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Physicochemical Properties and Determination of Some Bioactive Phytochemical Constituents From Linum Usitatissimum L Oil (Flaxseed) by Gas Chromatography-mass Spectrometry (GC-MS) and Atomic Absorption

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Abstract

Flaxseed (Linum usitatissimum L.) is an important source of oil, The dried seed of flaxseed 100 g, produced 38% of oil extracted by n-hexane, the physiochemical properties of flaxseed such as acid value (0.71 mg KOH/g) as oleic acid, peroxide value (2.69 meq O2/kg), saponification value (186.50 mg KOH/g oil), and iodine value (96.67 g/100 g), density (0.91 g/cm3), refractive index (1.39 and at 28°C), viscosity (CST) (68.33). Gas Chromatography-Mass Spectrometry (GC-MS) was used to identify bioactive phytochemical constituents it contained 26 compounds identified, the major compound were 9-Octadecenoic acid (Z)-, methyl ester (Oleic acid) (43.72) act as antioxidants, anti-cancer, Hexadecanoic acid, methyl ester (Palmitic acid) (17.36) act as Anti-oxidant, decrease blood cholesterol, anti-inflammatory, 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z) fatty acids:α-Linolenic acid, an omega-3 (n-3) fatty acid γ-Linolenic acid, an omega-6 (n-6) fatty acid (15.72), Octadecanoic acid Methyl stearate (13.13) used As a food additive, soaps, cosmetics, detergents lubricants, softening and release agents,13-Docosenoic acid, methyl ester, (Z)-(1.31), cis-11-Eicosenoic acid, methyl ester (0.96) act as Antibacterial, antitumor, antifungal, cytotoxic. Atomic absorption is used to identify minerals content it contains Calcium 1180ppm, Copper 4.1ppm, Ferric 15.7ppm, Zinc 34.58ppm.

Keywords: Flaxseed (*Linum usitatissimum* L); physicochemical properties. Gas Chromatography-Mass Spectrometry (GC-MS): Atomic absorption.

1. Introduction

Linum usitatissimum L. the common name flax or linseed belongs to the family Linaceae is an annual herb [1]. It is beneficial in the chemical composition used as food oil (36 to 40 % of oil) plant rich in essential fatty acids (e.g., a-linolenic acid) that reduce the risk of coronary heart diseases [2] and the prevention of breast and prostate cancers[3]. Flaxseed oil is the richest plant source of oleic, linoleic (omega-6) and linolenic (omega-3) polyunsaturated fatty acids (PUFA), which are essential for humans. It contains a lot of bioactive phenolic compounds, which have biological activity including antimicrobial, antiradical, antioxidant and anticancer effects [4, 5, 6, 7]. Flaxseed *Linum usitatissimum* L contains the lignan, secoisolariciresinoldiglucoside (SDG) these

lignan have biological activities as antioxidant and antibacterial [8]. Flaxseed is an important source of fiber (20%) and protein, (30%) [9]. they include mainly phenols, polyphenols, tocopherols, anthocyanins and carotenoids [10]. These compounds mainly antioxidants are synthetic or natural compounds that can delay some types of cell damage and quench reactive radical species formed during oxidative reactions in metabolism [11, 12].

2. Material and Methods

Material

Flaxseed (*Linum usitatissimum* L.) Purchase in the supermarket and identified in the Department of Biology, College of Science, Khartoum University.

Method

Extraction of flaxseed Oil

200 g of prepared seed samples were packed in extraction thimble and inserted into the Sox let extractor, the solvents used n-hexane temperature (40-60°C), (500 mL) were separately refluxed for 8 h. At the end of the period, each of the solvents was recovered by rotary evaporator and residual oil was oven- dried at 60°C for 30 min. The obtained oils were allowed to cold to room temperature before analysis [13].

Calculation using the equation:-
Yield % of oil= Mass of oil/ Mass of sample × 100%.

Chemical characters of the oil

Acid value

The acid value was determined according to the method of ISO 660:1996. Ten grams of oil were dissolved in 50 ml of the solvent and titrated against 0.1N sodium hydroxide with continuous shaking using phenolphthalein as an indicator until pink color appear.
The acid value was calculated as follows:

$$\text{Acid value (mg KOHg}^{-1}) = \frac{(v-b) \times N \times 56.1}{W}$$

Where: 56.1 = molecular weight of potassium hydroxide (g/mol); V = Volume in mL of standard potassium hydroxide used for sample titration; b = Volume in mL of standard potassium hydroxide used for blank titration; N = Normality of the potassium hydroxide solution; and W = Weight in g of the sample [14].

Saponification value

About 2.0 g of sample were weighed in a 200 ml conical flask, 20 mL of 0.5 mol/L alcoholic potassium hydroxide was added. The flask was gently heated and occasionally shaken for 30 minutes and cooled. The unreacted KOH was then back-titrated with 0.5 mol/L HCl. A blank titration was performed [15].

The saponification value (SV) in mg/g was calculated $\frac{(B-v) \times M \times 56.1}{}$

$$W$$

Where: *B* = blank titre volume (mL); *V* = Sample titre volume (mL); *M* = molarity of the HCl; 56.1 = the molecular weight of KOH; *W* = weight of sample (g).

Peroxide value

About 5 g of the sample were added to 30 mL of the solvent mixture (acetic acid: chloroform, 2:1 v/v) 5 mL of saturated potassium iodide were added; the mixture was gently shaken and boiled for one minute. The flask left at room temperature in dark, 30 mL of distilled water were added, and the flask sealed and stirred. Few drops of the starch solution were added to the mixture and the latter was titrated with 0.01N Na2S2O3 solution. A blank titration was performed [16]. The peroxide value, expressed as mille equivalent of peroxide oxygen per kg sample (meq/kg), was calculated using equation

$$\text{Peroxide value of the sample (Meq/kg)} = \frac{(A-B) \times N \times 100}{W}$$

Where: *A* = volume (mL) of Na2S2O3 solution consumed in sample titration; *B* = volume (mL) of
Na2S2O3 solution consumed in blank titration; *N* =Normality of sodium thiosulphate solution, *w* = weight of the sample (g) [16].

Iodine value

One gram of oil was accurately weighed and placed in a dry bottle. Twenty ml of carbon tetrachloride from a dry measuring cylinder was used to dissolve the oil. The content was mixed and exactly 25 ml of wijs solution from a pipette was added. And allowed to stand in dark for 30minutes.Twenty ml of 0.1N potassium iodide solution was added. The solution was swirled and titrated carefully with 0.1N sodium thiosulphate solution. With continuous shaking. Two ml of starch solution was added and the titration was continued. Until the endpoint was reached when the blue color disappeared:

$$\text{Iodine value} = (B - A) \times 12.69 \times N/W$$

Where:
B: Volume (ml) of 0.1N sodium thiosulphate used in blank
A: Volume (ml) of 0.1N sodium thiosulphate used in blank
W: Weight of oil in (g) [17]

Physical characters of the oil

Refractive index

The refractive index of oils was determined by the Abbe 60 Refract meter. After calibration of the instrument, few drops of the oil were placed between the prisms of the refract meter in such a way that the space between the prisms was completely filled. The oil was then allowed to assume the temperature of the prisms 32=2C. The refractive index was then read [18].

Relative density

The relative density was determined according to the method [19]. The relative density of the liquid (specific gravity) is the weight of a given volume of the liquid at the specified temperature, compared with the weight of an equal volume of water at the same temperature. All weighing being taken in the air [19].

Viscosity

The viscosity of the oil samples was determined by using Ostwald-U- tube viscometer according to the method [20].
Calculations:
Relative viscosity of the oil = T-T0 / T0
Where:
T: flow time of the oil (sec)
T0: flow time of the distilled water (sec).

Sample Preparation

Mixed thoroughly 2ml of the sample with 7ml of alcoholic sodium hydroxide (NaOH) that was prepared by dissolving 2 g in 100 ml methanol. 7 ml of alcoholic sulfuric acid (1ml H_2SO_4 to 100 ml methanol) was then added. Shaking the mixture for 5 minutes. Then the content of the test tube was left to stand overnight.1 ml of Supersaturated sodium chloride (NaCl) was then added and the contents being shaken. Add 2ml of normal hexane and the contents were shaking thoroughly for three minutes. Then the upper layer of the test tube (n-hexane layer) was taken using a disposable syringe. Diluted 5 µl from the n-hexane extract with 5 ml of diethyl ether. Then filter the mixture through a syringe filter 0.45 µm and use anhydrous sodium sulphate as drying agent, 1g of it.1µl of the diluted sample was injected in the GC.MS instrument.

Method of analysis:

GC/MS Conditions

GM/MS technique was used to determine the qualitative and quantitative analysis of the sample, model (GC/MS-QP2010-Ultra) from japans 'Simadzu Company, the serial number 020525101565SA and capillary column (Rtx-5ms-30m×0.25 mm×0.25µm). The injection of the sample by using the split mode, the carrier gas was helium passed with flow rate 1.61 ml/min, the program of temperature was started from 60c with rate 10c/min to 300c as final temperature degree with 3 minutes hold time, the ion source temperature was 200c, the injection port temperature was 300c, and the interface temperature was 250c. The analyzed sample by using scan mode in the range of m/z 40-500 charges to ratio and the total run time was 27 minutes. Identification of constituents for the sample was achieved by comparing their mass fragmentation patents and retention index and with those present in the library, the National Institute of Standards and Technology (NIST). , results were recorded.

Atomic Absorption Spectrophotometer (AA-7000-Shimadzu).

3. Results and Discussion

The amount of the flaxseed oil obtained were 38%, this result agrees with [21] recorded a percentage of flaxseed oil was 35.10%, this variation in the extraction percentages can be due to

the differences soil, differences in species of the plant and diversity of climate. The physicochemical properties of extracted flaxseed oil were investigated and the results obtained were shown in Table 1. chemical properties such as acid value (0.71 mg KOH/g) as oleic acid, the acid value is a measure of the free fatty acids content in oil, peroxide value (2.69 MEG O2/KG) a widely used test forgives an idea about the early stages of oil oxidation and detect the rancidity in oils, saponification value (186.50 mg KOH/g oil), a high saponification value of the oil can be used in soap making. and iodine value (96.67 g/100 which is considered as one of the parameters used to determine the quality of the oil, refers to the presence of unsaturated fatty acids in oils, which reflects the susceptibility of oil to oxidation. these obtained results were agree with the result obtained by [21,22,23,24,25]. Table.2 show the Physical properties such as density (0.91 g/cm3), refractive index (1.39 and at 28°C), viscosity (cSt) (68.33) these results agree with [21,22,23].Table.3 and figure(1-26) show the chemical constituents obtained by Gas Chromatography-Mass Spectrometry (GC-MS) it contained 26 compounds identified, the major compound are, 9-Octadecenoic acid (Z)-, methyl ester (Oleic acid) (43.72) act as antioxidant, anti-cancer, Hexadecanoic acid, methyl ester (Palmitic acid) (17.36) act as Anti-oxidants, decrease blood cholesterol, anti-inflammatory, 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z) fatty acids:α-Linolenic acid, an omega-3 (n-3) fatty acid γ-Linolenic acid, an omega-6 (n-6) fatty acid (15.72), Octadecanoic acid Methyl stearate (13.13) used As a food additive, soaps, cosmetics, detergents lubricants, softening and release agents,13-Docosenoic acid, methyl ester, (Z)-(1.31), cis-11-Eicosenoic acid, methyl ester (0.96) act as Antibacterial, antitumor, antifungal, cytotoxic [26,27,28,29,30]. Table.4 how Atomic absorption used to identify minerals content it contains Calcium 1180ppm, Copper 4.1ppm, Ferric 15.7ppm, Zinc 34.58ppm.

Tables and figures of flaxseed oil

Table.1.Chemical properties of flaxseed

Acid value (mg KOH/g oil)	0.71 mg KOH/g
Saponification value (mg KOH/g oil)	186.50 mg KOH/g oil
Peroxide value (meq O2/kg)	2.69 meq O2/kg
Iodine value (g/100g)	96.67 g/100 g

Table.2. Physical properties of flaxseed

Density (g/cm^3)	0.91 g/cm^3
viscosity (cSt)	68.33
Refractive index	1.39 and at 28°C

Table. 3. Chemical constituents of flaxseed obtained by Gas Chromatography-Mass Spectrometry (GC-MS)

	Name	Ret.Time	Area	Area%
1.	Methyl tetradecanoate	13.289	916164	0.19
2.	Pentadecanoic acid, methyl ester	14.360	344674	0.07
3.	7-Hexadecenoic acid, methyl ester, (Z)-	15.151	328194	0.07
4.	9-Hexadecenoic acid, methyl ester, (Z)-	15.191	1043203	0.21
5.	Hexadecanoic acid, methyl ester	15.408	84377342	17.36
6.	cis-10-Heptadecenoic acid, methyl ester	16.155	668876	0.14
7.	Heptadecanoic acid, methyl ester	16.365	1226357	0.25
8.	9-Octadecenoic acid (Z)-, methyl ester	17.140	212421520	43.72
9.	9-Octadecenoic acid (Z)-, methyl ester	17.188	3666857	0.75
10	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	17.240	76398640	15.72
11	Methyl stearate	17.342	63802824	13.13
12	11,14-Eicosadienoic acid, methyl ester	17.904	175052	0.04
13	11,14,17-Eicosatrienoic acid, methyl ester	18.731	2130390	0.44
14	cis-11-Eicosenoic acid, methyl ester	18.863	4655679	0.96
15	8,11,14-Eicosatrienoic acid, methyl ester	18.902	1328973	0.27
16	Eicosanoic acid, methyl ester	19.062	3013673	0.62
17	13-Docosenoic acid, methyl ester, (Z)-	20.506	6371600	1.31
18	Docosanoic acid, methyl ester	20.690	2565009	0.53
19	Octadecanoic acid, 6-hydroxy-, methyl ester	20.783	2076001	0.43
20	15-Tetracosenoic acid, methyl ester, (Z)-	22.034	1283185	0.26
21	Methyl 8-oxohexadecanoate	22.099	1055613	0.22
22	Tetracosanoic acid, methyl ester	22.187	2029432	0.42
23	Erucic acid	23.013	1568971	0.32
24	Hexacosanoic acid, methyl ester	23.587	495770	0.10
25	Stigmast-5-en-3-ol, oleate	25.072	1078478	0.22
26	9,19-Cyclolanost-23-ene-3,25-diol, 3-acetate, (3.beta.,23E)-	25.351	10948184	2.25

