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Instrument Instructions

Gas Chromatography-Mass Spectrometry Instrument Instructions

Introduction/Theory

Gas chromatography-mass spectrometry (GC-MS) is used to analyze complex organic and biochemical mixtures (1). The GC-MS instrument consists of two main components. The gas chromatography portion separates different compounds in the sample into "pulses" of pure chemicals based on their volatility (2) by flowing an inert gas (mobile phase), which carries the sample, through a stationary phase fixed in the column (1). Spectra of compounds are collected as they exit a chromatographic column by the mass spectrometer, which identifies and quantifies the chemicals according their mass-to-charge ratio (m/z). These spectra can then be stored on the computer and analyzed (1).

As stated above, the instrument is used to analyze complex organic and biochemical mixtures. The Agilent 5975C GC-MS can inject various kinds of samples, including microliter volumes of liquids, vapor from the headspace of a closed sample bottle, or chemicals adsorbed on a solid phase microextraction (SPME) fiber (3). The samples must be of a suitable size and are introduced as a "plug" of vapor. Sample sizes range from a few tenths of a microliter to 20 μL for ordinary packed analytical columns. Capillary columns, which require samples that are smaller by a factor of 100 or more, often require a sample splitter, which delivers a small known fraction of the injected sample, while the rest goes to waste. (1)

Block diagram/Explanation

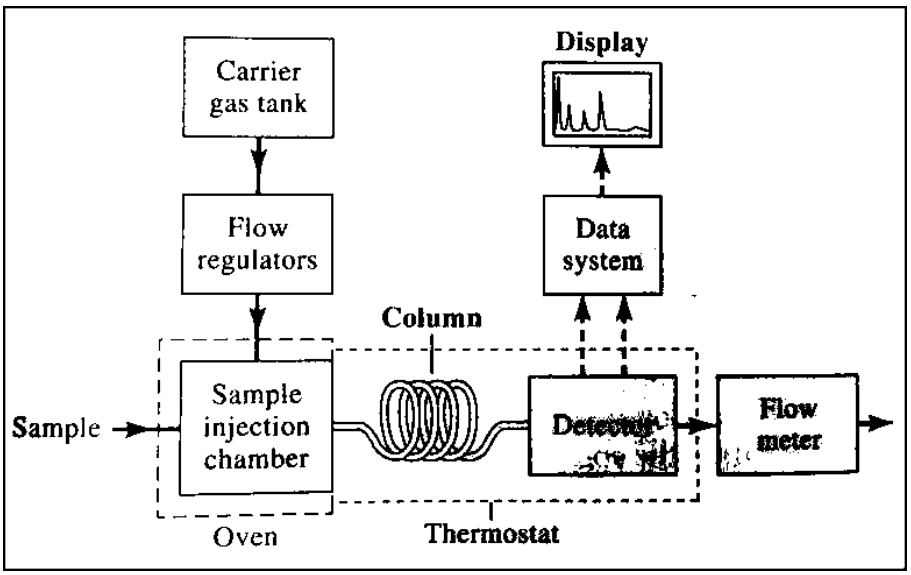


Figure 1 Block diagram of GC-MS (1)

Figure 1 shows a block diagram of the GC-MS. The mobile-phase gas is called the carrier gas, which must be chemically inert. This gas is most often helium, however, argon, nitrogen, and hydrogen are also used. These gases are held in pressurized tanks and use pressure regulators, gauges, and flow meters to control the flow rate of the gas. Flow rates usually range from 25-150 mL/min with packed columns and 1-25 mL/min for open tubular capillary columns, and are assumed to be constant if inlet pressure is constant. This is often accompanied by a molecular sieve to purify the gas before it is used. (1)

Samples are introduced as a "plug" of vapor. Liquid samples are introduced using calibrated micro syringes to inject sample through a septum and into a heated sample port which should be about 50°C above the boiling point of the least volatile constituent of the sample. After the sample is introduced, it is carried to the column by the mobile phase. (1)

Packed and open tubular or capillary columns are two types of columns used in gas chromatography. Packed columns are 1-5 m long, while capillary columns range from a few meters to 100 meters long. The Agilent 5975C GC-MS uses a 30 meter capillary column (4). Columns are usually constructed of fused silica or stainless steel, and coiled to fit into an oven, with the coils ranging from 10-30 cm in diameter. The temperature of the column is an important variable, so the oven is equipped with a thermostat that controls the temperature to a few tenths of a degree. Boiling point of the sample and the amount of separation required determines the temperature the sample should be run with. As a rough estimate, a temperature equal to or slightly above the average boiling point of the sample should give reasonable results. Temperatures can also be increased in steps as separation proceeds, for samples with a broad boiling range. As the mobile phase carrying the sample is passed through the stationary phase in the column, the different components of the sample are separated. (1)

After being separated, the sample is run through a detector. The detector in a GC-MS is, not surprisingly, a mass spectrometer. The type of mass spectrometer used in the Agilent 5975C GC-MS is a quadrupole mass analyzer (4), which ionizes the sample and then separates the ions based on their mass-to-charge ratio (1).

This data is then sent to a computer to be displayed and analysed. The computer linked to the Agilent 5975C GC-MS has a library of samples to help analyze this data.

Data for the GC-MS is displayed in several ways. One is a total-ion chromatogram, which sums the total ion abundances in each spectrum and plots them as a function of time. Another is the mass spectrum at a particular time in the chromatogram to identify the particular component that was eluted at that time. A mass spectra of selected ions with a specific mass to charge ratio, called a mass chromatogram, can also be used. (1)

Instrument parameters include flow rate, sample size, type of column, as well as column length and temperature, and the library used to analyze the data. As stated above, flow rates range from 25-150 mL/min with packed columns and 1-25 mL/min for open tubular capillary columns. Sample sizes range from a few tenths of a microliter to 20 μL for ordinary packed analytical columns. Capillary columns require samples that are smaller by a factor of 100 or more. The Agilent 5975C GC-MS uses a capillary column that is 30 meters long (4). The temperature of the oven can be varied depending on the boiling point of the sample. The column can withstand temperatures of -60°C to 360°C. There are also several different libraries available to analyze data. (1)

Samples can be both identified and quantified. The mass spectrum is used to identify various components of the sample based on mass to charge ratio, however, the components of the sample can also be quantified using the peak height or peak area of an eluate from the GC column. Under carefully controlled conditions and using a standard, an accuracy of 1% relative error can be obtained. (1)

