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Mass Spectra of Ricinoleic Acid and Ricinoleic Acid Methyl Ester Obtained From Ocimum Gratissimum Leaves Extract

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Abstract: *The mass spectra of Ricinoleic acid and Ricinoleic acid methyl ester were obtained from the gas chromatography-mass spectrometry of the n-Hexane extract of Ocimum gratissimum leaves. The method employed was extraction via maceration and gas chromatography-mass spectrometry technique was employed in separating the extract constituents. The extraction was carried out for eight days, in the first four days the extract obtained was labeled first n-Hexane extract of Ocimum gratissimum leaves, n-Hexane was added to the marc and left for another four days to get another extract labeled as second n-Hexane extract of Ocimum gratissimum leaves, having been evaporated to constant weight both extracts were analyzed using gas chromatography-mass spectrometry technique. Ricinoleic acid and Ricinoleic acid methyl ester were seen in the chromatogram of the second n-Hexane extract, with the ester emerging before the acid. The mass spectra were interpreted as the fragmentation patterns of the acid and its methyl ester which were given by accounting for the ions present in the mass spectra. The mass spectra therein obtained have been identified as those of Ricinoleic acid and Ricinoleic acid methyl ester. It can therefore be suggested that the mass spectra obtained in this study be included in National Institute of Standards and Technology Chemistry WebBook.*

Keywords: *Ocimum gratissimum, n-Hexane extract, gas chromatography-mass spectrometry, mass spectra, Ricinoleic acid, Ricinoleic acid Methyl Ester.*

I. Introduction

Ocimum gratissimum is a perennial herb of the genus *Ocimum* belonging to the family Lamiacea, it is widely distributed in tropical and temperate regions and has been used traditionally in the treatment of epilepsy, high fever and diarrhea [1], pneumonia and conjunctivitis [2]. It has been confirmed to be active against bacteria [3], virus [4], fungi [5] and parasites [6], it has also been found to possess antioxidant [7], antiulcer [8], antinociceptive [9], anti-inflammatory [10] and antiurolithiatic properties [11] as a result of the phytochemicals contained in the various extract of the plant such as alkaloids, flavonoids [12], saponins, terpenes and terpenoids, steroids and phenols [13], [14]. The gas chromatography-mass spectrometry analysis of the plant's essential oil has been found to contain eugenol as the most abundant compound, benzyl alcohol, sabene, camphor, thymol and other volatiles as minor components [15].

Hexadecanoic acid, dehydrodihydrodiisoeugenol, phytol, α-linolenic acid, linoleic acid and ricinoleic acid has also been found to be present in the n-Hexane extract of the leaves [16].

Gas chromatography-mass spectrometry is a powerful analytical tool; it is a hyphenated separation technique in which a gas chromatograph is interfaced to a mass spectrometer. The gas chromatograph separates the sample substance into its constituent compounds and produces peaks as shown in the chromatogram according to their retention times (R.T), while the mass spectrometer detects the ions present and produces the mass spectra which give the fingerprint of peaks at various mass to charge ratios (m/z) of the particular compound present [17]. Mass spectra are graphs that represent the mass to charge ratios on the x-axis and the percentage abundances on the y-axis, therefore, the mass spectra are characteristics of a compound [18] and are useful in determining the molecular formula of the compound by giving the exact molecular weight of the compound [19]. This present study is aimed at giving the mass spectra of Ricinoleic acid and Ricinoleic acid methyl ester obtained from the gas chromatography-mass spectrometry analysis of the n-Hexane extract of *Ocimum gratissimum* leaves and showing clearly their fragmentation patterns.

II. Materials And Methods

2.1 Collection and Extraction of Plant

Leaves of *Ocimum gratissimum* were collected in April 2013 in Jos North area of Plateau State and were identified in the Botany Department of University of Jos by Mr Agyeno. The leaves haven been rinsed and dried at room temperature, were crushed into powder, 50g of the powder was extracted by maceration for 4 days with 250ml research grade n-Hexane. The solvent was separated from the marc by decantation and labeled first n-Hexane extract of *Ocimum gratissimum* leaves (OGH_1E), another 250ml of n-Hexane was added to the marc and kept for another 4 days, decanted and labeled second n-Hexane extract of *Ocimum gratissimum* leaves (OGH_2E). The extracts were then evaporated to constant weight using a steam bath.

2.2 Chemical Analysis of Extracts

A QP2010 Plus SHIMADZU JAPAN gas chromatograph interfaced to a mass spectrometer was used to analyze the extracts in NARICT Zaria, Kaduna State Nigeria. The instrument was programmed as follows: flow rate of carrier gas (He) was 1ml/min, injection port temperature was 200^0c, initial column temperature was 50^0c increased and kept constant at 300^0c for 9mins with heating rate of 8^0c/min. The volume (1µl) injected was with split mode to avoid over flooding the column. The extracts constituents emerged according to their retention times (R.T).

III. Results

The percentage yields of the first extract (OGH_1E) and second extract (OGH_2E) were 3% and 2% respectively. The chromatograms of the first extract (OGH_1E) and second extract (OGH_2E) are as shown in Fig 1 and 2 respectively. The mass spectra of Ricinoleic acid methyl ester and Ricinoleic acid are as shown in fig 2a and 2b respectively.