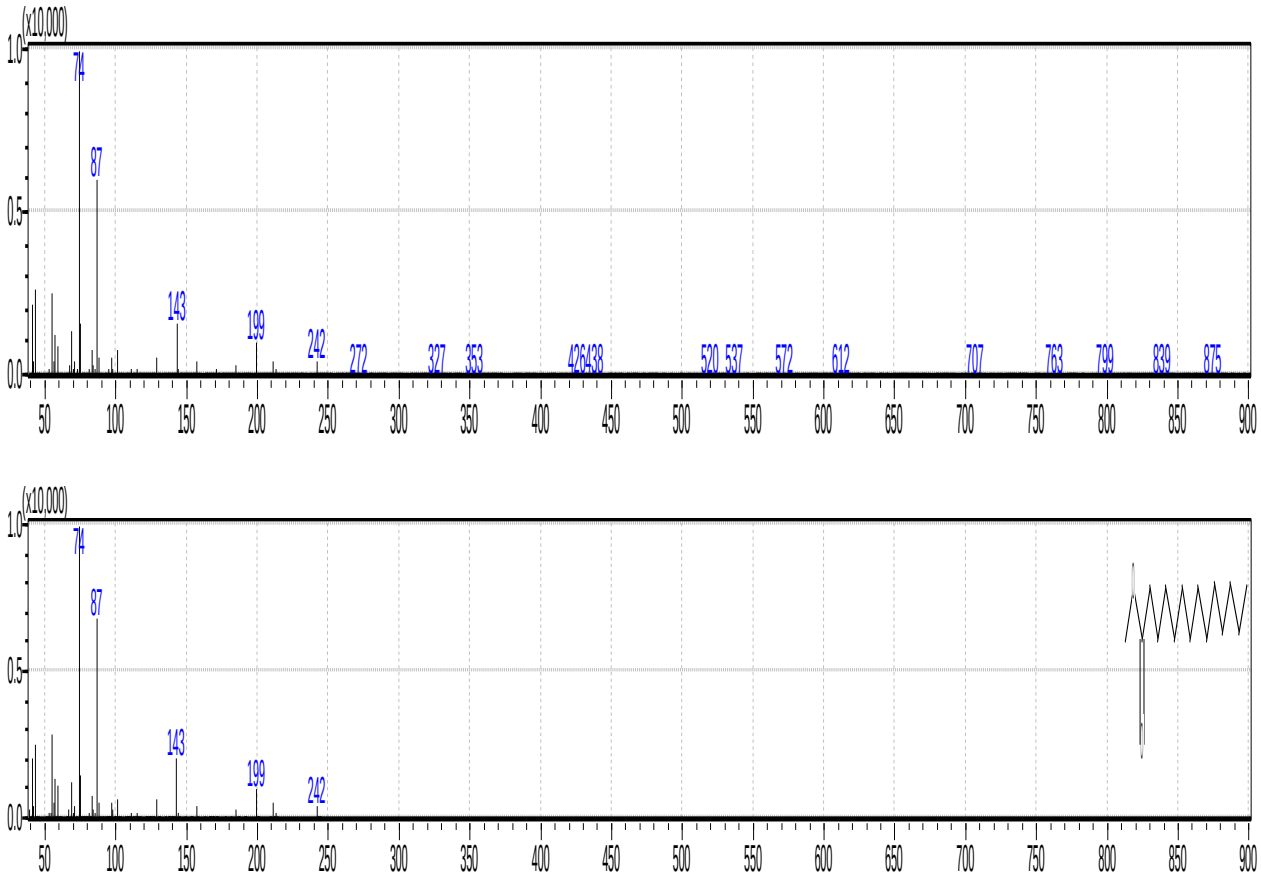
Table.4. Minerals of flaxseed

No	Elements	Amount in flaxseed
1	Ca ppm	1180
2	Cd ppm	NDT
3	Co ppm	NDT
4	Cr ppm	NDT
5	Cu ppm	4.1
6	Fe ppm	15.7
7	Pb ppm	NDT
8	Zn ppm	34.58

NDT=Not Detected

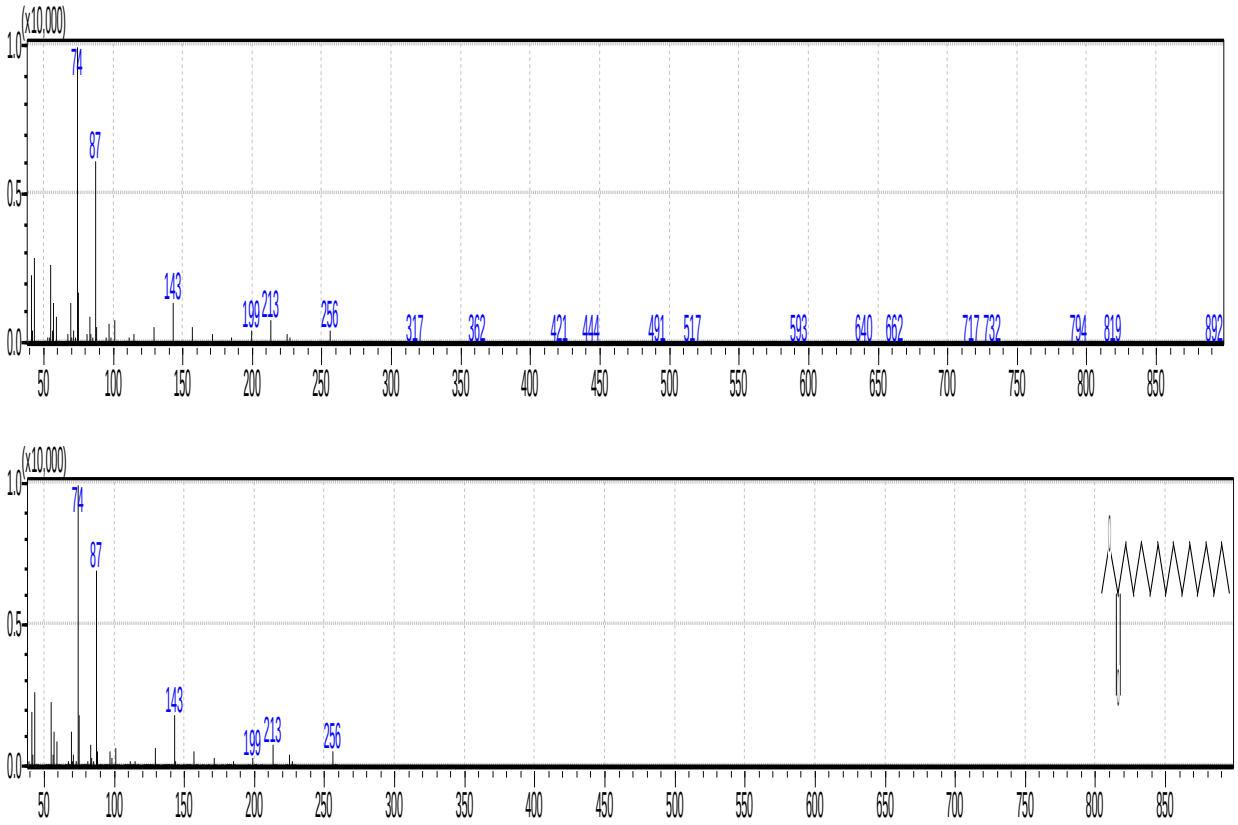
Methyl tetradecanoate $$ Tetradecanoic acid, methyl ester

Molecular weight 242. Formula C15H30O2 (Figure 1)



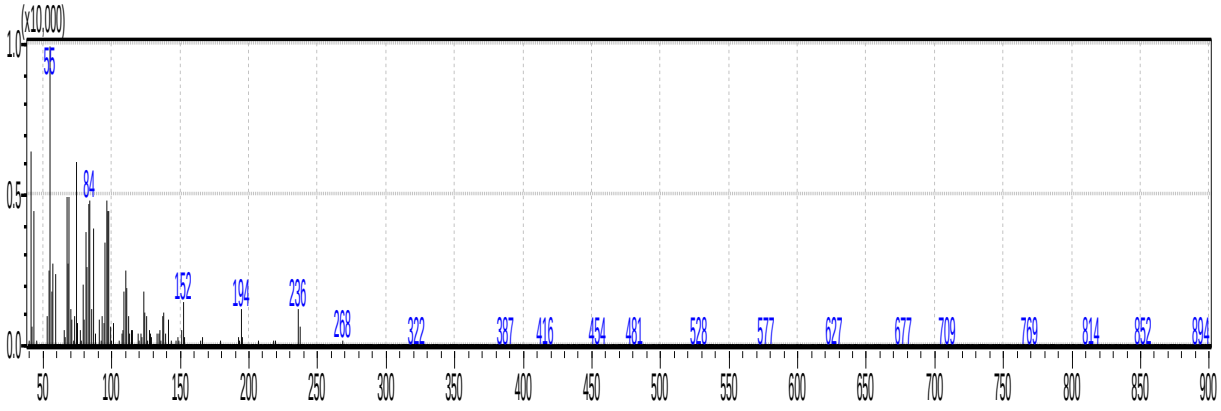
2/ Pentadecanoic acid, methyl ester

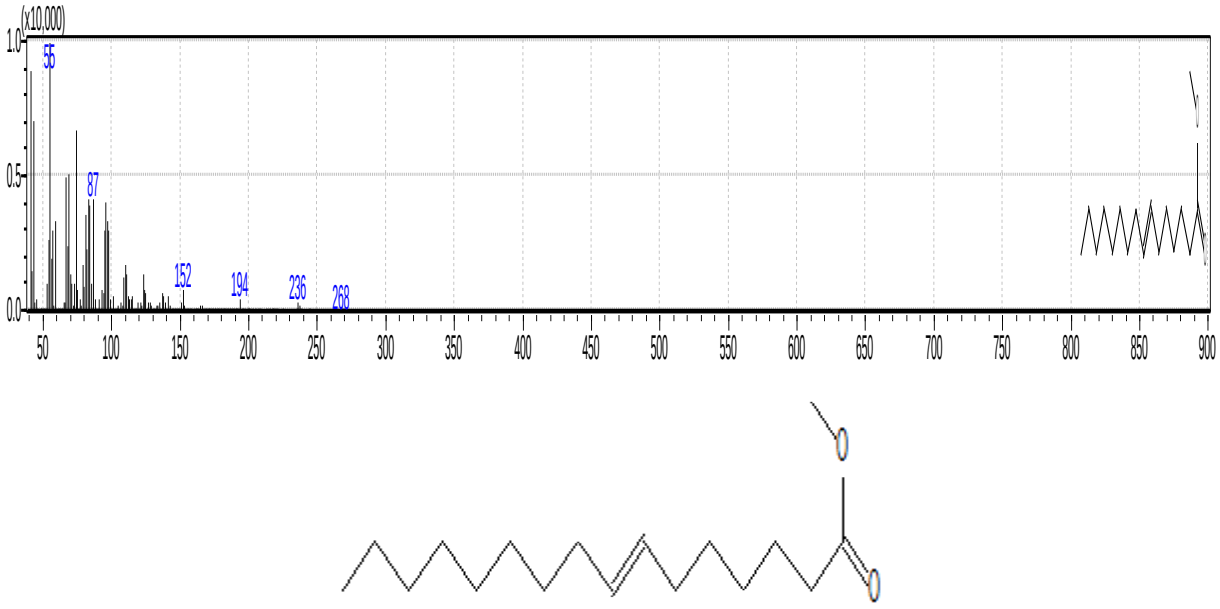
Molecular weight 256. Formula C16H32O2 (Figure2)



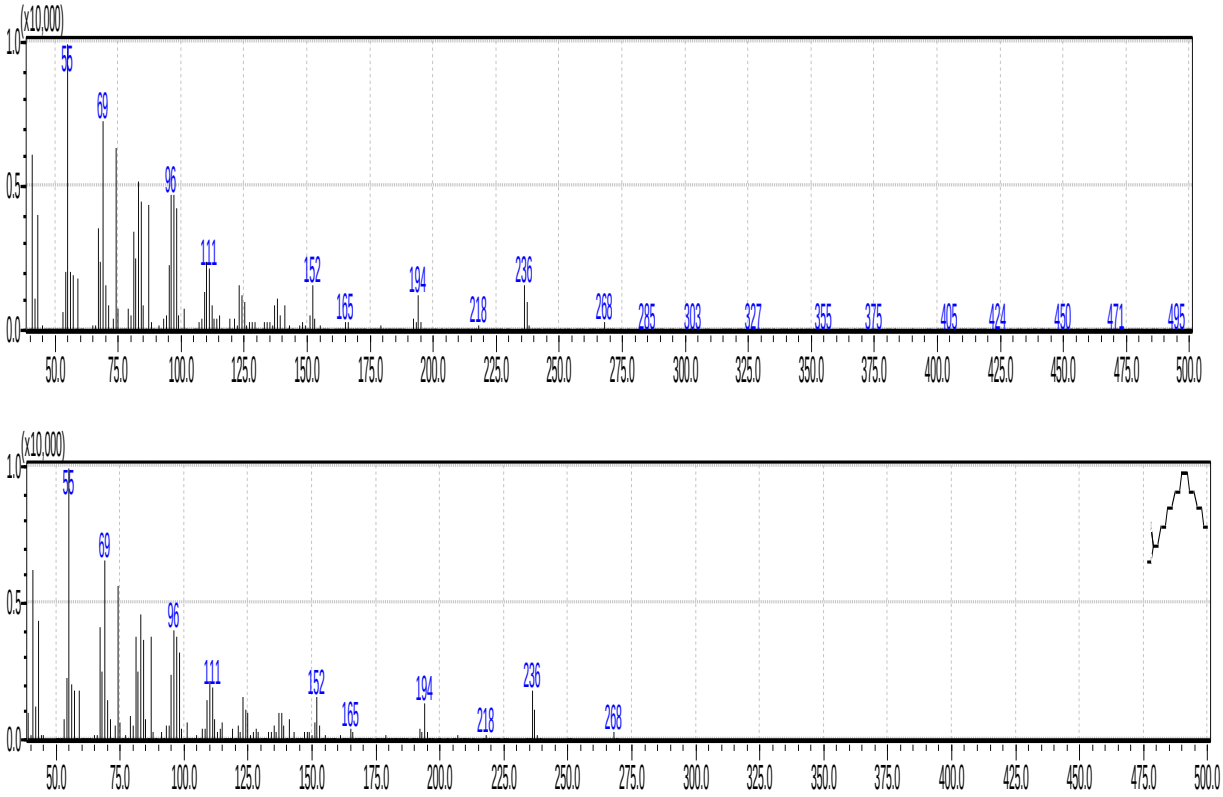
3/7-Hexadecenoic acid, methyl ester, (Z)-4/

