Sample Preparation

- Some things to consider when preparing your sample:
  - Make it as dry as possible. Any water remaining in solution will make it more difficult to vaporize your compound.
  - The boiling point of your compound should be low. Larger compounds such as proteins, polymers or other high molecular compounds are not volatile enough to ionize unless they are functionalized to be more volatile.
  - The compound should be able to completely dissolve in a weak polar solvent with a low boiling point. Examples include diethyl ether, dichloromethane, and hexane. This will allow the solvent to elute first and not interfere with the compound signals.
  - If the compound has a low boiling point, use a solvent that has a higher boiling point so that it will elute last.
  - NEVER inject a neat sample. Always use an appropriate solvent.

Initial Steps

- Check that the output pressure is around 80 psi.
- Check the gas cylinder level is sufficient for your run.
- The gas should be an inert gas that can carry the compound but not interact with it.
- He, N₂, H₂ and Ar are often used where He and N₂ are the most common.
- He is preferred because it can maintain a high velocity when travelling though the column reducing the analysis time. N₂ is cheaper but longer analysis is required since the velocity range of N₂ is narrow and slow.

- If the program is not open already, double clicking the icon located on the desktop to start the GCMS Real Time Analysis software.
- Most often the program is already open and in Ecology Mode. Click Cancel to start the instrument initialization. This may take a few minutes.

Data Acquisition Setup

- Once the program opens, on the left menu options, select to go to the acquisition setup window.
• On the right side of the window, check the GC Consumables by hovering over the icon. This will show how often the septum or glass tube have been used. If they are used more than their suggested usage, contact a SIF staff member for replacement.

• Open a previous method file that you or someone in your lab has made before.

  Click on or go through the menu File -> Open Method File. If you do not have one to start with, you can use a SIF default method file (see figure for options).

• This will read in the hardware configuration and acquisition settings.

Setting Autosampler Parameters

![Autosampler Parameters](image)

• Change the number of rinses that are appropriate for your sample. Setting 2 for each option is a good starting point.

• All other parameters should remain as shown.

Setting GC Parameters

• Column oven temperature – set the initial temperature of the column. Range is usually between 40 and 100°C. Set to 50°C.

• Injection temperature – this should be set based on the boiling point of the target compound. Range is usually between 200 to 300°C. 250°C is the default.

• Injection Mode –
  o Split – Use this mode if the target compound concentration is high (> 10 ng/µL). A high gas flow is used and will generate narrower peaks.
  o Splitless - Use this mode if the target compound concentration is low (< 10 ng/µL). The gas flow will be slower and a Sampling Time will be defined to dictate how much of the sample goes to the column. This could lead to peak broadening.

• Flow Control Mode – Most often, Linear Velocity is selected.
  o Pressure – the pressure setting of the carrier gas. Typical range is 75 to 150 kPa. Start by using 121.6 kPa.
  o Total Flow – the rate of the gas flow. The rate value will depend on whether Split or Splitless injection mode is selected.
  o Column flow – is the rate of the gas flow that goes to the column. This is much lower than the Total flow. 1 to 2 mL/min is a good starting point.
- **Linear Velocity** – the rate is automatically calculated
- **Split Ratio** - defines how much of the sample splits between the column and the split vent. This ratio can be between 5 to 500. The lower the ratio, more sample will pass to the column. For Split mode set the ratio to 50. For Splitless set the ratio to -1.0.

**Program Column Oven Temperature**
- To separate target compounds the column oven temperature can be programmed to be a specific temperature at certain time points.
  - **Rate** – this is the change in temperature per minute (°C/min)
  - **Final Temperature** – the desired temperature at a specific time point
  - **Hold Time** – the time to keep the column at the specific temperature
  - **Total Program Time** – will be calculated based on the above values.

