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BIODIESEL PRODUCTION FROM OILSEED PLANTS

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Fatty acid profiles of seed oils of 25 species having 10% or more fixed oil in their seed/ kernel were examined. The amount of oil, saponification value, iodine value and free fatty acid of oil of these plants were in the range of 10-76 %, 160.84-240.38 mg KOH/g, 5.16-147.4 mg I_2 /g and 0.35-25.23 %, respectively. By using the 1:6 ratio of crude oil and methanol for the free fatty acid content less than 3 %, or the 1:10 ratio followed by 1:6 ratio of crude oil and methanol for free fatty acid content more than 3 %, the biodiesel of oils of these plant species could be conveniently synthesized. Oils of 25 species have the methyl ester content (86.65-95.4%), viscosity (3-16.4 cSt), density (872.5-923 kg/m^3), acid value (0.01-0.49 mg KOH/g) and flash point (>120 °C). The fatty acid methyl esters of 16 species were found to have great potential of biodiesel and they met the most specification of biodiesel standard.

Field of study...Petrochemistry and Polymer Science…Student‘s signature..........................
Academic year..........................2007.............................Advisor’s signature..........................
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LIST OF SCHEMES

LIST OF ABBREVIATIONS

µl	microliter
µm	micrometer
ASTM	American Standard Test Method
°C	Degree Celsius
cSt	Centistroke
°F	Degree
FFA	Free fatty acid
FID	Flame Ionization Detector
FT-IR	Fourier Transform Infrared Spectroscopy
g	gram
GC	Gas-liquid chromatography
h	hour
NMR	Nuclear Magnetic Resonance Spectroscopy
kg/cm^3	Kilogram per cubic metre
L	Liter
MJ/kg	Millijule per kilogram
min	Minute
mg	Miligram
ml	Milliter
mm	Millimeter
nm	Nanometer
ppm	parts per million
rpm	Revolution per minute
v/v	Volume by volume
w/v	Weight by volume
%wt	percent weight
δ_H	Chemical shift of ^1H NMR
AA	*Acacia auricalifomis*
AI	*Azadirachta indica* Valeton
AM	*Acacia mangium*
AS	*Annona squamosa* Linn

CAP	*Carica papaya* L.
CI	*Calophyllum inophyllum*
CP	*Ceiba pentandra* Gaertn
CS	*Canarium subulatum*
CT	*Croton tiglium* L.
EA	*Eucalyptus camaldulensis*
GA	*Gmelina arborea* Roxb
HB	*Hevea brasiliensis*
HI	*Hydnocarpus ilicifolius*
HS	*Hyptis suaveolens*
IM	*Irvingia malayana*
LG	*Litsea glutinosa* Robins
MA	*Melia azadirachta* L.
PA	*Parinari anamense* Hance
PK	*Pinus kesiya*
PM	*Pinus merkusii*
SE	*Sapindus emarginatus*
SF	*Sterculia foetida* L.
TB	*Terminalia bellirica* Roxb
WT	*Wrightia tomentosa*
XK	*Xylia kerrii*

CHAPTER I

INTRODUCTION

Thailand was highly dependent on petroleum imports, and increasing world petroleum prices had a serious impact on the country's balance of payments. The demand for diesel fuel in Thailand consumed about 50 million liters per day of diesel in last year, which was 44.7 % of total consumption of petroleum product. The consumption of diesel fuel in Thailand is being continuously increased. Thus, looking for an alterative way to develop a substitute for diesel is an imperious task for the country. At the same time, air pollution is one of the most serious environmental problems all over the world [1]. The advantages of using this alternative fuel are its renewability, better quality of exhaust gas emissions, its biodegradability and, given that all the organic carbon present is photosynthetic in origin, it does not contribute to a net rise in the level of carbon dioxide in the atmosphere and, consequently, to the greenhouse effect [2].

Thai government had a policy is to use local feedstock such as vegetable oil, animal fats, waste food oils as fuel. Biodiesel is one of several alternative fuels, which is a renewable fuel made by a chemical reaction of alcohol and vegetable, animal oils, fats, or greases. Through a refinery process called transesterification. The proposed of the transesterification process is to lower the viscosity of the oil [3]. The reactions can be catalyzed by alkali, acid or enzymes, but the first two types have received more attention due to the short reaction times and low cost compared with the third one [4]. Thus, the alkaline and acid catalyzed transesterification process was determined for biodiesel productions in this research. Biodiesel can be used in any diesel engine in pure form or blended with petroleum diesel at any level. Even a blend of 20% bio- and 80% petroleum diesel will significantly reduce carcinogenic emissions and gases that may contribute to global warming [5].

The raw materials being exploited commercially by the biodiesel countries constitute the edible fatty oils derived from rapeseed, soybean, palm, sunflower, coconut, linseed, etc. Use of such edible oil to produce biodiesel in Thailand is not feasible in view of a big gap in demand and supply of such oils in the country.

Increased pressure to augment production of edible oil has also put limitation on the use of these oils for production of biodiesel. Beneath Thai conditions only such plants can be considered for biodiesel, which produce non-edible oil in appreciable quantity and can be grown in large scale on non-cropped marginal lands and wastelands [6].

There is a long list of trees, shrubs and herbs available plentifully in Thailand, which can be exploited for use as diesel fuel. This research concentrated on 25 oilseed plants, which contain 20% or more oil in their seed, fruit or nut.

Objectives of the research:

(1). To study the chemical composition of oil from oilseed plants.
(2). To synthesize methyl ester compounds from oilseed plants for using as biodiesel.
(3). To study the properties of biodiesel from oilseed plants.

CHAPTER II

THEORY AND LITERATURE REVIEWS

2.1 Diesel fuels

Diesel fuel is a fossil fuel that obtained from fractional distillation of the crude oil. The boiling range of distillate fuel is approximately 160-330°C (300-700°F), which is higher than the boiling range of gasoline (Figure 2.1). Diesel fuel is ignited in an internal combustion engine cylinder by the heat of air under high compression, which is any fuel suitable for burning in diesel engines.

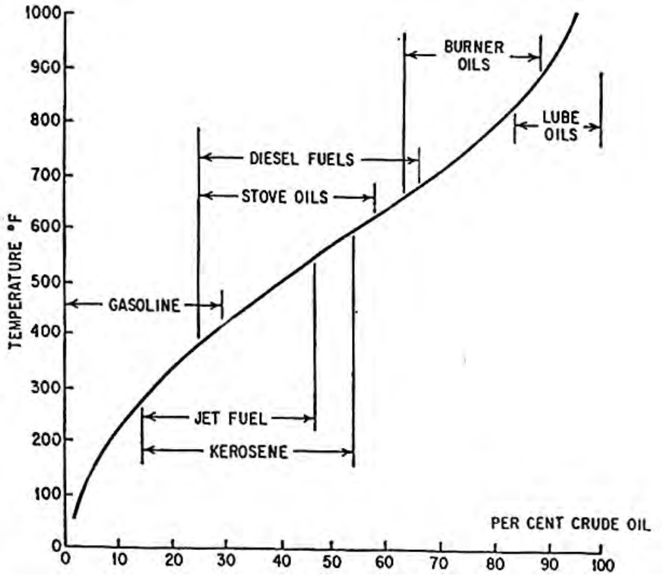


Figure 2.1 Proportion of diesel fuel and other petroleum products processed from crude petroleum [7].

2.1.1 Types of diesel fuels

There are three basic types of diesel fuels. High-speed diesel is normally used as a fuel for high-speed diesel engines operating above 1,000 rpm such as trucks, cars, buses, locomotives, and pumping sets *etc.* Gas turbine requiring distillate fuels normally make use of high-speed diesel as fuel.

Medium-speed diesel is used for a wide range of purpose including generation of electricity, stationary power generators, railroads, and pipeline pumps. It operates range of 450 to 1,000 rpm.

Low-speed diesel or marine diesel is commonly used on ships, fishing boats, and for generation of electricity. Low-speed diesel can operate below 300 rpm. Typical ranges of diesel engines are listed at Table 2.1.

