# THE UNIVERSITY GRANTS COMMISSION (UGC)
# NEW DELHI

## Final Progress Report of

## The Major Research Project
## On

***In-vitro*** **testing of the efficacy (individually and in combination) of different solvent extracts isolated from Astro-herbal/medicinal plants against synthetic gp120-CD4 interaction and HIV-1-RT'**

**Submitted**

**By**


## Prof. S.Y.Anwar

**DEPAPARTMENT OF GENETICS**
**OSMANIA UNIVERSIT**

**Annexure – IX**

**UNIVERSITY GRANTS COMMISSION**
**BAHADUR SHAH ZAFAR MARG**
**NEW DELHI – 110 002**

**PROFORMA FOR SUBMISSION OF INFORMATION AT THE TIME OF SENDING THE FINAL REPORT OF THE WORK DONE ON THE PROJECT**

1. TITLE OF THE PROJECT **:** *In-vitro* testing of the efficacy (individually and in combination) of different solvent extracts isolated from Astro-herbal/medicinal plants against synthetic gp120-CD4 interaction and HIV-1-RT'

2. NAME AND ADDRESS OF THE PRINCIPAL INVESTIGATOR : **Prof. S.Y.Anwar (Retd.)**
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**Telangana State – 500 007**

**3.** NAME AND ADDRESS OF THE INSTITUTION : **Dept. of Genetics**
University College of Science
**Osmania University, Hyderabad**
**Telangana State – 500 007**

**4.** UGC APPROVAL LETTER NO. AND DATE : **F. No. 42-709/2013 (SR) Dt.21-03-2013**

5. DATE OF IMPLEMENTATION : **01-04-2013**

6. TENURE OF THE PROJECT : **01-04-2013 to 30-03-2017**

7. TOTAL GRANT ALLOCATED : **Rs. 14,00,200/-**

8. TOTAL GRANT RECEIVED : **Rs. 10,76,000/-**

**9.** FINAL EXPENDITURE : **Rs.13,27,950/-**

10. TITLE OF THE PROJECT : *In-vitro* testing of the efficacy (individually and in combination) of different solvent extracts isolated from Astro-herbal/medicinal plants against synthetic gp120-CD4 interaction and HIV-1-RT'

## 11. OBJECTIVES OF THE PROJECT :

- **Objective – 1** Collecting and establishing 09 plants in Plant Genetics Experimental Farm, Department of Genetics, Osmania University
- **Objective – 2** Preparation of individual and Indian Traditional Knowledge based friendly, non-friendly and all-in-one polyherbal extracts from among 09 plants

  **Objective 3** - Isolation and characterization of bio-constituents from each extract through GCMS
- **Objective – 4** Anti-HIV activity of individual and polyherbal extracts

## 12. WHETHER OBJECTIVES WERE ACHIEVED : Yes

### Work done

- **Objective – 1:**

**Collecting and establishing 09 plants in Plant Genetics Experimental Farm of Department of Genetics, Osmania University:**

**Nine** plantlets viz. *Calotropis gigantea, Butea monosperma, Ficus religiosa, Ficus racemosa, Achyranthes aspera, Acacia catechu, Prosopis cineraria, Cynodon dactylon and Desmostachya bipinnata* were collected from Professor Jayashankar Telangana State Agricultural University of Hyderabad and Private Nurseries of Hyderabad. *Acacia catechu* was imported from Rayirath gardens, Thrissur, Kerala. Plants were placed in Plant Genetics Experimental Farm (PGEF) of Department of Genetics in a space of 09 **feet × 09 feet** with a distance of 1m between plants. *Calotropis gigantea* was placed in the middle, *Ficus racemosa* on east, *Butea monosperma* on south-east, *Acacia catechu* on south, *Cynodon dactylon* on south-west, *Prosopis cineraria* on west, *Desmostachya bipinnata,* on north-west, *Ficus religiosa* on north and *Achyranthes aspera* on north-east. This distribution is as according to the principles of Indian Traditional Knowledge and as follows –

EAST ↑

| | | |
|---|---|---|
| ***Achyranthes aspera*** | ***Ficus racemosa*** | ***Butea monosperma*** |
| ***Ficus religiosa*** | ***Calotropis gigantea*** | ***Acacia catechu*** |
| *Desmostachya bipinnata* | ***Prosopis cineraria*** | *Cynodon dactylon* |

- **Objective – 2:**

**Preparation of individual, friendly, non-friendly polyherbal and all-in-one polyherbal extracts**

Stem of *Calotropis gigantea, Acacia catechu, Achyranthes aspera, Ficus religiosa, Prosopis cineraria*, bark of *Ficus racemosa* and *Butea monosperma*, leaves of *Cynodon dactylon* and *Desmostachya bipinnata.*

**Sample preparation***:* Explants were cleaned, washed in running water then distilled water and naturally shade dried for a week and pulverized using mortar and pestle. **Extraction through Maceration**: 10 g of the homogenized explant was weighed using electronic balance (Shimadzu AUX220, Japan) and was soaked in closed sterile, flat-bottom glass container (Borosil, India) containing whole 100 ml of analytical grade Ethanol (Hymankimia, United Kingdom), a selective solvent known as menstrum. This non-flowing system of menstrum and powder was kept in contact with each other and incubates at room temperature, with vigorous shaking at regular intervals, for seven days. At the end of seventh day of maceration -after attaining equilibrium-the solution was, filtered through muslin cloth (DS Enterprises, India). The liquid extract thus expressed was known as macerate. The remained inert fibrous, insoluble and damp solid residue, called marc, was pressed (or) strained to recover as much occluded macerate as possible. The expressed and strained liquids are called miscella. The miscella was further filtered through a, Whattmann no.1 filter paper (Whatman,UK) was evaporated under reduced pressure, in vaccuo , at below 40°C using rotary evaporator (Supervac R/185, India), which yielded a gummy residue, the analyte.

**Objective – 3: Isolation and characterization of bio-constituents from each extracts**

**Metabolomic research using GCMS technology:**

GCMS technique was carried out at BA Laboratory, Hyderabad, and Telangana State, India. GCMS analysis was performed using Agilent gas chromatograph model 6890 N coupled to an Agilent 5973 N mass selective detector. The GC was fitted with a HP-5MS capillary column of 30 m X 250 µm X 0.25 µm. The temperature program was as follows: injector temperature 280 °C, initial oven temperature was at 60 °C, then 60 °C for 1 min, 40 °C / min to 170 °C for 0 min, 10 °C / min to 310 °C for 3 min. Helium was used as carrier gas at 8.2317 **psi** (pounds per square inch) pressure with flow 1 ml /min. Samples were solved in chloroform and 2 µl aliquot were injected automatically. Measurement of peak areas and data processing was carried out by GCMS solution ver.2. The spectrum of the unknown/test component was compared with the spectrum of the known components stored in the **NIST (National Institute of Standards and Technology) library**. The name and retention time, molecular weight and structure of the components of the test materials were ascertained.

## • Objective – 4.1 : Anti HIV-1 RT p66 Assay

Rotaevaporated extracts of Stem of *Calotropis gigantea, Acacia catechu, Achyranthes aspera, Ficus religiosa, Prosopis cineraria*, bark of *Ficus racemosa* and *Butea monosperma*, leaves of *Cynodon dactylon* and *Desmostachya bipinnata* were used evaluating their anti HIV-1 RT p66 potentiality using HIV-1 Reverse Transcriptase (p66) Capture ELISA kit and gp120-CD4 interaction inhibition through gp120 capture ELISA kit, from Immuno Diagnositcs (USA). Methodology for both kits was almost the same with minor differences as showed below.

**HIV-1 RT p66 capture ELISA kit**

**Flow chart**

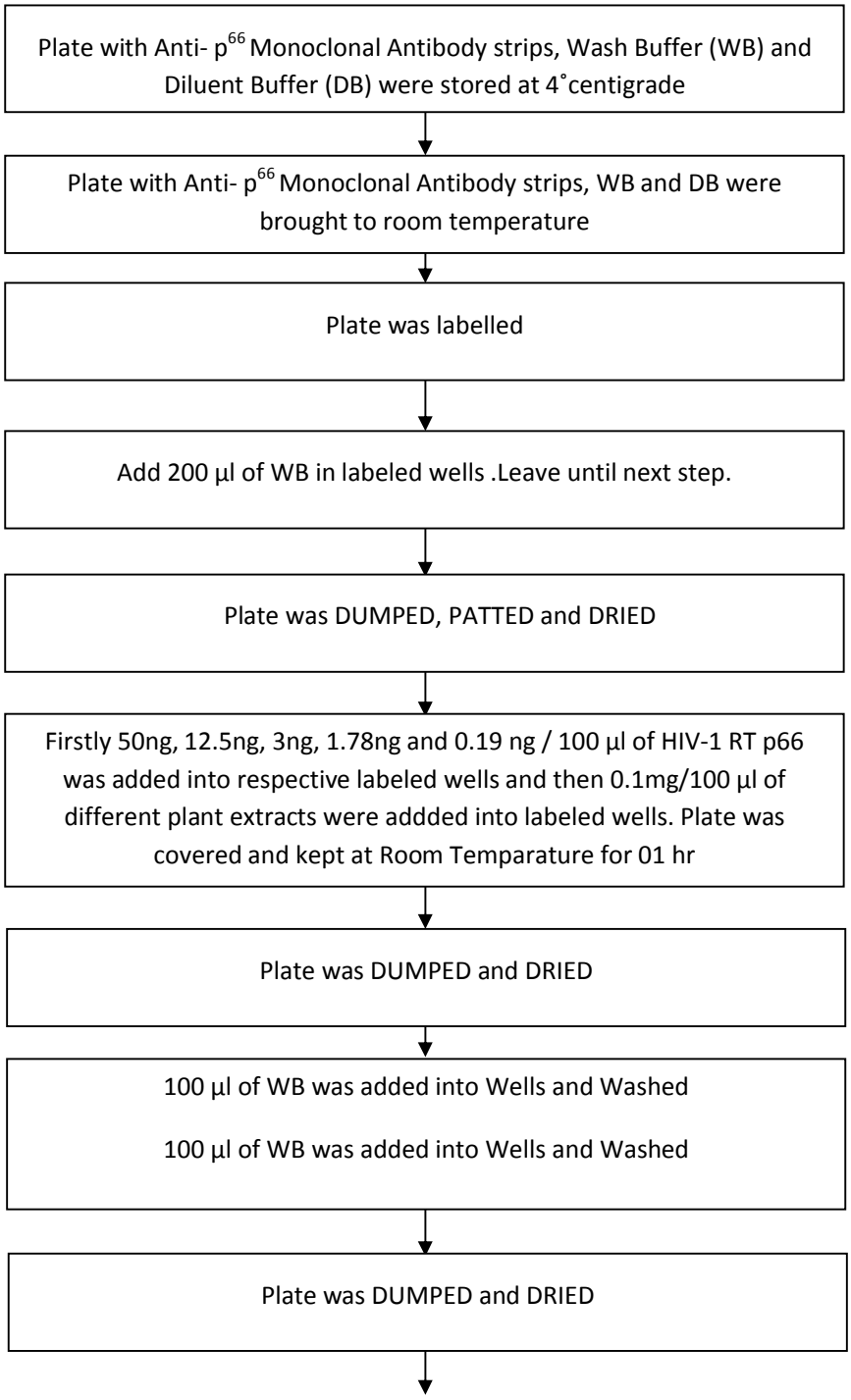
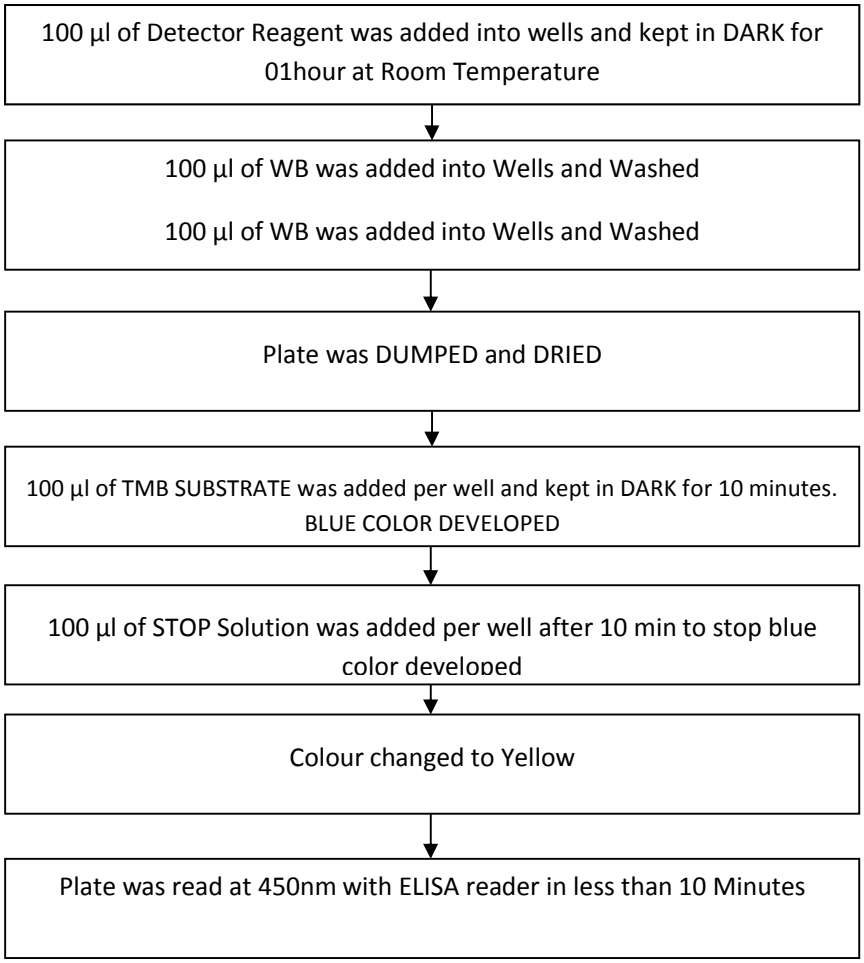


Plate with Anti- p66 Monoclonal Antibody strips, Wash Buffer (WB) and Diluent Buffer (DB) were stored at 4˚centigrade
Plate with Anti- p66 Monoclonal Antibody strips, WB and DB were brought to room temperature
Plate was labelled
Add 200 µl of WB in labeled wells .Leave until next step.
Plate was DUMPED, PATTED and DRIED
Firstly 50ng, 12.5ng, 3ng, 1.78ng and 0.19 ng / 100 µl of HIV-1 RT p66 was added into respective labeled wells and then 0.1mg/100 µl of different plant extracts were addded into labeled wells. Plate was covered and kept at Room Temparature for 01 hr
Plate was DUMPED and DRIED
100 µl of WB was added into Wells and Washed
100 µl of WB was added into Wells and Washed
Plate was DUMPED and DRIED

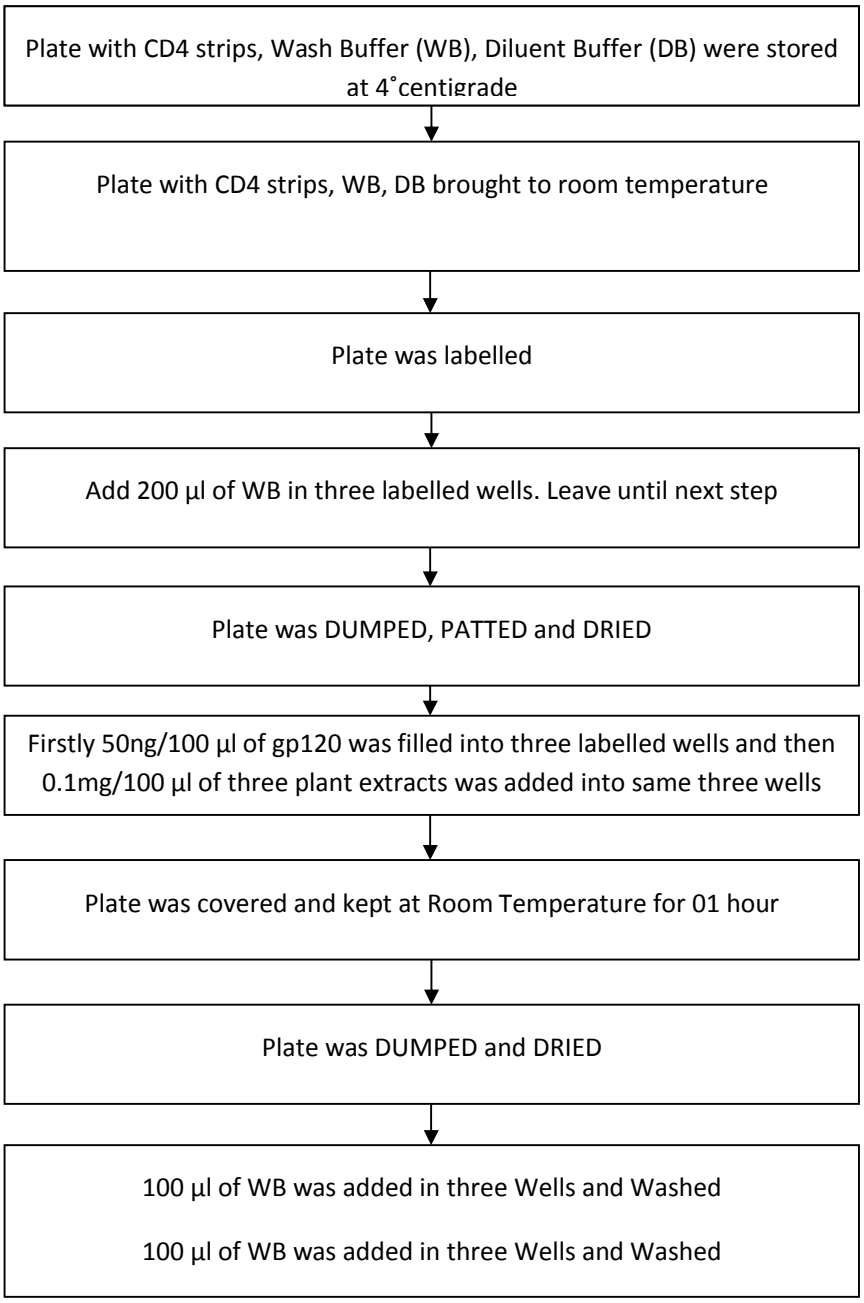


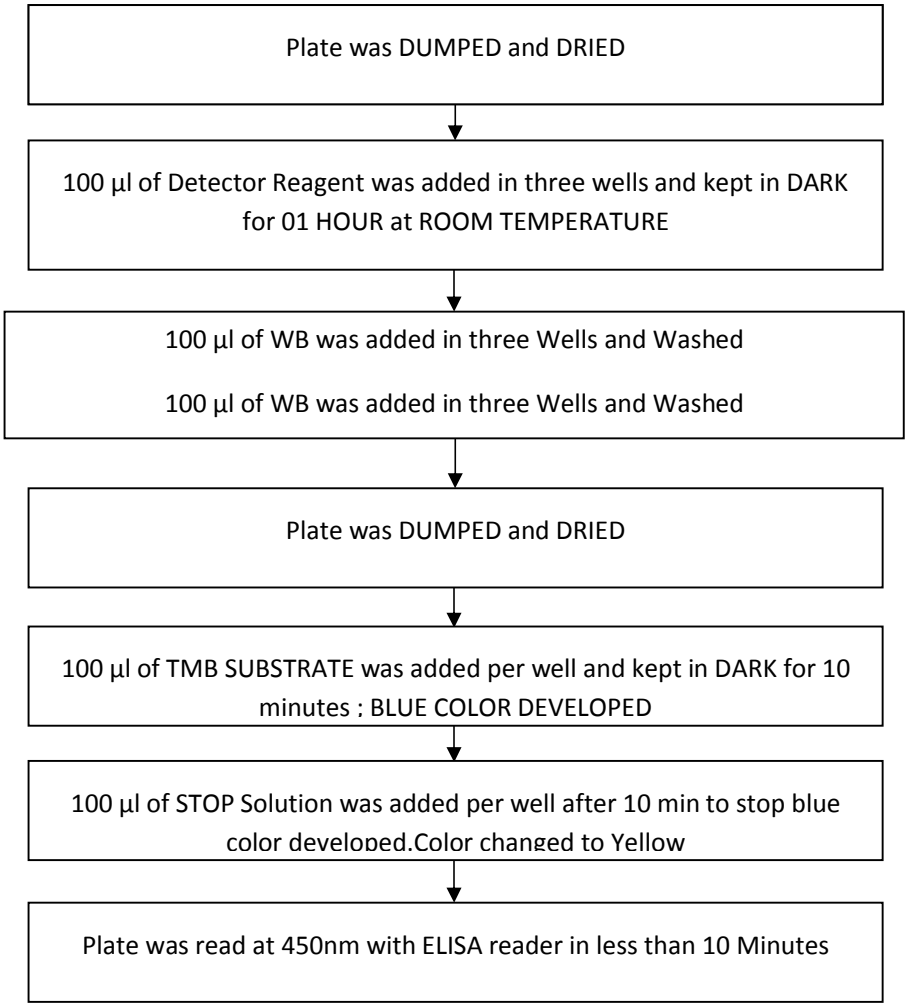

**Brief working procedure**

Wells of Plate supplied in kit, were coated with Anti-p66 Monoclonal Anti-body. HIV -1 RT $p^{66}$ antigen was also supplied in kit. Different dilutions of $p^{66}$ viz. 50 ng, 12.5 ng, 3.0 ng, 0.78 ng and 0.19 ng per 100 µl in Diluent Buffer was added into designated wells. 1mg/1ml of each plant extract or a total of 1 mg / 1 ml of different plant extract in diluents buffer was prepared.100 µl of plant extracts were added into their respective labeled wells. Zidovudine (AZT) was used as positive control. 1 mg / 1 ml of AZT was prepared using diluents buffer and 100 µl of AZT was added in respective wells. Percentage of inhibition was calculated as - % Inhibition = OD of Control – OD of plant sample / OD of control × 100

- **Objective – 4.2: gp120 Capture ELISA Kit**

Flow Chart






**Brief working procedure:**

Wells of Plate supplied in kit, were adsorbed with CD4. gp120 antigen was also supplied in kit. 50 ng / 100 µl of gp120 were added into designated wells of Plate. Three plant extracts were prepared at concentration of 1 mg / 1ml in diluent buffer. 100 µl of plant extracts were added into their respective labeled wells. Heparin was used as positive control 1 mg / 1 ml of Heparin was prepared and 100 µl of Heparin was added in respective wells. Percentage of inhibition was calculated as -%Inhibition = OD of Control – OD of plant sample/ OD of control × 1000

# RESULTS

**Objective – 1:**

Nine plants were collected and established in Plant Genetics Experimental Farm (PGEF), Department of Genetics, Osmania University (OU)

Demo / Trial Plot of 09 plants in Osmania University main campus

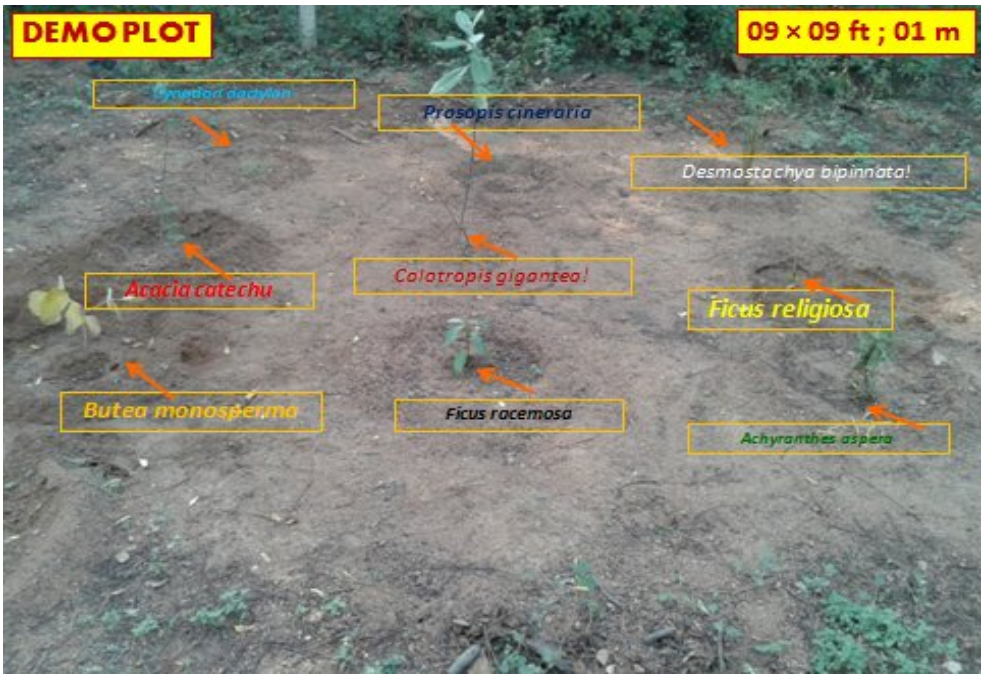



**Figure 1: Plantation in Dept. of Genetics, OU**

**Objective – 2:**

**In accordance principles of Indian Traditional Knowledge - I**ndividual, friendly, non-friendly polyherbal and all-in-one polyherbal extracts were prepared using leaves of Cynodon dactylon, Desmostachya bipinnata; stem bark of Butea monosperma and Ficus racemosa; and Stems of Calotropis gigantea, Acacia catechu, Achyranthes aspera, Ficus religiosa and Prosopis cineraria.

**Objective – 3:**

Isolation and characterization of bio-constituents from above each extracts was done.

**Overall results of GCMS analysis 09 individual plants and nine-in-one polyherbal extract:**

**Flow Chart**

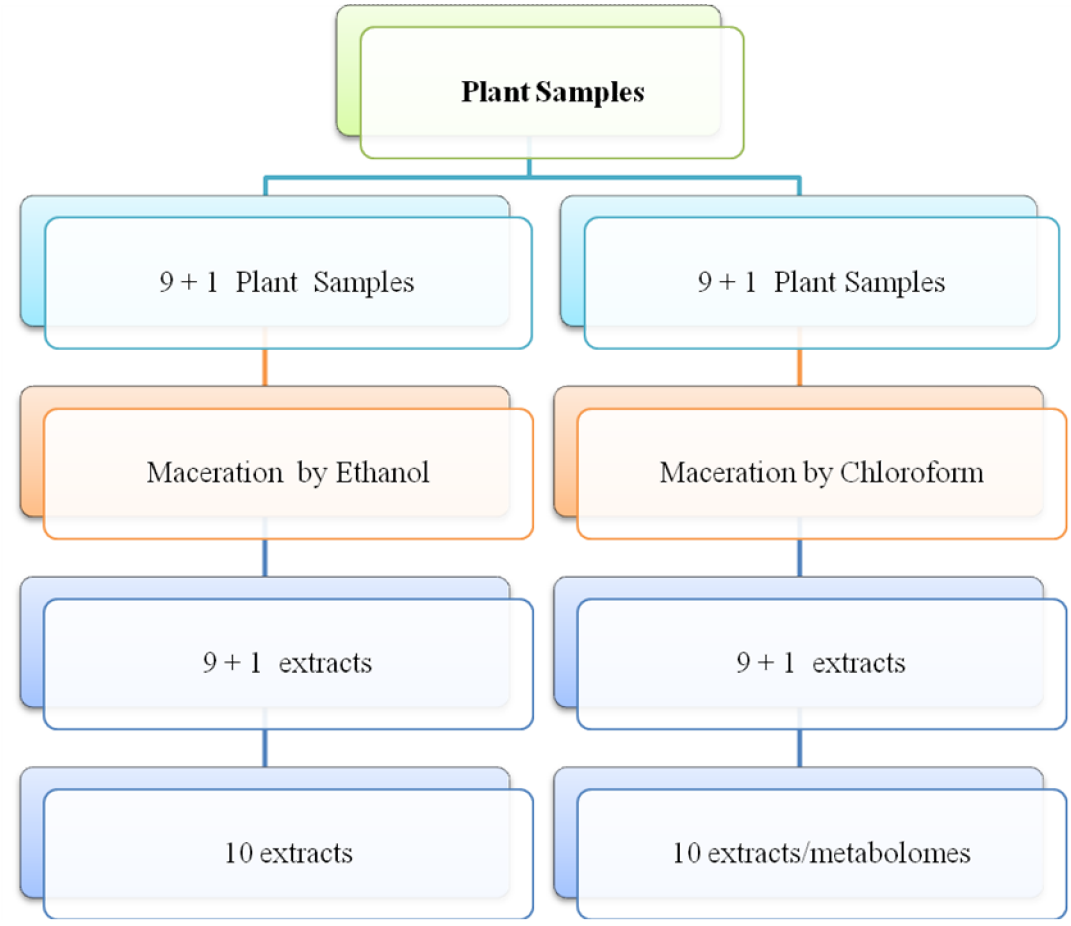



**Figure 2: Plant Samples and Extracts**

Nine Plants were extracted with cold maceration method using chloroform and ethanol along with 01 polyherbal formulation made of all nine plants. 9+1 extracts were subjected to GCMS analysis revealing compounds in 10 extracts.

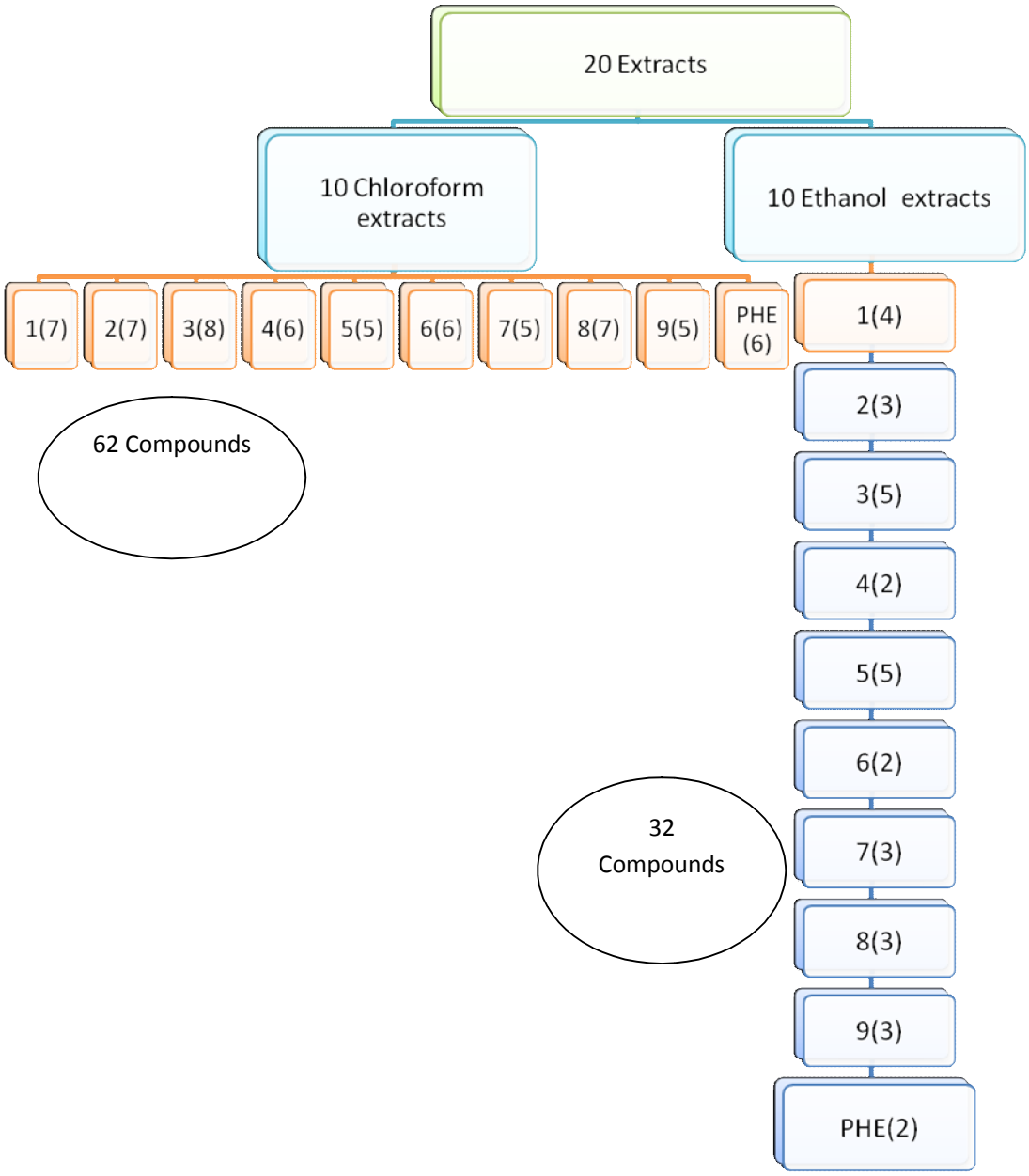



**Figure 3: Numerically designated Plant and no. of compounds identified in its ethanol and chloroform extract, as showed in parenthesis.**

***Legend:*** **Chloroform Extracts**: 1 (stem of *Calotropis gigantea*) showed 7 compounds; 2 (Bark of *Butea monosperma*) showed 7; 3(stem of *Ficus religiosa*) showed 8; 4(leaves of *Cynodon dactylon*) showed 6; 5 (stem of *Achyranthes aspera*)showed 5; 6(bark of *Ficus glomerata*) showed 6; 7(leaves of *Desmostachya bipinnata*) showed 5; 8 (stems of *Prosopis cineraria*)showed 7; 9(stem of *Acacia catechu*) showed 5 and nine-in-one polyherbal extract showed 6 compounds. Total of 62 compounds revealed through chloroform extracts.

**Ethanol Extracts:** 1 (stem of *Calotropis gigantea*) showed 4 compounds; 2 (Bark of *Butea monosperma*) showed 3; 3(stem of *Ficus religiosa*) showed 5; 4(leaves of *Cynodon dactylon*) showed 2; 5 (stem of *Achyranthes aspera*)showed 5; 6(bark of *Ficus glomerata*) showed 2; 7(leaves of *Desmostachya bipinnata*) showed 3; 8 (stems of *Prosopis cineraria*)showed 3; 9(stem of *Acacia catechu*) showed 3 and Polyherbal Extract (PHE) showed 2 compounds. Total of 32 compounds revealed through ethanol extracts.

**Conclusion:** 10 Chloroform extracts showed total of 62 compounds and 10 ethanol extracts reported 32 compounds (Fig.22).

