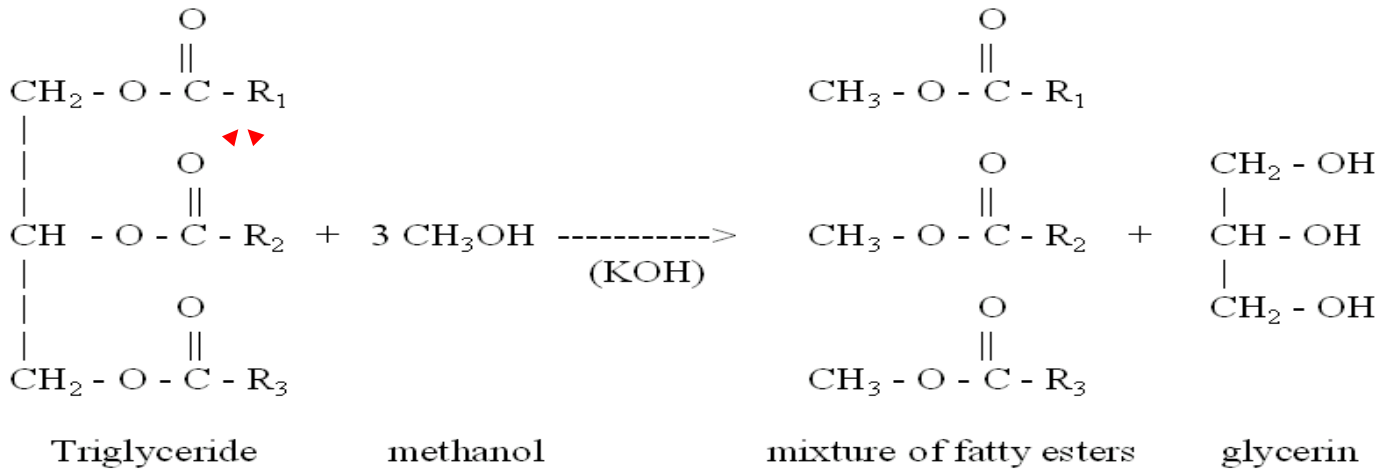
CHE246 Biodiesel Lab

In this lab four types of vegetable oil (peanut, canola, soy, and corn) are used to make biodiesel. The 4 types of biodiesel produced are not labeled and you must use GC-MS to determine the original vegetable oil. The choices are canola, corn, peanut, and soybean oil. See Table 1for the percentage of each fatty acid present in the oils. Please read the section in your text on biodiesel synthesis.

General Procedure

Biodiesel will be produced from virgin vegetable oils via a trans-esterification process. Each lab group will be assigned 2 unknown vegetable oils from which to make biodiesel. In this process sodium methoxide (50 mL) is added to the vegetable oil (250 mL) and reacted with constant stirring for an hour at ~55 C. In biodiesel synthesis, the volume of methanol used is usually 20% of the oil volume and 5.5 g of sodium hydroxide is used for each liter of oil and is mixed with the methanol prior to adding to the oil. The methoxide was prepared previously and is ready to use. After reacting, the biodiesel would need to be 'washed' and 'dried' prior to use in a vehicle, but we will analyze directly.



Analysis of Methylesters

Dilute the biodiesel by adding 10 uL to 5 mL of methanol in an amber vial. Each group needs to analyze their own biodiesel plus the other two unknowns. As exact quantitation in this lab is not criticial, we will not use an internal standard, but rather inject as close to 2 ul as possible into the GC-MS. Table 1lists the fatty acid content of each type of oil. By making biodiesel we remove an H and add a -CH_3 group, thus the new fatty acid methyl esters (FAMEs) are 14 amu heavier than the fatty acid. To identify oils compare the area counts of each methyl ester within and between samples to estimate the ratios of each that are present. The best strategy is usually to look at the area ratios of FAMEs within each sample and compare to the other samples. E

Palmitic Acid: $CH_3(CH_2)_{14}COOH$ (MW = 256.43)

Stearic Acid: $CH_3(CH_2)_{16}COOH$ (MW = 284.48)

Oleic Acid: (1 double bond at position 9 from the omega end – opposite the carboxylic acid) $CH_3(CH_2)_7CH=CH(CH_2)_7COOH$ (MW=282.46)

Linoleic Acid: (2 double bonds, one at 6 and one at 9; MW=280.45)

alpha-Linolenic Acid: (3 double bonds at 3, 6 and 9; MW = 278.44) An omega-3 fatty acid!