Table 2.1 Typical ranges of diesel engines

Type	Speed Range	Conditions	Typing application
Low Speed	<300 rpm	Heavy load, constant speed Fairy high load	Marine main propulsion; electric power generation
Medium Speed	300-1000 rpm	Relatively constant speed	Marine auxiliaries; Stationary power
High Speed	>1000 rpm	Frequent and wide variation in load and speed	Generators; pumping units Road transport vehicles; diesel locomotives

2.2 Vegetable oils as engine fuels [3, 8, 9, 10]

The use of vegetable oils as alternative fuels has been around for 100 years when the inventor of the diesel engine Rudolf Diesel first tested peanut oil, in his compression ignition engine. In the 1930s and 1940s, vegetable oils were used as diesel substitutes from time to time, but usually only in emergency situations. Recently, because of increase in crude oil prices, limited resources of fossil oil and environmental concerns, there has been a renewed focus on vegetable oils and animal fats to make biodiesel. Continued and increasing use of petroleum will intensify local air pollution and magnify the global warming problems caused by carbon dioxide. In a particular case, such as the emission of pollutants in the closed environment of underground mines, biodiesel has the potential to reduce the level of pollutants and the level of potential for probable carcinogens.

The advantages of using vegetable oils as fuels are:

- Vegetable oils are liquid fuels from renewable sources.
- They do not over-burden the environment with emissions.
- Vegetable oils have potential for making marginal land productive by their property of nitrogen fixation in the soil.
- Vegetable oil's production requires lesser energy input in production.
- Vegetable oils have higher energy content than other energy crops like alcohol. Vegetable oils have 90% of the heat content of diesel and they have a favorable output/input ratio of about 2–4:1 for un-irrigated crop production.
- The current prices of vegetable oils in world are nearly competitive with petroleum fuel price.
- Vegetable oil combustion has cleaner emission spectra
- Simpler processing technology.

Due to the rapid decline in crude oil reserves, the use of vegetable oils as diesel fuels is again promoted in many countries. Depending upon climate and soil conditions, different nations are looking into different vegetable oils for diesel fuels. For example, soybean oil in the USA, rapeseed and sunflower oils in Europe, palm oil in Southeast Asia (mainly Malaysia and Indonesia), and coconut oil in Philippines are being considered as substitutes for mineral diesel.

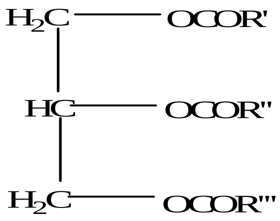
An acceptable alternative fuel for engine has to fulfill the environmental and energy security needs without sacrificing operating performance. Vegetable oils can be successfully used in CI engine through engine modifications and fuel modifications. Engine modifications include dual fueling, injection system modification, heated fuel lines etc. Fuel modifications include blending of vegetable oils with diesel, transesterification, cracking/pyrolysis, micro-emulsion, and hydrogenation to reduce polymerization and viscosity.

From amongst the large number of vegetable oils available in the world, if any specific oil needs to be adopted as a continuing energy crop, it is then essential that an oilseed variety having higher productivity and oil content must be produced. Nevertheless, technologies must be developed for the use of vegetable oils as an alternative diesel fuel that will permit crop production to proceed in an emergency situation. Vegetable oil in its raw form cannot be used in engines. It has to be converted to a more engine-friendly fuel called biodiesel. System design approach has taken care to see that these modified fuels can be utilized in the existing diesel engine without substantial hardware modification. It will be expensive and time-consuming to incorporate even a minor design alteration in the system hardwarebof a large number of existing engines operating in the rural agricultural sector of any country.

In its simplest form, the carbon cycle of vegetable oil consists of the fixation of carbon and the release of oxygen by plants through the process of photosynthesis and then combining of oxygen and carbon to form CO_2 through processes of combustion. It is appropriate to mention here that the CO_2 released by petroleum diesel was fixed from the atmosphere during the formative years of the earth, whereas the CO_2 released by biodiesel gets continuously fixed by plants and may be recycled by the next generation of crops. The carbon cycle time for fixation of CO_2 and its release after combustion of biodiesel is quite small as compared (few years) to the cycle time of petroleum based fuels (few million years). It is well known that petroleum refiners are now facing new sulfur and aromatic compound specifications. Since biodiesel is a fuel made up of esters derived from oils and fats from renewable biological sources, it has been reported to emit substantially lower quantities of most of the regulated pollutants compared to mineral diesel. Biodiesel has comparable energy density, cetane number, heat of vaporization, and stoichiometric air/fuel ratio with mineral diesel. The large molecular size of the component triglycerides result in the oil having higher viscosity compared with that of mineral diesel. Viscosity affects the handling of the fuels by pump and injector system, and the shape of fuel spray. The high jet penetration and poor atomization results in larger droplets. The fuel Jet tends to be a solid stream instead of spray of small droplets hence the fuel does not get mixed with air required for burning. Larger droplets have poor combustion leading to loss of engine power and fuel economy. In small engines, the fuel spray may even impinge upon the cylinder walls, washing away the lubricating oil film and causing the dilution of crank case oil leading to excessive wear of moving parts.

2.2.1. Vegetable oil chemistry

The petroleum diesel fuel molecules are saturated non-branched molecules with carbon atoms ranging between 12 and 18, whereas vegetable oils are the mixture of organic compounds ranging from simple straight chain compound to complex structure of proteins and fat-soluble vitamins. Fats and oils are primarily water-insoluble, hydrophobic substances in the plant and animal kingdom that are made up of one mole of glycerol and three moles of fatty acids and are commonly referred to as triglycerides. Vegetable oils are usually triglycerides generally with a number of branched chains of different lengths, and have structure notation as shown in Scheme 2.1.



Scheme 2.1 Structure of a triglyceride.

where R’, R’’, R’’’ represent hydrocarbon chain of fatty acids. Fatty acids vary in carbon chain length and in the number of unsaturated bonds (double bonds). The structures of common fatty acids are given in Table 2.2, and fatty acid compositions of some vegetable oils are given in Table 2.3.

Vegetable oils have about 10% less heating value than diesel due to the oxygen content in the molecule and the viscosity of vegetable oil is several times higher than that of mineral diesel due to large molecular weight and complex chemical structure. The fuel related properties (Physical and Thermal) of some of the vegetable oils are listed in Table 2.4.

Table 2.2 Chemical structure of common fatty acids [9]

Fatty acid	Systermatic name	Structure	Formular
Lauric	Dodecanoic	12:0	$C_{12}H_{24}O_2$
Myristic	Tetradecanoic	14:0	$C_{14}H_{28}O_2$
Palmitic	Hexadecanoic	16:0	$C_{16}H_{32}O_2$
Stearic	Octadecanoic	18:0	$C_{18}H_{36}O_2$
Arachidic	Eicosanoic	20:0	$C_{20}H_{40}O_2$
Behenic	Docosanoic	22:0	$C_{22}H_{44}O_2$
Lignoceric	Tetracosanoic	24:0	$C_{24}H_{48}O_2$
Oleic	cis-9-Octadecenoic	18:1	$C_{18}H_{34}O_2$
Linoleic	cis-9,cis-12Octadecadienoic	18:2	$C_{18}H_{32}O_2$
Linolenic	cis-9,cis-12,cis-15-Octadecatrienoic	18:3	$C_{18}H_{30}O_2$
Erucic	cis-13-Docosenoic	22:1	$C_{22}H_{42}O_2$

Table 2.3 Chemical composition of vegetable oils [9]

