

Introduction-

Mass spectrometry is a very sensitive analytical technique. It is possible to perform routine analyses on as little as 1 picogram of sample. That's 0.000000000001 g or 10^{-12} g! In fact, it is easy to use too much sample, in which case the excess material may interfere with subsequent analyses. In GC-MS work, a solution of the sample to be analyzed is injected into a gas chromatograph. As the components of the sample elute from the chromatographic column, each is directed into the mass spectrometer. It is important in doing GC-MS work that the solutions you prepare contain the appropriate concentration range. As a rule of thumb, the concentration of the sample you will analyze should be approximately 10^{-8} g/ μ L. Since the nominal sample size will be 1 μ L, this means that you will be analyzing approximately 10^{-8} g of material.

Since MS is so sensitive, it is also important that you minimize the presence of contaminants in your samples. One of the most common contaminants is the plasticizer used in disposable pipet tips, plastic tubing, plastic caps, etc. To avoid such contaminants you should use glass pipets and syringes when you prepare your samples. Once you have prepared a sample of the appropriate concentration, you should store it in a glass vial with a teflon-lined cap.

Sample Preparation

Stock Solution A

1. Transfer 3-4 mL of the appropriate solvent into a clean, dry vial. This should be enough solvent to prepare your sample and to clean the syringe after you have finished.
2. Add 10 mg (0.0050-0.0150 g) of sample to another clean, dry 4 dram vial.
3. Using a clean, dry syringe, add 1 mL of solvent to your sample. Cap the vial and mix thoroughly. The nominal concentration of this sample is 10^{-5} g/ μ L.

Sample Solution B

1. Using a clean, dry 10 μ L syringe, transfer 1 μ L of stock solution A to a clean, dry 4-dram screw-capped vial with a teflon-lined cap.
2. Using a clean, dry syringe, add 1 mL of solvent to to the 1 μ L of stock solution A. The nominal concentration of this sample is 10^{-8} g/ μ L. Cap the vial and mix thoroughly.
3. Label the vial with your name and sample identification.

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Before you record any spectra, you have to have a place to store the data that you will collect. That means you will have to create a personalized folder. **All data files are stored on the D drive** of this computer. The path to the directory in which you will create your personal folder is **D:\Xcalibur\Sprectra2006**. If you look in the Spectra2001 directory, you will see several folders, one of which belongs to Polly Ester. The name of Polly's folder is EsterP. Since Polly is an OChem prodigy, the spectra she has recorded will serve as reference spectra for you to compare to your spectra.

Creating Your Own Folder-

1. Shrink this window and position it so you can see the My Computer icon on the desktop.
2. Open My Computer and navigate to **D:\Xcalibur\Sprectra2006**.
3. Open the Spectra2006 folder.
4. Select New/Folder from the File menu.
5. Follow Polly Ester's example and assign a name to the new folder, i.e. your folder name should follow the convention LastF, where Last is your last name and F is the initial of your first name.
6. Close all the windows that you opened in order to create your folder.
You are now ready to collect data. The directions for doing so are listed under the heading Setting up a sequence.

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Adjust the size and position of this window so that you can see the icons on the left side of the desktop.

These directions are specific for the analysis of volatile samples. Separate instructions are available for the analysis of non-volatile samples.

In the context of this course a compound is considered volatile if it is a liquid or a solid that has a reasonable vapor pressure at temperatures normally encountered in gas chromatography. By this measure stilbene and diphenylacetylene are volatile. Gas chromatography is the method of choice for introducing volatile materials into a mass spectrometer. This combination of techniques is called tandem gas chromatography-mass spectrometry (GC-MS). In GC-MS a solution of the sample of interest is injected into the injection port of a GC. The components of the sample flow through the column at different rates. When a compound exits the column, it is directed into the mass spectrometer where its mass spectrum is recorded.

Getting Started

The program that controls the GC and the MS is called Xcalibur. The directions that follow assume that Xcalibur is not running. If any Xcalibur windows are open, close them before you attempt to set up your operating procedures.

Setting Up Your Operating Parameters

1. Position this window near the bottom of the screen. Adjust its height so that it fills the bottom half of the screen.
This will allow you to view these directions and the Sequence Setup window at the same time. You will want to arrange the Sequence Setup window so that it is visible above this window.
2. Start the program by double clicking the Xcalibur icon on the desktop.
After a brief interval this will open a 2-frame window called "Roadmap-Homepage". The left hand frame of this window provides information about the status of the GC and the MS. The right hand portion of this window contains six icons. The one you are interested in initially is called "Sequence Setup".
3. Click on the Sequence Setup icon.
A spreadsheet containing 9 columns will appear. You will have to scroll to the right to see the headings of all 9 columns. Steps 4-9 involve entering information into the appropriate rows of most of these columns.
4. Select Open from the File menu and navigate along the path
D:\Xcalibur\CHY252meth.
Inside the CHY252meth folder is a set of sequence files, each of which defines specific operating parameters for a specific sample. The file named Stilbene100_225, for example, is the file you would use to analyze a sample of the stilbene you prepared.
5. Select the appropriate sequence file and click Open.