Annotated picture of the instrument

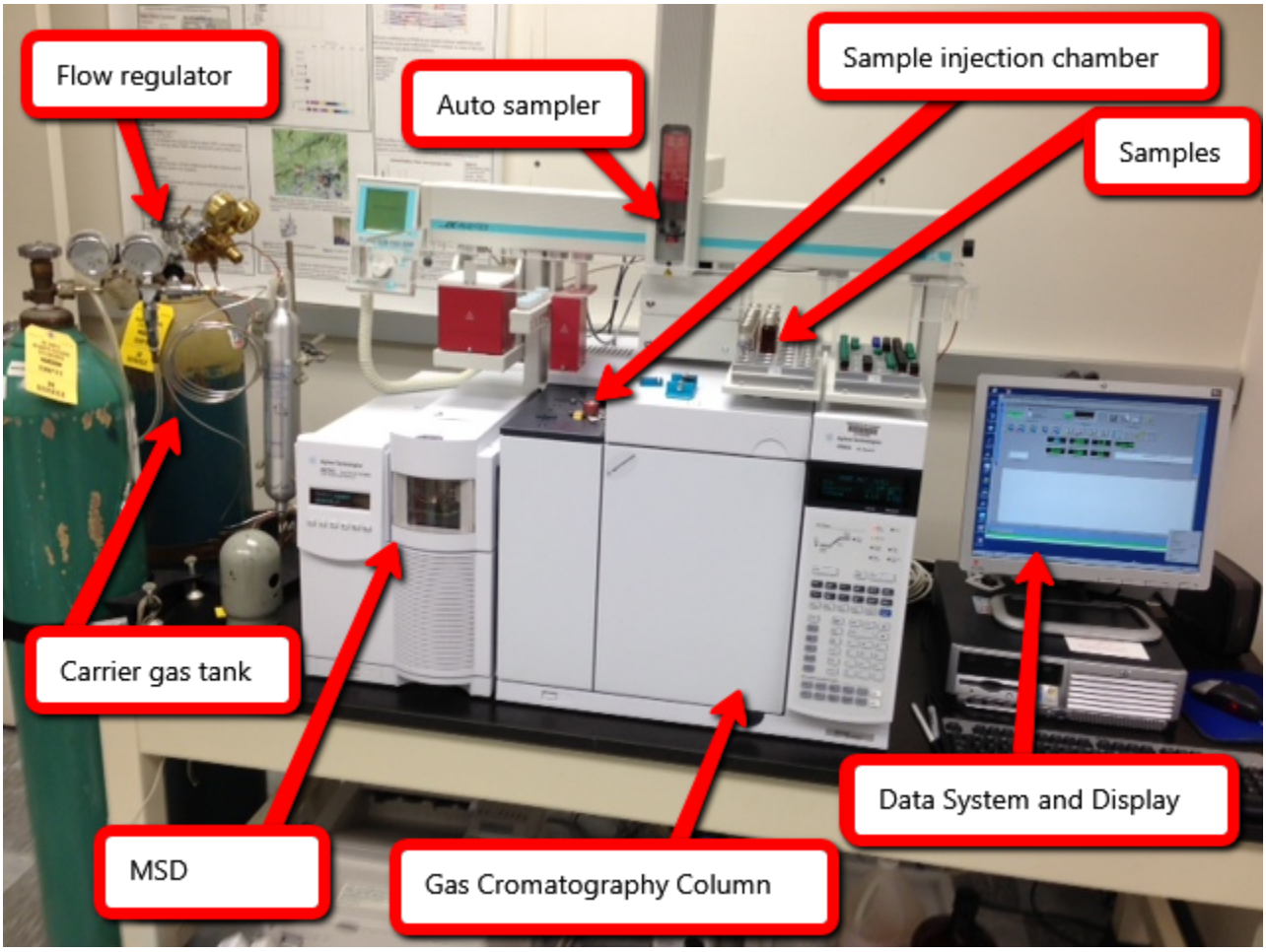


Figure 2 Annotated picture of the Agilent 5975C GC-MS

Individual component diagrams:

The capillary column

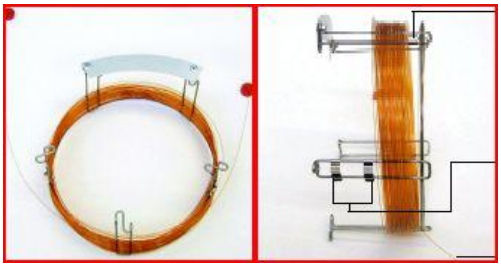


Figure 3 and 4 Front and side view of capillary column (4)

In column chromatography, the stationary phase (which in the Agilent is cross-linked/surface bonded 100% dimethylpolysiloxane (5)) is held in a narrow tube, seen as the orange line in Figures 3 and 4, through which a mobile phase is forced through under pressure. The sample is eluted through the stationary phase by the continuous addition of fresh mobile phase, causing the sample to separate into its component parts. As the sample travels down the column, it transfers between the mobile and stationary phase. The rate at which the sample separates depends on the fraction of time it spends in the mobile phase. For solutes strongly retained by the stationary phase this fraction is small, while solutes strongly retained by the mobile phase this fraction is large. Different components should have different rates, which separate into bands as the sample is eluted down the column. The different components are separated and passed out the end of the column where they are detected and collected to be sent to the mass spectrometer. (1)

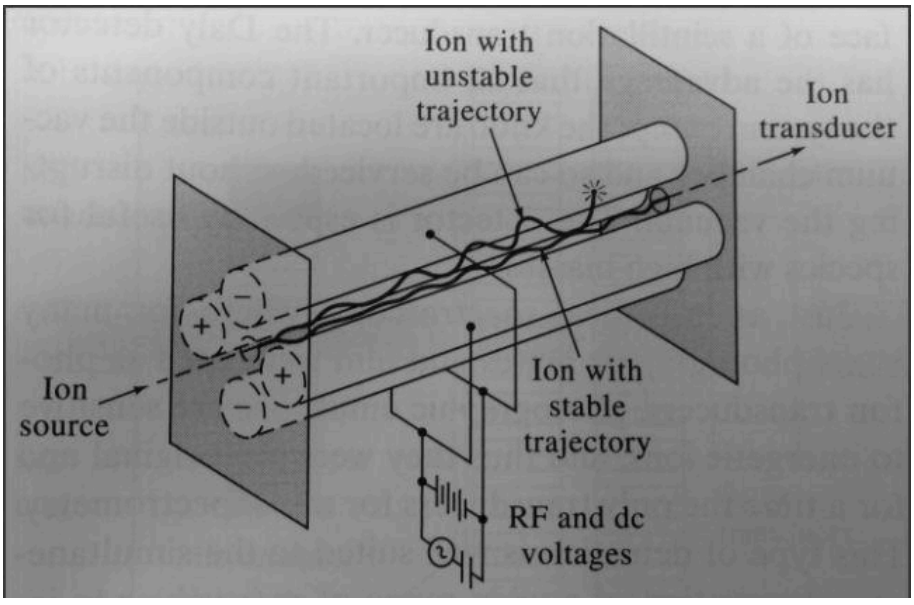


Figure 5 Quadrupole mass spectrometer (1)

The quadrupole mass analyzer, a diagram of which is shown in Figure 5, is composed of four parallel cylindrical rods which function as electrodes. Opposite rods are connected to either

a positive or negative variable DC source. The rods also have variable radio-frequency AC voltages, which are 180° out of phase, applied to each pair. After the sample is converted into gaseous ions by bombardment with electrons, ions are accelerated into the space between the rods using a potential difference of 5 to 10V, while the AC and DC voltages on the rods are increased concurrently, while keeping their ratio constant. When the voltage is varied, only certain ions with a particular mass to charge ratio will make it through to the transducer, while the others will strike the rods and become neutral. A transducer converts the beam of ions into an electrical signal that can be processed and displayed as a mass spectra on a computer. (1)

Instrument operation:

Safety precautions

There are several safety precautions that should be kept in mind while using the GC-MS. Different hazards are labeled with warning symbols, shown in Figure 6, in the manual or on the instrument itself. The machine is connected to a power source, and many of the internal parts carry a dangerous voltage, even when the power switch is off. These are shielded with covers, which should not be removed. Many parts of the machine get hot and should not be touched. Do not turn the mass spectrometer off, or bring food/drinks into the instrument room, as they may damage it. It is also recommended that the room is kept ventilated, and you wear long sleeves, gloves, and safety glasses, as some of the parts use refractory ceramic fibers. In general, do not mess with or remove parts. When the machine is on, keep hands out of the plastic guard, as this is where the autosampler moves. (4)

Symbols

Warnings in the manual or on the instrument must be observed during all phases of operation, service, and repair of this instrument. Failure to comply with these precautions violates safety standards of design and the intended use of the instrument. Agilent Technologies assumes no liability for the customer's failure to comply with these requirements.

