Getting low-resolution GC-MS data on the JEOL GCMate

Introduction

GC-MS is a technique that combines gas phase separation and mass-based detection/characterization of analytes. It's a <u>very</u> sensitive method that is limited to reasonably volatile compounds that are thermally stable. Compounds that readily form hydrogen bonds with themselves (e.g. amides and carboxylic acids) are especially poor candidates for GC-MS analysis in their native form. However, even highly polar compounds, such as carbohydrates or peptides can be made suitable for GC analysis by derivatization. Ask us if you want more info.

<u>Separation of analytes</u> is achieved by controlled heating of the column in the GC oven. In a typical temperature program the oven is heated to an initial set point and that temperature is maintained for a few minutes to drive the solvent off the column. The oven temperature is then heated up (ramped) to a final temperature causing the analytes to advance on the column and separating them based on their volatility. The final temperature is typically held for a period of time to ensure all analytes are driven off the column. You control the heating and thus separation by selecting an appropriate inlet procedure when setting up your experiments.

<u>Detection of analytes</u> is accomplished by repeatedly scanning through the desired mass (or more precisely mass to charge ratio, m/z) range during the inlet program and counting the number of ions that hit the detector. Adding up all ions generates the total ion chromatogram (TIC).

In your lab

To minimize chemical contamination in the MS room you MUST transfer your sample into the autosampler vial in your own lab and you MUST label all vials with your name!

For low resolution MS you should make a up a solution of your compound in any solvent you'd like as long as that solvent's boiling point is significantly less than the boiling point of your analyte. The concentration should be around 0.1 mg/ml for the standard GC programs. If you can't weigh your compound accurately err on the side of too little sample rather than too much. You will only need 0.5-1 ml of sample, so don't dissolve 10 mg in 100 ml of solvent! It's a waste of compound and solvent. Use successive dilutions if you need to (if you don't know what "successive dilution" means ask us and we'll explain). Place 0.5–1 ml of your sample in an autosampler vial and seal it with a septum cap. The color of the cap doesn't matter. You may pick up vials from the MS room (B-22) or Tony's office. Feel free to pick up a few extra for future samples or you can also order your own if you desire. *Please let us know if the vials or caps are running low!*

In the GC-MS room

<u>Optional</u>: To purge accumulated gunk from the GC column, on the GC keypad press [OVEN TEMP] [3] [0] [0] [OK] to heat the oven up to 300°C. The oven will cool down automatically when you start your acquisition.

<u>Insert your sample</u> into an open position on the sample tray of the autosampler. The tray has four removable quadrants (Q1–Q4 in diagram). The position numbers increase from the inside to the outside and counter-clockwise around the tray. Determine the positions based on the diagram and **do NOT trust the numbers printed on the quadrants**! They are removable and someone may have put them back in the wrong position, i.e. the quadrant closest to you is always positions 1–25 even if the plastic has 75–99

Sample tray

Injector tower

Q3
51-75

Q4
76-99

Q2
26-50

Q1
1-25
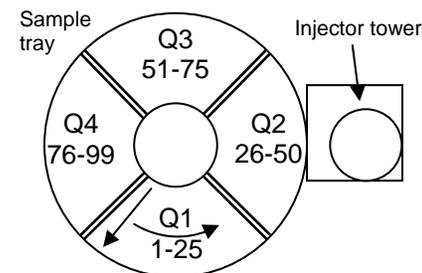
You stand here!

printed on it. Note the position of your sample and enter the appropriate information in the logbook and the white board mounted on the wall (coming soon).

Check the solvent level in wash bottle A1 (position 12) and B1 (position 8) in the small carousel mounted directly on the injector tower of the autosampler. If the level is at or below the "minimum solvent level" mark, fill wash A with methanol and wash B with hexane from the solvent bottles standing next to the GC. Pipettes and bulbs should be on the prep table and please let us know if we they are running low. You may now sit in front of the computer.

