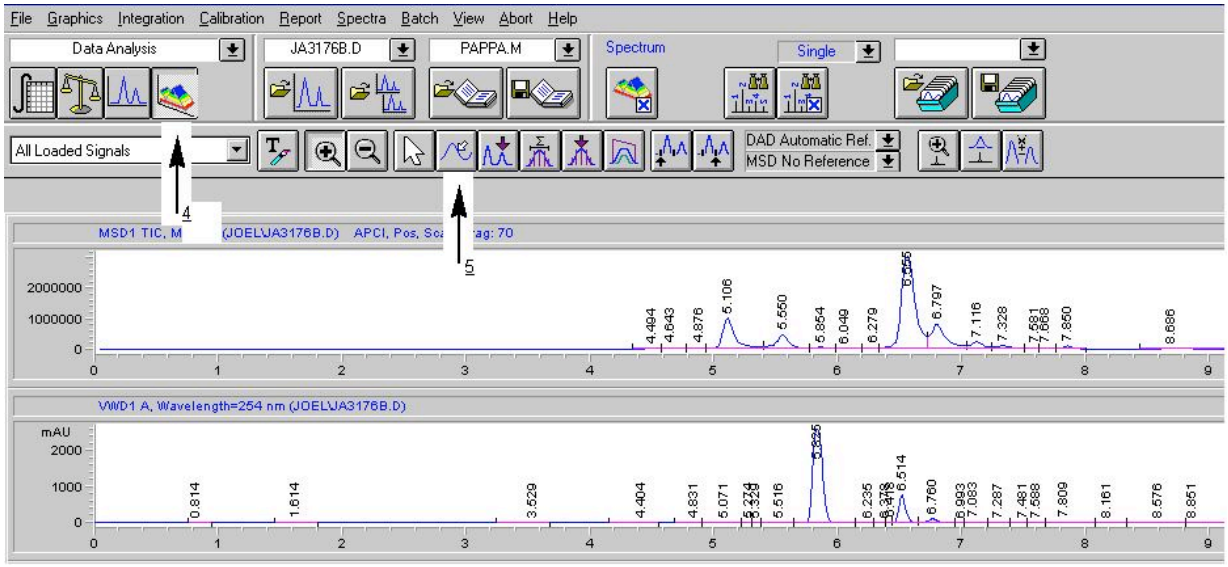
Polar Compounds		Nonpolar Compounds	
0 min	5% MeCN	0 min	5% MeCN
6 min	100% MeCN	4 min	100% MeCN
7 min	100% MeCN	10 min	100% MeCN
8 min	5% MeCN	11 min	5% MeCN
10 min	5% MeCN	13 min	5% MeCN

  - Fifth letter is A, B, or C. This indicates the mass range that you are searching in. Mass range A is 100-500 amu, mass range B is 400-800 amu, mass range C is 100-1000 amu.
- For example **PESPA** stands for Positive ionization, Electrospray mode, for Polar Compounds in mass range 100-500 amu.
- **IF A METHOD IS NOT WORKING OR IS CORRUPTED DO NOT TRY TO FIX IT YOURSELF**. Tell the person who is in charge of the LC/MS.
- And as a reminder, please check and fill the wash vial (number 81) with the 50:50 H_2O/IPA squirt bottle next to the LC/MS. Also please fill the solvent reservoirs when they are low.