See accompanying instructions for more information.

Indicates a hot surface.

Indicates hazardous voltages.

Indicates earth (ground) terminal.

Indicates potential explosion hazard.

or

Indicates radioactivity hazard.

Indicates electrostatic discharge hazard.

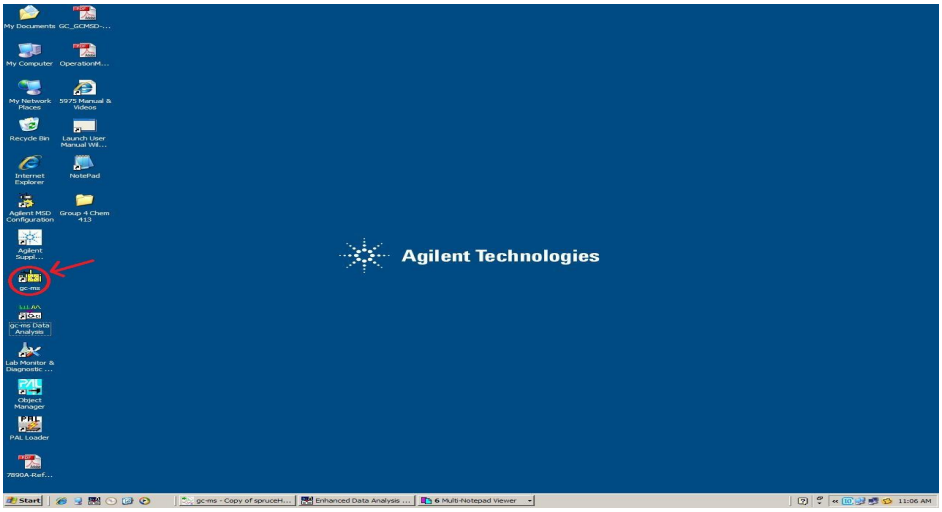
Indicates that you must not discard this electrical/electronic product in domestic household waste.

Figure 6. Warning symbols and meanings (4)

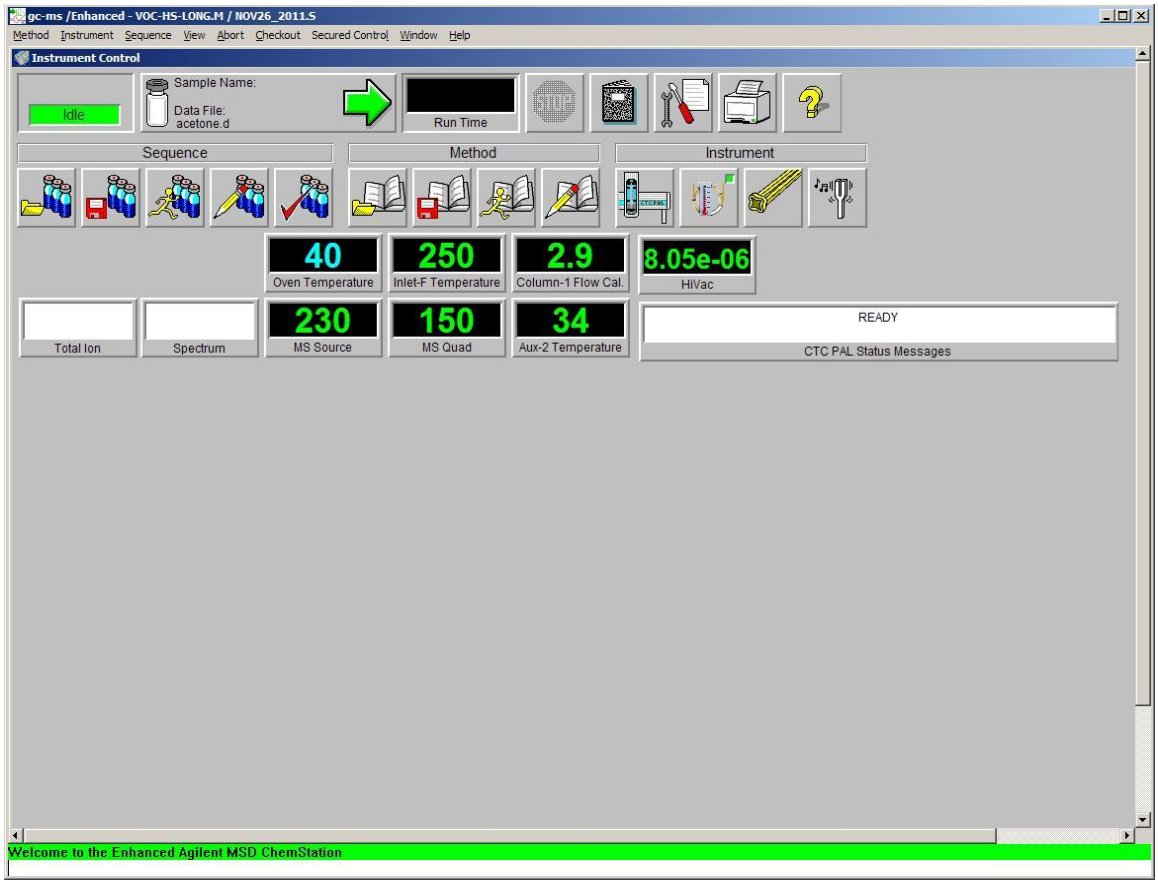
Instrument startup.

The instrument should be running when you come in the room, so it should not need to be turned on. The computer should also be on, however if it is not, it can be turned on like any other computer, or powered down and restarted if it is having trouble. The username to login is Administrator, and the password is 3000hanover. The login and password can also be found on a sticker located under the computer stand on the hard drive. Once you are logged in, open the gc-ms program with a double click (this may take 5-10 minutes), and you are ready to go.