Vegetable oil	Fatty acid composition (wt%)									
	C14:0	C16:0	C18:0	C20:0	C22:0	C24:0	C18:1	C22:1	C18:2	C18:3
Corn	0	12	2	*Tr*	0	0	25	0	6	*Tr*
Cottonseed	0	28	1	0	0	0	13	0	58	0
Crambe	0	2	1	2	1	1	19	59	9	7
Linseed	0	5	2	0	0	0	20	0	18	55
Peanut	0	11	2	1	2	1	48	0	32	1
Rapeseed	0	3	1	0	0	0	64	0	22	8
Safflower	0	9	2	0	0	0	12	0	78	0
Sesame	0	13	4	0	0	0	53	0	30	0
Soya bean	0	12	3	0	0	0	23	0	55	6
Rice-bran	0.4-0.6	11.7-16.5	1.7-2.5	0.4-0.6	—	0.4-0.9	39.2-43.7	—	26.4-35.1	—
Mahua	—	16-28.2	20-25.1	0.0-3.3	—	—	41.0-51.0	—	8.9-13.7	—
Neem	0.2-0.26	13.6-16.2	14.4-24.1	0.8-3.4	—	—	49.1-61.9	—	2.3-15.8	—
Karanja	—	3.7-7.9	2.4-8.9	—	—	1.1-3.5	44.5-71.3	—	10.8-18.3	—

Tr : Trace

Table 2.4 Physical and thermal properties of vegetable oils [9]

Vegetable oil	Kinematic viscosity	Cetane number	Heating value (MJ/kg)	Cloud point (°C)	Pour point (°C)	Flash point (°C)	Density (Kg/l)	Carbon residue (wt%)	Ash (wt%)
Corn	34.9	37.6	39.5	−1.1	−40.0	277	0.9095	0.24	0.01
Cotton seed	33.5	41.8	39.5	1.7	−15.0	234	0.9148	0.24	0.01
Cramble	53.6	44.6	40.5	10.0	−12.2	274	0.9044	0.23	0.05
Linseed	22.2	34.6	39.3	1.7	−15.0	241	0.9236	0.22	<0.01
Peanut	39.6	41.8	49.8	12.8	−6.7	271	0.9026	0.24	0.005
Rapeseed	37.0	37.6	39.7	−3.9	−31.7	246	0.9115	0.30	0.054
Safflower	31.3	41.3	39.5	18.3	−6.7	260	0.9114	0.25	0.006
Sesame	35.5	40.2	39.3	−3.9	−9.4	260	0.9133	0.25	<0.01
Soyabean	32.6	37.9	39.6	−3.9	−12.2	254	0.9138	0.27	<0.01
Sunflower	33.9	37.1	39.6	7.2	−15.0	274	0.9161	0.23	<0.01
Palm	39.6	42.0	—	31.0	—	267	0.9180	—	—
Tallow	—	—	40.0	—	—	201	—	6.21	—

The high viscosity of vegetable oil, 35–60 cSt compared to 4 cSt for diesel at 40 °C, leads to problem in pumping and spray characteristics (atomization and penetration etc.). The inefficient mixing of oil with air contributes to incomplete combustion. High flash point attributes to its lower volatility characteristics. This results in high carbon deposit formation, injector coking, piston ring sticking and lubrication oil dilution and oil degradation. The combination of high viscosity and low volatility of vegetable oils cause poor cold starting, misfire and ignition delay. Some of the short- and long-term problems associated with utilization of vegetable oils in engine are shown in Table 2.5.

This table also discusses probable reasons and potential solutions for these problems. Polyunsaturated natures of the vegetable oils cause long-term problems such as gum formation, ring sticking etc. Because of these problems, vegetable oils must be chemically modified to a more suitable and compatible furl for existing engines.

Table 2.5 Problems and potential solutions for using vegetable oils as engine fuels [3]

Problem	Probable cause	Potential solution
Short-term		
1. Cold weather starting	High viscosity, low cetane, and low flash point of vegetable oils	Preheat fuel prior to injection. Chemically alter fuel to an ester
2. Plugging and gumming of filters, lines and injectors	Natural gums (phosphatides) in vegetable oil. Ash.	Partially refine the oil to remove gums. Filter to 4 microns
3. Engine knocking	Very low cetane of some oils. Improper injection timing	Adjust injection timing. Preheat fuel prior to injection. Chemically alter fuel to an ester
Long-term		
4. Coking of injectors and carbon deposits on piston and head of engine	High viscosity of vegetable oil, incomplete combustion of fuel. Poor combustion at part load.	Heat fuel prior to injection. Switch engine to diesel when operating at part load. Chemically alter the vegetable oil to an ester
5. Excessive engine wear	High viscosity, incomplete combustion of fuel. Poor combustion at part load. Possibly free fatty acids in vegetable oil. Dilution of engine lubricating oil due to blow-by of vegetable oil.	Heat fuel prior to injection. Switch engine to diesel when operating at part load. Chemically alter the vegetable oil to an ester. Increase lubricating oil changes. Lubricating oil additives to inhibit oxidation.
6. Failure of engine lubricating oil due to polymerization	Collection of poly-unsaturated vegetable oil blow-by in crank-case to the point where polymerization occurs	Same as in 5.

2.2.2. Vegetable oil utilization as engine fuel

Neat vegetable oils are not suitable as fuel for diesel engines; hence they have to be modified to bring their combustion-related properties closer to those of mineral diesel. This fuel modification is mainly aimed at reducing the viscosity to get rid of flow and combustion-related problems. Considerable efforts have been made to develop vegetable oil derivatives that approximate the properties and performance of HC-based fuels. Vegetable oils can be used through at least four ways:

- Direct use and blending
- Micro-emulsion
- Pyrolysis (thermal cracking).
- Transesterification

2.2.3. Direct use and blending

Caterpillar (Brazil) in 1980 used pre-combustion chamber engines with the mixture of 10% vegetable oil to maintain total power without any alterations or adjustments to the engine. At that point, it was not practical to substitute 100% vegetable oil for diesel fuel, but a blend of 20% vegetable oil and 80% mineral diesel was successful. Some short-term experiments used up to a 50/50 ratio. Pramanik et al. [11] found that 50% blend of Jatropha oil can be used in diesel engine without any major operational difficulties but further study is required for the long-term durability of the engine. Direct use of vegetable oils and/or the use of blends of the oils have generally been considered to be not satisfactory and impractical for both direct and indirect to oxidation, polymerization during storage and diesel engines. The high viscosity, acid composition, combustion, carbon deposits and lubricating oil free fatty acid content, as well as gum formation due thickening are obvious problems. The probable reasons for the problems and the potential solutions are shown in Table 2.5.

2.2.4. Micro-emulsions

To solve the problem of the high viscosity of vegetable oils, micro-emulsions with solvents such as methanol, ethanol and 1-butanol have been investigated. A micro-emulsion is defined as a colloidal equilibrium dispersion of optically isotropic fluid microstructures with dimension generally in the 1–150 nm range, formed spontaneously from two normally immiscible liquids. They can improve spray characteristics by explosive vaporization of the low boiling constituents in the micelles. Short-term performance of micro-emulsions of aqueous ethanol in soybean oil was nearly as good as that of no. 2 diesel, in spite of the lower cetane number and energy content.

2.2.5. Pyrolysis (thermal cracking)

Pyrolysis is the conversion of one substance into another by means of heat or by heat in presence of a catalyst. The paralyzed material can be vegetable oils, animal fats, natural fatty acids or methyl esters of fatty acids. The pyrolysis of fats has been investigated for more than 100 years, especially in those areas of the world that lack deposits of petroleum. Many investigators have studied the pyrolysis of triglycerides to obtain products suitable for diesel engine. Thermal decomposition of triglycerides produces alkanes, alkenes, alkadines, aromatics and carboxylic acids.

2.2.6. Transesterification

In organic chemistry, transesterification is the process of exchanging the alkoxy group of an ester compound by another alcohol. The reactions are often catalyzed by an acid or a base. Transesterification is crucial for producing biodiesel from biolipids. The transesterification process is the reaction of a triglyceride (fat/oil) with a bio-alcohol to form esters and glycerol [12]. The details of the process of transesterification and biodiesel production are given in following paragraphs.

2.3 Sources of biodiesel [4]

There are more than 350 oil-bearing crops identified, among which only soybean, palm, sunflower, safflower, cottonseed, rapeseed and peanut oils are considered as potential alternative fuels for diesel engines. Worldwide consumption of soybean oil is the highest in 2003 (27.9 million metric tons). Table 2.6 are shows the world vegetable and marine oil consumption between 1998 and 2003. Vegetable oil is one of the renewable fuels. Vegetable oils have become more attractive recently because of their environmental benefits and the fact that these are made from renewable resources. Vegetable oils are a renewable and potentially inexhaustible source of energy with energy content close to diesel fuel. On the other hand, extensive use of vegetable oils may cause other significant problems such as starvation in developing countries. The vegetable oil fuels were not acceptable because they were more expensive than petroleum fuels.