Table 1. Average Percent by weight of total fatty acids.

Oil or Fat	Unsat./Sat. ratio	Saturated					Mono unsaturated	Poly unsaturated	
		Capric Acid C10:0	Lauric Acid C12:0	Myristic Acid C14:0	Palmitic Acid C16:0	Stearic Acid C18:0	Oleic Acid C18:1	Linoleic Acid (ω6) C18:2	Alpha Linolenic Acid (ω3) C18:3
Almond Oil	9.7	-	-	-	7	2	69	17	-
Beef Tallow	0.9	-	-	3	24	19	43	3	1
Butterfat (cow)	0.5	3	3	11	27	12	29	2	1
Butterfat (goat)	0.5	7	3	9	25	12	27	3	1
Butterfat (human)	1.0	2	5	8	25	8	35	9	1
Canola Oil	15.7	-	-	-	4	2	62	22	10
Cocoa Butter	0.6	-	-	-	25	38	32	3	-
Cod Liver Oil	2.9	-	-	8	17	-	22	5	-
Coconut Oil	0.1	6	47	18	9	3	6	2	-
Corn Oil (Maize Oil)	6.7	-	-	-	11	2	28	58	1
Cottonseed Oil	2.8	-	-	1	22	3	19	54	1
Flaxseed Oil	9.0	-	-	-	3	7	21	16	53
Grape seed Oil	7.3	-	-	-	8	4	15	73	-
Illipe	0.6	-	-	-	17	45	35	1	-
Lard (Pork fat)	1.2	-	-	2	26	14	44	10	-
Olive Oil	4.6	-	-	-	13	3	71	10	1
Palm Oil	1.0	-	-	1	45	4	40	10	-
Palm Olein	1.3	-	-	1	37	4	46	11	-
Palm Kernel Oil	0.2	4	48	16	8	3	15	2	-
Peanut Oil	4.0	-	-	-	11	2	48	32	-
Safflower Oil*	10.1	-	-	-	7	2	13	78	-
Sesame Oil	6.6	-	-	-	9	4	41	45	-
Shea nut	1.1	-	1	-	4	39	44	5	-
Soybean Oil	5.7	-	-	-	11	4	24	54	7
Sunflower Oil*	7.3	-	-	-	7	5	19	68	1
Walnut Oil	5.3	-	-	-	11	5	28	51	5

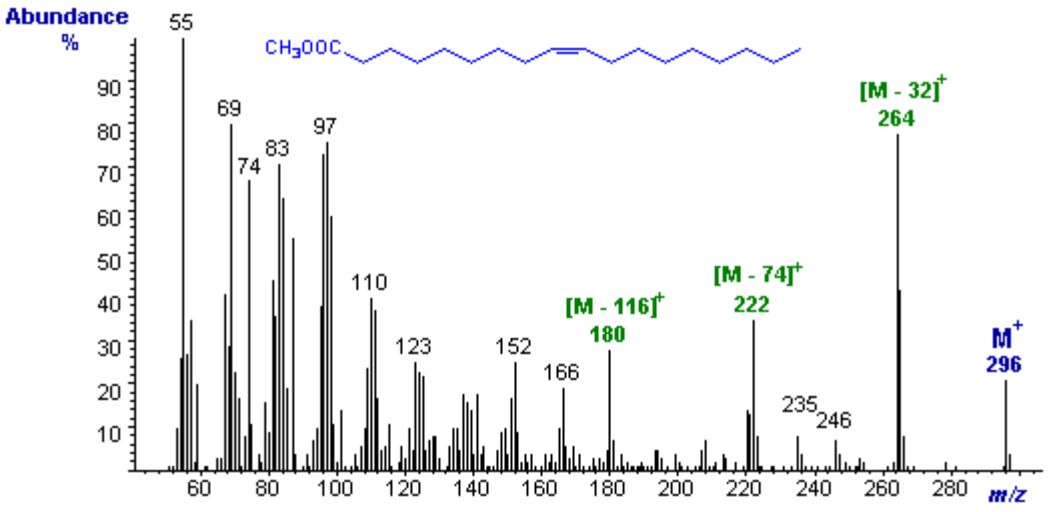
* Not high-oleic variety.