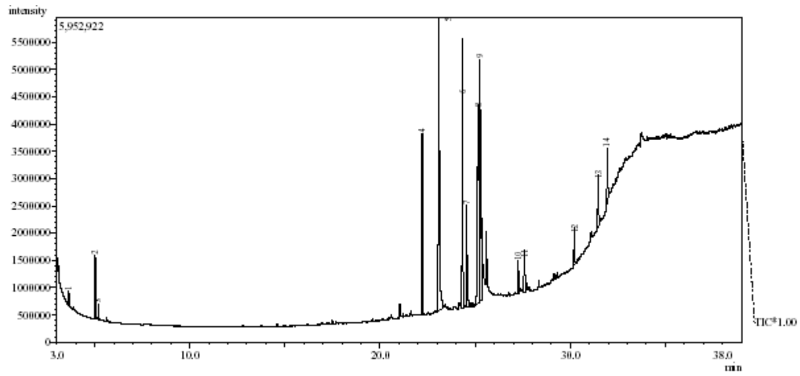


Figure 1: chromatogram of OGH_1E

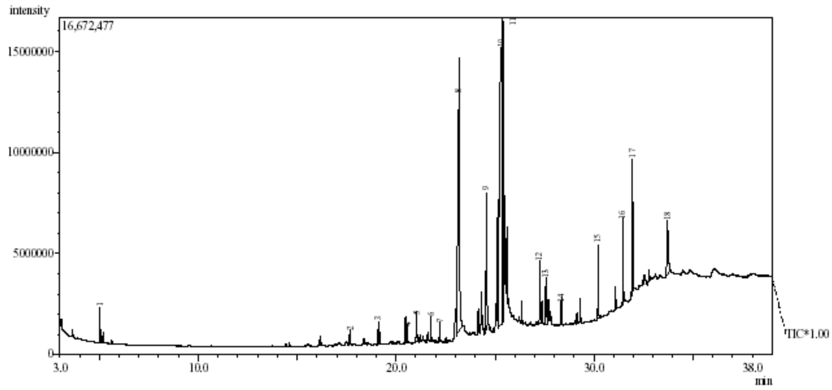


Figure 2: chromatogram of OGH_2E

R.T 25.308min

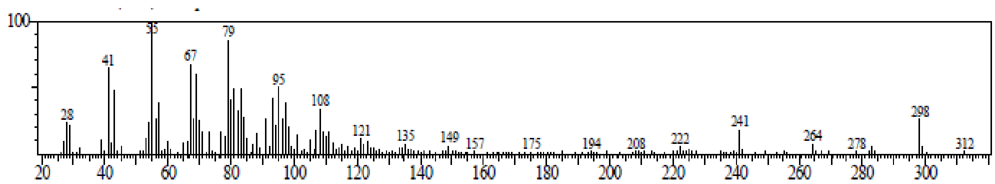


Figure 2a: mass spectrum of Ricinoleic acid methyl ester ($C_{19}H_{36}O_3$)

R.T 25.400min

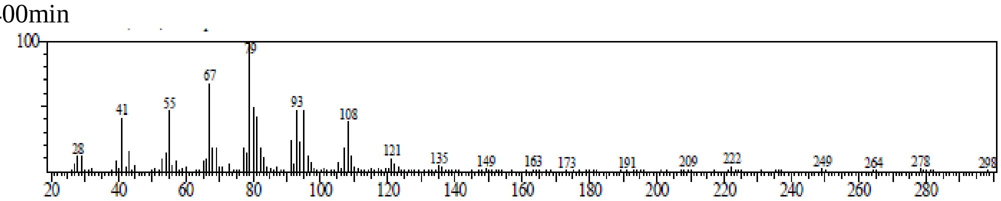


Figure 2b: mass spectrum of Ricinoleic acid ($C_{18}H_{34}O_3$)

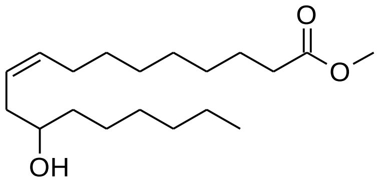
IV. Discussion

In the present study, the yield of the first n-Hexane extract of *Ocimum gratissimum* leaves was higher than that of the second extract. Ricinoleic acid and Ricinoleic acid methyl ester were not seen in the first extract but rather were seen in the second extract of the leaves, this could be as a result of the exhaustive extraction carried out, more so, the extracts were not combined. In the chromatogram of (OGH_2E), peak 10 is Ricinoleic acid methyl ester while peak 11 is Ricinoleic acid, the chromatogram also shows that the acid and its ester were in high concentrations with the acid (peak 11) being the highest. It is evident that the ester is more volatile than the acid as it emerged first from the column with retention times (R.T) of 25.308min and 25.400min respectively. The mass spectra showed clearly the molecular weights of the ester and the acid as 312 and 298 respectively and were similar as they contained almost the same fragmented ions except for 157,175,194, 208 and 241 in that of the ester while for the acid the different ions were 163,173,191, 209 and 249.

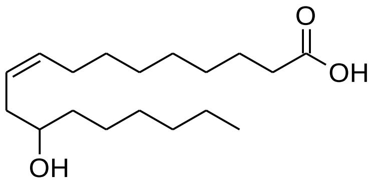
The system in which the identification of the compound constituents is taking place (mass spectrometer) is rich in ions and so many reactions take place to form more of stable ions than unstable ones.

4.1 Fragmentation Patterns of Ricinoleic acid methyl ester and Ricinoleic acid

Molecular structure of Ricinoleic acid methyl ester with molecular weight of 312 as shown in the mass spectrum is shown below.



Molecular structure of Ricinoleic acid with molecular weight of 298 as shown in the mass spectrum is shown below.



4.1.1 Fragmentation pattern of Ricinoleic acid methyl ester

The structure of Ricinoleic acid methyl ester could be rewritten as:

$$CH_3O-\overset{O}{\overset{/\!/}{C}}-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-\overset{H}{\overset{|}{C}}=\overset{H}{\overset{|}{C}}-CH_2-\underset{\underset{OH}{|}}{\overset{H}{\overset{|}{C}}}-CH_2-CH_2-CH_2-CH_2-CH_2-CH_3$$

The ions to be accounted for therefore include 298, 278, 264, 241, 222, 208, 194, 175, 157,149, 135, 121, 108, 79, 67, 55, 41 and 28 as shown in the spectra.

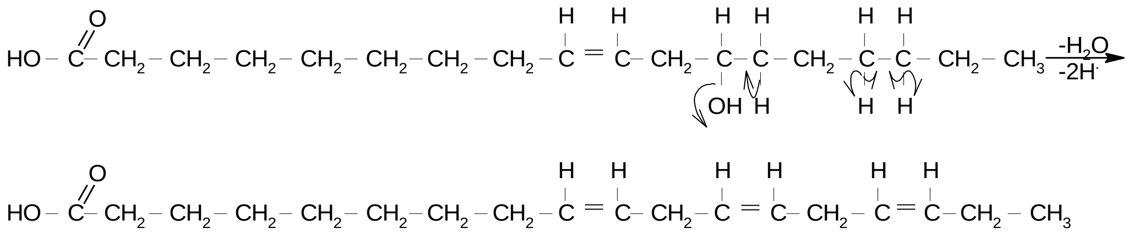
Now there is a loss of the methyl group attached to the oxygen and then H is attached to O to give the ion with m/z of 298 which is Ricinoleic acid.