Table 2.6 World vegetable and marine oil consumption (million metric tons) [4]

Vegetable Oil	**1998**	**1999**	**2000**	**2001**	**2002**	**2003**
Soybean	23.5	24.5	26	26.6	27.2	27.9
Palm	18.5	21.2	23.5	24.8	26.3	27.8
Rapeseed	12.5	13.3	13.1	12.8	12.5	12.1
Sunflower seed	9.2	9.5	8.6	8.4	8.2	9.0
Peanut	4.5	4.3	4.2	4.7	5.3	5.8
Cottonseed	3.7	3.7	3.6	4	4.4	4.9
Coconut	3.2	3.2	3.3	3.5	3.7	3.9
Palm kernel	2.3	2.6	2.7	3.1	3.5	3.7
Olive	2.2	2.4	2.5	2.6	2.7	2.8
Fish	1.2	1.2	1.2	1.3	1.3	1.4
Total	80.7	85.7	88.4	91.8	95.1	98.3

A variety of biolipids can be used to produce biodiesel. These are (a) virgin vegetable oil feedstock; rapeseed and soybean oils are most commonly used, though other crops such as mustard, palm oil, sunflower, hemp and even algae show promise; (b) waste vegetable oil; (c) animal fats including tallow, lard and yellow grease and (d) non-edible oils such as Jatropha, neem oil, castor oil, tall oil, etc. Soybeans are commonly used in the United States for food products which has led to soybean biodiesel becoming the primary source for biodiesel in this country.

In Malaysia and Indonesia palm oil is used as a significant biodiesel source. In Europe, rapeseed is the most common base oil used in biodiesel production. In India and Southeast Asia, the Jatropha tree is used as a significant fuel source.

Algae can grow practically in every place where there is enough sunshine. Some algae can grow in saline water. The most significant difference of algal oil is in the yield and hence its biodiesel yield. According to some estimates, the yield (per acre) of oil from algae is over 200 times the yield from the best-performing plant/vegetable oils. Microalgae are the fastest growing photosynthesizing organisms. They can complete an entire growing cycle every few days. Approximately 46 ton of oil/hectare/year can be produced from diatom algae. Different algae species produce different amounts of oil. Some algae produce up to 50% oil by weight. The production of algae to harvest oil for biodiesel has not been undertaken on a commercial scale, but working feasibility studies have been conducted to arrive at the above number.

Specially bred mustard varieties can produce reasonably high oil yields, and have the added benefit that the meal leftover after the oil has been pressed out can act as an effective and biodegradable pesticide.

2.4. Advantages of biodiesel as diesel fuel [4, 13]

The advantages of biodiesel as diesel fuel are liquid nature portability, ready availability, renewability, higher combustion efficiency, lower sulfur and aromatic content, higher cetane number and higher biodegradabilit. Main advantages of biodiesel given in the literature include domestic origin, reducing the dependency on imported petroleum, biodegradability, high flash point and inherent lubricity in the neat form.

2.4.1. Availability and renewability of biodiesel

Biodiesel is the only alternative fuel so that low concentration biodiesel–diesel blends run on conventional unmodified engines. It can be stored anywhere where petroleum diesel fuel is stored. Biodiesel can be made from domestically produced, renewable oilseed crops such as soybean, rapeseed and sunflower. The risks of handling, transporting and storing biodiesel are much lower than those ones, associated with petrodiesel. Biodiesel is safe to handle and transport because it is as biodegradable as sugar and has a high flash point compared to petroleum diesel fuel. Biodiesel can be used alone or mixed in any ratio with petroleum diesel fuel. The most common blend is a mix of 20% biodiesel with 80% petroleum diesel, or B20 under recent scientific investigations; however, in Europe the current regulation foresees a maximum 5.75% biodiesel.

2.4.2. Higher combustion efficiency of biodiesel

Oxygen content of biodiesel improves the combustion process and decreases its oxidation potential. Structural oxygen content of a fuel improves combustion efficiency due to the increase of the homogeneity of oxygen with the fuel during combustion. Because of this the combustion efficiency of biodiesel is higher than petrodiesel as well as the combustion efficiency of methanol/ethanol is higher than that of gasoline. A visual inspection of the injector types would indicate no difference between the biodiesel fuels when tested on petrodiesel. The overall injector coking is considerably low. Biodiesel contains 11% oxygen by weight and contains no sulfur. The use of biodiesel can extend the life of diesel engines because it is more lubricating than petroleum diesel fuel. Biodiesel has got better lubricant properties than petrodiesel.

The higher heating values (HHVs) of biodiesels are relatively high. The HHVs of biodiesels (39–41 MJ/kg) are slightly lower than that of gasoline (46 MJ/kg), petrodiesel (43 MJ/kg) or petroleum (42 MJ/kg), but higher than coal (32–37 MJ/kg).

2.4.3. Lower emissions by using biodiesel

Combustion of biodiesel alone provides over a 90% reduction in total unburned hydrocarbons (HC), and a 75–90% reduction in polycyclic aromatic hydrocarbons (PAHs). Biodiesel further provides significant reductions in particulates and carbon moNOxide than petroleum diesel fuel. Biodiesel provides a slight increase or decrease in nitrogen oxides depending on engine family and testing procedures.

Sulfur content of petrodiesel is 20–50 times those of biodiesels. Several municipalities are considering mandating the use of low levels of biodiesel in diesel fuel on the basis of several studies which have found HC and particulate matter (PM) benefits from the use of biodiesel. The use of biodiesel to reduce N_2O is attractive for several reasons. Biodiesel contains little nitrogen, as compared with petrodiesel which is also used as a reburning fuel. The N_2O reduction was strongly dependent on initial N_2O concentration and only slightly dependent upon temperature, where increased temperature increased N_2O reduction. This results in lower N_2O production from fuel nitrogen species for biodiesel. In addition, biodiesel contains virtually trace amount of sulfur, so SO_2 emissions are reduced in direct proportion to the petrodiesel replacement. One of the most common blends of biodiesel contains 20 vol% biodiesel and 80 vol% conventional diesel. The use of blends of biodiesel and diesel oil is preferred in engines, in order to avoid some problems related to the decrease of power and torque and to the increase of NOx emissions (a contributing factor in the localized formation of smog and ozone) with increasing content of pure biodiesel in the blend. Emissions of all pollutants except NOx appear to decrease when biodiesel is used. The fact that NOx emissions increase with increasing biodiesel concentration could be a detriment in areas that are out of attainment for ozone. Reductions in net carbon dioxide emissions are estimated at 77–104 g/MJ of diesel displaced by biodiesel. These reductions increase as the amount of biodiesel blended into diesel fuel increases. The best emissions reductions are seen with biodiesel.

The exhaust emissions of commercial biodiesel and petrodiesel were studied in a 2003 model year heavy-duty 14 L six-cylinder diesel engine with EGR. The commercial biodiesel fuel significantly reduced PM exhaust emissions (75–83%) compared to the petrodiesel base fuel. However, NOx exhaust emissions were slightly increased with commercial biodiesel compared to the base fuel. The chain length of the compounds had little effect on NOx and PM exhaust emissions, while the influence was greater on HC and CO, the latter being reduced with decreasing chain length. Unsaturation in the fatty compounds causes an increase in NOx exhaust emissions.

