

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. What is the difference between full scan, single ion monitoring, multiple reaction (ms-ms) monitoring?
3. The mass analyzers in the instrument are Quadrupoles. The configuration is called a Triple Quad. Describe briefly the purpose of the three quads.
4. What is the advantage of using the mass spectrometer (MS) as a detector rather than a traditional flame ionization detector (FID).

INTRODUCTION:

An **essential oil** is defined as a concentrated hydrophobic liquid containing volatile aromatic compounds from plants. Essential oils can be named from the plant from which they were extracted, such as oil of clove. An essential oil contains the characteristic fragrance of the plant from which it is derived. Two oils that will be observed in this experiment are peppermint oil and lavender oil. These oils contain compounds which give them their distinctive characteristics, and these compounds can be separated using gas chromatography. Gas chromatography is used to separate volatile, non-polar compounds, which these oils contain. Longer chain oils which would be less volatile would have to be derivatized, to increase their volatility for their analysis. You will compare the constituents of both oils and their properties. The constituents in the oils that are separated by GC can be broken into fragments which act as a fingerprint of the compound. The parent compounds and/or the fragments can be used in quantitation of the compounds if standards are available.

In the first week you will use the full scan mode to perform qualitative analysis of lavender and peppermint essential oils. A chemical database (NIST) 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 quantitative analysis.

OBJECTIVES:

- 1) To gain familiarity with the functions and capabilities of the Varian (Bruker) 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 NIST.

- 3) To compare three techniques for identification and quantitation of target molecules.

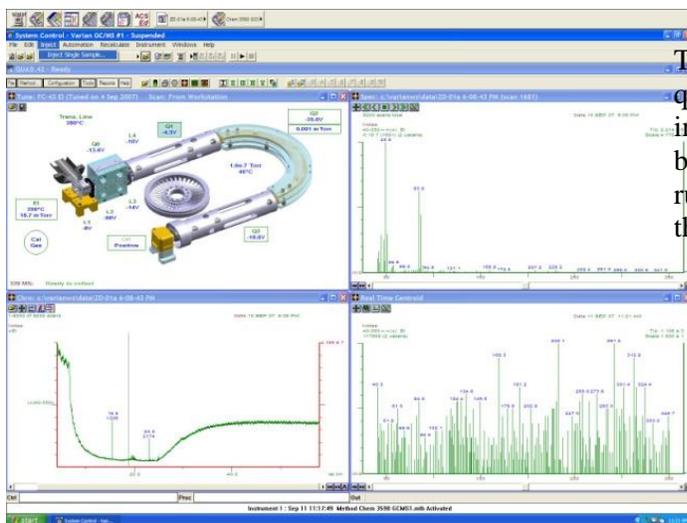
WEEK 1: CHARACTERIZATION OF COMPOUNDS IN PEPPERMINT AND LAVENDAR ESSENTIAL OILS

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 μL injection is sufficient for good results.

Samples of lavender essential and peppermint oil are provided for you. You will find them in the auto sampler tray of the GC-MS. They are diluted 1:100 in absolute ethanol.

Below is an image of the interface for the Varian Software:



The top left screen indicates the triple quadrupole MS status and the top right indicating the current data 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.

A) Create the GC-MS Method:

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

In the method builder, the components of the GC and MS are segregated into compartments. The ones you will set are the column oven, injection temperature, flow rate and the MS (acquisition).

GC Components:

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

Column Oven Coolant: On Off

Enable Coolant at (C):

Coolant Timeout (min):

Stabilization Time (min):

	Temp (C)	Rate (C/min)	Hold (min)	Total (min)
1	75		5.00	5.00
2	265	10.0	40.00	64.00
3				
4				
5				
6				
7				
8				

Buttons: Add, Insert, Delete

- Change the start temperature to 50°C and hold for 5 min.
- Ramp at a rate of 10°C up to 250 and hold for another 5 min

4. Select **Injector** :

Front Injector | Middle Injector | Rear Injector

Front Injector Type: Injector Oven: On Off

Temperature (C):

Split Ratio...

- set the injector temperature to 200.
- Set the split ratio to “On” under initial and a ratio of 20.