$$CH_3-O-\overset{O}{\overset{/\!/}{C}}-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-\overset{H}{\overset{|}{C}}=\overset{H}{\overset{|}{C}}-CH_2-\underset{\underset{OH}{|}}{\overset{H}{\overset{|}{C}}}-CH_2-CH_2-CH_2-CH_2-CH_2-CH_3\xrightarrow[-CH_3^{\cdot}]{+H^{\cdot}}$$

$$HO-\overset{O}{\overset{/\!/}{C}}-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-\overset{H}{\overset{|}{C}}=\overset{H}{\overset{|}{C}}-CH_2-\underset{\underset{OH}{|}}{\overset{H}{\overset{|}{C}}}-CH_2-CH_2-CH_2-CH_2-CH_2-CH_3$$

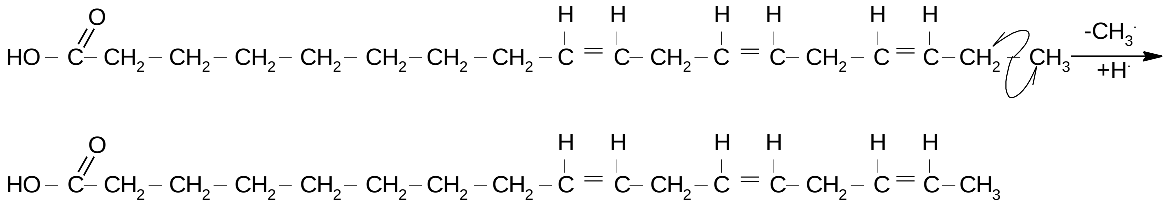
Ricinoleic acid (m.w 298)

There is a loss of a water molecule and two hydrogen to form two double bonds at the 12th and 15th positions to give a thermodynamically stable ion which is α-linolenic acid (9,12,15-Octadecatrienoic acid) with m/z of 278.



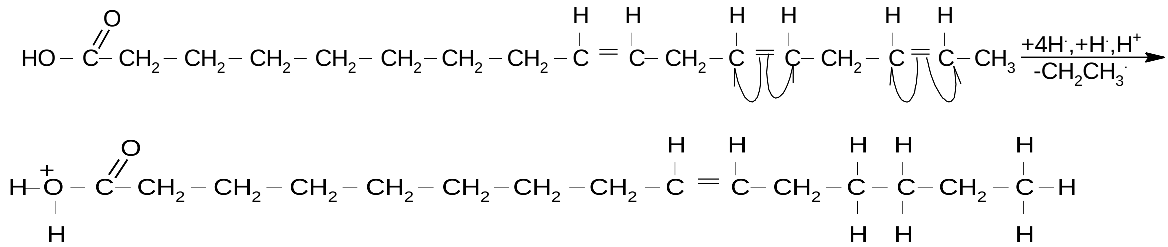
α-linolenic acid (m.w 278)

There is a loss of the terminal methyl group and addition of $H^{\cdot}$ to give an ion with m/z of 264 (9,12,15-Heptadecatrienoic acid).



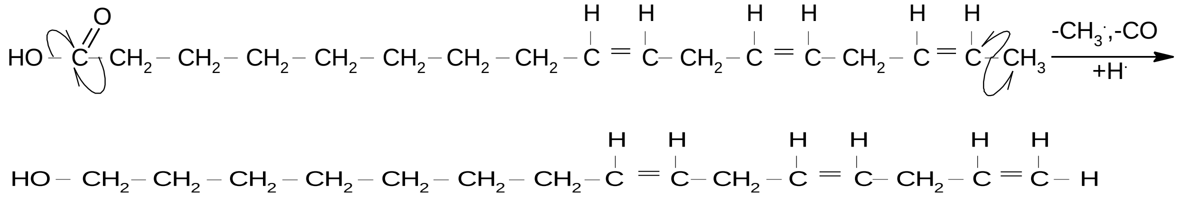
9,12,15-Heptadecatrienoic acid (m.w 264)

There is now addition of $4H^{\cdot}$ to saturate the double bonds at positions 12 and 15, then a loss of the terminal $CH_2CH_3^{\cdot}$ occurs with the addition of $H^{\cdot}$ and protonation of the OH to give an ion with m/z of 241.



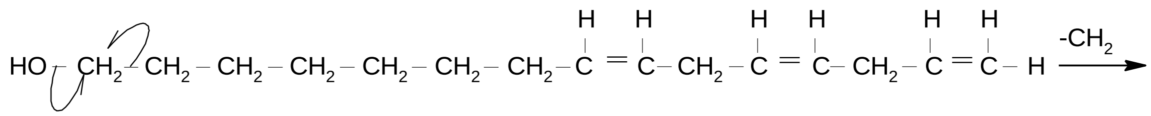
Protonated 9-Pentadecenoic acid (m.w 241)

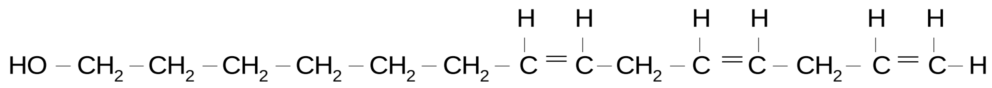
There could also be formation of an ion with m/z of 222 from the ion with molecular weight of 264 (9,12,15-Heptatrienoic acid), where there is a loss of CO and $CH_3^{\cdot}$ with the addition of $H^{\cdot}$.



8,11,14-Pentadecatrienol (m.w 222)

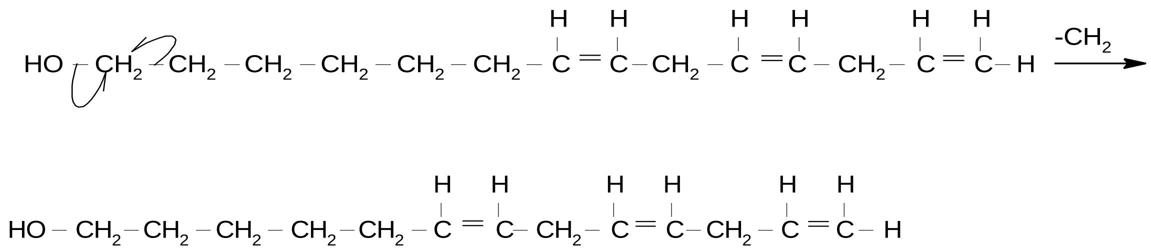
From the ion with molecular weight of 222, there is a loss of methylene group to give an ion with m/z of 208.