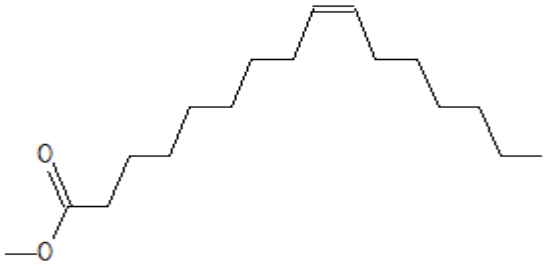
Molecular weight 268. Formula C17H32O2 (Figure 3)





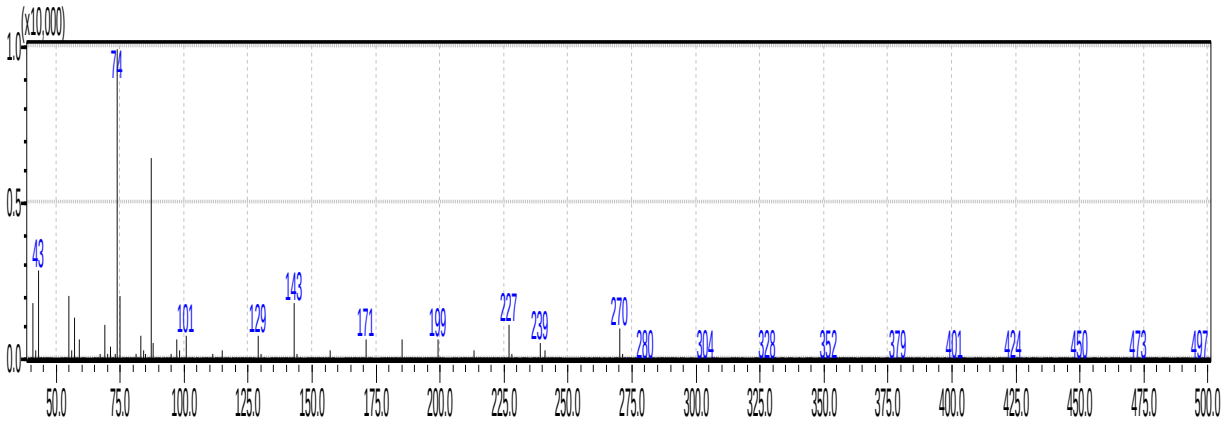
4/ 9-Hexadecenoic acid, methyl ester, (Z)- $$ Methyl palmitoleate
Molecular weight 268. Formula C17H32O2 (Figure.4.)

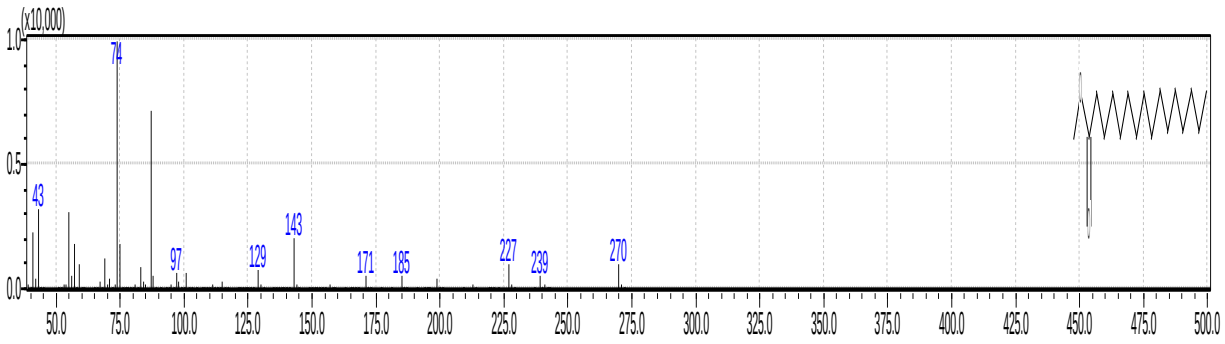


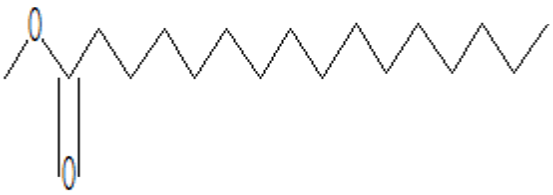


5/ Hexadecanoic acid, methyl ester $$ Palmitic acid, methyl ester

Molecular weight 270. Formula C17H34O2 (Figure.5)

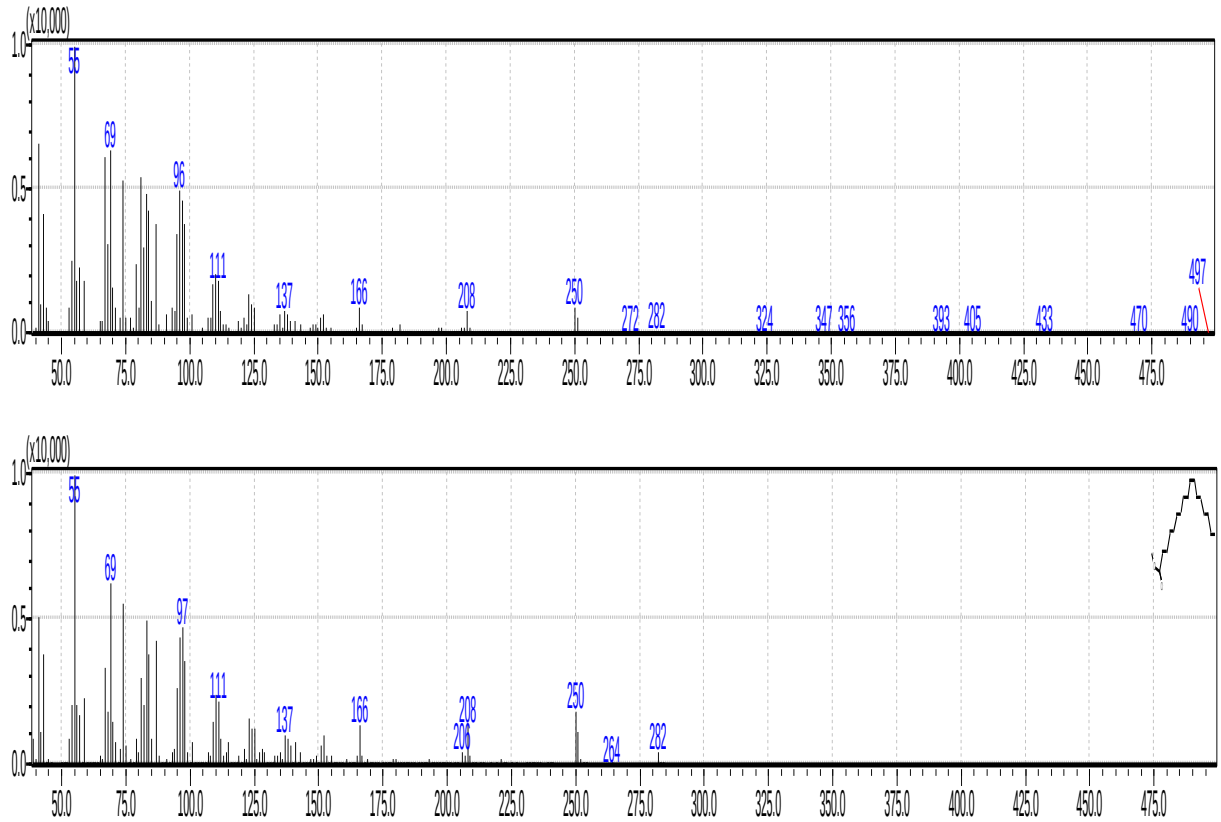


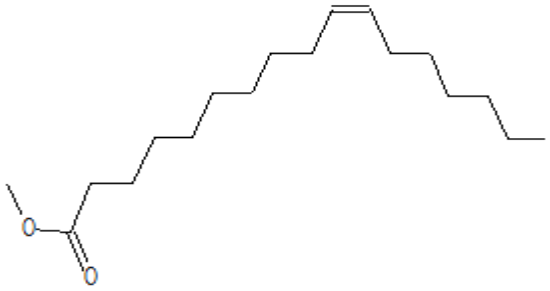




6/ cis-10-Heptadecenoic acid, methyl ester

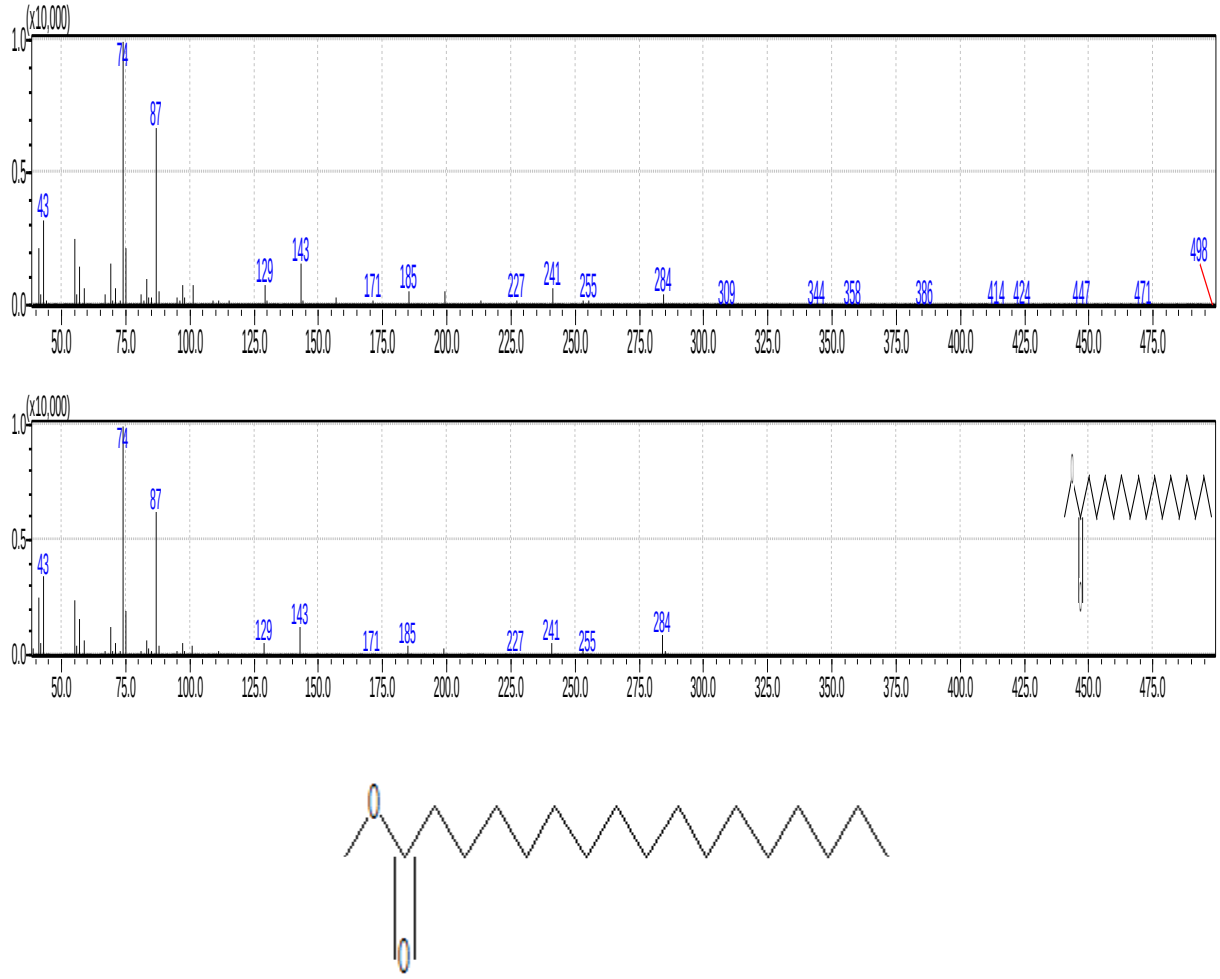
Molecular weight 282. Formula C18H34O2 (Figure.6)





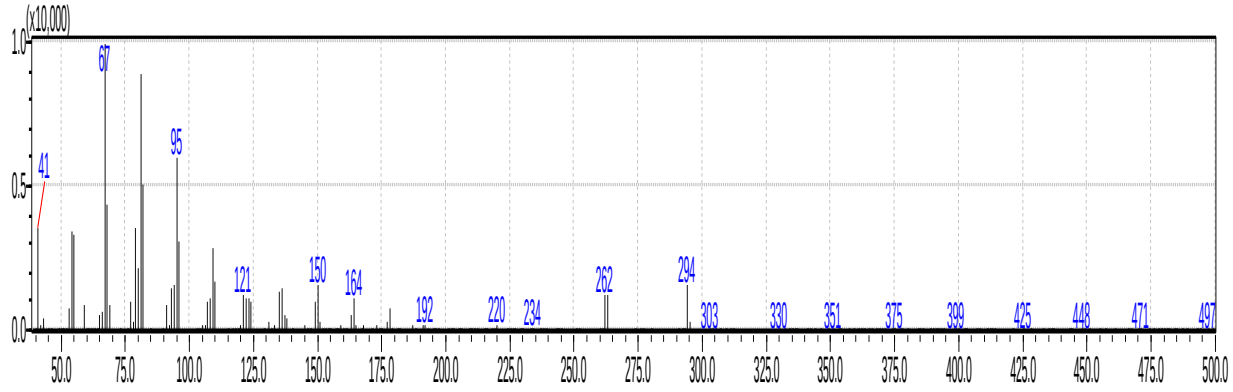
7/ Heptadecanoic acid, methyl ester $$ Margaric acid methyl ester