5. Select **Column Flow**

- Set the column flow rate to 1.0 ml/min
- Make sure the mode it set to constant flow

Front EFC | Middle EFC | Rear EFC

Front EFC Type:

	Pressure (psi)	Rate (psi/min)	Hold (min)	Tot
1				
2				
3				
4				

Buttons: Add, Insert, Delete

Constant Column Flow Mode

Constant Flow: Off On

Pressure Pulse: No Yes

Column Flow (ml/min):

Pulse Pressure (psi):

Pulse Duration (min):

MS Acquisition:

6. In the left hand navigation pane, select ms acquisition method.

Method Specs.

Model: 320GC&LC

Ionization: EI

Method run time: 58.0

Data type: Centroid

Collect delay: 4.0 Min.

Detector: Use EDR

Scan width in SIM and MRM mode: 0.70 amu

No overrides in effect

Advanced Options

Time segment 1 of 1

Start at retention time: 0.00 Min.

Collect Data: CID gas on:

Scan Time (in Seconds): 0.500

Mass peak width in amu: Quad 1: Calibrated, Quad 3: Calibrated

Copy to all

Mass List

	Polarity	Q1 First Mass	Q1 Last Mass	Q3 First Mass	Q3 Last Mass	Capillary	Collision Energy	Req. Dwell Time
1	Pos	40.00	350.00					0.500
2	Pos							
3	Pos							
4	Pos							
5	Pos							
6	Pos							
7	Pos							
8	Pos							
9	Pos							
10	Pos							
11	Pos							
12	Pos							
13	Pos							
14	Pos							
15	Pos							
16	Pos							
17	Pos							
18	Pos							

- Set Q1 first mass to 50
- Set Q1 last mass to 400
- Make sure the Collect Data box is checked
- Set the delay to 2 min.

7. Click File > Save method. Method is done!

8. 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.

B) Set Up the Automated Injection and Start the Run:

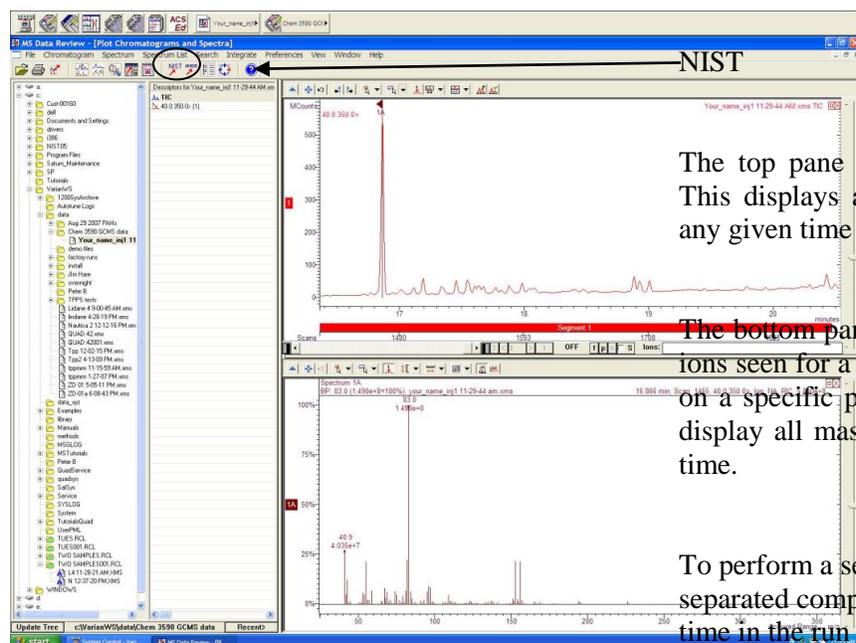
In the software, the sample list controls the automated run and provides a link of communication between the instrument and the auto sampler. Under the Varian workstation window, click File > New Sample List > and name accordingly to your day and week and name. Make sure you know where it's saved and what it's called so that you can refer back to it if needed.

- Enter the sample name and vial number. 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 > 3590, and 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 in Screen III "MS DATA REVIEW" of the bottom left quadrant of the screen in the

Varian workstation window and select your run. The screen shown should be similar to the one below:



The top pane is the Total Ion Chromatogram. This displays a count of the total ions seen at any given time in the run.