Double click GC-MS to start program



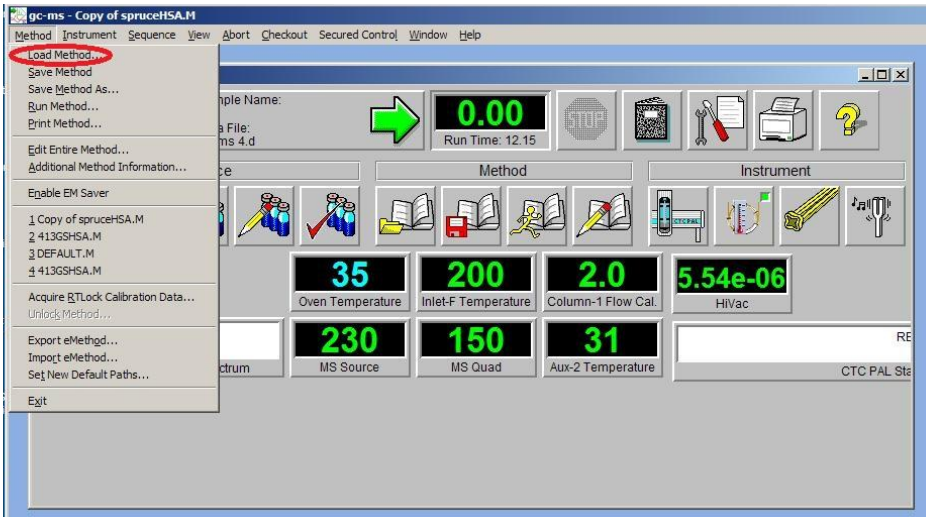
How screen should look when you come in or after program is started.



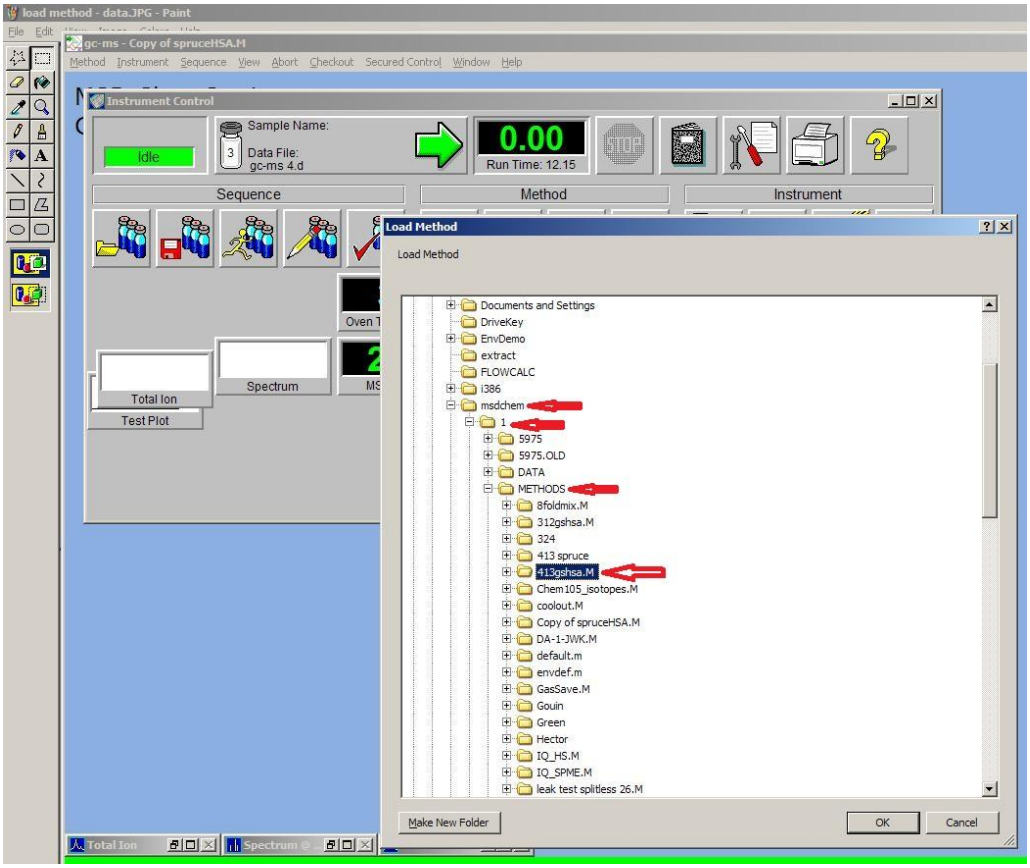
Instrument setup.

To prepare the instrument to run samples you should follow the below steps.

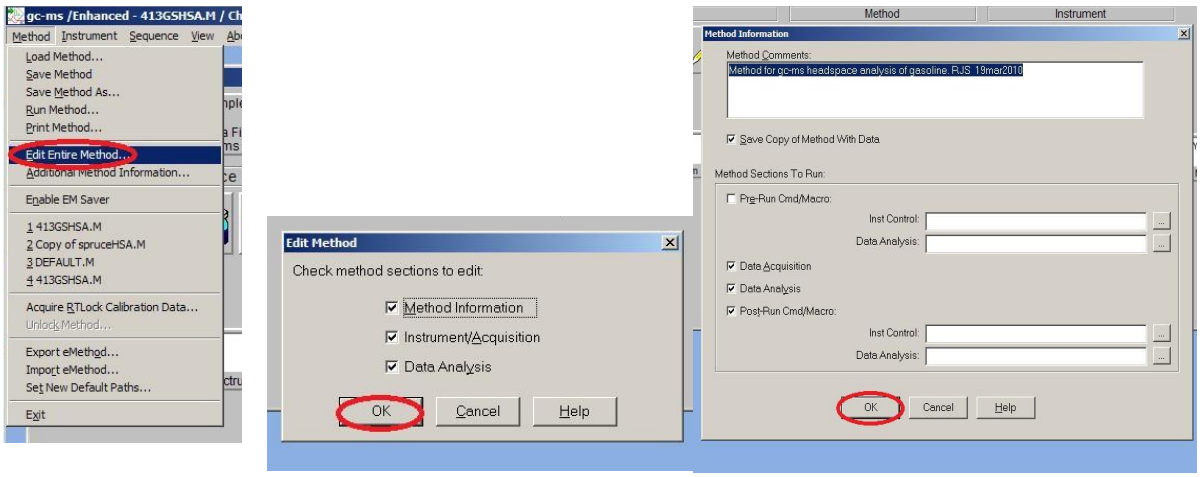
Under Method, select Load Method



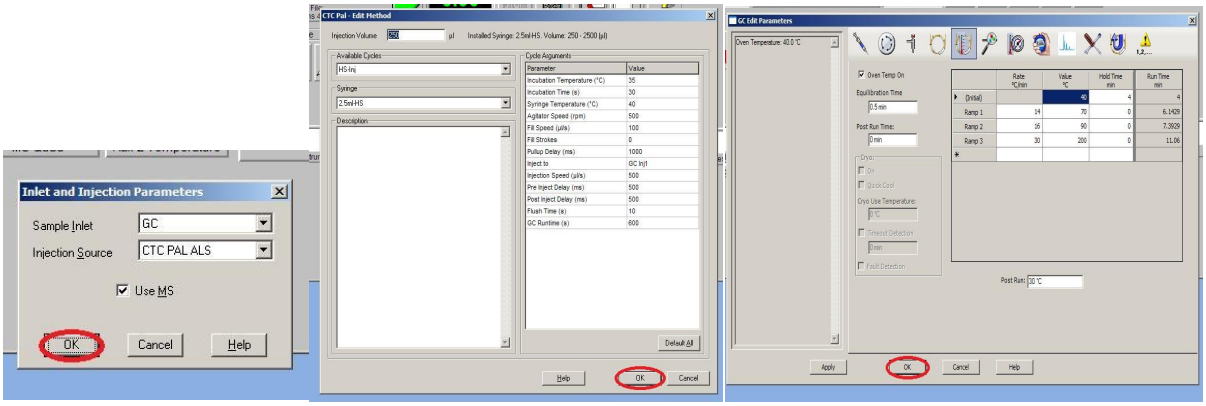
Choose 413gshsa.M under msdchem>1>METHODS