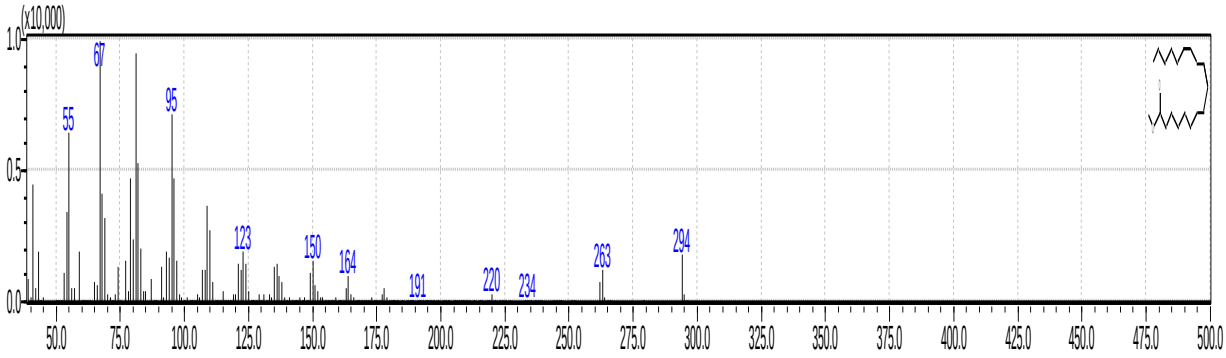
Molecular weight 284. Formula C18H36O2 (Figure.7)

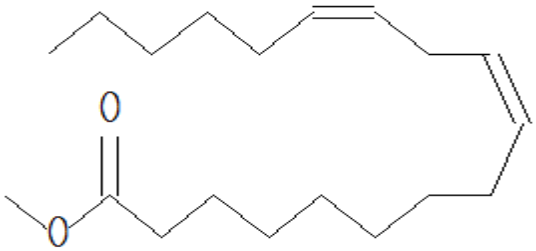


8/ 9,12-Octadecadienoic acid (Z,Z)-, methyl ester $$ Linoleic acid, methyl ester

Molecular weight 294. Formula C19H34O2 (Figure.8)

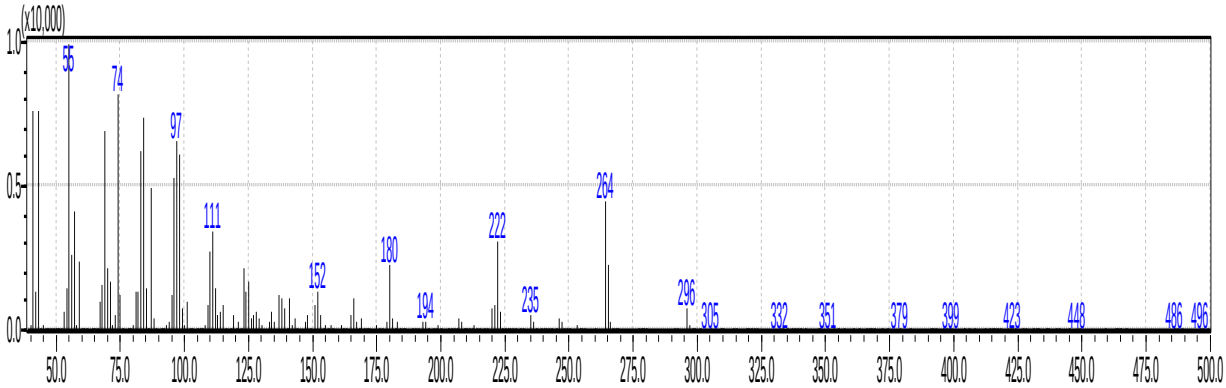


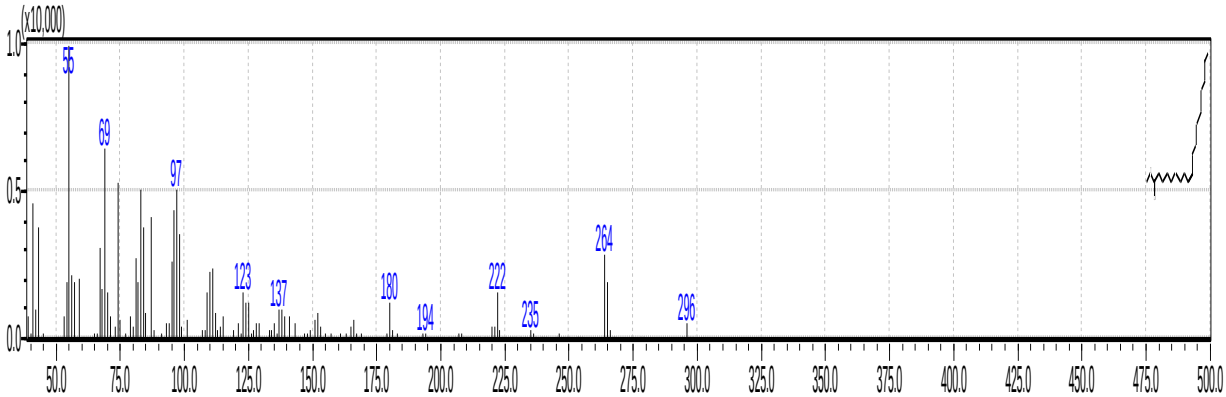


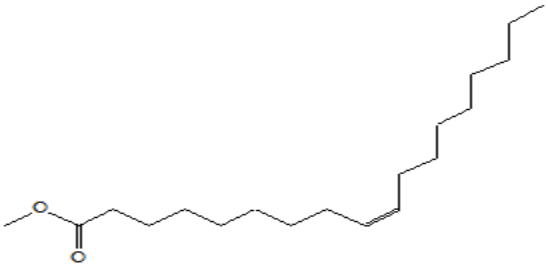


9/ 9-Octadecenoic acid (Z)-, methyl ester $$ Oleic acid, methyl ester

Molecular weight 296. Formula C19H36O2 (Figure.9)

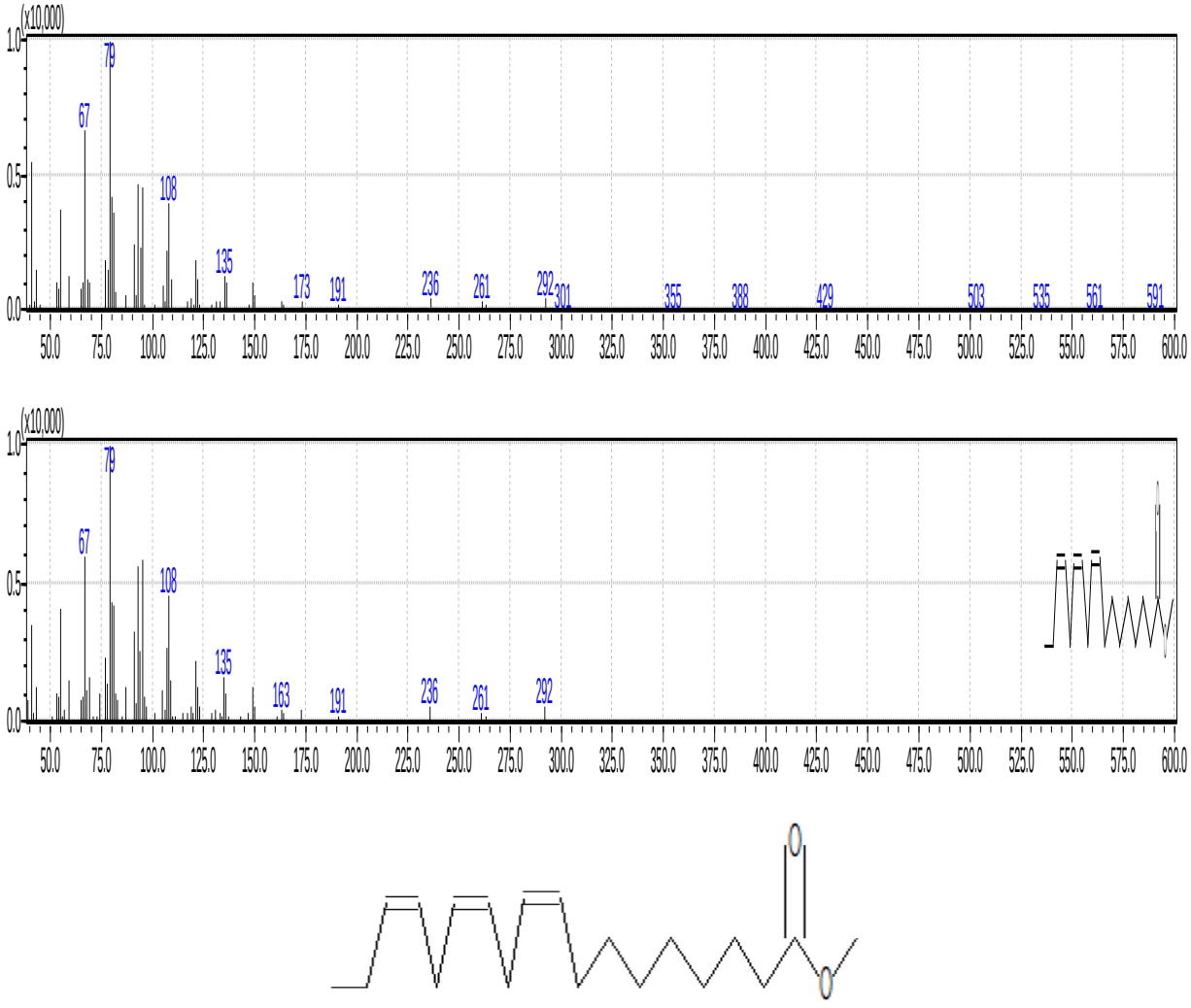






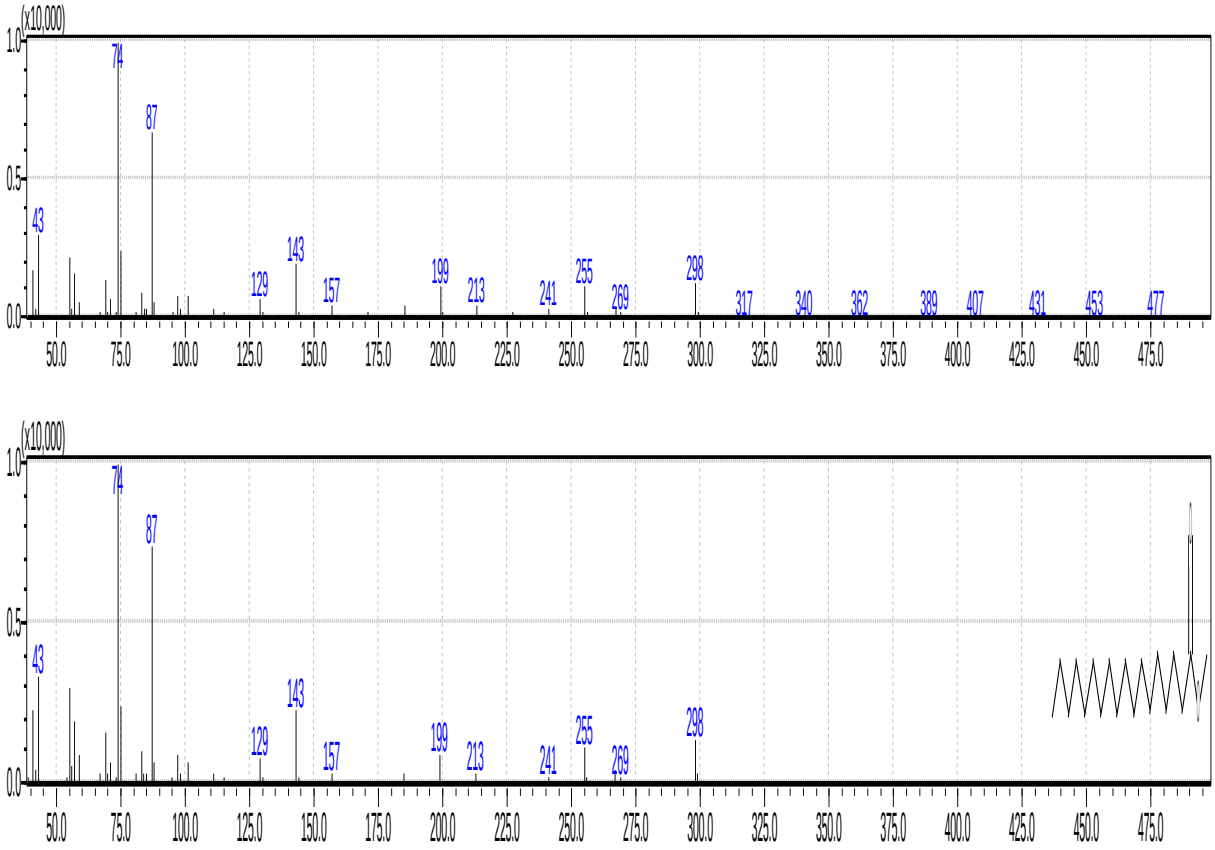
10/ 9, 12, 15-Octadecatrienoic acid, methyl ester, (Z, Z, Z) - $$ Linolenic acid, methyl ester

Molecular weight 292. Formula C19H32O2 (Figure.10)



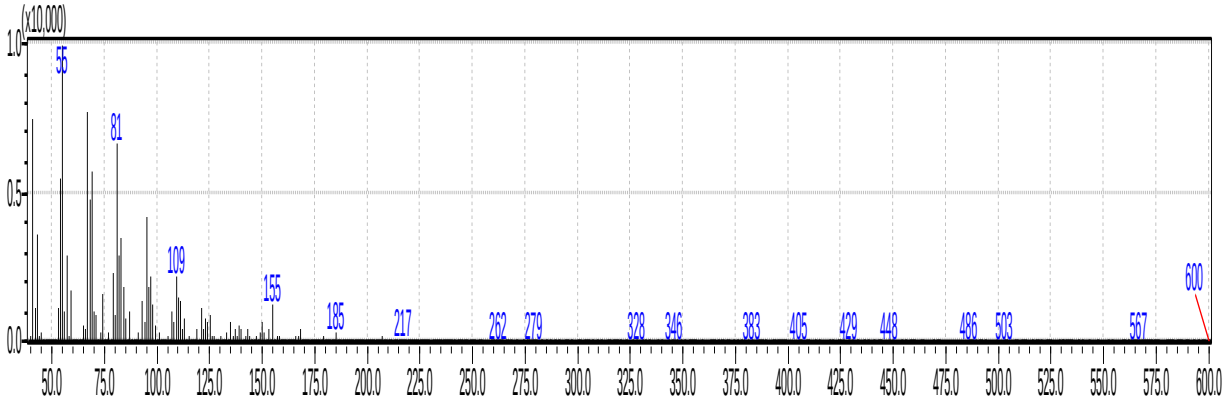
11/ Methyl stearate $$ Octadecanoic acid, methyl ester $$ Stearic acid, methyl ester

