

Starting procedure: by Vicky

- is the vacuum pump running (should run all time, otherwise it'll need 4-5 hours to have a adequate vacuum for the MS) *otherwise something has gone wrong- F.T*
- Remove plug from ion inlet of mass spec making sure to hold needle at the end (Figure 1)



Figure 1: Plug in ion inlet of mass spec

- Turn on power buttons on all machine components (Figure 2) starting from the right top side (**red arrow**) and finishing at the interface (**blue arrow**).



Figure 2: Turning on computer components from right to left

- Turn on computer (Login: admin, password: admin)
- Click on software icon – labsolutions (login: admin, no password)
- Click on LCMS – 2020 (Figure 3) – Make sure a beep is heard when software opens (indicating a connection between LC-MS and software)

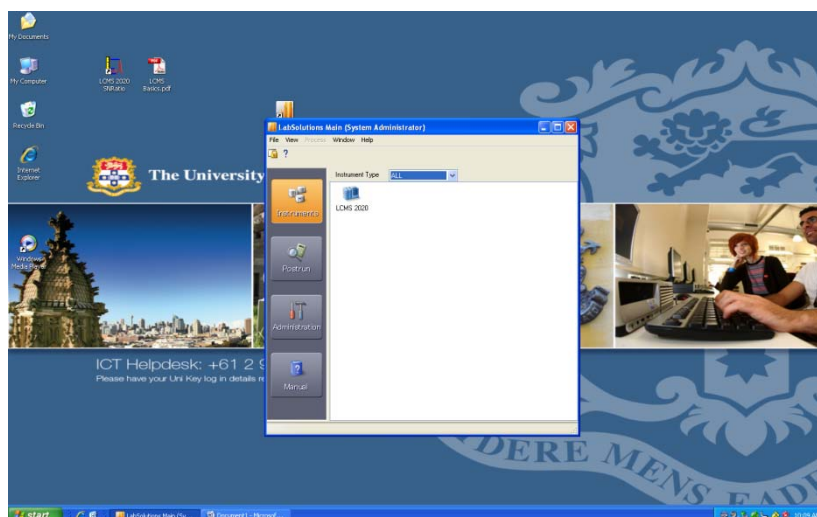


Figure 3: Labsolutions software – LCMS 2020

- Turn nebulizer gas on (Gas on icon – Figure 3), make sure nebulizing gas says 1.5L per min in right hand window (Figure 3)

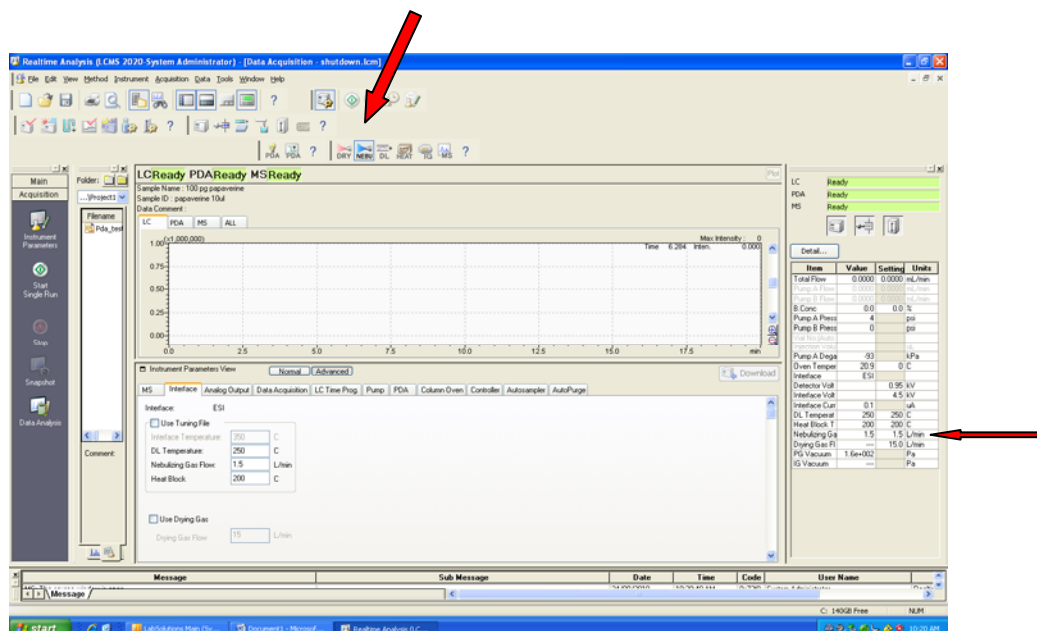


Figure 3: Press NEB icon on, make sure gas reads 1.5L/min

Purge:

- Manual purge - Only necessary if machine has been idle for 2 days or more, you can check this by visually checking solvent lines for air bubbles. Turn black wheel 90° anticlockwise on Pump A and B, remove about 10 - 15 mL solvent with the syringe from both solvent lines A and B at the bottom of the left tower. (Figure 4) NB: **When finished purging manually and purging via pressing the machine's button (see 'auto purge' instructions below) turn the black wheel back to the closed position.** (Figure 5), *Otherwise your compound will go through the waste bin (happened to me) & you won't get a spectrum of your compound as it would not have passed through the column. Please do not tighten the tap too tight, Cedric said that's not good- F.T*



Figure 4: Turn black wheels on Pump A and Pump B 90° to the left



Figure 5: Syringe out 10-15 mL solvent from line A and line B

- Auto Purge tubes/pumps - To be done every morning before start-up. (The nebulizer gas must be on otherwise machine will not allow purging as the computer has been turned on. The typical flow

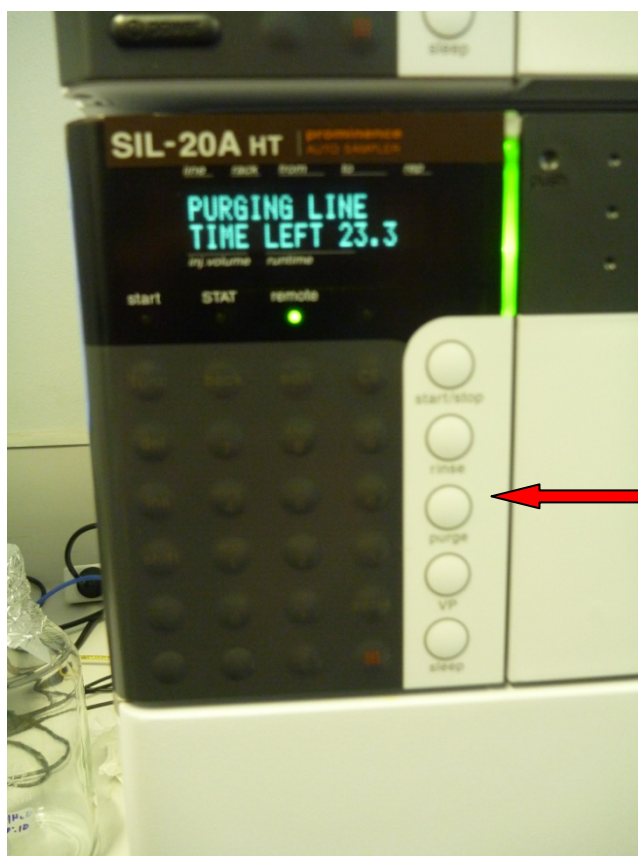
setting for the gas is 1.5 mL/min - F.T) usually it's good to have drying gas on too when you are using the MS. (Figure 6).

- Turn black wheel 90° anticlockwise on pump A and pump B. Press “purge” and let run for about 3-4 min. Then press “purge” again to stop it (*unless it has stopped itself already*). (Default value is 8 mins but 3-4mins is enough). NB: When finished turn the black wheel back to closed position.



• **Figure 6: Press purge on Pump A and Pump B**

- Purge the auto sampler (default 25 min, but 10 - 15 min should be enough): press “purge” on front of machine and leave running for about 10-15 mins. NB: Make sure there is enough rinsing solution (Solvent C) before purging. (Figure 7),



- **Figure 7: Press purge on front of autosampler**

Note: Cedric said the pumps can withstand pressures up to 6000 psi. Usually the pump pressure is way below 4000 psi and if it is at an instance > 4000psi then the pump is blocked. Usually, if solvent is not flowing through the column yet, the pressure is about 200-300 psi but when the solvent is flowing through the column the pressure is between ~300 and ~2500 psi depending on the solvent used and column density - F.T.

Mass Spec: It is essential to remove the plug from ion inlet before using the MS (don't touch plug / ion source plug at the tip), check that the vacuum light is green on MS (after closing the warning window, which appears every time when the MS chamber is opened).

Turn Nebulizer gas on (*if off - F.T.*), (Gas on icon). Click download. Click instrument icon to activate pumps and column oven. Green lights will be seen on front of machine parts when activated.

Sample preparation

This is absolutely crucial. The instrument will block up if a sample is badly prepared

- use only HPLC/MS grade solvents to dissolve sample
- *Any solvents that are miscible with the solvent used for the run can be used. E.g. MeOH - F.T.*
- centrifuging and/or filtering the sample (pipette with cotton wool plug) to remove particles / solids (*the best way to filter solvents is to centrifuge first and then filter using the 0.2uM filters- F.T*)

How much to inject?

If in doubt always start with a sample that is very dilute. If your signal is weak, you can increase the concentration or injector volume. Standards (things that ionize well) can be analysed at concentrations

of XXX. As a guide: maximum concentration that should be employed is: 1mg / 10 mL
- minimum concentration: 10 pg/ μ L (1 mg / 100 mL)