At the computer

If nobody is logged in, log in as user "Ivan Keresztes" with the password Kersztes (notice missing 'e'). Do NOT log in as gcms.
If somebody is already logged in

1. Somebody is logged in and the instrument is idle, or
2. Somebody is logged in and the instrument is running a sample.

You should see a window that looks like this:
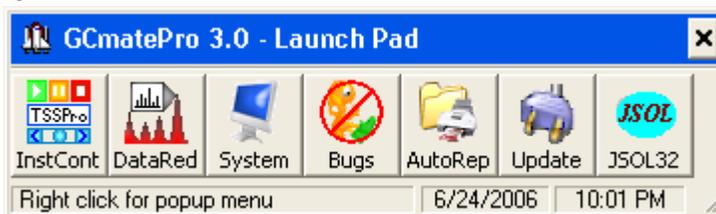
(If you don't see Launch Pad click Start Menu → All Programs → TSS Pro 3 → Launchpad.) The first button starts "instrument control" which is equivalent to data acquisition. The second button starts "data reduction" which is mass-spec-speak for data processing. You will **never** use any of the other buttons.

Start "Instrument Control" by clicking the InstCont icon. You should get a window that looks like this:

Make sure the "Tune Mode" button is depressed on the graphical menubar.
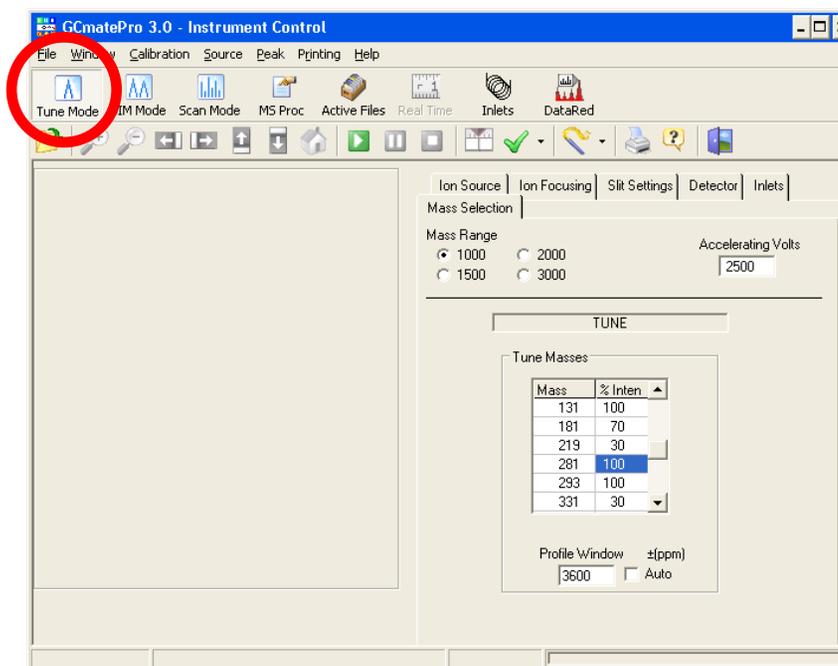
Click File→Open File…
The file open dialogue appears.
Select LOAD_ME_LR from the list.
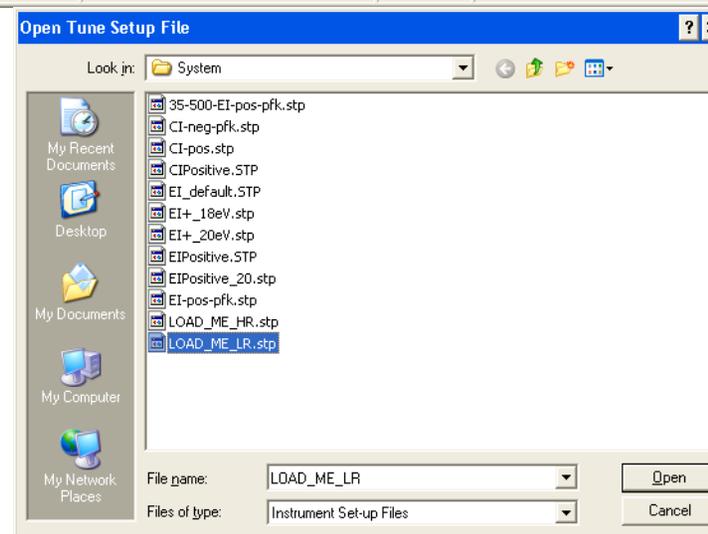
Press the green "Play" button:

Click the "Mass Selection" tab…
…then select 281 from the "Tune Masses" list by *single* clicking it. (If you accidently double clicked it just click "Cancel" in the window that pops up.)
You should see a single peak somewhere in the window. If you don't, find Ivan or Tony.
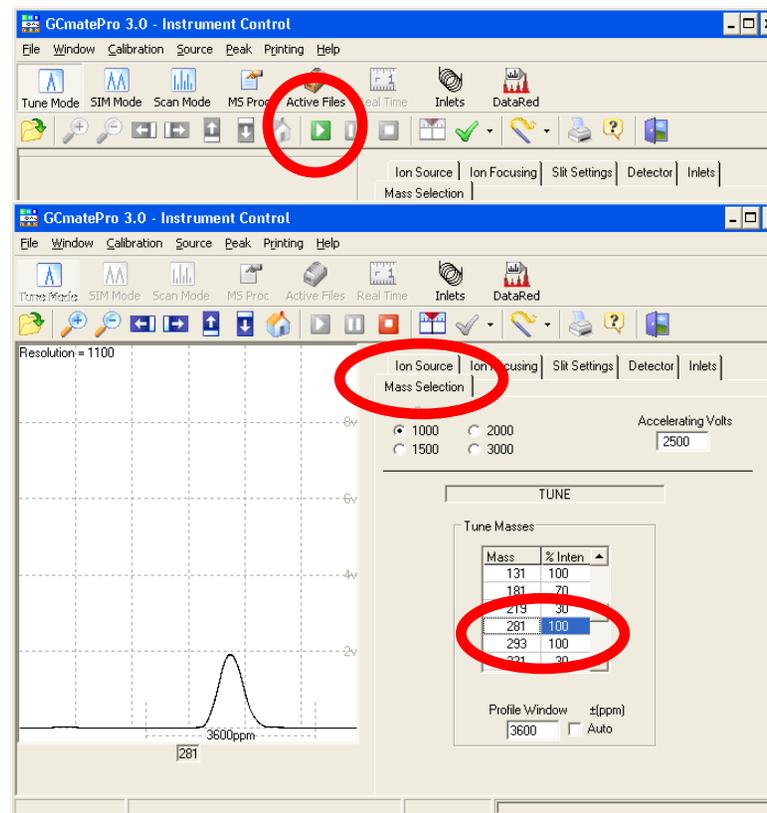
The peak will probably be off-center. Double click the top of the peak to center it.
Click the "wizard's wand" icon to start the automatic tuning routine.
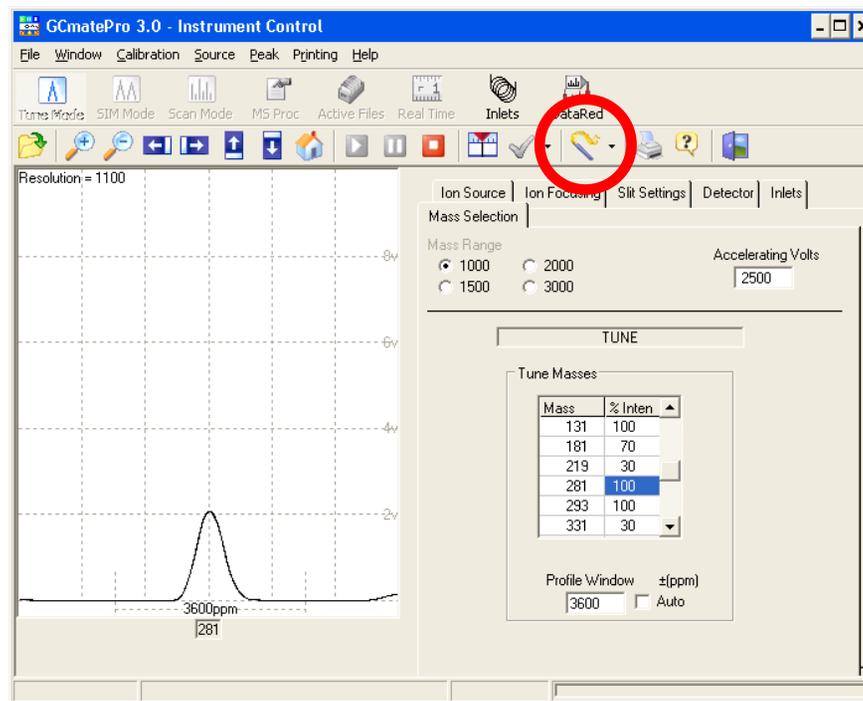
A new window pops up and the display goes blank.
