

## Varian 3800/2000 GC/MS Operating Instructions

### General Notes:

- \* All mouse functions are accomplished with the left button unless specified.
- \* The function of each icon can be listed by placing the cursor over the icon and waiting a second or two. The icon label will be listed on the screen.
- \* Don't use the go-away box on the 3800.44 or 2000.40 control windows. Minimize them instead.
- \* Do use the go-away box for the Review/Process Data- SatView window.

### Procedure:

1. Check that the helium pressure gauge on the wall reads 80 psi.
3. On the computer, note the Varian Star Toolbar. This is a wide toolbar that runs down the left side of the screen. If the Varian Star toolbar runs across the bottom of the screen, click on the background of the toolbar and drag it to the left. Click on the System Control icon. This icon is at the top.
4. Pull down the Windows menu and select 3800.44 to display the GC control panel.
5. To select the desired method, click on the "Activate a Method" icon. This icon is an "open file folder" icon in the middle of the top icon bar, just to the right of the method pull down list. Find the folder for your course or research group; you may need to go up a level. In this folder, the methods "m50t200m12", "m50t200m12CI", or "m50t200m12mw" are good general methods. See the notes at the end of these instructions for a discussion of molecular weight determination. This method runs a temperature gradient from 50 to 200°C in 12 minutes. Click Open to continue.
6. Check the GC temperature zones, which are listed in the middle of the screen. The column oven should be 50°C, the front injector 225°C, and the middle injector 50°C. We will use the front injector for this lab. The oven temperature gradient is set for 50-200°C at 15°C/min. With an initial hold at 50°C for 2.0 min, the total run time is 12 min. Check for the total run time, which is listed as the "End Time." Note the two status indicator lights: the Equilibrating/Ready light and the Fault/No Fault lights should both be green before beginning a run. These status indicators are in the upper left-hand block of the control window.
7. Minimize the 3800 control window.
8. Pull down the Windows menu and select 2000.40 to display the MS control panel.
9. Click on the Auto Tune button.

9. The tuning procedure calibrates the mass axis. Choose the “FC-43 mass calibration” tuning mode in the pull-down list box in the middle of the window. Make sure the check box to the left of the pull-down list box is checked, and the other three check boxes are cleared. Click on Start Auto Tune. The system will open the calibration valve on the MS. This valve allows the vapor from a fluorinated organic liquid, FC-43, to enter the MS. The text box across the bottom the screen shows the progress of the Auto Tune procedure. After the procedure is complete, check to see that “Multipoint mass cal: completed” has been printed in the text box.

10. Click on the Acquisition button, at the far right of the MS control window.

### RUNNING A SAMPLE

1. The status indicators should both be green and be labeled Ready and No Faults.
2. The system is ready to begin. The start of data acquisition is signaled by the mechanical switch on the top of the injection port. When you press down on this switch with the syringe, data acquisition will begin.
3. Remember to use only a blunt tipped, conical needle syringe, to avoid destroying the Merlin GC inlet. Before the first use, flush out a syringe with methylene chloride. Be sure to eject the cleaning solvent into a waste container. Do not eject back into the source bottle as it will contaminate the entire bottle. Flush out the syringe at least 50 times with air to remove traces of solvent.

**Ion Trap Mass specs are very sensitive. You must be very careful not to overload the instrument.**

If you are injecting just gases, no liquids, use step 4.  
If you are injecting liquids skip to step 5.

4. **Head Space Analysis:** If you are doing head-space analysis flush out the syringe with your sample vapor at least three times. Inject 2-3 uL of your sample by gently pushing the syringe needle into the injection port on the top of the GC. Be sure to push straight down and guide the needle in as it is very fragile and easily bent. Remove the syringe immediately after injection.
5. **Solution Analysis:** Inject 0.2-0.3 uL of your sample by gently pushing the syringe needle into the injection port on the top of the GC. That’s correct—the syringe has just a tiny amount of solution in it. Be sure to push straight down and guide the needle in as it is very fragile and easily bent. Remove the syringe immediately after injection.
6. Note the data file name. This name will be listed in the upper-right corner of the acquisition dialog window. It will be in a form such as “2000.40012.SMS.”

7. To view the data while it is being taken, click on the Hide Keypad button, which is on the left side in the middle of the control window. Pull down the adjacent list box and select Spectrum and Chromatogram. Both the yellow Spectrum and Chromatogram windows should be displayed. The relative heights of these windows can be adjusted by dragging the border between the two windows up and down. (Sometimes, this border is left too low so that only one window shows, even though you have chosen to display "Spectrum and Chromatogram." If this happens, move the mouse to the bottom of the yellow window until the cursor changes to "=". You can then drag the border up to reveal the second yellow window.)