## 3.1 GCMS Chromatogram of Chloroform extracts of Nine Plants and Nine-in-One Polyherbal extract (Fig 4-13)

### 3.1.1 *Calotropis gigantea* (L.) (Stems)

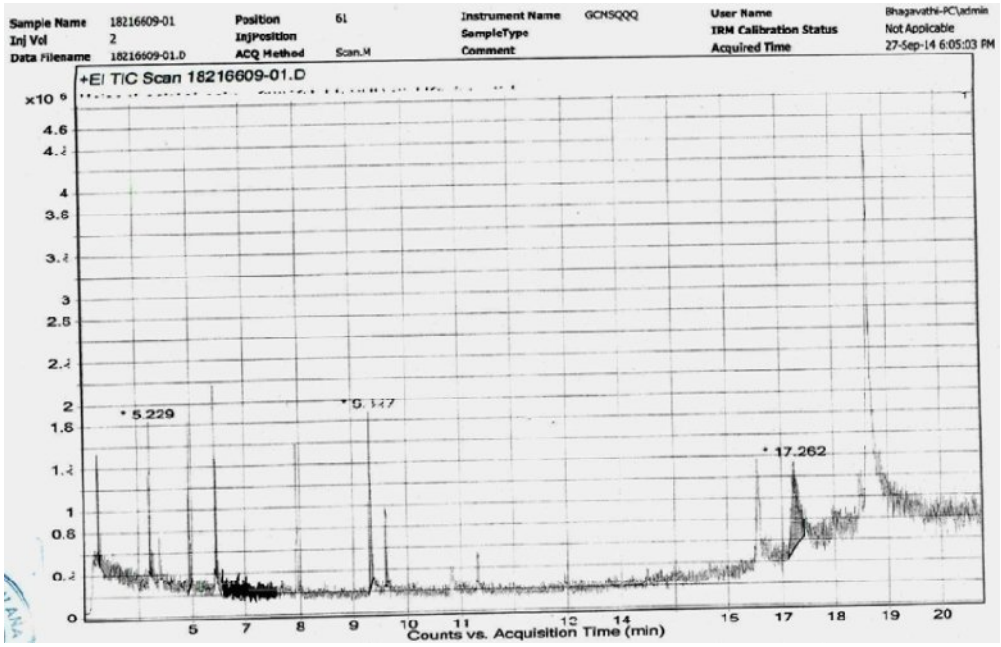



**Figure 4: GCMS Chromatogram of Chloroform extract of stems of *Calotropis gigantea* (L.)**

### 3.1.2 *Butea monosperma* (L.) (Stem Bark)

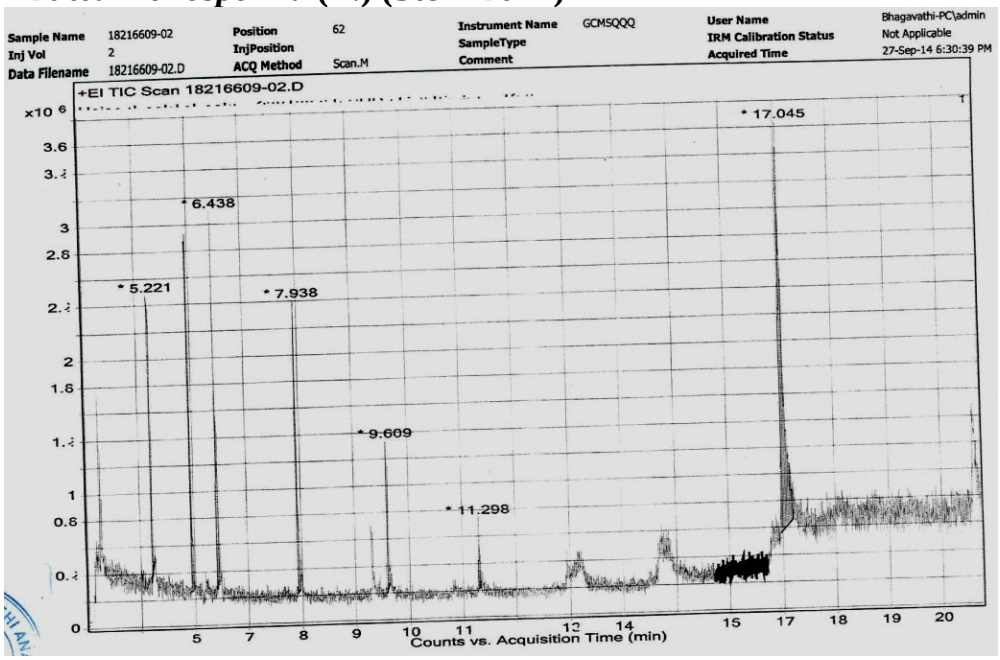



**Figure 5: GCMS Chromatogram of Chloroform extract of stem bark of *Butea monosperma* (L.)**

### 3.1.3 *Ficus religiosa* (L.) (Stems)

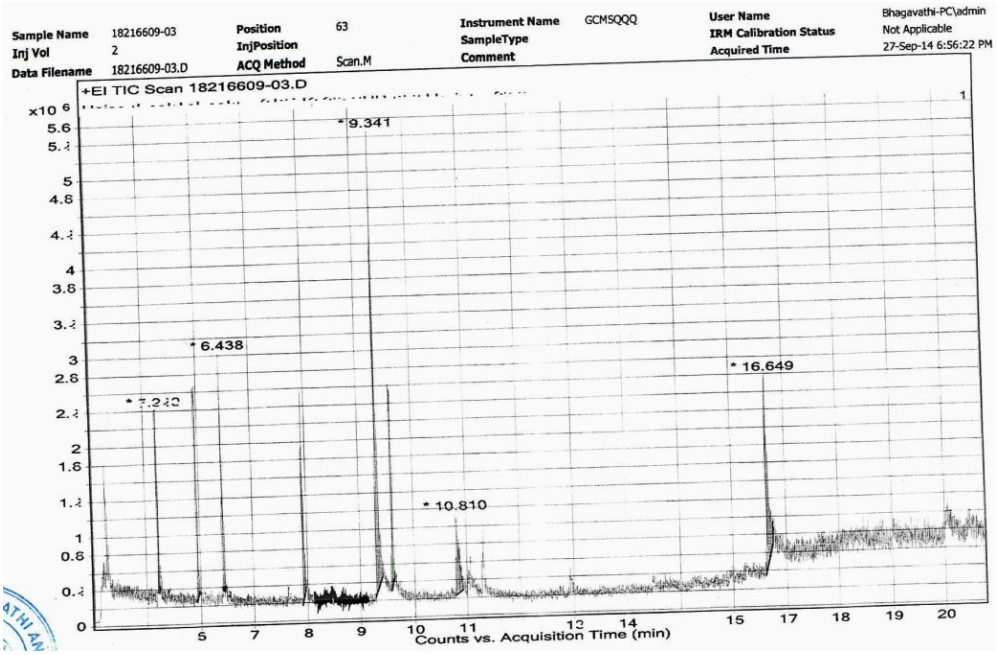



**Figure 6: GCMS Chromatogram of Chloroform extract of stems of *Ficus religiosa* (L.)**

### 3.1.4 *Cynodon dactylon* (L.) pers

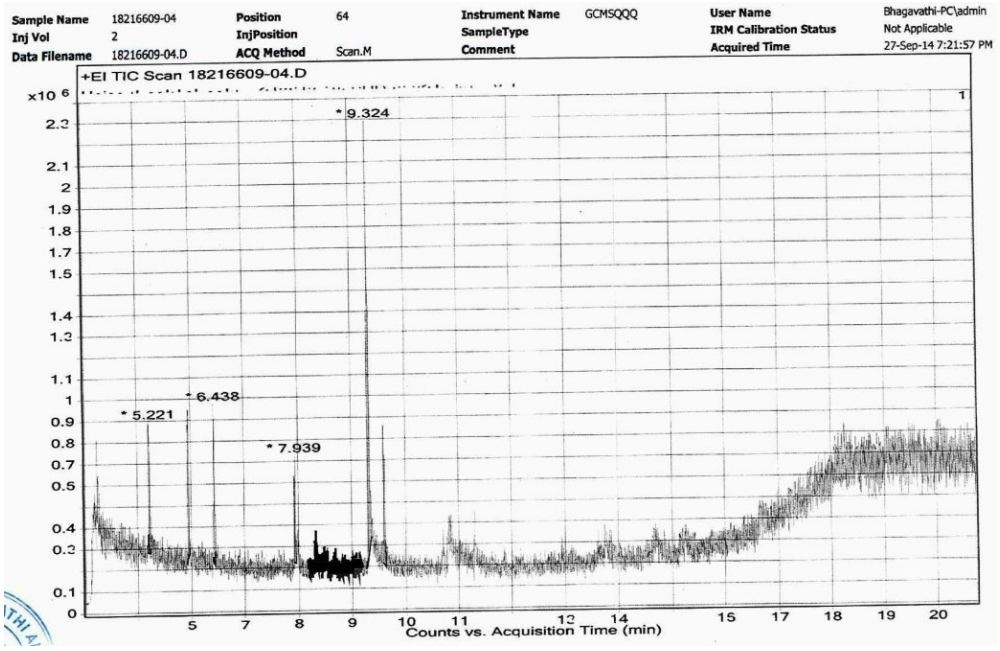



**Figure7: GCMS Chromatogram of Chloroform extract of leaves of *Cynodon dactylon* (L.) pers**

### 3.1.5 *Achyranthes apera* (L.) (Stems)

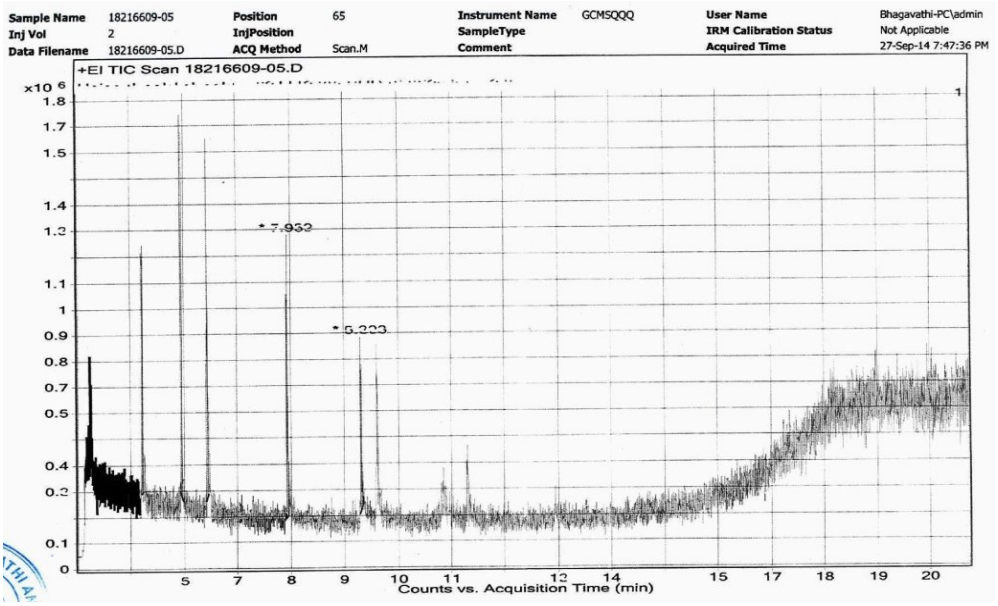



**Figure 8 : GCMS Chromatogram of Chloroform extract of stems of *Achyranthes apera*(L.)**

### 3.1.6 *Ficus racemosa* (L.) (Stem Bark)

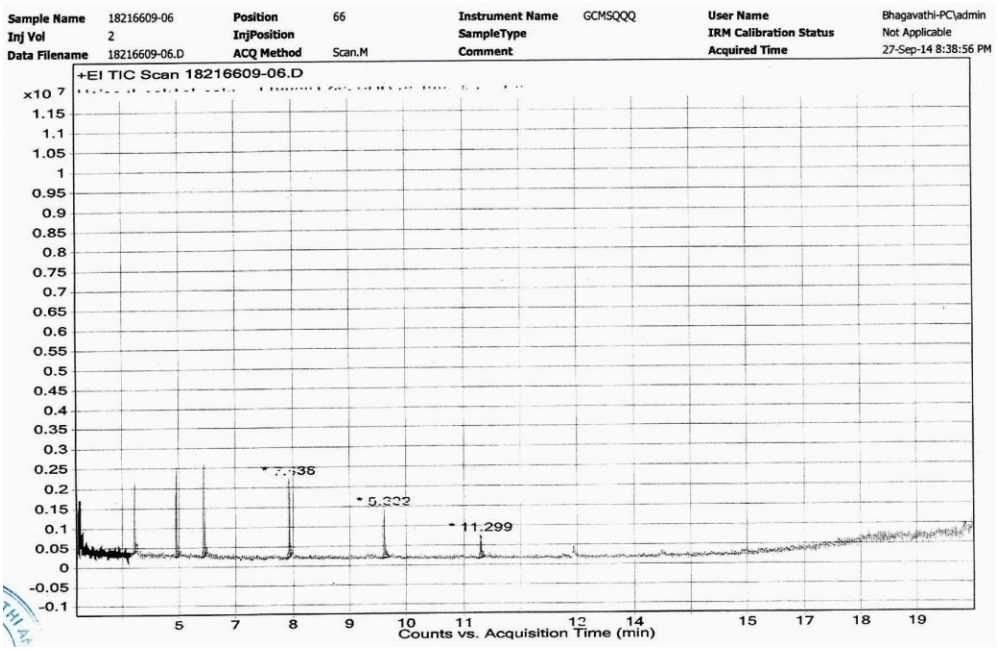



**Figure 9: GCMS Chromatogram of Chloroform extract of stem bark of *Ficus racemosa* (L.)**

### 3.1.7 *Desmostachya bipinnata* (stapf.) (Leaves)

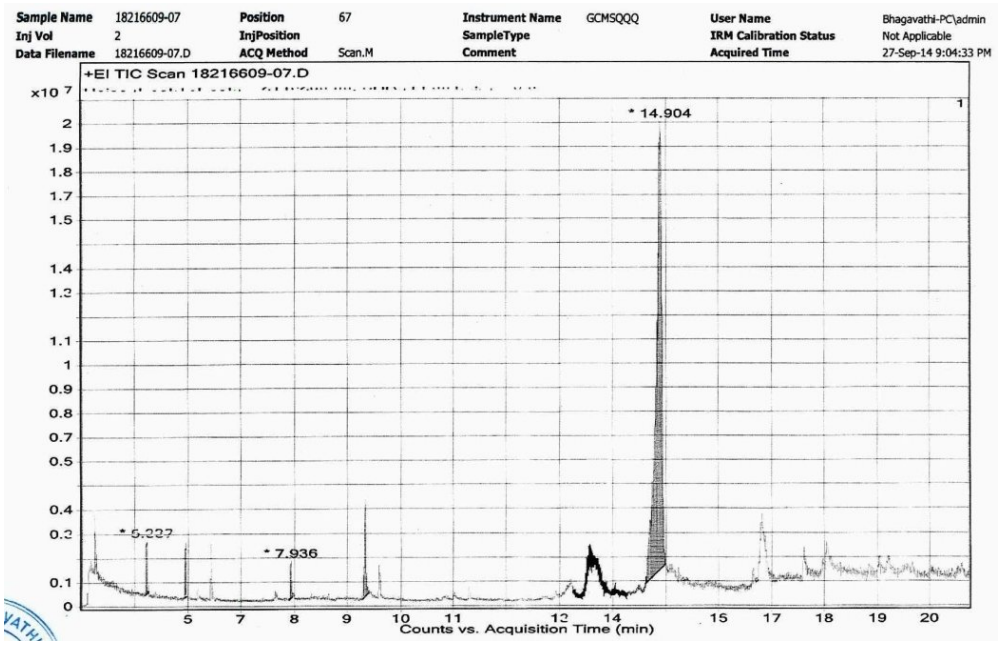



**Figure 10: GCMS Chromatogram of Chloroform extract of leaves of *Desmostachya bipinnata* (stapf.)**

### 3.1.8 *Prosopis cineraria* (L.) (Stem)

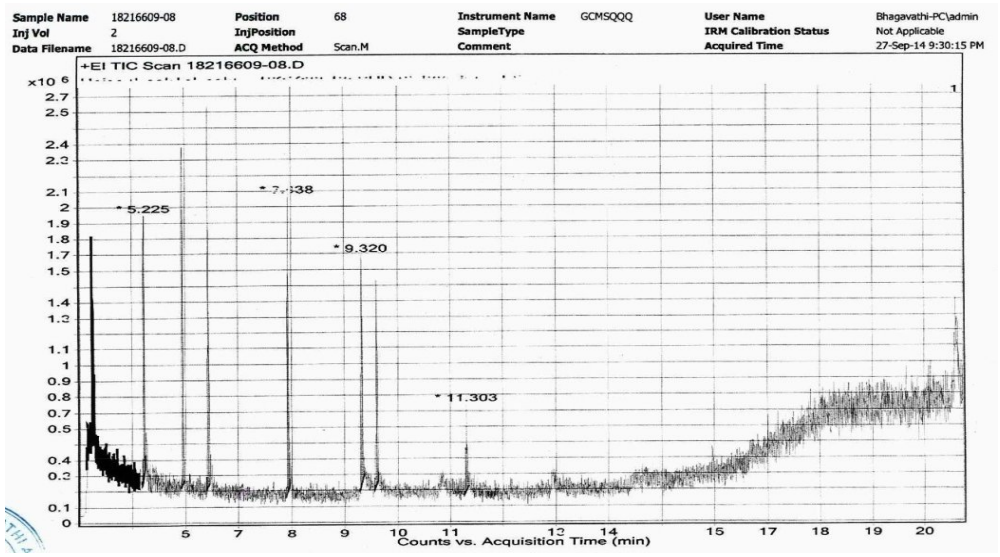



**Figure 11: GCMS Chromatogram of Chloroform extract of stems of *Prosopis cineraria* (L.)**

### 3.1.9 Acacia *catechu* Rottler (Willd.) (Stem)

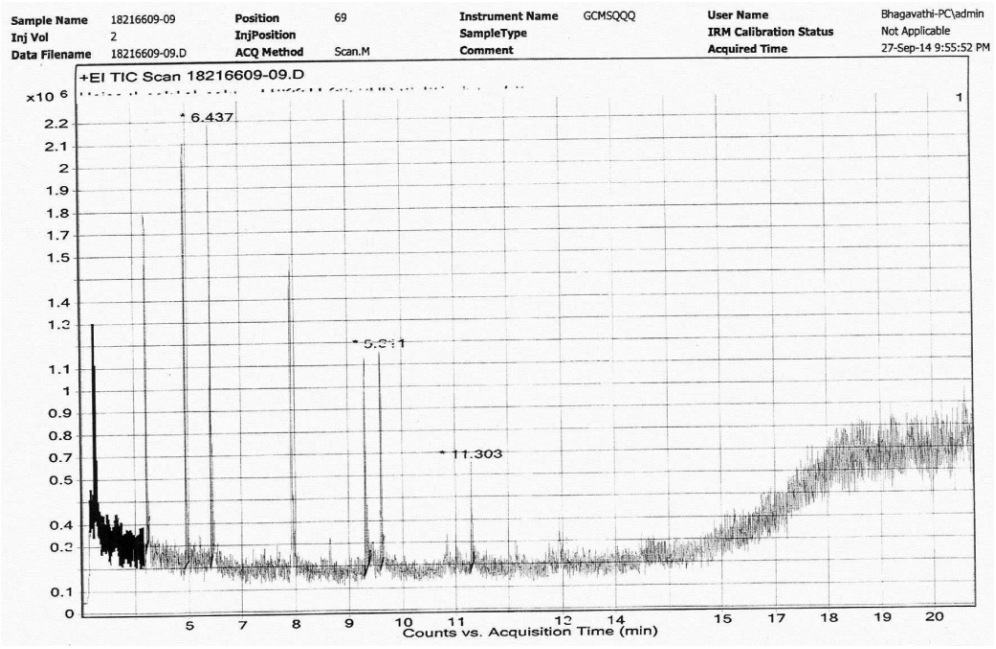



**Figure 12: GCMS Chromatogram of Chloroform extract of stems of *Acacia catechu* Rottler (Willd.)**

### 3.1.10 Polyherbal powder

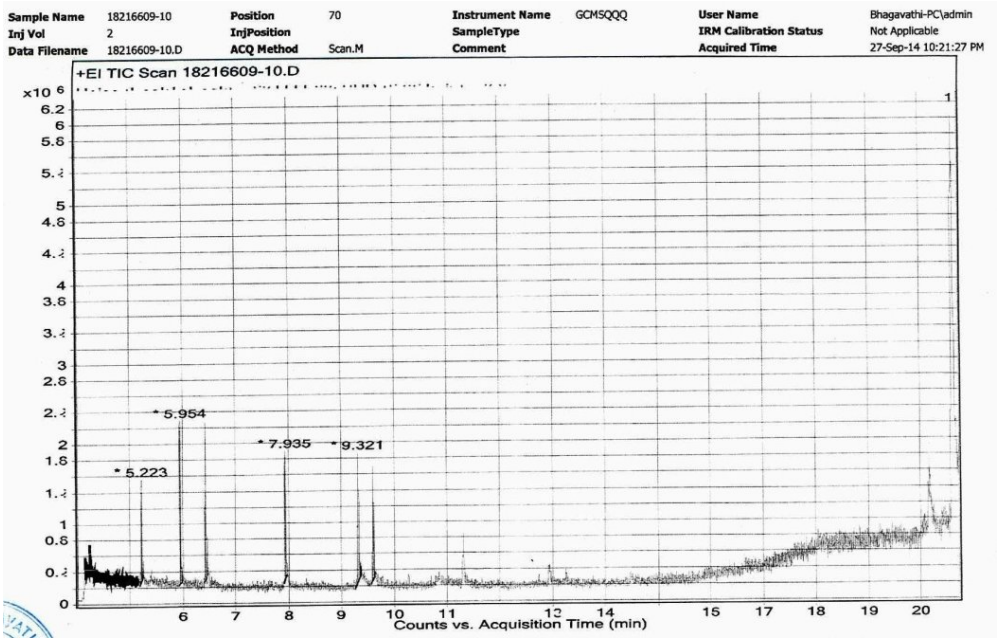



**Figure 13: GCMS Chromatogram of Chloroform extract of Polyherbal powder**

Overview of GCMS analysis of nine explants using chloroform was as in table 1 -

Table 1: Plant, Plant location, Plant processing, Compounds

| Name of the Plant | Explant | Solvent | Method of Extraction | No. of Compounds | Name of the Identified Compounds |
|---|---|---|---|---|---|
| *Calotropis gigantea* Linn. | Stem | Chloroform | Maceration | 7 | 1. 1-Dodecanethiol<br>2. 4-Octadecenal<br>3. 6-(Diethylamino)benzofuran-3(2H)-one<br>4. 1-Eicosanol<br>5. 1-Nitro-beta—d-arabinofuranose,tetraacetate<br>6. 9-Hexadecenoic acid, 9-octadecenyl ester, (Z,Z)-<br>7. 2-Thiazolamine, 4-(3,4 – dimethoxyphenyl)-5-methyl- |
| *Butea monosperma* | Bark | Chloroform | Maceration | 7 | 1. 1-Undecanol<br>2. 6-(Diethylamino)benzofuran-3(2H)-one<br>3. 1-Hexadecanol<br>4. Behenic alcohol<br>5. 4-Octadecenal<br>6. 9-Hexadecenoic acid, 9-octadecenyl ester, (Z,Z)- (or) Oleyl palmitoleate<br>7. Betulin |
| *Ficus religiosa* L. | Stem | Chloroform | Maceration | 8 | 1. 1-Hexadecanol<br>2. 6-(Diethylamino)benzofuran-3(2H)-one<br>3. Dodecyl fluoroacetate /Fluroacetic acid, dodecyl ester<br>4. 1-Eicosanol<br>5. n-Hexadecanoic acid / Palmitic acid<br>6. 4-Octadecenal<br>7. 9-Cycloheptadecen-1-ol<br>8. Octadecane, 3-ethyl-5-(2-ethylbutyl)- |
| *Cynodon dactylon* | Leaves | Chloroform | Maceration | 6 | 1. Cyclohexane,1'-dodecylidenebis(4-methyl-<br>2. 6-(Diethylamino)benzofuran-3(2H)-one<br>3. 9-Octadecene,1,1'-(1,2-ethanediylbis(oxy))bis-, (z,z)-<br>4. 9-Hexadecenoic acid, 9-octadecenyl ester, (Z,Z)- (or) Oleyl palmitoleate<br>5. 1-Nitro-beta—d-arabinofuranose,Tetraacetate<br>6. 9-Hexadecenoic acid |
| *Achyranthes aspera* | Stems | Chloroform | Maceration | 5 | 1. 1-Hexadecanol<br>2. 6-(Diethylamino)benzofuran-3(2H)-one<br>3. 4-Octadecenal<br>4. 9-Hexadecenoic acid, 9-octadecenyl ester, (Z,Z)- (or) |

| Name of the Plant | Explant | Solvent | Method of Extraction | No. of Compounds | Name of the Identified Compounds |
|---|---|---|---|---|---|
| | | | | | Oleyl palmitoleate<br>5. Decanoic acid, 1, 1a, 1b, 4, 4b,5,7a,7b,8,9-decahydro-4a,7b-dihydroxy-3-(hydroxymethyl)-1,1,6,8-tetramethyl-5-oxo-9Ah-cyclopropa(3,4)benz(1,2-e)azulene-9,9a-diyl ester,(1a R-(1aα,1bβ,4aβ,7aα,7bα,8α,9β,9aα))- |
| *Ficus racemosa* | Bark | Chloroform | Maceration | 6 | 1. 1-Hexadecanol<br>2. 6-(Diethylamino)benzofuran-3(2H)-one<br>3. Acetic acid, chloro-, octadecyl ester,<br>4. 4-Octadecenal<br>5. Pentafluoropropionic acid, octadecyl ester<br>6. 9-Hexadecenoic acid, 9-octadecenyl ester, (Z,Z)- (or) Oleyl palmitoleate |
| *Desmostachya bipinnata* | Leaves | Chloroform | Maceration | 5 | 1. 1-Hexadecanol<br>2. 6-(Diethylamino)benzofuran-3(2H)-one<br>3. E-7-Octadecene<br>4. 1-Nitro-beta—d-arabinofuranose,tetraacetate<br>5. Lup-20(29)-en-3-ol, acetate,(3β)- (or)<br>6. 3-acetyllupeol |
| *Prosopis cineraria* | Stems | Chloroform | Maceration | 7 | 1. 1-Nonadecene<br>2. 6-(Diethylamino)benzofuran-3(2H)-one<br>3. Cyclotridecane<br>4. 4-Octadecenal<br>5. 1, 2, 5-Azoniadiboratole, 2, 2, 3, 4, 5-pentaethyl, 2, 5-dihydro-1-trimethylsilyl<br>6. 5-Octadecenal<br>7. 9-Hexadecenoic acid, 9-octadecenyl ester, (Z,Z)- (or) Oleyl palmitoleate |
| *Acacia catechu* | Stem | Chloroform | Maceration | 5 | 1. 2-Heptadecenal<br>2. 6-(Diethylamino)benzofuran-3(2H)-one<br>3. Acetic acid, chloro-, octadecyl ester,<br>4. Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester (or) 1,2-Dipalmitin<br>5. 9-Hexadecenoic acid, 9-octadecenyl ester, (Z,Z)- (or) Oleyl palmitoleate |
| Nine-in-one polyherbal | | Chloroform | Maceration | 6 | 1. 4-Octadecenal<br>2. 6-(Diethylamino)benzofuran-3(2H)-one |

| Name of the Plant | Explant | Solvent | Method of Extraction | No. of Compounds | Name of the Identified Compounds |
|---|---|---|---|---|---|
| (NIO) extract | | | | | 3. 1-Eicosanol<br>4. 10-Heneicosene (c,t)<br>5. 1-Nitro-beta—d-arabinofuranose,tetraacetate<br>6. 9-Hexadecenoic acid, 9-octadecenyl ester, (Z,Z)- (or) Oleyl palmitoleate |
| **10**<br><br>**(Total No's)** | | | | **62**<br><br>**(Total M)** | |

***Legend:*** 3= *Ficus religiosa*; 1= *Calotropis gigantea*; 2= *Butea monosperma*; 5= *Achyranthes aspera*; 6= *Ficus racemosa*; 9= *Acacia catechu*; 8= *Prosopis cineraria*; 4= *Cynodon dactylon*; 7= *Desmostachya bipinnata*

## ETHANOL EXTRACTS

**3.2 GCMS Chromatogram of Ethanol extracts of Nine Plants and Nine-in-One Polyherbal extract (Fig.14-23)**

### 3.2.1 *Calotropis gigantea* (L.)(Stems)

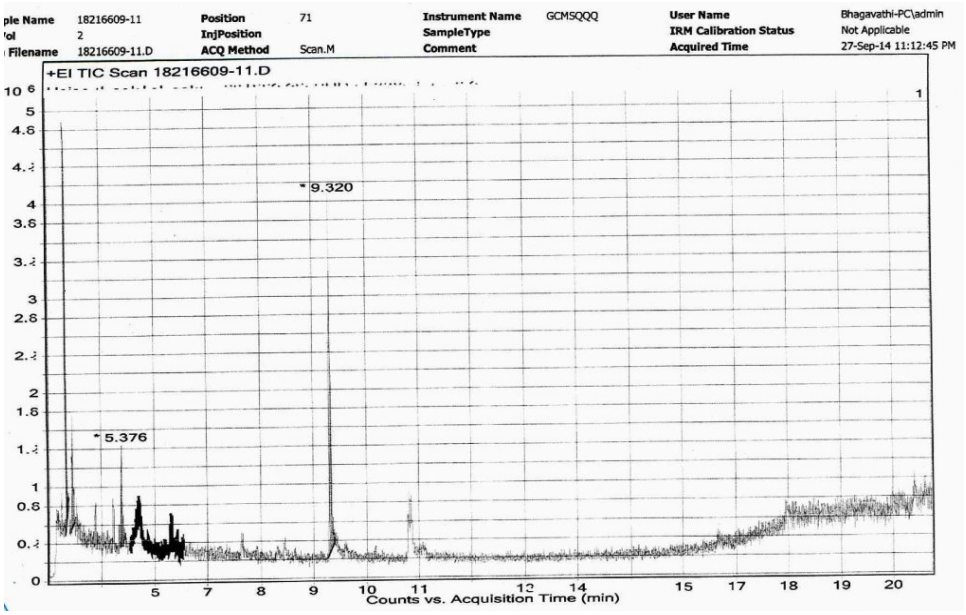



**Figure 14: GCMS Chromatogram of ethanol extract of stems of *Calotropis gigantea* (L.)**

### 3.2.2 *Butea monosperma* (L.) (Stem Bark)

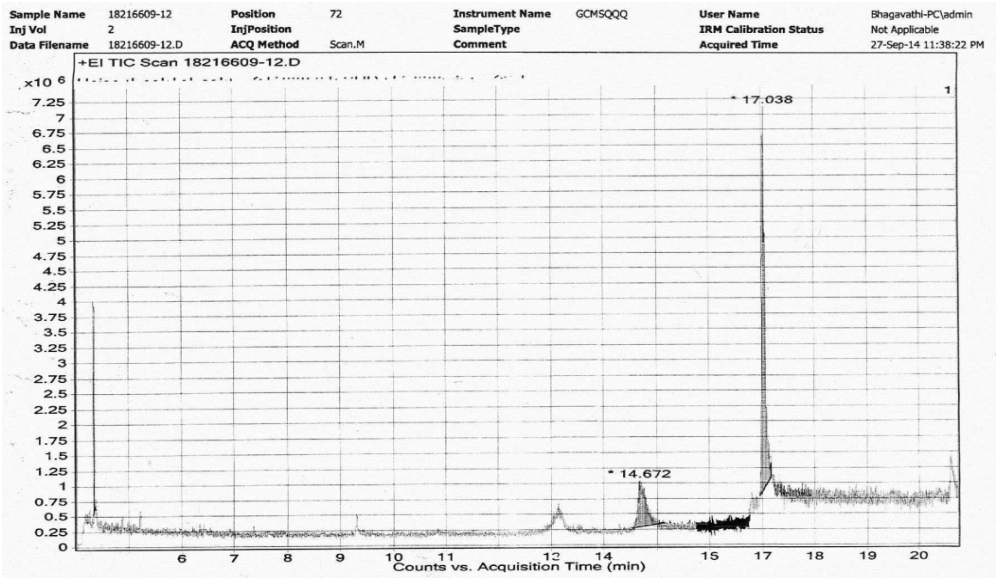



**Figure 15: GCMS Chromatogram of ethanol extract of stem bark of *Butea monosperma* (L.)**

### 3.2.3 *Ficus religiosa* (L.) (Stem)

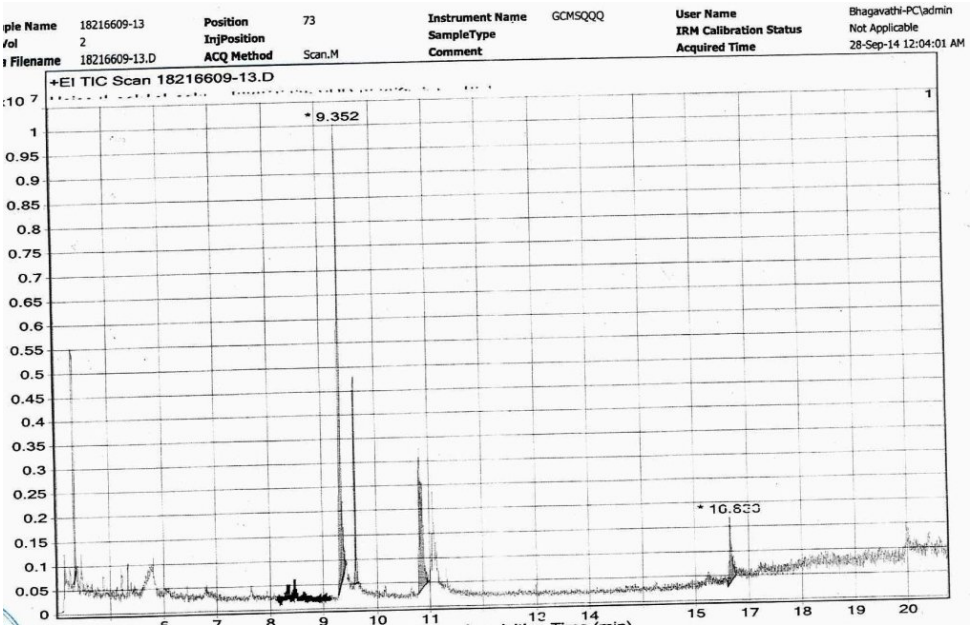



**Figure 16: GCMS Chromatogram of ethanol extract of stems of *Ficus religiosa* (L.)**

### 3.2.4 *Cynodon dactylon (*L.) pers (Leaves)

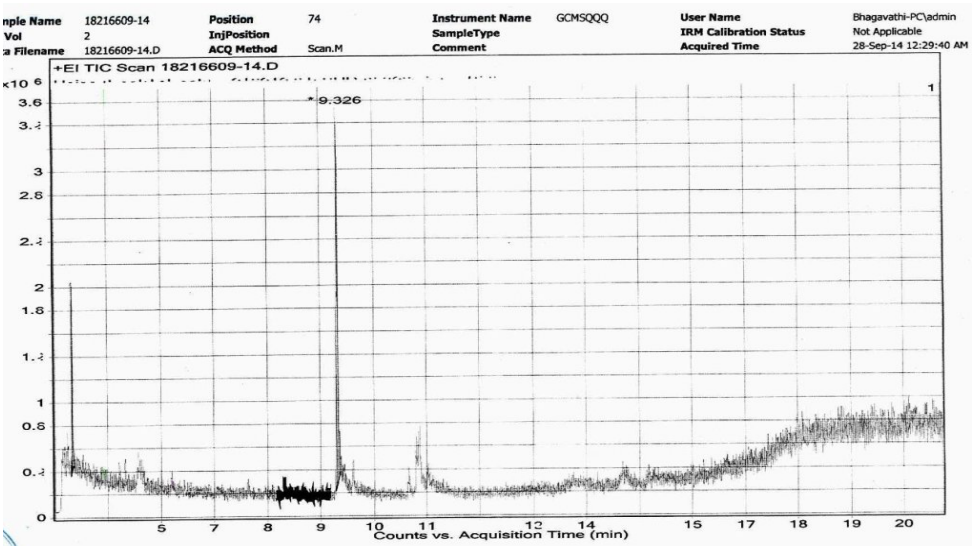



**Figure 17: GCMS Chromatogram of ethanol extract of leaves of *Cynodon dactylon (*L.) pers**

### 3.2.5 *Achyranthes aspera* (L.) (Stems)

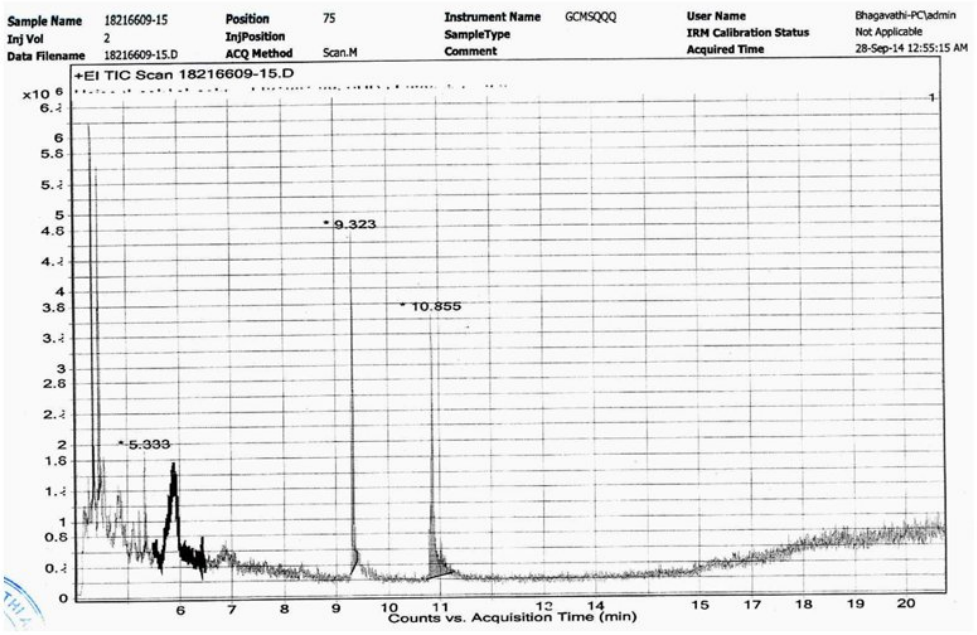



**Figure 18: GCMS Chromatogram of ethanol extract of stems of *Achyranthes aspera* (L.)**

### 3.2.6 Ficus *racemosa* (L.) (Stem Bark)

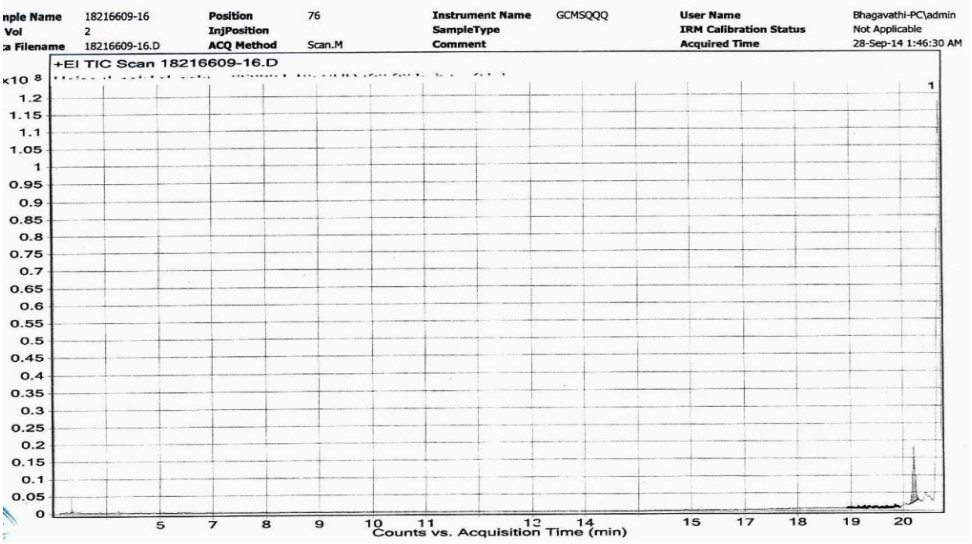



**Figure 19: GCMS Chromatogram of ethanol extract of stem bark of *Ficus racemosa* (L.)**

### 3.2.7 *Desmostachya bipinnata* (L.) *S*tapf (Leaves)

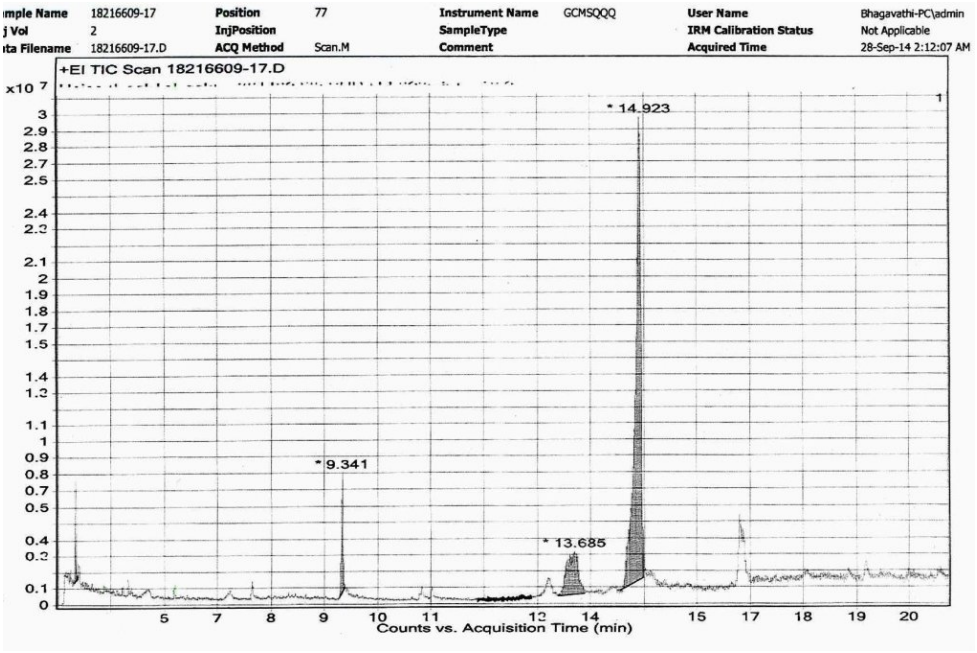



**Figure 20: GCMS Chromatogram of ethanol extract of leaves of *Desmostachya bipinnata* (L.) Stapf**

### 3.2.8 *Prosopis cineraria* (L.) (Stems)

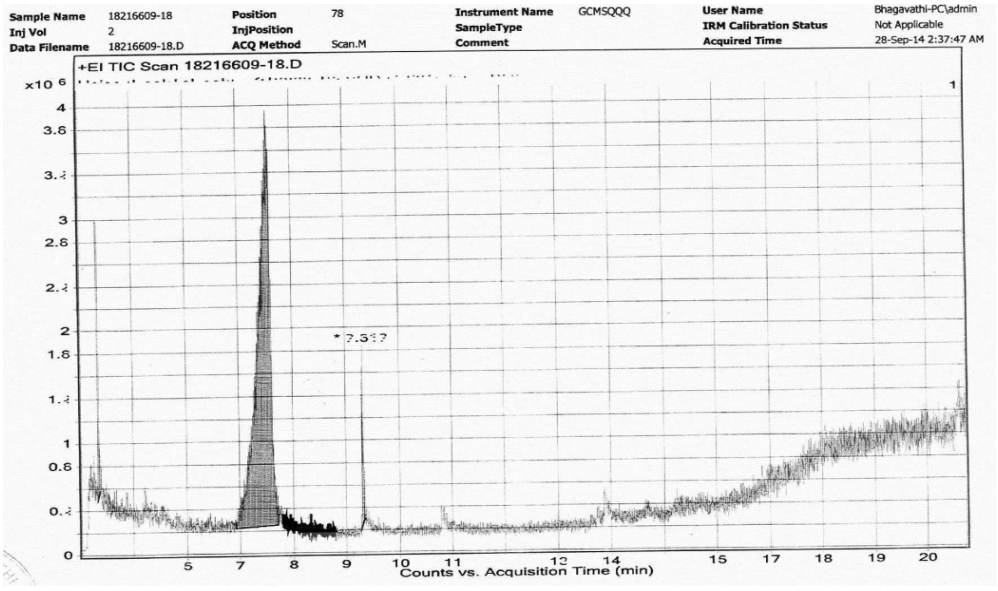



**Figure 21: GCMS Chromatogram of ethanol extract of stems of *Prosopis cineraria* (L.)**

### 3.2.9 *Acacia catechu* Rottler (Willd.) (Stems)

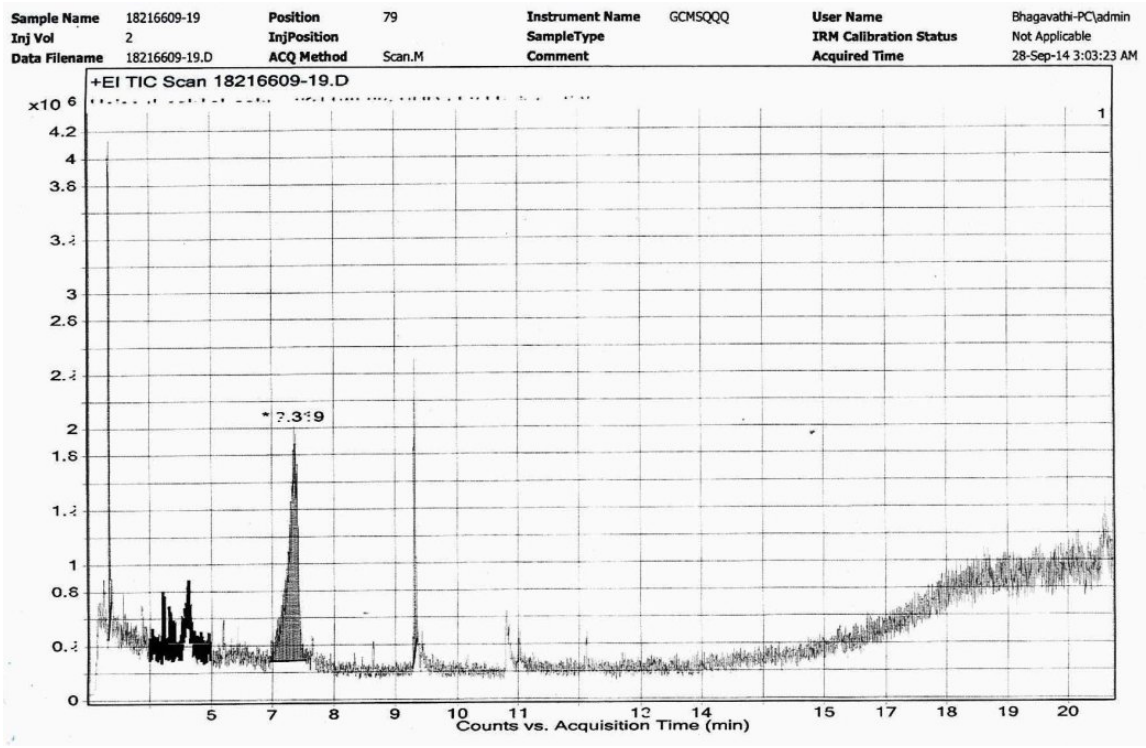



**Figure 22: GCMS Chromatogram of ethanol extract of stems of *Acacia catechu* Rottler (Willd.)**

### 3.2.10 Polyherbal powder

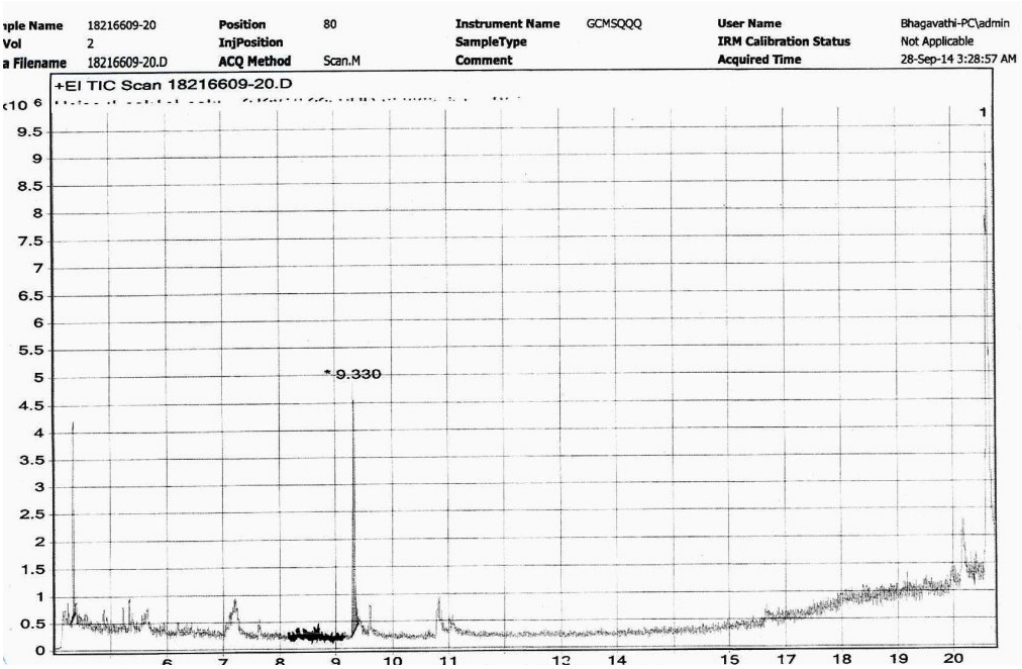



**Figure 23: GCMS Chromatogram of ethanol extract of Polyherbal pow**

Overview of GCMS analysis of nine explants using ethanol was as in table 2

Table 2: Plant, Plant location, Plant processing, Compounds

| Name of the Plant | Explant | Solvent | Method of Extraction | No. of Compounds | Name of the Compounds |
|---|---|---|---|---|---|
| *Calotropis gigantea* (L). | Stem | Ethanol | Maceration | 04 | 1. Naphthalene<br>2. 6-Acetyl-1,7-dimethy-8-propyldecahydro-1,6-napthyridine<br>3. Trans-3-Oxooctahydro-4a(2H)-naphthalene carboxylic acid<br>4. 4-Octadecenal |
| *Butea monosperma* (L). | Bark | Ethanol | Maceration | 03 | 1. Naphthalene<br>2. Haematoporphyrin<br>3. Betulin |
| *Ficus religiosa* (L). | Stem | Ethanol | Maceration | 05 | 1. Naphthalene<br>2. 1-Nitro-beta—d-arabinofuranose,tetraacetate<br>3. Nonadecanoic acid, ethyl ester<br>4. Z,Z-4,16-Octadecadien-1-ol acetate<br>5. 1-chlorooctadecane |
| *Cynodon dactylon* (L).pers | Leaves | Ethanol | Maceration | 02 | 1. Naphthalene<br>2. 1-Nitro-beta—d-arabinofuranose,tetraacetate |
| *Achyranthes aspera*(L). | Stems | Ethanol | Maceration | 05 | 1. Naphthalene<br>2. 2,3-Bis(1-methylallyl) pyrrolidine<br>3. Trimethylene borate<br>4. 9-Hexadecenoic acid, eicosyl ester, (Z)-<br>5. Oleyl palmitoleate |
| *Ficus racemosa*(L). | Bark | Ethanol | Maceration | 02 | 1. Naphthalene<br>2. Beta.-Amyrin |
| *Desmostachya bipinnata* (stapf). | Leaves | Ethanol | Maceration | 03 | 1. Naphthalene<br>2. 1-Nitro-beta—d-arabinofuranose,tetraacetate<br>3. Fenretinide |
| *Prosopis cineraria*(L). | Stems | Ethanol | Maceration | 03 | 1. Naphthalene<br>2. 3,6,9,12,15,18,21,24-octaoxabicyclo(12.10.0) tetracosane<br>3. 1,2-Dipalmitin |
| *Acacia Catechu* Rottler (Willd). | Stem | Ethanol | Maceration | 03 | 1. Naphthalene<br>2. 1-Isopropyl-4-methyl-acridone<br>3. Oleic acid |
| Nine-in-one polyherbal extract | Mix of above explants | Ethanol | Maceration | 02 | 1. Naphthalenen<br>2. Bicyclo (3.3.1) nonane-2, 4-dione, 9, 9-dimethoxy- |
| **10 (Total no. samples)** | | | | **32 (Total compounds)** | |

According to Indian Traditional Knowledge, compatible plants were *Ficus religiosa, Acacia catechu, Calotropis gigantea and Butea monosperma*; and non-compatible plants were *Ficus religiosa* and *Ficus racemosa.*

**3.3 GCMS analysis of Tetraherbal extract made of traditional compatible plants i.e. *Calotropis gigantea, Butea monosperma, Ficus religiosa and Acacia catechu*** (Table 9)

**3.3 (a) GCMS chromatogram and compounds in chloroform extract of Tetraherbal extract**

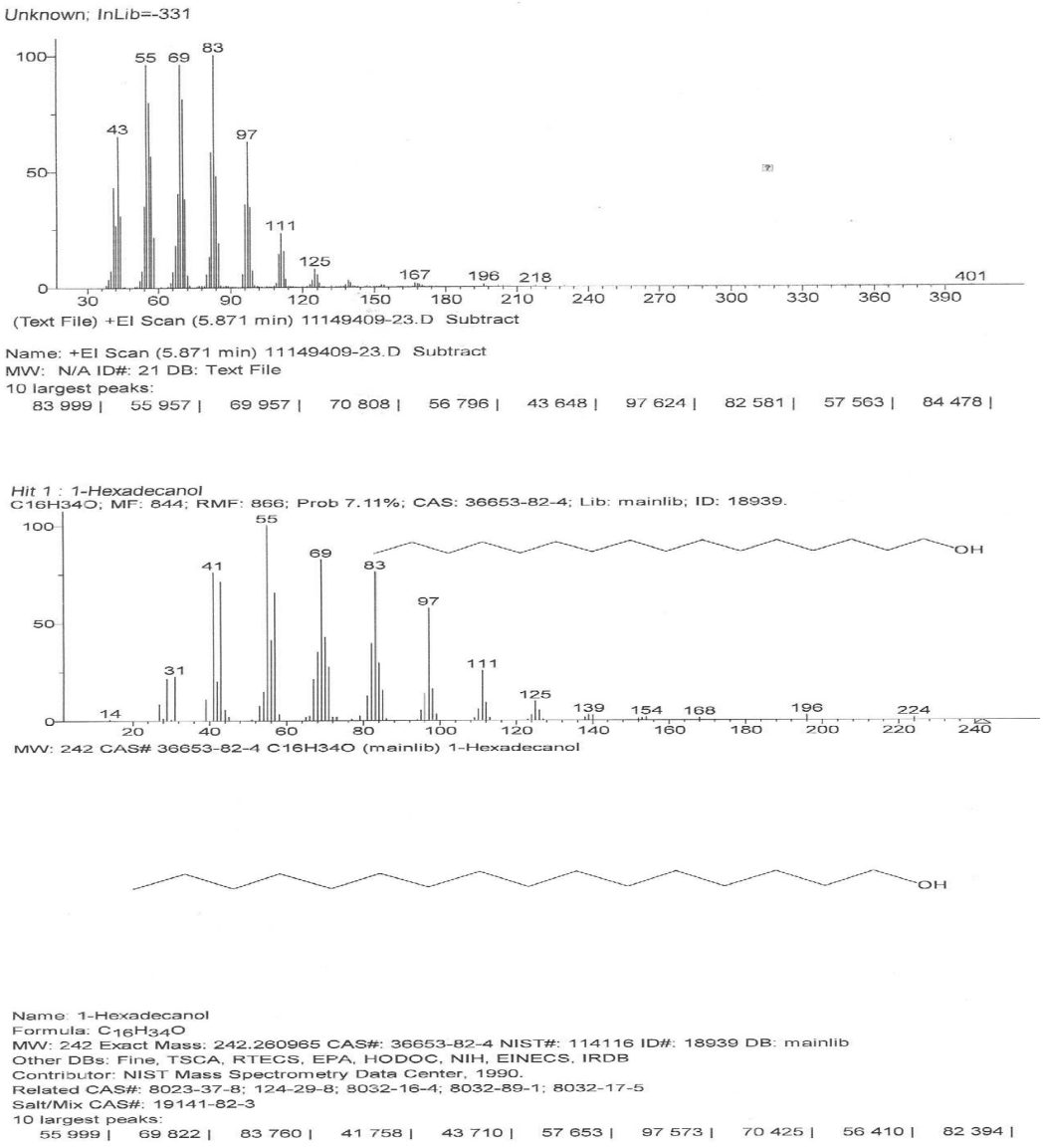
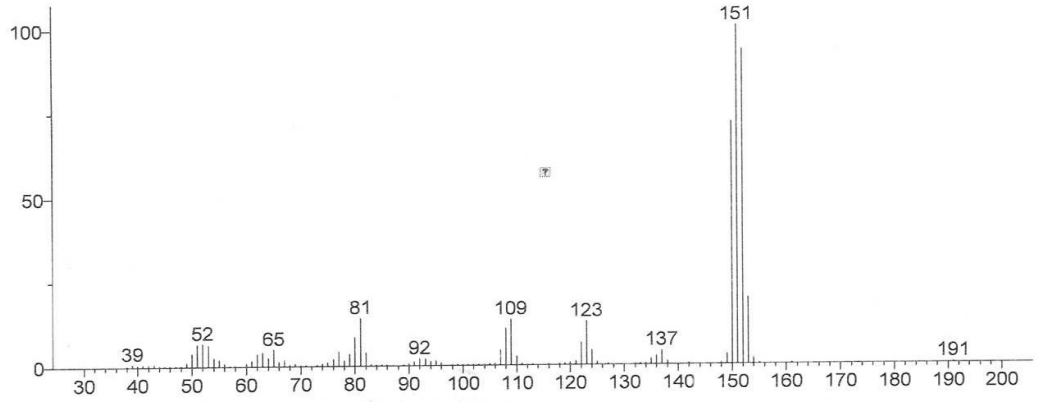



**Figure 23a: 1- Hexadecanol**

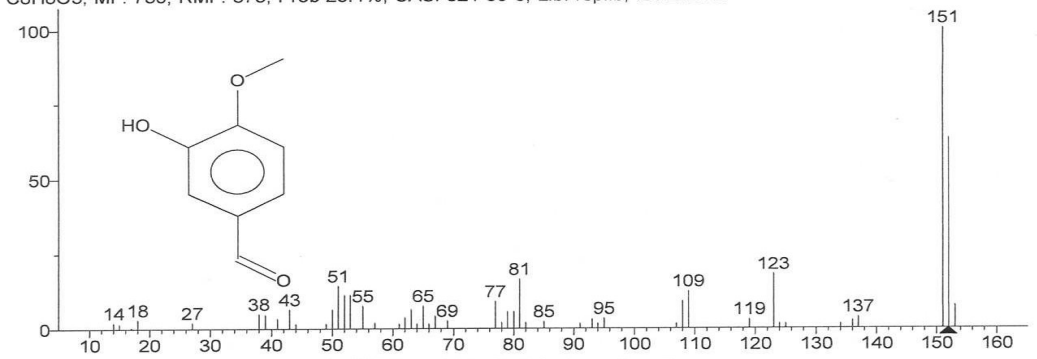
Unknown; InLib=-752



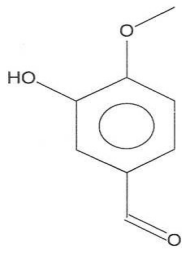

(Text File) +EI Scan (6.041 min) 11149409-23.D Subtract

Name: +EI Scan (6.041 min) 11149409-23.D Subtract
MW: N/A ID#: 22 DB: Text File
10 largest peaks:
151 999 | 152 929 | 150 715 | 153 195 | 81 139 | 109 133 | 123 127 | 108 106 | 80 83 | 52 66 |

Hit 1 : Benzaldehyde, 3-hydroxy-4-methoxy-
C8H8O3; MF: 730; RMF: 873; Prob 23.1%; CAS: 621-59-0; Lib: replib; ID: 22030.