**Setting GC Flame Ionization Detector Parameters**

- **Temperature** – set the inlet temperature of the sample
- **Do not change the Flow Rates**
- **Check the Signal Acquire box if the GC signal is to be recorded**
  - **Sampling Rate** – The amount of time to record a signal. **40 msec is the default.**
  - **Stop Time** – this will automatically use the value from the GC program set in the GC tab

**Setting MS Parameters**

- **Ionization Mode**
  - **Negative Chemical Ionization (NCI)** – produces negative ions using electron capture process with a buffer gas such as methane. Electron capture is a very low energy process and the recorded mass spectra contain little or no fragmentation.
  - **Semi-Chemical Ionization (SCI)** – ionization is achieved through gas-phase chemical reactions between the analyte and ions generated from a reagent gas such as methane. Molecules are ionized by transfer of an electron, a proton or other charged species.
Semi-Electron Ionization (SEI) – electron impact ionization is performed. When an electron generated from a filament comes close to neutral molecules, it has enough energy to pull/remove outer shell electrons producing additional free electron and positive ions.

- Ion Source Temperature – This is the temperature used to create ions. Typically set between 200 and 300°C, start with 225°C. Higher temperature will result in better resolution, but more fragmentation.
- Detector Voltage – Is used to direct the ions to the detector. Use the default value (0.9 kV)
- Interface Temperature – the interface temperature is the temperature at the stage between the GC and MS. It is often used to remove the carrier gas.
- Solvent Cut Time – The amount of time to allow the solvent signal to pass prior to starting the detector. This is usually set to 0.5 minutes less than the start time setting.

**Fill in the MS table settings**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Start Time (min)</th>
<th>End Time (min)</th>
<th>Acq. Mode</th>
<th>Event Time(s)</th>
<th>Scan Speed</th>
<th>Start m/z</th>
<th>End m/z</th>
<th>Q1 Resolution</th>
<th>Q3 Resolution</th>
<th>Ch1 CE</th>
<th>Ch1 CE</th>
<th>Ch2 CE</th>
<th>Ch2 CE</th>
<th>Ch3 CE</th>
<th>Ch3 CE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/1</td>
<td>0.25</td>
<td>0.50</td>
<td>Q3 Scan</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>High</td>
<td>Low</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>1/2</td>
<td>0.00</td>
<td>0.00</td>
<td>Q3 SIM</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>Unit</td>
<td>Unit</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>1/3</td>
<td>0.00</td>
<td>0.00</td>
<td>Product Ion Scan</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>Unit</td>
<td>Unit</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>1/4</td>
<td>0.00</td>
<td>0.00</td>
<td>Neutral Loss Scan</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>Unit</td>
<td>Unit</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

- First Column (1-1, 1-2, 2-1 etc.) – First number refers to the group of events occurring in the same time range. The second number is the event number within the group. Events will the same start time will automatically be placed in the same group.
- Compound Name – Label the compound that you expect to observe with these settings
- Start/End Time – Set the time of the flow. If you know when the solvent peak appears you can adjust the start time to ignore this peak. To find out, run one analysis of the solvent and determine when the solvent peak appears.
- Acquisition Mode
  - Q1/Q3 Scan
    - These perform only one stage of mass analysis. Q1 or Q3 is used as the mass analyzer scanner to obtain a complete mass spectrum. The other assemblies are used as ion transmission devices.
    - This method is used to determine or confirm the m/z (identity) of unknown compounds or the m/z of each component in a mixture of unknown compounds.
    - Full scans can give more information about an analyte than SIM scans but has lower sensitivity.
  - Q1/Q3 Selected Ion Monitoring (SIM)
    - This method is used to monitor a particular ion or set of ions. Q1 or Q3 is used to selectively record an ion from the column.
    - This is useful in trace analysis and in rapid screening of a large number of samples for a target compound.
    - Since only a few ions are monitored this will have lower detection limits and faster to perform compared to full-scan modes.
    - Must know what ions or reactions you are looking for.
    - **Change the Absolute Detector Voltage to 0.2 kV to start.** Typically set between 0.1 and 0.3 kV.
- Multiple Reaction Monitoring (MRM)
A specific precursor ion is selected in Q1 among various ions, fragmented in the collision cell (Q2), and a specific ion among the fragments is then detected in Q3.

This is less affected by impurities compared to SIM, enabling quantitative analysis with high selectivity.