8. After the solvent delay has passed, you will see the Total Ion Chromatogram accumulating on the screen. While it is running, you may adjust the graph parameters, including the vertical scaling using the slider at the far right of each yellow window. If you need to stop the run while it is in progress, click on the Show Keypad button, and then select Reset. To end early you will also need to reset the GC. Pull down the Windows menu and choose 3800.40, and again click on Reset, and finally minimize the GC control window to return to MS data acquisition.

9. You can display information about the current MS scan by clicking the Scan Information icon. This icon looks like a small piece of white paper with tiny lettering. Clicking again hides this information box. You can display the current cursor m/z position and the corresponding data value by selecting the Cursor Display icon. This icon looks like a small mass spectrum with one red peak. Clicking again hides this information box.

### *Data Analysis*

10. When the chromatographic peak returns to baseline or the run is completed, you can display the data in the Review/Process MS Data window. To open your data in the Review/Process MS window click on the small black icon that has a representation of the chromatogram in yellow and green. This small icon is in the middle of the screen above the yellow chromatogram window. (There are two other larger icons on the screen with the same symbol. These larger icons are used to enter the Review/Process MS Data application with a previously saved data file.)

11. In the SatView application window, pull down the Spectra menu and make sure that "Background Correct Spectra" is not checked. If it is checked selecting that entry will clear this mode.

12. Move the cursor over the chromatogram trace. Clicking the mouse will load the mass spectrum that was taken at that time into the window, on the left. Individual mass spectra or several consecutive spectra that have been averaged can be displayed. Pull down the Spectra menu, slide right on Spectrum Averaging and choose Single spectra. Click on the chromatogram trace to load single spectra for viewing. Now return to the Spectra menu, slide right on Spectrum Averaging and choose 5 point spectrum. Now clicking on the chromatogram trace will display the averaged result of 5 spectra centered on the time that you selected.

13. Areas of either the chromatogram or mass spectrum can be enlarged by highlighting a box around the area by holding the left mouse button down and dragging around the area. Clicking the red A icon, above the spectrum window, will return to the full X and Y expansion.

14. To prepare for printing, press the right mouse button with the mouse in the mass spectrum window and slide right on Options. If you want a listing of the ion fragments and their abundances, make sure “print ions and intensities” is checked. Normally, we don’t need a listing and “print ions and intensities” should not be checked. To print the mass spectrum, click right again and choose “Print Spectrum.” In the print preview screen, click on the “print page” icon. This icon looks like a printer with a single sheet of paper. Click on the “Exit!” menu item to return to the data viewer.

15. When you are finished with data analysis, click the go-away box on the MultiChrom window and the SatView window.

16. You should now see the 2000.40 MS acquisition window. If necessary, click on the Show Keypad button to see the acquisition controls. Check the status indicators. If they are both green, the system is ready for the next sample.

17. Rinse the injection syringe 25 times with methanol.

#### SHUT DOWN PROCEDURE

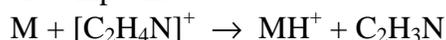
1. Minimize the 2000.44 acquisition control window.

2. Maximize the 3800.44 control window, or alternatively pull down the Windows menu and choose 3800.44.

3. Click on the Activate a Method icon. (This icon is an “open file folder” icon in the middle of the top icon bar.) If you are in the CH341 folder; you will need to go up a level. Select the “standby” method. Click Open to continue. The standby method sets the oven at 50°C, the front injector at 200°C, and the middle injector to 50°C. The split vents are turned off to conserve on helium.

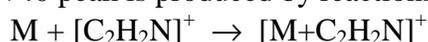
### **Note on Molecular Weight Determination**

Compounds often don't give a large parent ion,  $M^+$ , peak. Alcohols, for example readily lose  $H_2O$  and give no parent ion. Auto-ionization also can give a parent peak for the protonated molecular ion,  $MH^+$ , which occurs at 1 m/z higher than the parent mass,  $M^+$ . (See the note on Auto-Ionization, below.) Chemical ionization, CI, is a very useful ionization method to circumvent these problems. In CI a low pressure of a reagent gas is admitted to the source of the mass spectrometer. The electron beam of the source ionizes this reagent gas. Ions from the reagent gas then react chemically in the gas phase with your sample to generate  $MH^+$  ions. Acetonitrile,  $C_2H_3N$ , is a common reagent gas for CI. The electron impact produces a number of reagent ions including the protonated acetonitrile ion,  $[C_2H_4N]^+$ . This reagent ion then reacts with your compound:

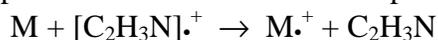


Therefore, the peak has a mass of one greater than your molecular weight. CI is a "soft ionization" technique. That is, the ionization process is very gentle and therefore produces little or no fragmentation. A CI spectrum is then very simple. There will usually be one prominent peak, the  $MH^+$  peak, and maybe a few much lower intensity fragment ions. The "m50t200m12CI" method is set up to easily do CI for your sample.