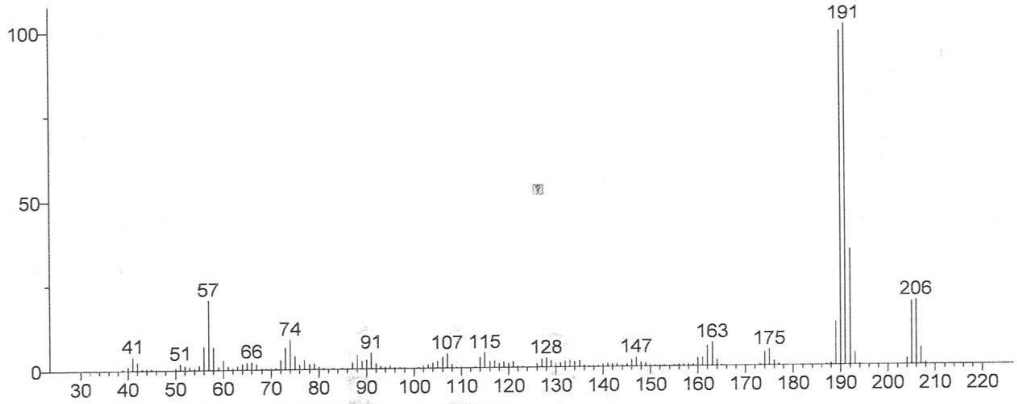

MW: 152 CAS# 621-59-0 C8H8O3 (replib) Benzaldehyde, 3-hydroxy-4-methoxy-




Name: Benzaldehyde, 3-hydroxy-4-methoxy-
Formula: $C_8H_8O_3$
MW: 152 Exact Mass: 152.047344 CAS#: 621-59-0 NIST#: 4908 ID#: 22030 DB: replib
Other DBs: Fine, TSCA, RTECS, HODOC, NIH, EINECS
10 largest peaks:
151 999 | 152 630 | 123 178 | 81 163 | 51 141 | 109 122 | 52 111 | 53 111 | 77 89 | 108 89 |

**Figure 24: Benzaldehyde,3-Hydroxy-4-methoxy**

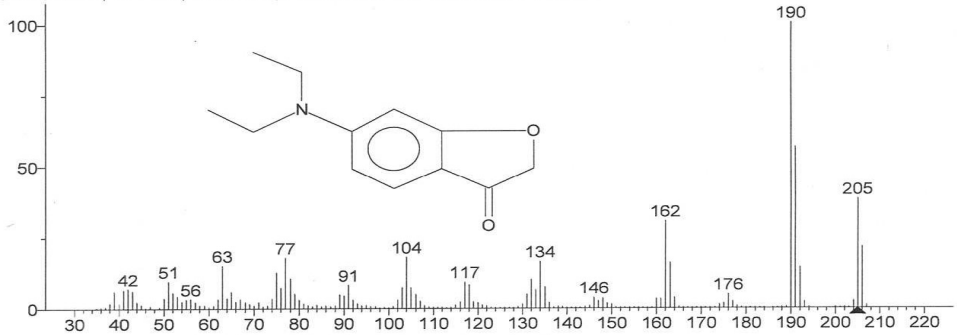
Unknown; InLib=-744



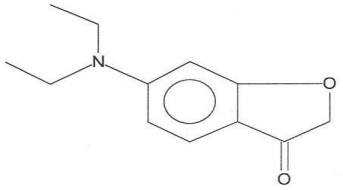

(Text File) +EI Scan (6.684 min) 11149409-23.D Subtract

Name: +EI Scan (6.684 min) 11149409-23.D Subtract
MW: N/A ID#: 23 DB: Text File
10 largest peaks:
191 999 | 190 980 | 192 342 | 57 203 | 206 187 | 205 183 | 189 124 | 74 86 | 56 67 | 163 67 |

*Hit* 1 : 6-(Diethylamino)benzofuran-3(2H)-one
C12H15NO2; MF: 734; RMF: 734; Prob 51.1%; Lib: mainlib; ID: 156101.



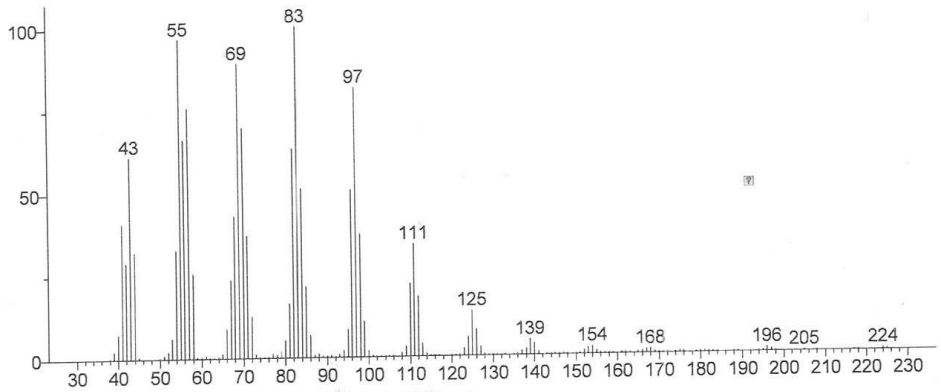

MW: 205 C12H15NO2 (mainlib) 6-(Diethylamino)benzofuran-3(2H)-one




Name: 6-(Diethylamino)benzofuran-3(2H)-one
Formula: $C_{12}H_{15}NO_2$
MW: 205 Exact Mass: 205.110279 NIST#: 210066 ID#: 156101 DB: mainlib
Contributor: Chemical Concepts
10 largest peaks:
190 999 | 191 563 | 205 379 | 162 303 | 206 213 | 104 179 | 77 177 | 134 162 | 163 158 | 63 150 |

**Figure 25 6-(Diethylamino)benzofuran-3(2H)-one**

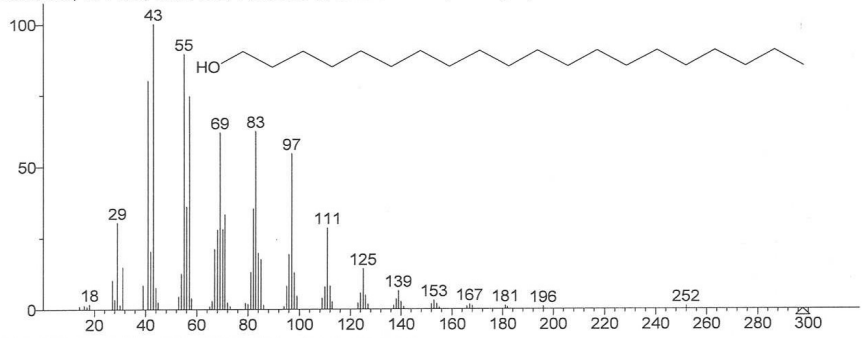
Unknown; InLib=-454




(Text File) +EI Scan (7.190 min) 11149409-23.D Subtract

Name: +EI Scan (7.190 min) 11149409-23.D Subtract
MW: N/A ID#: 24 DB: Text File
10 largest peaks:
83 999 | 55 965 | 69 890 | 97 813 | 57 756 | 70 694 | 56 660 | 82 631 | 43 609 | 84 509 |

Hit 1 : 1-Eicosanol
C20H42O; MF: 845; RMF: 862; Prob 6.65%; CAS: 629-96-9; Lib: replib; ID: 2006.

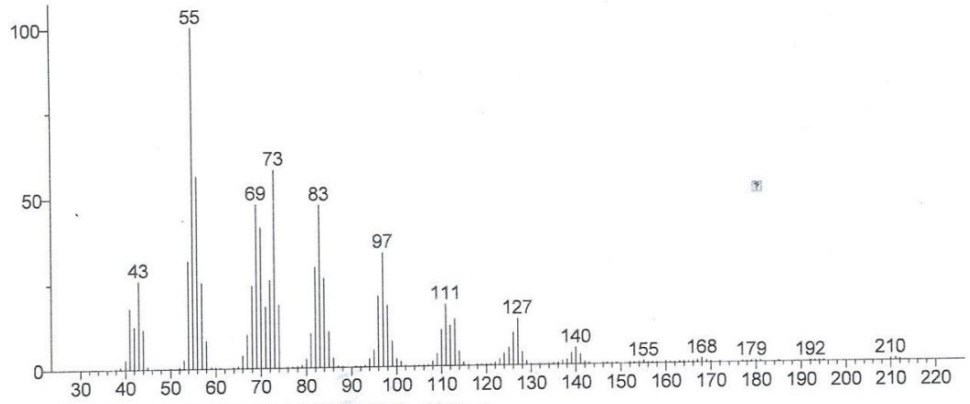



MW: 298 CAS# 629-96-9 C20H42O (replib) 1-Eicosanol


Name: 1-Eicosanol
Formula: $C_{20}H_{42}O$
MW: 298 Exact Mass: 298.323566 CAS#: 629-96-9 NIST#: 113075 ID#: 2006 DB: replib
Other DBs: Fine, TSCA, HODOC, NIH, EINECS, IRDB
Contributor: NIST Mass Spectrometry Data Center, 1990.
10 largest peaks:
43 999 | 55 893 | 41 799 | 57 743 | 83 620 | 69 617 | 97 542 | 56 358 | 82 350 | 71 330 |

**Figure 26 1-Eicosanol**

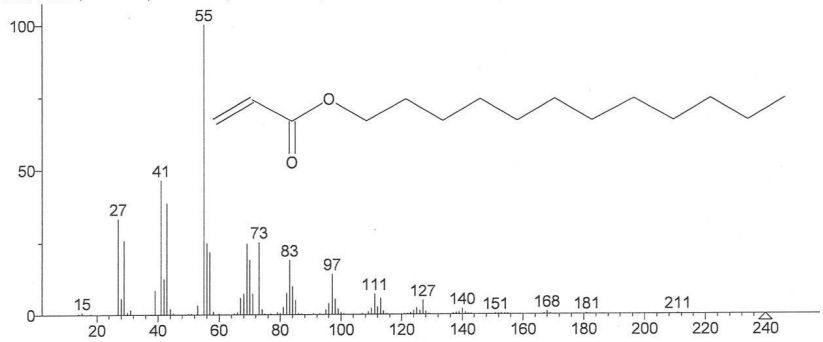
Unknown; InLib=-464



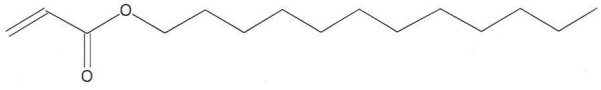

(Text File) +EI Scan (7.975 min) 11149409-23.D Subtract

Name: +EI Scan (7.975 min) 11149409-23.D Subtract
MW: N/A ID#: 25 DB: Text File
10 largest peaks:
55 999 | 73 578 | 56 560 | 69 478 | 83 473 | 70 411 | 97 333 | 54 315 | 82 293 | 84 261 |

Hit 1 : Dodecyl acrylate
C15H28O2; MF: 791; RMF: 829; Prob 15.3%; CAS: 2156-97-0; Lib: replib; ID: 4392.




MW: 240 CAS# 2156-97-0 C15H28O2 (replib) Dodecyl acrylate




Name: Dodecyl acrylate
Formula: $C_{15}H_{28}O_2$
MW: 240 Exact Mass: 240.20893 CAS#: 2156-97-0 NIST#: 343283 ID#: 4392 DB: replib
Other DBs: Fine, TSCA, HODOC, NIH, EINECS
Contributor: NIST Mass Spectrometry Data Center
Related CAS#: 199685-42-2
10 largest peaks:
55 999 | 41 461 | 43 384 | 27 330 | 29 257 | 73 250 | 56 247 | 69 245 | 57 216 | 70 189 |

**Figure 27 Dodecyl acrylate**

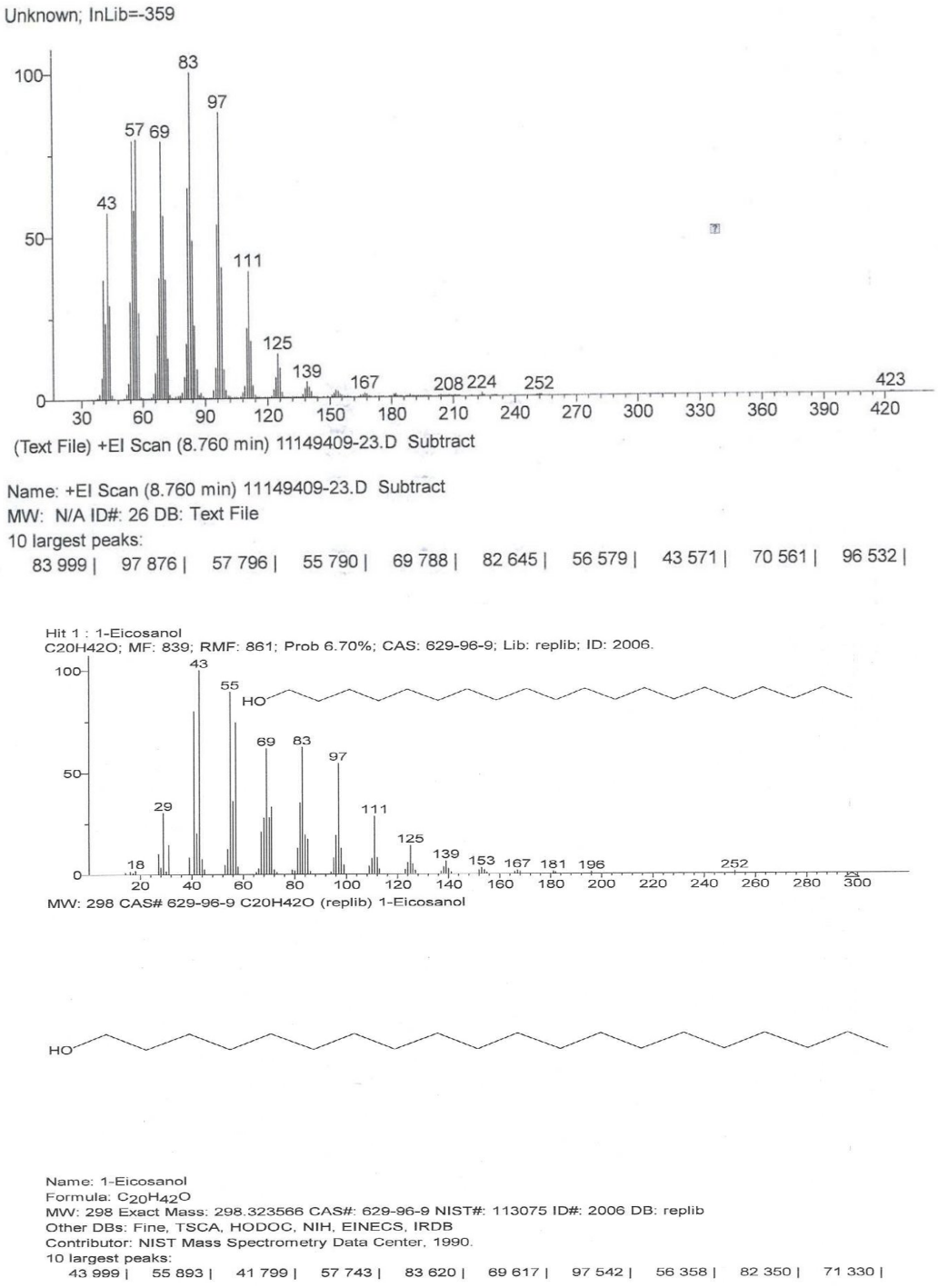



**Figure 28** 1-Eicosanol

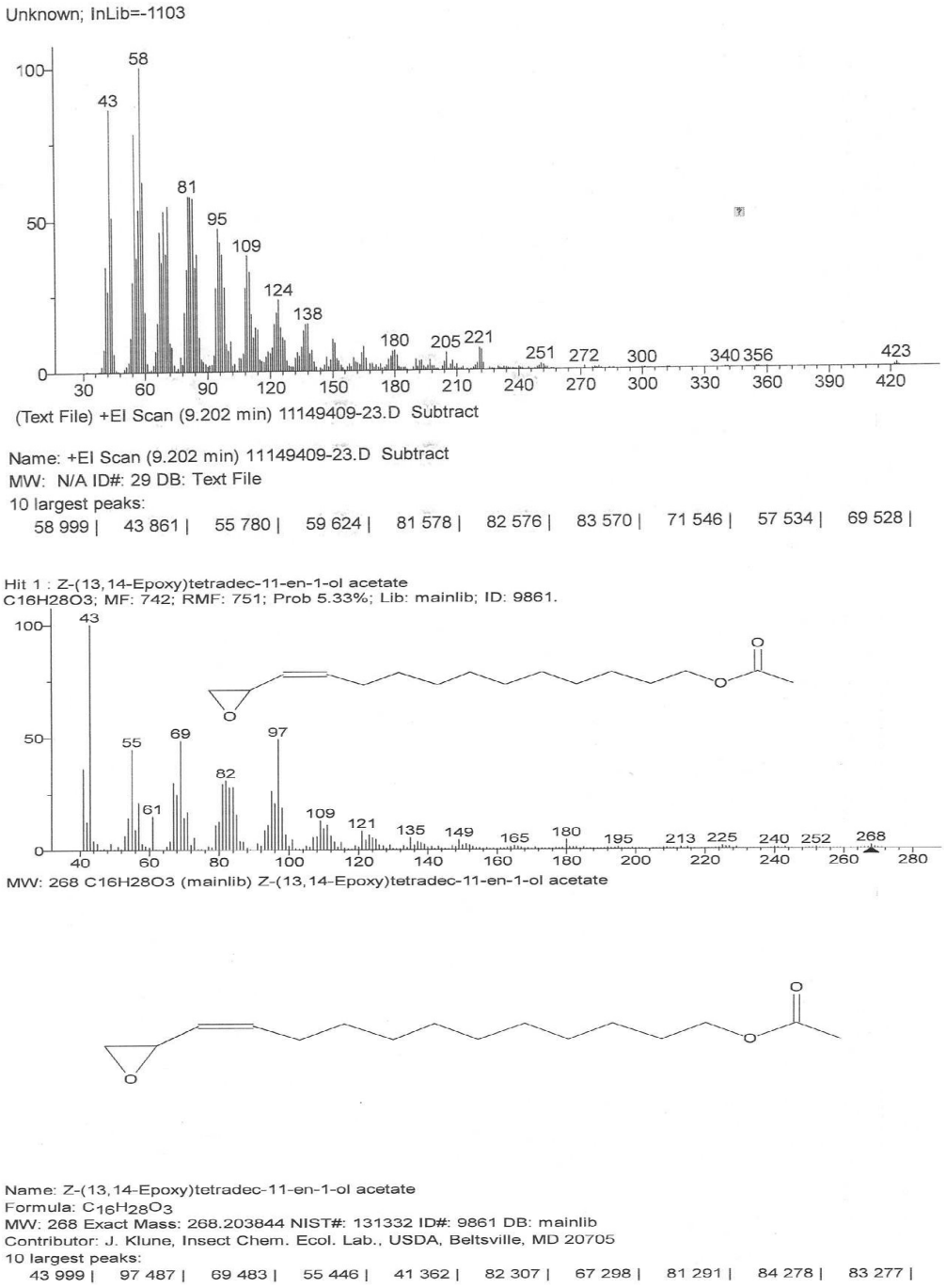
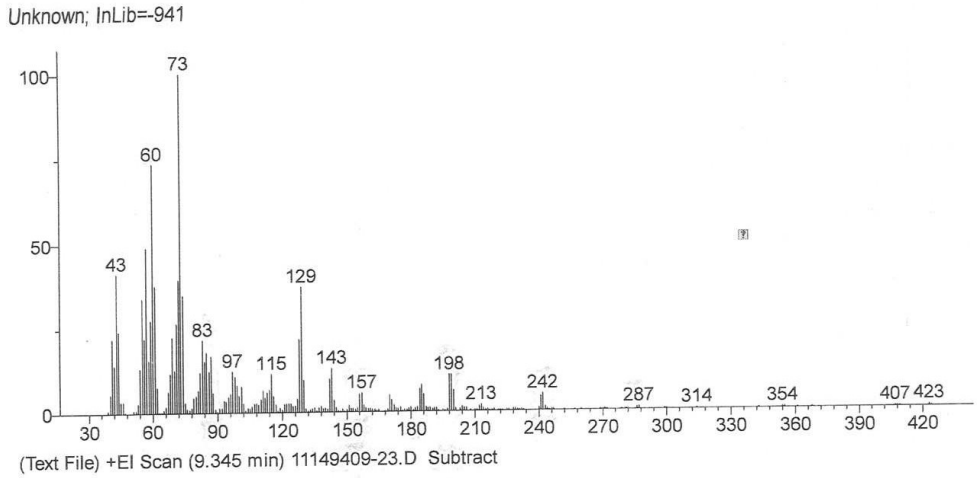


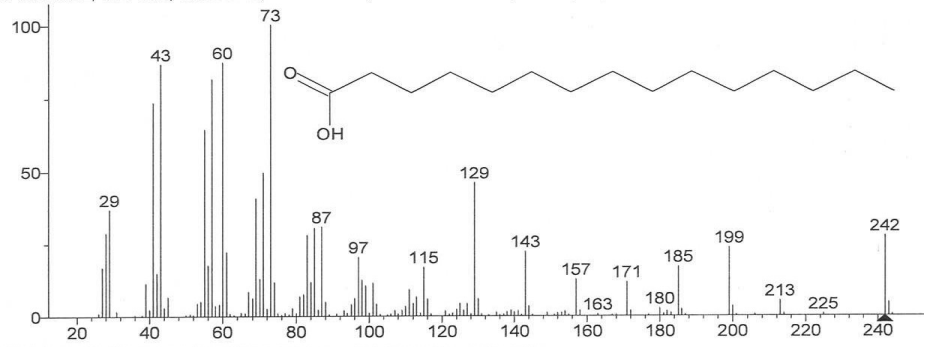

**Figure 29 Z-(13,14-Epoxy) tetradec-11-en-1-ol acetate**



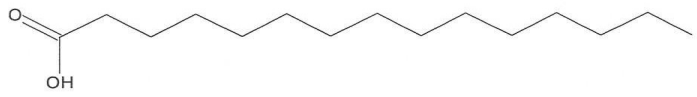

(Text File) +EI Scan (9.345 min) 11149409-23.D Subtract

Name: +EI Scan (9.345 min) 11149409-23.D Subtract
MW: N/A ID#: 30 DB: Text File
10 largest peaks:
73 999 | 60 733 | 57 487 | 43 411 | 72 392 | 61 375 | 129 369 | 74 346 | 55 338 | 59 273 |

Hit 1 : Pentadecanoic acid
C15H30O2; MF: 689; RMF: 749; Prob 24.5%; CAS: 1002-84-2; Lib: replib; ID: 9038.



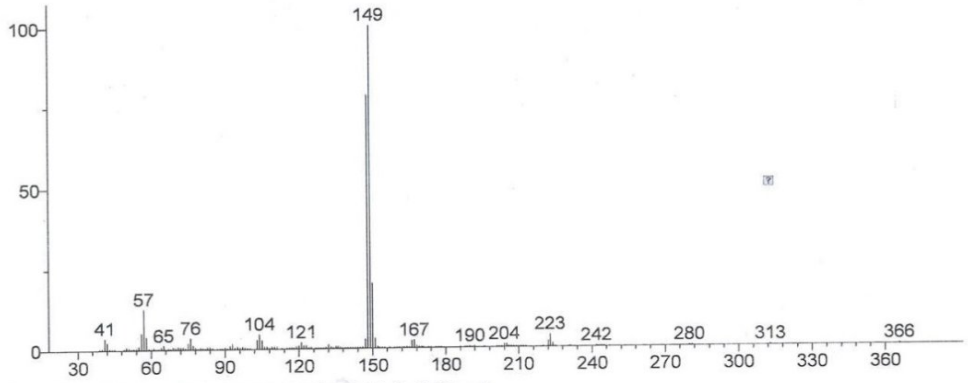

MW: 242 CAS# 1002-84-2 C15H30O2 (replib) Pentadecanoic acid




Name: Pentadecanoic acid
Formula: $C_{15}H_{30}O_2$
MW: 242 Exact Mass: 242.22458 CAS#: 1002-84-2 NIST#: 221146 ID#: 9038 DB: replib
Other DBs: Fine, TSCA, RTECS, HODOC, NIH, EINECS, IRDB
Contributor: Chemical Concepts
10 largest peaks:
73 999 | 60 871 | 43 865 | 57 814 | 41 734 | 55 641 | 71 493 | 129 457 | 69 405 | 29 367 |

**Figure 30** Pentadecanoic acid

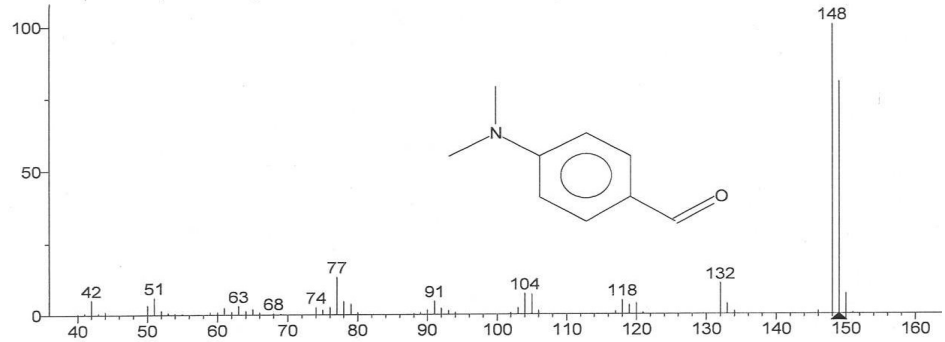
Unknown; InLib=-941



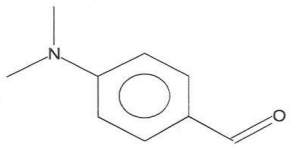

(Text File) +EI Scan (9.472 min) 11149409-23.D Subtract

Name: +EI Scan (9.472 min) 11149409-23.D Subtract
MW: N/A ID#: 31 DB: Text File
10 largest peaks:
149 999 | 148 782 | 150 201 | 57 125 | 56 52 | 104 45 | 58 40 | 223 38 | 41 36 | 76 36 |

Hit 1 : Benzaldehyde, 4-(dimethylamino)-
C9H11NO; MF: 683; RMF: 767; Prob 12.7%; CAS: 100-10-7; Lib: replib; ID: 21626.




MW: 149 CAS# 100-10-7 C9H11NO (replib) Benzaldehyde, 4-(dimethylamino)-




Name: Benzaldehyde, 4-(dimethylamino)-
Formula: $C_9H_{11}NO$
MW: 149 Exact Mass: 149.084064 CAS#: 100-10-7 NIST#: 335242 ID#: 21626 DB: replib
Other DBs: Fine, TSCA, RTECS, HODOC, NIH, EINECS, IRDB
Contributor: Drug Lab
10 largest peaks:
148 999 | 149 802 | 77 131 | 132 106 | 104 71 | 150 70 | 105 69 | 51 58 | 42 50 | 118 48 |

**Figure 31** Benzaldehyde,4-(dimethylamino)

### 3.3 (b) GCMS chromatogram and compounds in ethanol extract of Tetraherbal extract

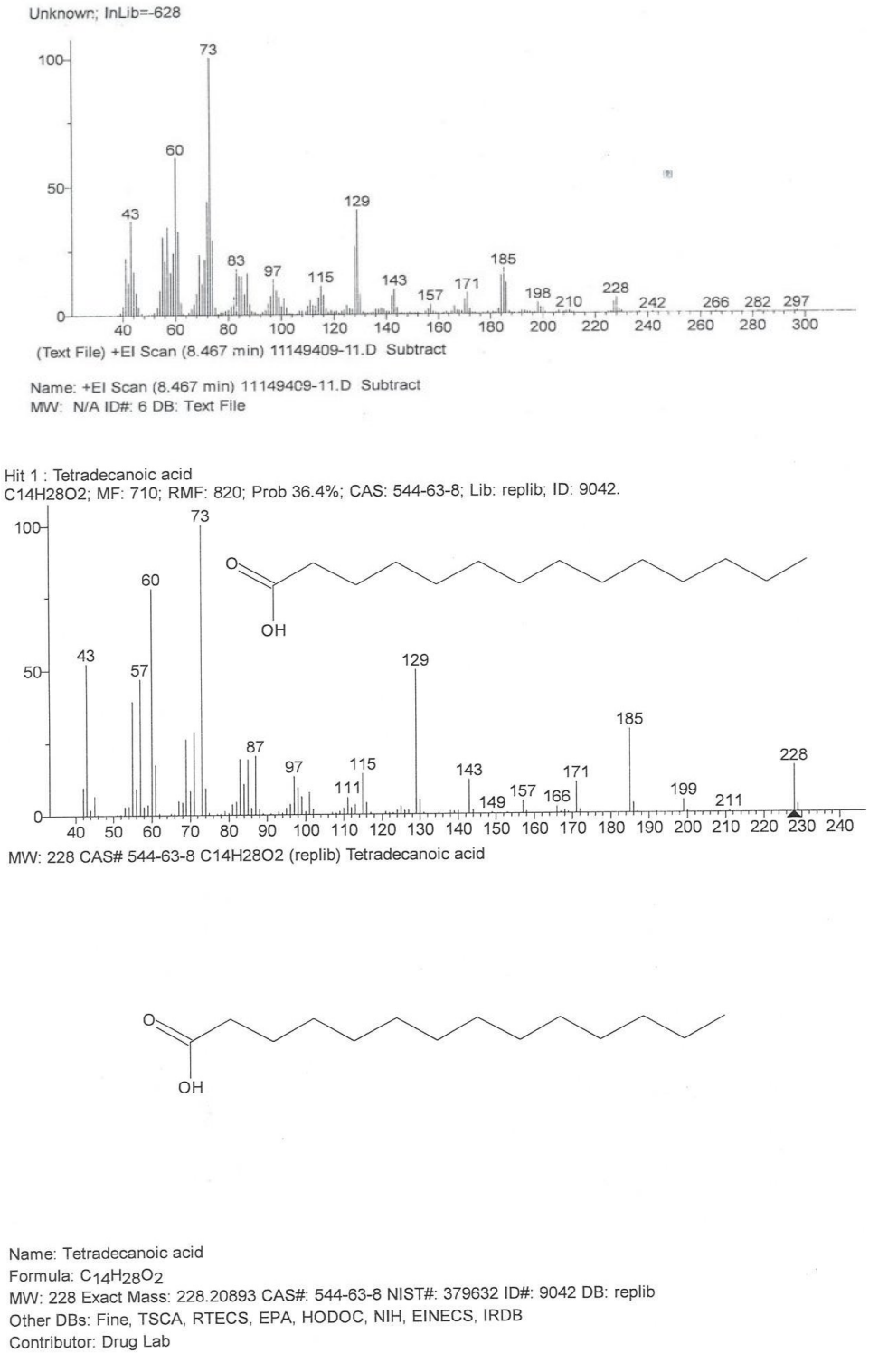
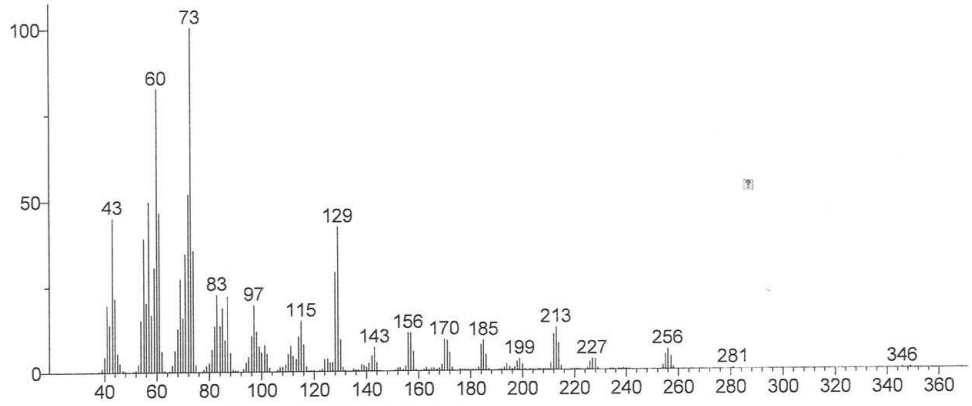



**Figure 32** Tetradecanoic acid

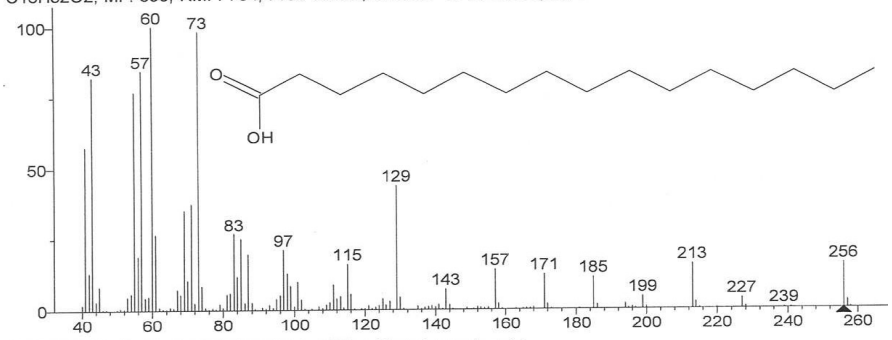
Unknown; InLib=-918



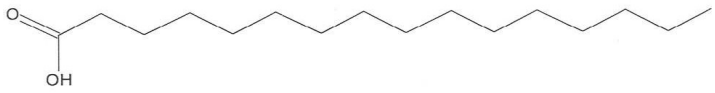

(Text File) +EI Scan (10.235 min) 11149409-11.D Subtract

Name: +EI Scan (10.235 min) 11149409-11.D Subtract
MW: N/A ID#: 7 DB: Text File

*Hit 1 : n-Hexadecanoic acid*
C16H32O2; MF: 699; RMF: 764; Prob 36.9%; CAS: 57-10-3; Lib: replib; ID: 7156.



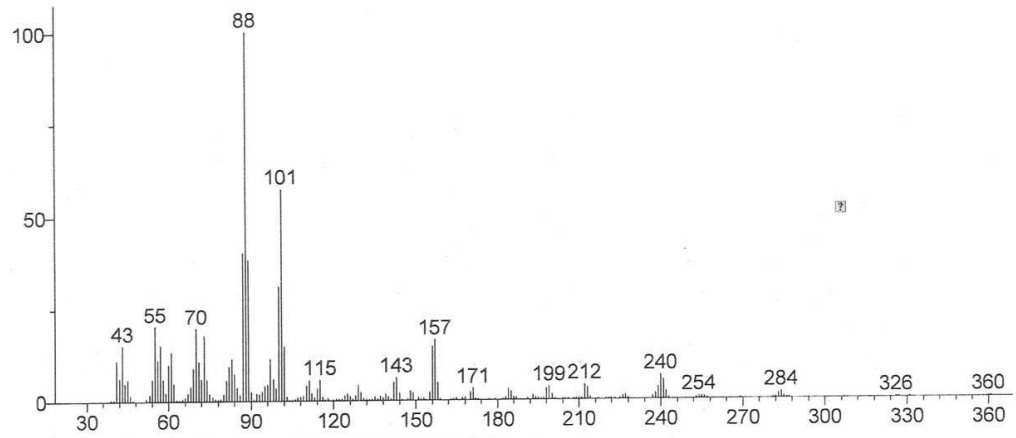

MW: 256 CAS# 57-10-3 C16H32O2 (replib) n-Hexadecanoic acid




Name: n-Hexadecanoic acid
Formula: $C_{16}H_{32}O_2$
MW: 256 Exact Mass: 256.24023 CAS#: 57-10-3 NIST#: 335494 ID#: 7156 DB: replib
Other DBs: Fine, TSCA, RTECS, EPA, HODOC, NIH, EINECS, IRDB
Contributor: Drug Lab
Related CAS#: 60605-23-4; 116860-99-2; 212625-86-0

**Figure 33** n-Hexadecanoic acid

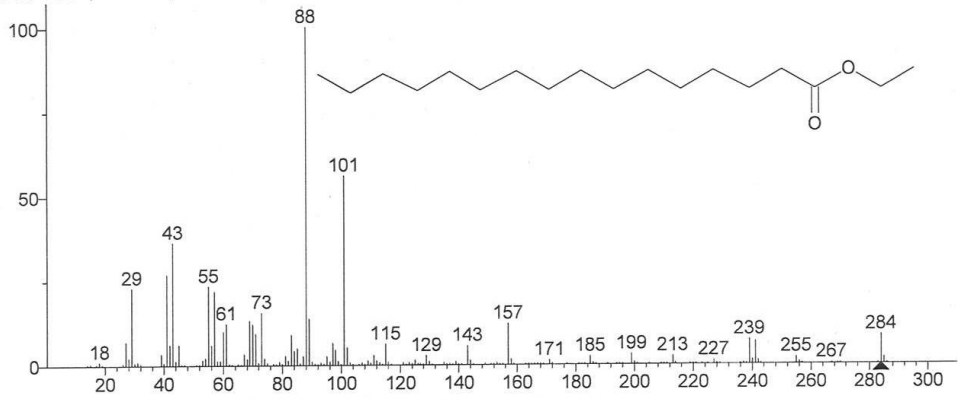
Unknown; InLib=-642



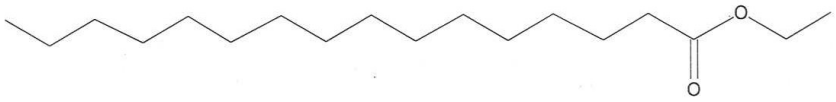

(Text File) +EI Scan (10.438 min) 11149409-11.D Subtract

Name: +EI Scan (10.438 min) 11149409-11.D Subtract
MW: N/A ID#: 8 DB: Text File

*Hit 1 : Hexadecanoic acid, ethyl ester*
C18H36O2; MF: 696; RMF: 739; Prob 37.9%; CAS: 628-97-7; Lib: mainlib; ID: 52733.