At the very bottom of the Instrument Control window you will see a message saying "Waiting for signal to stabilize" or "Autotune in progress".
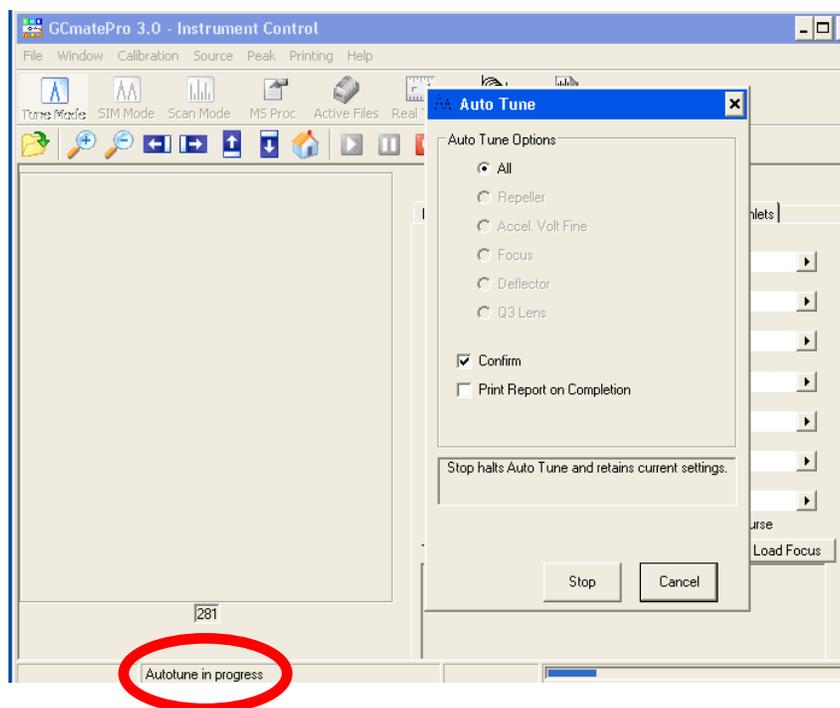
When Auto Tune is done it will display the new and old peak shapes. If the new one looks better than or the same as the old (which should almost always be the case) click the OK button in the Auto Tune window.
If the new peak shape looks worse than the old one, please tell Ivan or Tony, because something is wrong with the instrument.
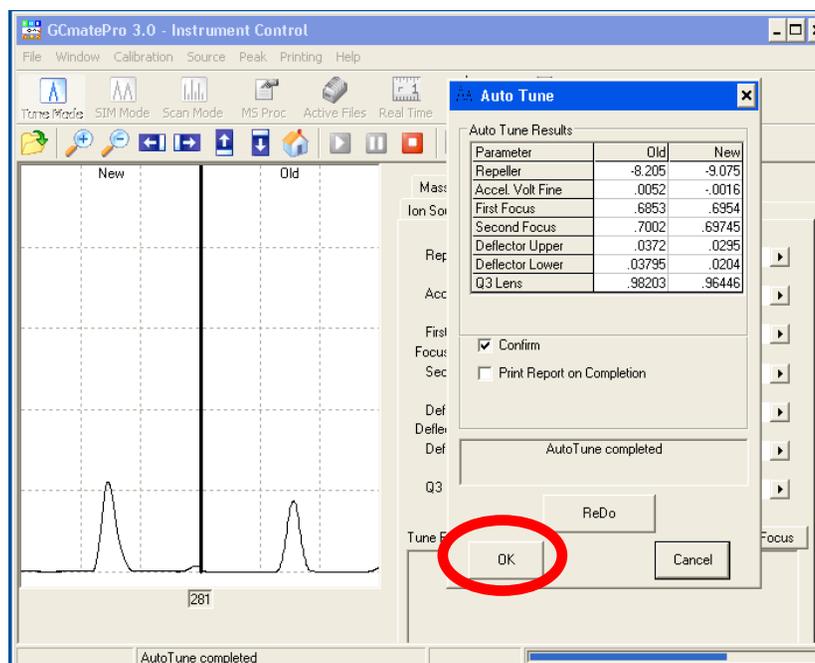
Click the red "stop" button.