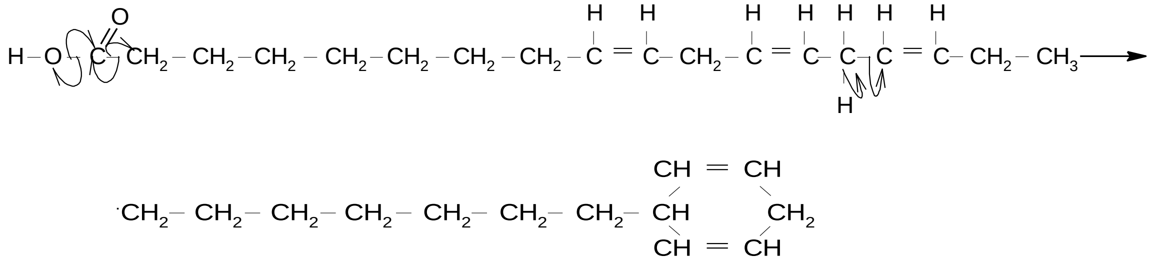
7,10,13-tetradecatrienol (m.w 208)

There is a loss of another methylene group to give an ion with m/z of 194.

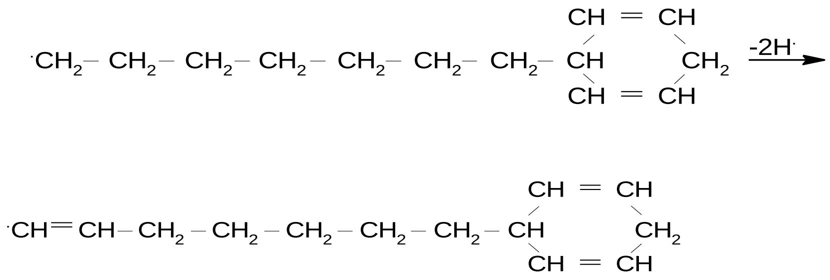


6,9,12-tridecatrienol (m.w 194)

Now from the α-linolenic acid (9,12,15-Octadecatrienoic acid), there could be losses of these ions/molecules CO, $OH^{\cdot}$, H^+, and $CH = CH–CH_2–CH_3^-$, to give a thermodymically stable cyclic ion with molecular weight of 177. The ion with the molecular weight of 177 loses two hydrogen to give an ion with m/z of 175.

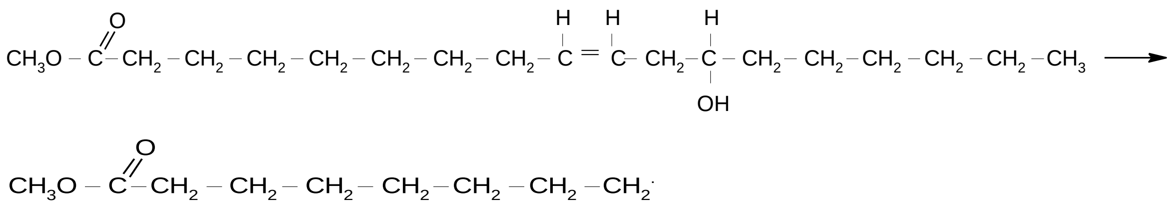


The ion with the molecular weight of 177 loses two hydrogen to give an ion with m/z of 175.



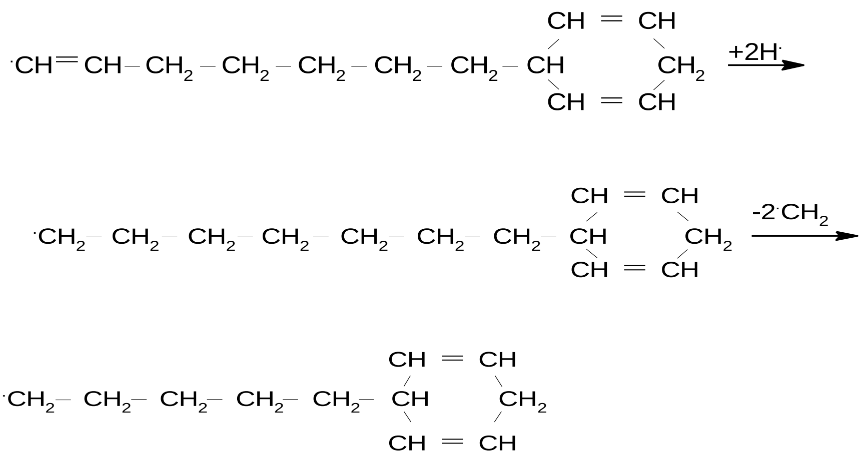
m.w 175

Now from the Ricinoleic acid methyl ester ion there is a cleavage of bond at the 8th position to give an ion with m/z of 157.



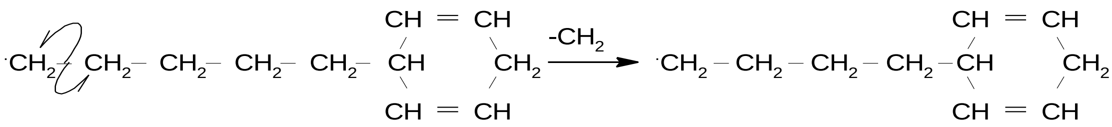
Methylheptyl ester radical (m.w 157)

Now from the ion with molecular weight of 175, there is addition of two hydrogen to saturate the bond, the saturated ion then loses two methylene fragments to form an ion with m/z of 149.



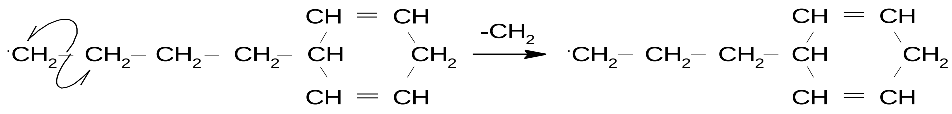
m.w 149

A loss of a methylene group will give an ion with m/z of 135.



m.w 149 m.w 135

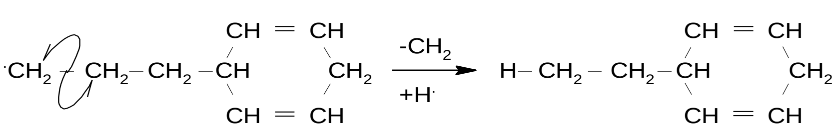
There is another loss of a methylene group to give an ion with an m/z of 121.



m.w 135 m.w 121

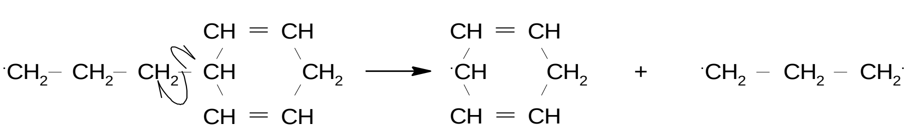
Now from the ion with m/z of 121, two possible cleavages of ions could occur to give the ions with m/z of 108 and 79 respectively.