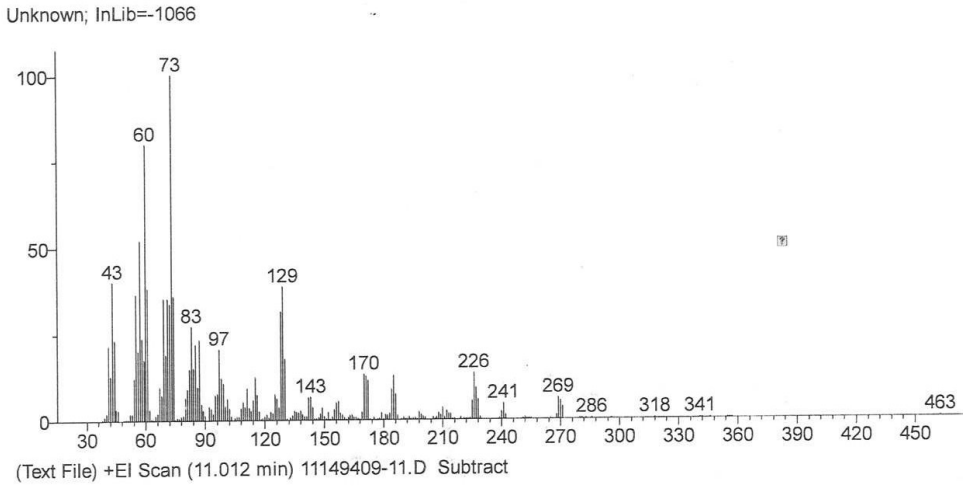



MW: 284 CAS# 628-97-7 C18H36O2 (mainlib) Hexadecanoic acid, ethyl ester

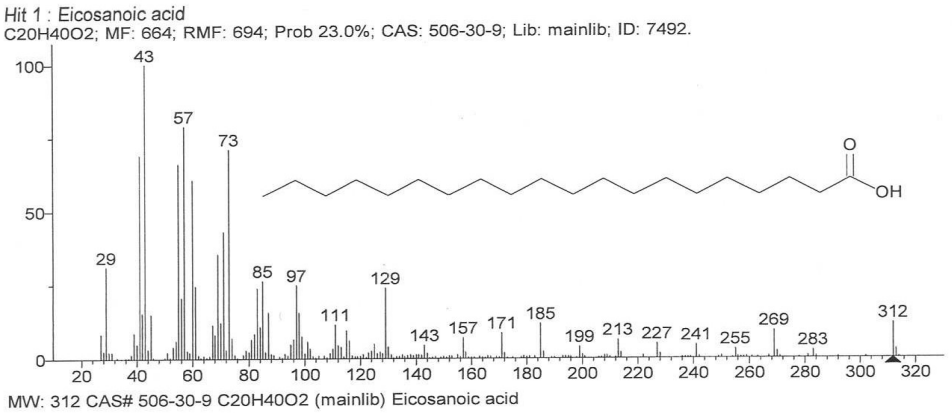


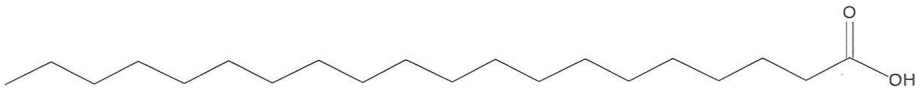

Name: Hexadecanoic acid, ethyl ester
Formula: $C_{18}H_{36}O_2$
MW: 284 Exact Mass: 284.27153 CAS#: 628-97-7 NIST#: 233204 ID#: 52733 DB: mainlib
Other DBs: Fine, TSCA, EPA, HODOC, NIH, EINECS, IRDB
Contributor: Japan AIST/NIMC Database- Spectrum MS-NW-5396

**Figure 34 Hexadecanoic acid, ethyl ester**



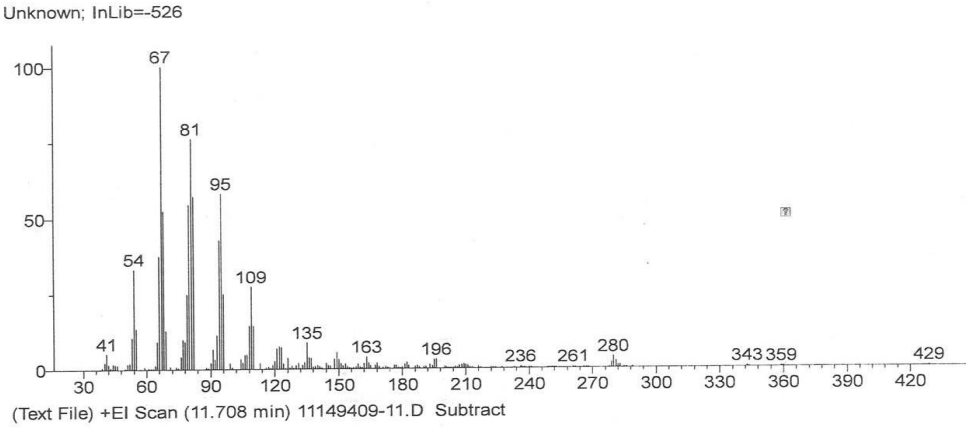

Name: +EI Scan (11.012 min) 11149409-11.D Subtract
MW: N/A ID#: 9 DB: Text File



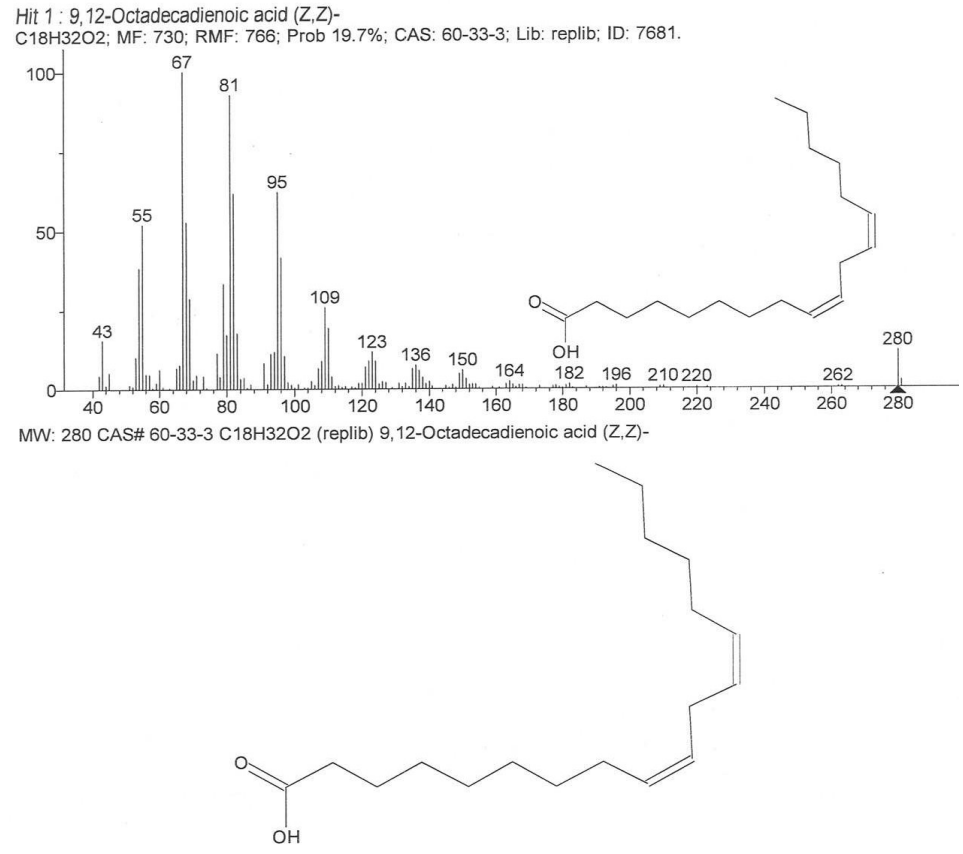




Name: Eicosanoic acid
Formula: $C_{20}H_{40}O_2$
MW: 312 Exact Mass: 312.30283 CAS#: 506-30-9 NIST#: 160470 ID#: 7492 DB: mainlib
Other DBs: Fine, TSCA, RTECS, HODOC, NIH, EINECS, IRDB
Contributor: Chemical Concepts

**Figure 35 Eicosanoic acid**




Name: +EI Scan (11.708 min) 11149409-11.D Subtract
MW: N/A ID#: 11 DB: Text File



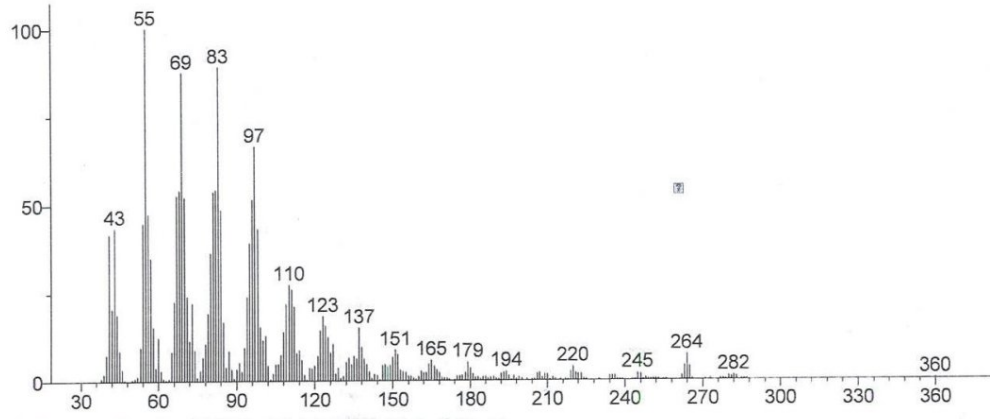

MW: 280 CAS# 60-33-3 C18H32O2 (replib) 9,12-Octadecadienoic acid (Z,Z)-




Name: 9,12-Octadecadienoic acid (Z,Z)-
Formula: $C_{18}H_{32}O_2$
MW: 280 Exact Mass: 280.24023 CAS#: 60-33-3 NIST#: 379359 ID#: 7681 DB: replib
Other DBs: Fine, TSCA, RTECS, HODOC, NIH, EINECS
Contributor: Drug Lab
Related CAS#: 8024-22-4; 949900-18-9

**Figure 36 9,12- Octadecadienoic acid (Z,Z)-**

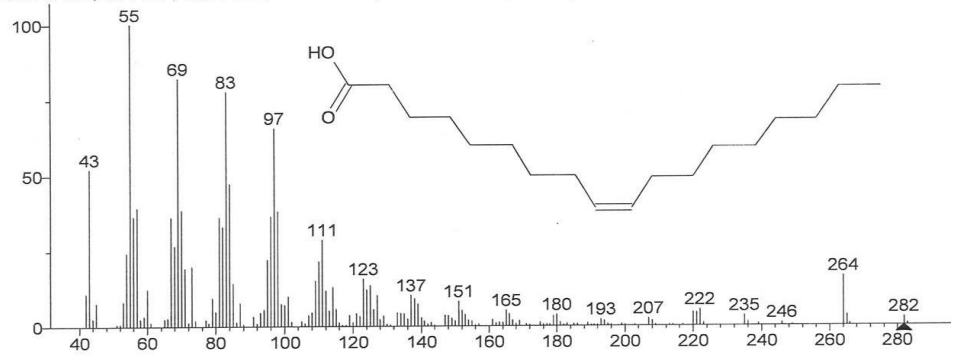
Unknown; InLib=-407



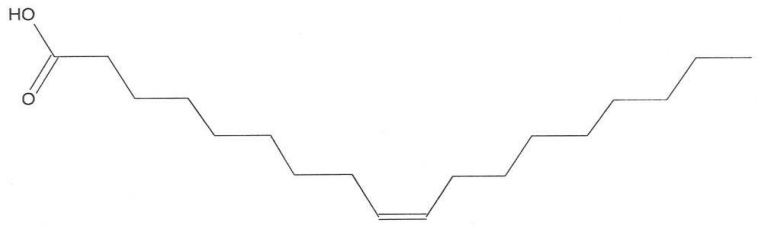

(Text File) +EI Scan (11.745 min) 11149409-11.D Subtract

Name: +EI Scan (11.745 min) 11149409-11.D Subtract
MW: N/A ID#: 10 DB: Text File

Hit 1 : Oleic Acid
C18H34O2; MF: 817; RMF: 844; Prob 11.8%; CAS: 112-80-1; Lib: replib; ID: 4760.



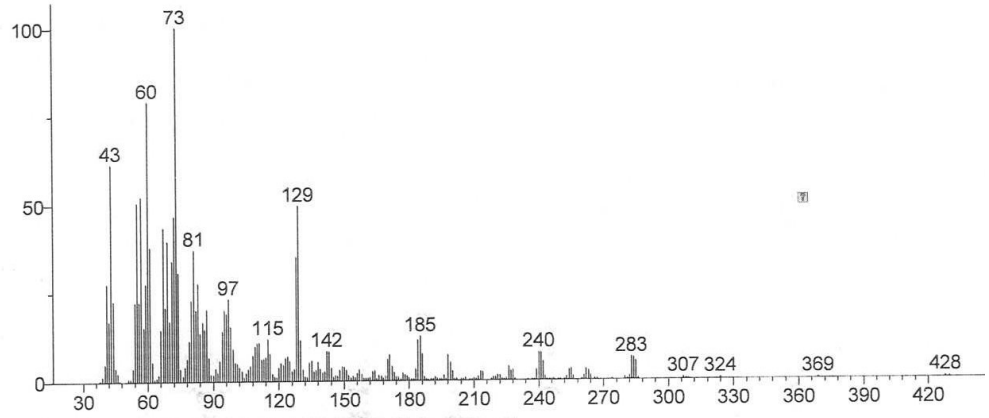

MW: 282 CAS# 112-80-1 C18H34O2 (replib) Oleic Acid




Name: Oleic Acid
Formula: $C_{18}H_{34}O_2$
MW: 282 Exact Mass: 282.25588 CAS#: 112-80-1 NIST#: 379354 ID#: 4760 DB: replib
Other DBs: TSCA, RTECS, USP, HODOC, NIH, EINECS, IRDB
Contributor: Drug Lab
Related CAS#: 56833-51-3; 8046-01-3; 949900-16-7

**Figure 37 Oleic acid**

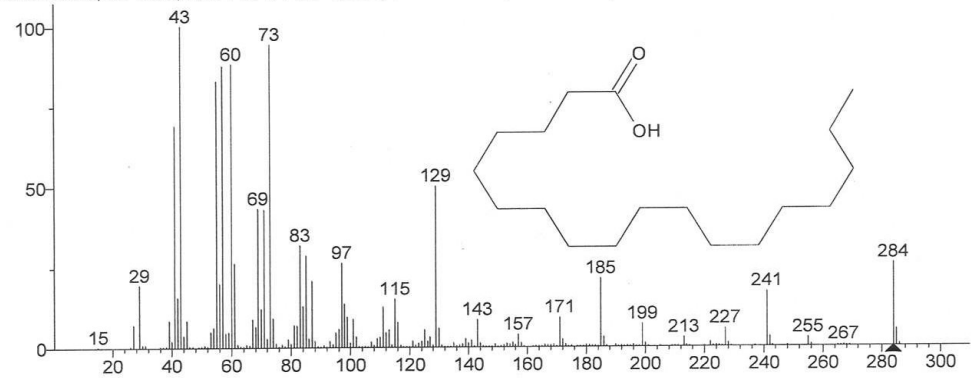
Unknown; InLib=-1169



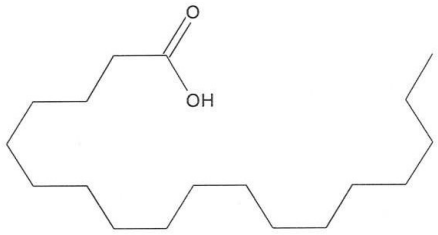

(Text File) +EI Scan (11.904 min) 11149409-11.D Subtract

Name: +EI Scan (11.904 min) 11149409-11.D Subtract
MW: N/A ID#: 12 DB: Text File

Hit 1 : Octadecanoic acid
C18H36O2; MF: 668; RMF: 694; Prob 17.6%; CAS: 57-11-4; Lib: mainlib; ID: 8691.

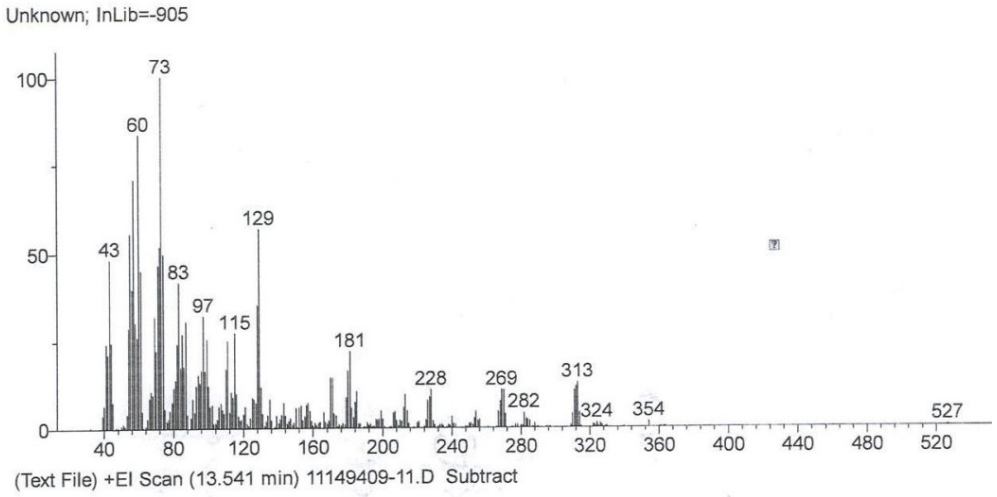



MW: 284 CAS# 57-11-4 C18H36O2 (mainlib) Octadecanoic acid



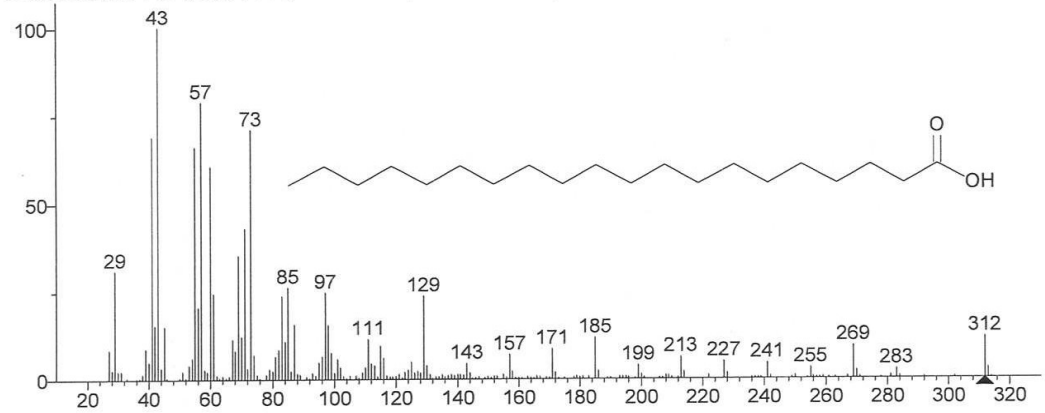

Name: Octadecanoic acid
Formula: $C_{18}H_{36}O_2$
MW: 284 Exact Mass: 284.27153 CAS#: 57-11-4 NIST#: 290961 ID#: 8691 DB: mainlib
Other DBs: Fine, TSCA, RTECS, EPA, HODOC, NIH, EINECS, IRDB
Contributor: NIST Mass Spectrometry Data Center, 1998.
Related CAS#: 8037-40-9; 197923-10-7; 294203-07-9; 39390-61-9; 58392-66-8; 8013-28-3; 8023-06-1; 8037-83-0; 8039-51-8; 8039-52-9; 8039-53-0; 8039-54-1

**Figure 38 Octadecanoic acid**

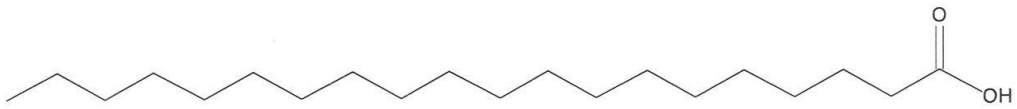



Name: +EI Scan (13.541 min) 11149409-11.D Subtract
MW: N/A ID#: 13 DB: Text File

Hit 1 : Eicosanoic acid
C20H40O2; MF: 664; RMF: 714; Prob 24.4%; CAS: 506-30-9; Lib: mainlib; ID: 7492.




MW: 312 CAS# 506-30-9 C20H40O2 (mainlib) Eicosanoic acid




Name: Eicosanoic acid
Formula: $C_{20}H_{40}O_2$
MW: 312 Exact Mass: 312.30283 CAS#: 506-30-9 NIST#: 160470 ID#: 7492 DB: mainlib
Other DBs: Fine, TSCA, RTECS, HODOC, NIH, EINECS, IRDB
Contributor: Chemical Concepts

**Figure 39 Eicosanoic acid**

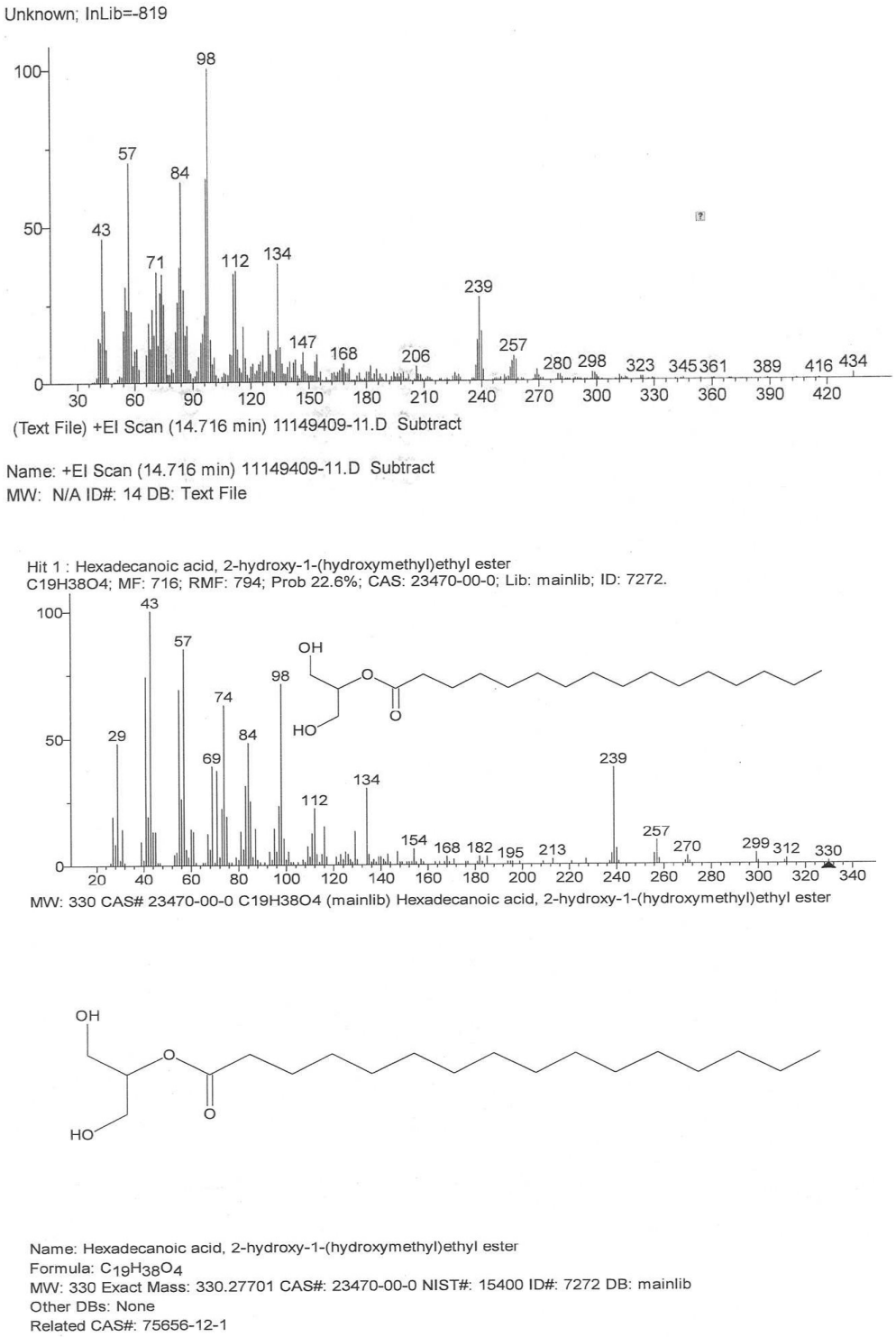
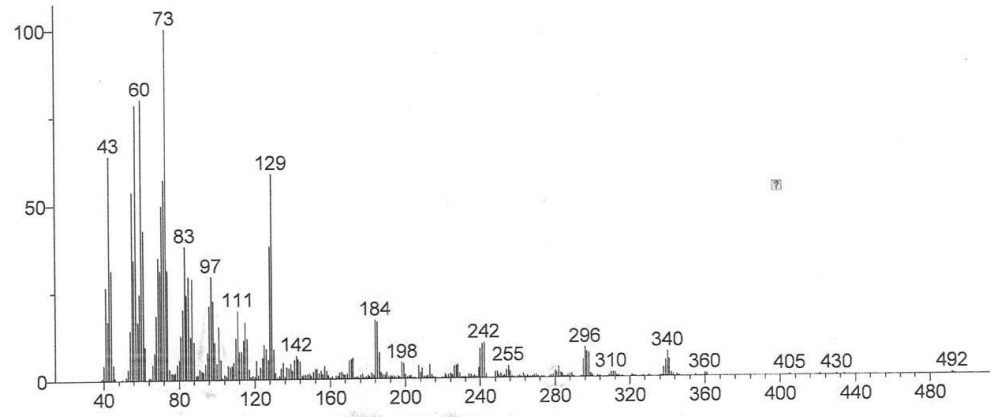



**Figure 40 Hexadecanoic acid, 2- hydroxyl-1-(hydroxymethyl)ethyl ester**

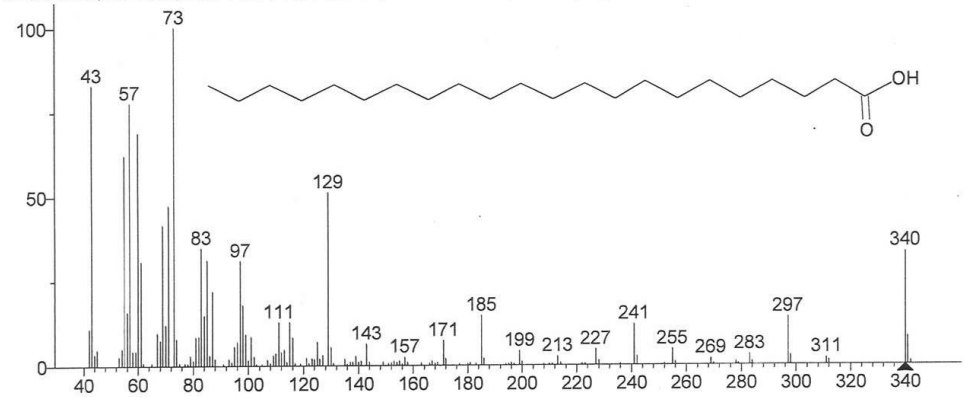
Unknown; InLib=-1032



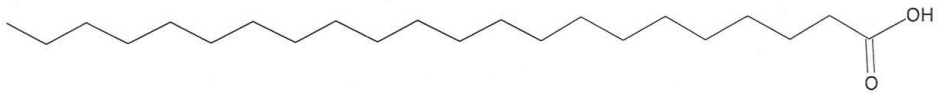

(Text File) +EI Scan (15.119 min) 11149409-11.D Subtract

Name: +EI Scan (15.119 min) 11149409-11.D Subtract
MW: N/A ID#: 15 DB: Text File

Hit 1 : Docosanoic acid
C22H44O2; MF: 682; RMF: 807; Prob 22.2%; CAS: 112-85-6; Lib: replib; ID: 8936.

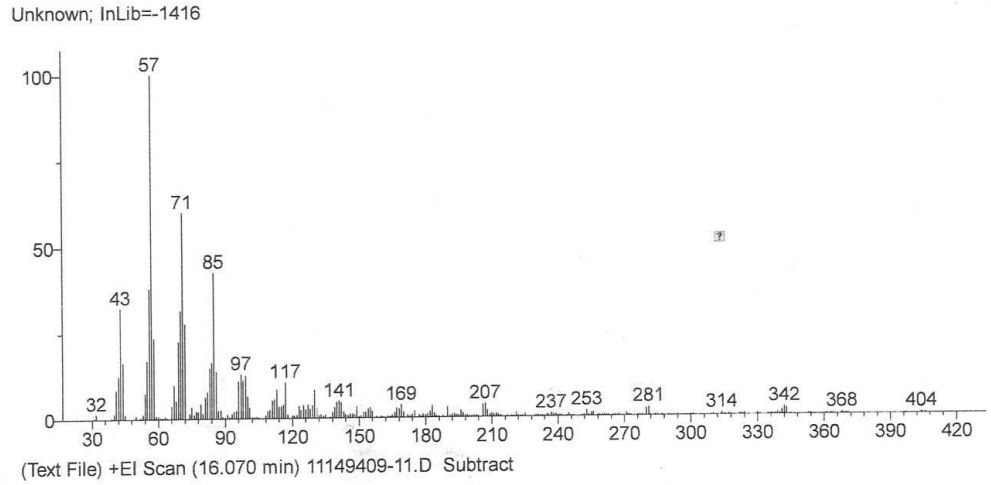



MW: 340 CAS# 112-85-6 C22H44O2 (replib) Docosanoic acid



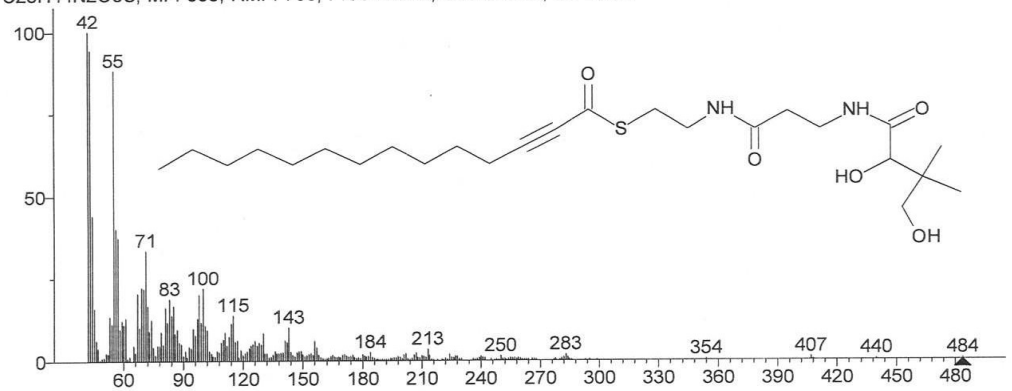

Name: Docosanoic acid
Formula: $C_{22}H_{44}O_2$
MW: 340 Exact Mass: 340.334131 CAS#: 112-85-6 NIST#: 379420 ID#: 8936 DB: replib
Other DBs: Fine, TSCA, HODOC, NIH, EINECS, IRDB
Contributor: Drug Lab

**Figure 41 Docosanoic acid**

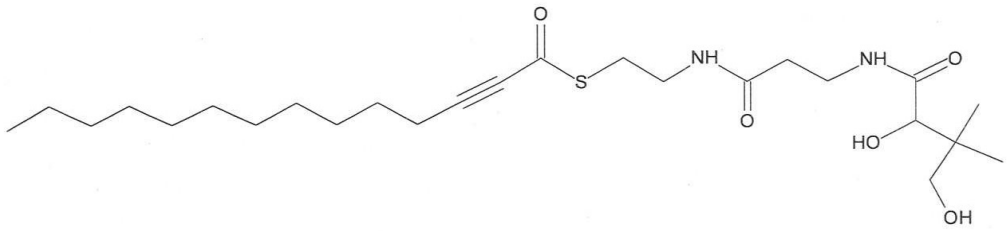



Name: +EI Scan (16.070 min) 11149409-11.D Subtract
MW: N/A ID#: 16 DB: Text File

Hit 1 : 2-Myristynoyl pantetheine
C25H44N2O5S; MF: 683; RMF: 706; Prob 7.00%; Lib: mainlib; ID: 4307.

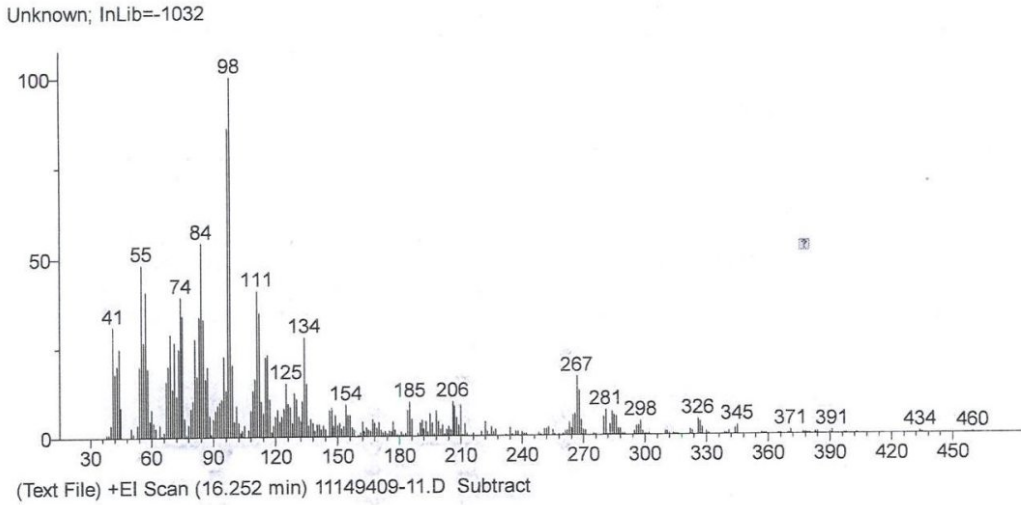



MW: 484 C25H44N2O5S (mainlib) 2-Myristynoyl pantetheine



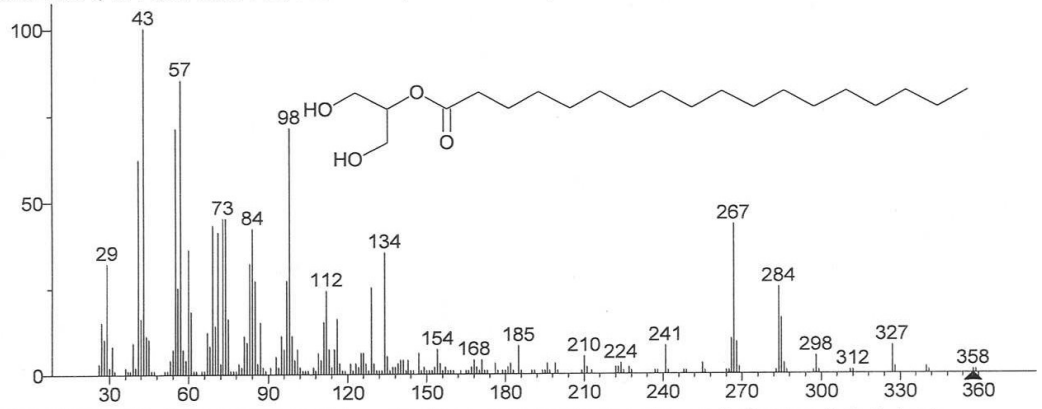

Name: 2-Myristynoyl pantetheine
Formula: $C_{25}H_{44}N_2O_5S$
MW: 484 Exact Mass: 484.297094 NIST#: 111636 ID#: 4307 DB: mainlib
Contributor: S. MILLER, DMNB, NINDS, NIH, Bethesda, MD 20892

**Figure 42 2-Myristynoyl pantetheine**

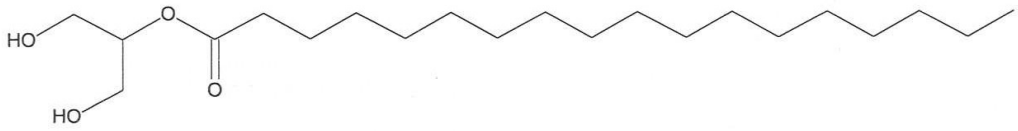



Name: +EI Scan (16.252 min) 11149409-11.D Subtract
MW: N/A ID#: 4 DB: Text File

Hit 1 : Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
C21H42O4; MF: 682; RMF: 757; Prob 19.4%; CAS: 621-61-4; Lib: mainlib; ID: 7334.

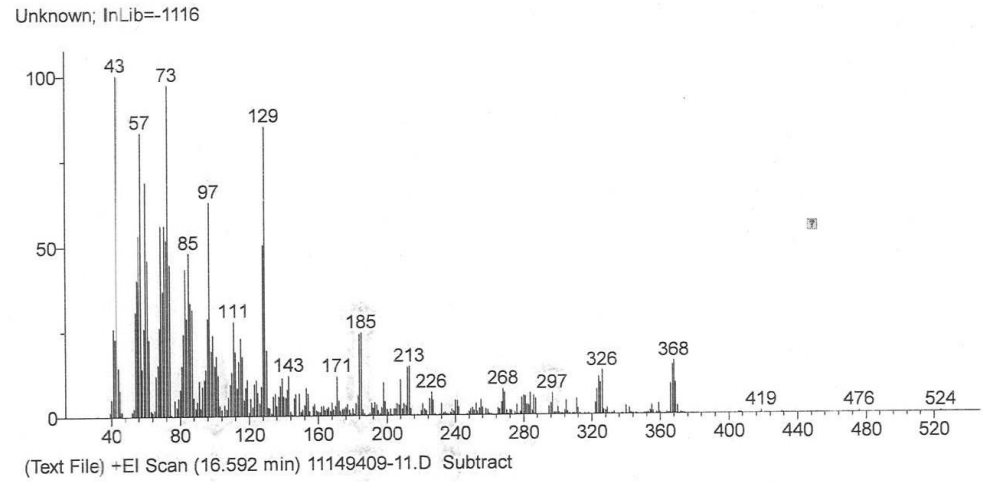



MW: 358 CAS# 621-61-4 C21H42O4 (mainlib) Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester




Name: Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
Formula: $C_{21}H_{42}O_4$
MW: 358 Exact Mass: 358.30831 CAS#: 621-61-4 NIST#: 16116 ID#: 7334 DB: mainlib
Other DBs: None

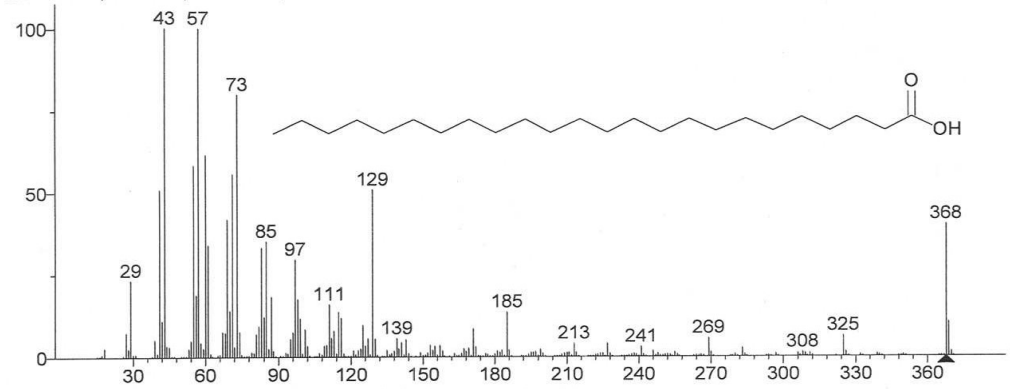
**Figure 43 Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester**



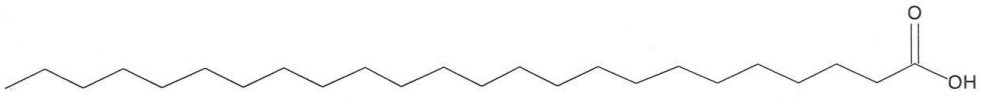

(Text File) +EI Scan (16.592 min) 11149409-11.D Subtract

Name: +EI Scan (16.592 min) 11149409-11.D Subtract
MW: N/A ID#: 5 DB: Text File

Hit 1 : Tetracosanoic acid
C24H48O2; MF: 645; RMF: 683; Prob 27.0%; CAS: 557-59-5; Lib: mainlib; ID: 7491.

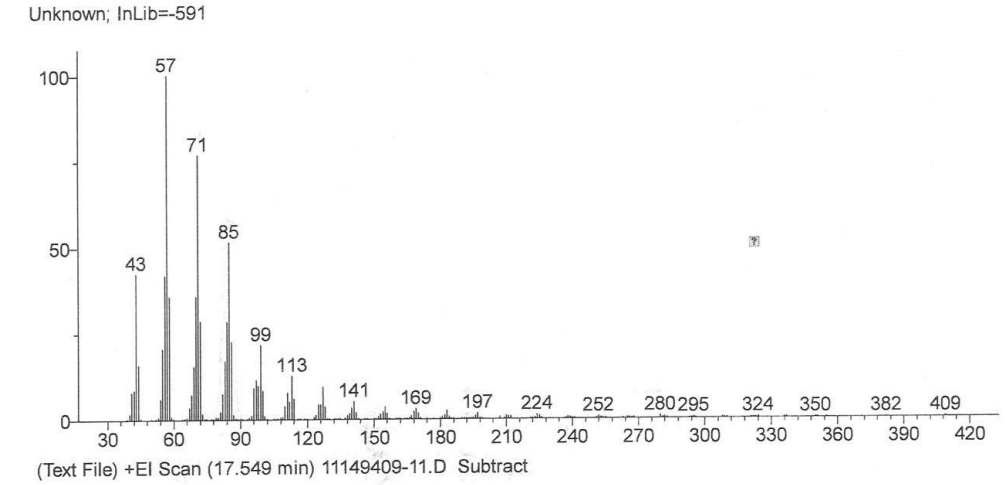



MW: 368 CAS# 557-59-5 C24H48O2 (mainlib) Tetracosanoic acid



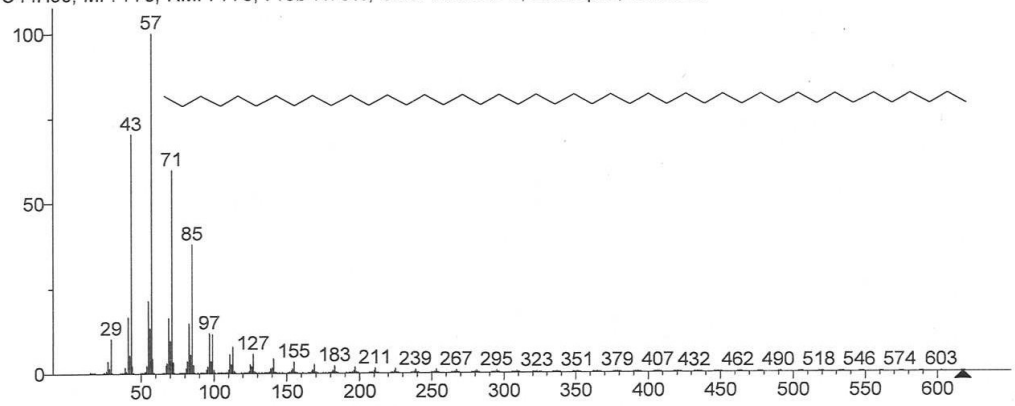

Name: Tetracosanoic acid
Formula: $C_{24}H_{48}O_2$
MW: 368 Exact Mass: 368.36543 CAS#: 557-59-5 NIST#: 231005 ID#: 7491 DB: mainlib
Other DBs: Fine, TSCA, HODOC, EINECS
Contributor: Japan AIST/NIMC Database- Spectrum MS-NW-2569

**Figure 44 Tetracosanoic acid**




Name: +EI Scan (17.549 min) 11149409-11.D Subtract
MW: N/A ID#: 6 DB: Text File

Hit 1 : Tetratetracontane
C44H90; MF: 775; RMF: 778; Prob 7.73%; CAS: 7098-22-8; Lib: replib; ID: 5823.