Percentages may not add to 100% due to rounding and other constituents not listed.

Where percentages vary, average values are used.

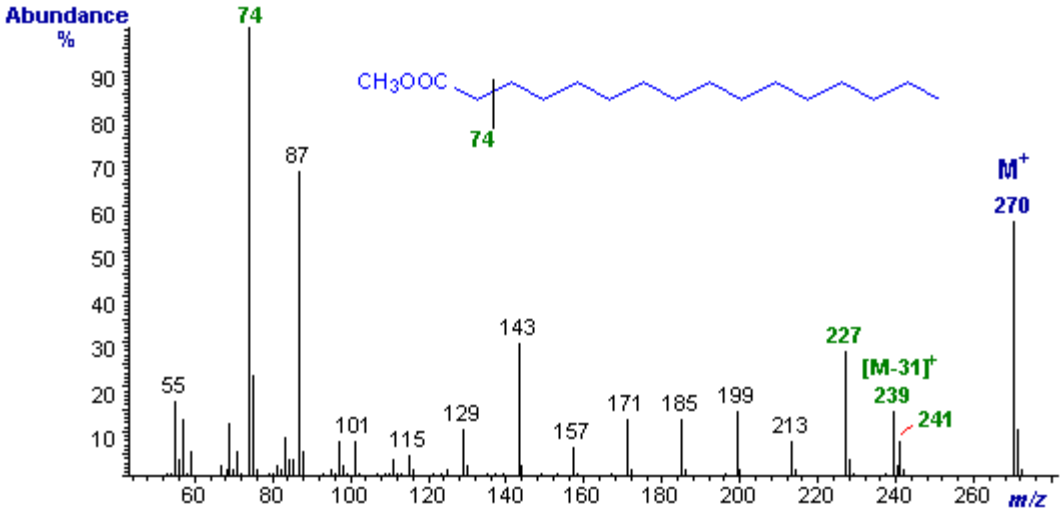
Below are mass spectra found at http://lipidlibrary.aocs.org/

The mass spectrum of methyl oleate:



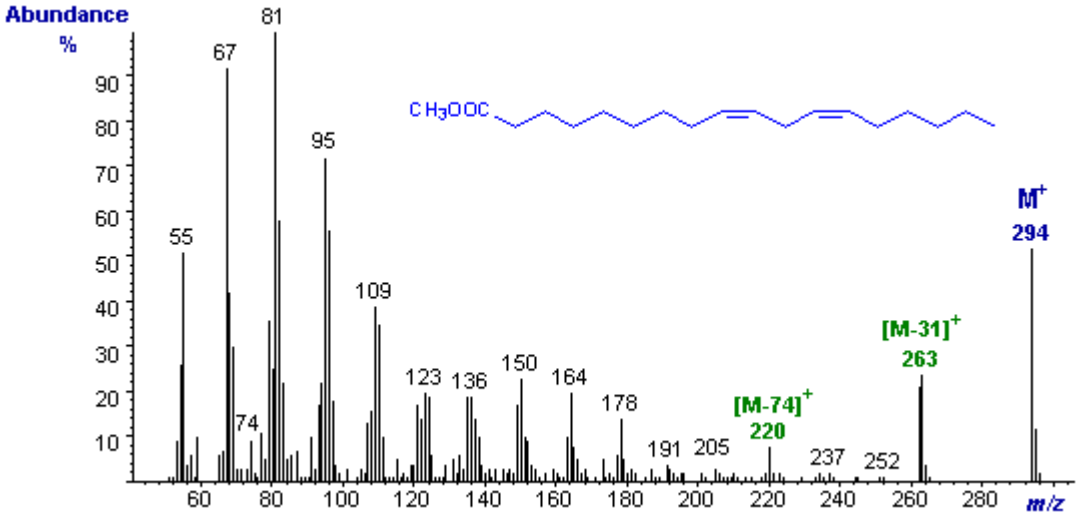
The molecular ion (*m/z* = 296) is clearly seen, and ions representing loss of the elements of methanol (*m/z* = 264 or $[M-32]^+$), i.e. a methoxyl group plus a hydrogen atom, and the loss of the McLafferty ion (*m/z* = 222) are abundant, as is the McLafferty ion *per se* (*m/z* = 74). A characteristic ion at $[M-116]^+$ (*m/z* = 180 in this instance), together with homologous ions at 166, 152, etc, are also diagnostic. The first of these ions is formed by loss of a fragment containing the carboxyl group by cleavage between carbons 5 and 6 with addition of a rearranged hydrogen atom. Thus, in contrast to the spectra of methyl esters of saturated fatty acids, hydrocarbon ions (general formula $[C_nH_{2n-1}]^+$) dominate the spectrum, with *m/z* = 55 as the base peak usually. The relative abundances of all of these ions tends to be appreciably greater than in the mass spectra of dienes and polyenes. However, there is no feature that permits location of the double bond, because this can migrate to any position when the alkyl chain is ionized in the mass spectrometer. Thus, nearly all the *cis*- and *trans*-18:1 isomers have virtually identical spectra.