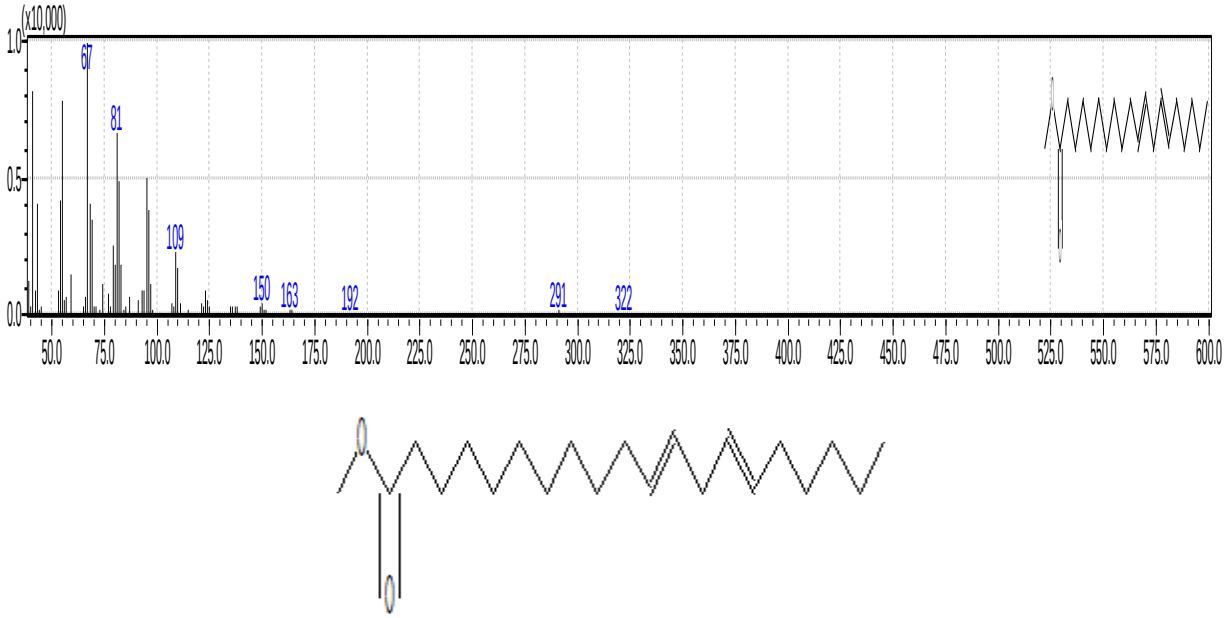
Molecular weight 298. Formula C19H38O2 (Figure.11)



12/ 11, 14-Eicosadienoic acid, methyl ester

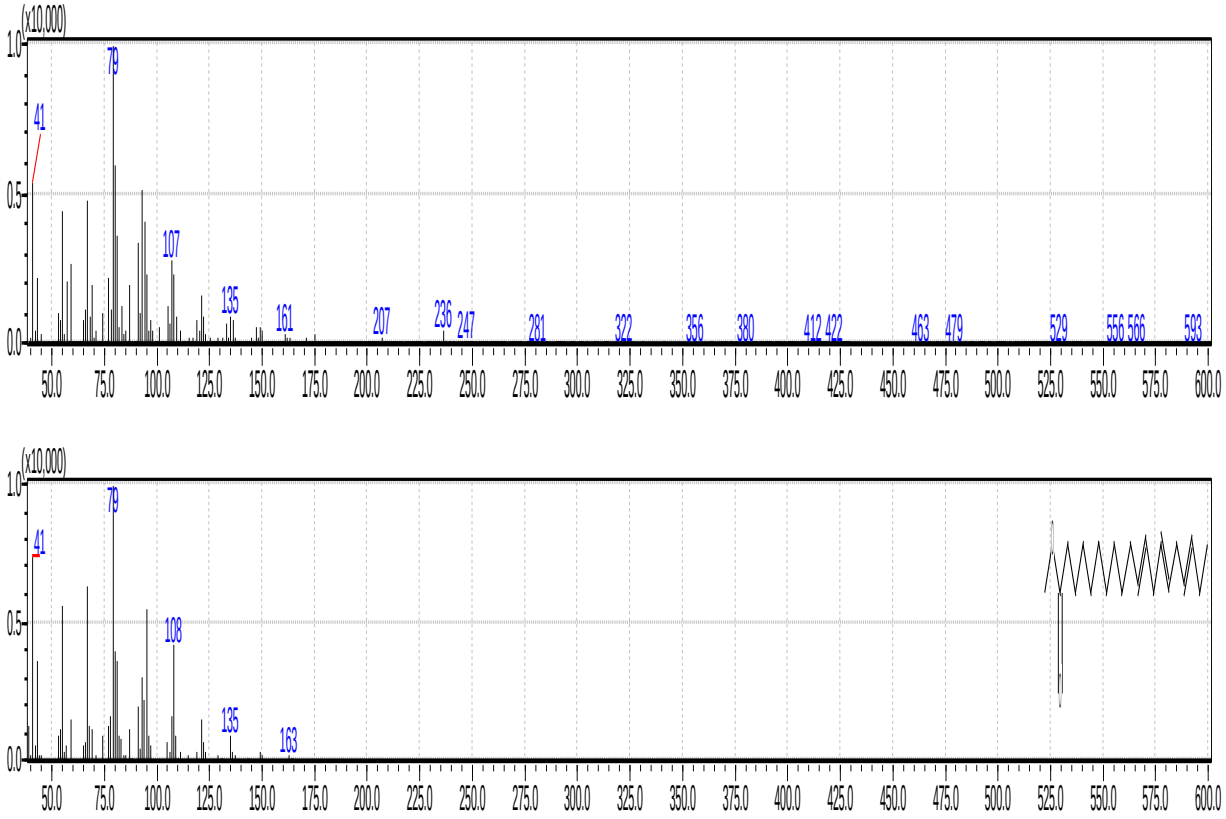
Molecular weight 322. Formula C21H38O2 (Figure.12)

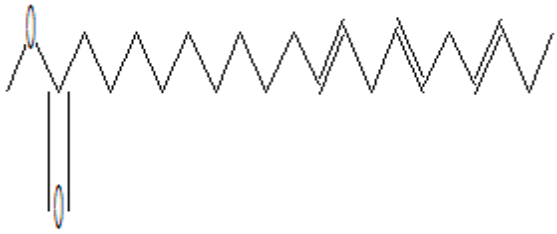




13/ 11, 14, 17-Eicosatrienoic acid, methyl ester

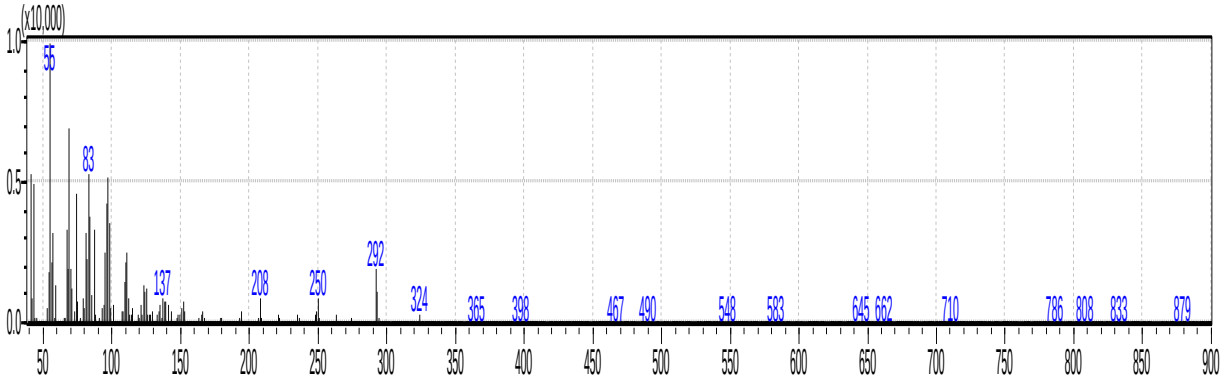
Molecular weight 320. Formula C21H36O2 (Figure13)

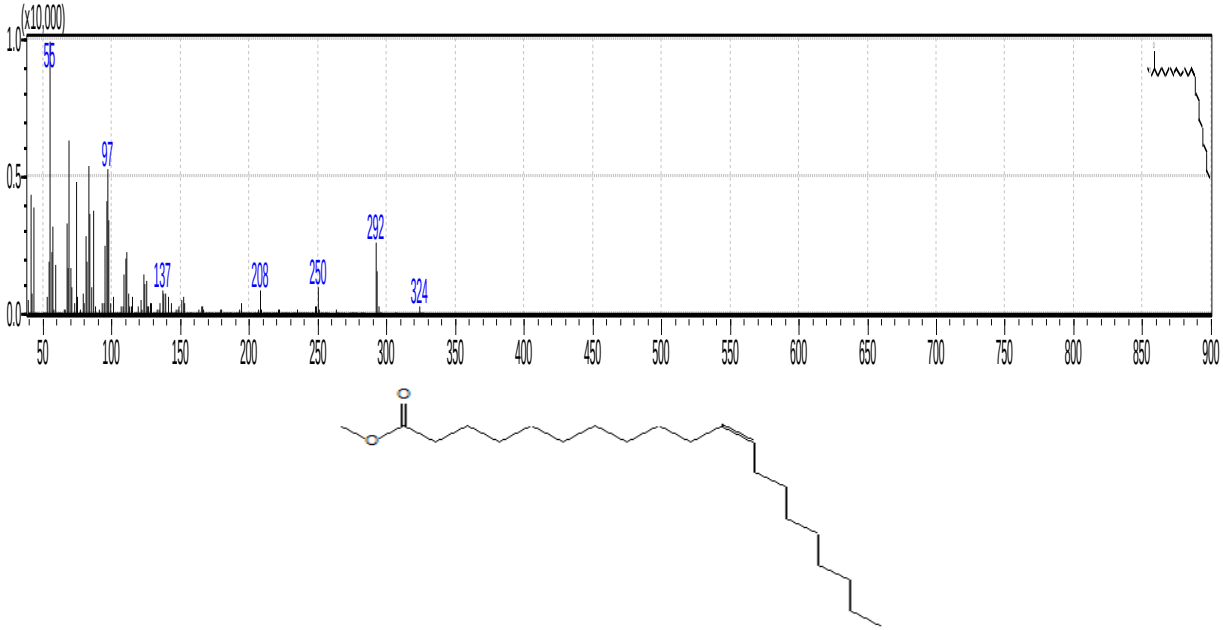




14/ cis-11-Eicosenoic acid, methyl ester

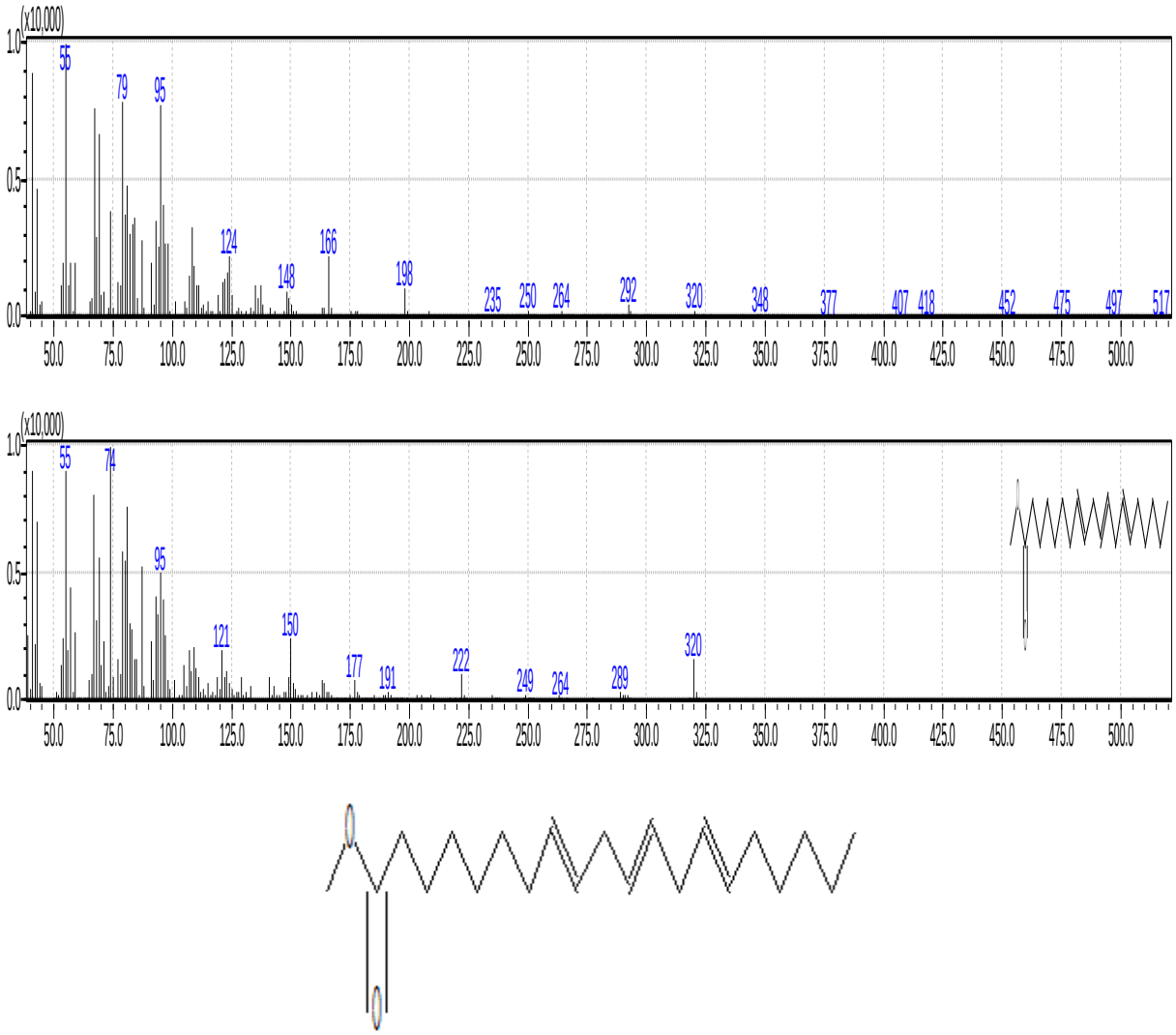
Molecular weight 324. Formula C21H40O2 (Figure.14)