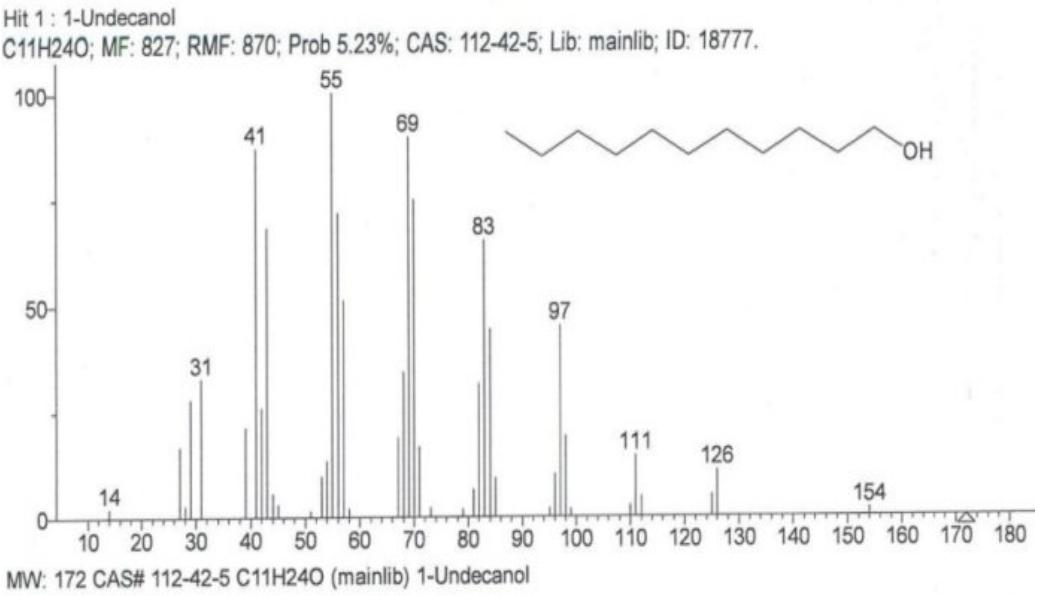

MW: 618 CAS# 7098-22-8 C44H90 (replib) Tetratetracontane

Name: Tetratetracontane
Formula: $C_{44}H_{90}$
MW: 618 Exact Mass: 618.704254 CAS#: 7098-22-8 NIST#: 23773 ID#: 5823 DB: replib
Other DBs: Fine, TSCA, HODOC, NIH, EINECS

**Figure 45 Tetratetracontane**

## 3.4 GCMS analysis of a biherbal extract made of traditional non-compatible plants i.e. *Ficus religiosa* and *Ficus racemosa* (Table 9)

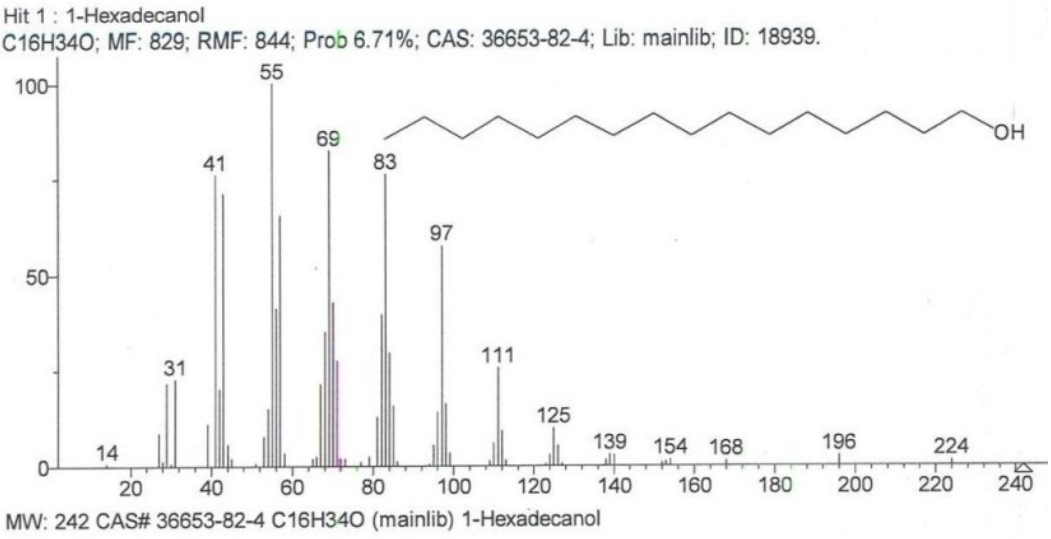
## 3.4 (a) GCMS chromatogram and compounds in chloroform extract of biherbal extract





Name: 1-Undecanol
Formula: $C_{11}H_{24}O$
MW: 172 Exact Mass: 172.182715 CAS#: 112-42-5 NIST#: 114087 ID#: 18777 DB: mainlib
Other DBs: Fine, TSCA, RTECS, EPA, HODOC, NIH, EINECS, IRDB
Contributor: NIST Mass Spectrometry Data Center, 1990.
10 largest peaks:
55 999 | 69 894 | 41 868 | 70 746 | 56 714 | 43 680 | 83 649 | 57 510 | 97 448 | 84 440 |

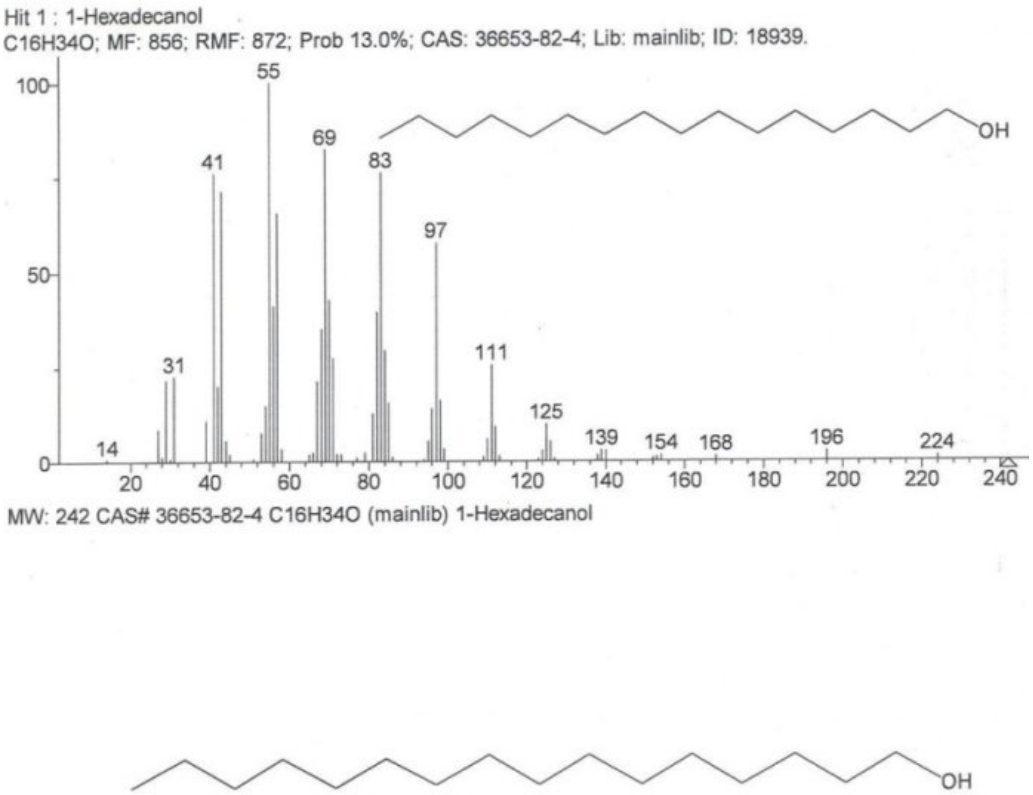
**Figure 46 1-Undecanol**





Name: 1-Hexadecanol
Formula: $C_{16}H_{34}O$
MW: 242 Exact Mass: 242.260965 CAS#: 36653-82-4 NIST#: 114116 ID#: 18939 DB: mainlib
Other DBs: Fine, TSCA, RTECS, EPA, HODOC, NIH, EINECS, IRDB
Contributor: NIST Mass Spectrometry Data Center, 1990.
Related CAS#: 8023-37-8; 124-29-8; 8032-16-4; 8032-89-1; 8032-17-5
Salt/Mix CAS#: 19141-82-3
10 largest peaks:
55 999 | 69 822 | 83 760 | 41 758 | 43 710 | 57 653 | 97 573 | 70 425 | 56 410 | 82 394

**Figure 47 1-Hexadecanol**







Name: 1-Hexadecanol
Formula: $C_{16}H_{34}O$
MW: 242 Exact Mass: 242.260965 CAS#: 36653-82-4 NIST#: 114116 ID#: 18939 DB: mainlib
Other DBs: Fine, TSCA, RTECS, EPA, HODOC, NIH, EINECS, IRDB
Contributor: NIST Mass Spectrometry Data Center, 1990.
Related CAS#: 8023-37-8; 124-29-8; 8032-16-4; 8032-89-1; 8032-17-5
Salt/Mix CAS#: 19141-82-3
10 largest peaks:
55 999 | 69 822 | 83 760 | 41 758 | 43 710 | 57 653 | 97 573 | 70 425 | 56 410 | 82 394

**Figure 48 1-Hexadecanol**

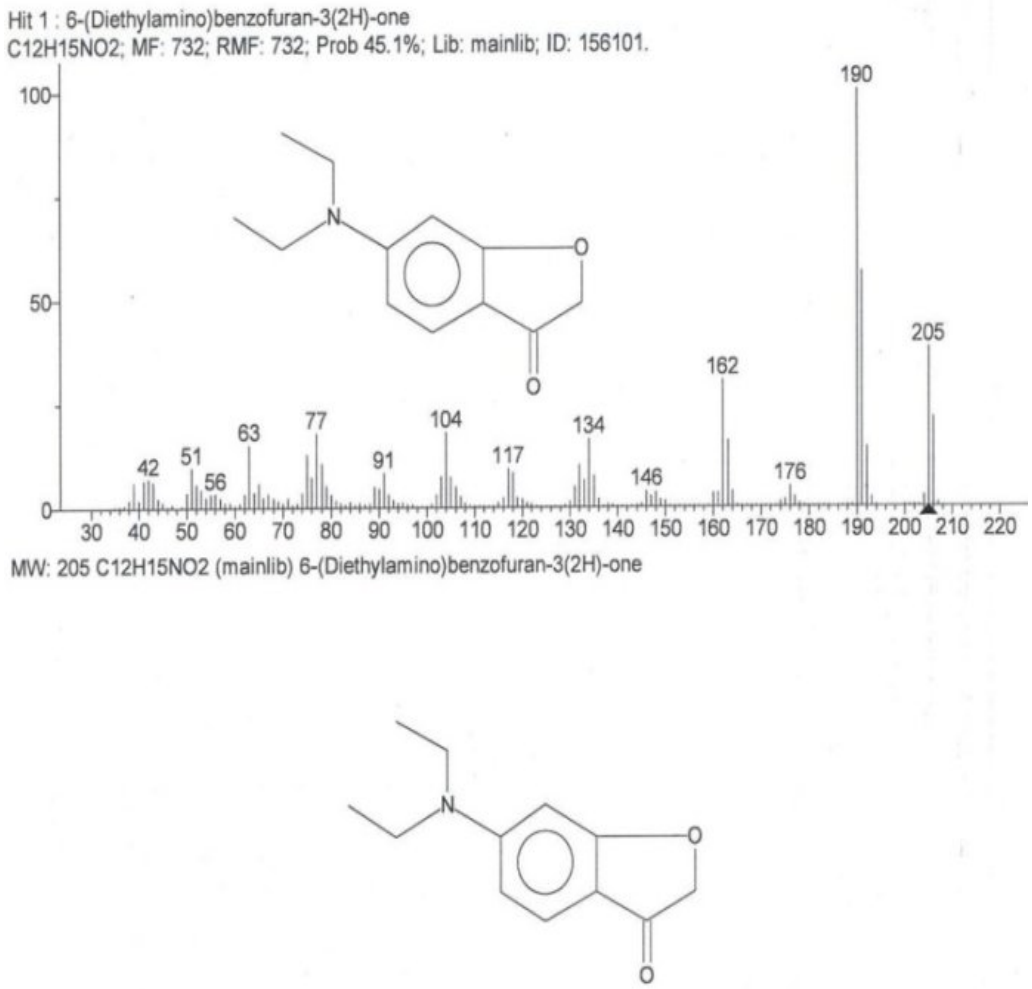
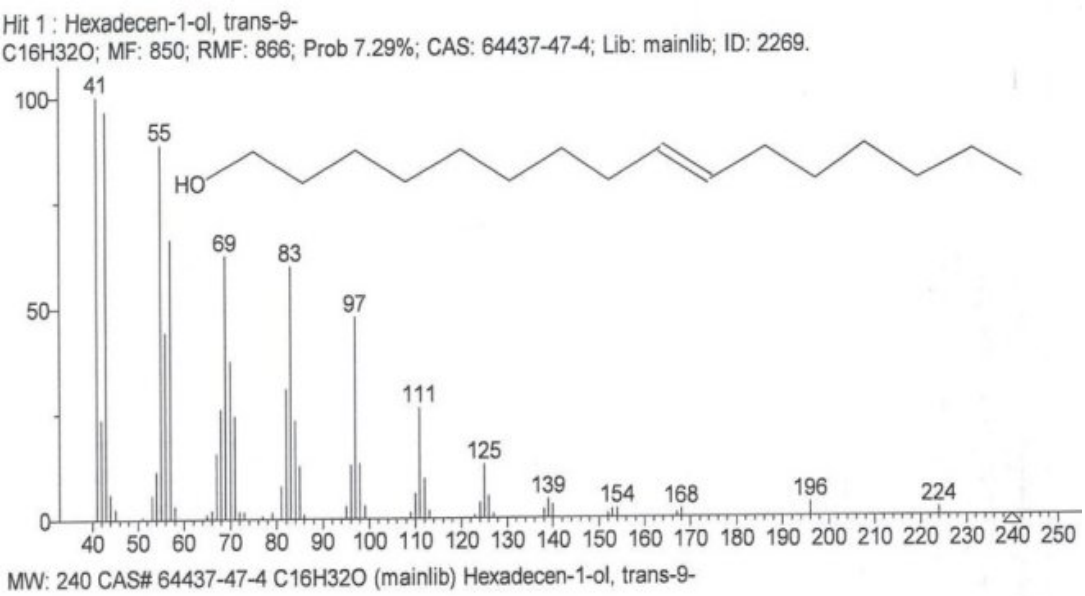



**Figure 49 6-(Diethylamino)benzofuran-3(2H)-on**





Name: Hexadecen-1-ol, trans-9-
Formula: $C_{16}H_{32}O$
MW: 240 Exact Mass: 240.245316 CAS#: 64437-47-4 NIST#: 141055 ID#: 2269 DB: mainlib
Other DBs: None
Contributor: Mark Whitten, Florida Museum of Natural History, U. of Florida
10 largest peaks:
41 999 | 43 965 | 55 885 | 57 660 | 69 620 | 83 594 | 97 474 | 56 439 | 70 372 | 82 306 |

**Figure 50 Hexadecen-1-ol, trans-9-**

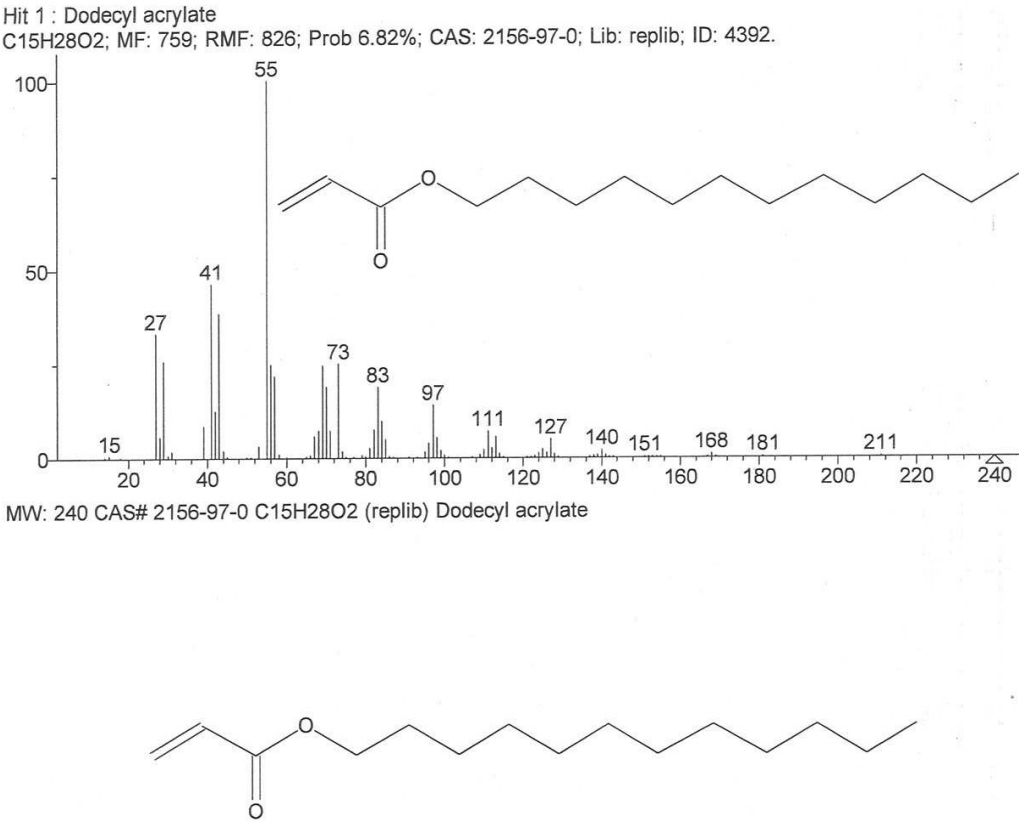
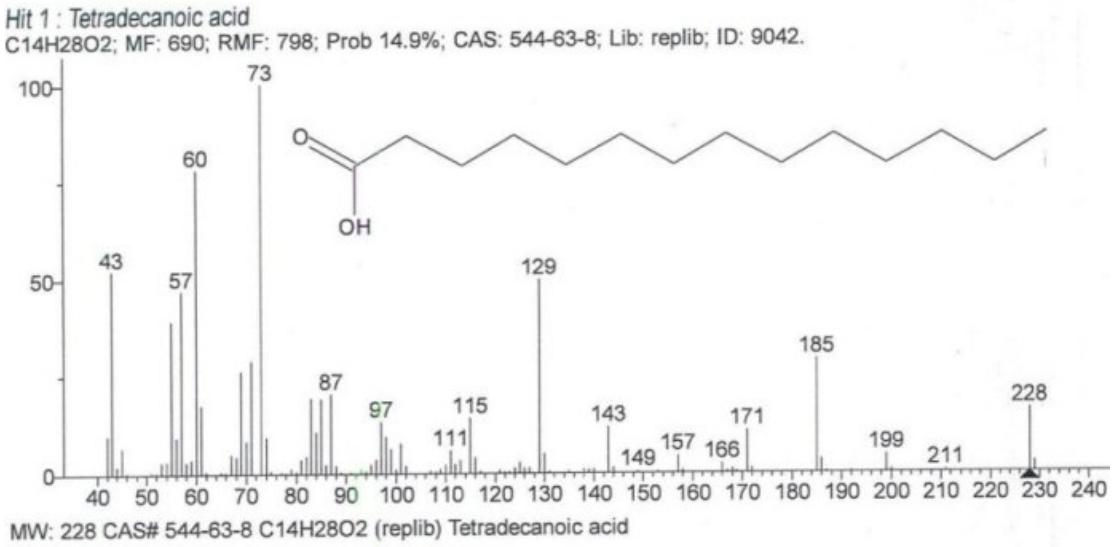
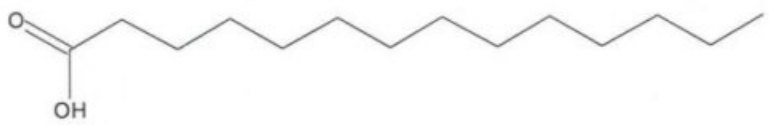


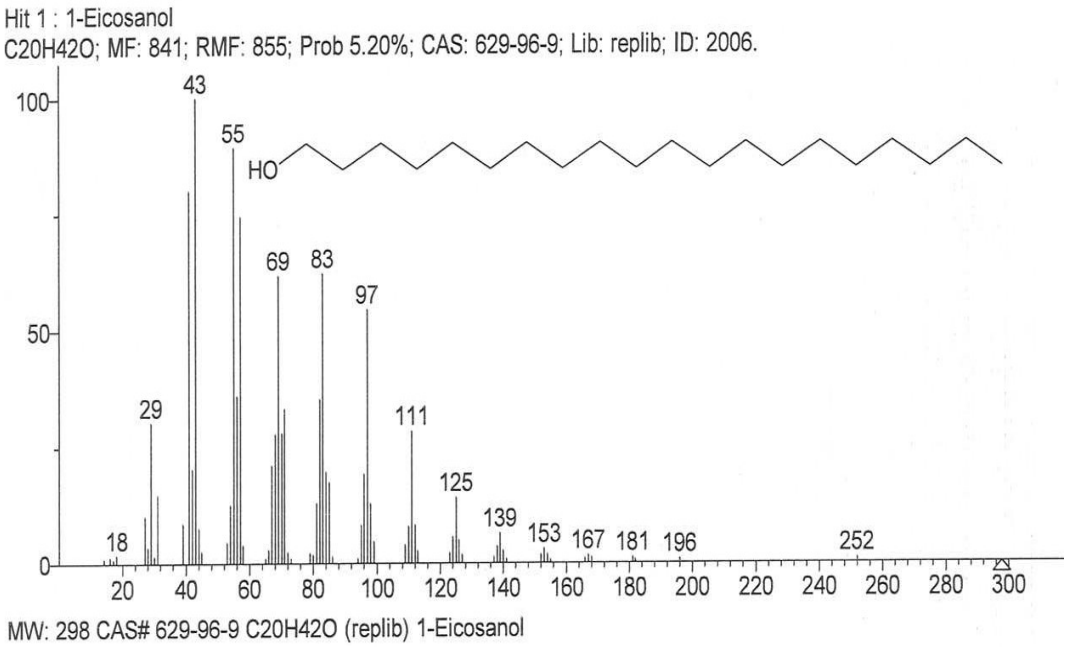

**Figure 51 Dodecyl acrylate**




Name: Tetradecanoic acid
Formula: $C_{14}H_{28}O_2$
MW: 228 Exact Mass: 228.20893 CAS#: 544-63-8 NIST#: 379632 ID#: 9042 DB: replib
Other DBs: Fine, TSCA, RTECS, EPA, HODOC, NIH, EINECS, IRDB
Contributor: Drug Lab
10 largest peaks:
73 999 | 60 778 | 43 520 | 129 495 | 57 467 | 55 391 | 185 288 | 71 287 | 69 261 | 87 201 |

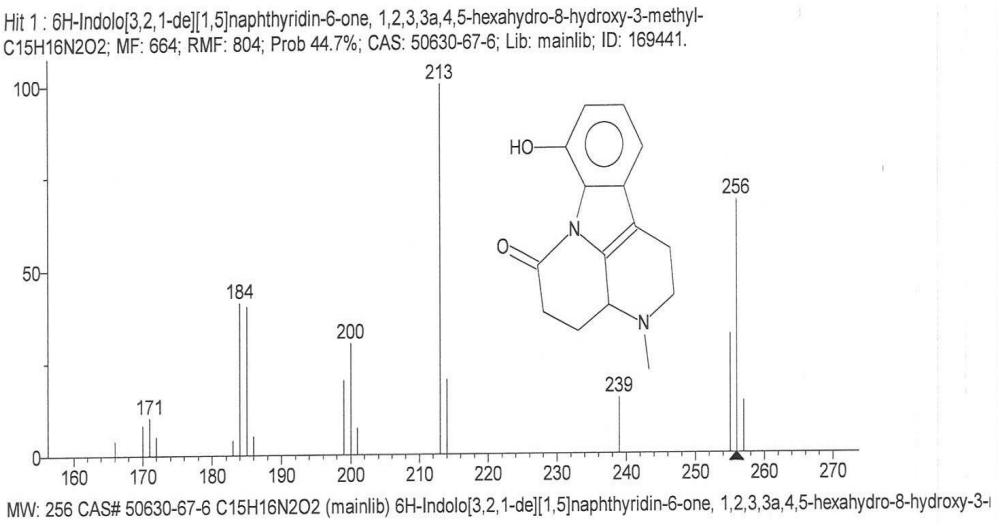
**Figure 52 Tetradecanoic acid**

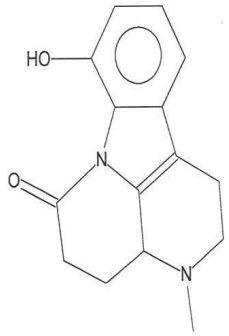




Name: 1-Eicosanol
Formula: $C_{20}H_{42}O$
MW: 298 Exact Mass: 298.323566 CAS#: 629-96-9 NIST#: 113075 ID#: 2006 DB: replib
Other DBs: Fine, TSCA, HODOC, NIH, EINECS, IRDB
Contributor: NIST Mass Spectrometry Data Center, 1990.
10 largest peaks:
43 999 | 55 893 | 41 799 | 57 743 | 83 620 | 69 617 | 97 542 | 56 358 | 82 350 | 71 330 |

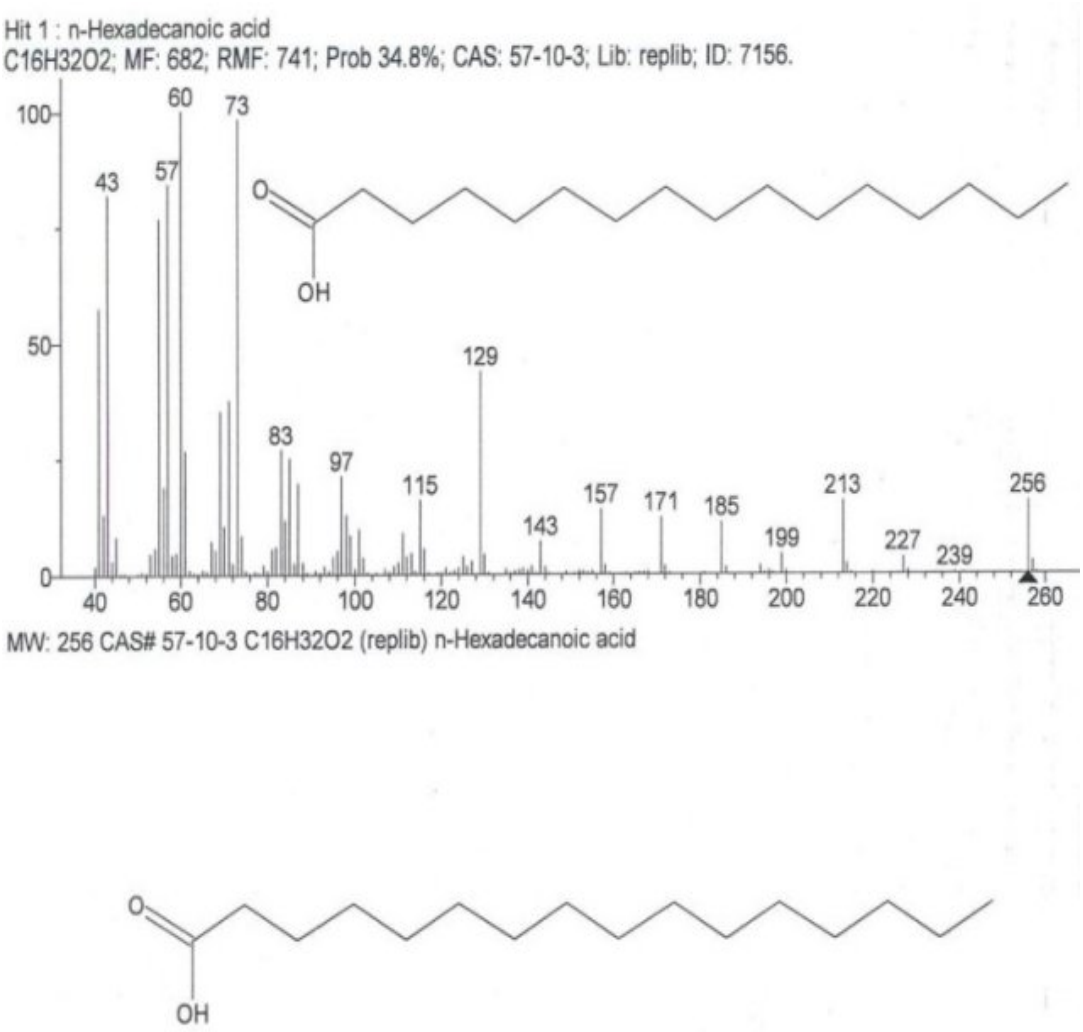
**Figure 53 1-Eicosanol**




Name: 6H-Indolo[3,2,1-de][1,5]naphthyridin-6-one, 1,2,3,3a,4,5-hexahydro-8-hydroxy-3-methyl-
Formula: $C_{15}H_{16}N_2O_2$
MW: 256 Exact Mass: 256.121178 CAS#: 50630-67-6 NIST#: 32664 ID#: 169441 DB: mainlib
Other DBs: None
10 largest peaks:
213 999 | 256 679 | 184 410 | 185 400 | 255 320 | 200 300 | 199 200 | 214 200 | 239 150 | 257 140 |

**Figure 54 6H-Indol(3,2,1-de)(1,4)naphthyridin-6-one, 1,2,3,3a,4,4-hexahydro-8-hydroxy-3-methyl-**





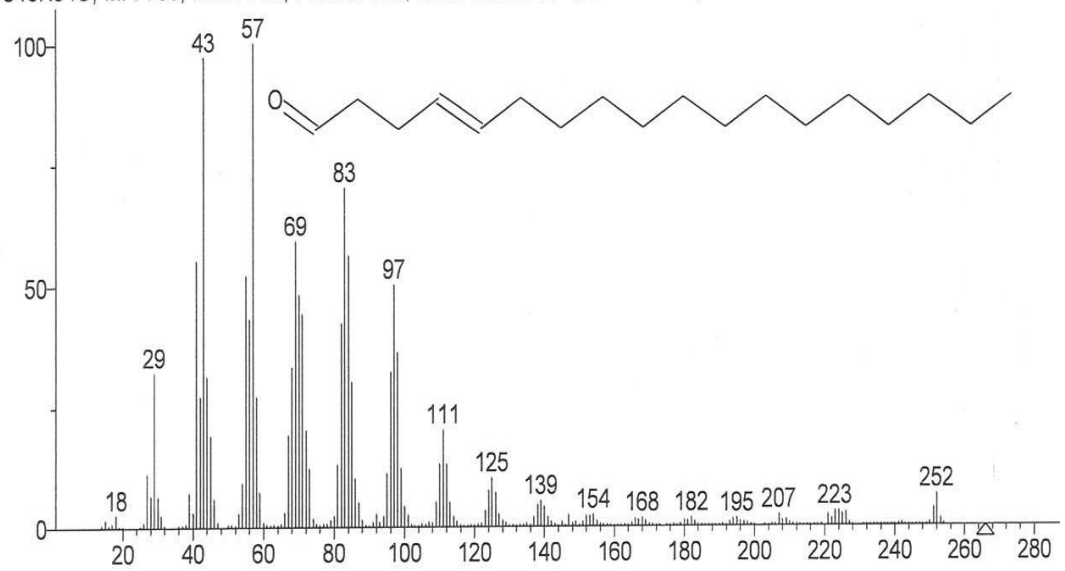


Name: n-Hexadecanoic acid
Formula: $C_{16}H_{32}O_2$
MW: 256 Exact Mass: 256.24023 CAS#: 57-10-3 NIST#: 335494 ID#: 7156 DB: replib
Other DBs: Fine, TSCA, RTECS, EPA, HODOC, NIH, EINECS, IRDB
Contributor: Drug Lab
Related CAS#: 60605-23-4; 116860-99-2; 212625-86-0
10 largest peaks:
60 999 | 73 980 | 57 840 | 43 817 | 55 767 | 41 574 | 129 435 | 71 373 | 69 351 | 83 267

**Figure 55 n-Hexadecanoic acid**

Hit 1 : 4-Octadecenal

C18H34O; MF: 795; RMF: 798; Prob 6.49%; CAS: 56554-98-4; Lib: mainlib; ID: 22760.




MW: 266 CAS# 56554-98-4 C18H34O (mainlib) 4-Octadecenal


Name: 4-Octadecenal

Formula: $C_{18}H_{34}O$

MW: 266 Exact Mass: 266.260965 CAS#: 56554-98-4 NIST#: 36166 ID#: 22760 DB: mainlib

Other DBs: None

Contributor: R.T.HOLMAN,UNIVERSITY OF MINNESOTA

10 largest peaks:

57 999 | 43 970 | 83 700 | 69 590 | 84 560 | 41 550 | 55 520 | 97 500 | 70 480 | 71 440 |

**Figure 56 4-Octadecenal**

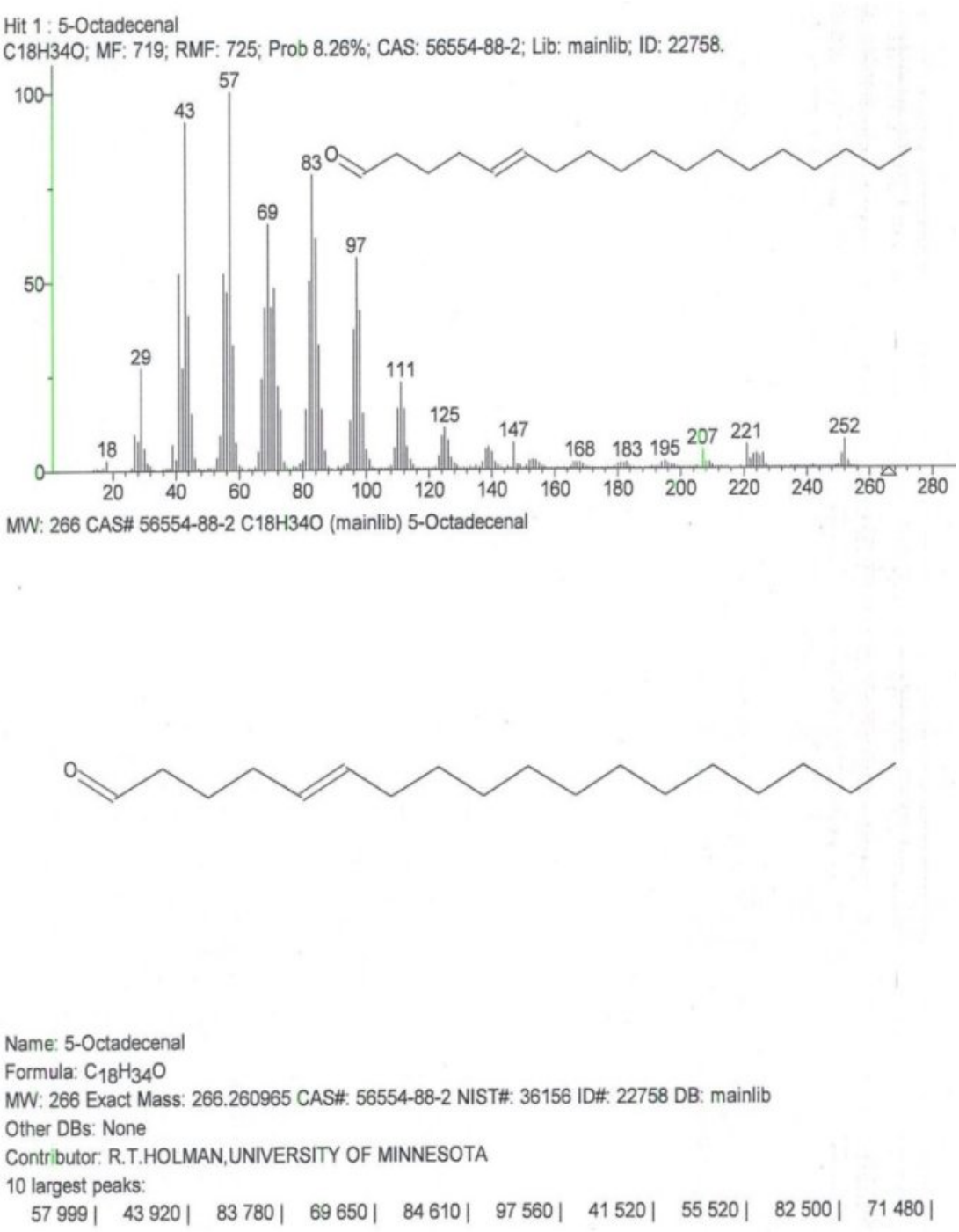
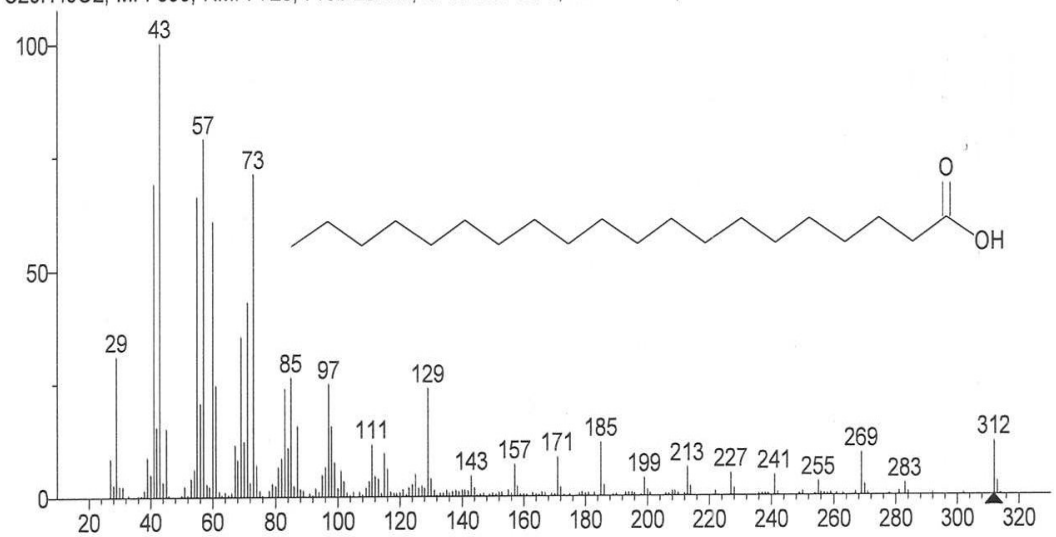
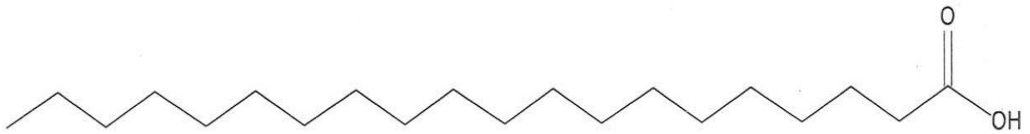



**Figure 57 4-Octadecenal**

Hit 1 : Eicosanoic acid
C20H40O2; MF: 699; RMF: 728; Prob 29.3%; CAS: 506-30-9; Lib: mainlib; ID: 7492.




MW: 312 CAS# 506-30-9 C20H40O2 (mainlib) Eicosanoic acid




Name: Eicosanoic acid
Formula: $C_{20}H_{40}O_2$
MW: 312 Exact Mass: 312.30283 CAS#: 506-30-9 NIST#: 160470 ID#: 7492 DB: mainlib
Other DBs: Fine, TSCA, RTECS, HODOC, NIH, EINECS, IRDB
Contributor: Chemical Concepts
10 largest peaks:
43 999 | 57 787 | 73 709 | 41 688 | 55 659 | 60 605 | 71 429 | 69 352 | 29 308 | 85 261 |

**Figure 58 Eicosanoic acid**

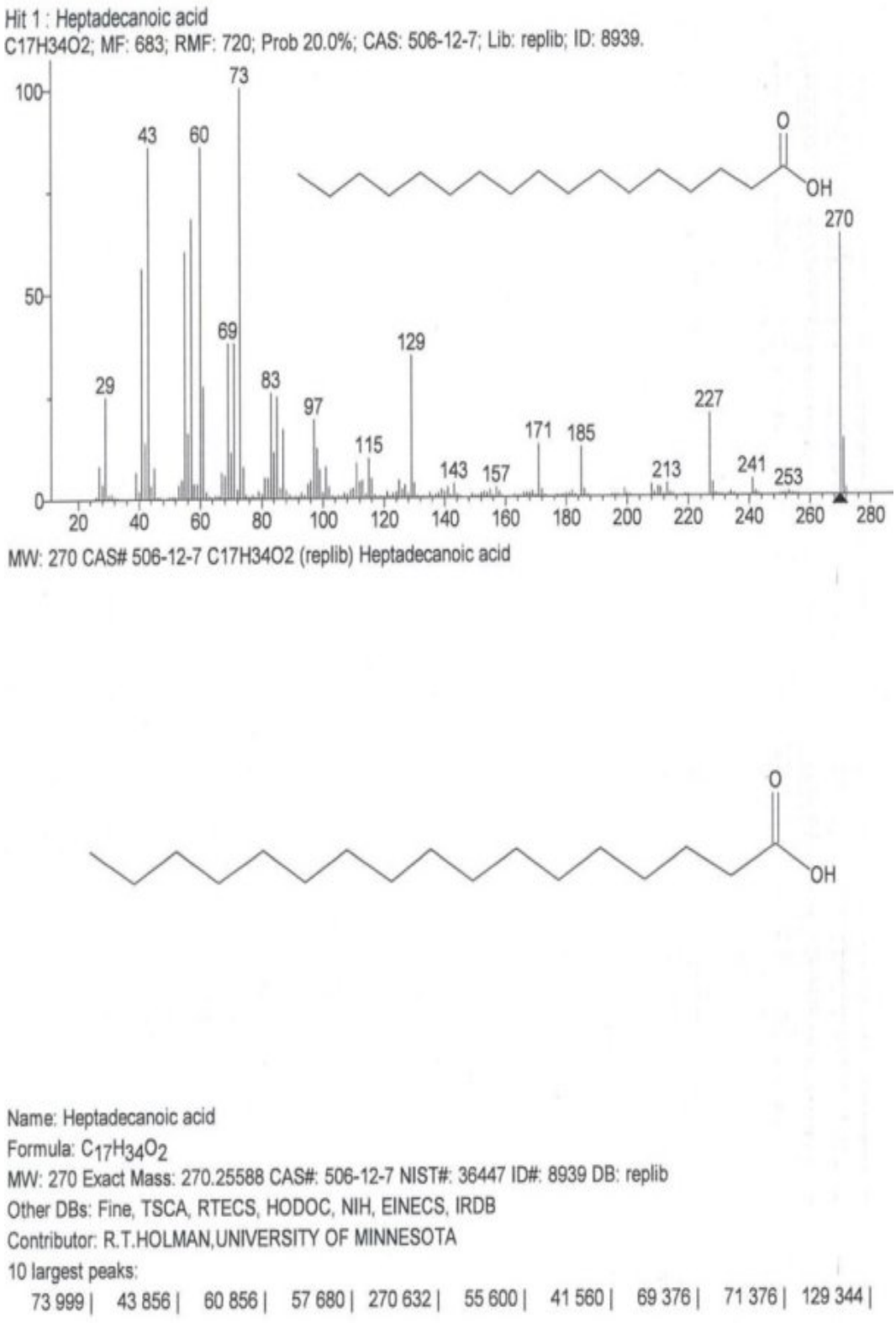
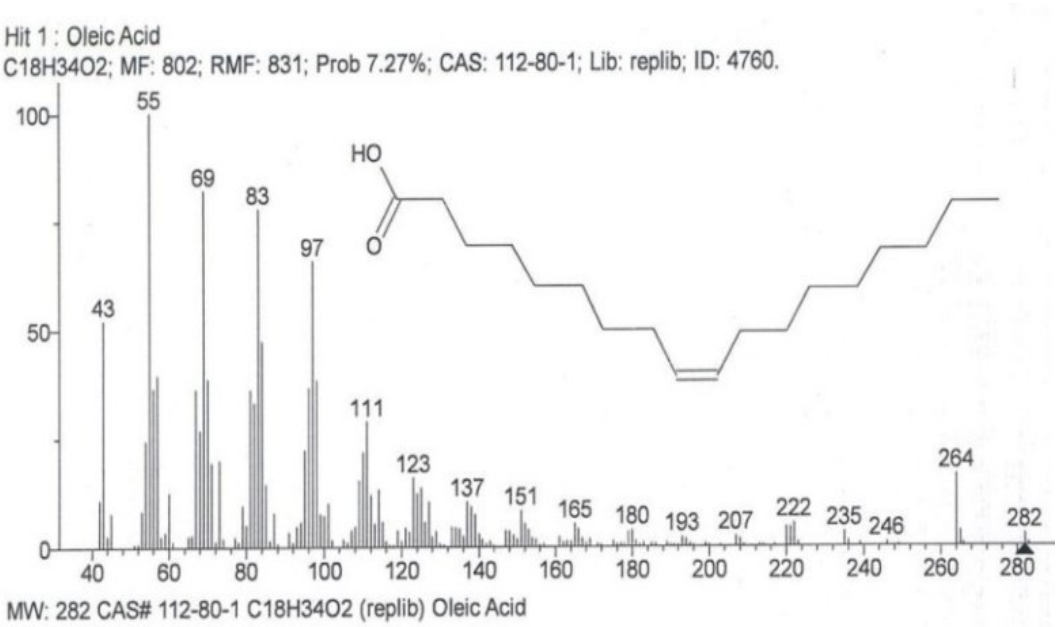
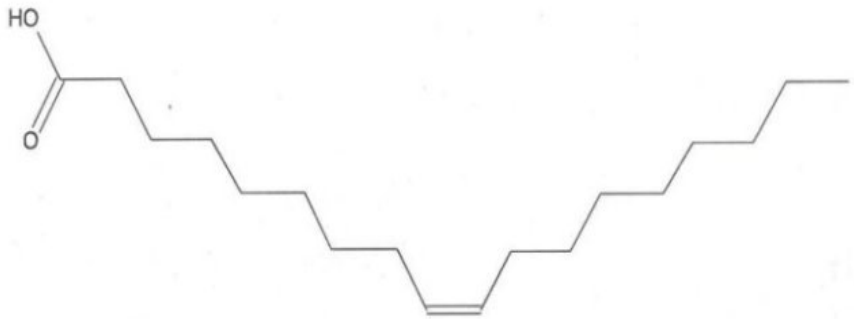


