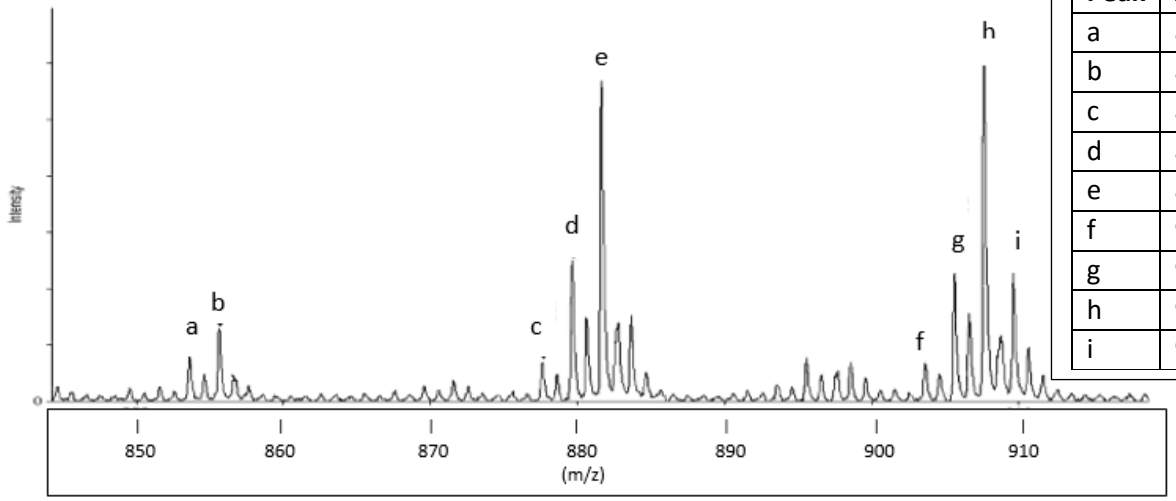
MALDI Activity 4

MALDI-TOF Mass Spectrometry: Data Analysis

Model 1: Introduction to Triacylglycerides (TAGs)

In MALDI Activity 3 you learned how to use the MMass program to plot an intensity versus *m/z* graph (a mass spectrum). The next step is for you to learn how to determine possible identities for the peaks in a mass spectrum of an oil or fat. Figure 1 illustrates a mass spectrum of olive oil in which a number of the largest peaks have been labeled. **All of the peaks are [M+Na]$^+$ adducts only**, due to the method of sample preparation used to obtain the data. You will use an Excel TAG data analysis table to help you with this task.



Peak	*m/z*
a	853.2
b	855.3
c	877.3
d	879.3
e	881.4
f	903.3
g	905.4
h	907.4
i	909.5

Figure 1: Sodium adduct olive oil MALDI-TOF data reproduced from Chapagain and Wiesman.[2]

TAG is an abbreviation for a **T**ri**A**cyl**G**lyceride, which are more commonly called an oil or fat. TAGs are a major component of plant seed oils or animal fats. Triacylglycerides are triesters formed from glycerol and three free fatty acids, as can be seen in the esterification reaction shown below in figure 2.

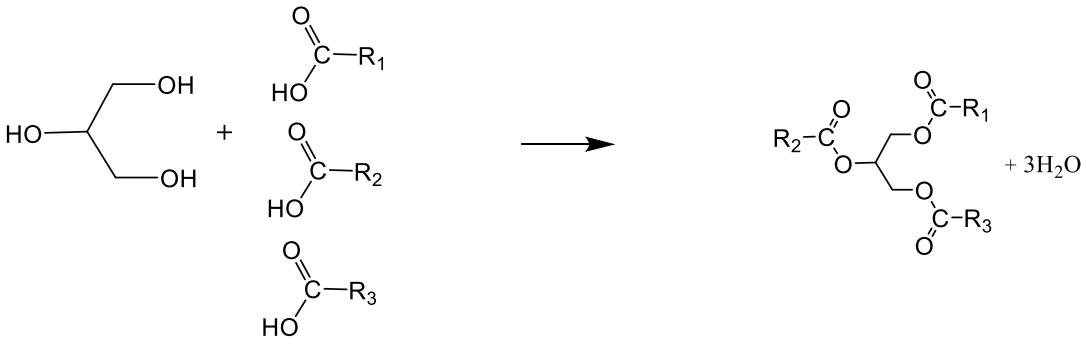


Figure 2: Condensation of Glycerol and Three Fatty Acids to Form a TAG.

All TAGs have the same glycerol backbone, but they vary in the identity of the R groups, which are derived from the fatty acid. All the R groups in TAGs have long chains, ranging from 10 – 28 carbons. Some of these R groups are totally saturated while others have one or more units of unsaturation. There are many different types of TAGs because there are many different free fatty acids that can condense with glycerol to form a TAG.