The mass spectrum of methyl palmitate is shown below:



The molecular ion at *m/z* = 270 is clearly seen, as is an ion at 239 ($[M-31]^+$) representing loss of a methoxyl group, and confirming that it is indeed a methyl ester. An ion at *m/z* = 227 ($[M-43]^+$) represents loss of a C_3 unit (carbons 2 to 4), via a complex rearrangement, while that at *m/z* = 74 is the McLafferty rearrangement ion. The latter has a special significance (see below), not least in that it confirms that the spectrum is that of a methyl ester. An ion at *m/z* = 241 ($[M-29]^+$) is also diagnostic and worthy of note. The long homologous series of related ions (14 amu apart) at *m/z* = 87, 101, 115,129, 143, 157, 199, etc. of general formula $[CH_3OCO(CH_2)_n]^+$ is evidence that there are unlikely to be other functional groups in the chain.

The mass spectrum of methyl linoleate (9,12-18:2 or 18:2(*n*-6)) is illustrated below (Hallgren *et al*., 1959) -



It has an abundant molecular ion (*m/z* = 294) and a prominent ion for loss of the McLafferty ion (*m/z* = 220), although the McLafferty ion *per se* (*m/z* = 74) is small. The ion representing $[M-31]^+$ is higher than that for $[M-32]^+$ (although this may not be true for all isomers). Hydrocarbon ions of general formula $[C_nH_{2n-3}]^+$ dominate in the lower mass range (*m/z* = 67, 81, 95, 109, 123, etc). As with the monoenes, there is little evidence for any ions that might serve to locate the double bonds. For example, an ion at *m/z* = 150 is a useful diagnostic aid for methylene-interrupted polyenoic (3 to 6 double bonds) fatty acids with an (*n*-6) or ω6 structure, but not for dienes.