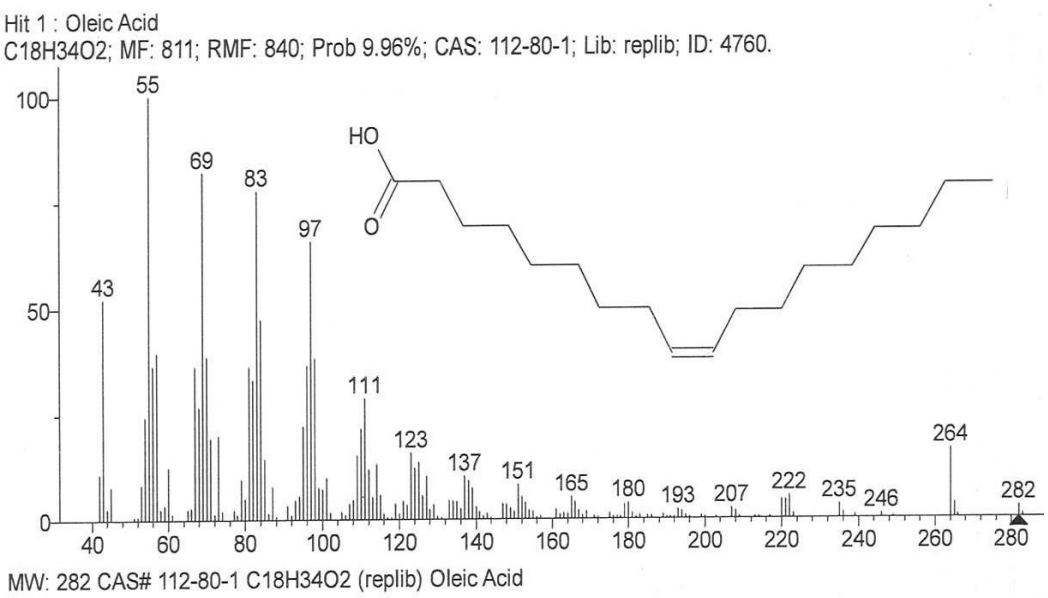

**Figure 59 Heptadecanoic acid**

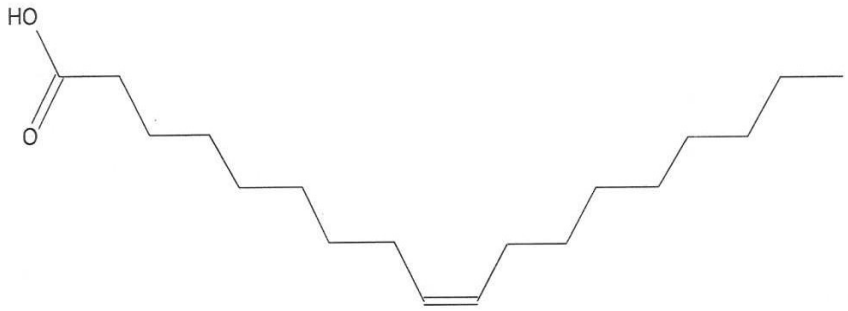



Name: Oleic Acid
Formula: $C_{18}H_{34}O_2$
MW: 282 Exact Mass: 282.25588 CAS#: 112-80-1 NIST#: 379354 ID#: 4760 DB: replib
Other DBs: TSCA, RTECS, USP, HODOC, NIH, EINECS, IRDB
Contributor: Drug Lab
Related CAS#: 56833-51-3; 8046-01-3; 949900-16-7
10 largest peaks:
55 999 | 69 819 | 83 775 | 97 656 | 43 519 | 84 470 | 57 392 | 70 383 | 98 381 | 96 364 |

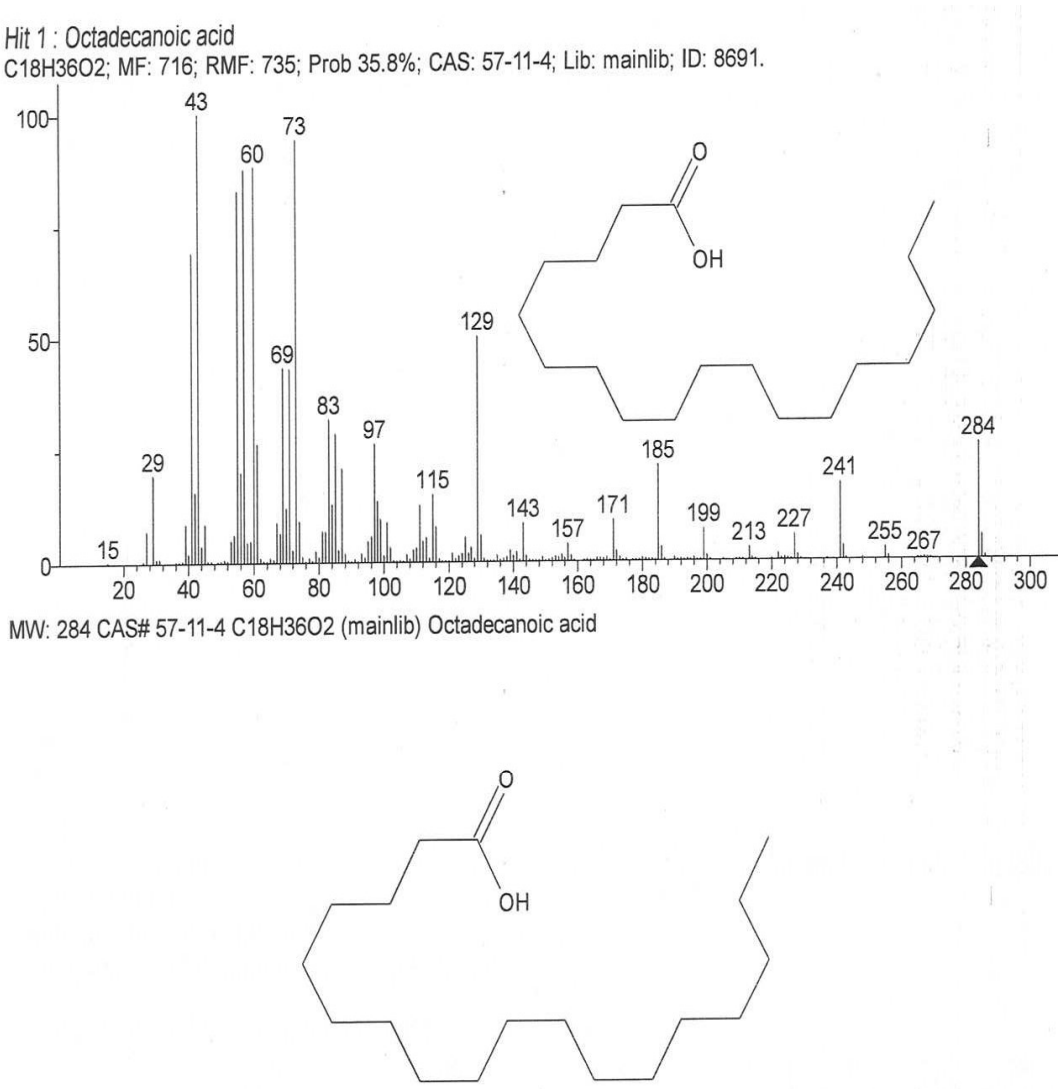
**Figure 60 Oleic acid**




Name: Oleic Acid
Formula: $C_{18}H_{34}O_2$
MW: 282 Exact Mass: 282.25588 CAS#: 112-80-1 NIST#: 379354 ID#: 4760 DB: replib
Other DBs: TSCA, RTECS, USP, HODOC, NIH, EINECS, IRDB
Contributor: Drug Lab
Related CAS#: 56833-51-3; 8046-01-3; 949900-16-7
10 largest peaks:
55 999 | 69 819 | 83 775 | 97 656 | 43 519 | 84 470 | 57 392 | 70 383 | 98 381 | 96 364

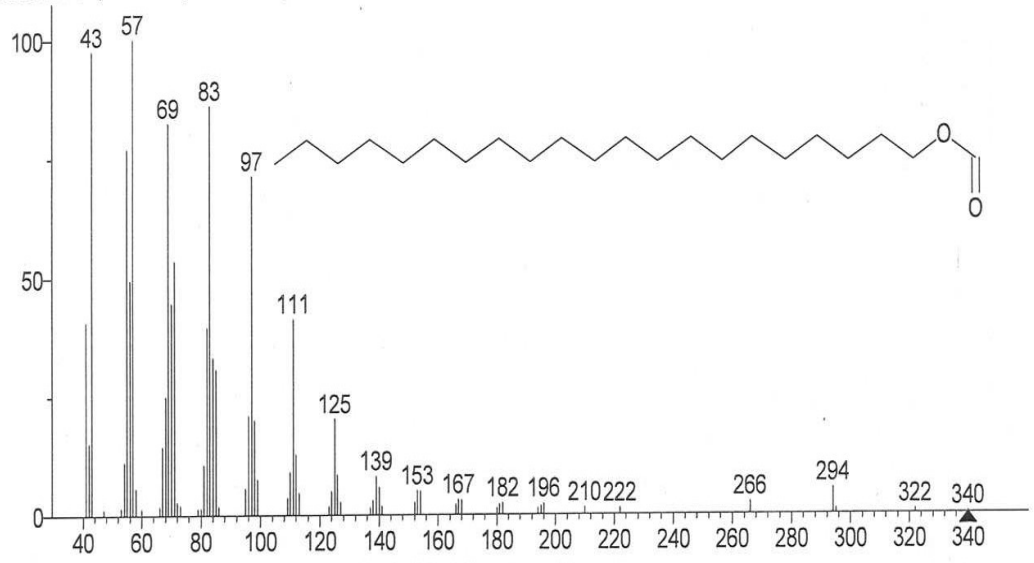
**Figure 61 Oleic acid**



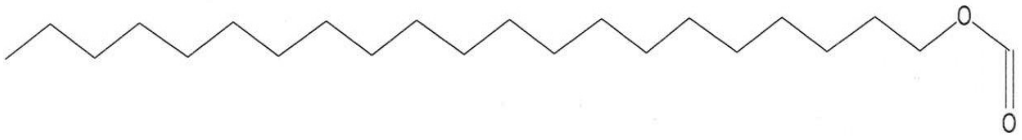

Name: Octadecanoic acid
Formula: $C_{18}H_{36}O_2$
MW: 284 Exact Mass: 284.27153 CAS#: 57-11-4 NIST#: 290961 ID#: 8691 DB: mainlib
Other DBs: Fine, TSCA, RTECS, EPA, HODOC, NIH, EINECS, IRDB
Contributor: NIST Mass Spectrometry Data Center, 1998.
Related CAS#: 8037-40-9; 197923-10-7; 294203-07-9; 39390-61-9; 58392-66-8; 8013-28-3; 8023-06-1; 8037-83-0; 8039-51-8; 8039-52-9; 8039-53-0; 8039-54-1
10 largest peaks:
43 999 | 73 942 | 60 881 | 57 875 | 55 827 | 41 688 | 129 500 | 69 432 | 71 429 | 83 317 |

**Figure 62 Octadecanoic acid**

Hit 1 : 1-Heneicosyl formate
C22H44O2; MF: 818; RMF: 867; Prob 4.08%; CAS: 77899-03-7; Lib: mainlib; ID: 22753.




MW: 340 CAS# 77899-03-7 C22H44O2 (mainlib) 1-Heneicosyl formate

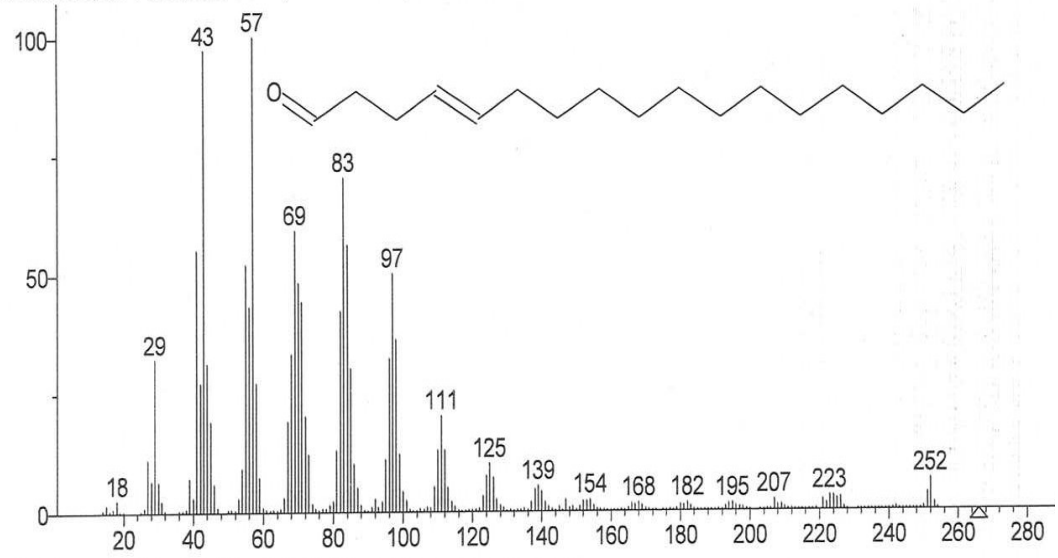



Name: 1-Heneicosyl formate
Formula: $C_{22}H_{44}O_2$
MW: 340 Exact Mass: 340.334131 CAS#: 77899-03-7 NIST#: 72853 ID#: 22753 DB: mainlib
Other DBs: None
Contributor: B.V. BURGER, DEP. CHEM., UNIV. STELLENBOSCH, SOUTH AFRIC
10 largest peaks:
57 999 | 43 973 | 83 859 | 69 823 | 55 768 | 97 710 | 71 533 | 56 492 | 70 444 | 111 411 |

**Figure 63 1-Heneicosyl formate**

Hit 1 : 4-Octadecenal

C18H34O; MF: 805; RMF: 812; Prob 10.0%; CAS: 56554-98-4; Lib: mainlib; ID: 22760.




MW: 266 CAS# 56554-98-4 C18H34O (mainlib) 4-Octadecenal

Name: 4-Octadecenal

Formula: $C_{18}H_{34}O$

MW: 266 Exact Mass: 266.260965 CAS#: 56554-98-4 NIST#: 36166 ID#: 22760 DB: mainlib

Other DBs: None

Contributor: R.T.HOLMAN,UNIVERSITY OF MINNESOTA

10 largest peaks:

57 999 | 43 970 | 83 700 | 69 590 | 84 560 | 41 550 | 55 520 | 97 500 | 70 480 | 71 440 |

**Figure 64 4-Octadecenal**

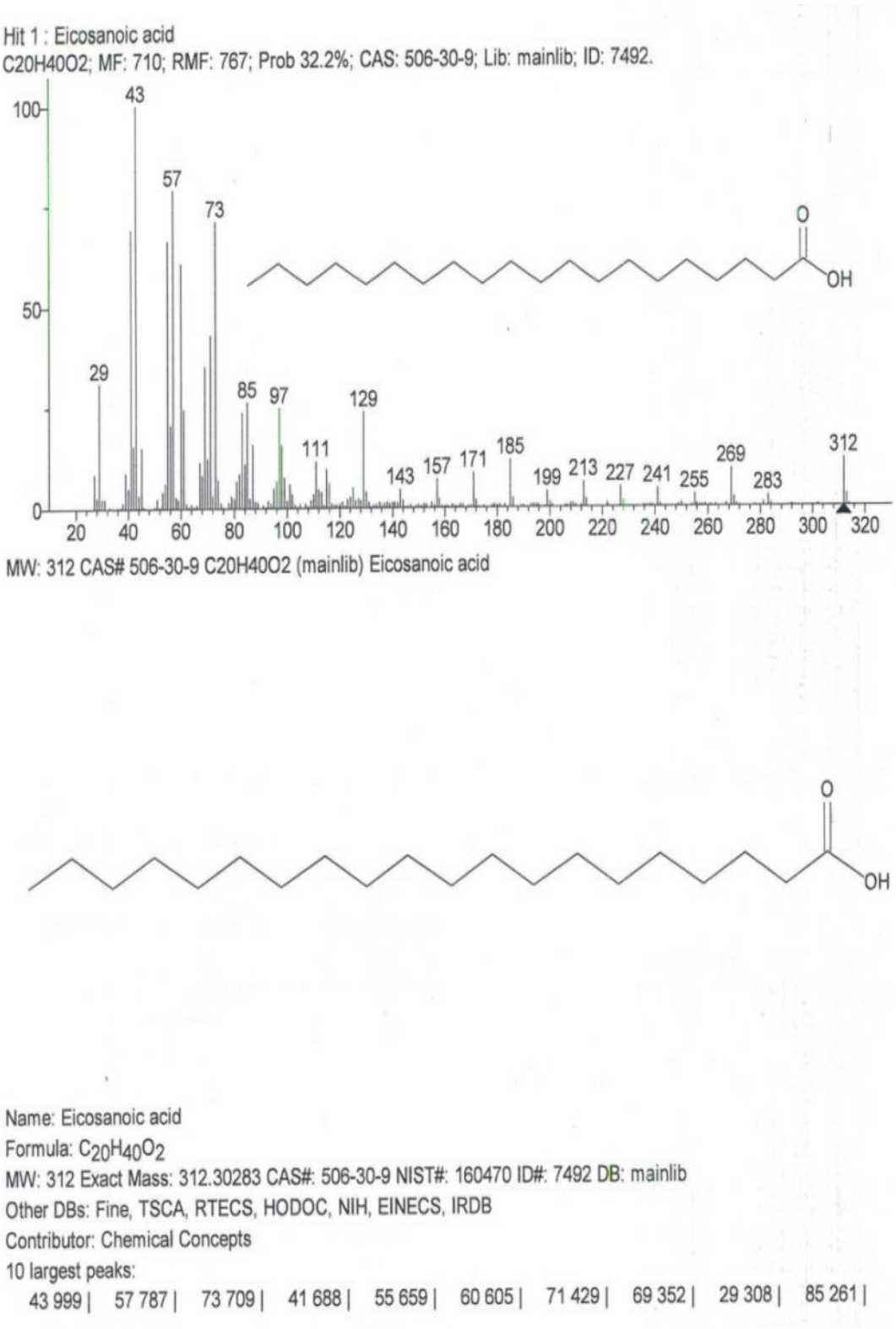
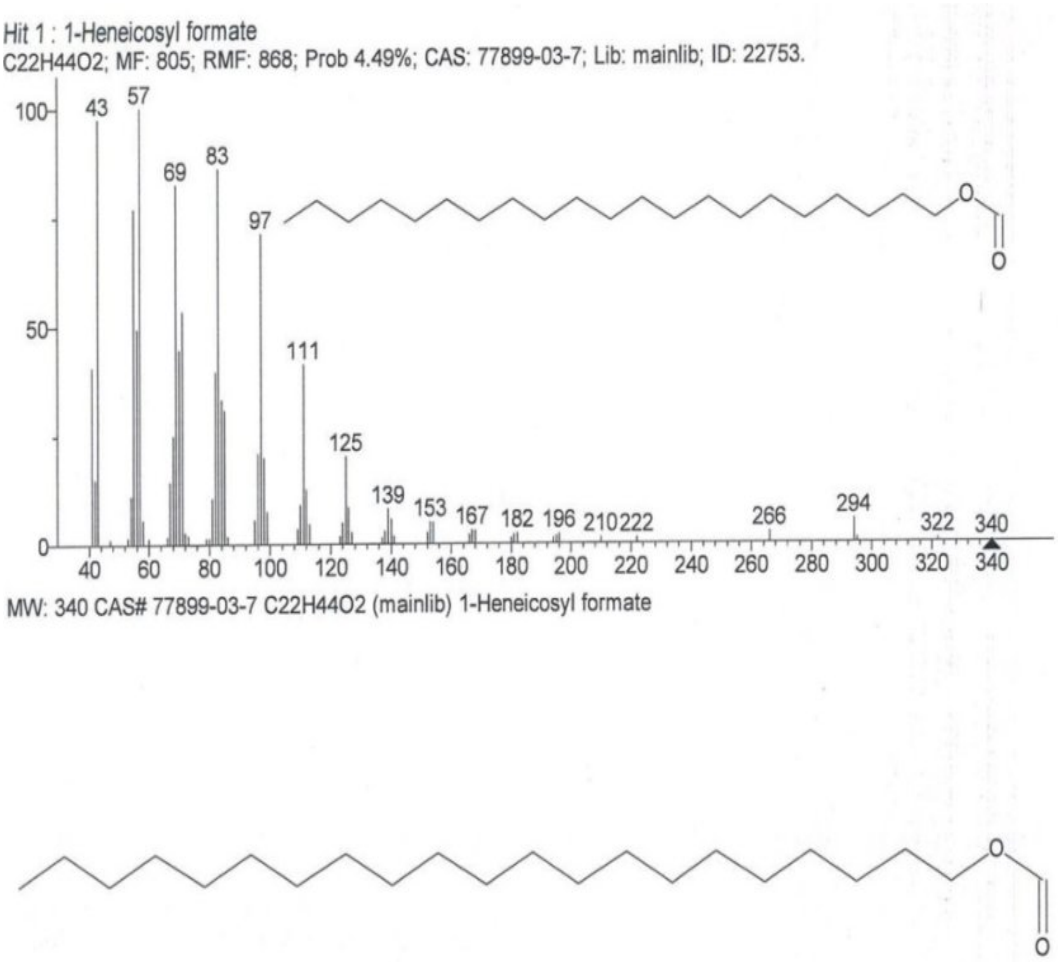
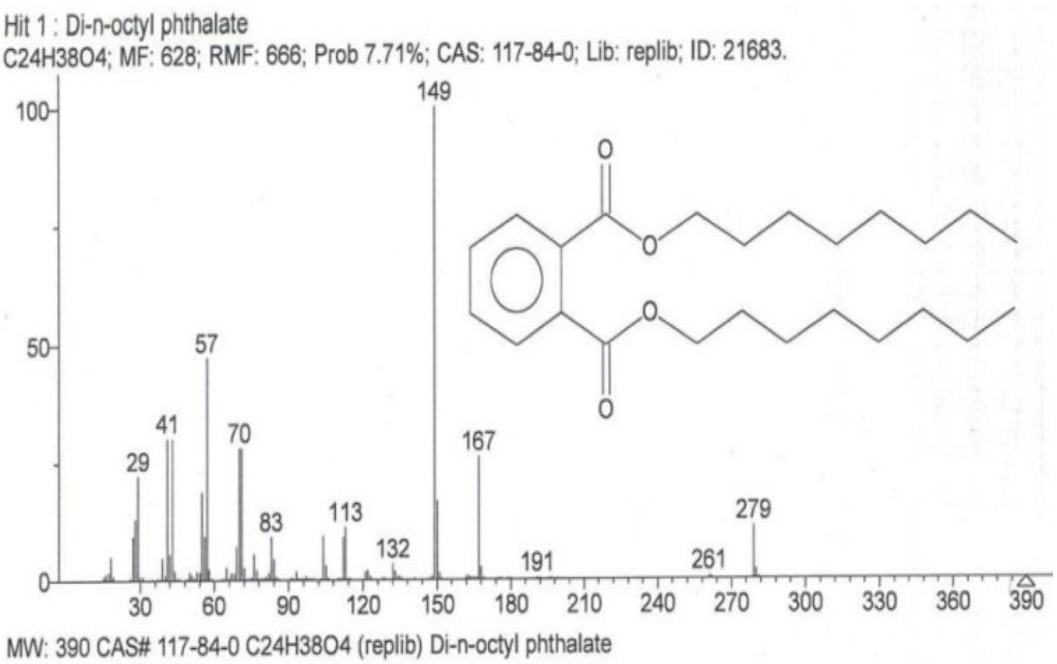


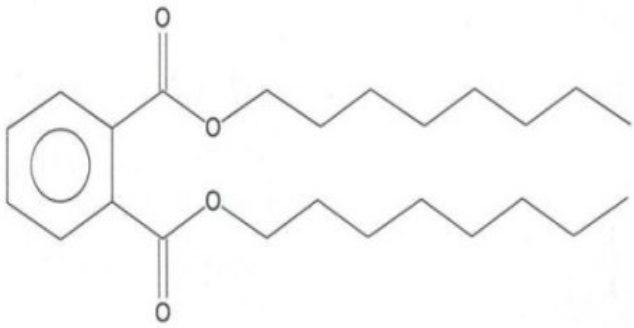

**Figure 65 Eicosanoic acid**




Name: 1-Heneicosyl formate
Formula: $C_{22}H_{44}O_2$
MW: 340 Exact Mass: 340.334131 CAS#: 77899-03-7 NIST#: 72853 ID#: 22753 DB: mainlib
Other DBs: None
Contributor: B.V. BURGER, DEP. CHEM., UNIV. STELLENBOSCH, SOUTH AFRIC
10 largest peaks:
57 999 | 43 973 | 83 859 | 69 823 | 55 768 | 97 710 | 71 533 | 56 492 | 70 444 | 111 411 |

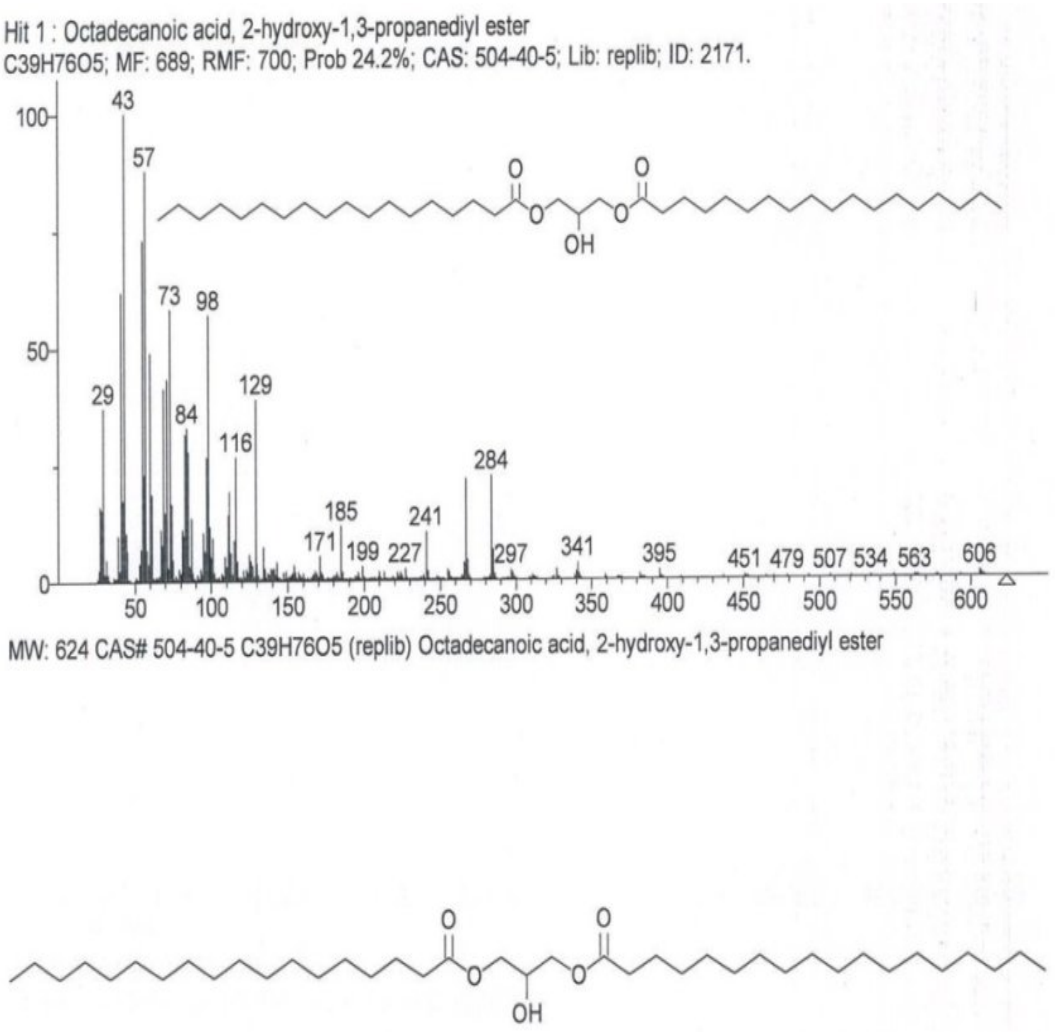
**Figure 66 1-Heneicosyl formate**




Name: Di-n-octyl phthalate
Formula: $C_{24}H_{38}O_4$
MW: 390 Exact Mass: 390.27701 CAS#: 117-84-0 NIST#: 23606 ID#: 21683 DB: replib
Other DBs: Fine, TSCA, RTECS, EPA, HODOC, NIH, EINECS, IRDB
10 largest peaks:
149 999 | 57 470 | 41 300 | 43 300 | 70 280 | 71 280 | 167 260 | 29 220 | 55 185 | 150 165 |

**Figure 67 Di-n-octyl phthalate**



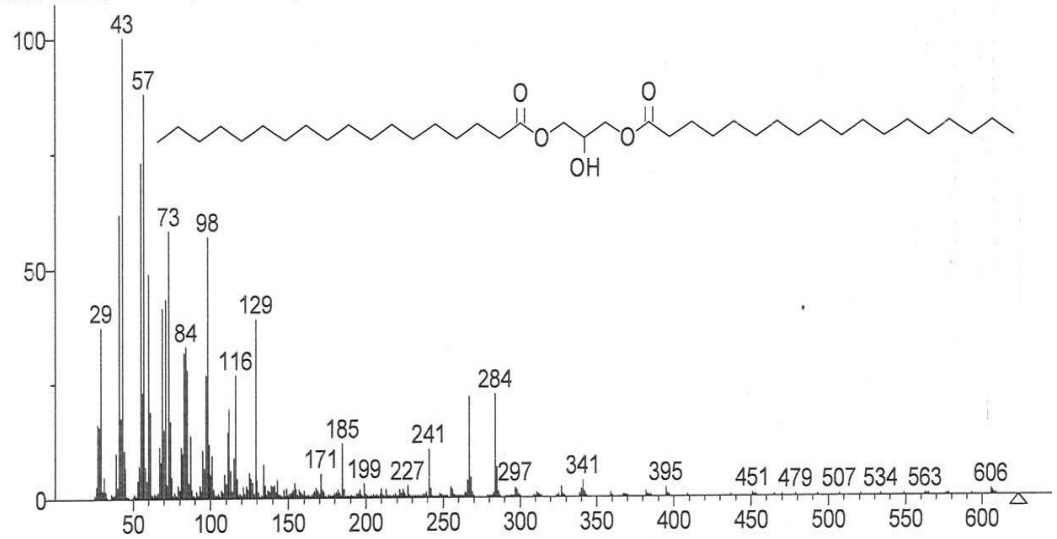




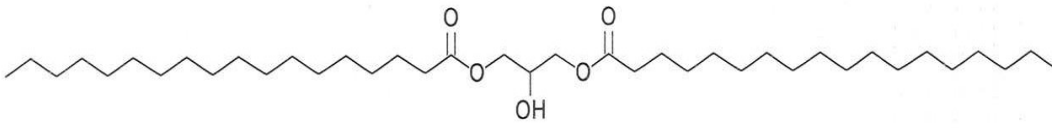
Name: Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester
Formula: $C_{39}H_{76}O_5$
MW: 624 Exact Mass: 624.569275 CAS#: 504-40-5 NIST#: 36227 ID#: 2171 DB: replib
Other DBs: HODOC, NIH, EINECS
Contributor: R.T.HOLMAN,UNIVERSITY OF MINNESOTA
10 largest peaks:
43 999 | 57 876 | 55 728 | 41 617 | 73 580 | 98 567 | 60 487 | 71 432 | 69 413 | 129 388 |

**Figure 68 Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester**

Hit 1 : Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester
C39H76O5; MF: 695; RMF: 704; Prob 20.5%; CAS: 504-40-5; Lib: replib; ID: 2171.




MW: 624 CAS# 504-40-5 C39H76O5 (replib) Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester

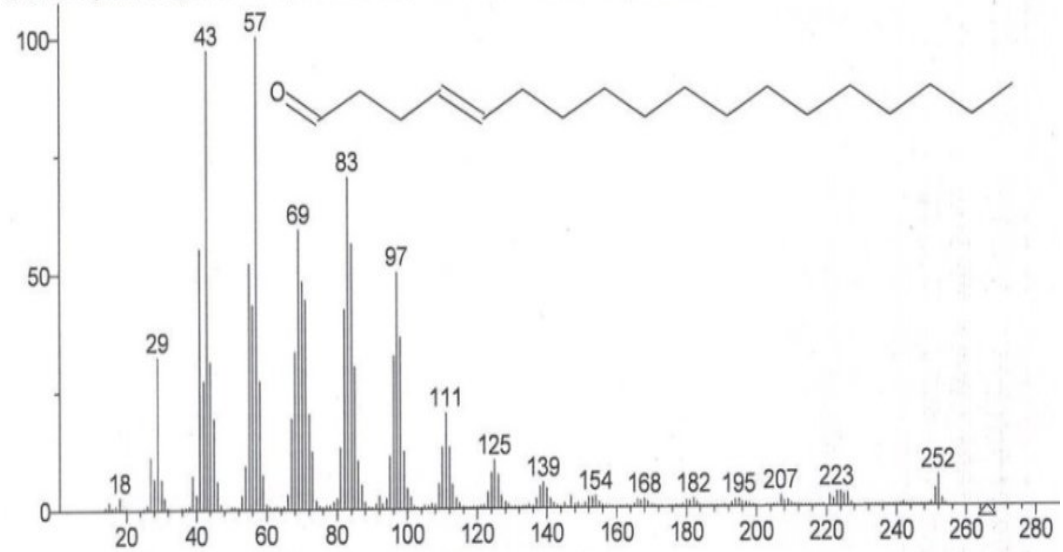



Name: Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester
Formula: $C_{39}H_{76}O_5$
MW: 624 Exact Mass: 624.569275 CAS#: 504-40-5 NIST#: 36227 ID#: 2171 DB: replib
Other DBs: HODOC, NIH, EINECS
Contributor: R.T.HOLMAN,UNIVERSITY OF MINNESOTA
10 largest peaks:
43 999 | 57 876 | 55 728 | 41 617 | 73 580 | 98 567 | 60 487 | 71 432 | 69 413 | 129 388 |

**Figure 69 Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester**

Hit 1 : 4-Octadecenal

C18H34O; MF: 788; RMF: 810; Prob 6.26%; CAS: 56554-98-4; Lib: mainlib; ID: 22760.




MW: 266 CAS# 56554-98-4 C18H34O (mainlib) 4-Octadecenal

Name: 4-Octadecenal

Formula: $C_{18}H_{34}O$

MW: 266 Exact Mass: 266.260965 CAS#: 56554-98-4 NIST#: 36166 ID#: 22760 DB: mainlib

Other DBs: None

Contributor: R.T.HOLMAN,UNIVERSITY OF MINNESOTA

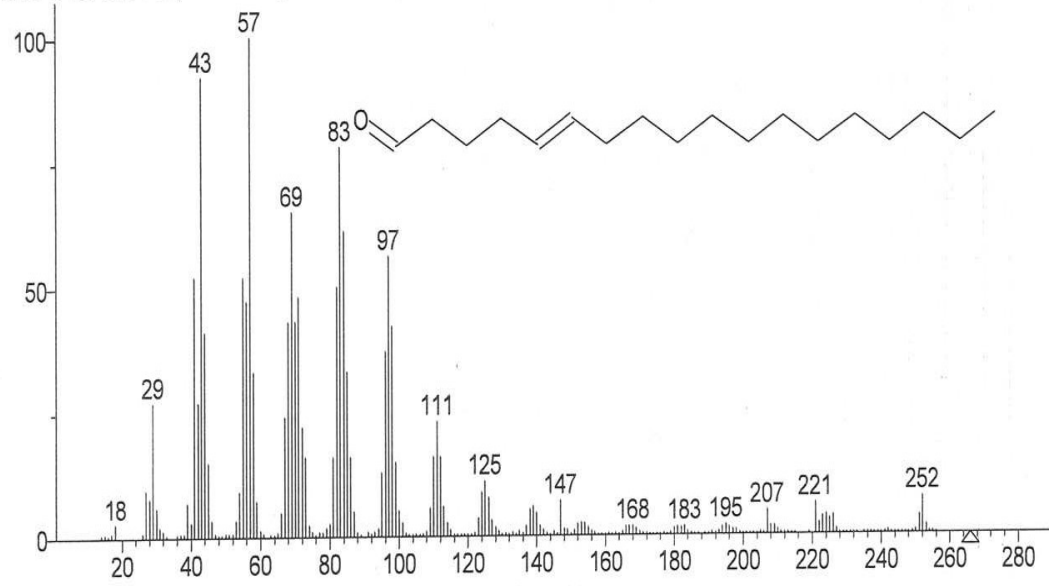
10 largest peaks:

57 999 | 43 970 | 83 700 | 69 590 | 84 560 | 41 550 | 55 520 | 97 500 | 70 480 | 71 440 |

**Figure 70 4-Octadecenal**

Hit 1 : 5-Octadecenal

C18H34O; MF: 767; RMF: 792; Prob 3.70%; CAS: 56554-88-2; Lib: mainlib; ID: 22758.




MW: 266 CAS# 56554-88-2 C18H34O (mainlib) 5-Octadecenal

Name: 5-Octadecenal

Formula: $C_{18}H_{34}O$

MW: 266 Exact Mass: 266.260965 CAS#: 56554-88-2 NIST#: 36156 ID#: 22758 DB: mainlib

Other DBs: None

Contributor: R.T.HOLMAN,UNIVERSITY OF MINNESOTA

10 largest peaks:

57 999 | 43 920 | 83 780 | 69 650 | 84 610 | 97 560 | 41 520 | 55 520 | 82 500 | 71 480 |

**Figure 71 4-Octadecenal**

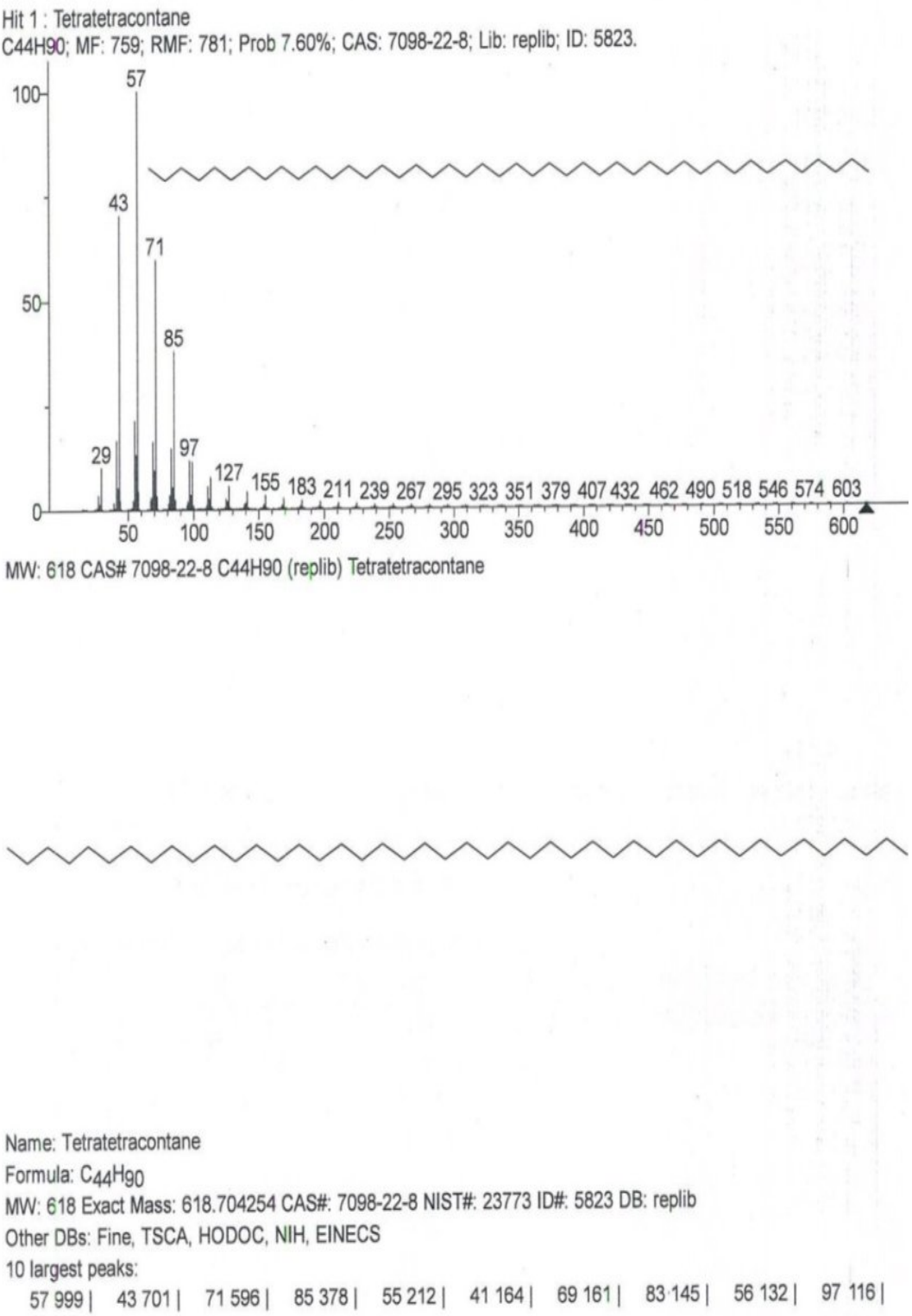



**Figure 72 Tetratetracontane**

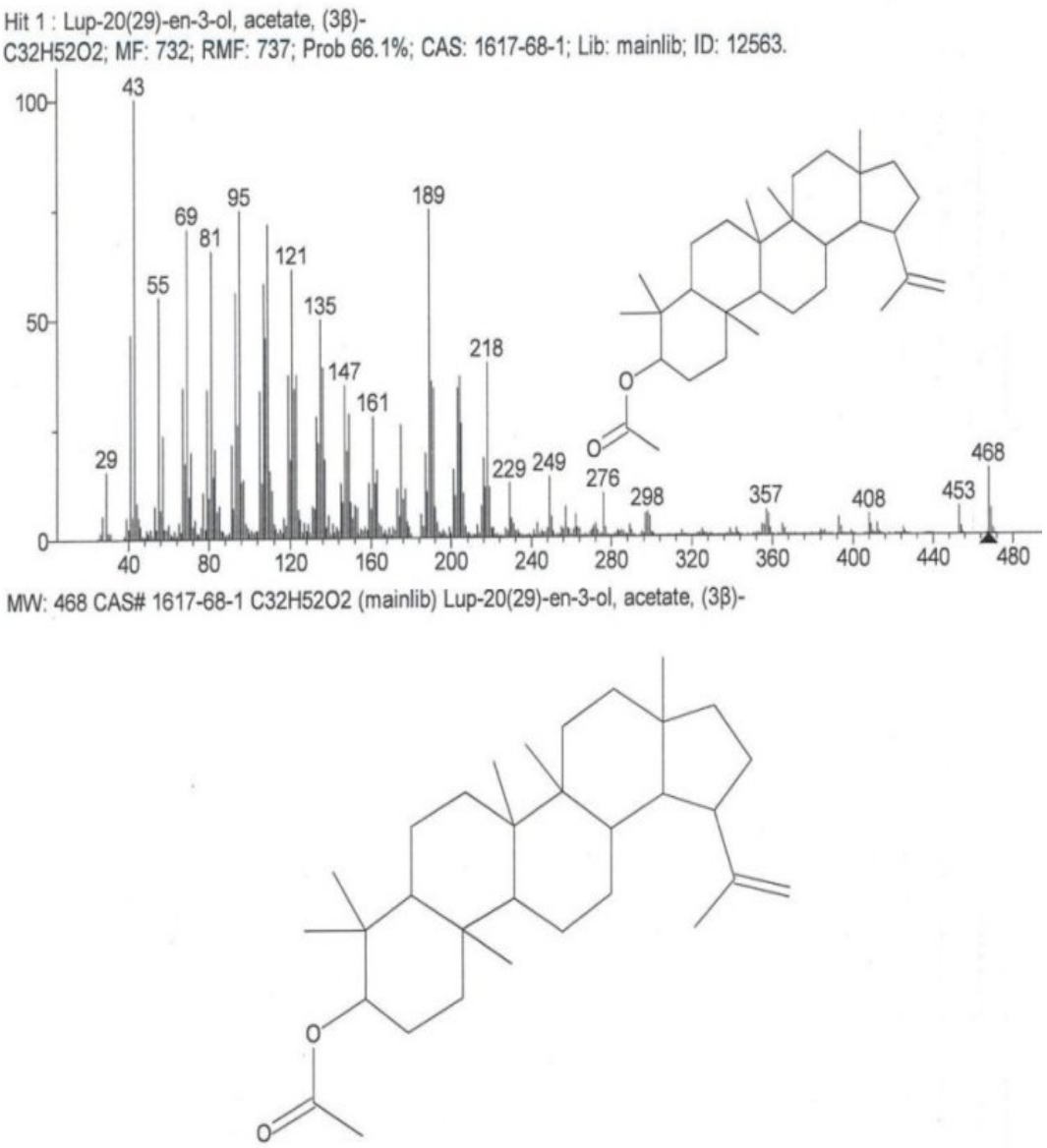



**Figure 73 Lup-20(29)-en-3-ol,acetate,(3beta)**

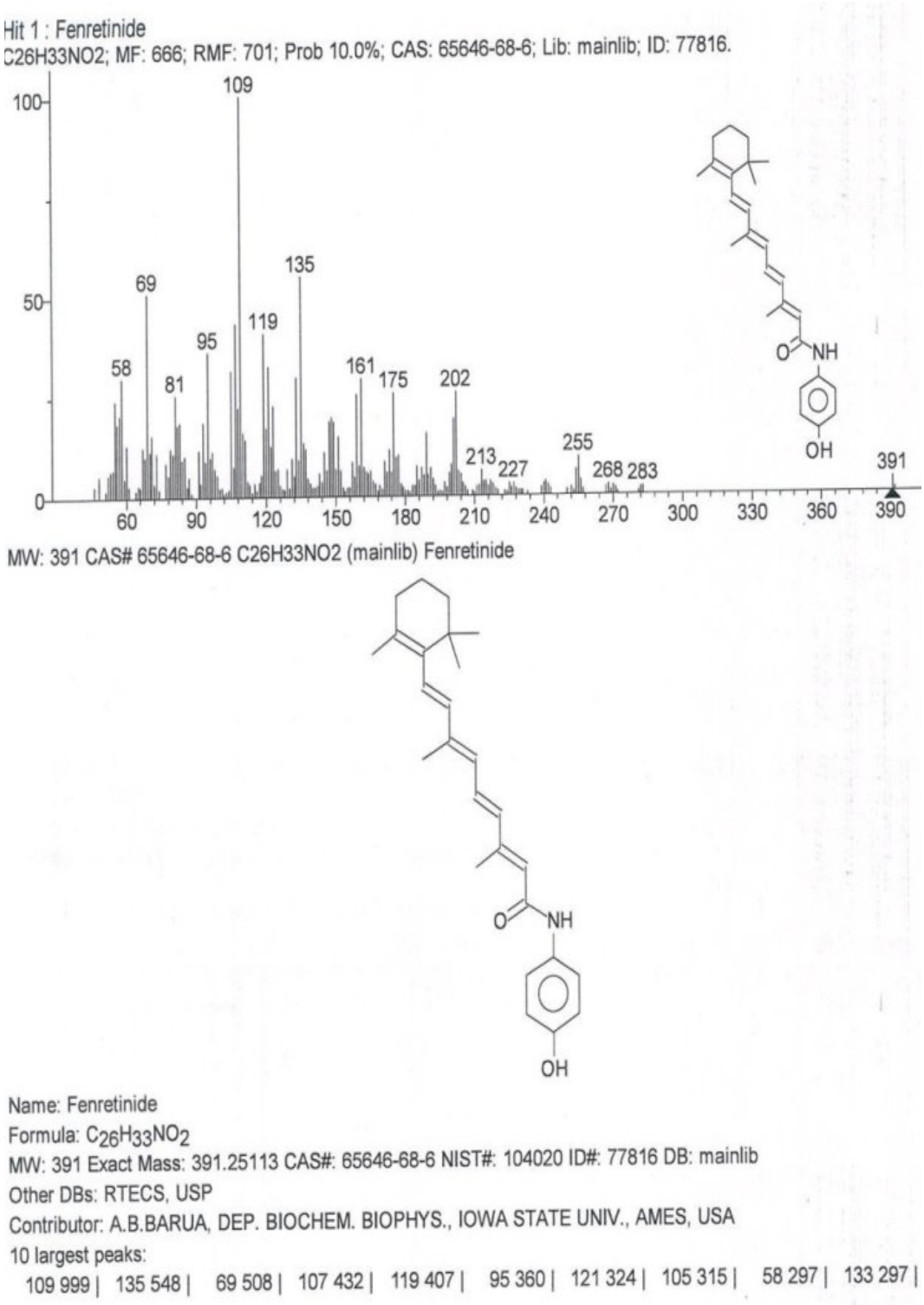
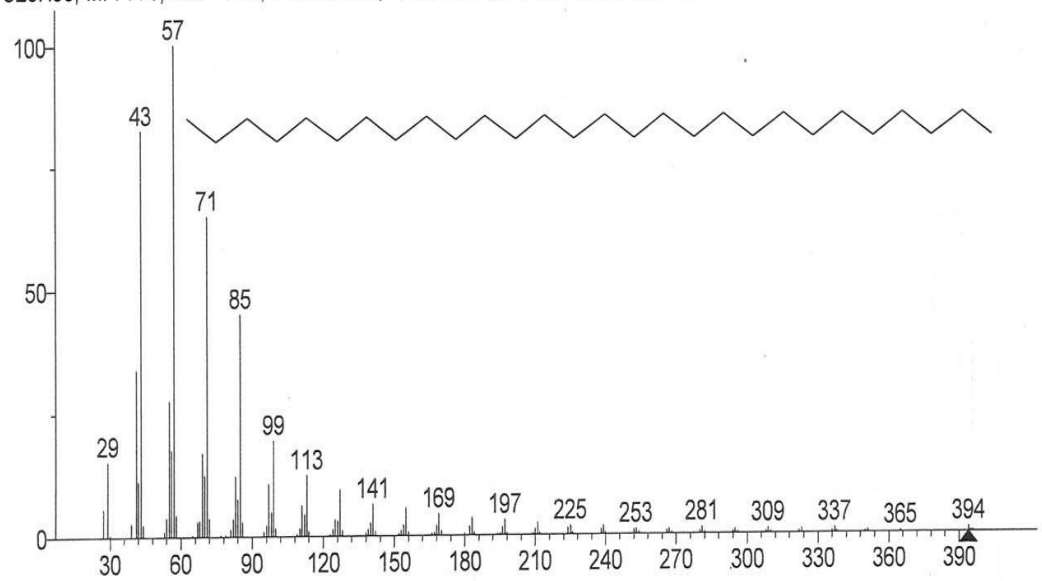



**Figure 74 Fenretinide**

Hit 1 : Octacosane

C28H58; MF: 776; RMF: 785; Prob 8.30%; CAS: 630-02-4; Lib: replib; ID: 5774.




