

GC-MS protocol and Qualitative Analysis:

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Creating a method file:

- i) On the desktop click "Real Time Analysis"
- ii) Click on 'Data Acquisition' at the top left corner
- iii) Select 'New method File' on the 'File' menu.

GC parameters _ click on 'GC' tab and set the analysis conditions.

1. Column oven temperature (45°) - this shows initial column oven temperature and can vary (40-100°C) depending upon the samples and methods. The sample could be run at a constant column temperature it can be ramped up depending upon the requirement. For example, initial final temperature could be 65 °C for 1 min (hold time) and then it can be ramped up to 300 °C at 12 °C/min or 15 °C/ min and then hold for 2 min at 300 °C.
2. Injection temperature (250 °C) – it is based on consideration of the boiling point of the target compound (200 - 300 °C)
3. Injection mode – select 'split' or 'splitless'.
 - Split mode (20) simply represents that your sample is splitting and not all of it is getting through the system. This mode is used if the concentration of target compound is high. As a rough guideline, select this mode when the target compound concentration is greater than 10 ng / μ L. This is commonly used mode. Select splitless if the concentration of target compound is low, below 10ng / μ L.
4. Flow control mode – Select 'pressure' when the method calls for a constant pressure mode and select 'liner Velocity' when the method calls for the constant linear velocity mode for the carrier gas. When no reference method is available, select 'linear velocity'.
5. Pressure Setting for Carrier Gas – Carrier gas has been already set up at around 60 kPa pressure (Refer to GC-MS operation guide for detail information).
6. Linear Velocity – it will be automatically set up according to pressure value for the carrier Gas.

NOTE: "Use default values for parameters that are not covered by the above explanation."

MS Parameters – click the 'MS' tab and set the analysis conditions.

1. Ion Source Temp : 200 °C (Do not change this)
2. Interference Temp : 250 °C (200-300 °C)
3. Solvent cut time: 2.5 min
 - Depends upon retention time of the solvent peak. In the absence of information about the elution time of the solvent peak, set 'Start Time' to zero minute and set 'End Time' to the 'GC Program Time' value. After one analysis of the solvent, and obtaining the solvent peak profile, change the 'Start Time' to the time after the end of the solvent peak. For example we have set a 'Start Time' of 2.5 min for hexane solvent.

NOTE: hexane peak comes before 2.5mins, Do not use lower than 2.5mins, it may saturate the machine. For gas sample do not put any cut-off time.

4. Click 'Relative to the Tuning Result'
5. Select 'Scan'
6. Enter the mass range to be measured, where 'Start m/z' is the lower mass limit, and 'End m/z' is the upper mass limit. In this system we have set up 50 for 'Start m/z' and 1090 for 'End m/z' (for detail refer to GC-MS operation guide).

NOTE: Use default values for parameters that are not covered by the above explanation.

Saving the method file:

1. Select 'Save Method File As' on the 'File' menu.
2. Enter a file name and click 'Save'.

Running a sample on GC-MS:

Sample preparation:

Sample could be head space air for volatiles study or liquid like lipids or terpenes etc. there are different syringe for air and for liquid sampling. For volatile study, use the paper syringe with valve.

Volatile samples:

Cultures grown or standard prepared in an air tight system with the septum. Head space air then sampled by the help of syringe through the septum without touching the liquid broth.

Liquid sample preparation:

In our lab, we always prepare our sample in hexane (a solvent). Once lipids or terpenes are extracted from the respective samples, it should be first dried out under N₂ gas to remove all the water. Then it dissolved in the solvent, hexane (20-200 µL or more).

NOTE: Do not inject any water, phenol, chloroform or any other samples or any other things into GC-MS. Please contact Dr.Basu : 970 313-7441

Method file set up for GC-MS run:

1. Open the method file from the file menu.
2. Click on 'sample log in' to label your data file.
3. Click on 'Download' – after you hit the download, wait until system becomes ready.
 - Check on the top right corner. It should show GC- Ready (color should be green) and MS-ready (color should be green). Also, On GC there are small bulbs for 'status', 'temperature' and 'Flow'. All should be green. Once you check all these, you are ready to inject your sample into GC-MS.

If your sample is gas, you can load up to 1-25µL (10µL) of the head space air sample from the vial depending upon the concentration of the target compounds.

If your sample is liquid, then take 1µL of the prepared liquid sample by the help of syringe.

NOTE: In the liquid sampling, it is very common to have air bubble in the syringe. Be very careful and remove all the air bubbles before sample injection. Also be sure to use right syringe for different samples.

Sample Injection and the GC-MS run:

Once you have extract amount of sample in the syringe, you can inject the sample through the injector port on the top of GC. Be very careful, you need to finish the injection and right away you have to hit the start button for a better result. Many peaks, single peak or no peak at all can be resulted depending upon the samples. The peak will appear at a certain retention time (RT).

Peak Identification

Peak identification can be done either during the sample run or after the run.

During run: One can check the similarity of the certain peak which appeared during the run by clicking in the middle of the peak by keeping the cursor in the center of the peak and hit on 'Similarity Search'. It will come up with at least 20 compounds with different mass spectra similarity for the peak. Only the compounds with more than 90% similarity with the mass spectra are good enough for the consideration.

After run: Once your run is finished, you won't be able to perform 'Similarity Search'. In this case, you need to go back to the desktop

- Double click on the 'Post Run Analysis'

- Open your file (go to file and click on 'Open a file' and then go to data or certain window where you have saved your file.
- After opening your file, you will be able to see full length of your GC run on the screen. You can scroll it to scan the whole length. You can also scroll up and down to see the whole length of the peak.
- Then, select your peak, click in the center of the peak and hit 'Similarity Search' as described above.

NOTE: Once you have the certain identity match to your compound of interest. Then, for the final confirmation you need to run a standard for your compound of interest in the exact same condition.

Quantification:

Once you know the retention time and MS match for your compound of interest. To quantify how much of your compound of interest is present in the sample, your compound of interest. To quantify how much of your compound of interest is present in the sample, you can note down the peak area of the compound of interest. While taking peak area, run triplicate of your sample and then take average peak area. Do the same thing for standard (triplicate run and take average). Then you can compare peak area of your sample and the standard. Since, the concentration of the standard is known, you can calculate the concentration of the compound of interest by using simple formula.

$$A_1C_1=A_2C_2$$

Where, A_1 and C_1 are average peak area and the concentration of the standard respectively and A_2 and C_2 are the average peak area and the concentration of the compound in sample respectively.

Reference:

Gas Chromatograph Mass Spectrometer GCMS-QP2010 Operation Guide for GCMS solution Ver. 2.6, SHIMADZU

