GAS CHROMATOGRAPHY –MASS SPECTROMETRY

Pre-Lab Questions

Questions to be answered before doing the experiment. **The answers are due at the beginning of each experiment without exception (the questions are for credit and some may appear on your final exam). Refer to Skoog et al., 2007, Principles of Instrumental Analysis, 6th edition, Chapters 20 & 27.**

1. How does electronic impact work as an ionization technique?

2. Why use electron impact rather than another ionization technique for characterizing small volatile compounds such as found in perfume (Mol. wt ~ 50-500 molecules)?

3. What type of mass analyzer will be used here and how does it work (detailed)?

4. Comments on the advantages of using the mass spectrometer (MS) as a detector rather than a traditional flame ionization detector (FID).

Week 1: CHARACTERIZATION OF UNKNOWN COMPOUNDS IN PERFUMES

INTRODUCTION:

Perfumes are solutions of various fragrant essential oils, aroma compounds and fixers. A particular perfume can be identified or differentiated from others perfumes by its GC trace, which constitutes a fingerprint. Because perfume makeup compounds are volatile, they are well suited for GC analysis. In this first GC/MS laboratory, you will perform only qualitative analysis. The second laboratory, however, will initiate you to quantitative analysis by GC/MS. The mass spectrometer (MS) used in these experiments is equipped with a triple quadrupole analyzer and allows several types of mass detection to be performed. In this first experiment, only the conventional (direct MS) mode will be used.

OBJECTIVE:

The main objective of this experiment is to gain familiarity with this particular GC-MS system, including the Varian (Bruker) GC (CP-380)0 with respect to qualitative analysis of complex mixtures of volatile organic compounds that are amenable to gas chromatographic (GC) analysis.

The initial analysis will be used as a non-optimized GC-MS run. For this first injection a general GC column oven program is already set up and will be used. Upon examination of your first chromatogram, the GC oven program will be adjusted to give a more efficient separation method, which will be used for the rest of the measurements. The collected data of the various GC peaks will be examined to determine compounds that are common to all samples and also those that differ from one perfume to another. The compounds in the mixture upon GC separation will then be characterized by electron impact MS. The MS of interest maybe compared within the NIST library spectra for possible identification. Reference to journal articles will prove beneficial when analyzing these compounds.

SAMPLE PREPARATION:

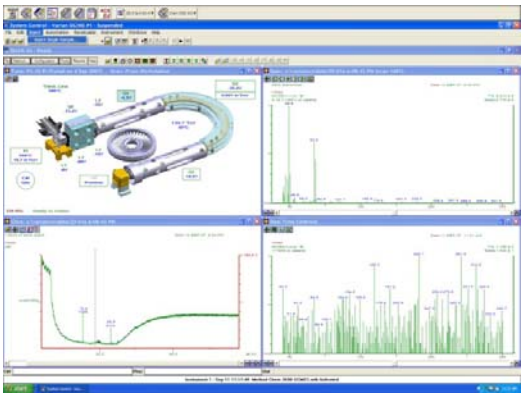
GC-MS is a very sensitive technique and it is thus very important that the samples injected into the GC be very dilute, usually a few hundred picograms of each constituent in a 1 or 2 µL injection is quite sufficient for good results.

The samples that will be examined are various formulations of commercially available perfumes and colognes. These are solutions of fragrant essential oils, aroma compounds and fixers. These compounds are dissolved in ethanol to some unknown concentration, so it is necessary to do a dilution before injecting a sample to avoid overloading and thus contaminating the instrument.

Make 5 dilute samples (ask your instructor for the concentrated samples and note which ones are provided to you). In each of the 5, 2-mL GC vials, pipette 15 µL~3 drops of perfume samples. Fill each vial with 1.5 mL of hexane, close the vials and mix them thoroughly.