MW: 394 CAS# 630-02-4 C28H58 (replib) Octacosane

Name: Octacosane

Formula: $C_{28}H_{58}$

MW: 394 Exact Mass: 394.453852 CAS#: 630-02-4 NIST#: 134306 ID#: 5774 DB: replib

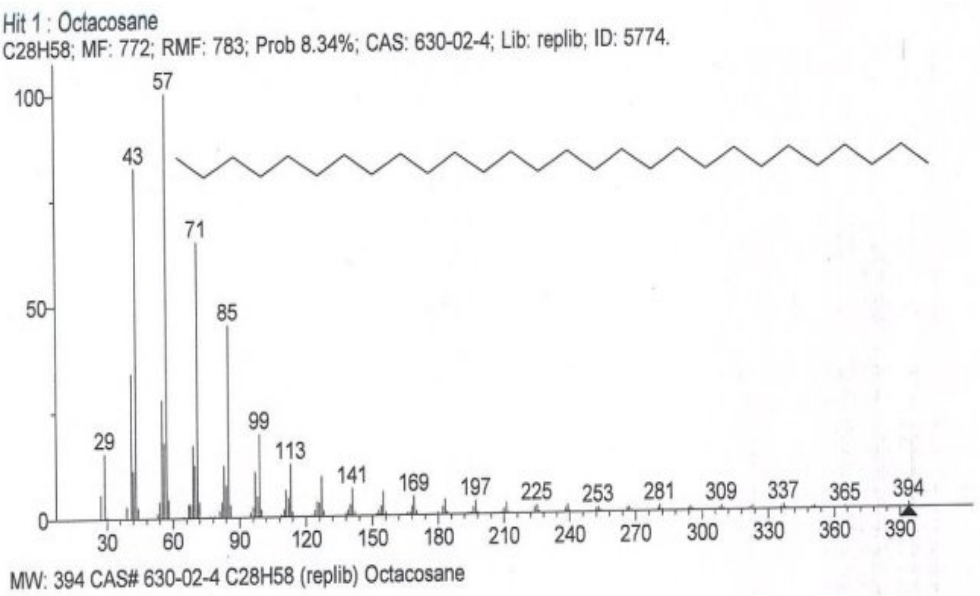
Other DBs: Fine, TSCA, EPA, HODOC, NIH, EINECS, IRDB

Contributor: NIST Mass Spectrometry Data Center, 1994

10 largest peaks:

57 999 | 43 823 | 71 647 | 85 449 | 41 337 | 55 274 | 99 193 | 56 174 | 69 168 | 29 151 |

**Figure 75 Octacosane**




Name: Octacosane
Formula: $C_{28}H_{58}$
MW: 394 Exact Mass: 394.453852 CAS#: 630-02-4 NIST#: 134306 ID#: 5774 DB: replib
Other DBs: Fine, TSCA, EPA, HODOC, NIH, EINECS, IRDB
Contributor: NIST Mass Spectrometry Data Center, 1994
10 largest peaks:
57 999 | 43 823 | 71 647 | 85 449 | 41 337 | 55 274 | 99 193 | 56 174 | 69 168 | 29 151 |

**Figure 76 Octacosane**

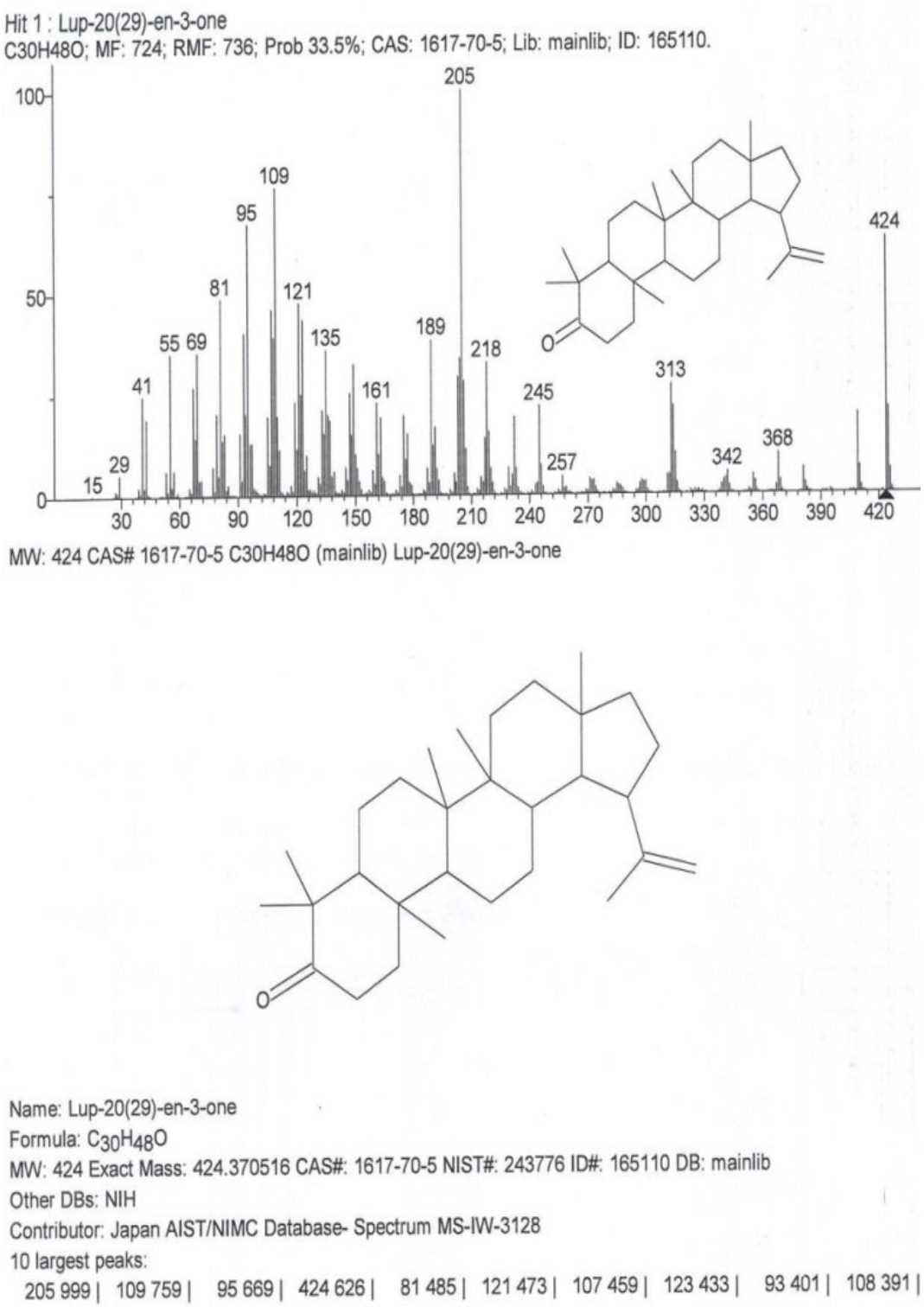
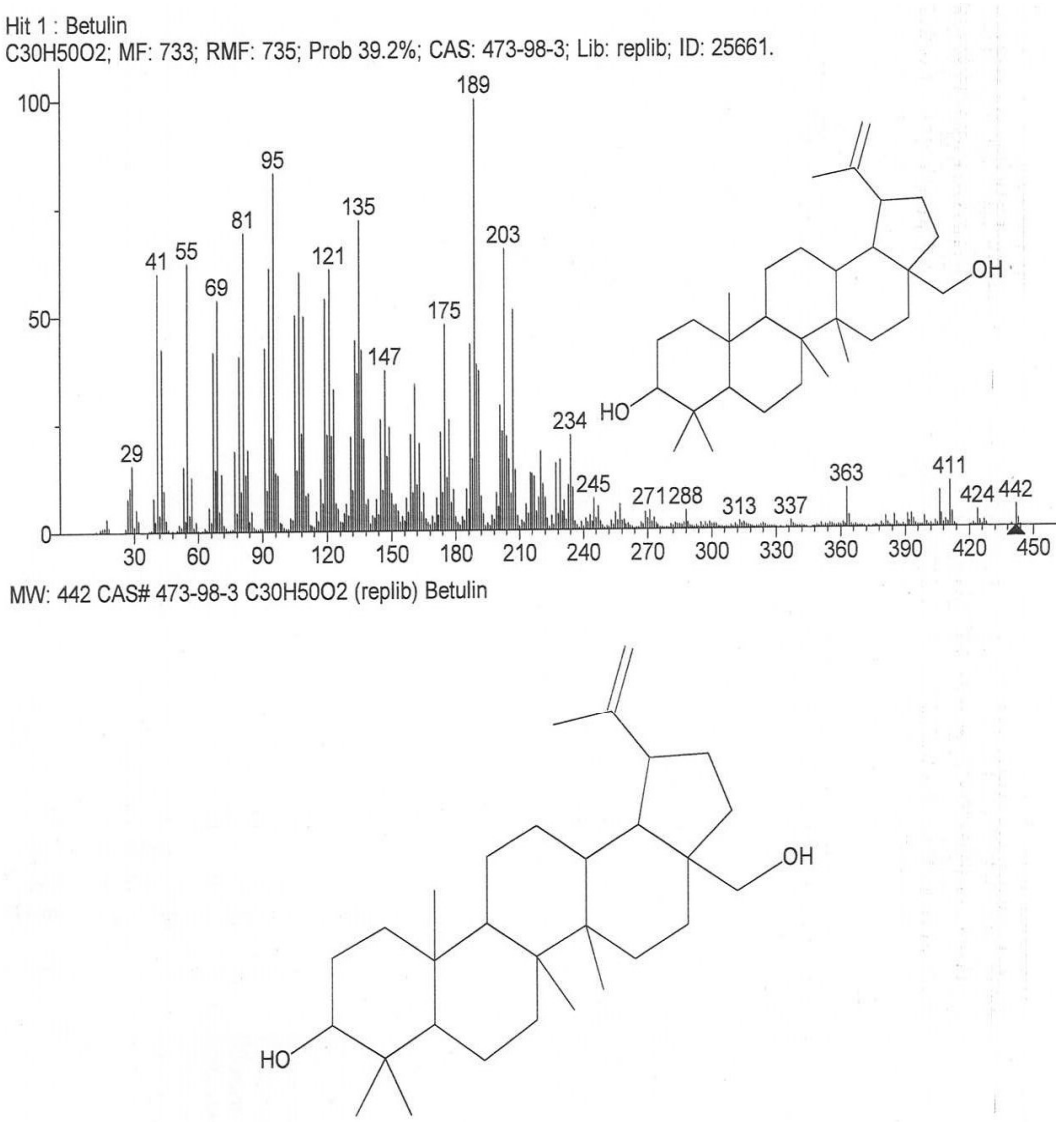


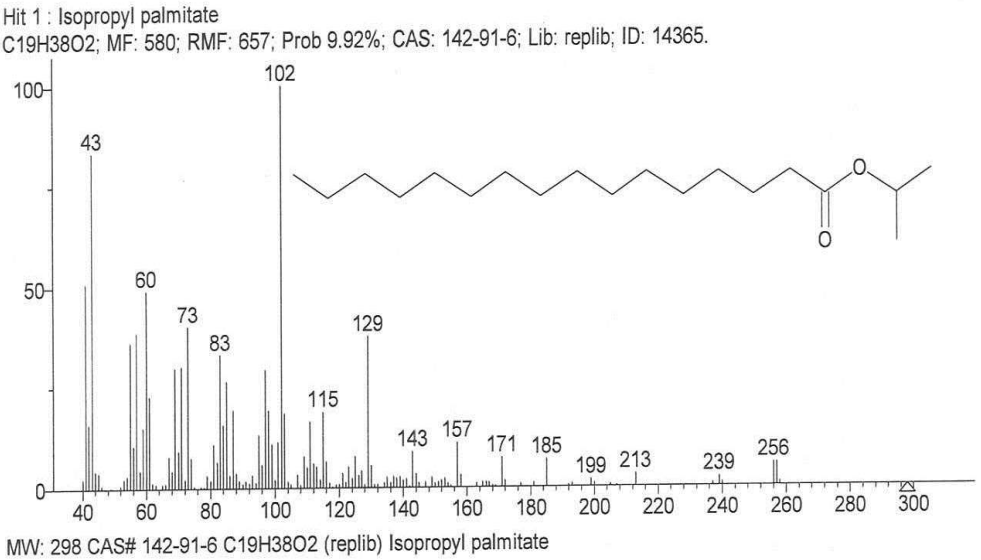

**Figure 77 Lup-20(29)-en-3-one**

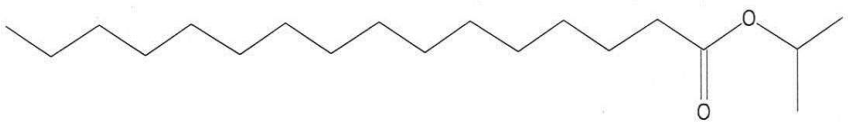



Name: Betulin
Formula: $C_{30}H_{50}O_2$
MW: 442 Exact Mass: 442.38108 CAS#: 473-98-3 NIST#: 234218 ID#: 25661 DB: replib
Other DBs: Fine, HODOC, NIH, EINECS
Contributor: Japan AIST/NIMC Database- Spectrum MS-NW-2414
Related CAS#: 1406-58-2
10 largest peaks:
189 999 | 95 830 | 135 720 | 81 691 | 203 653 | 55 621 | 93 609 | 121 606 | 107 601 | 41 598 |

**Figure 78 Betulin**

### 3.4 (b) GCMS chromatogram and compounds in ethanol extract of biherbal extract

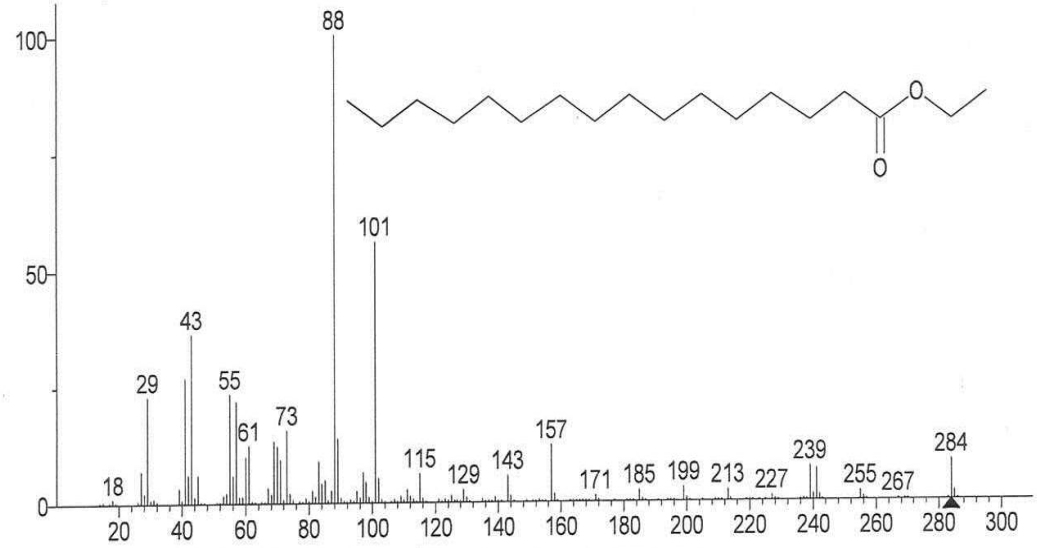


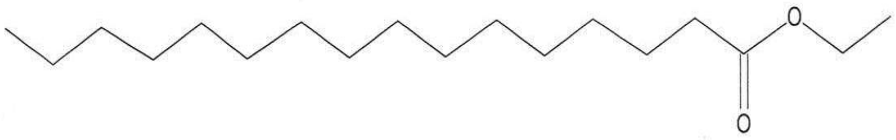

Name: Isopropyl palmitate
Formula: $C_{19}H_{38}O_2$
MW: 298 Exact Mass: 298.28718 CAS#: 142-91-6 NIST#: 70630 ID#: 14365 DB: replib
Other DBs: Fine, TSCA, RTECS, USP, HODOC, NIH, EINECS
Contributor: L.E. Slivon, Battelle Columbus Laboratories, Columbus, Ohio 43201

**Figure 79** Isopropyl Palmitate

Hit 1 : Hexadecanoic acid, ethyl ester
C18H36O2; MF: 694; RMF: 729; Prob 35.6%; CAS: 628-97-7; Lib: mainlib; ID: 52733.



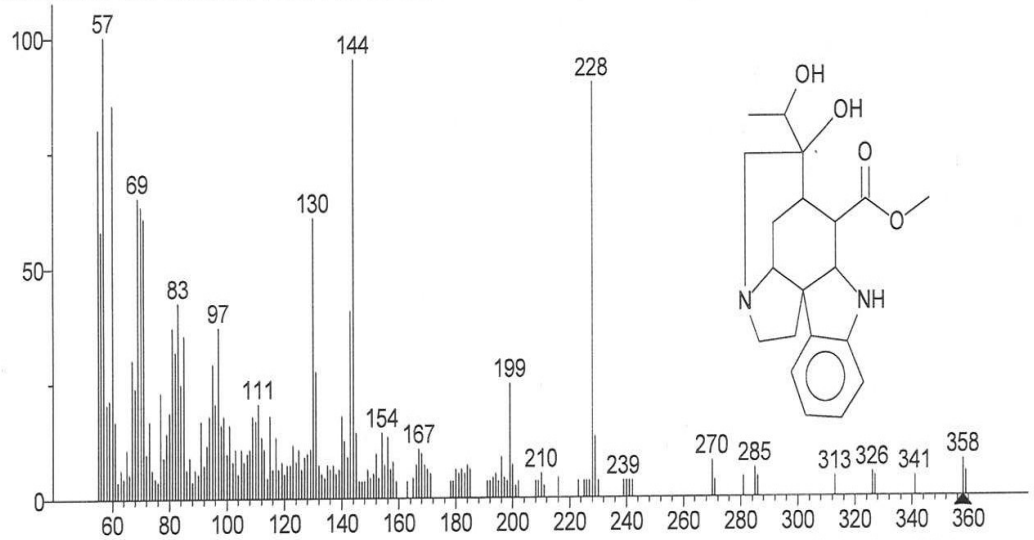

MW: 284 CAS# 628-97-7 C18H36O2 (mainlib) Hexadecanoic acid, ethyl ester



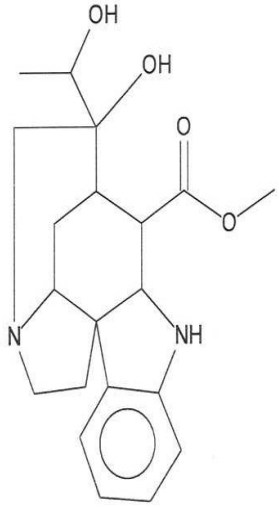

Name: Hexadecanoic acid, ethyl ester
Formula: $C_{18}H_{36}O_2$
MW: 284 Exact Mass: 284.27153 CAS#: 628-97-7 NIST#: 233204 ID#: 52733 DB: mainlib
Other DBs: Fine, TSCA, EPA, HODOC, NIH, EINECS, IRDB
Contributor: Japan AIST/NIMC Database- Spectrum MS-NW-5396

**Figure 80 Hexadecanoic acid ethyl ester**

Hit 1 : Curan-17-oic acid, 19,20-dihydroxy-, methyl ester, (19S)-
C20H26N2O4; MF: 656; RMF: 693; Prob 9.58%; CAS: 2111-90-2; Lib: mainlib; ID: 24769.




MW: 358 CAS# 2111-90-2 C20H26N2O4 (mainlib) Curan-17-oic acid, 19,20-dihydroxy-, methyl ester, (19S)-




Name: Curan-17-oic acid, 19,20-dihydroxy-, methyl ester, (19S)-
Formula: $C_{20}H_{26}N_2O_4$
MW: 358 Exact Mass: 358.189257 CAS#: 2111-90-2 NIST#: 48471 ID#: 24769 DB: mainlib
Other DBs: None
Contributor: CARL DJERASSI DEPT OF CHEM STANFORD UNIV STANFORD CALIF 94305

**Figure 81** Curan-17 oic acid- 19,20 –dihydroxy-, methyl ester, (19s)

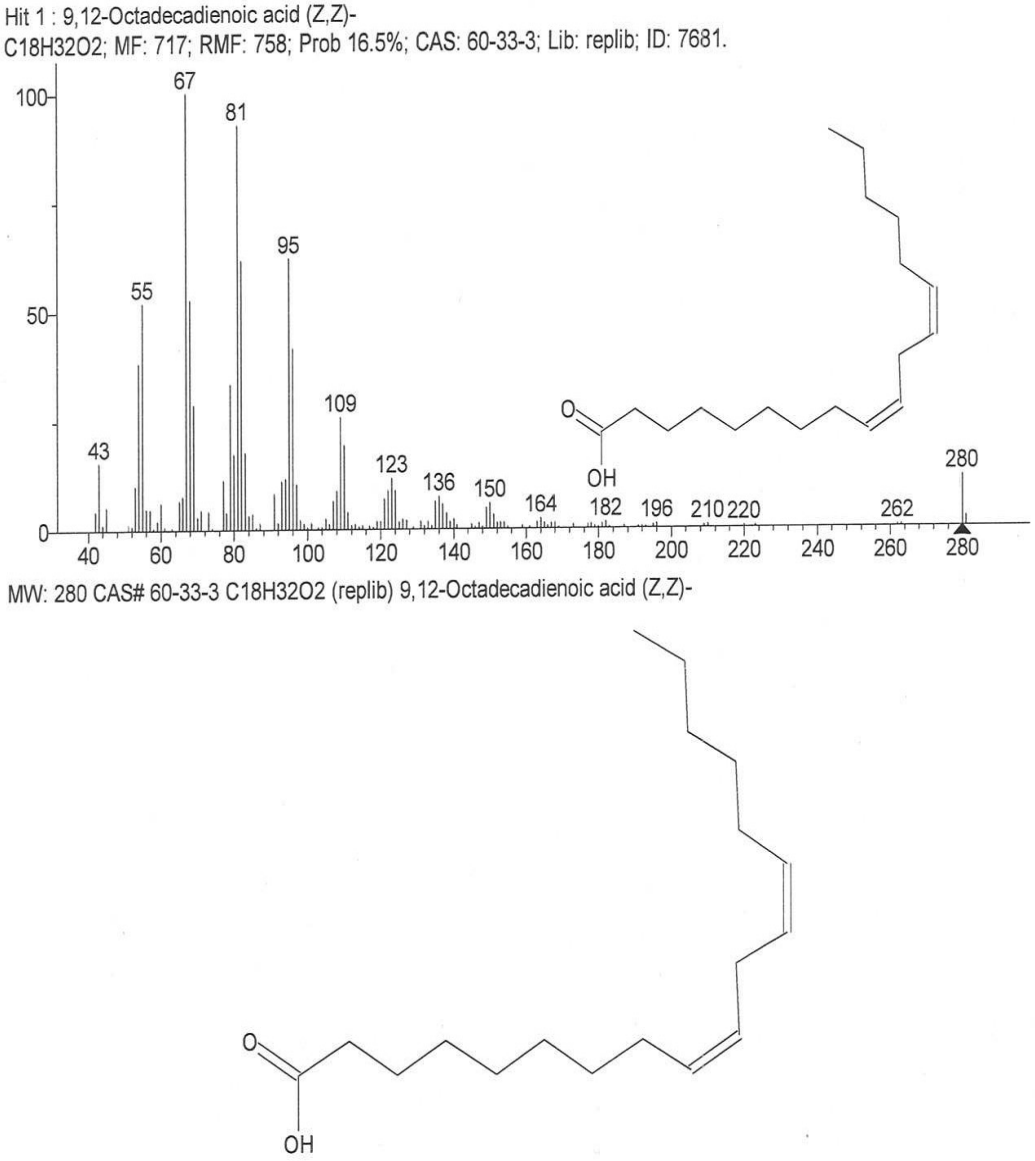
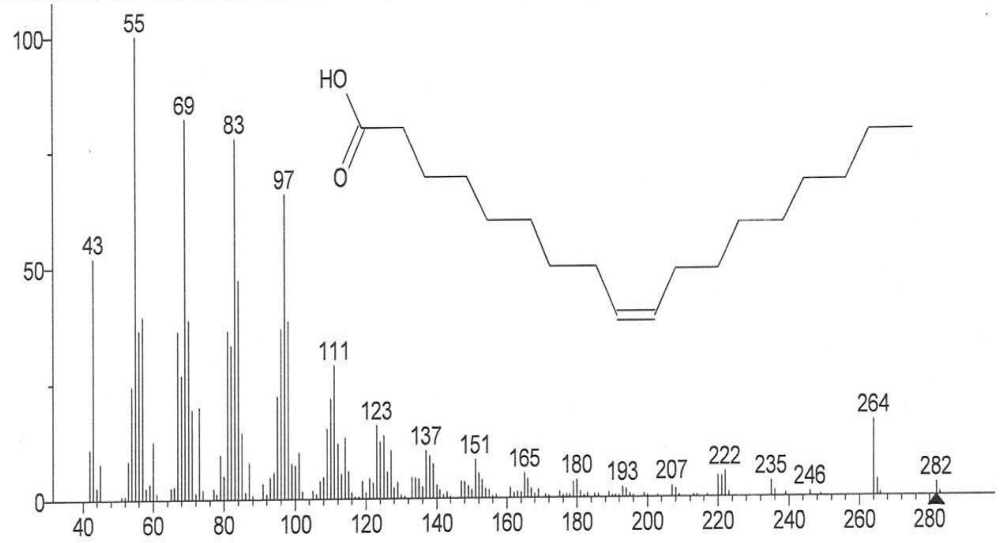


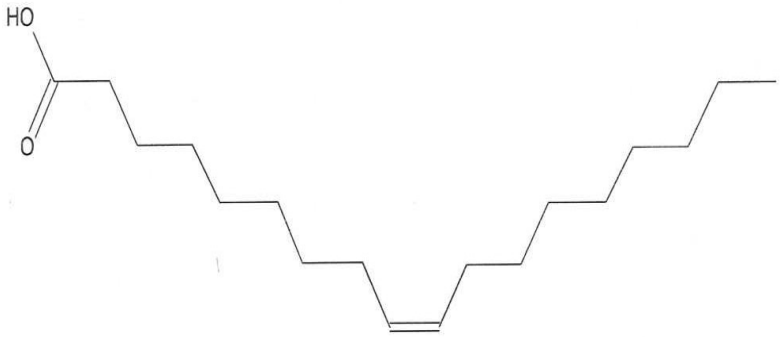

**Figure 82** 9,12 – Octadecadienoic acid (z,z)-

Hit 1 : Oleic Acid

C18H34O2; MF: 818; RMF: 847; Prob 9.60%; CAS: 112-80-1; Lib: replib; ID: 4760.




MW: 282 CAS# 112-80-1 C18H34O2 (replib) Oleic Acid




Name: Oleic Acid

Formula: $C_{18}H_{34}O_2$

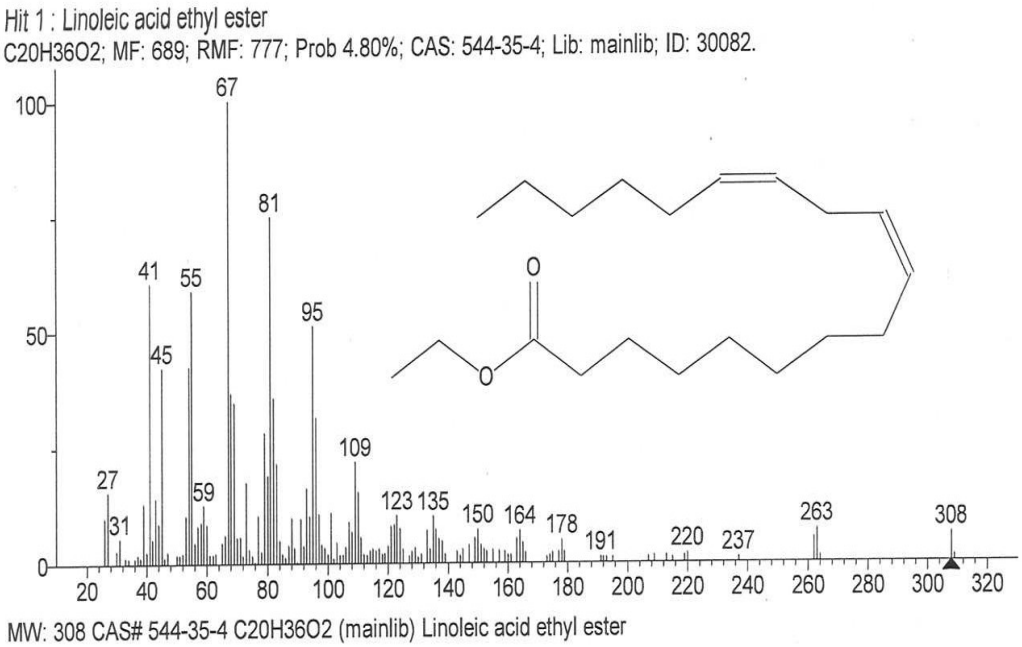
MW: 282 Exact Mass: 282.25588 CAS#: 112-80-1 NIST#: 379354 ID#: 4760 DB: replib

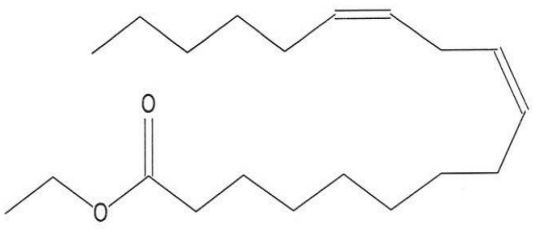
Other DBs: TSCA, RTECS, USP, HODOC, NIH, EINECS, IRDB

Contributor: Drug Lab

Related CAS#: 56833-51-3; 8046-01-3; 949900-16-7

**Figure 83 Oleic acid**



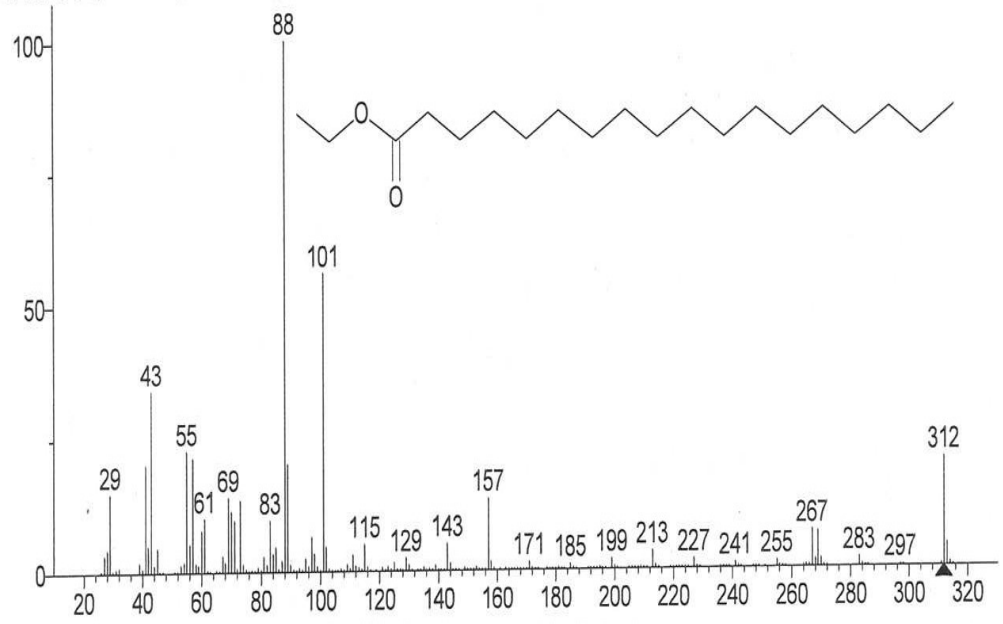




Name: Linoleic acid ethyl ester
Formula: $C_{20}H_{36}O_2$
MW: 308 Exact Mass: 308.27153 CAS#: 544-35-4 NIST#: 155747 ID#: 30082 DB: mainlib
Other DBs: Fine, TSCA, HODOC, NIH, EINECS
Contributor: Chemical Concepts

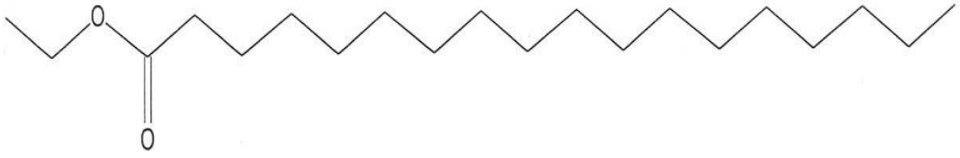
**Figure 84 Linoleic acid ethyl ester**

Hit 1 : Octadecanoic acid, ethyl ester

C20H40O2; MF: 670; RMF: 690; Prob 21.7%; CAS: 111-61-5; Lib: replib; ID: 12004.




MW: 312 CAS# 111-61-5 C20H40O2 (replib) Octadecanoic acid, ethyl ester




Name: Octadecanoic acid, ethyl ester

Formula: $C_{20}H_{40}O_2$

MW: 312 Exact Mass: 312.30283 CAS#: 111-61-5 NIST#: 36393 ID#: 12004 DB: replib

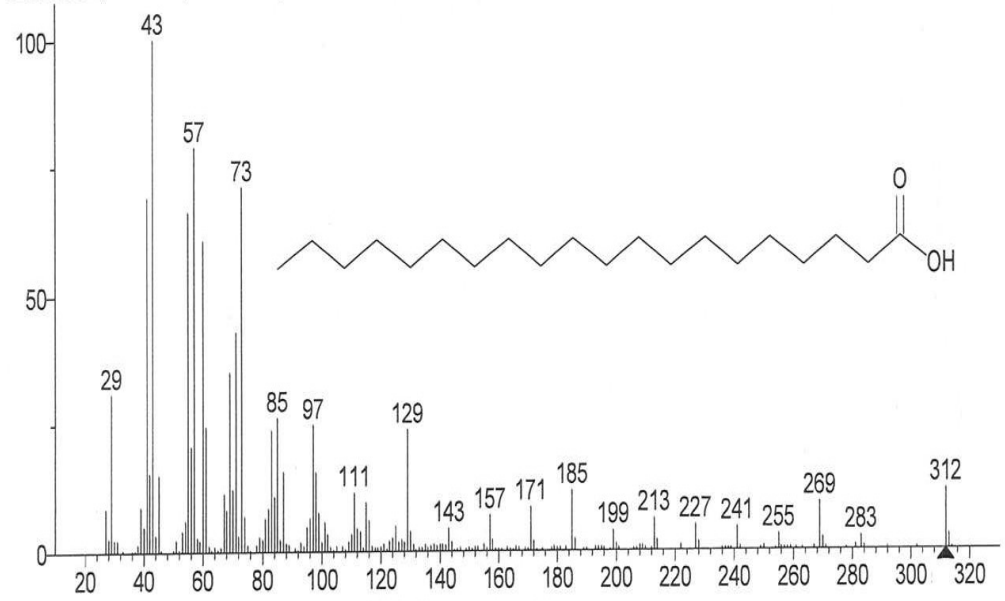
Other DBs: Fine, TSCA, RTECS, HODOC, NIH, EINECS, IRDB

Contributor: R.T.HOLMAN,UNIVERSITY OF MINNESOTA

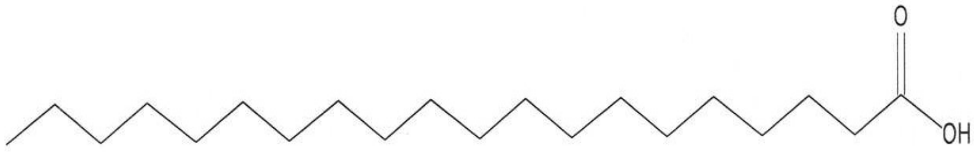
**Figure 85 Octadecanoic acid, ethyl ester**

Hit 1 : Eicosanoic acid

C20H40O2; MF: 710; RMF: 760; Prob 38.2%; CAS: 506-30-9; Lib: mainlib; ID: 7492.




MW: 312 CAS# 506-30-9 C20H40O2 (mainlib) Eicosanoic acid




Name: Eicosanoic acid

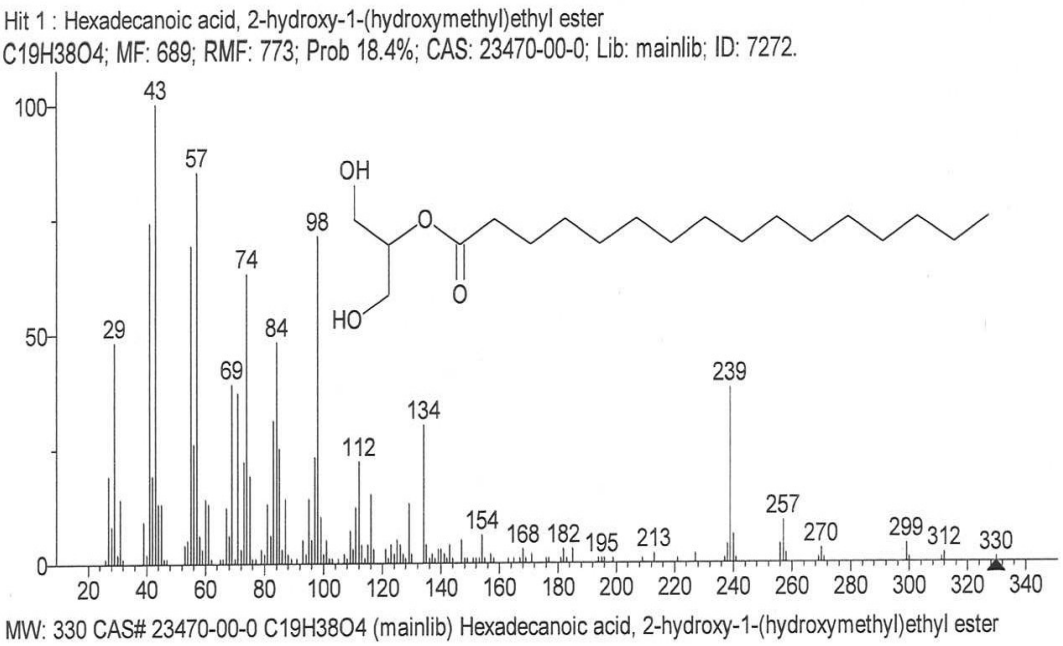
Formula: $C_{20}H_{40}O_2$

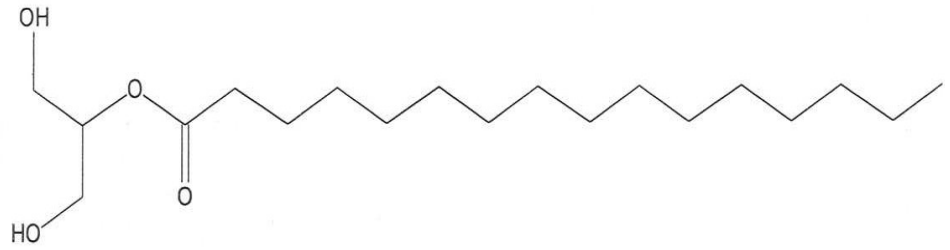
MW: 312 Exact Mass: 312.30283 CAS#: 506-30-9 NIST#: 160470 ID#: 7492 DB: mainlib

Other DBs: Fine, TSCA, RTECS, HODOC, NIH, EINECS, IRDB

Contributor: Chemical Concepts

**Figure 86 Eicosanoic acid**



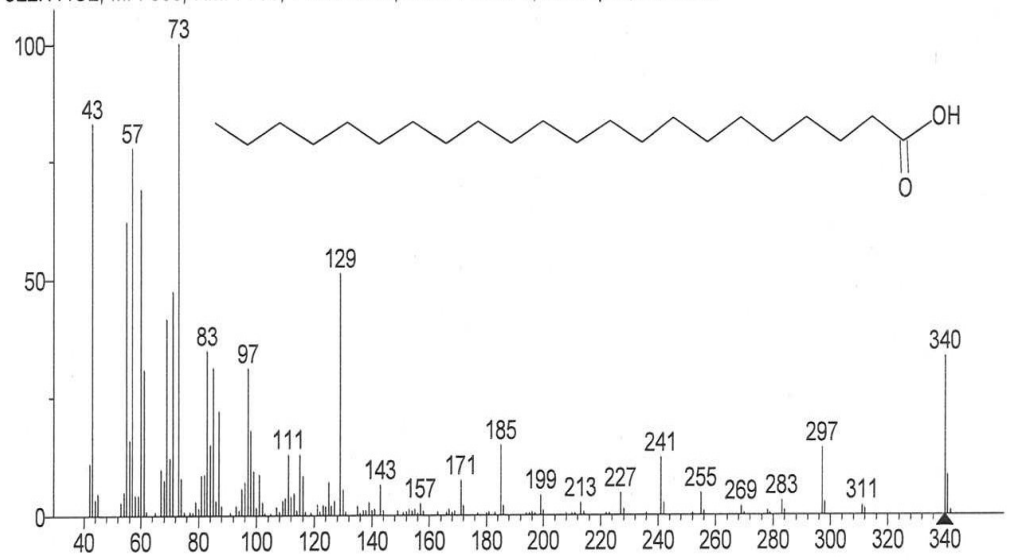




Name: Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
Formula: $C_{19}H_{38}O_4$
MW: 330 Exact Mass: 330.27701 CAS#: 23470-00-0 NIST#: 15400 ID#: 7272 DB: mainlib
Other DBs: None
Related CAS#: 75656-12-1

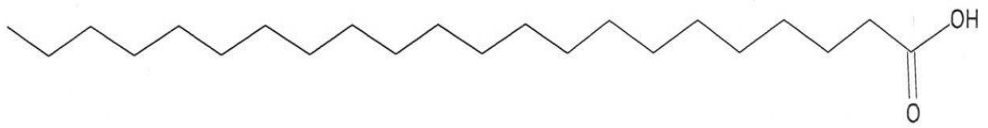
**Figure 87** Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester

Hit 1 : Docosanoic acid

C22H44O2; MF: 666; RMF: 790; Prob 24.4%; CAS: 112-85-6; Lib: replib; ID: 8936.