Click the "MS Proc" button.

In the unlikely event that a pop up window opens with a list of calibration files select 35-500-03-B.cal and click Open. **Otherwise ignore this step!**
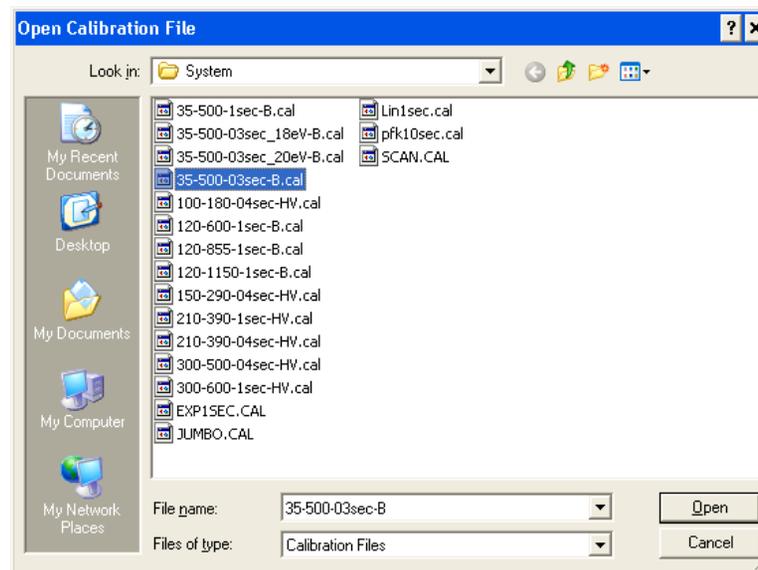
Click File→Open File