2.4.4. Biodegradability of biodiesel

Biodiesel is non-toxic and degrades about 4 times faster than petrodiesel. Its oxygen content improves the biode biodegradation process, leading to a decreased level of quick biodegradation. In comparison with petrodiesel, biodiesel shows better emission parameters. It improves the environmental performance of road transport, including decreased greenhouse emissions (mainly of carbon dioxide). As biodiesel fuels are becoming commercialized their existence in the environment is an area of concern since petroleum oil spills constitute a major source of contamination of the ecosystem. Among these concerns, water quality is one of the most important issues for living systems. It is important to examine the biodegradability of biodiesel fuels and their biodegradation rates in natural waterways in case they enter the aquatic environment in the course of their use or disposal. Chemicals from biodegradation of biodiesel can be released into the environment. With the increasing interest in biodiesel, the health and safety aspects are of utmost importance, including determination of their environmental impacts in the transport, storage or processing [3]. The biodegradabilities of several biodiesels in the aquatic environment show that all biodiesel fuels are readily biodegradable. After 28 days all biodiesel fuels were 77%–89% biodegraded, diesel fuel was only 18% biodegraded. The enzymes responsible for the dehydrogenation/oxidation reactions that occur in the process of degradation recognize oxygen atoms and attack them immediately.

2.4.5. Disadvantages of biodiesel as diesel fuel

Major disadvantages of biodiesel are higher viscosity, lower energy content, higher cloud point and pour point, higher nitrogen oxides (NOx) emissions, lower engine speed and power, injector coking, engine compatibility, high price and higher engine wear. Important operating disadvantages of biodiesel in comparison with petrodiesel are cold start problems, the lower energy content, higher copper strip corrosion and fuel pumping difficulty from higher viscosity. This increases fuel consumption when biodiesel is used in comparison with application of pure petrodiesel, in proportion to the share of the biodiesel content. Taking into account the higher production value of biodiesel as compared to the petrodiesel, this increase in fuel consumption raises in addition to the overall cost of application of biodiesel as an alternative to petrodiesel. Biodiesel has higher cloud point and pour point compared to conventional diesel. Neat biodiesel and biodiesel blends increase nitrogen oxides (NOx) emissions compared with petroleum-based diesel fuel used in an unmodified diesel engine. Peak torque is less for biodiesel than petroleum diesel but occurs at lower engine speed and generally the torque curves are flatter. The biodiesels on the average decrease power by 5% compared to that of diesel at rated load.

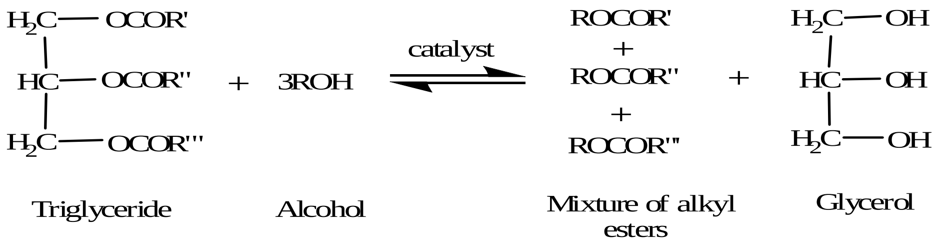
2.5 Transesterification [3, 9, 14, 15]

Transesterification or alcoholysis is the displacement of alcohol from an ester by another in a process similar to hydrolysis, except than alcohol is used instead of water. This process has been widely used to reduce the high viscosity of triglycerides. The transesterification reaction is represented by the general equation as Scheme 2.2.

$$\mathrm{RCOOR'} + \mathrm{R''OH} \xrightleftharpoons{\text{catalyst}} \mathrm{RCOOR''} + \mathrm{R'OH}$$

Scheme 2.2 General equation of transesterification.

In the transesterification of vegetable oils, a triglyceride reacts with an alcohol in the presence of strong acid or base to give a mixture of fatty acids alkyl esters and glycerol as shown in Scheme 2.3.



Scheme 2.3 General equation for transesterification of vegetable oils.

2.5.1 Transesterifcation kinetics and mechanism

Transesterification of triglycerides produce fatty acid alkyl esters and glycerol. The glycerol layer settles down at the bottom of the reaction vessel. Diglycerides and monoglycerides are the intermediates in this process. The mechanism of transesterification is described in Scheme 2.4. The first step is the conversion of triglycerides to diglycerides followed by the conversion of diglycerides to monoglycerides and of monoglycerides to glycerol yielding one methyl ester molecule from each glycerides at each step. The step wise reactions are reversible and a little excess of alcohol is used to shift the equilibrium towards the formation of esters. In presence of excess alcohol, the foreword reaction is pseudo-first order and the reverse reaction is found to be second order. The transesterification reaction can be catalyzed by both homogeneous and heterogeneous catalysts. In turn, the homogeneous catalysts include alkalis and acids. The most commonly used alkali catalysts are sodium hydroxide, sodium methoxide and potassium hydroxide. In this sense, numerous references can be found in the background literature. Sulfuric acid, hydrochloric acid and sulfonic acid are usually preferred as acid catalysts. Finally, the heterogeneous catalysts include enzymes, titanium silicates, alkaline earth metal compounds, anion exchange resins and guanadines heterogenized on organic polymers. The Alkali-catalyzed transesterification is much faster than acid –catalyzed transesterification and is most often used commercially.

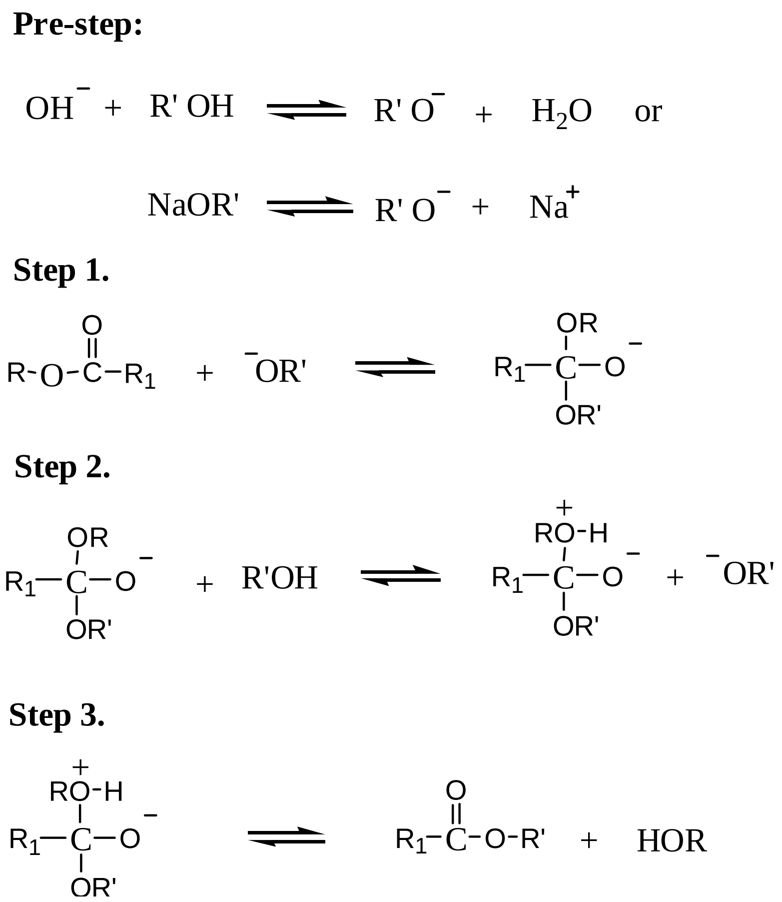
1. $\text{Triglyceride(TG)} + \text{R'OH} \underset{K_2}{\overset{K_1}{\rightleftharpoons}} \text{Diglyceride(DG)} + \text{R'COOR}_1$

2. $\text{Diglyceride(DG)} + \text{R'OH} \underset{K_4}{\overset{K_3}{\rightleftharpoons}} \text{Monoglyceride(MG)} + \text{R'COOR}_2$

3. $\text{Monoglyceride(MG)} + \text{R'OH} \underset{K_6}{\overset{K_5}{\rightleftharpoons}} \text{Glycerol(GL)} + \text{R'COOR}_3$