For some types of compounds CI also produces adduct ions. These ions are at  $M+13$  or  $M+40$ . The  $M+40$  peak is produced by reactions with the  $[C_2H_2N]^+$  cation:



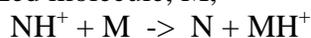
The adduct peaks are usually much lower in intensity than your parent  $MH^+$  peak. For some types of samples the normal  $M^+$  ion is also produced by a charge transfer process:



For example, acetonitrile CI works well with compounds containing nitrogen atoms, producing a characteristic  $MH^+$  ion and a confirmatory  $M+13$  adduct ion (probably from the reaction with  $[CH_3CNCH_2]^+$ ). Saturated hydrocarbons also produce abundant  $[M+40]^+$  adducts, and alkenes give both  $[M+40]^+$  and  $[M+54]^+$  ions.

### **Note on Auto Ionization**

The  $M+1/M$  ratios for various peaks are very helpful for predicting the formula for the ion. However, the ionization process in some mass spectrometers can produce inaccurate ratios for the parent ion. This problem is caused by auto ionization, which is particularly prevalent in internal ionization ion trap mass spectrometers. The ions in an ion trap are held for relatively long periods (10 msec) in the trap before being scanned out to determine the mass spectrum. During this time ion-molecule reactions can occur. An ion in the trap can transfer a proton to an unionized molecule,  $M$ ,



Unfortunately, this alternative ionization mechanism produces an  $M+1$  peak that can be much larger than the  $M+1$  isotope peak from the  $M^+$  ion. Therefore, you should always be careful to consider auto-ionization when using an internal ionization ion trap mass spectrometer. Auto-ionization is expected for basic compounds, like amines, and to a lesser extent esters and ketones.

The prevalence of auto-ionization depends on several factors. Auto-ionization increases as the number of ions in the trap increases and as the time in the trap increases. Both of these

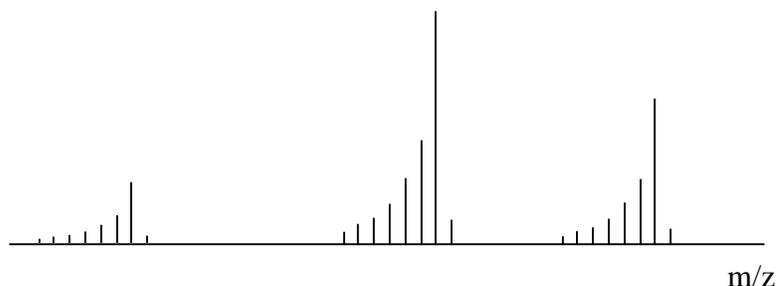
parameters may be easily controlled. In addition to auto-ionization, overfilling the trap decreases mass resolution. Ion traps have an automatic mechanism for controlling the number of ions in the trap, called automatic gain control, or AGC. The system does a pre-scan to determine the signal strength and then adjusts the hold time in the trap to avoid filling the trap with too many ions. The pre-scan signal is compared to a target ion count. The hold time is adjusted to match this target. If there is insufficient sample to meet the target, the hold time is increased. However, for very low sample concentrations the hold time may be impractical so a maximum hold time is specified to avoid slowing down the data rate unduly. To avoid auto-ionization, the target ion count and the maximum hold time may be decreased. For example, the default target ion count is 25000 and the default maximum hold time is 25000 us. To avoid auto-ionization the target ion count can be decreased to 500 and the maximum hold time in the trap may be decreased to 2000 us or less. The method "" is set up in this way. Sample amounts can also be decreased to cut down on the concentration of ions in the trap.

Another advantage of using decreased target ion counts and maximum hold times is resolution is improved. The disadvantage of using decreased target ion counts and maximum hold times is less sensitivity. Since ion trap mass spectrometers are about ten times as sensitive as quadrupole mass analyzers, even when operating with decreased source controls, you can still have comparable sensitivity to a quadrupole mass spectrometer.

How do you know if auto-ionization is a problem for your sample? Compare the mass spectra on the leading and trailing edge of your chromatogram peak with the spectra near the apex. If the  $M+1/M$  ratio changes drastically, with the spectra near the apex having much larger  $M+1/M$  ratios, then auto-ionization is a problem. For approximate  $M+1/M$  ratios, you can then just use the spectra from the leading or trailing edge.

Another way to tell if auto-ionization is a problem is to compare peaks in the spectrum. If your compound has an even molecular weight, most of the fragment peaks will have odd masses. Conversely, if your molecular weight is odd, most of the fragment ions will have even masses. If you have good signal to noise, you should do another run with much smaller amounts of material. However, to be careful, you should repeat your run with much smaller target ion counts and maximum hold times, in addition to smaller amounts using the m50t200m12mw method.

Another indicator that auto-ionization is a problem is the appearance of a large number of trailing peaks:



If your spectrum takes on this appearance, you need to dilute your sample by a factor of 50-100.