PROCEDURE:

What you should see on the screen when you look at the instrument is a control screen, which allows you to see MS operation parameters (image of the instrument) and edit a method, as shown below:



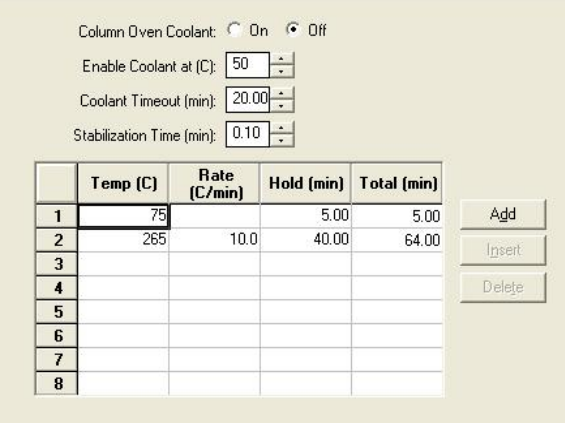
The top left screen indicates the triple quadropole MS status and the top right indicating the current data on analysis. In the bottom left window signals and data from a last run are shown, in the bottom right is live data of the MS.

There are two autosampler methodologies for injecting sample onto the GC. Sample can also be injected manually using a hand held syringe. Both automated methods use the Combi Pal autosampler. One method is for liquid samples and the other is for volatile head space analysis. In this experiment liquid injection will be used for both week 1 and week 2 experiments.

A generic method has been set up to start this experiment. Find the tab labeled "**Method"** and open the file name called "**Chem 3590 GCMS1.mth**". Make sure with your instructor or demonstrator that this method has been activated in the system. This method controls all experimental parameters of importance during a run, including MS data acquisition and GC oven temperature. This method is not optimized, but will be adequate for the preliminary injection.

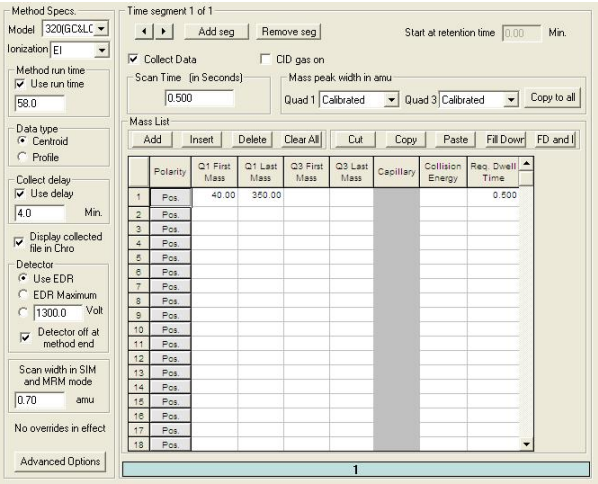
Make sure that the GC and MS parameters are correct by right clicking on the **Chem 3590 GCMS1** method and opening "**view/edit method**" which will bring up the "**Method Builder**" screen.

Select **Column Oven**, and you should see a window similar to the one below:



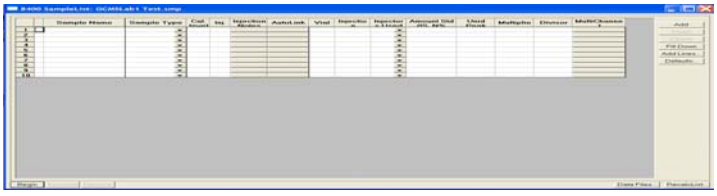
Change the program so that the oven is thus programmed to start at 75^{o}C and stay at that temperature for 5 minutes, and then the temperature will increase by 20 ^{o}C/min until it reaches 265^{o}C, at which it will stay for 2 min. This should give a total run time of 16.5 min.

Go back to "**Method Builder**" screen and select the **MS Acquisition Method** parameters. On this page it is important to note: the mass range (40-350) and the **Collect data** box which need to be checked.