MW: 340 CAS# 112-85-6 C22H44O2 (replib) Docosanoic acid




Name: Docosanoic acid

Formula: $C_{22}H_{44}O_2$

MW: 340 Exact Mass: 340.334131 CAS#: 112-85-6 NIST#: 379420 ID#: 8936 DB: replib

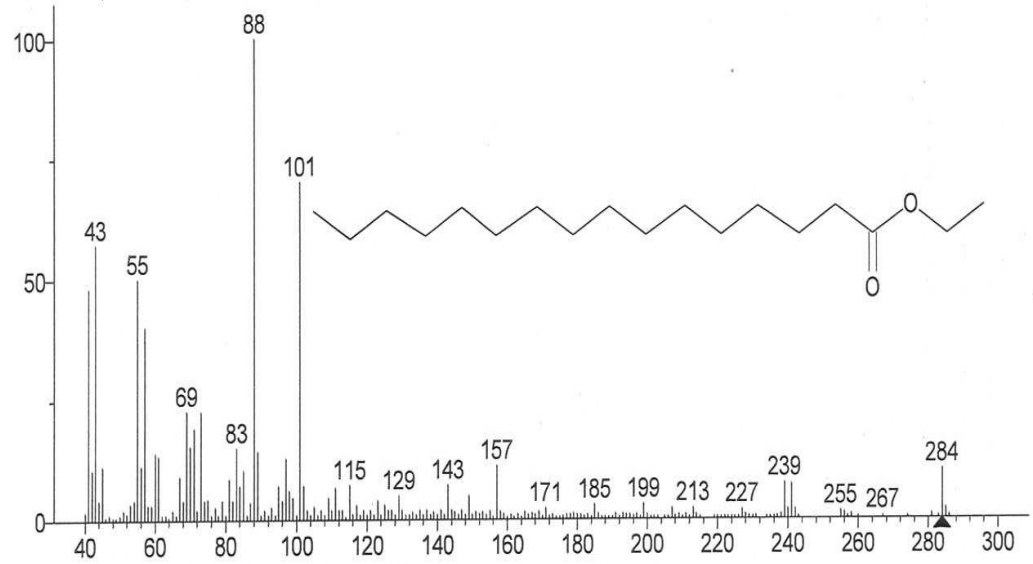
Other DBs: Fine, TSCA, HODOC, NIH, EINECS, IRDB

Contributor: Drug Lab

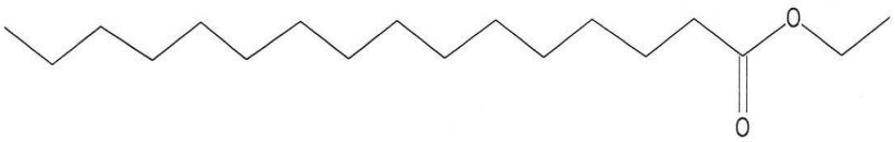
**Figure 88 Docosanoic acid**

Hit 1 : Hexadecanoic acid, ethyl ester

C18H36O2; MF: 642; RMF: 703; Prob 20.6%; CAS: 628-97-7; Lib: replib; ID: 12003.




MW: 284 CAS# 628-97-7 C18H36O2 (replib) Hexadecanoic acid, ethyl ester




Name: Hexadecanoic acid, ethyl ester

Formula: $C_{18}H_{36}O_2$

MW: 284 Exact Mass: 284.27153 CAS#: 628-97-7 NIST#: 43659 ID#: 12003 DB: replib

Other DBs: Fine, TSCA, EPA, HODOC, NIH, EINECS, IRDB

Contributor: ACIR ALIP ETHY SE30 05 1980 E M.HORN

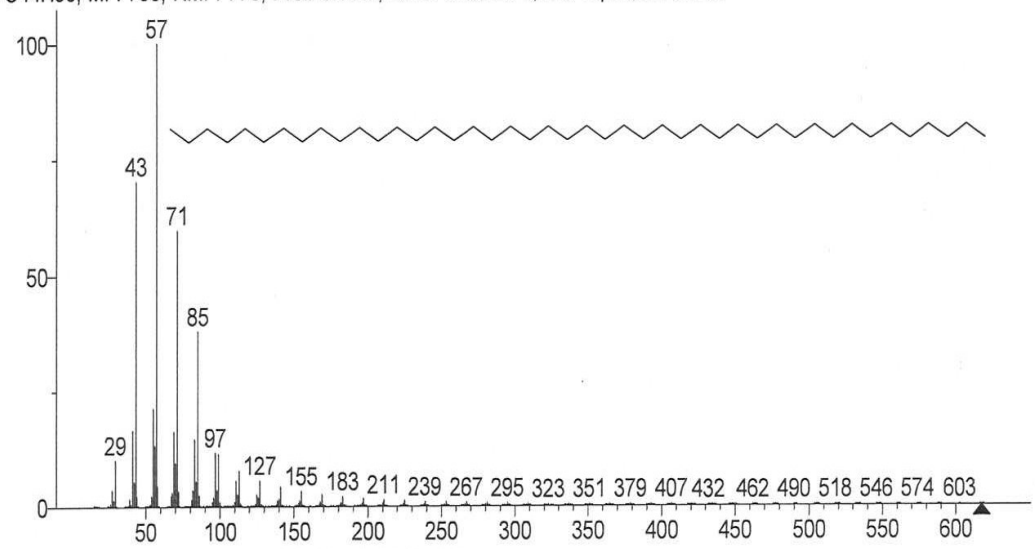
10 largest peaks:

88 999 | 101 700 | 43 570 | 55 500 | 41 480 | 57 400 | 69 226 | 73 224 | 71 190 | 70 152 |

**Figure 89 Hexadecanoic acid, ethyl ester**

Hit 1 : Tetratetracontane

C44H90; MF: 750; RMF: 770; Prob 6.50%; CAS: 7098-22-8; Lib: replib; ID: 5823.




MW: 618 CAS# 7098-22-8 C44H90 (replib) Tetratetracontane

Name: Tetratetracontane

Formula: $C_{44}H_{90}$

MW: 618 Exact Mass: 618.704254 CAS#: 7098-22-8 NIST#: 23773 ID#: 5823 DB: replib

Other DBs: Fine, TSCA, HODOC, NIH, EINECS

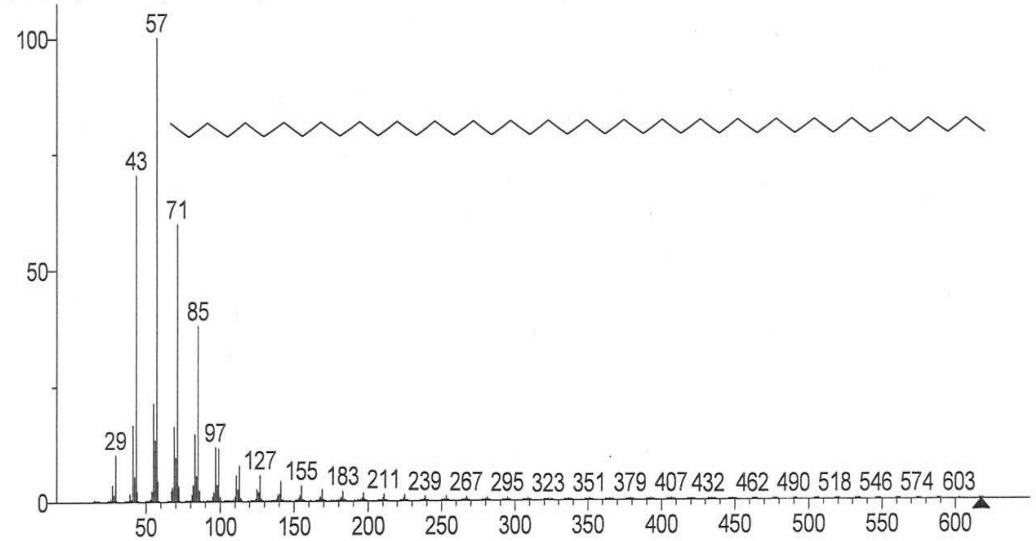
10 largest peaks:

57 999 | 43 701 | 71 596 | 85 378 | 55 212 | 41 164 | 69 161 | 83 145 | 56 132 | 97 116 |

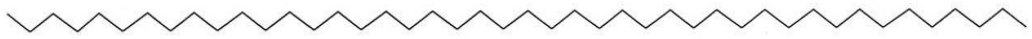
**Figure 90 Tetratetracontane**

Hit 1 : Tetratetracontane

C44H90; MF: 773; RMF: 792; Prob 11.6%; CAS: 7098-22-8; Lib: replib; ID: 5823.




MW: 618 CAS# 7098-22-8 C44H90 (replib) Tetratetracontane



Name: Tetratetracontane

Formula: $C_{44}H_{90}$

MW: 618 Exact Mass: 618.704254 CAS#: 7098-22-8 NIST#: 23773 ID#: 5823 DB: replib

Other DBs: Fine, TSCA, HODOC, NIH, EINECS

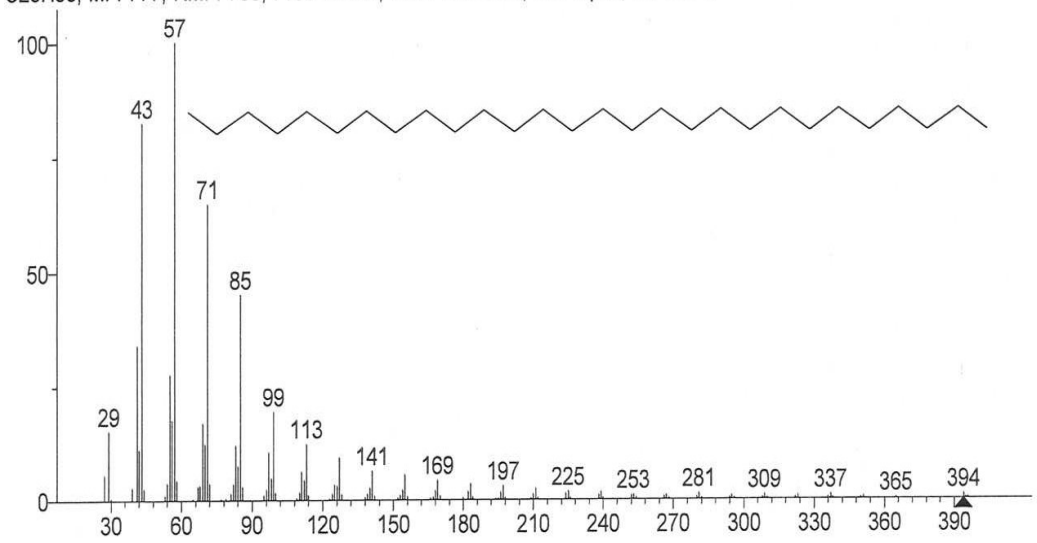
10 largest peaks:

57 999 | 43 701 | 71 596 | 85 378 | 55 212 | 41 164 | 69 161 | 83 145 | 56 132 | 97 116 |

**Figure 91 Tetratetracontane**

Hit 1 : Octacosane

C28H58; MF: 777; RMF: 789; Prob 7.88%; CAS: 630-02-4; Lib: replib; ID: 5774.




MW: 394 CAS# 630-02-4 C28H58 (replib) Octacosane

Name: Octacosane

Formula: $C_{28}H_{58}$

MW: 394 Exact Mass: 394.453852 CAS#: 630-02-4 NIST#: 134306 ID#: 5774 DB: replib

Other DBs: Fine, TSCA, EPA, HODOC, NIH, EINECS, IRDB

Contributor: NIST Mass Spectrometry Data Center, 1994

10 largest peaks:

57 999 | 43 823 | 71 647 | 85 449 | 41 337 | 55 274 | 99 193 | 56 174 | 69 168 | 29 151 |

**Figure 92 Octacosane**

**Table 3: List of compounds, their retention time and probability in GCMS report of ethanol and chloroform extracts of a traditional unique Tetraherbal and a biherbal powder**

| Name of the Sample | Solvent used | No. of Compound | Retention time | Name of the Compound | Probability |
|---|---|---|---|---|---|
| Vedic Compatible Plants (Tetraherbal) | Ethanol | 14 | 8.46 | Tetradecanoic acid | 36.4 |
| | | | 10.234 | n-Hexadecanoic acid | 36.9 |
| | | | 10.438 | Hexadecanoic acid, ethyl ester | 37.9 |
| | | | 11.012 | Eicosanoic acid | 23.00 |
| | | | 11.708 | 9,12- Octadecadienoic acid (z,z)- | 19.7 |
| | | | 11.744 | Oleic acid | 11.8 |
| | | | 11.904 | Octadecanoic acid | 17.6 |
| | | | 13.441 | Eicasanoic acid | 24.4 |
| | | | 14.716 | Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester | 22.6 |
| | | | 14.119 | Docosanoic acid | 22.2 |
| | | | 16.070 | 2-Myristynoyl pantetheine | 7.00 |
| | | | 16.242 | Octadecanoic acid, 2-hydroxy-1-(hydromethyl)ethyl ester | 19.4 |
| | | | 16.492 | Tetracosanoic acid | 27 |
| | | | | | |
| | | | 17.449 | Tetratetracontane | 7.73 |
| Vedic non-compatible plants (Biherbal) | Ethanol | 14 | 10.264 | Isopropyl Palmitate | 9.92 |
| | | | 10.444 | Hexadecanoic acid, ethyl ester | 34.6 |

| Name of the Sample | Solvent used | No. of Compound | Retention time | Name of the Compound | Probability |
|---|---|---|---|---|---|
| | | | 11.031 | Curan-17 oic acid- 19,20 – dihydroxy-, methyl ester, (19s) | 9.48 |
| | | | 11.734 | 9,12 – Octadecadienoic acid (z,z)- | 16.4 |
| | | | 11.772 | Oleic acid | 9.60 |
| | | | 11.914 | Linoleic acid, ethyl ester | 4.80 |
| | | | 12.146 | Octadecanoic acid, ethyl ester | 21.7 |
| | | | 13.444 | Eicosanoic acid | 38.2 |
| | | | 14.719 | Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester | 18.4 |
| | | | 14.124 | Docosanoic acid | 24.4 |
| | | | 14.324 | Hexadecanoic acid, ethyl ester | 20.6 |
| | | | 16.074 | Tetratetracontane | 6.40 |
| | | | 16.080 | Tetratetracontane | 11.6 |
| | | | 17.486 | Octacosane | 7.88 |
| | | | | | |
| Vedic Compatible Plants (Tetraherbal) | Chloroform | 20 | 4.871 | 1-Hexadecanol | 7.11 |
| | | | 6.041 | Benzaldehyde, 3-hydroxy-4-methoxy | 23.1 |
| | | | 6.684 | 6-(Diethylamino)Benzofuran-3- (2H)-one | 41.1 |
| | | | 7.190 | 1- Eicosanol | 6.64 |
| | | | 7.974 | Dodecyl acrylate | 14.3 |
| | | | 8.760 | 1-Eicosanol | 6.70 |

| Name of the Sample | Solvent used | No. of Compound | Retention time | Name of the Compound | Probability |
|---|---|---|---|---|---|
| | | | 9.202 | Z-(13,14-Epoxy) tetradec-11-en-1-ol acetate | 4.33 |
| | | | 9.344 | Pentadecanoic acid | 24.4 |
| | | | 9.472 | Benzaldehyde, 4-(dimethylamino)- | 12.7 |
| | | | 10.439 | 6H-Indol (3,2,1-de)napthyridin – one, 1,2,3,3a,4,4 – hexahydro-beta- hydroxyl -3- methyl | 44.7 |
| | | | 10.474 | 4-Octadecenal | 6.49 |
| | | | 11.112 | Eicosanoic acid | 26.3 |
| | | | 11.924 | Oleic acid | 9.96 |
| | | | 12.074 | Octadecanoic acid | 34.3 |
| | | | 12.174 | 1-Heneicosyl formate | 4.80 |
| | | | 13.809 | n-Tetracosanol -1 | 4.44 |
| | | | 14.188 | Octadecanoic acid, 2-hydroxy -1,3 propanediyl ester | 20.4 |
| | | | 14.349 | 4- octadecenal | 3.70 |
| | | | 17.104 | Lup-20 (29)-en-3-ol, acetate, (3beta) | 66.1 |
| | | | 17.920 | Octacosane | 8.30 |
| | | | | | |
| Vedic non-compatible plants (Biherbal) | Chloroform | 24 | 4.763 | 1-Undecanol | 4.23 |
| | | | 4.764 | 1-Hexadecanol | 6.71 |
| | | | 4.877 | 1-Hexadecanol | 13 |
| | | | 6.694 | 6-(Diethylamono)benzofuran-3-(2H)-one | 44.1 |

| Name of the Sample | Solvent used | No. of Compound | Retention time | Name of the Compound | Probability |
|---|---|---|---|---|---|
| | | | 7.201 | Hexadecen-1-ol, trans-9- | 7.29 |
| | | | 7.940 | Dodecyl acrylate | 6.82 |
| | | | 8.432 | Tetradecanoic acid | 14.9 |
| | | | 8.772 | 1-Eicosanol | 4.2 |
| | | | 10.494 | n-hexadecanoic acid | 34.8 |
| | | | 10.494 | 4-octadecenal | 8.26 |
| | | | 11.`127 | Heptadecanoic acid | 20.0 |
| | | | 11.912 | Oleic acid | 7.27 |
| | | | 12.074 | Octadecanoic acid | 34.8 |
| | | | 12.189 | 4-octadecenol | 10.0 |
| | | | 13.639 | Eicosanoic acid | 32.2 |
| | | | 13.818 | 1- Heneicosyl formate | 4.49 |
| | | | 14.072 | Di-n-octyl phthalate | 7.71 |
| | | | 14.184 | Octadecanoic acid, 2-hydroxy, 1,3- propanediyl ester | 24.2 |
| | | | 14.349 | 4-octadecenal | 6.26 |
| | | | 16.108 | Tetratetracontane | 7.60 |
| | | | 17.104 | 7,8-Epoxylanostan-11-ol, 3-acetoxy- | 9.74 |
| | | | 17.292 | Fenretinide | 10.00 |
| | | | 17.639 | Octacosane | 8.34 |
| | | | 18.007 | Lup-02(29)-en-3-one | 33.4 |
| | | | 18.444 | Betulin | 39.2 |

# Results

## Objective – 4.1 : Anti HIV-1 RT p66 Assay

**Table 4: Conc. and OD of HIV-1 RT p66**

| Conc. Of HIV-1 RT p66 (ng/100µL) | 0.09 | 0.39 | 1.56 | 6.25 | 25 |
|---|---|---|---|---|---|
| Absorbance (OD) | 0.002 | 0.015 | 0.029 | 0.045 | 0.089 |

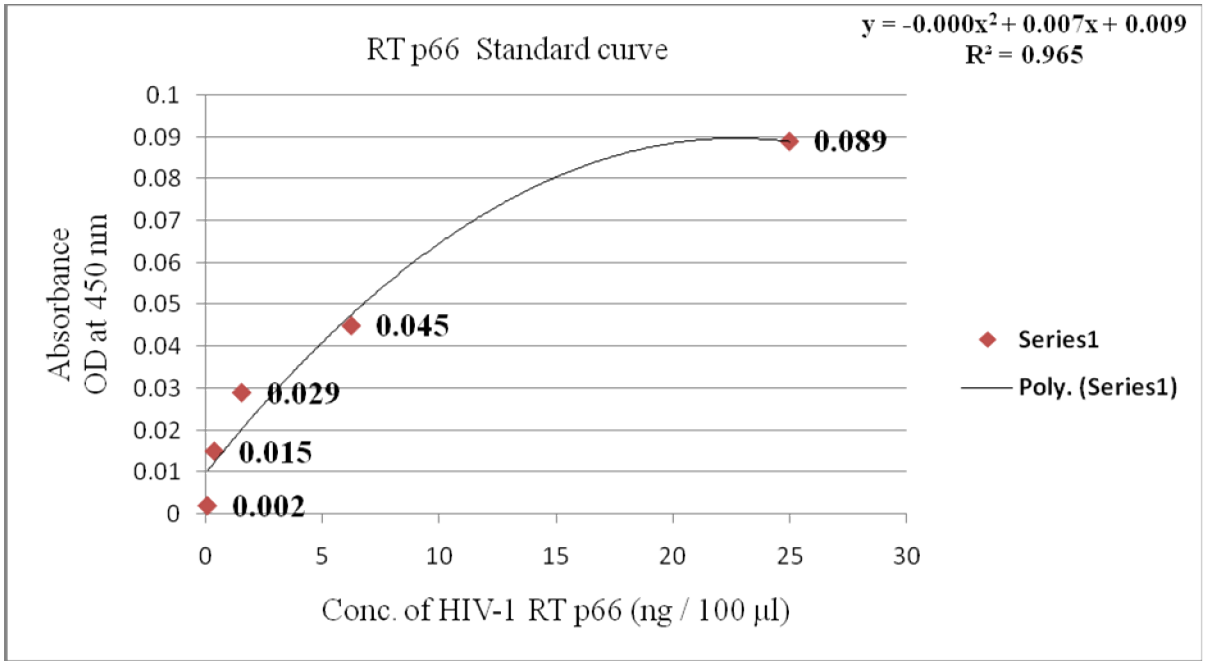



Figure 93: HIV-1 RT p66 Standard Curve

## 4.1 (a) Inhibition of extracts of Individual 09 plants

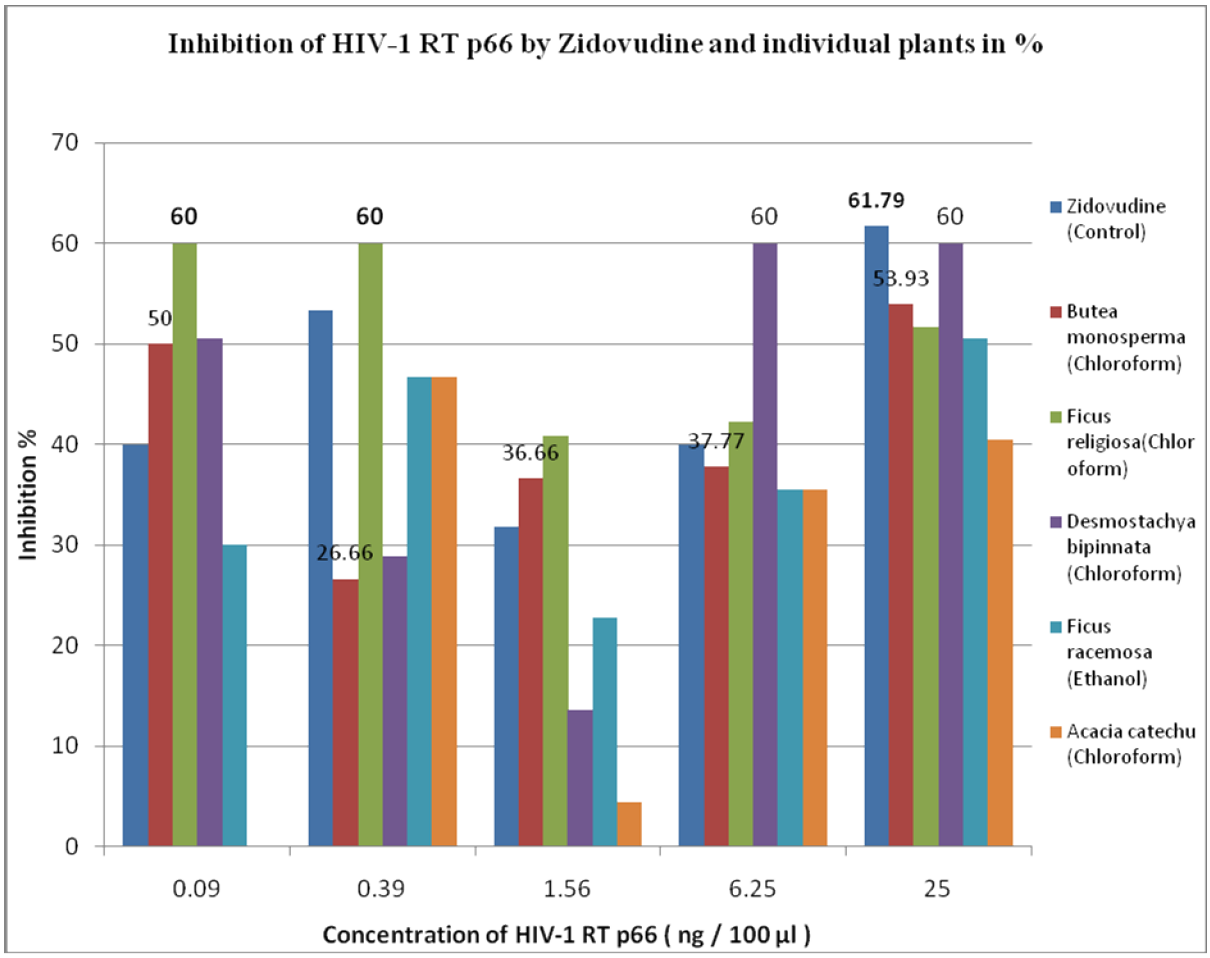



Figure 94 Inhibition of HIV-1 RT p66 by Zidovudine and 09 individual plants

4.1 (b) Inhibition of extracts of Bi, Tri and Pentaherbal extracts

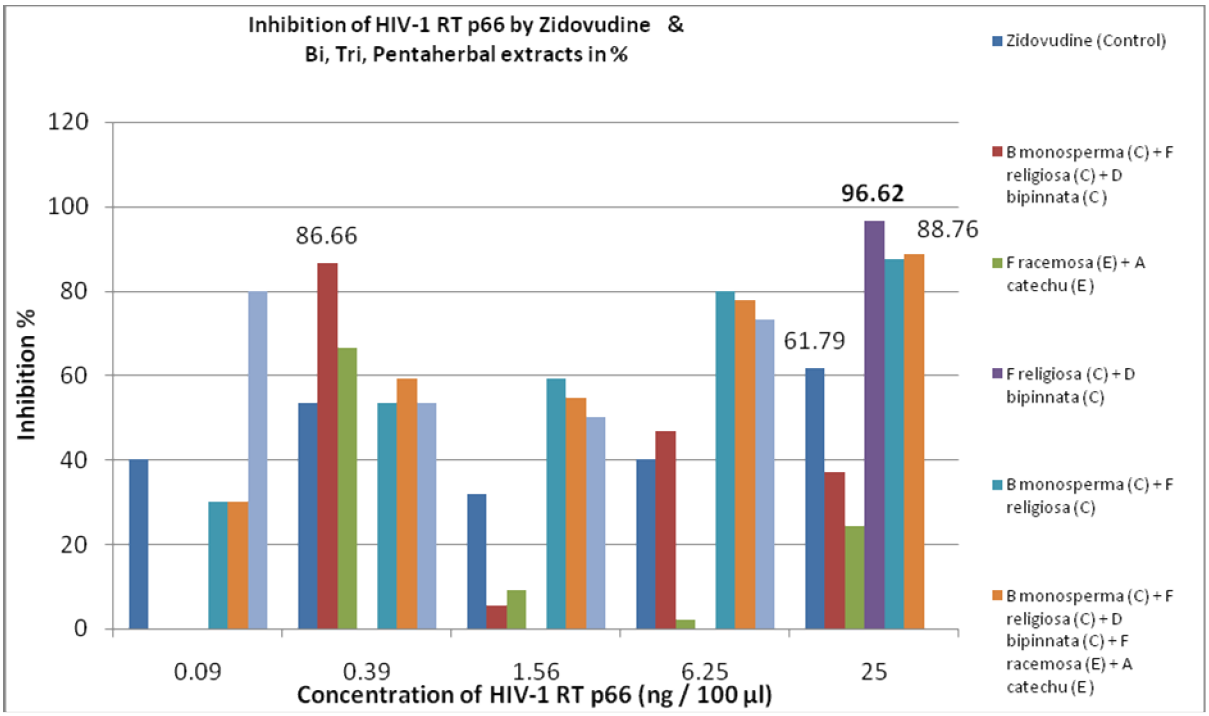



Figure 95 Inhibition of HIV-1 RT p66 by Zidovudine and Bi, Tri and Pentaherbal extracts

4.1 (c) Inhibition of Unique Polyherbal extracts

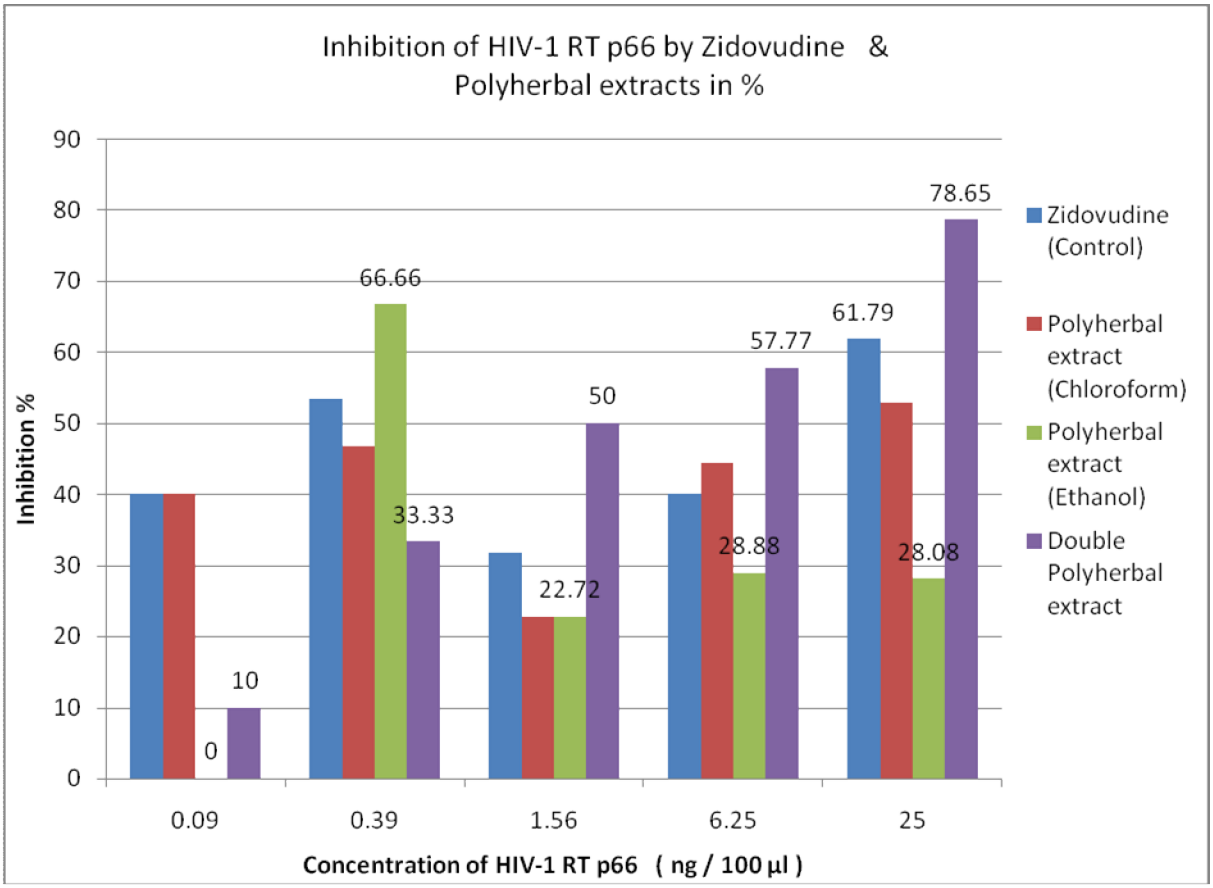



Figure 96 Inhibition of HIV-1 RT p66 by Polyherbal extracts

# Objective – 4.2: Anti HIV-1 gp120 assay

**Table 5: Conc. and OD of HIV-1** gp120

| Conc. Of HIV-1 gp 120 (ng/100µL) | 3.12 | 6.25 | 12.5 | 25 | 50 |
|---|---|---|---|---|---|
| Absorbance (OD) | 0.012 | 0.025 | 0.09 | 0.19 | 0.39 |

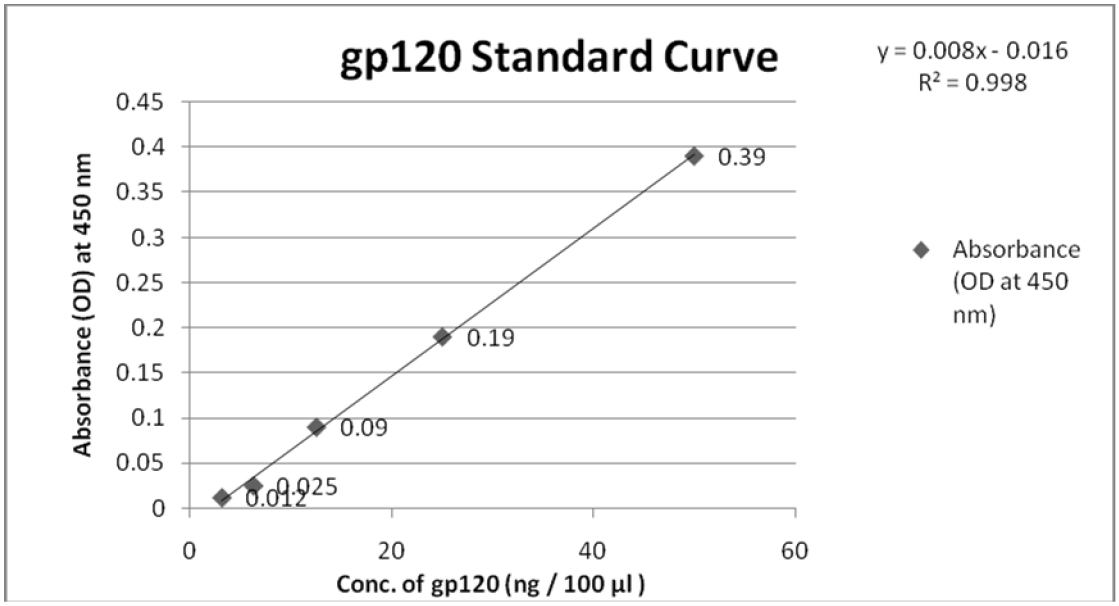



Figure 97 HIV-1 RT gp120 Standard Curve

Concentration of different plant extracts from their respective OD values was as in table 6.

**Table 6: 'x' value (Conc.) from 'y' values (OD)**

| SN | Plant | Absorbance at 450 nm at 50 ng / 100 µl ('y' value) | Conc. Of plant extract in (ng / 100 µl ) ('x' value) |
|---|---|---|---|
| 1 | Butea monosperma(E) | 0.13 | 18.25 |
| 2 | Ficus religiosa (E) | 0.14 | 19.5 |
| 3 | Ficus racemosa (E) | 0.16 | 22 |
| 4 | Butea monosperma(C) | 0.31 | 40.75 |
| 5 | Desmostachya bipinnata (E) | 0.37 | 48.25 |
| 6 | Nine-in-one (C) | 0.38 | 49.5 |
| | Heparin (Control) | 0.10 | 14.5 |

## 4.2 (a) Inhibition of individual extracts and polyherbal extracts

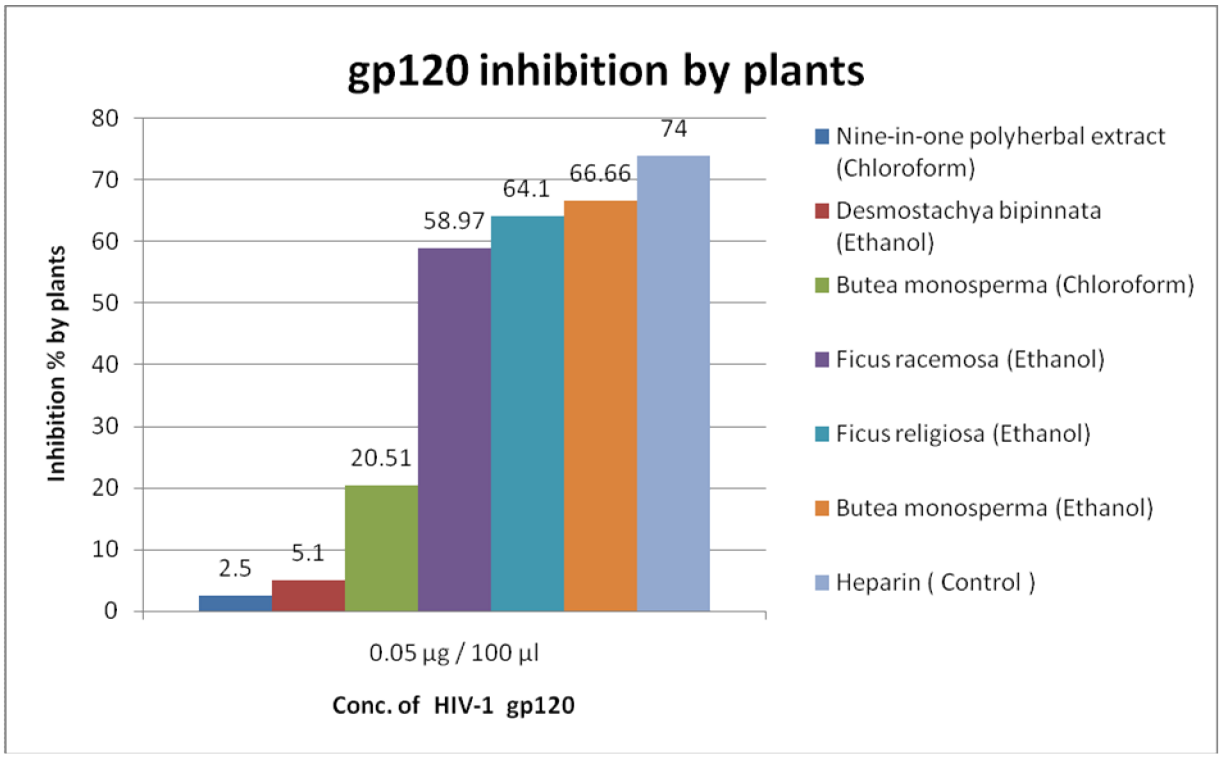



Figure 98 Inhibition of HIV-1 gp120-CD4 interaction by individual plant extracts

## Table 7 Overall results of anti-HIV activity

| SN | **Individual Plant extracts** | **Inhibition% (HIV-1 RT p66)** |
|---|---|---|
| 1 | *Butea monosperma* (Chloroform) | **50.00 – 53.93** |
| 2 | *Ficus religiosa* (Chloroform) | 51.68 - 60.00 |
| 3 | *Desmostachya bipinnata* (Chloroform) | 50.56 - 60.00 |
| 4 | *Ficus racemosa* (Ethanol) | 50.56 |
| | **Bi, Tri, Pentaherbal Extracts** | |
| 5 | *Ficus racemosa* (Ethanol) + *Acacia catechu* (Ethanol) | 66.66 |
| 6 | *Butea monosperma* (Chloroform) + *Ficus religiosa* (Chloroform) | **53.33 - 87.64** |
| 7 | *Ficus religiosa* (Chloroform) + *Desmostachya bipinnata* (Chloroform) | **96.62** |
| 8 | *Butea monosperma* (Chloroform) + *Ficus religiosa* (Chloroform) + *Desmostachya bipinnata* (Chloroform) | **86.66** |
| 9 | *Butea monosperma* (Chloroform) + *Ficus religiosa* (Chloroform) + *Desmostachya bipinnata* (Chloroform) + *Ficus racemosa* (Ethanol) + *Acacia catechu* (Ethanol) | **54.54 – 86.76** |
| 10 | *Prosopis cineraria* (Chloroform) + *Desmostachya bipinnata* (Chloroform) | **50.00 - 80.00** |
| | **Polyherbal extracts** | |
| 11 | Nine-in-one (Chloroform) | 52.80 |
| 12 | Nine-in-one (Ethanol) | 66.66 |
| 13 | Nine-in-one (Chloroform) + Nine-in-one (Ethanol) | 50.00 - 78.65 |
| | | |
| | Individual Plant extracts | **Inhibition% (HIV-1 gp120 )** |
| 1 | ***Butea monosperma* (Ethanol)** | **66.66** |
| 2 | *Ficus religiosa* (Ethanol) | 64.1 |
| 3 | *Ficus racemosa* (Ethanol) | 58.97 |

## 13. ACHIEVEMENTS FROM THE PROJECT:

1. Nine plants were established in Dept of Genetics, Osmania University
2. Different extracts consisting of seven plants were prepared
3. GCMS analysis of different extracts were carried out
4. Anti-HIV 1 RT p66 and Anti gp120-CD4 interaction of different extracts was studied.

## 14. SUMMARY OF THE FINDINGS ( IN 500 WORDS ) :

Plantlets were collected from Prof. Jayashankar Telangana State Agriculture University (Hyderabad) and Rayirath gardens (Thrissur, Kerala) and plantation of plantlets was done in Plant Genetics Experimental Farm of Department of Genetics, Osmania University, and Hyderabad in accordance with Indian Traditional Knowledge. 09 individual plant powders representing 09 planets were extracted with ethanol and chloroform separately. 09 individual plant powders were mixed to form a nine-in-one polyherbal powder which was also extracted with ethanol and chloroform separately. GCMS of these 20 extracts was done. After eliminating common compounds among twenty extracts, 62 individual compounds were identified. With the aid of ChemDB NIAID database it was known that a total of 13 microbes (Virus, Bacteria, and Fungi) were susceptible to the compounds identified in 20 extracts. Nine-in-one polyherbal powder was also digested with distilled water and subjected to GCMS identifying 19 compounds. According to the principles of Indian Traditional Knowledge, following two types of Astro- polyherbal powders were prepared –

1. Compatible (a combination of *Calotropis gigantea, Butea monosperma, Acacia catechu, Ficus religiosa* powders)
2. Non-Compatible ( a combination of *Ficus religiosa, Ficus racemosa* powders)

Above two types of polyherbal powders were subjected to extraction with ethanol and chloroform separately. GCMS of these 04 extracts was done. 72 compounds were identified. Thus through GCMS, from among 25 different extracts a total of 153 compounds were identified.

**Anti-HIV activity of 09 plants**

ImmunoDx IIC – USA kits were used for knowing anti HIV activity of 09 plants. A biherbal extract made of chloroform extracts of stems of *Ficus religiosa* and leaves of *Desmostachya*

*bipinnata* induced 96.62 % of inhibition of HIV-1 RT p66. Ethanol extract of stem bark of *Butea monosperma* induced maximum inhibition of gp120-CD4 interaction i.e. 66.66%.

## 15. CONTRIBUTION TO THE SOCIETY:

a) Demo plantation lead to the education, information and awareness on seven plants representing seven traditional planets
b) Certain compounds identified through GCMS when cross checked with Chemdb NIAID database found to be potent anti HIV drugs. Preliminary screening of anti-HIV drugs was thus accomplished
c) Biherbal extract made of *Ficus religiosa* and *Desmostachya bipinnata* induced highest Anti HIV-1 RT p66 activity

## 16. WHETHER ANY Ph. D. ENROLLED / PRODUCED OUT OF THE PROJECT:

YES. One PhD have been produced.

## 17. NO. OF PUBLICATIONS OUT OF THE PROJECT

Two Research Publications (Reprints are herewith enclosed at the end of the report)

1. Ameer Jani Shaik, Yousuf Anwar Syed, 2018. *In-vitro* anti HIV activity of plants of nine-planet forest, *Jetir,* 5:(8) 297-303

2. Ameer Jani Shaik, Yousuf Anwar Syed, 2018. GCMS analysis of an unique biherbal extract, *Int.J.Pharm.Bio.Sci,* 8: (3) 241-257

**(PRINCIPAL INVESTIGATOR)** **(REGISTRAR)**