Student Prelab Manual

Problem: A historian from the National Museum of Civil War Medicine in Frederick, Maryland, discovers what he believes to be a mid-1800's medical kit on a Civil War battlefield near Knoxville in eastern Tennessee. Inside the kit is a small vial of liquid whose identifying label can no longer be read due to faded ink. Suspecting that the vial contains an anesthetic used in the Civil War, he sends the liquid to a laboratory to be examined. What is in the vial?

Consider: Formulate a strategy to solve the problem before going to the virtual lab. ***Some*** of the points you will wish to consider follow.

Undoubtedly you will want to start by reading the General Anesthetics case study single web page once you get to the web site. Additional detail about the history of general anesthetics, as well as other information, is available via the "Detailed Background" link if you care to access it. (If you go straight to the Lab without first picking a case study to read, you will not be able to complete the assignment.)

A valid assumption to make is that the unknown is pure and is only one of the compounds listed in the "Table of Some General Anesthetics" on the web site. Remember that this is an assumption, and can in fact be wrong. The data you collect may support or disprove the assumption (as well as provide the evidence you need to unequivocally identify the compound).

One of the earliest decisions you will have to make is "What type of mass spectrometer should I use?" If you need an introduction, a general refresher, or a specific reminder about mass spectrometry techniques (such as GC/MS) or some aspect of it (such as what a TIC is), visit the Tutorial link and the links thereupon.

After selecting the instrument you plan to use (by clicking on the picture of that instrument) you will need to prepare your sample for the instrument, and this is accomplished in the wet lab. Then proceed to the instrument.

Set the instrumental conditions necessary to provide the best data to answer the question (upper left quadrant of the four panel instrument screen); if you don't know what these conditions are, pick some, collect data, and come back to collect data with different parameters if necessary. For example, some things to consider:

- The mass range to scan should extend somewhat above the nominal mass of the compound, and as low as the smallest fragment of interest, both constrained by the limits of the instrument.
- The relative size of sizes of the four panes can be changed by dragging either the horizontal or vertical divider bars; these adjustments allow you to custom views as necessary.

Once you have the instrument configured as you wish, inject your sample - this automatically initiates data collection. Once the entire set of data has been collected, the total ion chromatogram (TIC) will be displayed in the bottom right pane.

Inspect the chromatogram, looking carefully to identify any peaks. Use the Data Analysis controls (upper right pane) to expand either the time range or the intensity scale of the TIC in order to aid your inspection (be sure to click the "Chromatogram" button after you change a parameter in order to see the new plot).

The chromatogram may contain solvent or air peaks. In addition there is always some background. How do you determine if a peak in the TIC is distinct compound or is simply due to background or the solvent? For example, some things to consider:

- The TIC is a subset of the data you collected; what else is available for your inspection/interpretation?
- Pay attention to the base peak in any mass spectrum you examine (the one carrying the "100% = " value) to help decide if its contribution to the TIC is significant or not.
- What is retention time and is it useful?
- Based on what you did in the wet lab, what did you inject?

If the data you collected is not the best, you can change the instrument settings and inject the sample again, or even go back to the wet lab to prepare a different sample (of the same compound or of a different one). You should ensure that you collect the best quality data possible, in order to justify whatever assignment you make. Feel free to copy the TIC, the mass spectrum (or spectra), and Table(s) and to paste them into your report (e.g., a MS-Word document), if one is needed. (The spectra are gifs, and the Table is a text file – all three are easily copied and pasted; if you wish to capture the instrument settings, after resizing the panes as appropriate, using a screen capture, Alt-PrntScrn, or do a copy and paste from the "Display Settings" button.)

Other: You have no option to change the GC settings, these have been fixed for you as indicated. The type of column used is identified as being nonpolar. Compounds migrate through this column with a speed inversely related to their molecular weight. The lower the molecular weight of the compound the faster it will move through the column. Under a given set of GC conditions, each unique compound emerges from the column at a 'characteristic' time, known as the retention time.

Since the mass spectrometer measures mass-to-charge ratio, it is necessary to ionize the sample; electron ionization (EI) is the most common ionization method. The mass spectrum obtained depends on the energy of the electron beam used in ionizing the gaseous sample. The lower the electron beam energy, the less likely fragmentation of the parent molecule will occur and the greater the likelihood of observing a molecular ion in the mass spectrum. The greater the ei energy, the more fragmentation, and hence structural detail, will be available from inspection of the mass spectrum. In general, 70 eV has been commonly used in ei-based mass spectrometry.

Your sample needs to be gaseous in order to be analyzed by the mass spectrometer. As a compound comes off the GC column and enters the ion source of the MS, it is important to ensure that it is in the vapor phase. However, too high a temperature can also be detrimental by "pyrolyzing" the molecule before it undergoes analysis

The scan rate sets the amount of time it takes the instrument to collect one mass spectrum. For example, a high scan rate (2.38 scans per second) means a complete mass spectrum is obtained every 420 milliseconds. Likewise, the lower the scan time, the lower the time resolution of the TIC and vice versa. Try different scan rate in order to pick the best one for your unknown.