Table 1: Examples of Seed Oil Free Fatty Acids

Acid Name	Abbreviation	Structure Symbol *	Monoisotopic mass (Da)
Palmitic	P	C16:0	256.24
Linoleic	L	C18:2 9c 12c	280.24
Oleic	O	C18:1 8c	282.26
Steric	S	C18:0	284.27

* C18 means 18 carbons, C18:2 means 2 double bonds. 9c means a cis double bond at position 9

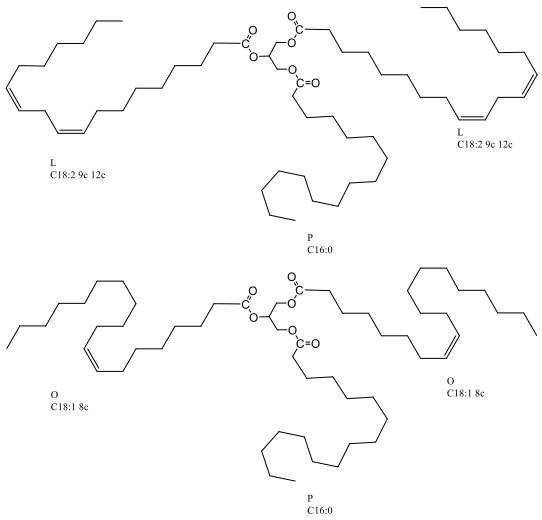


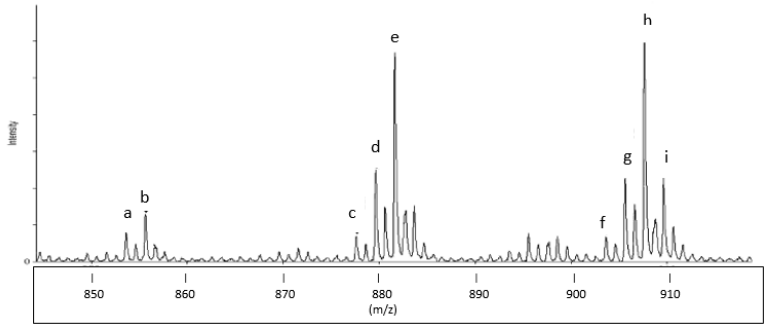
Figure 3: Two examples of triacylglycerides found in olive oil.

Figure 3 shows two examples of Triacylglycerides (TAGs) that are found in olive oil. Figure 3a shows a TAG, called **LPL**, composed of a two linoleic (**L**) and one palmitic (**P**) fatty acid linked to glycerol through an esterification reaction. Figure 3b shows a TAG, called **OPO**, composed of two oleic (**O**) and one palmitic (**P**) fatty acid linked to glycerol through an esterification reaction.

In model 2 (on the next page) you will determine the identity of a TAG that is present in olive oil, similar to what we will do with our seed oil samples in lab. To accomplish this task, you will need to download the TAG Data Analysis Table from the D2L site for this course. From this course's D2L site, go to the Content tab, scroll down and open the MALDI Mass Spectrometry Folder. In this folder download the "TAG Data Analysis Table".

Model 2: Identifying a TAG from Olive Oil using the TAG Data Analysis Table

Figure 1 has been reproduced here for convenience.



Peak	*m/z*
a	853.2
b	855.3
c	877.3
d	879.3
e	881.4
f	903.3
g	905.4
h	907.4
i	909.5

Figure 1: Sodium adduct olive oil MALDI-TOF data reproduced from Chapagain and Wiesman.[2]

In figure 1, find peak e and the corresponding *m/z* for that peak.

1. What is the *m/z* value for peak e? _____

2. Open the Excel TAG Data Analysis Table, then open the triglycerides sheet tab at the bottom left. Find the portion of this sheet that matches figure 4 to the right. The blue cell of the TAG Data Analysis Table is where you will enter in the *m/z* value for peak e. Do that right now. You will notice that the values for the green, pink and yellow cells will update once you enter in a value in the blue cell. The updated cells calculate the *m/z* value of the TAG, [M], if the value you entered for the ion mass was actually $[M+H]^+$ (displayed in green), $[M+Na]^+$ (pink), or $[M+K]^+$ (yellow), respectively. We will focus on the pink values only, which correspond to $[M+Na]^+$, because figure 1 displays only sodium adducts.

	A	B	C	D
7	Type in the mass of the ion signal that you			
8				
9		877.3		
10				
11	if your peak was $[M+H]^+$, then [M] is			
12		876.3		
13				
14	if your peak was $[M+Na]^+$, then [M] is			
15		854.3		
16				
17				
18				
19	if your peak was $[M+K]^+$, then [M] is			
20		838.3		
21				

Figure 4: The entry portion for *m/z* peaks in the TAG table.