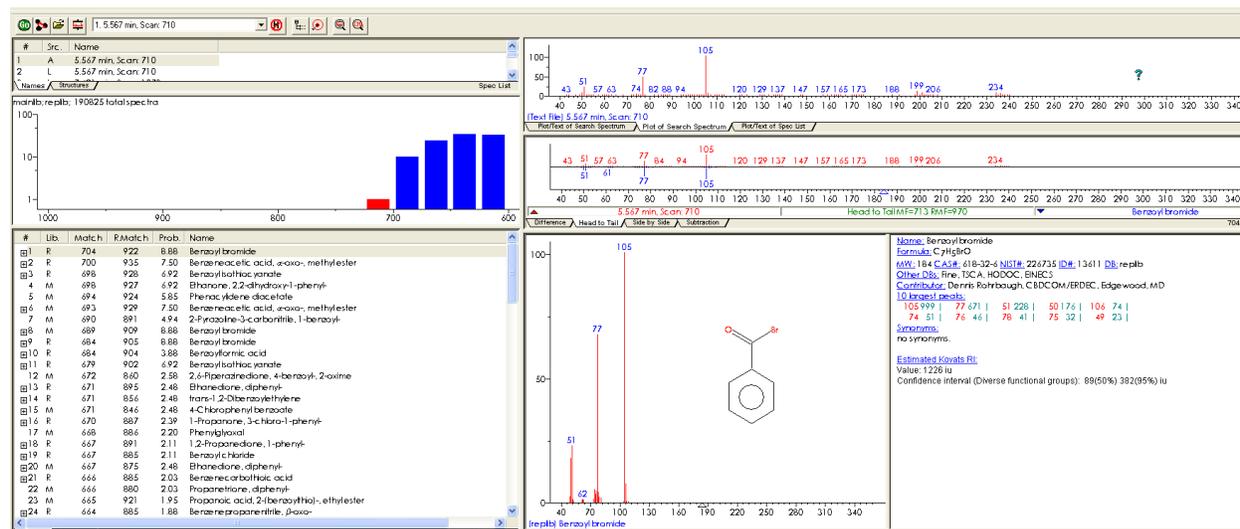
The bottom pane displays the masses of specific ions seen for a designated time in the run. Click on a specific peak in the TIC and the RIC will display all masses of all ions for that particular time.

To perform a search of the identity of your separated compounds, select the compounds time in the run and click the NIST icon.

Qualitative Analysis of the essential oils:

There are two aspects of the analysis. The first is to use the NIST database to identify the separated components of each oil, and second to identify specific ions in the spectra that will be used for further quantitative analysis and comparison of scan modes next week.

1. Open the MS Data review and locate your file(s).
2. Go through each major peak in the spectrum using the cursor. For each peak, perform a search by clicking the NIST button.



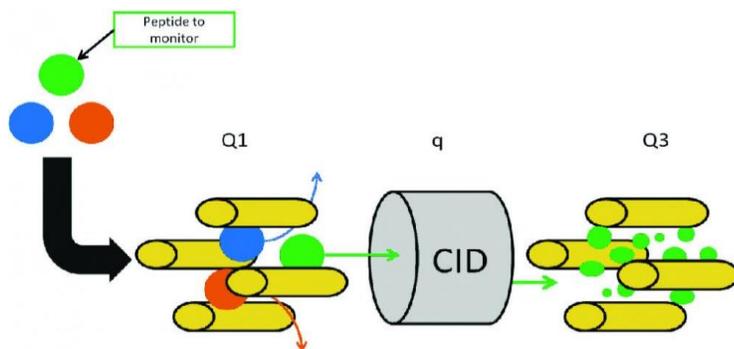
The bottom left pane displays the compound(s) that are matched with your spectra in order of confidence. Record the first 1 or 2 (depending on the scores). Do not pay any attention to a score below 800. You can refer to the top pane, which displays visual representation of the ions matched to the database as well as the middle 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. **ALSO write down the retention time, which is displayed in the very top left pane. This is important when trying to decide whether the compound is unique to or common to both oils**

Each peak represents a different molecule separated from the essential oil by the GC. Integrate each peak by holding down the "I" key and run a line across the base of each peak or use the integrate in the "action" buttons. Record the integration values. This data will give you a comparison of the levels of each compound in the individual oils. This can be tabulated in a table and the percent of the compounds compared in the results section and compared with literature values in the discussion. The ratio would depend on the plant variety the oil comes from and possibly could be used to identify a variety.