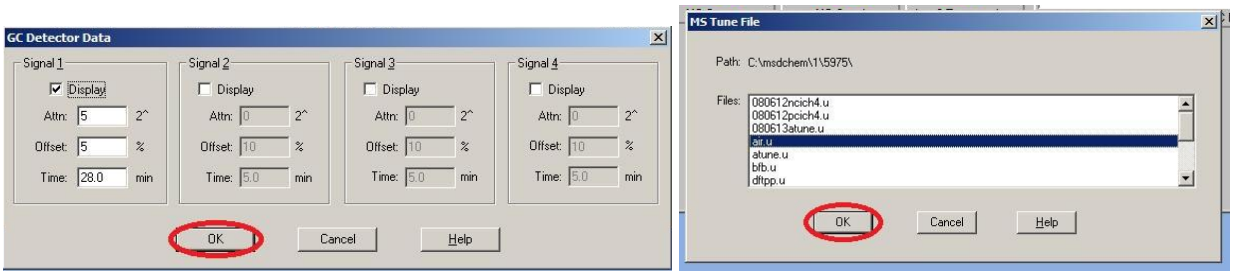
Click Method, Edit Entire Method, Click OK, Click OK,



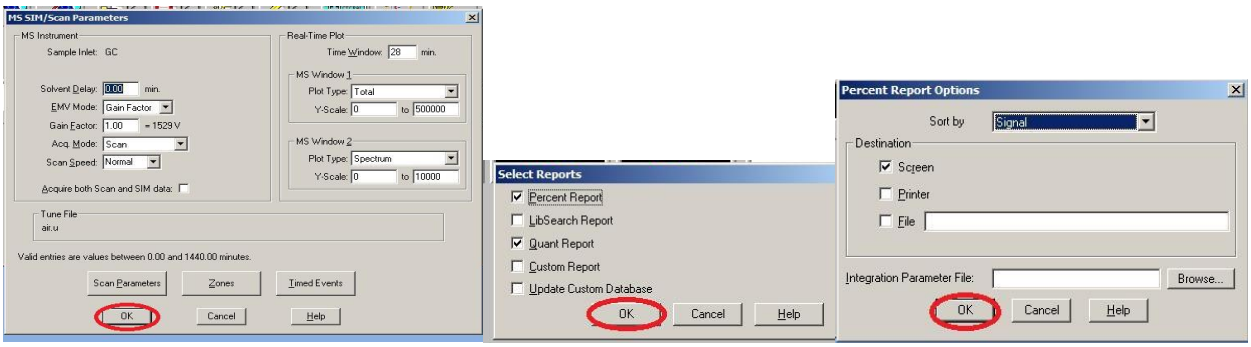
Click OK, Click OK. Record these parameters. Click OK



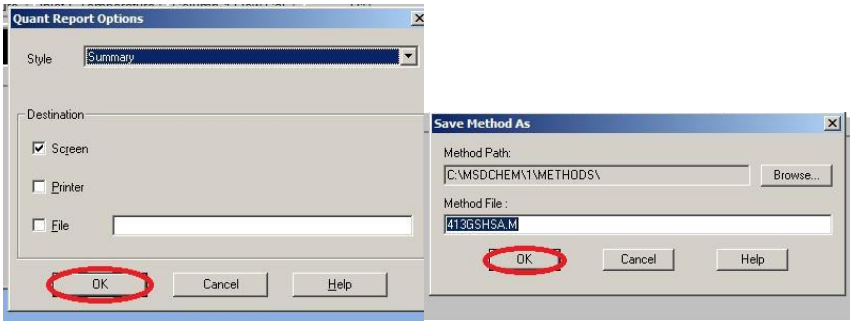
Click OK, Click OK



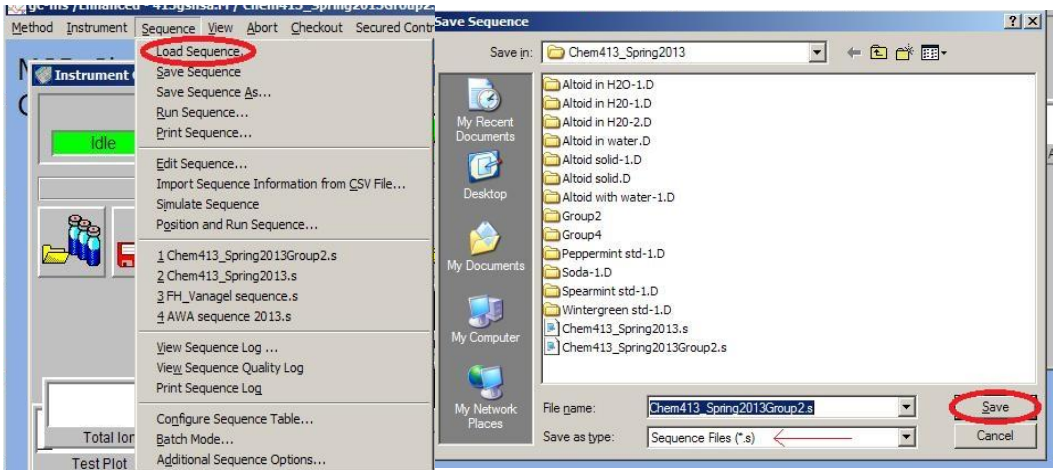
Click OK, Click OK, Make sure screen is checked but not printer. Click OK



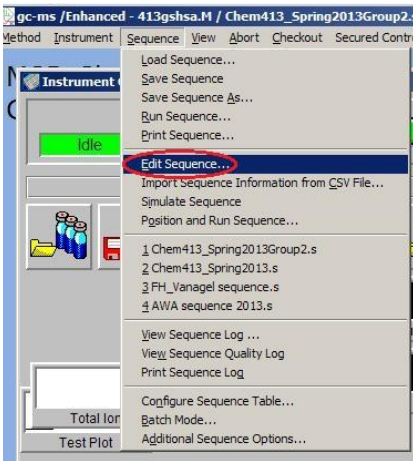
Make sure screen is checked but not printer, click OK. Click OK



Go to Sequence, Load Sequence. Save under Chem413_Spring2013 as a .S file.