3. In the TAG Data Analysis Table, find the table listed as "Table 2" on the right side of the Excel file. You will notice that there are many cells that are highlighted in pink and that each cell is the same value. This is because all of the highlighted values corresponds to different potential TAGs that have the same *m/z* ratio and would yield the $[M+Na]^+$ peak value that you entered. To confirm this idea for yourself, use the monatomic mass entries from "***Table 1:** Examples of Seed Oil Free Fatty Acids*" from this activity (on the previous page) to check that a TAG for PLS would have the same mass as OOP. (**Hint:** a calculator may be helpful here.)

PLS _____ OOP _____

Check your work. Are the numbers you calculated above within ±1 *m/z* of each other? **Yes** or **No**

If you circled "Yes", you move on to 4. If you circled "No", consult further with your group or ask another group or your instructor for help.

4. Notice that in "Table 2" in the TAG Data Analysis Table, the column farthest to the left (highlighted in blue) has a listing of both the molecular weight and the structure symbol for many different fatty acids. Each of these structure symbols correspond to a fatty acid abbreviation that can be seen in figure 5.

5. Due to the way the table calculates matches, approximately half of the matches are duplicates. The duplication begins past the value that touches only two black squares, as can be seen below in figure 6. **You will ignore the values that appear inside the black block of cells.**

Fatty acid Names, Abbreviations and Symbols		
Abbr.	*Fatty acid name*	*Structure symbol*
M	Myristic acid	C14:0
P	Palmitic acid	C16:0
Pe	Palmitoleic acid	C16:1 9c
S	Stearic acid	C18:0
O	Oleic acid	C18:1 8c
El	9-elaidic acid	C18:1 9c
Va	vaccenic acid	C18:1 11c
L	Linolec acid	C18:2 9c. 12c
Le	α-Linolenic acid	C18:3 9c, 12c, 15c
E	Eiconanoic	C20:0
Ee	Eicosenoic acid	C20:1 11c
Ed	Eicosadienoic acid	C20:2 11c, 14c
D	Docosanoic	C22:0
Er	Erucic acid	C22:1 13c
Dd	Docosadienoic acid	C22:2 13c, 16c
Dt	Docosatrienoic acid	C22:3 13c, 16c, 19c
N	Nervonic acid	C24:1 14c

Figure 5: Abbreviations, names and structure symbols for some fatty acids.

Table 2	This table requires that one FA be C16:0														
C16:0	256.2402	256.2402	256.2402	256.2402	256.2402	256.2402	256.2402	256.2402	256.2402	256.2402	256.2402	256.2402	256.2402	256.2402	256.2402
	228.2089	256.2402	254.2246	284.2715	282.2559	280.2402	278.2246	312.3028	310.2872	308.2715	340.3341	338.3185	336.3028	334.2872	366.3498
	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C20:2	C22:0	C22:1	C22:2	C22:3	C24:1
228.2089 C14:0	750.67												858.77		
256.2402 C16:0	778.70	806.74								858.77					
254.2246 C16:1	776.69	804.72	802.71						858.77						
284.2715 C18:0	806.74	834.77	832.75	862.80		858.77									
282.2559 C18:1	804.72	832.75	830.74	860.78	858.77										
280.2402 C18:2	802.70	830.74	828.72	858.77	856.75	854.74									
278.2246 C18:3	800.69	828.72	826.71	856.75	854.74	852.72	850.71			880.75					
312.3028 C20:0	834.77	862.80	860.78	890.83	888.81	886.80	884.78	918.86							
310.2872 C20:1	832.74	860.77	858.76	888.81	886.79	884.77	882.76	916.84	914.82						
308.2715 C20:2	830.74	858.77	856.75	886.80	884.78	882.77	880.75	914.83	912.81	910.80					
340.3341 C22:0	862.80	890.83	888.81	918.86	916.85	914.83	912.81	946.89	944.88	942.86	974.92				
338.3185 C22:1	860.78	888.81	886.80	916.85	914.83	912.81	910.80	944.88	942.86	940.85	972.91	970.89			
336.3028 C22:2	858.77	886.80	884.78	914.83	912.81	910.80	908.78	942.86	940.85	938.83	970.89	968.88	966.86		
334.2872 C22:3	856.75	884.78	882.77	912.81	910.80	908.78	906.77	940.85	938.83	936.81	968.88	966.86	964.85	962.83	
366.3498 C24:1	888.81	916.85	914.83	944.88	942.86	940.85	938.83	972.91	970.89	968.88	1000.94	998.92	996.91	994.89	1026.96

Figure 6: Highlighted unique values from TAG table 2.