There is a loss of another methylene group and an addition of $H^{\cdot}$ to give an ion with m/z of 108.



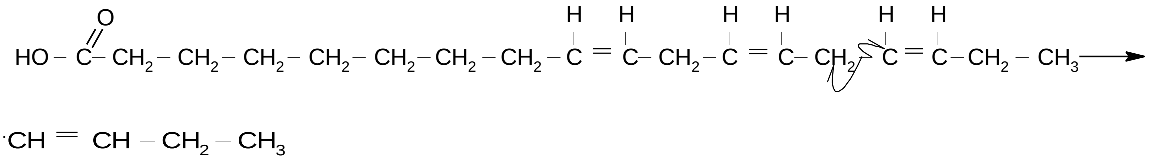
m.w 121 m.w 108

The cleavage of the cyclic fragment (m.w 79) occurs as shown below.



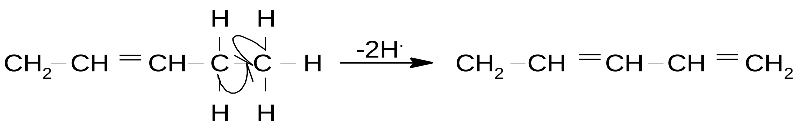
m.w 121 m.w 79 m.w 42

Now from the ion with molecular weight of 278 (α-linolenic acid), there could be a cleavage of bond from the right hand side to give an ion with m/z of 55 as shown below.



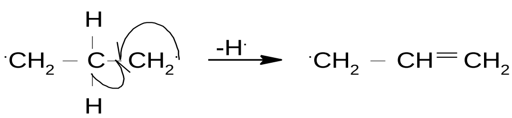
m.w 55

Now there is an addition of methylene fragment to that with the molecular weight of 55 to give an ion with molecular weight of 69 which in turn loses two hydrogen to give an ion with m/z of 67.
s



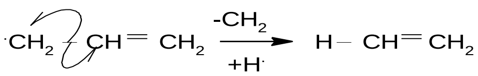
m.w 67

The ion with molecular weight of 42 loses a H· to form an ion with m/z of 41 as shown in the spectra above.



m.w 42 m.w 41

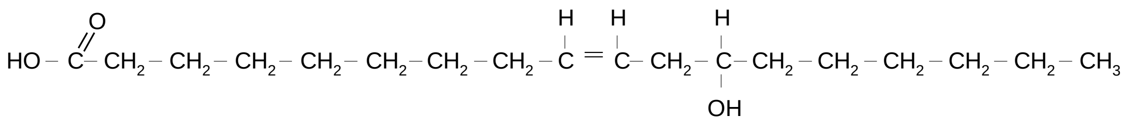
Finally, a loss of methylene group occurs with the addition of H· to give an ion with the m/z of 28.



m.w 41 m.w 28

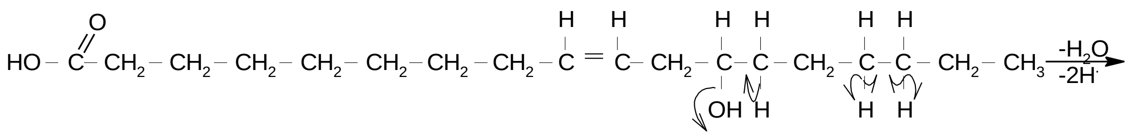
4.1.2 Fragmentation pattern of Ricinoleic acid

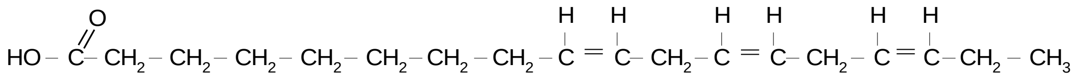
The structure of Ricinoleic acid can be rewritten as



The ions to be accounted for in the spectrum of the acid include 278, 264, 249, 222, 209, 191, 173, 163, 149, 135, 121, 108, 93, 79, 67, 55, 41 and 28.

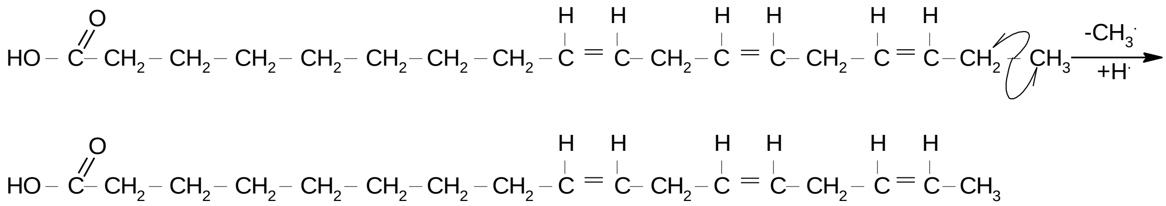
There is a loss of water molecule and two hydrogen to form two double bonds at the 12th and 15th positions to give a thermodynamically stable ion which is α-linolenic acid (9,12,15-Octadecatrienoic acid) with a m/z of 278.





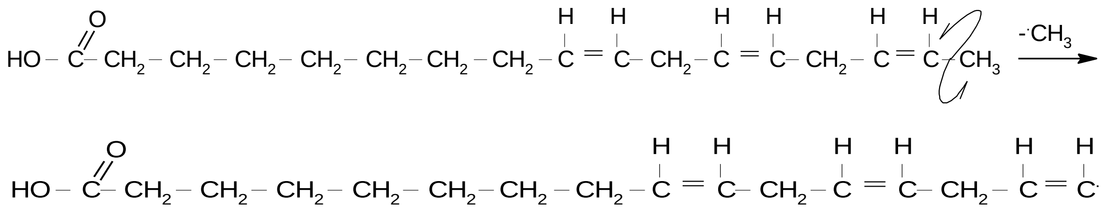
α-linolenic acid (m.w 278)

There is a loss of the terminal methyl group and addition of $H^{\cdot}$ to give an ion with m/z of 264 (9,12,15-Heptadecatrienoic acid).