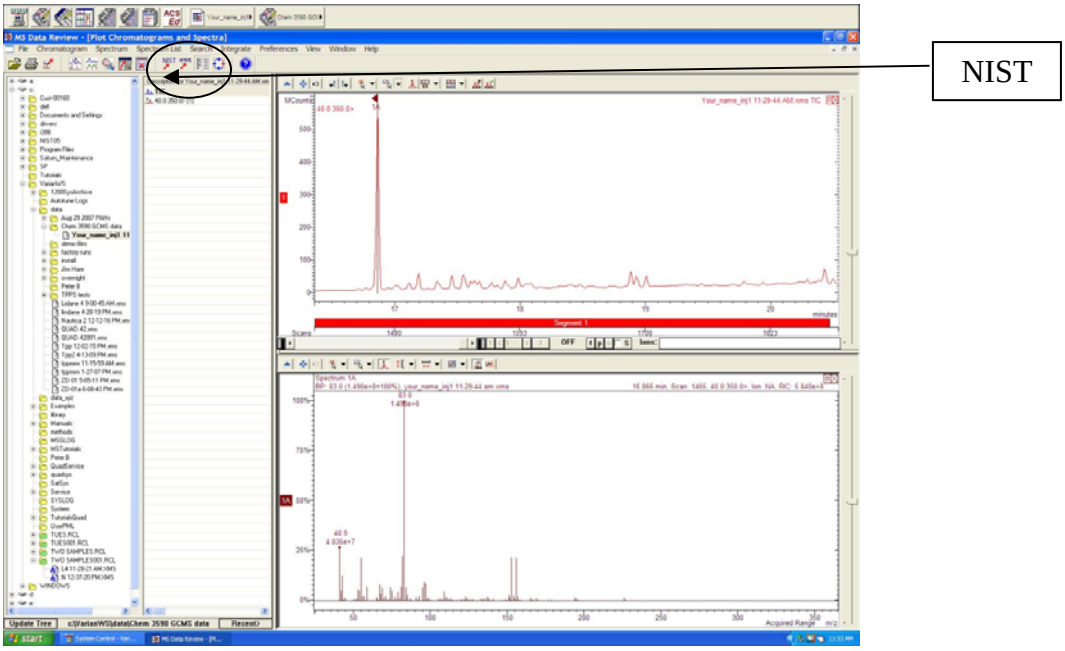
Setting Up The Automated Injection:

Creating a sample list: You will use the auto injector to perform successive analyses, you will need to establish a sample list for automation. Under **File**, click on **New sample list**, create a save file name "**Your group number and day_GCMS1.smp**" and click Ok, you will then see an empty table.



The first run is used to optimize the instrument so only include one perfume sample in the sample list and place this sample in the autosampler tray in the appropriate position.

While your run is proceeding, you may look at the data **"live"**. To open and analyze the live data, click the fourth icon of the bottom left screen on the main screen (see very first picture) and select your run. The screen shown should be similar to the one below:



The software gives you the possibility of focusing on a section of the chromatogram (drag and drop the cursor for the area of interest) and also by clicking on the apex of a peak to obtain its mass spectrum (bottom). A **NIST** search (see bottom on second row from the top) will allow you to identify the substance corresponding to this peak with a high level of certainty.

Qualitative Analysis Using Optimized Conditions and Automated Injections:

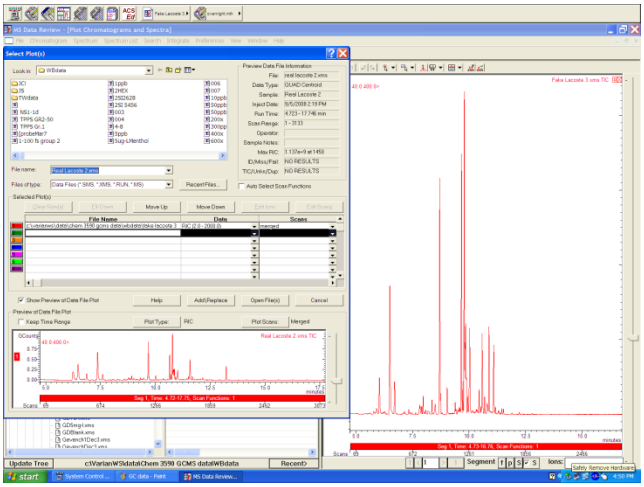
The program you used was not optimized for running your samples. Because you will not have time to go through several optimization steps, an optimized method has been set up for you. You will have to modify the current GC column oven temperature program. Adjust the temperature program as follows:

Open "**view/edit method**" and bring up the "**Method Builder**" screen. Select **Column Oven**. **Start at 80ºC, leave at 80ºC for 3 min, then increase temperature to 270ºC at a rate of 15ºC/min; leave at 275ºC for 1 min.** Save (save as) the method under "Optimized and your group and date" and activate it.