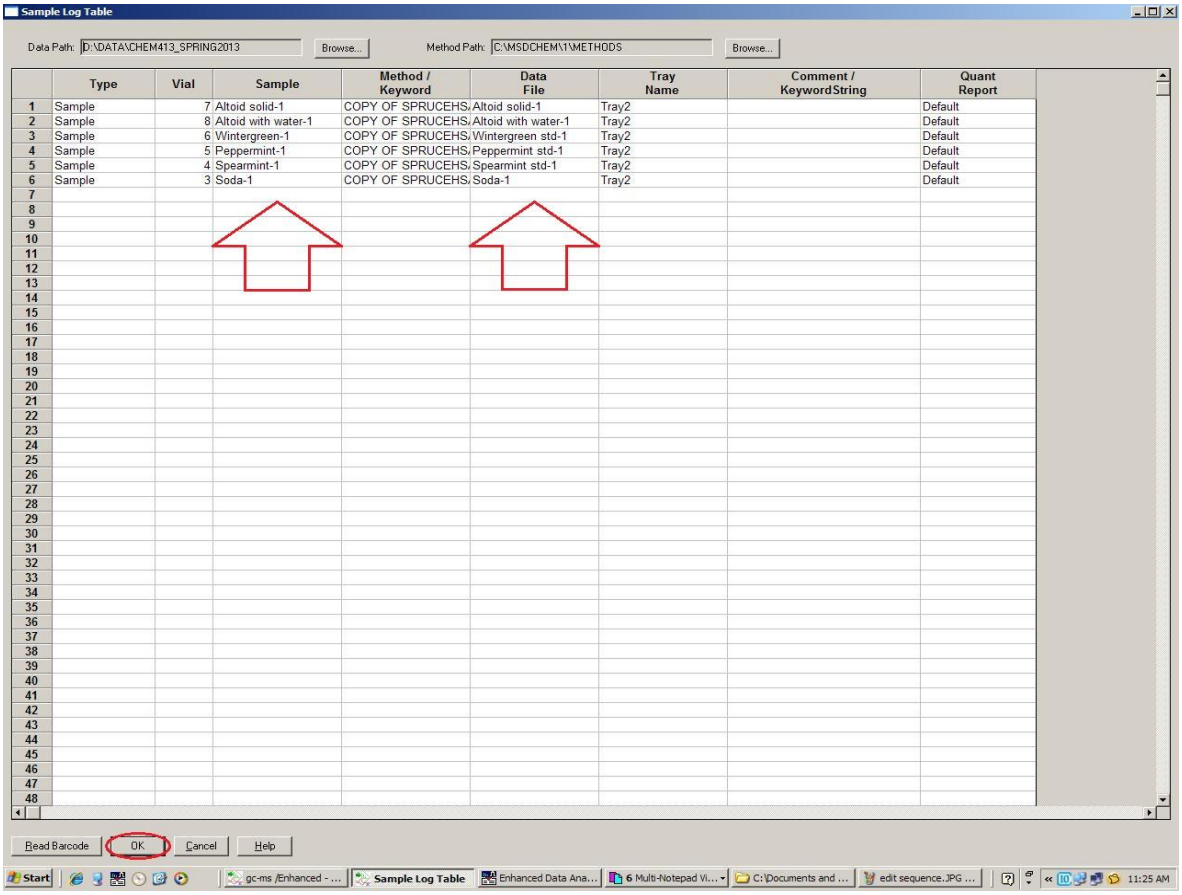


Go to Sequence, Edit Sequence

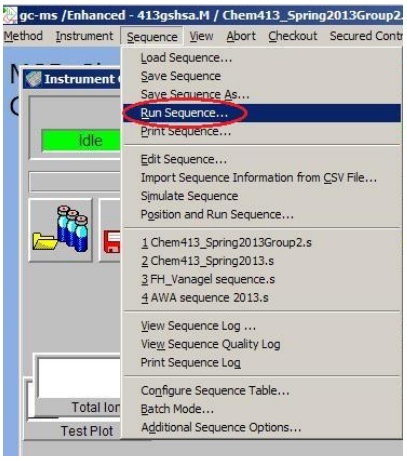


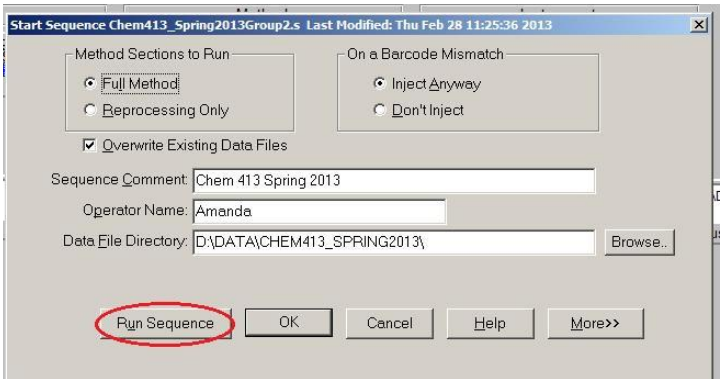
This is where you tell the autosampler what to sample, the name of your sample, and the method to run it under. The sample column and the Data File must be the same. The gc-ms will not automatically run triplicate, so do do this you must write each out each sample three times, labeling them something like sample1, sample2, sample3 to tell them apart, so that the machine will sample from that vial three times.

When you are done click OK.