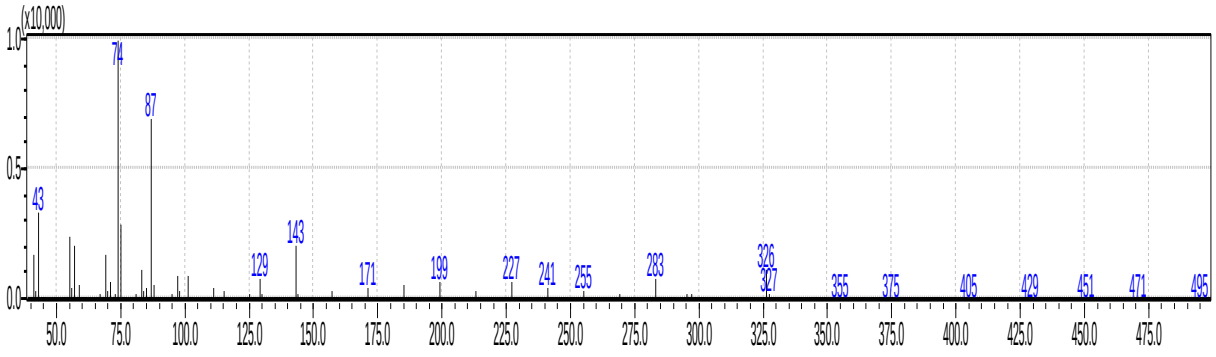
15/ 8, 11, 14-Eicosatrienoic acid, methyl ester

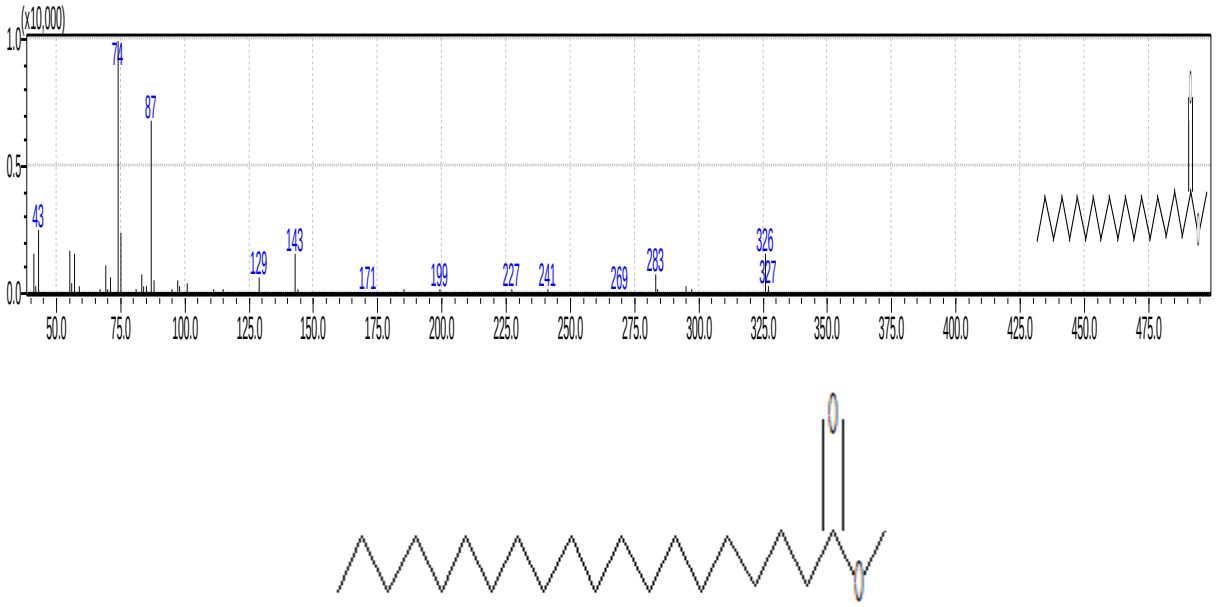
Molecular weight 320. Formula C21H36O2 (Figure.15)



16/ Eicosanoic acid, methyl ester $$ Methyl arachisate $$ Methyl eicosanoate $$ Arachidic acid methyl ester

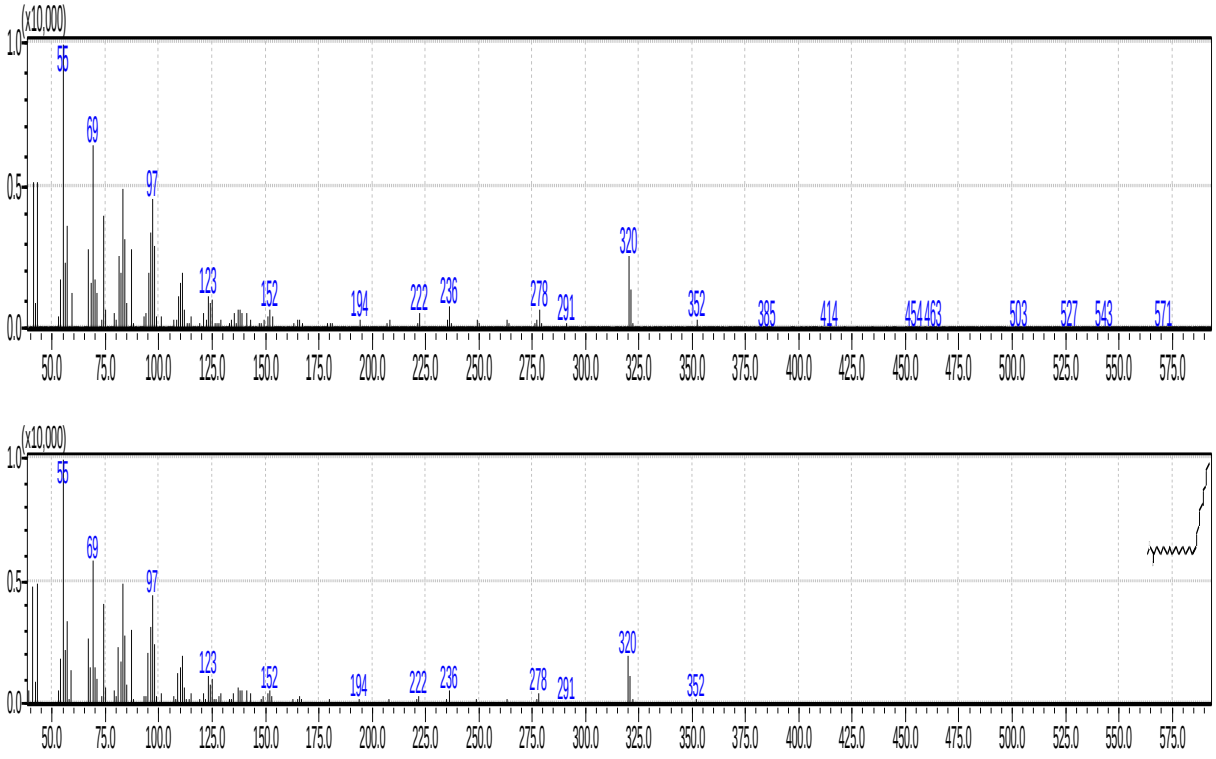
Molecular weight 326. Formula C21H42O2 (Figure.16)

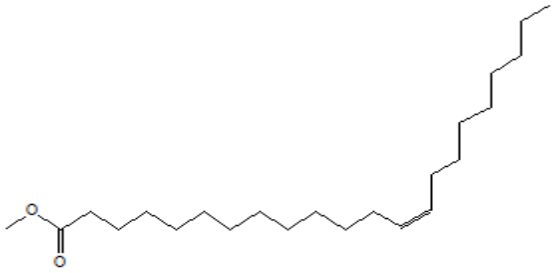




17/ 13-Docosenoic acid, methyl ester, (Z)-

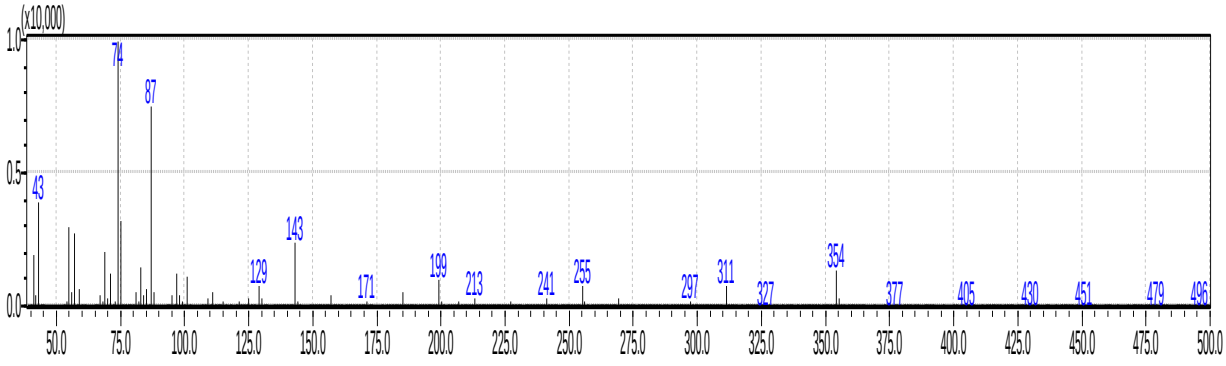
Molecular weight 340. Formula C22H44O2 (Figure.17)

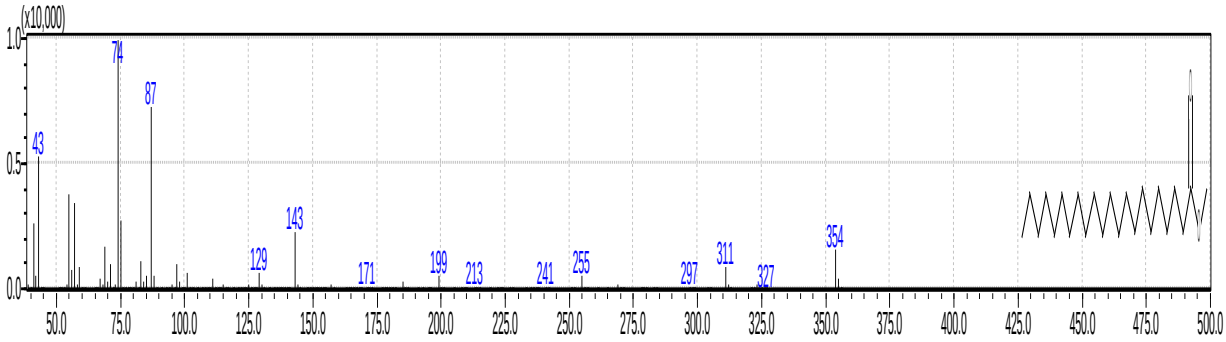




18/ Docosanoic acid, methyl ester $$ Behenic acid, methyl ester

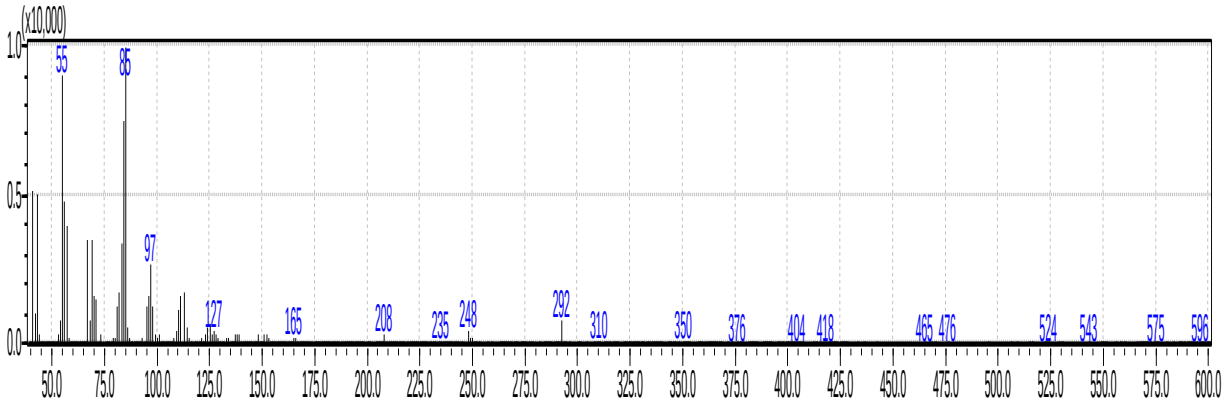
Molecular weight 326. Formula C21H42O2 (Figure.18)

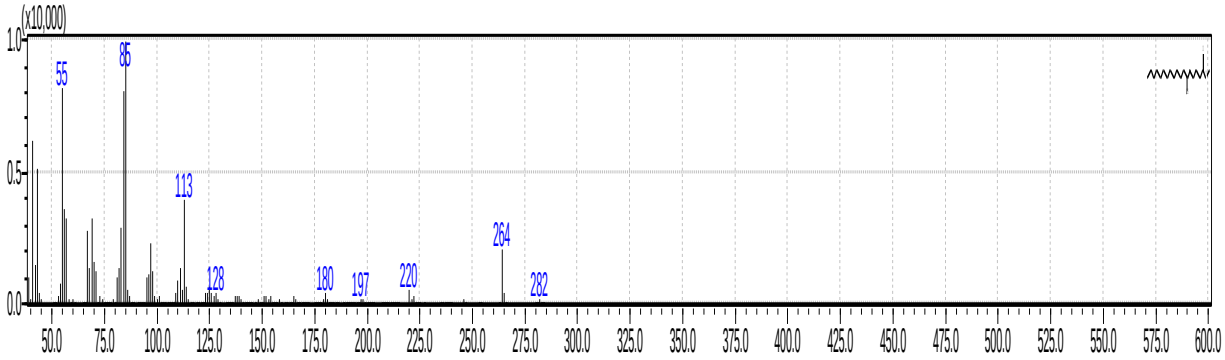


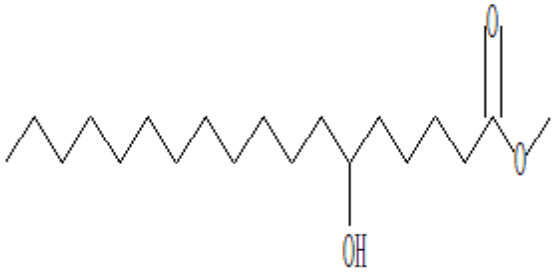


19/ Octadecanoic acid, 6-hydroxy-, methyl ester

Molecular weight 314. Formula C19H38O3 (Figure.19)