This loads into the spreadsheet all of the information you need to perform an analysis except the File Name, Sample ID, and Path

6. Select the appropriate cell in the File Name column and assign a name to the data file that the program will create once you have recorded the GC-MS.
Your file name should be descriptive of the sample you are analyzing; for example, Wittig reaction, or stilbene, or diphenylacetylene, etc.
7. In the Sample ID column enter the identification code for your sample:
Use the convention FLddmmyy_nn, where F and L are the initials of your first and last names, dd, mm, and yy refer to the date, and nn indicates the number of the sample, i.e. first, second, etc. As an example, the Sample ID for the second sample that Polly Ester ran on September 13, 2001 would be PE091301_02.
8. Double click the appropriate cell of the Path column.
A window called Select Directory will open.
9. Navigate to D:\Xcalibur\Spectra2001, select your folder, and click OK.
This tells the computer to store the data that you collect in the appropriate place. This completes the set up. You are now ready to inject your sample into the GC. However, the instrument is not. If you look near the bottom of the Status window, you should see a message saying that the GCQ/Polaris MS is ready to download. You should see the same message beneath the Trace GC 2000 heading.
10. Select "Run This Sample" from the Actions menu and click OK in the window that opens.
The status of the GCQ/Polaris MS at this point should read Ready to Run. The status of the Trace GC 2000 may read Ready to Run, or it may read Preparing for Run. Once both the MS and the GC are ready, the status messages will both change to Waiting for Contact Closure. This means that the instrument is ready for you to inject your sample.

Injecting Your Sample

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Injecting your sample is the easiest part of the entire process. The injection port is located on the top of the Trace GC unit. While making an injection is very simple, there is a proper technique to filling a 10 μL syringe. **It is important to remember that these syringes are both delicate and expensive. Handle them with care. Take precautions not to kink the plunger of the syringe.**

Filling the Syringe

1. Immerse the tip of the needle of the syringe into your sample.
You should be able to hold your sample vial and the barrel of the syringe in one hand while manipulating the plunger with the other.
2. Slowly draw the plunger back until you have approximately 1 μL of liquid in the barrel.
3. Remove the needle from the sample and pull the plunger back until you have about 3-4 μL of air in front of your sample.
At this point you should have about 1 μL of sample sandwiched between two layers of air.

Injecting Your Sample

1. Insert the needle of the syringe through the septum in the injection port.
Do not force the needle. If you feel resistance, back off and try again.
2. Depress the plunger and withdraw the needle from the injection port.
You should try to do this in one smooth, continuous motion.
3. Press the Start button.
The status window should indicate that both the GCQ/Polaris MS and the Trace GC 2000 are running. A complete run takes about 10 minutes.
4. While you are waiting, clean the syringe (click instructions below).

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Once data collection is complete, the data is stored in your folder in the file that you specified in the File Name column of the spreadsheet that you filled in during the Sequence Setup. In order to view this data you use a program called Qual Browser. This program will also allow you to print your spectra as well as to compare your mass spectrum to a library of mass spectra of known compounds.

Instructions

Displaying your data

1. Click on the Roadmap View icon in the upper left corner of the Sequence Setup window. On the Roadmap, Click Qual Browser.

A new window called Qual Browser will open.

2. Select Open from the File menu.
3. Navigate to D:\Xcalibur\Spectra2001\<<your folder>\<your file>.
4. Select your file and click Open.

This loads a window called Raw Data into the Qual Browser window. The Raw Data window contains the GC and the MS data in two separate cells. The abscissa of the MS graph is labeled m/z. The abscissa of the GC data is labeled Time(min). Each peak in the GC cell corresponds to a compound that is present in your sample.

5. Click on the “push pin” in the upper right hand corner of the cell containing the MS data.
6. Position the cursor over the tallest peak in the GC frame and click the mouse.

This command tells the program to display the mass spectrum that corresponds to the peak that you clicked on.

Printing Your Spectra

1. Select Print from the File menu of the Qual Browser window.
The options “All Cells in the selected window” and “One Page” should be selected in the Print window that opens.
2. Click OK, then click OK again in the next Print window.

Performing a Library Search

1. From the Actions menu of the Qual Browser window, select Library/Search.
This action initiates a search of the NIST library of MS data files. After a brief delay the program returns a prioritized list of structures whose MS spectra are most similar to your spectrum. The structure of the first compound is shown in a frame adjacent to the listing of search results.
2. Click on any row of the “Hit” column to display the structure of the corresponding compound.

CHY 252/254
Cleaning 10 μ L Syringes

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An apparatus for cleaning 10 μ L syringes is set up by the side of the sink in the **Demonstration Area** in the lab. (This is outside the door to your right.) These instructions are posted there as well.

Instructions-

1. Remove the plunger from the syringe and gently wipe it with a Kim Wipe that you have moistened with the solvent you used for your sample. Take care not to bend or kink the plunger.
2. Turn on the aspirator.
3. Insert the syringe into the hole in the stopper in the suction flask.
4. Using a Pasteur pipet, add 5-6 drops of solvent to the top of the syringe, letting the suction draw air through it between drops.
5. Remove the syringe from the stopper after the barrel is dry.
This generally takes about 30 seconds.
6. Turn off the aspirator.