**Change the Absolute Detector Voltage to 1.8 kV.** Typical range is between 1.6 and 2.0 kV.

### Product Ion Scan
- Performs two stages of analysis:
  - Ions formed by the ion source enter Q1 which is set to transmit ions of one m/z (precursor ions). These ions then transfer to Q2 which is surrounded by the collision cell.
  - The ions in the collision cell can fragment the precursor further to produce product ions. The product ions then go into Q3 for mass analysis.
- The mass spectrum shows the product ions produced for the fragmentation of the selected precursor ion.
- This scan determines the m/z values of all the product ions from a specific precursor.

### Precursor Ion Scan
- Performs two stages of analysis:
  - Ions formed by the ion source enter Q1 which is set to transmit ions of one m/z (precursor ions). These ions then transfer to Q2 which is surrounded by the collision cell.
  - In the collision cell precursor ions can fragment produce product ions by unimolecular decomposition of metastable ions or by CID. The ions from the collision cell then moves into Q3 which then transmits selected product ion for mass analysis.
- The final spectrum shows all the precursor ions that fragment to produce the selected product ion.

### Neutral Loss Scan
- Q1 and Q3 scan at the same rate and the same mass range width. The mass ranges are offset by a selected mass so that the product mass analyzer scans a selected number of mass units lower or higher than the precursor mass analyzer.
- Q1 separates ions that from in the ion source by their m/z values, which then passes into the collision cell. Here the ions fragment further and go into Q3 which separates the product ions by their m/z value.
- For neutral loss mass spectrum, the detectable ion must lose a neutral moiety whose mass is equal to the difference in mass ranges being scanned.
- For a neutral gain (association) mass spectrum, the detectable ion must gain a neutral moiety whose mass is equal to the difference in mass ranges being scanned.

- **Event Time** – the time at which data will start acquiring at each event.
- **Scan Speed** – the rate at which ions coming out of the trap are recorded. **Set to 10000.**
- **Start/End m/z** – The mass range limits to detect.
- **Q1 and Q3 Resolution** – The resolution of the Q1 and/or Q3 channel. Use a low setting for faster results.
- **Ch1/2/3 m/z (MRM)** – the mass of the precursor ion > mass of the product ion m/z. Up to 16 ions can be set.
- **Ch1/2/3 CE** – The collision energy of the channel. **A good starting point is 5.** Increase if compound is less labile.

**Save the Method File**

- Go to the File menu and select “Save Method File As…”
- Navigate to “C:\GCMSolution\Data\NYUid\”
- Enter a File Name and press Save.

**Acquire Data**

- Create a batch file by opening the Batch Assistant bar by pressing the icon on the left. If you are still in the Data Acquisition Menu on the left press the icon to return to the main menu.
- Create a new batch file through the menu File -> New Batch File.
- Open the Batch Wizard by clicking on the icon and follow the wizard to setup the batch table

**Batch Table**

- **Batch Table** – Select if you are going to create a new table or append to the currently open table
- **Batch Type** – Always select **Line1+Line2** to acquire both FID and MS data.
- **Sample Type** – **Select Unknown**.
- **Method** – Select the NYU standard method or the method you have created previously.
- **Data Processing** – Select Qualitative for analysis to be performed after acquisition.
Sample Information
- Here you will specify the starting vial number location, Sample Count (number of samples you have), Injection Volume, and Sample Name/ID
- If multiple samples are analyzed you can have the name and ID automatically incremented.

Data Information
- Here you will input the file name of the data and select if a Report is to be generated at the end.
- Press Finish when done.

- Once done, a batch table should show with the first line populated. You can switch to the batch table using the tabs in the Data Acquisition window.

- To add another sample to the table you can either go through the wizard again and append to the table or right-click on the table and select Insert Row.
- If adding a row, you can double click on a field to edit the entry. To change the data file location double click the Data file field and change as needed.
- Once completed, you can save the batch file through File -> Save Batch File As...

- Press the Start icon on the left assistant bar to start the analysis.
- You can add samples while the analysis is already running, by creating a new batch file following the above steps, and in the menu select Batch -> Batch Queue and add the batch file you just made.

Ending Your Session
- Once the acquisition and analysis are completed remove your sample and put the instrument in Ecology Mode by pressing the icon in the right status section.