

## **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, 6<sup>th</sup> edition, Chapters 20 & 27.**

1. How does electronic impact work as an ionization technique?
2. Explain the terms: product ion scan, single ion monitoring, multiple reaction monitoring?
3. The mass spectrometer in the GC/MS instrument has three Quadrupoles. Explain the function of this system.
4. The GC separates your sample based on different retention times on the column. Comment on GC-MS separation.

### **INTRODUCTION:**

Essential oils and their components have widespread application, from medicine, aromas and fragrance, flavor, agriculture, and general health and wellbeing. A particular essential oil can be differentiated from the “fingerprint” of molecules provided by GC analysis. Because essential oil compounds are volatile, they are well suited and easily analyzed by Gas Chromatography methods. Recently there has been increasing demand for the quickest, most economical and efficient analysis of oils.

In the first week you will use the full scan mode to perform qualitative analysis of lavender and peppermint essential oils. A chemical database is available which has thousands of electron impact (EI) spectra making it possible to identify molecules detected in each oil. Data analysis will also involve comparing the two oils for common and unique components. During the second week, you will explore the different scan modes available within the Triple Quad Mass spectrometer to improve sensitivity and selectivity for qualitative and quantitative analysis.

### **OBJECTIVES:**

- 1) To gain familiarity with the functions and capabilities of the Varian 3800 GC coupled to the Varian 320 triple quadrupole mass spectrometer in the MCAL laboratory.
- 2) To gain a general understanding of using a chemical database (with spectral reference) to identify unknown compounds and the limitation and advantages of those libraries. You will specifically use the National Institute of Standards (NIST) library.

## **WEEK 1: CHARACTERIZATION OF COMPOUNDS IN PEPPERMINT AND LAVENDAR ESSENTIAL OILS**

## PROCEDURE

*Note: 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  $\mu$ L injection is sufficient for good results.*

Samples of lavender and peppermint essential oils are provided. Linalool and Caryophyllene are labelled and are also provided. They should be diluted 1:100 in absolute ethanol.

### **Create the GC-MS Method:**

In the GC-MS screen the top left screen indicates the triple quadrupole MS status and the top right indicates the current data being analysed. In the bottom left window signals and data from the last run are shown and in the bottom right is live data from the MS.

Locate the method macro at the top left of the screen. Left click and select view/edit method which opens the method builder. Name your file with your group and day. Save as your name your method with your group #, names and date. Make sure you know the title so that you can later locate it.

### **Select Injector :**

2. Set the injector temperature to 200<sup>0</sup>C. Set the split ratio to “On” with a split of 20.

### **Select Column Flow:**

3. Set column flow to a rate of 1.0 ml/min.

### **MS Acquisition:**

Save method. Make your method the active method by clicking the folder icon next to the method macro in the Varian workstation. Select your method from the list and open.

### **Set Up the Automated Injection and Start the Run:**

In the method builder, the components of the GC and MS are segregated into compartments. You can access the method input for each component by using the left hand navigation window. The windows you will need to program are shown below:

### **GC Components:**

1. Selecting Column Oven, you should see a window of variables. Set the start temperature to 50<sup>0</sup>C and hold for 5 min. Ramp at a rate of 10<sup>0</sup>C per min. up to 250<sup>0</sup>C and hold for 5 min. The total method run time will be displayed.

4. Select MS acquisition method. Set Q1 first mass to 50. Set Q1 last mass to 400. Make sure the Collect Data box is checked. Masses will be collected between 50 and 400 daltons.

In the galaxie software, the sample list controls the automated run and provides a link of communication between the instrument and the autosampler. Under the Varian workstation window, click File > New Sample List. This will open a new sample list. Name the sample similar to

your method. Make sure you know where it's saved and what it's called so that you can refer back to when needed.

In the sample list enter the sample name and vial number of the four compounds analysed. Ensure Tray 2 is selected in the dropdown. Enter the injection volume to be 1 ul. In the bottom right corner, click Data Files. This allocates where your data will be saved. Select Varian WS > Data > 4590 2018` and create a new folder with your group name. Click Ok. Double Check that everything is entered in the sample list correctly. Click "Begin" to start the run.

*Note: Depending on the previous settings, the instrument may take some time to stabilize or reach specific temperatures and settings of your method. You will see the status change from Not Ready to Waiting for injection to Ready. If after a few minutes*

*this doesn't seem to be progressing, seek help from instructor.*

While your run is proceeding, you may look at the data "live". To open and analyze the live data, click the fourth icon "MS DATA REVIEW" of the bottom left quadrant of the screen in the Varian workstation window and select your run. The top pane is the Total Ion Chromatogram (TIC). This displays a count of the total ions seen at any given time in the run.

The bottom pane displays the masses of specific ions seen for a designated time in the run. Click on a specific peak in the TIC and the RIC will display all masses of all ions for that particular time.