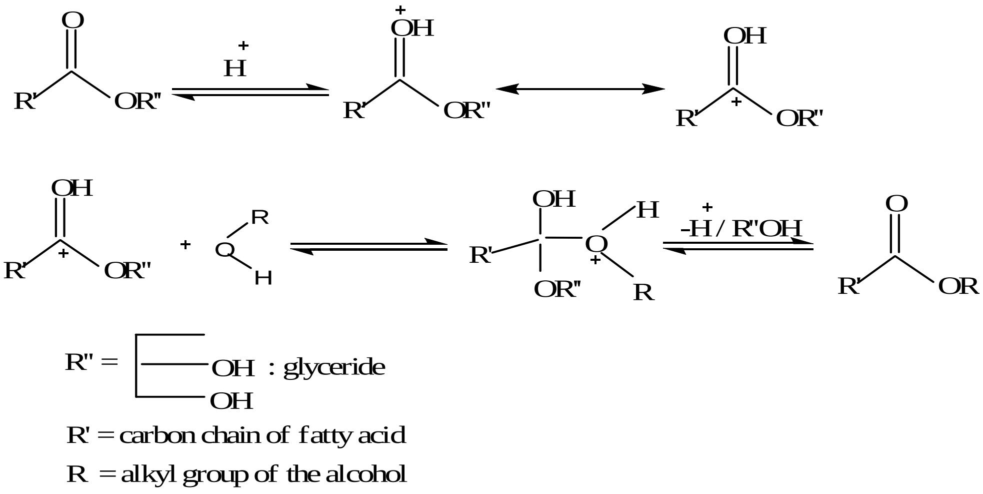
Scheme 2.4 General equation for transesterification of triglycerides.

The mechanism of alkali-catalyzed transesterification is described in Scheme 2.5. The first step involves the attack of the alkoxide ion to the carbonyl carbon of the triglyceride molecule, which results in the formation of a tetrahedral intermediate. The reaction of this intermediate with an alcohol produces the alkoxide ion in the second step. In the last step the rearrangement of the tetrahedral intermediate gives rise to an ester and a diglyceride.



Scheme 2.5 Mechanism of base catalyzed transesterification.

Transesterification can be catalyzed by Brownsted acids, preferably by sulfonic and sulfuric acids. The mechanism of acid catalyzed transesterification of vegetable oil (for a monoglyceride) is shown in Scheme 2.6. However, it can be extended to di-and tri-glycerides. The protonation of carbonyl group of the ester leads to the carbocation, which after a nucleophilic attack of the alcohol produces a tetrahedral intermediate. This intermediate eliminates glycerol to form a new ester and to regenerate the catalyst. The process of transesterification is affected by various factors depending upon the reaction condition used. The effects of these factors are described below.



Scheme 2.6 Mechanism of acid catalyzed transesterification.

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2.5.2. Effect of free fatty acid and moisture [16, 17, 18]

The free fatty acid and moisture content are key parameters for determining the viability of the vegetable oil transesterification process. To carry the base catalyzed reaction to completion; an free fatty acid (FFA) value lower than 3% is needed. The higher the acidity of the oil, smaller is the conversion efficiency. Both, excess as well as insufficient amount of catalyst may cause soap formation.

If the low qualities of beef tallow or vegetable oil with high FFA are used to make biodiesel fuel, they must be refined by saponification using NaOH solution to remove free fatty acids. Conversely, the acid catalyzed process can also be used for esterification of these free fatty acids. The starting materials used for base catalyzed alcoholysis should meet certain specifications. The triglycerides should have lower acid value and all material should be substantially anhydrous. The addition of more sodium hydroxide catalyst compensates for higher acidity, but the resulting soap causes an increase in viscosity or formation of gels that interferes in the reaction as well as with separation of glycerol. When the reaction conditions do not meet the above requirements, ester yields are significantly reduced. The methoxide and hydroxide of sodium or potassium should be maintained in anhydrous state. Prolonged contact with air will diminish the effectiveness of these catalysts through interaction with moisture and carbon dioxide. Most of the biodiesel is currently made from edible oils by using methanol and alkaline catalyst. However, there are large amounts of low cost oils and fats that could be converted to biodiesel. The problems with processing are low cost oils and fats are that they often contain large amounts of free fatty acids that cannot be converted to biodiesel using alkaline catalyst. Therefore, two-step esterification process is required for these feed stocks. Initially the FFA of these can be converted to fatty acid methyl esters by an acid catalyzed pretreatment and in the second step transesterification is completed by using alkaline catalyst to complete the reaction.

2.5.3 Catalyst type

Catalysts are classified as alkali, acid, or enzymes. Alkali-catalyzed transesterification is much faster than acid-catalyzed reaction. However, if a vegetable oil has high free fatty acid and water content, acid-catalyzed transesterification reaction is suitable. Partly due to faster esterification and partly because alkaline catalysts are less corrosive to industrial equipment than acidic catalysts, most commercial transesterification reactions are conducted with alkaline catalysts. Sodium methoxide was found to be more effective than sodium hydroxide. Sodium alkoxides are among the most efficient catalysts used for this purpose, although NaOH, due to its low cost, has attracted its wide use in large-scale transesterification. The alkaline catalyst concentrations in the range of 0.5–1% by weight yield 94–99% conversion of vegetable oils into esters. Further increase in catalyst concentration does not increase the conversion and it adds to extra costs because it is necessary to remove the catalyst from the reaction products at the end. Methanol can quickly react with triglycerides and NaOH is easily dissolved in it. The reaction can be catalyzed by alkalis, acids, or enzymes. The alkalis include NaOH, KOH, carbonates and corresponding sodium and potassium alkoxides such as sodium methoxide, sodium ethoxide, sodium propoxide and sodium butoxide. Sulfuric acid, sulfonic acids and hydrochloric acid are usually used as acid catalysts. Lipases also can be used as biocatalysts [8].

2.5.4 Molar ratio of alcohol to oil

One of the most important variables affecting the yield of ester is the molar ratio of alcohol to vegetable oil. The stoichiometry of the transesterification reaction requires 3 mole of alcohol per mole of triglyceride to yield 3 mole of fatty esters and 1 mole of glycerol. To shift the transesterification reaction to the right, it is necessary to use either a large excess of alcohol or remove one of the products from the reaction mixture continuously. The second option is preferred wherever feasible, since in this way, the reaction can be driven towards completion. When 100% excess methanol is used, the reaction rate is at its highest. A molar ratio of 6:1 is normally used in industrial processes to obtain methyl ester yields higher than 98% by weight. Freedman et al. [19] studied the effect of molar ratio (from 1:1 to 6:1) on ester conversion with vegetable oils. Soybean, sunflower, peanut and cottonseed oils behaved similarly and achieved highest conversions (93–98%) at a 6:1 molar ratio. Ratios greater than 6:1 do not increase yield (already 98–99%), however, these interfere with separation of glycerol.

2.5.5 Effect of reaction time and temperature

The conversion rate increases with reaction time. Freedman et al. [19] transesterified peanut, cotton-seed, sunflower and soybean oil under the condition of methanol–oil molar ratio 6:1, 0.5% sodium methoxide catalyst and 60 °C. An approximate yield of 80% was observed after 1 min for soybean and sunflower oils. After 1 h, the conversion was almost the same for all four oils (93–98%). Ma et al. [20] studied the effect of reaction time on transesterification of beef tallow with methanol. The reaction was very slow during the first minute due to mixing and dispersion of methanol into beef tallow. From one to 5 min, the reaction proceeds very fast. The production of beef tallow methyl esters reached the maximum value at about 15 min. Transesterification can occur at different temperatures, depending on the oil used. For the transesterification of refined oil with methanol (6:1) and 1% NaOH, the reaction was studied with three different temperatures. After 0.1 h, ester yields were 94, 87 and 64% for 60, 45 and 32 °C, respectively. After 1 h, ester formation was identical for 60 and 45 °C runs and only slightly lower for the 32 °C run. Temperature clearly influenced the reaction rates and yield of esters [19].