6. All of the TAGs in figure 6 have one palmitic acid (P) as one of their acyl groups, as can be seen in the structure symbol C16:0 listed below the heading "**Table 2**" above (or in your TAG Data Analysis Table). This same pattern can be seen in the rest of the tables in the TAG Data Analysis Table. For example, in table 1 all of the TAGs have one myristic acid (M) as one of their acyl groups, as can be seen in the structure symbol C14:0 listed below the heading **"Table 1"**.

7. Use the fatty acid abbreviations in figure 5 to identify all possible unique TAGs **highlighted in pink** from Figure 6 (or in your TAG Data Analysis Table) for peak value e from figure 1. Note that the structure symbol C18:1 has multiple possible identities.

TAG number	Fatty acid abbreviations
TAG 1	R_1= __P__ R_2 = __Dd_ R_3= __M___
TAG 2	R_1= __P__ R_2 = ____ R_3= _____
TAG 3	R_1= __P__ R_2 = ____ R_3= _____
TAG 4	R_1= __P__ R_2 = ____ R_3= _____
TAG 5A	R_1= __P__ R_2 = _Va__ R_3= __Va__
TAG 5B	R_1= __P__ R_2 = _Va_ R_3= __El__
TAG 5C	R_1= __P__ R_2 = ____ R_3= _____
TAG 5D	R_1= __P__ R_2 = ____ R_3= _____
TAG 5E	R_1= __P__ R_2 = ____ R_3= _____
TAG 5F	R_1= __P__ R_2 = ____ R_3= _____

TAGs 5A through 5F should show all unduplicated combinations of the C18:1 structure symbol. 5A and 5B have been done for you

8. Scientific studies of the olive oil lipid profile show that the TAG that corresponds to peak e in the MALDI mass spectrum has a palmitic acid (P) and oleic acid (O). Given this information, which TAGs listed above could correspond to peak e?

9. *Check your work.* Do you have 3 TAGs listed above? **Yes** or **No**

   If you circled "Yes", you move on to question 10. If you circled "No", consult further with your group or ask another group or your instructor for help.

10. The actual TAG that corresponds to peak e is **POO**. Is this TAG included in your list above in 8? **Yes** or **No**

    If you circled "Yes", you move to the next page. If you circled "No", consult further with your group or ask another group or your instructor for help.

In the Excel TAG Mass Calculator file you were working only with Table 2 to determine what your possible TAGs could be for peak e (*m/z* = 881.4) in the MALDI Mass Spectrum of Olive Oil, with your TAG analysis focused on the potential $[M+Na]^+$ adducts. If it was not known that this peak was due to a

$[M+Na]^+$ adduct, then an analysis of potential $[M+H]^+$ adducts (displayed in green) and the $[M+K]^+$ adducts (displayed in yellow) would also need to be done in Table 2. This would add to the list of possible TAG identities for peak e. (Note: Table 2 has no potential $[M+K]^+$ adducts since no cells are displayed in yellow). The additional analysis for peak e doesn't end with Table 2 and the other potential $[M+H]^+$ and $[M+K]^+$ adducts found in this table. **Notice that Table 2 is one of 15 Tables in the Excel TAG Mass Calculator file**. In order to do a complete analysis of TAGs that could correspond to peak e, the same process that you carried out using Table 2 would also need to be carried out using Tables 1, 3, 4, 5, 6, etc. As you can imagine, the process of analyzing all the major peaks in a MALDI-TOF mass spectrum of any oil sample is no small task.

Fortunately, the analysis process that you just completed for peak e using Table 2 is the same process that can be used with **ALL** fifteen TAG Mass Calculator tables to analyze other peaks in an oil sample to determine possible TAG identities for their $[M+H]^+$, $[M+Na]^+$ and $[M+K]^+$ adducts. The process is somewhat simplified by the fact that all TAGs will appear on more than one table. For example, the TAG **POO** will show up in both table 2 (where all the TAGs contain the **P** acyl group) and in table 5 (where all the TAGs contain the **O** acyl group) and can be listed only once in the possible matches.

Exercises for MALDI Activity 4:

1. Using table 2 in the TAG Mass Calculator file, identify possible $[M+Na]^+$ sodium adduct TAGs for peaks **b** (m/z = 855.3) and **d** (m/z = 879.3) in figure 1.

2. Which peak would also have a sodium adduct found on table 6?

3. Additional research showed that the actual TAG appears on both tables. Identify the three possible TAGs for this peak based on your comparison of results from table 2 and table 6.

References:

1. http://www.webelements.com/ accessed 1/18/2016
2. Chapagain, B., P.; Wiesman, Z. *J. Agric. Food Chem.* **2009**, *57*, 1135–1142

Funding Provided by the National Science Foundation

"MALDI-based Research-like Experiences in a 2YC/4YC Collaboration with a Renewable Fuels Industry Partner" NSF ATE #1400885