Select GC_35-500-LR_10min.MSM from the list regardless of what you are doing later.
[The purpose of this step is to make sure you have all the relevant settings available to you when you set up your GC-MS experiments. If the last person did direct probe work or did not use the autosampler, some settings would be grayed out.]
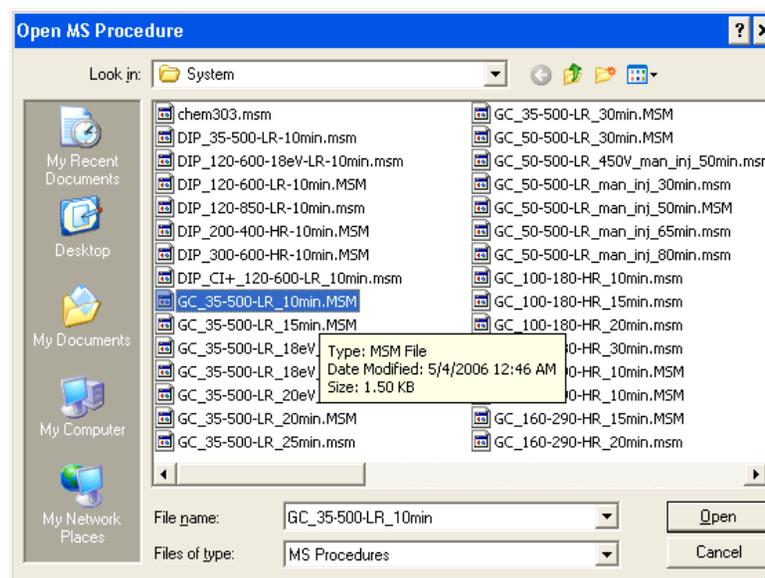
Click the "Active Files" button…
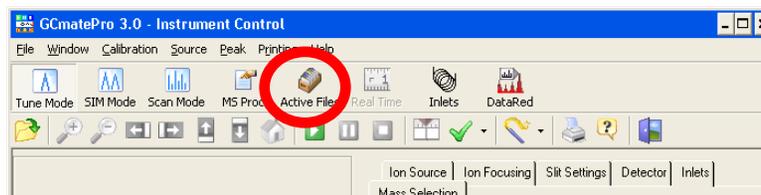
…and the active file list opens.
Click Edit→Add Line
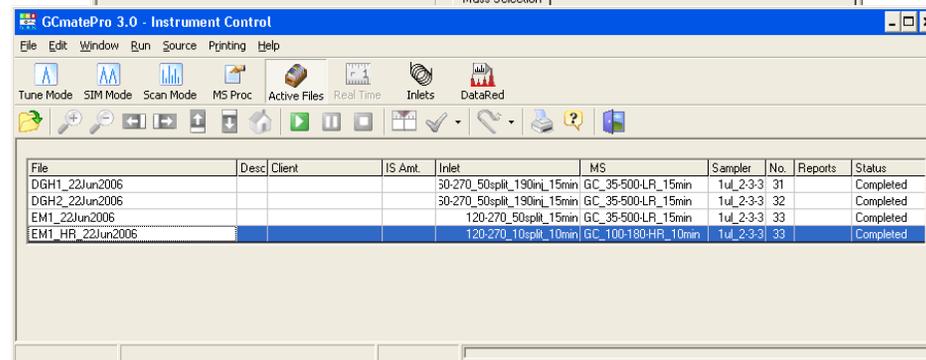
Find your directory and enter the name of your file
(preferably including your initials).
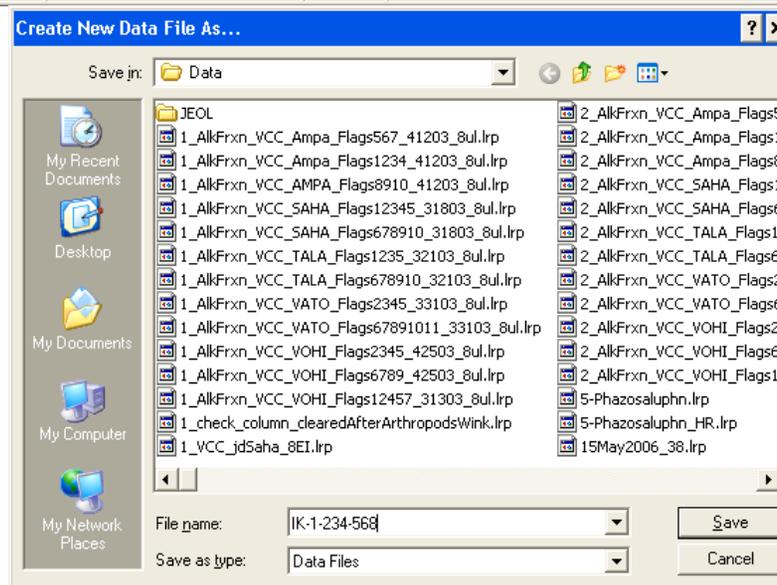
Fill in the form that opens:
File: Clicking on the name re-opens the "Create new data file as…" dialogue.
Sample Description: This is a pair of text fields, each 256 characters long. The content of the first box is printed in the plot headers.
Client: Enter you name.
Internal Std Amount: Ignore this one.
Inlet procedure: Click the text box to get a list of GC methods and select the appropriate method. The naming convention for inlet procedures is as follows: The first number is the initial oven temperature; the second number is the final temperature. An additional number means that there are two different ramp rates in the program. The number followed by "split" is the split ratio. Use lower split ratios for more dilute samples. If there is a number followed by "inj", it represents a non-standard injector temperature (default is 230 °C). Use lower injector temperatures if your sample is showing signs of decomposition in the injector. The last number is the total length of the program in minutes.
Example #1: 120-270_50split_20min starts at 120 °C and ramps to 270 °C with 50 split in the injector and a total duration of 20 minutes.
Example #2: 60-100-300_splitless_190inj_45min starts at 60 °C, heats to 100 °C, changes the ramp rate and heats to 270 °C. The entire sample volume is pushed onto the column (splitless injection) with a 190 °C injector temperature. Program duration is 45 minutes.
MS Procedure: Click the text box to get a list of MS methods. Select GC_35-500-LR_NNmin, where NN is the length of your GC method. The length of the GC method and MS method **MUST** match each other.
Autosampler Procedure: Click and click cancel to set it to 'Current'.
Location: Enter the number of the autosampler position that holds your sample
Click OK.
The "Create new data file as…" dialogue opens again. If you want to run more than one experiment, enter the name for the next sample. Otherwise, Click Cancel to close it.

There should be a new line(s) at the end of the active file list corresponding to the analysis (analyses) you just set up. Double check that everything is set up the way anted it. If it isn't, double click the line to bring up the "Data File Description" dialogue. To remove an analysis from the queue, left click the file name so that the name is highlighted with a dashed line, then right click and select "Delete Line" from the dropdown list
If everything is fine press the green "play" button to start the data acquisition…

…which switches you over to "Real Time Display" mode.
The status bar tells you what the instrument is doing. Initially, it will likely tell you that it's "Waiting for Inlet T = …". Once the GC is ready the autosampler will inject your sample and the MS will turn on automatically after a 3.5 minute solvent delay.
If you changed your mind, click the "stop" button and click "Yes" when asked if you want to cancel the injection.
After the injection has been made, you must wait until the GC program is complete before you can do anything else.

If you set up multiple analyses they will run automatically in the order that they appear in the active file list. To change the order of the samples, left click on the line you want to move, then right click and select "Move Line" from the dropdown list. Click the line above which you wish to insert the line you are moving.