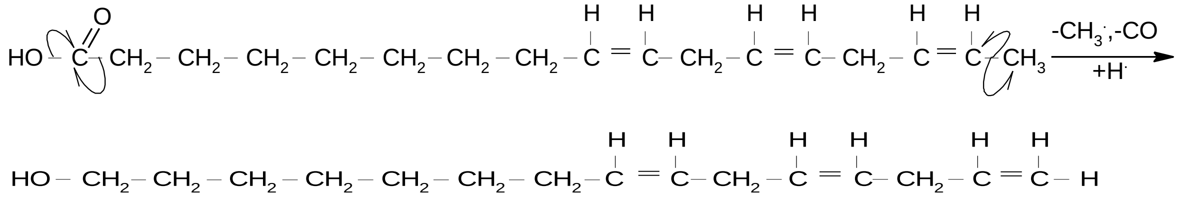
9,12,15-Heptadecatrienoic acid (m.w 264)

Now there is a loss of the terminal methyl group to form an ion with m/z of 249.



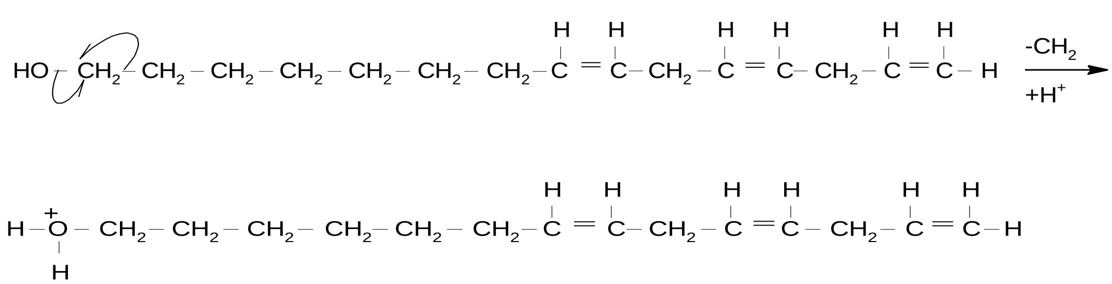
9,12,15-hexadecatrienoic acid radical (m.w 249)

Now there is a formation of an ion with m/z of 222 from the ion with the molecular weight of 264 where there are losses of CO molecule and $CH_3^{\cdot}$ with addition of $H^{\cdot}$ as shown below.



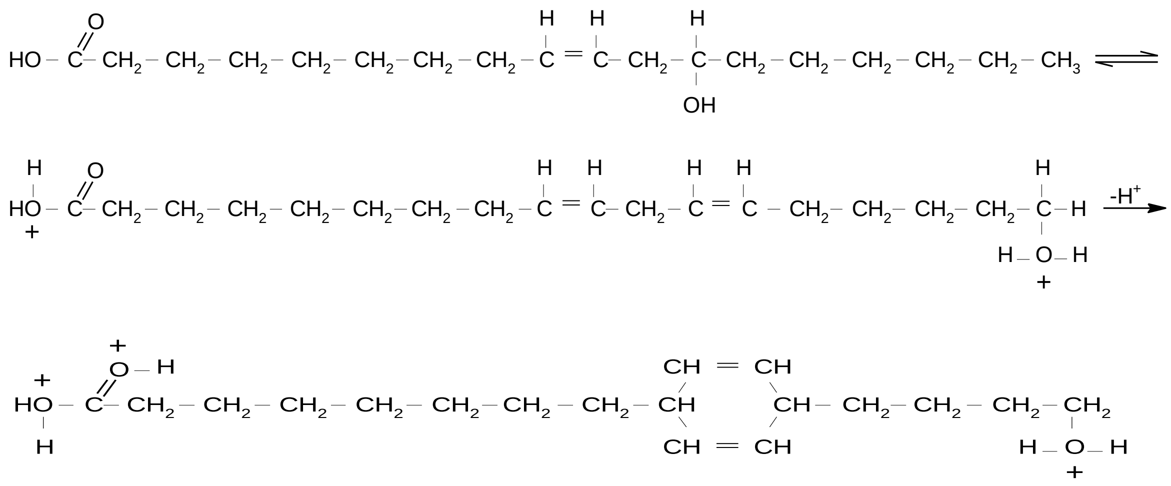
8,11,14-pentadecatrienol (m.w 222)

From the ion with molecular weight of 222, there is a loss of methylene group and protonation of the alcoholic group to give an ion with m/z of 209.

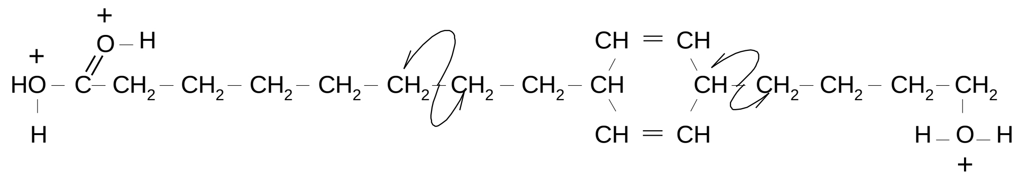


protonated 7,10,13-tetradecatrienol (m.w 209)

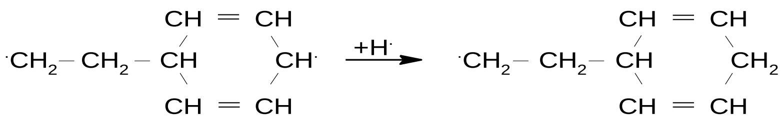
For the formation of the ion with an m/z of 191, the following reactions will have to take place. First, the Ricinoleic acid will isomerize as shown below and there will be formation of a cyclic ion.