2.6 Properties and specification of biodiesel [2, 9, 15]

The characteristics of biodiesel are close to mineral diesel, and, therefore, biodiesel becomes a strong candidate to replace the mineral diesel if the need arises. The conversion of triglycerides into methyl or ethyl esters through the transesterification process reduces the molecular weight to one-thirds that of the triglycerides, the viscosity by a factor of about eight and increases the volatility marginally. Biodiesel has viscosity close to mineral diesel. These vegetable oil esters contain 10–11% oxygen by weight, which may encourage combustion than hydrocarbon-based diesel in an engine. The cetane number of biodiesel is around 50. Biodiesel has lower volumetric heating values (about 10%) than mineral diesel but has a high cetane number and flash point. The esters have cloud point and pour points that are 15–25 °C higher than those of mineral diesel. The properties of biodiesel from different oils and diesel fuels are compared in Table 2.7.

Table 2.7 Properties of biodiesel from different oils [2, 9]

Properties	Biodiesel (vegetable oil methyl ester)					
	Palm	Linseed	Sunflower	Tallow	Peanut	Diesel
Viscosity at 37.8 °C (mm2/s)	5.7	3.59	4.6	—	4.9	3.06
Cetane number	62	52	49	—	54	50
Lower heating value (MJ/kg)	33.5	35.3	33.5	—	33.6	43.8
Cloud point (°C)	13	—	1	12	5	—
Pour point (°C)	—	-15	—	9	—	-16
Flash point (°C)	164	172	183	96	176	76
Density (kg/l)	0.88	0.874	0.86		0.883	0.855
Carbon residue (wt%)	—	1.83	—	—	—	—

2.6.1 Specification for biodiesel

Biodiesel is defined in the standard as a fuel comprised of mono-alkyl esters of long chain fatty acids derived from vegetable oils or animal fats, designated B100.The fuel properties of the biodiesel were measured specifications according to the ASTM and EN standards in Table 2.8.

Table 2.8 Specification for biodiesel [15]

Property	**Method**	**Limits**	**Units**
Viscosity at 40 °C	ASTM D 445	3.5-5.0	cSt
Acid number	ASTM D 664	Max 0.5	KOH/g
Flash point	ASTM D 93	Min 120	°C
Methyl ester	EN 14103	Min 96.5	% wt
Density at 15 °C	ASTM D 1298	860-900	Kg/m^3
Cetane number	ASTM D 613	Min 51	
Water and sediment	ASTM D 2709	Max 0.05	% wt
Total contaminate	ASTM D 5452	Max 0.0024	% wt
Total sulfur	ASTM D 5453	Max 0.05	% wt
Carbon residue	ASTM D 4530	Max 0.3	% mass
Sulfated ash	ASTM D 874	Max 0.02	% wt
Triglyceride	EN 14105	Max 0.2	%wt
Total glycerin	EN 14105	Max 0.25	% wt

The flash point is defined as the “lowest temperature corrected to a barometric pressure of 101.3 kPa (760 mm Hg), at which application of an ignition source causes the vapors of a specimen to ignite under the specified conditions of test.” This test, in part, is a measure of residual alcohol in the B100. The flash point is also a determinant for flammability classification of materials. B100’s typical flash point is > 200 ° C, classifying it as “non-flammable”.

Methyl ester: The purpose of this EN is to determine the ester content of fatty acid methyl ester intended for use as pure biofuel or as a blending component for heating and diesel fuels. Determination of the percentage of methyl ester of fatty acid present in the sample by gas chromatography with internal calibration.

Water and sediment is a test that "determines the volume of free water and sediment in middle distillate fuels having viscosities at 40 °C in the range 1.0 to 4.1 mm2/s and densities in the range of 700 to 900 kg/m^3." This test is a measure of cleanliness of the fuel. For B100 it is particularly important because water can react with the esters, making free fatty acids, and can support microbial growth in storage tanks.

Kinematic viscosity – "the resistance to flow of a fluid under gravity". [Equal to the dynamic viscosity/density.] The kinematic viscosity is a basic design specification for the fuel injectors used in diesel engines. Too high a viscosity and the injectors do not perform properly.

Density – "the mass per unit volume of a substance at a given temperature."

Sulfated ash – "The residue remaining after a sample has been carbonized, and the residue subsequently treated with sulfuric acid and heated to a constant weight." This test monitors the mineral ash residual when a fuel is burned. For biodiesel, this test is an important indicator of the quantity of residual metals in the fuel that came from the catalyst used in the esterification process.

Total sulfur – "This method covers the determination of total sulfur in liquid hydrocarbons, boiling in the range from approximately 25 to 400 ° C, with viscosities between approximately 0.2 and 20 cSt (mm^2/s) at room temperature." Vegetable oil feedstocks typically have very little sulfur, but this test is an indicator of contamination of protein material and/or carryover catalyst material or neutralization material from the production process.

Cetane number – "a measure of the ignition performance of a diesel fuel obtained by comparing it to reference fuels in a standardized engine test." Cetane for diesel engines is analogous to the octane rating in a spark ignition engine. It is a measure of how the fuel will ignite in the engine. For B100, the cetane number can be very accurately predicted using the ester's composition. It is unlikely that an individual producer will ever run cetane tests on-site.

Cloud point – “The temperature at which a cloud of wax crystals first appears in a liquid when it is cooled down under conditions prescribed in this test method.” The cloud point is a critical factor in cold weather performance for all diesel fuels. The chemical composition of some biodiesel feedstocks leads to a B100 that may have higher cloud points than desired. The cloud point, however, is another parameter that can be predicted accurately with knowledge of the esters composition, but producers are advised to be able to perform this test.

Carbon residue – “In petroleum products, the part remaining after a sample has been subjected to thermal decomposition.” The carbon residue is a measure of how much residual carbon remains after combustion. This is particularly important in diesel engines because of the possibility of carbon residues clogging the fuel injectors. The most common cause of excess carbon residues in B100 is an excessive level of total glycerin. Total glycerin is also measured directly using an additional procedure specified in this Standard.

Acid number – “The quantity of base, expressed as milligrams of potassium hydroxide per gram of sample, required to titrating a sample to a specified end point.” The acid number is a direct measure of free fatty acids in B100. The free fatty acids can lead to corrosion and may be a symptom of water in the fuel.

Total glycerin – “is the sum of free and bonded glycerin.” Bonded glycerin – “is the glycerin portion of the mono-, di-, and triglyceride molecules.” Elevated total glycerin values are indicators of incomplete esterification reactions and predictors of excessive carbon deposits in the engine. The terms “glycerin” and “glycerol” are used interchangeably.

Phosphorus – “This test covers the quantitative determination of barium, calcium, copper, magnesium, phosphorus, sulfur, and zinc in unused lubricating oils and additive packages.” In the case of B 100, phosphorus can come from incomplete refining of the vegetable oil and from bone and proteins encountered in the rendering process.

2.7 Combustion, emission, engine problems and deposit [21]

Generally, similar types of compound are observed in the exhaust emission of conventional Diesel Fuel (DF) and vegetable oil-derived fuels. This is proof of the suitability of fatty compounds as diesel because there presumably are similarities in their combustion behavior.

Emissions from any kind of engine are the result of the proceeding combustion within in the engine. The combustion process, in relation to the properties of the fuel, and its completeness are responsible for any problems associated with the use of biodiesel, such as formation of deposits, etc. To comprehend the formation of emissions, deposits, and possibly direct the combustion to suppress undesirable emissions and deposits.

Ideally, the products of complete combustion of hydrocarbons are CO_2 and water according to the equation which show for alkanes (saturated hydrocarbon):

$$\mathbf{C_nH_{2n+2} + (1.5n+0.5)O_2 \longrightarrow nCO_2 + (n+1)H_2O}$$

Combustion in a diesel engine occurs mainly through a diffusion flame and is therefore incomplete. This causes the formation of partiallyNOxidized materials such as carbon moNOxide (CO), other oxygenated species (aldehydes, etc.) and hydrocarbon.

In the case of biodiesel, release of CO_2, as indicated above, from the ester moiety of methyl ester occurs besides combustion formation of CO_2 from hydrocarbon portions of biodiesel. The formation of CO_2, an incombustible compound despite its high oxygen content (although mistakenly assumed that it can serve as a combustion enhancer because of its high oxygen content), shows that one has judicious in choosing oxygenated compound as combustion enhancer for the combustion-enhancing properties will depend on the nature of the oxygen (such as bonding, etc.) in those compounds. So, the higher oxygen content of biodiesel does not necessarily imply improved combustion compared to conventional DF because of removal of this oxygen from the combustion process by liberation of CO_2, but CO_2 may contribute to combustion in other ways.