Under Automation, open your previous sample list used for your first injection but now add and fill the list with the perfume samples and place all samples into the autosampler to be injected and analyzed automatically. Also to put the instrument into standby mode after all injections are done add another sample but this time click on the drop down list of the column "**sample type**" selecting **method**, and then select "**overnight**".

Follow the same analysis method for each sample run as you did for your optimized run. Once you are able to get the complete GC for each sample you can overlay the data comparing the different types of perfumes. To do this open up the GC sample file and then go to **File** then open **Select Plot(s)** (shown

below).



You can add up to 7 overlays by going to each slot selecting the file you want to analyze and clicking **add/replace**. When you have all the files you want to analyze click on the button that says **Open file(s)** and all the files of interest should open up.

When you find a GC peak of interest go to the NIST library and identify that compound. Try to find a compound know to be a perfume compound. Specific reference to a journal article on perfumes should provide this information.

OBSERVATIONS AND CONCLUSIONS:

Your observations and conclusions should include the following items:

1. Differences observed between optimized and non-optimized runs? Why would you optimize a run and how would you go about doing this?

2. Your report should include a brief general discussion of your observations of differences and/or similarities of the substances examined from one perfume to another.

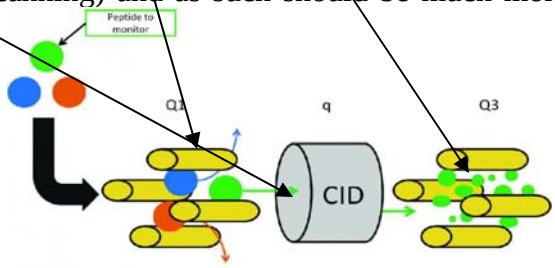
Week 2: MRM AND SIM METHODS FOR DETERMINATION OF MENTHONE

OBJECTIVE:

The objective of this experiment is to demonstrate the unique ability of the Varian (Bruker) GC triple quadrupole mass spectrometer for the sensitive detection of compounds using Single Ion Monitoring (SIM) and Multiple Reaction Monitoring (MRM), also called MS/MS.

INTRODUCTION:

The equipment you are using is a triple quadrupole mass spectrometer, which has two analyzers and a collision cell. The first analyzer or quadrupole is tuned to pass only one m/z value (green ball), in this case we will use the molecular ion (which will be determined from the full scan run). The second quadrupole, which will have no mass specific analytical function, will act as a collision cell to enhance the breakdown of the molecular ion to its product ions. A major (abundant) product ion will be determined with the third quadrupole. This technique will be virtually a non dispersive technique (no scanning) and as such should be much more sensitive than the dispersive full scanning technique.



The GC-MS will be optimized, and then the MS will be utilized in two sensitive ways to determine the presence of the target compound. These techniques are known as tandem mass spectrometry (MS/MS or MRM) and single ion monitoring (SIM).

EXPERIMENTAL:
SAMPLE PREPARATION:

A menthone diluted stock solution (1 drop menthone to 2 ml with iso-octane) is provided. Run the full scan (using week 1 full scan setup) of the menthone using the method (Menthone 2013) found in the methods file.

MS/MS mode:

A GC-MS run, in full scan mode, of menthone solution is used to determine the molecular ion of interest and the GC retention time of this ion. In MS data review you can see the mass spectrum of interest at the molecular ion retention time and you can determine which ion or very narrow range of ions to use in your refined MS/ MS experiment.