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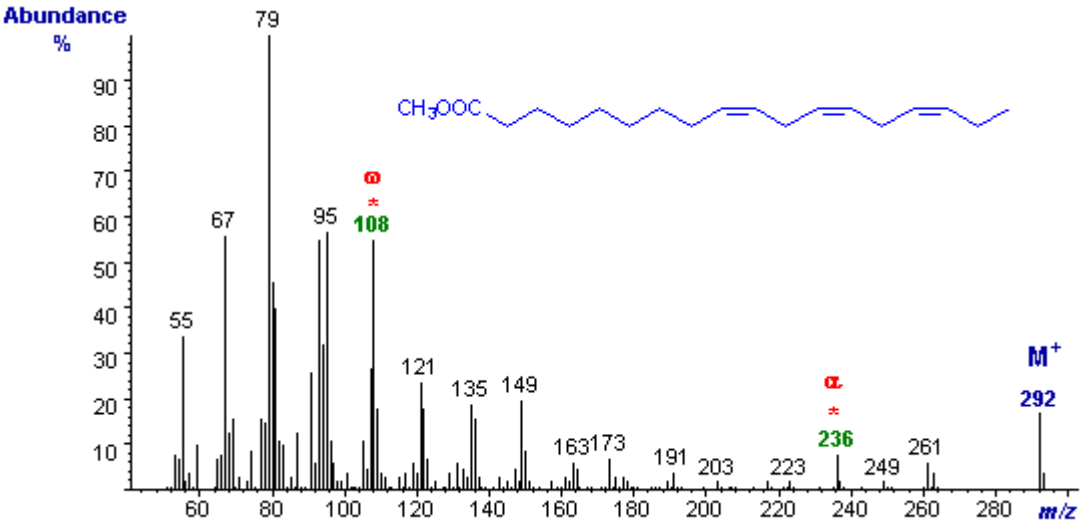
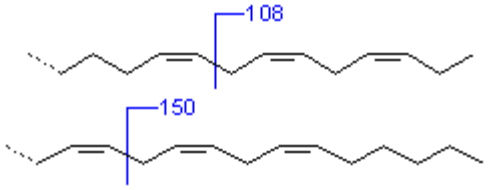
methyl 9,12,15-octadecatrienoate (α-linolenate or 18:3(*n*-3)) (Hallgren et al., 1959))-

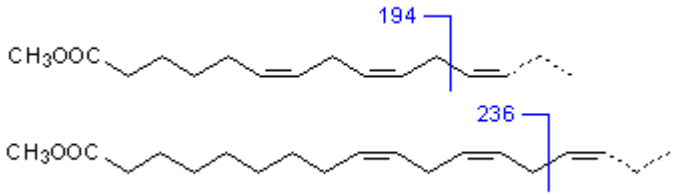


Both spectra are rather similar, but there are features that make them something more than simply 'fingerprints' for identification purposes. For example, a peak at *m/z* = 150 is characteristic for methyl esters of polyunsaturated fatty acids with an *n*-6 terminal moiety (see the spectrum above), while one at *m/z* = 108 defines an *n*-3 terminal group (Holman and Rahm, 1971; Brauner *et al.*,1982; Fellenberg *et al.*, 1987)). For the minor (*n*-9) and (*n*-4) families the relevant ions are at *m/z* = 192 and 122, respectively. They are formed by cleavage in the positions shown.



My impression is that each of these ions occurs with reasonable consistency in the spectra of fatty acid methyl esters from both biochemical families, although they are not necessarily unique to such acids. It should be noted that these ions, which are sometimes termed the '*omega*' ions, are relevant only for fatty acids with three or more double bonds, not for dienes.

In addition, there are small ions formed by a similar cleavage at the carboxyl end of the molecule giving a fragment containing the first two double bonds and the second methylene group (minus a proton) that could be termed the '*alpha*' ion, as illustrated. Thus in the mass spectrum of methyl 6,9,12-octadecatrienoate, this ion is at *m/z* = 194, and it appears to be present in the spectra of all conventional polyenoic acids to which we have access with the first double bond in position 6. The corresponding ion in the spectrum of methyl 9,12,15-octadecatrienoate is at *m/z* = 236. This ion was first noted by Holman and Rahm (1971) and was studied more systematically via chemical ionization methods in a paper by others that appears to have been largely overlooked since (Brauner *et al*., 1982)).



Analogous ions are seen in spectra of methyl esters of most methylene-interrupted polyunsaturated fatty acids (three or more double bonds) as listed in **Table 1**. The *alpha* ions are not always as easily distinguished as the omega ions, but they do appear always to be present other than in some of the fatty acids of the minor (n-1) family) (author, unpublished).

Table 1. *Alpha* ions in the mass spectra of methyl esters of polyunsaturated fatty acids.

First double bonds	Δ4,7	Δ5,8	Δ6,9	Δ7,10	Δ8,11	Δ9,12	Δ10,13	Δ11,14
Ion (*m/z*)	166	180	194	208	222	236	250	264