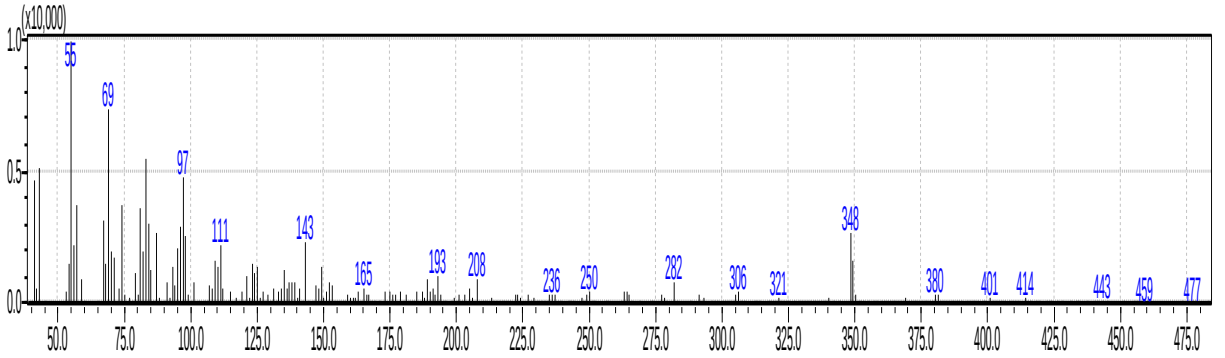


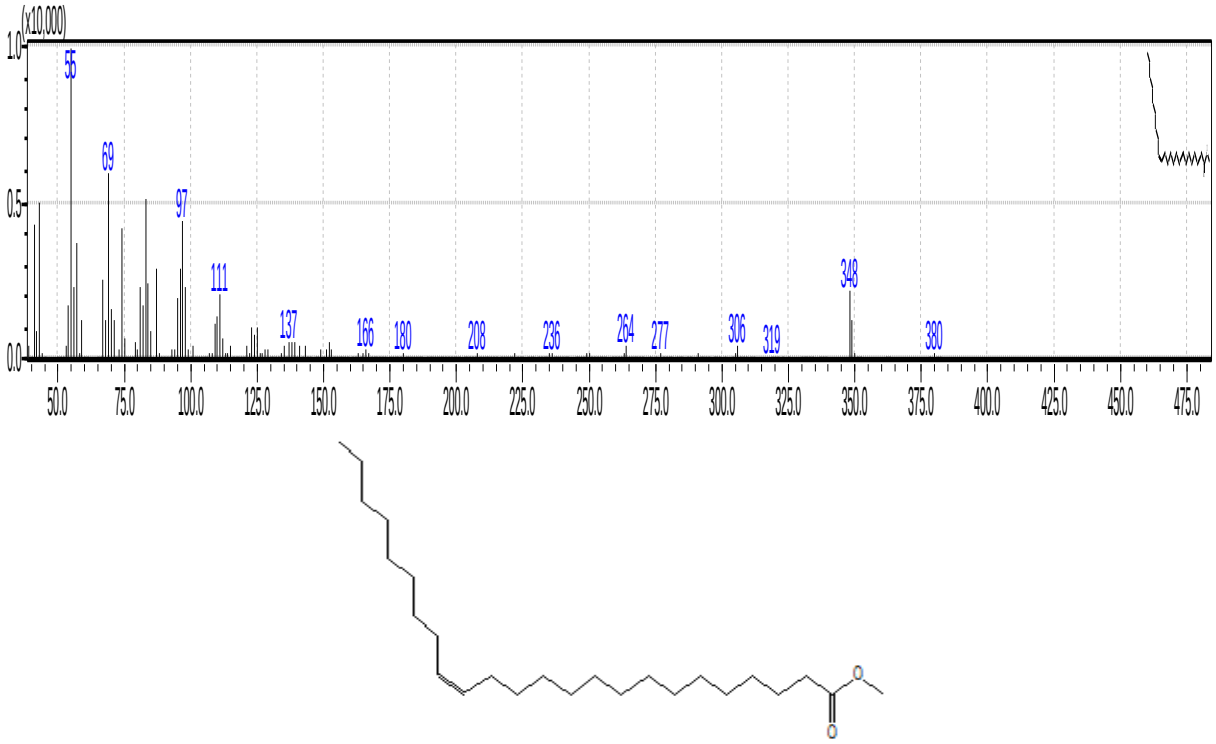




20/ 15-Tetracosenoic acid, methyl ester, (Z)-

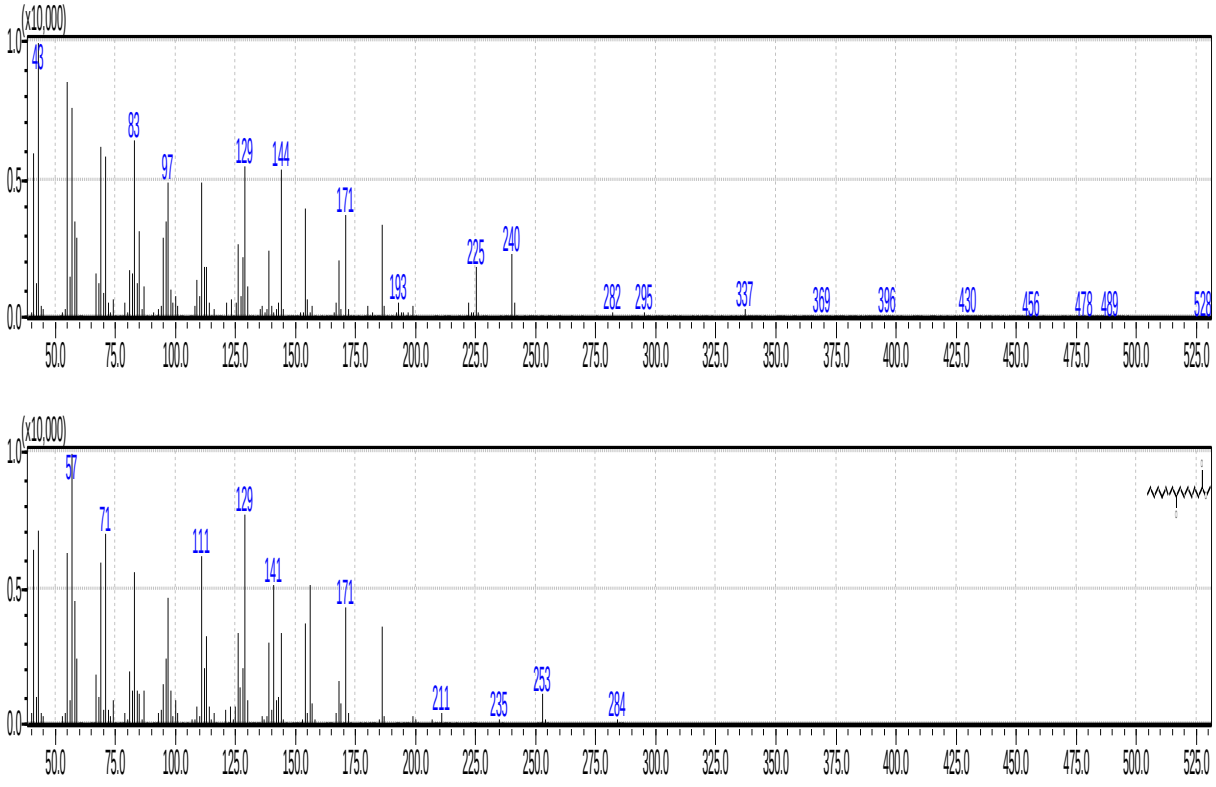
Molecular weight 368. Formula C24H48O2 (Figure.20)

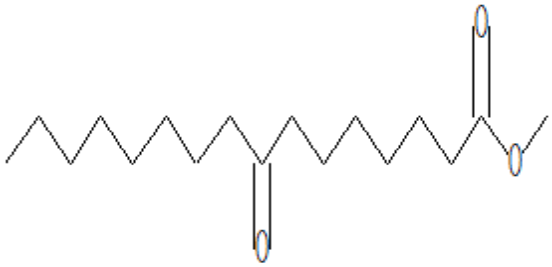




21/ Methyl 8-oxohexadecanoate

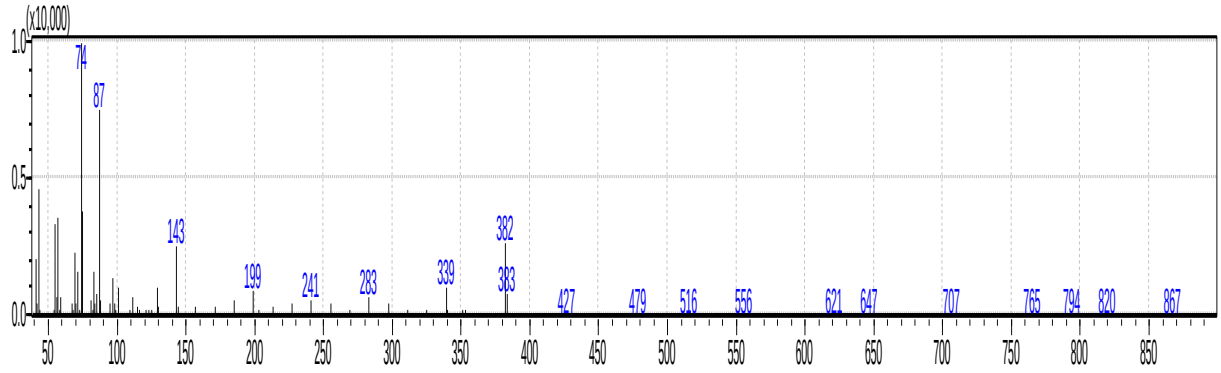
Molecular weight 284. Formula C17H32O3 (Figure21)

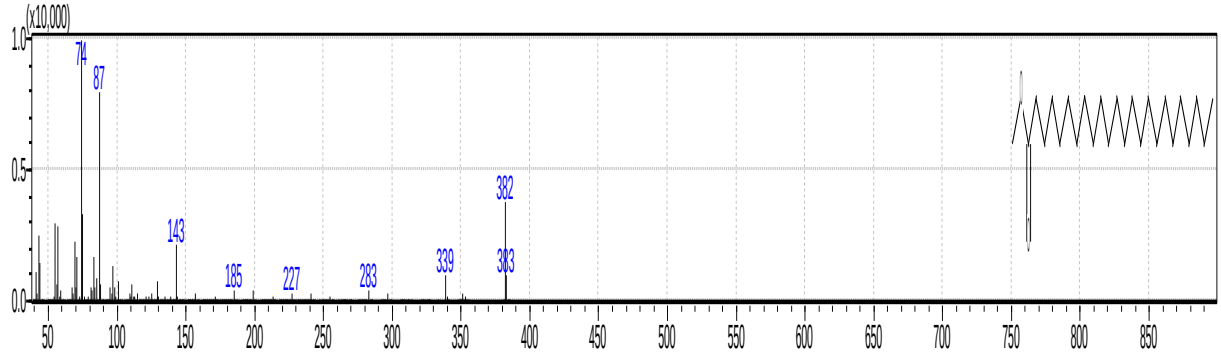




22/ Tetracosanoic acid, methyl ester

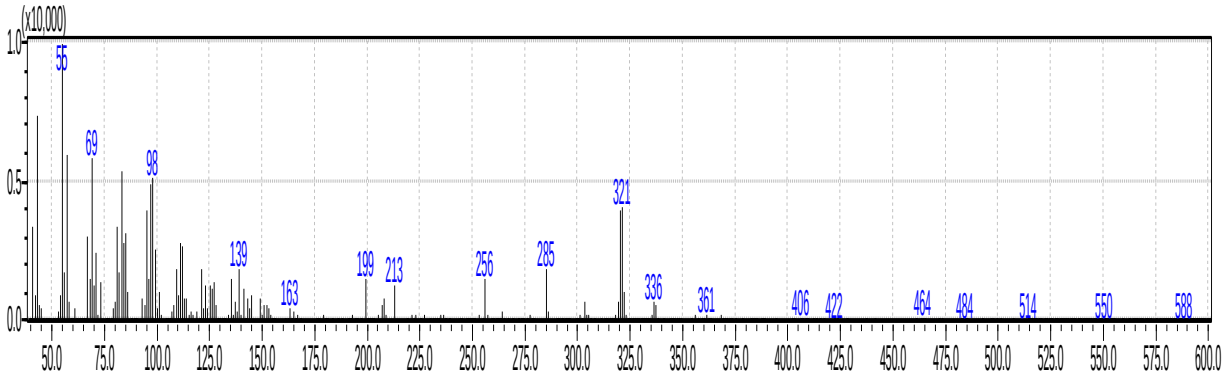
Molecular weight 382. Formula C25H50O2 (Figure.22)

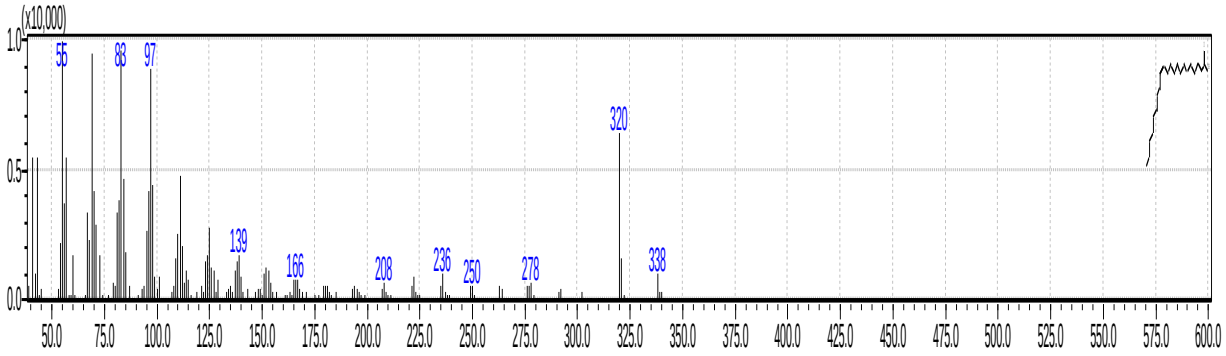


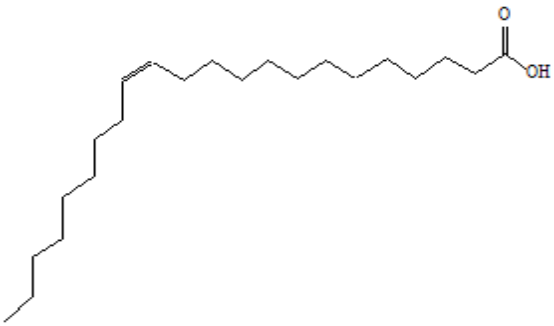


23/ Erucic acid

Molecular weight 338. Formula C22H42O2 (Figure.23)