Click Sequence, Run Sequence

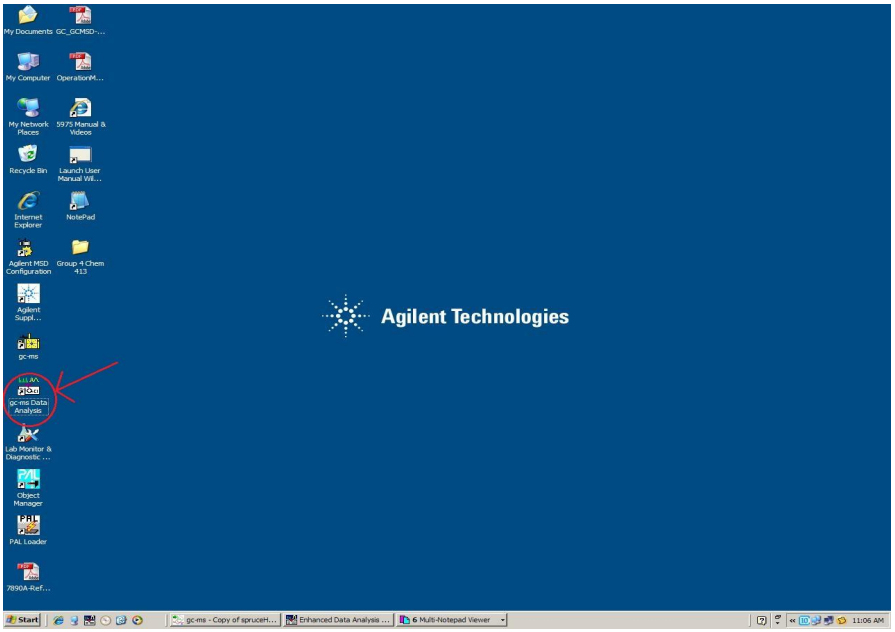




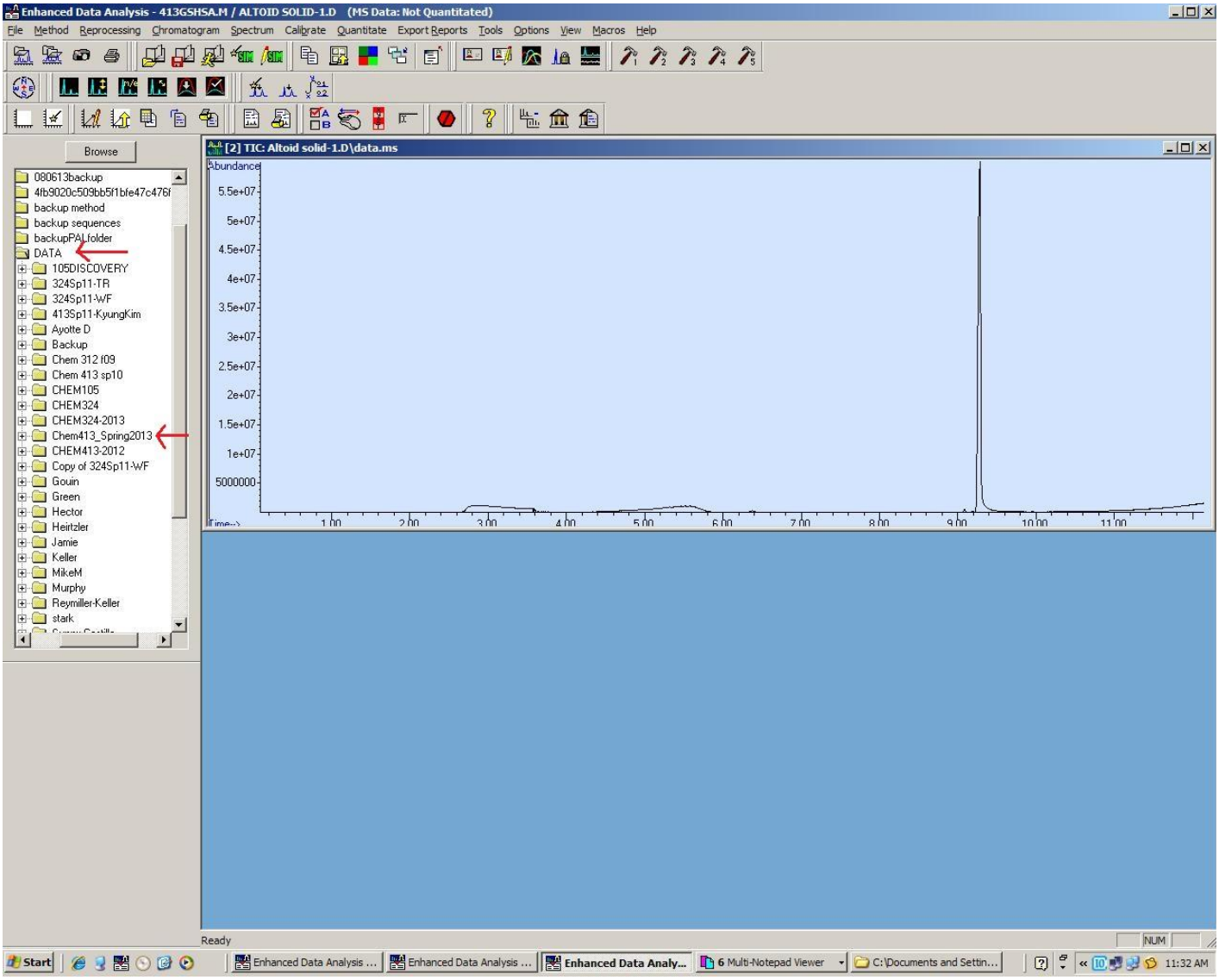
Make sure Full Method and Inject Anyway is selected, then click Run Sequence. The instrument will make a loud noise and the autosampler will start collecting your first sample.

Data Analysis

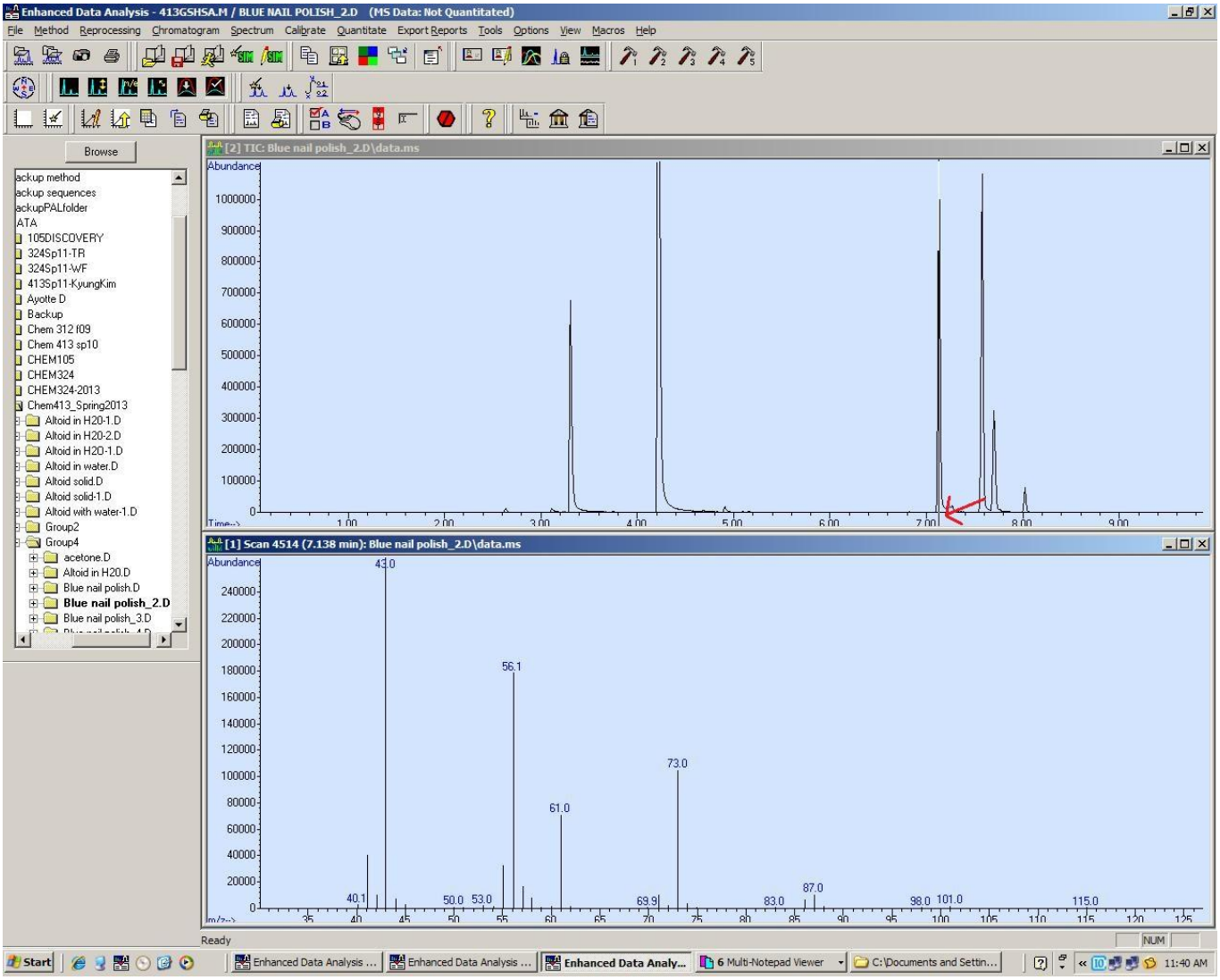
To analyze data after your samples have been run, click on gc-ms Data Analysis icon.