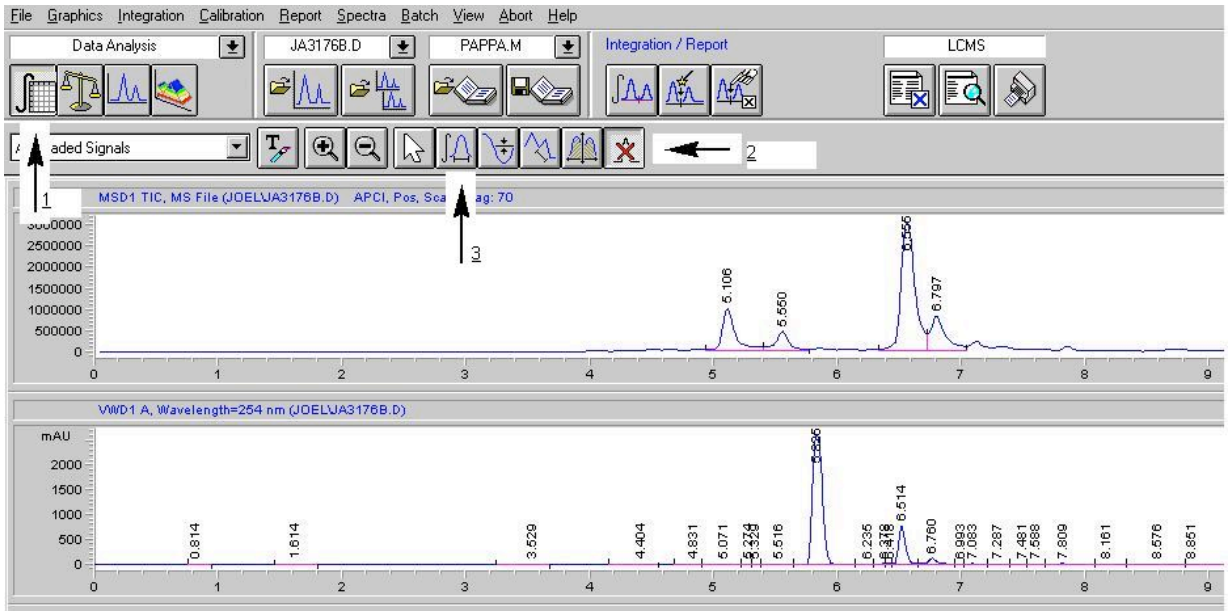
SECTION 2: Data analysis

Data analysis should be done offline or when the LC/MS is not running. You need to be in "Data Analysis" rather than "Method and Run Control."

- Load your data. The screen will perhaps look like the picture below. If not the click on button 4.



- By clicking button 5, then on a peak, a pop up window containing the molecular ion for that peak will appear. Keep doing this until you find the one you want.
- By going to File, "extract ions", then typing in the ion you are looking for, you can have the computer search for you.
- If you click button 1 the tool bar will appear like the picture below.

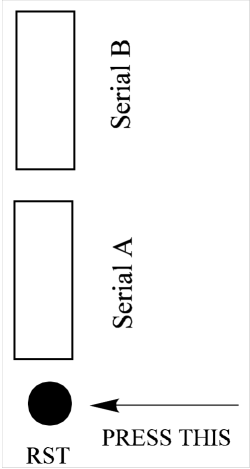


- Clicking button 2 allows you to remove unwanted integrations. This is important in case you decide to print. If you do not do this then WAY too much information will be printed off and it will waste time and supplies.
- By clicking on button 3 you can re-integrate any peaks that you may want.
- To print off your data, go to File, Print, the destination is "printer"; the report style is "LCMS". Then click OK.

SECTION 3: Restart protocol

Occasionally the LC/MS will not want to work, this is generally after the nitrogen dewar is out but occasionally will happen for no apparent reason. Here is a step by step sequence of what to do to get it working again.

- Whatever you do, **DO NOT EVER PRESS THE RED BUTTON** that has a piece of tape on it saying "DON'T TOUCH." This is an emergency shut off, if it is pressed too many times then an explosion may occur.
- Close Chemstation offline and online.
- Close the "GAG bootp server".
- Press [ctrl][alt][del].
- Click on "Task Manager".
- Click on "Hpmsln32.exe" and then "end task".
- Click on "NTVDM.exe" and then "end task".
- Close the open window then restart the computer.
- While the computer is restarting open the side panel on the left side of the MS. Press a pen into the hole labeled "RST", see figure below, this resets the computer on the MS. This is not 100% necessary but increases your chances of the system working.