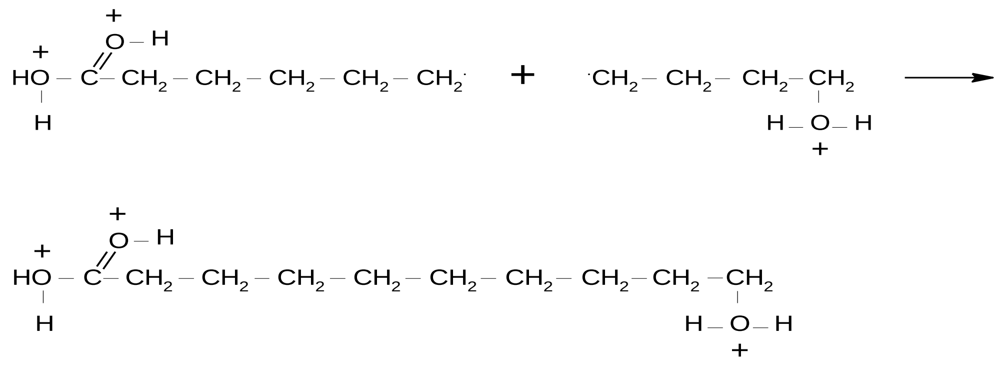
Bond cleavage occurs and the remaining fragments combine to give the ion with the molecular weight of 191.



The cleavage of the cyclic component on addition of a H·, forms an ion with molecular weight of 107 as shown below

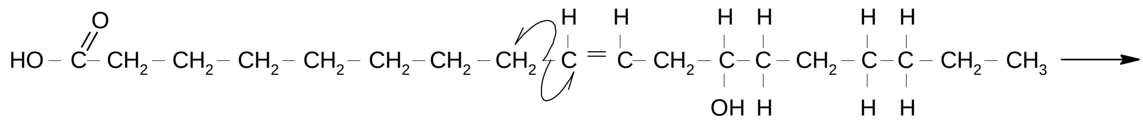


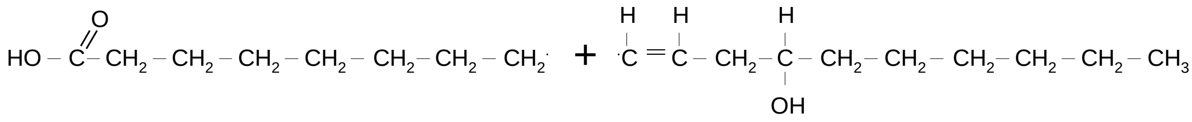
Here, there is combination of the remaining fragments to give the ion with the molecular weight of 191 as shown below



m.w 191

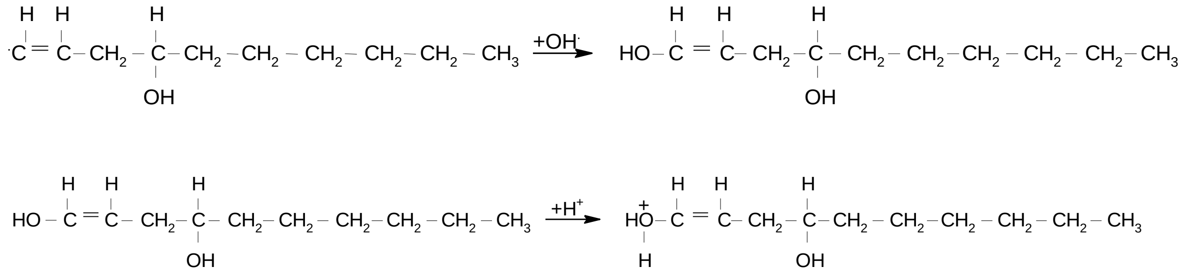
Still from the acid, there is a cleavage of bond after the 8th carbon.





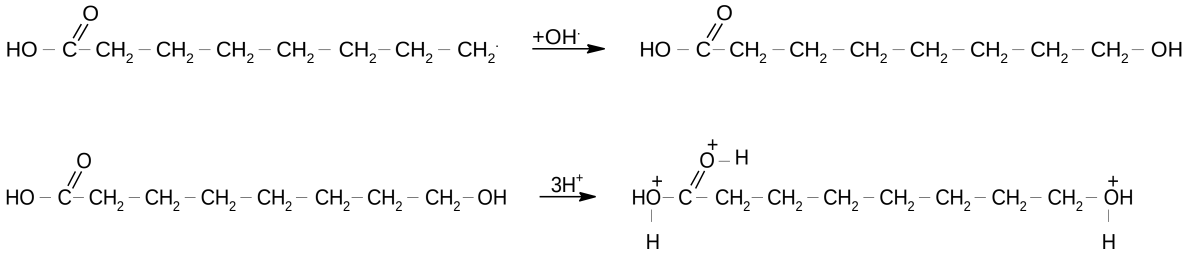
m.w 143 m.w 155

Now there is an addition of OH· to the ion with the molecular weight of 155 with further protonation to give an ion with the m/z of 173 as shown below.



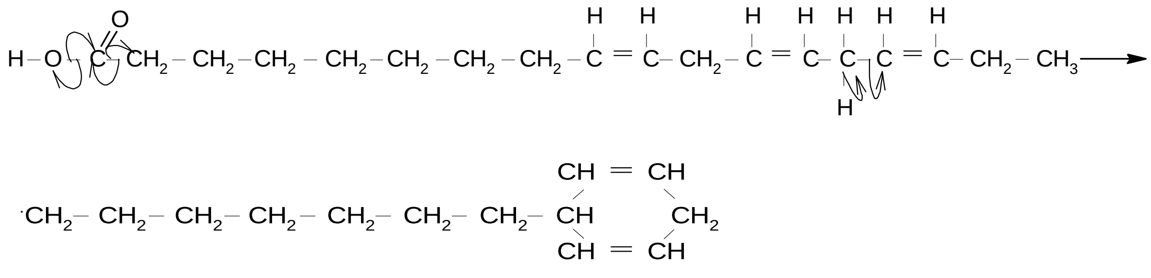
m.w 173

There is also another addition of OH· to the ion with the molecular weight of 143 with further protonation of the two hydroxyl groups and the carboxylic group to give an ion with the m/z of 163.

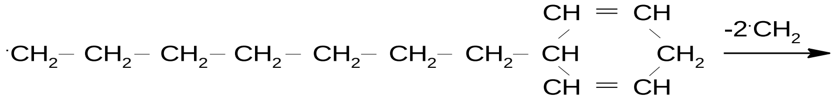


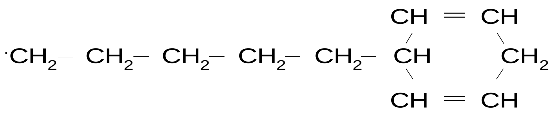
m.w 163

Now from the α-linolenic acid (9,12,15-Octadecatrienoic acid), there could be losses of these ions/molecules CO, OH·, H^+, and CH = CH–CH_2–CH_3^- , to give a thermodymically stable cyclic ion.