In your **laboratory write-up** you can list in results the component oils of each oil and discuss the components and how they affect the characteristics of each essential oil.

Week 2: MS-MS AND SIM METHODS COMPARISON IN ESSENTIAL OILS

The equipment you are using is a triple quadrupole mass spectrometer, which has two analyzers and a collision cell. The first 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 a mass analyzer, and can be set to measure one specific ion or a range of fragment ions. The end goal in developing a method depends on what you are interested in and the sample you are working with, but ultimately a GC analyst would strive for those parameters which provide best selectivity and sensitivity and the least analysis time.



Procedure

You will be working with Linalool, and Caryophyllene. You should have found these compounds in the oil's. You will need to go back and search for them in your full scan spectra to verify **their specific** retention time(s).

For time purposes, you will set up the runs to go overnight. This should only consume about 1-1.5 hours of the lab period, but you will need to come back to collect your data at a time when your schedule allows after the samples are finished running.

Standards:

The samples examined are Lavender, Peppermint oil diluted 1:100 in absolute alcohol, and 4 dilutions of Linalool, and Caryophyllene in concentration of 1:100, 1:200, 1:400 and 1:800.

MS-MS Mode: Edit Method

1. Open the method you made for full scan mode and use it as a template to make new methods using "save as", indicating in the name that it is your new (MS-MS for example) method.
2. Turn on the CID gas in the method builder for the ms-ms run. **Make sure the CID gas tank is on – it is argon located by the fume hood.** The regulator should be set to 90 psi.
3. Change the Q1 mass to a major the ion of interest (from the previous week's full scan mode) **for your compound (Linalool, Caryophyllene) that eluted first.**
4. Set Q3 first mass to 50 and Q3 last ion mass to match Q1 first mass value. Use 15 ev.
5. Click **add segment** at the top. This will split the run into two time segments and allow different settings for each. Add 15 ev to each segment.
6. Repeat Steps 3 and 4 for time segment #2.
7. Change the time in the box at the top to 1-2 minutes before your RT for compound #2.
8. Save the method and open it in the Varian Workstation to make it active.
9. Add to sample list the diluted essential oils and linalool and caryophyllene oils to run with this method. Place appropriate auto sampler number and tray (tray 2).

SIM mode: Edit method:

1. Open your full scan method, "Save As" and name as SIM method.
2. Change the Q1 mass to the most abundant ion for your earlier compound from full scan mode. Leave Q3 blank.
3. 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.
4. Save your method.
5. Add the SIM samples to your sample list:
 - a. After the last MS-MS sample, in the **Sample type** column select **Activate method** in the drop down.
 - b. Click the same line under the **autolink** column and a window will appear for your to enter your method path.

- c. Browse and find your SIM method. This will make the instrument switch to that method after the MS-MS run in the middle of your sample list.
6. 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. Make sure you come back soon to get your data – Do not leave it until 1 day or 2 before your report is due.

Data:

You have data from the MS-MS and SIM runs that enables you to develop standard curves. You can integrate the area under the peaks of interest by holding down the “I” key and place your pointer between points at the base of the peaks that you wish to integrate or use the integrate function in the above “ribbon”. Develop these standard curves and discuss the advantages of the different methods referring to the standard curves, etc. You can use the SIM and MS-MS curves to calculate approximate concentration of Linalool and Caryophalline in peppermint and lavender oils. Discuss whether the GC-MS is a good instrument for quantitation as observed in your analysis and referring to the literature.

QUESTIONS

1. Why can you have a NIST library for the GC-MS and not for a LC-MS?
2. What is one major disadvantage of the GC-MS compared to an LC-MS?
3. What is advantage of SIM and MS-MS over full scan method in GC-MS?
4. Which is more sensitive method SIM or MS-MS? Why?