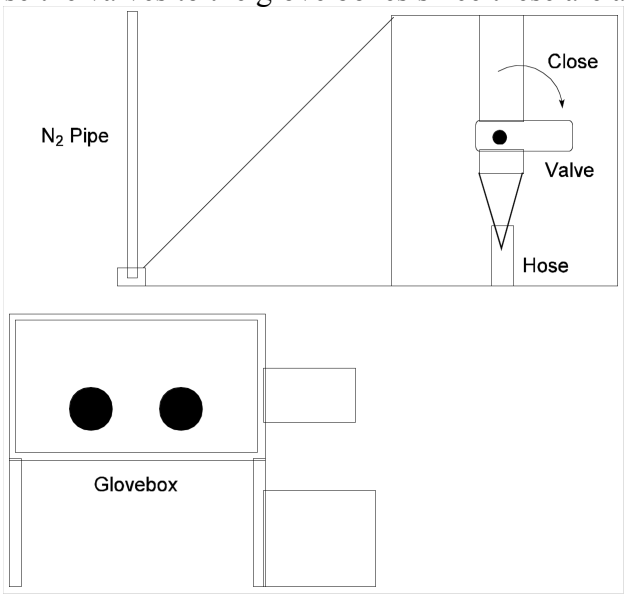


- Wait for the computer to prompt you for the password, the password is 3000Hanover.
- Once windows boots up DO NOT click on Chemstation for 2 minutes, this is very important in that it gives the system time to for the "GAG bootp server" to talk to the pc.
- Once that has been established you can click on Chemstation online.
- If this doesn't work, try it again. After 2 tries find whoever is in charge of the LC/MS.

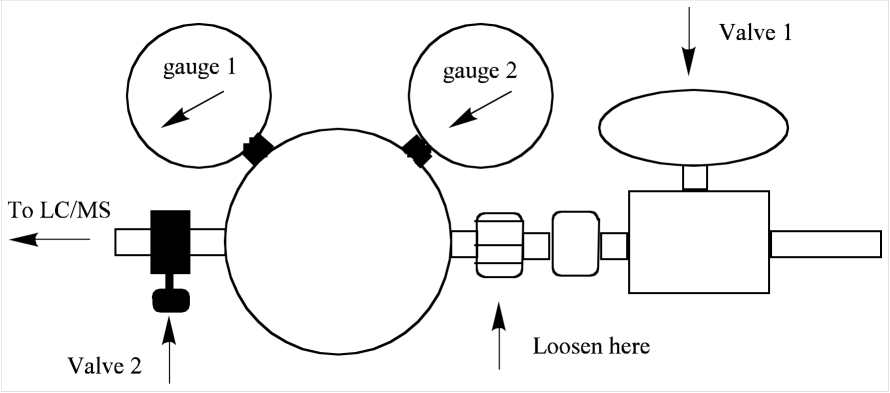
SECTION 4: How to change the nitrogen dewar.

If the LC/MS suddenly goes red, and there is no pressure indicated in the "MSD Status" box then the nitrogen is probably out. You will have to call to have a new dewar delivered. When the delivery person comes do the following.

- Get the appropriately sized wrench from the tool chest.
- Go to room 11 and close the valves to the glove boxes since these are also on the dewars.



- Close valves number 1 and 2 (valve 2 has a sticker on it that says which way to turn it close).



- Loosen the pressure valve by turning counter clockwise at the position directed on the figure.
- Allow the delivery person the change the tank.
- Place the pressure valve onto the new tank.
- Open valves 1 and 2.
- Open the valves in room 11 that are to the glove boxes.
- Check the pressure on gauge 1, if the pressure is less that 80 then the LC/MS won't work. Give it at least a minute to build pressure. If pressure still has not climbed high enough then open the pressure release valve (on the dewar) a bit.
- Go to the LC/MS.
- Since all the icons will be red, you have to left click on the MS, go to "control", then click "on", then "ok".
- Do this for the pumps and UV detector.
- Left click on the injector and go to reset injector.

SECTION 5: General reminders

- Check the solvent levels.
- Make sure to click the "Post-Sequence CMD/Macro" and select "standby." When starting a new sequence.
- Write your name and compound in the log book, the m/z observed and the abundance observed.