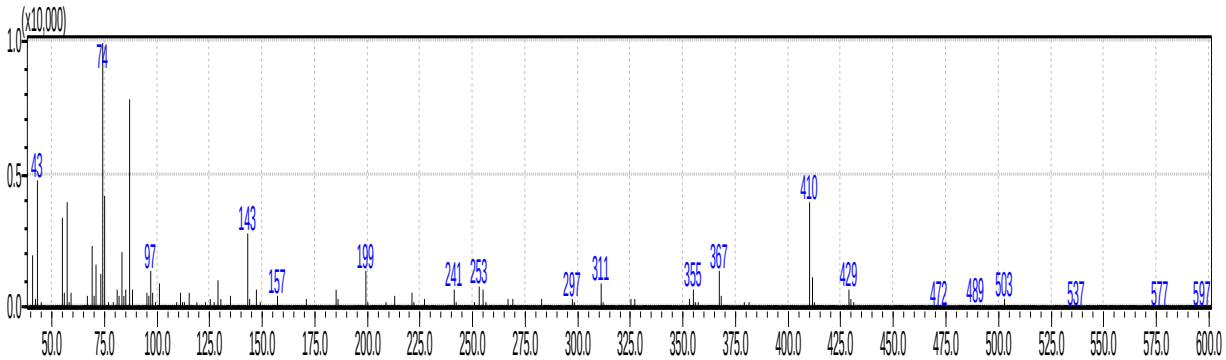


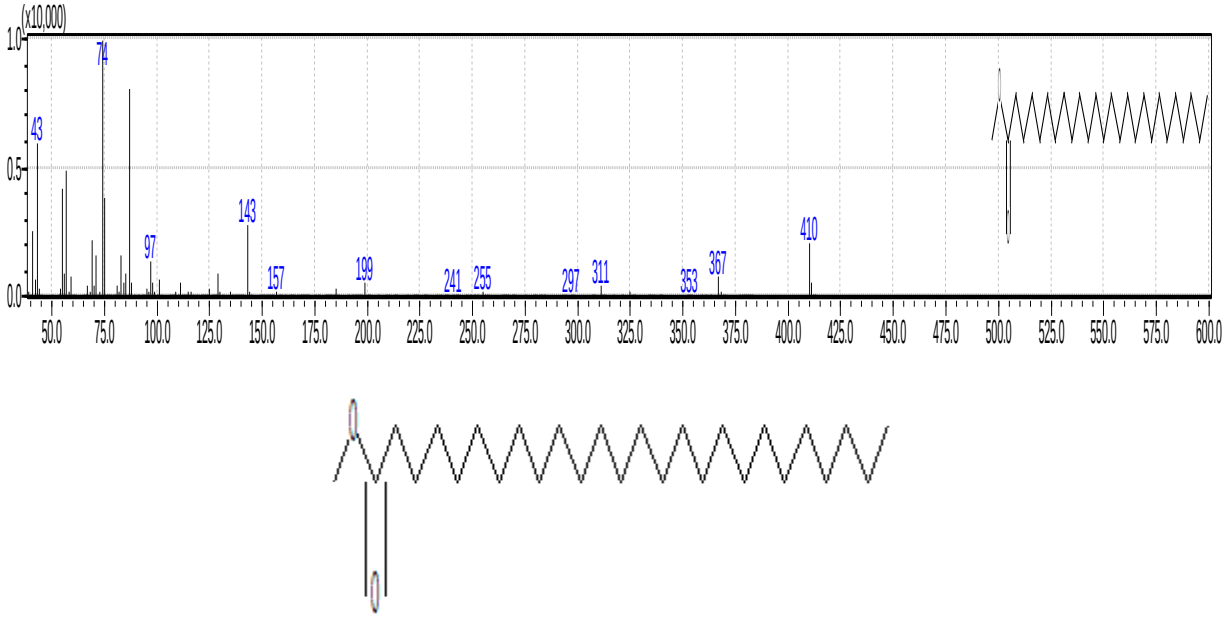




24/ Hexacosanoic acid, methyl ester

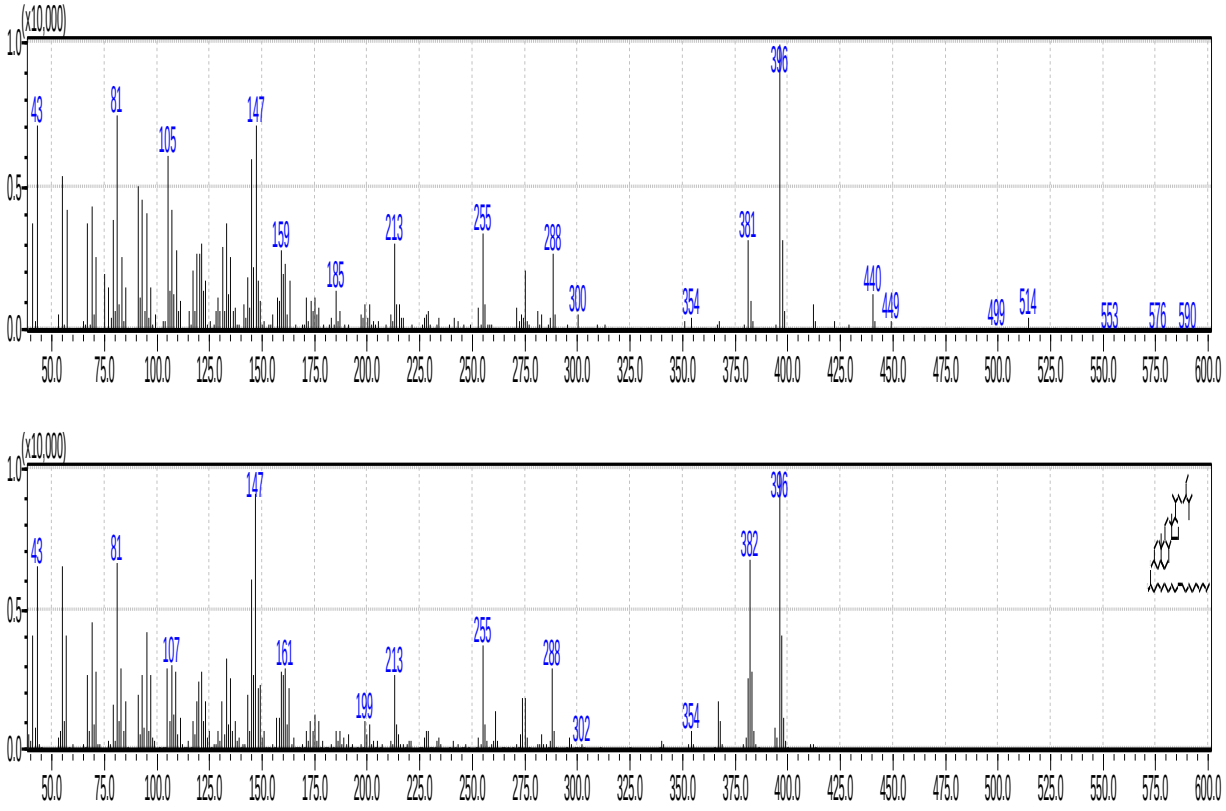
Molecular weight 410. Formula C27H54O2 (Figure.24)

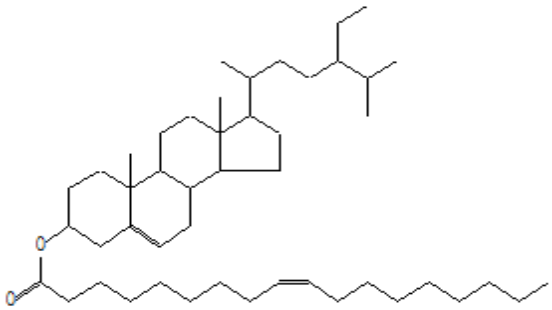




25/ Stigmast-5-en-3-ol, Oleate

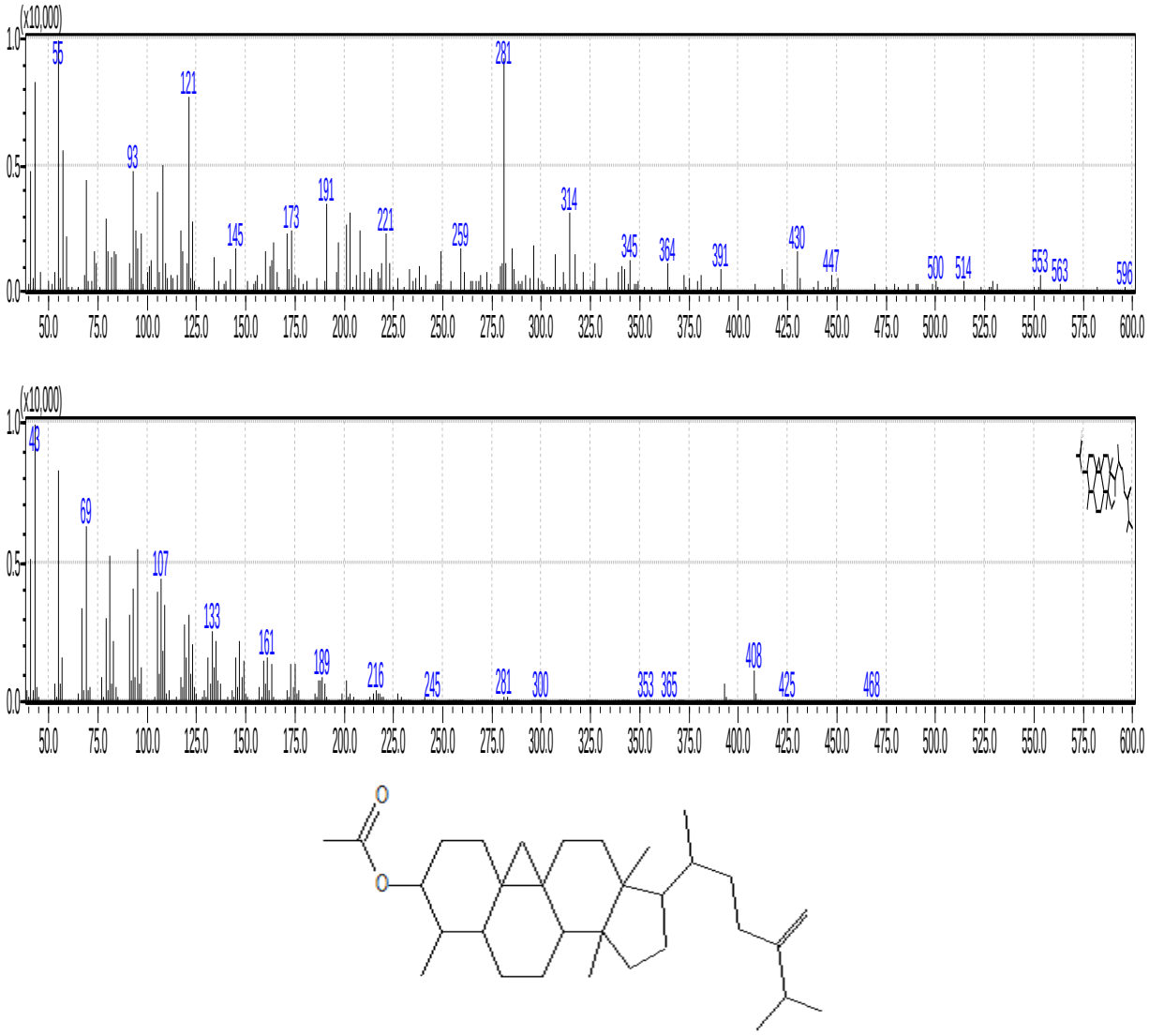
Molecular weight 678. Formula C47H82O2 (Figure.25)





26/ 9, 19-Cycloergost-24(28)-en-3-ol, 4, 14-dimethyl-, acetate, (3.beta. 4. alpha., 5.alpha.)-

Molecular weight 468. Formula C32H52O2 (Figure.26)



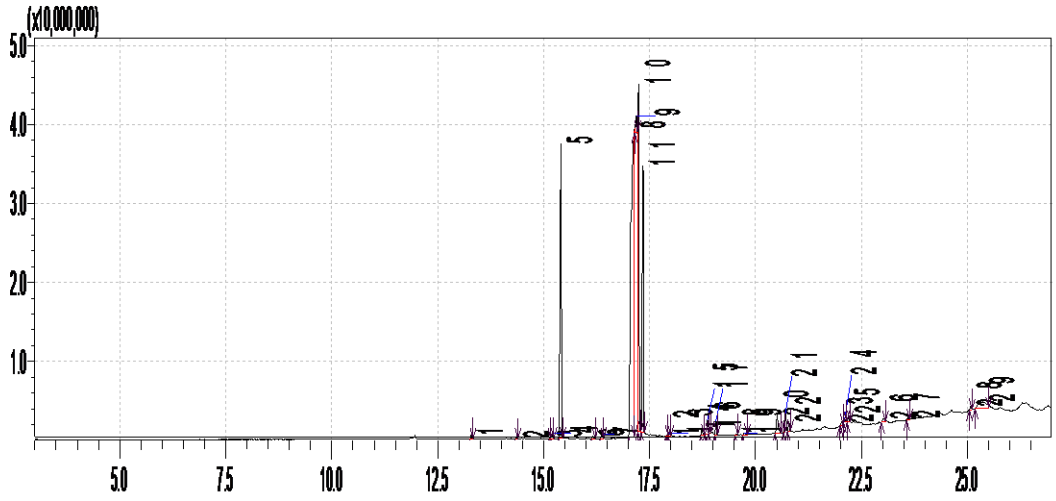


Figure.27.

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