The masse(s) of the molecular ion or the most abundant fragment ion(s) you will use for MS/MS

analysis. You will pass the molecular ion through the 1st quod, break the ion into product ions in the 2nd quod (which is only a collision cell) and measure specific product ion(s) in the 3rd quod.

In the **Method Builder** the **Acquisition Method** parameters need to be changed from those used in the full scan method to those appropriate for an MS/MS experiment.

In the **Acquisition Method** you must set the parameter called Q1 First Mass. This is the mass of the molecular ion that will be broken down in Q2. The masses of the products of the decomposition that takes place in the second qradrupole (Q2), the collision cell, you will scan in the third quadrupole from ~ 50 Th to ~ 310 Th. These masses are entered in the Q3 first mass column and the Q3 last mass column. This will cover the masses of all the major decomposition products produced in the collision cell (Q2).

To modify the full scan method to the MS/MS method: turn the CID (argon) gas on and set the collision energy to 15V. DO NOT CHANGE ANY GC PARAMETERS HERE AS WE DO NOT WANT TO ALTER ANY RETENTION TIMES.

You will then run a MS/MS scan allowing only the ion of interest (molecular ion in this case) through Q1, fractionating the ion in Q2 and measuring a specific ion in Q3. This is very specific for the Q3 daughter ion, since this ion can only be produced by collision induced dissociation (CID) of the molecular ion.

Quantitation of the Product Ion:

Dilute the sample you have just run by a factor of 20 (i.e. 20:1). Run the MS/MS method and save this sample and do a further dilution of 10:1 (total dilution 200:1) on this sample and repeat the experiment with no change other than the data file name. Be sure that you save the two diluted samples 20:1 and 200:1 as you will need these two samples to do the next part of the experiment.

In the next part we will use another sensitive GC-MS technique, namely SIM (selected ion monitoring), sometimes called SIR (selected ion recording)

In this method we go back to the to **Method Builder, Acquisition Method** page and change the MS/MS method to a SIM method. To make the **SIM method** the CID gas needs to be turned off. Enter the mass of the ion that you wish to monitor under **Acquisition, Q1 first mass** column and make sure all other columns are blank. (You could also have entered the mass of the ion of interest in the Q3 first mass column and leave all other columns blank as well i.e. you can use either the first quad or the last one). Contrast this with MS/MS where you use all three quads. whereas in SIM the second quad. simply passes the ions from the first to the third quad. without altering them in any way (no collision gas, no collision energy).

Run the two samples, the 20:1 and the 200:1 with the SIM method, again not changing GC parameters.

When you have used both of the more sensitive methods, MS/MS and SIM on the two diluted

samples you can open the chromatograms and select **integrate, plot 1** and the program will integrate the peaks and give you the signal to noise ratio. You can integrate the appropriate GC peaks by hand by holding down the "I" key and connect the start and finish of the peak using the "mouse". You should relate these integrated areas to the actual sample concentrations. You can do further dilutions using both methods and determine estimated LOD (sensitivity) for each method.

OBSERVATIONS AND CONCLUSIONS

Your observations and conclusions should include the following items:

- The specific difference between full scan techniques and SIM, and MS/MS i.e., MRM techniques.
- The reasons for choosing one technique over the other.
- The sensitivity of one technique over the other.
- Tables and/or graphs of the LOD of the GC-MS.

QUESTIONS

1) When comparing the cost for inexpensive and expensive perfumes/colognes there is a considerable price difference. From the experiment conducted and your personal opinion what are the causes of the major price difference of these samples? (Chemical and non chemical)

2) Every instrument used in the lab is currently available in industry. Understanding what the strengths and weaknesses of the GC-MS are, where specifically would an instrument of this caliber be used to a high degree? Explain.

3) The GC-MS can be used simply as either a GC or an MS but here they are coupled as one instrument. What is the overall benefit of combining the GC and MS as one instrument compared to using each component separately? When would one choose to either use only a GC or MS?

4) The LC-MS uses a trap instead of a quatrapole. Compare.

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