To perform a NIST search of the component being selected, select a peak in the run and click the NIST icon. This will give you a search of the data base of all EI spectra similar to your spectra.

### **Qualitative Analysis of the essential oils:**

There are two aspects of the analysis. The first is to use the NIST database to identify the components of lavender and peppermint oils which have been separated by the GC column. The second aspect is to identify specific ions in the spectra of these subcomponents (Linalool and Caryophyllene) that can be used for quantitative analysis and comparison of scan modes in week 2. Open the MS Data review and locate your file(s). Go through each major peak in the spectrum of the peppermint and lavender oils using the cursor (best to expand peak). For each peak, perform a search by clicking the NIST icon.

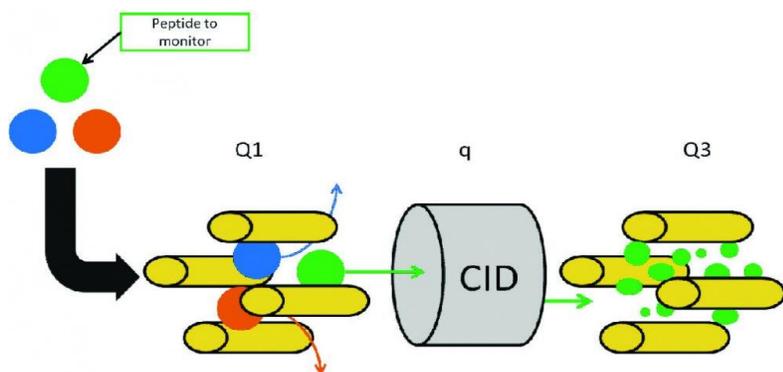
The NIST search displays the compound(s) that are matched between your spectra in order of confidence. Record the first two compounds with highest probability scores. Do not pay any attention to a score below 800. You can refer to the pane, which displays visual representation of the ions matched to the database as well as the pane which displays the structure of the compound identified. Also note any synonyms in the right hand pane, sometimes these are simplified and can be more useful. Write down the retention time of the compounds of interest which is important when trying to decide whether the compound is unique to one or common to both oils.

### **Week 2:**

### **MS-MS and Single Ion Monitoring (SIM) Methods Comparison in Essential Oils**

#### **Introduction:**

The equipment you are using is a triple quadrupole mass spectrometer, which has two analyzers (Q1 and Q3) and a collision cell. The first analyzer or quadrupole can be tuned to pass only one  $m/z$  value (which will be determined from the full scan run). The second quadrupole, which has no analytical function acts as a collision cell to fragment the molecular ion into smaller product (daughter) ions. The third quadrupole is also a mass analyzer, and can be set to measure 1 specific ion or a range of fragment ions from the collision cell. The end goal in developing a method depending on what you are interested in and the sample you are working with, but ultimately an GC analyst would strive for those parameters which provide best selectivity and sensitivity and the least time.



### Procedure:

You will be working with the compounds Linalool and Caryophyllene. You should have found both these compounds in the Peppermint and Lavender oils. You will need to go back and search for them in your full scan spectra to verify their specific retention times.

### MS-MS Mode: Edit Method:

Open the method you made for full scan mode and “save as”, indicating in the name that it is your MS-MS method. Turn on the CID gas in the method builder. Make sure the CID gas tank is on – it is argon located by the fume hood. The regulator should be set to 90 psi. Change the Q1 mass to the ion of interest (from the previous week’s full scan mode) for your compound that eluted first, (step 1). Set Q3 first mass to 50 and Q3 last ion mass to match Q1 first mass and set the collision energy to 15 ev. (step 2). Click add segment at the top. This will split the run in to two time segments and allow different settings for each.

Repeat Steps 1 and 2 above for time segment #2. Change the time in the box at the top to a time before your RT for compound #2. Save the method and open it in the Varian Workstation to make it active. Set up the Linalool and Caryophyllene samples and the two essential oils to run with this method in the sample list.

### SIM mode: Edit method:

Open your full scan method, "Save As" and name as SIM method. Change the Q1 mass to the most abundant ion for your earlier compound from full scan mode. Leave Q3 blank. Add a segment and enter the most abundant ion for compound #2. Change the time for the segment to be prior to your retention time of compound 2. Save your method.

**To run samples overnight:**

After the last MS-MS sample, in the Sample type column select Activate method in the drop down. Click the same line under the autolink column and a window will appear for you to enter your method path. Browse and find your method. This will make the instrument switch to that method in the middle of your sample list. Add the standards and essentials to your list again. It will evaluate using the SIM method.

Consult with an instructor before you leave to make sure everything is okay. Collect your data the following day or when you have time.

**Questions:**

- 1) What characteristics make an oil unique or different than other oils. Give an example.
- 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) What is the overall benefit of using MS-MS compared to using full scan analysis in the MS.
- 4) What is the advantage of SIM compared to full scan MS analysis.