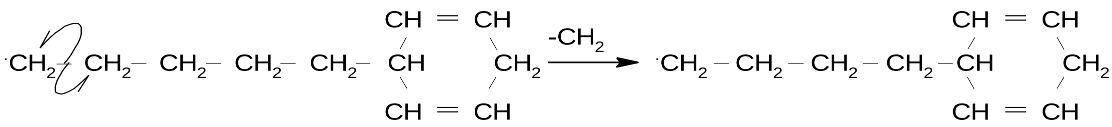
This ion then loses two methylene fragments to give the ion with the m/z of 149.





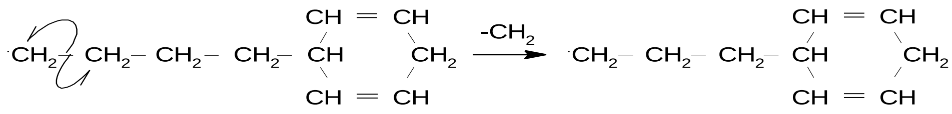
m.w 149

A loss of a methylene group will give the ion with m/z of 135.



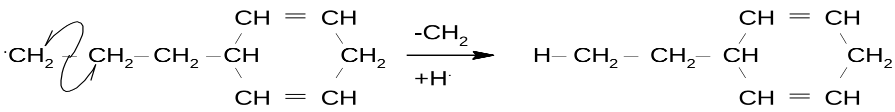
m.w 149 m.w 135

Another loss of a methylene group will give the ion with m/z of 121.



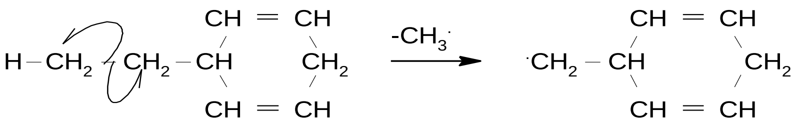
m.w 135 m.w 121

Now there is a loss of another methylene group and an addition of H· to give an ion with m/z of 108.



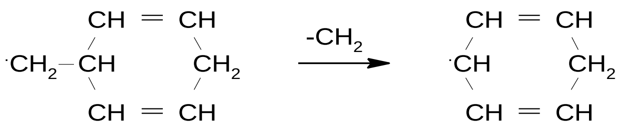
m.w 121 m.w 108

There is a loss of the methyl fragment to give an ion with m/z of 93.



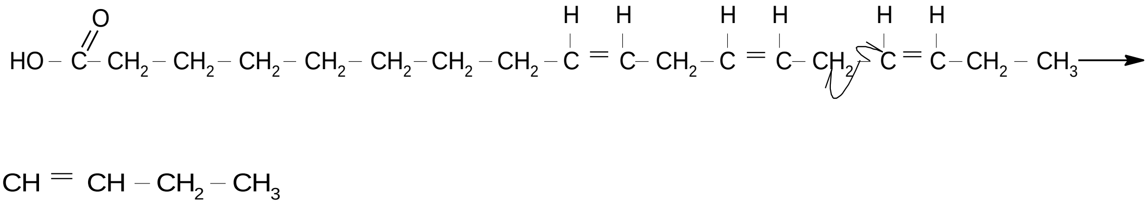
m.w 108 m.w 93

From the ion with m/z of 93, there is a loss of methylene group to give an ion with m/z of 79.



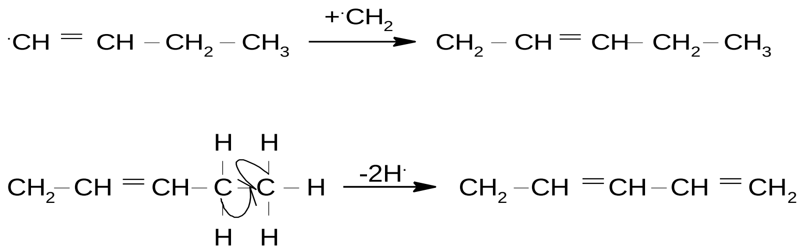
m.w 93 m.w 79

Now from the ion with molecular weight of 278 (α-linolenic acid), there could be a cleavage of bond from the right hand side to give an ion with m/z of 55 as shown below.



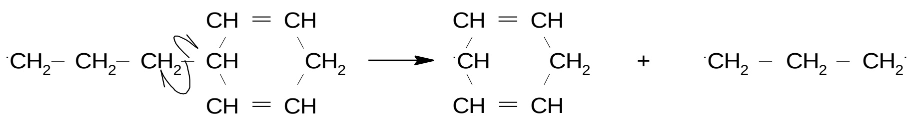
m.w 55

Now there is an addition of methylene fragment to the ion with m/z of 55 to give an ion of molecular weight of 69 which in turn loses two hydrogen to give an ion with m/z of 67.

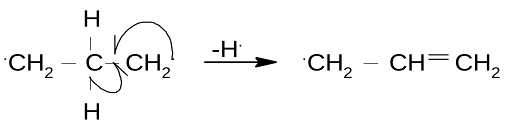


m.w 67

From the ion with m/z of 121, there could be a cleavage of the cyclic ion and the remaining fragment gives an ion with m/z of 42 which in turn loses H· to form a more stable ion with m/z of 41.

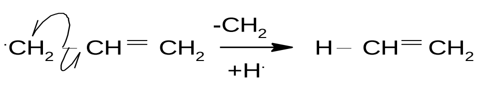


m.w 42



m.w 41

Finally, a loss of a methylene group occurs with the addition of H· to give an ion with the m/z of 28.



m.w 28

V. Conclusion

This study has successfully interpreted the mass spectrum of Ricinoleic acid and its methyl ester. It has also been able to distinguish the fragmentation patterns of the acid and the ester as the different ions contained in the ester (157, 175, 194, 208 and 241) and the acid (163, 173, 191, 209 and 249) were clearly accounted for.

Therefore, the spectra therein haven been identified as shown by the fragmentation patterns are possibly those of the Ricinoleic acid methyl ester and Ricinoleic acid. Hence it is suggested that their respective mass spectrum be uploaded into NIST (National Institute of Standards and Technology) so as to aid other researchers as this study is the first to report the presence of Ricinoleic acid and Ricinoleic acid methyl ester in *Ocimum gratissimum*. These compounds haven been found to possess anti-viral, anti-inflammatory, analgesic [20], [21] antifungal, antibacterial and antioxidant properties [22] compensates for the usefulness of the plant in the treatment of bacterial, fungal and viral infections.

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