Under DATA find CHEM413_Spring2013 folder and click on the sample you want to analyze.



Double right click on a peak to view the mass spec of sample under peak.

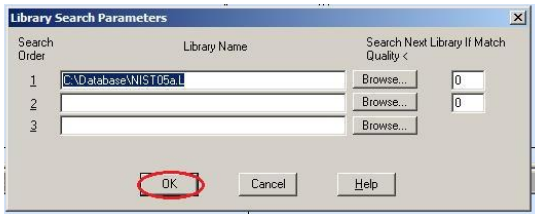


It is also possible to match using the library.

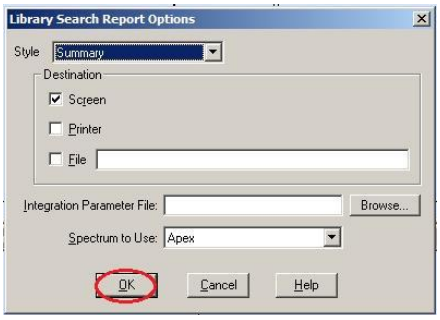
Click the Library icon to choose library.

Browse and find the NISTO library to upload.

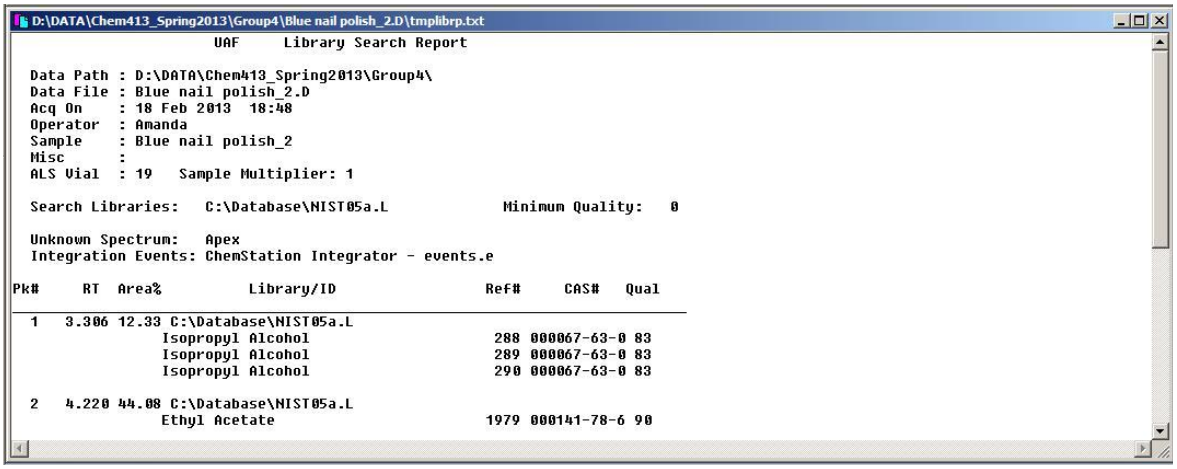
Click OK.



Double click on library icon with paper next to it to search the library. When this screen comes up make sure screen is selected and not printer. Click OK.



A screen will appear under your data with library matches.



Sample preparation

The Agilent 5975C GC-MS is capable of injecting various kinds of samples such as microliter volumes of liquids, vapor from a headspace vial, or chemicals adsorbed on a solid phase microextraction (SPME) fiber (3) but only headspace analysis will be discussed since all other methods will not be used during this analytical class.

There are a few guidelines on how a sample should be prepared for headspace GC-MS. All compounds must be volatile enough to pass through the chromatographic column when heated to 300°C. Non-polar compounds with up to 500 g/mol molecular weight and polar compounds with up to 300 g/mol molecular weight should make it through. Samples should NOT contain non-volatile salts or metals or polymers since these substances are harmful to the injector and/or subsequent injections. Samples should be prepared in high volatile solvents such

as dichloromethane, hexane, or ether at a concentration of 1 mg to 10 mg samples per mL of solvent (6).

Instrument shutdown

Instrument should not be shut down, but left on after your samples are finished running. You should leave it as you found it, hopefully looking like the second screen capture of Instrument Startup.

Troubleshooting

If the program won't open, or the machine is offline, try restarting the computer and reopening the program. For further problems or questions, contact Professor Iceman or Professor Heirtzler. **Do not turn off the mass spectrometer** or the instrument, and make sure samples are placed correctly in the autosampler, or you may end up with something similar to Figure 7.

Figure 7 WHAT NOT TO DO

Instrument maintenance

General maintenance tasks are important to perform, and doing so regularly can reduce operating problems and costs, as well as prolong the life of the GC-MS. A record should be kept of all maintenance tasks that are performed. Figure 8 shows many of the common maintenance tasks that should be performed and when. Remember to use the proper precautions while performing maintenance. Other tasks can be performed as needed.

Table 21 Maintenance schedule

Task	Every week	Every 6 months	Every year	As needed
Tune the MSD				X
Check the foreline pump oil level	X			
Check the calibration vial(s)		X		
Replace the foreline pump oil*		X		
Replace the diffusion pump fluid			X	
Check the dry foreline pump				X
Clean the ion source				X
Check the carrier gas trap(s) on the GC and MSD				X
Replace the worn out parts				X
Lubricate sideplate or vent valve O-rings†				X
Replace CI Reagent gas supply				X
Replace GC gas supplies				X

* Every 3 months for CI MSDs using ammonia reagent gas.

† Vacuum seals other than the side plate O-ring and vent valve O-ring do not need to be lubricated. Lubricating other seals can interfere with their correct function.

Figure 8 Common maintenance tasks and schedule (4)

Resources

1.Skoog, D. A., Holler, F. J., Crouch, S. R. (2007). Principles of Instrumental Analysis. 6th Edition. Brooks/Cole Cengage Learning. Chapters 11, 20, 26 and 27.

2. Oregon State University. (2012). GC-MS: How does it Work?. Environmental Health Sciences Center. Corvallis OR, 97331. <http://www.unsolvedmysteries.oregonstate.edu/MS_05>

3. University of Alaska Fairbanks. (2013). Instrumentation and Computers. Department of Chemistry. <http://www.uaf.edu/chem/instrumentation/>

4. Agilent Technologies. (2012). 5975 Series MSD Operation Manual. 4th Edition. <http://www.cmtc.psu.edu/patterson/facilities/agilent%20597UserManual.pdf>

5. Neobits. (2009). Agilent Technologies - 122-1052 - Agilent J&W DB -1 GC Columns - Standard 17.8 Cm (7) Columns (Each). <http://shop.neobits.com/agilent_technologies_122_1052_agilent_j_w_db_1_gc_columns_standard_17_8_cm_7_columns_each_1032672824.php>

6. Penn State. (2013). Sample preparation for small molecules for MS analysis. The Huck Institutes of the Life Sciences. University Park, PA 16802. <http://www.huck.psu.edu/facilities/proteomics-mass-spectrometry-up/protocols/sample-preparation-for-ms-analysis-of-small-molecules>