Exhaust emissions observed in the combustion of conventional DF and biodiesel are smoke, particulates polyaromatic hydrocarbons (PAHs), hydrocarbons, CO and oxides of nitrogen (NOx; referred to as nitrous oxides or nitrogen oxides). Important differences are sulfur-containing emissions which are not formed from biodiesel due to its lack of sulfur.

Engine problem is used neat vegetable oil fuel. At least in short-term trials, neat oils gave satisfactory engine performance and power output, often equal to or even slightly better than DF. However, vegetable oils cause engine problems. This was recognized in the early stages of renewed interest in vegetable oil-based alternative DFs.

Generally, most emissions observed for DF are reduced when using esters. In early reporting emissions with methyl and ethyl soyate as fuel, it was found that CO and hydrocarbons were reduced while NOx were produced consistently at a higher level than the reference DF. The differences in exhaust gas temperatures corresponded with the differences in NOx levels. Similar results were obtained on the emissions of rapeseeds oil methyl ester. NOx emissions were slightly increased, while hydrocarbon, CO, particulate and PAH emissions were in ranges similar to the DF reference. The study on PAH emissions, where also the influence of various engine parameters was explored, found that the PAH emissions of sunflower ethyl ester were situated between DF and the corresponding neat vegetable oil.

2.8 Economic feasibility of biodiesel

Economical feasibility of biodiesel depends on the price of the crude petroleum and the cost of transporting diesel long distances to remote markets. It is certain that the cost of crude petroleum is bound to increase due to increase in its demand and limited supply. Further, the strict regulations on the aromatics and sulfur contents in diesel will result in higher cost of production of diesel fuels. The cost of chemicals, mainly alcohol and catalyst, depends on the process, as well as the unit prices for the chemicals. The cost of producing methyl or ethyl esters from edible oils is currently much more expensive than hydrocarbon-based diesel fuel. Due to the relatively high costs of vegetable oils (about 1.5 to two times the cost of diesel), methyl esters produced from it cannot compete economically with hydrocarbon-based diesel fuels unless granted protection from considerable tax levies applied to the latter. In absence of tax relief, there is a need to explore alternate feedstock for production of biodiesel**.**

The cost of biodiesel can be reduced if we consider non-edible oils and used-frying oils instead of edible oils. Non-edible oils such as mahua, karanja, babassu, Jatropha, neems etc. are easily available in many parts of the world, and are cheaper compared to edible oils. Most of these non-edible oils are not used to their potential and in fact produced in surplus quantities. Several countries including Netherlands, Germany, Belgium, Austria, USA, Japan and India discard used frying oils. With the mushrooming of fast food centers and restaurants in the world, it is expected that considerable amounts of used-frying oils will be discarded. This oil can be used for making biodiesel, thus helping to reduce the cost of water treatment in the sewerage system and in the recycling of resources.

Most of the biodiesel that is currently made uses soybean oil, methanol and an alkaline catalyst. The high value of soybean oil as a food product makes production of a cost-effective fuel very challenging. However, there are large amounts of low cost oils and fats, such as restaurant waste and animal fats that could be converted to biodiesel [8]. The problem with processing these low-cost oils and fats is that they often contain large amounts of FFA that cannot be converted to biodiesel using an alkaline catalyst. Currently, there are seven producers of biodiesel in the USA. Pure biodiesel (100%) sells for about \$1.50–\$2.00 per gallon before taxes. Fuel taxes will add approximately \$0.50 per gallon. A mix of 20% biodiesel and 80% diesel will cost about 15–20b more per gallon over the cost of mineral diesel. The cost of biodiesel production results in a generally accepted view of the industry in Europe that biodiesel production is not profitable without fiscal support from the government.

A review of 12 economic feasibility studies shows that the projected costs for biodiesel from oil seed or animal fats have a range US\$0.30–0.69/l, including meal and glycerin credits and the assumption of reduced capital investment costs by having the crushing and/or esterification facility added onto an existing grain or tallow facility. Rough projections of the cost of biodiesel from vegetable oil and waste grease are, respectively, US\$0.54–0.62/l and US\$0.34–0.42/l. With pre-tax diesel priced at US\$0.18/l in the US and US\$0.20–0.24/l in some European countries, biodiesel is thus currently not economically feasible, and more research and technological development will be needed [22].

The total energy use for biodiesel production in the common method is 17.9 MJ/l biodiesel. Transesterification process alone consumes 4.3 MJ/l, while from our calculation,the supercritical methanol method requires as much as 3.3 MJ/l, or energy reduction of 1.0 MJ for each liter of biodiesel fuel. In the common catalyzed method, mixing is significant during the reaction. In our method, since the reactants are already in a single phase, mixing is not necessary. Since our process is much simpler, particularly in purification step which only needs a removal of unreacted methanol, it is further expected that about 20% of cost reduction can be realized from transesterification process. Therefore, the production cost for biodiesel fuel from rapeseed oil falls to be US$0.59/l, compared to US$0.63/l for the common catalyzed method [23].

The brake power of biodiesel was nearly the same as with petrodiesel, while the specific fuel consumption was higher than that of petrodiesel. Carbon deposits inside the engine were normal, with the exception of intake valve deposits. The results showed the transesterification treatment decreased the injector coking to a level significantly lower than that observed with petrodiesel. Although most researchers agree that vegetable oil ester fuels are suitable for use in CIE, a few contrary results have also been obtained. The results of these studies point out that most vegetable oil esters are suitable as diesel substitutes but that more long-term studies are necessary for commercial utilization to become practical.

2.9 Literature reviews

In 1970, Kaimal *et al*. [24] studied fatty acid compostions of lipids isolated from *Ceiba pentandra, Sterculia foetida* and *Hydnocarpus weightiana*. The main components of the seed lipids of the *Sterculia foetida* and *Hydnocarpus weightiana* were sterculic acid and hydnocapic acid.

In 2002, Antolin *et al*. [25] studied the optimization of biodiesel production of sunflower oil tranesterification. The reaction was achieved at 70 ºC using 0.28% w/w of potassium hydroxide as a catalyst with an excess amount of methanol. By this condition 96% of methyl ester was achieved. According to European standard, the sunflower methyl esters could be used as diesel fuels.

In 2004, Vincente *et al*. [26] studied the integrated biodiesel production by used different basic catalyst (sodium methoxide, potassium methoxide, sodium hydroxide and potassium hydroxide) for transesterification of sunflower oil. All the experiments were carried out under the same reaction condition. The reactions were carried out at 65 °C with 6:1 molar ratio of methanol to oil and 1% basic catalyst. The yield of biodiesel using sodium hydroxide, potassium hydroxide, sodium methoxide and potassium methoxide were 85.9, 91.67, 99.33 and 98.46 %, respectively. The yield of biodiesel from methoxide catalyst was higher because the basic methoxides only have the trace hydroxide ion as an impurity. In this sense, they do not produce soap through triglyceride saponification.

In 2005, Ghadge *et al*. [27] studied a technique to produe biodiesel from mahua oil (*Madhuca indica*) having high free fatty acids. The high FFA (19%) level of crude mahua oil can be reduced to less than 1% in a 2-step pretreatment process of esterification using acid catalyzed(1% v/v H_2SO_4) reaction with methanol (0.3-0.35 v/v) at 60 °C and 1 h reaction time. The second step used alkali-catalyzed (0.7% w/v KOH) transesterification reaction with methanol (0.25 v/v) to produce biodiesel. This process gave a yield of 98 % mahua biodiesel.

In 2005, Mohibbe Azam *et al*. [6] studied fatty acid profiles of seed oils of 75 plant species in India which having 30% oil in their seed or kernel. Fatty acid compositions, iodine value, cetane number, were used to predict the quality of fatty acid methyl esters of oil for use as biodiesel. Fatty acid methyl esters of oils of 4 species were found most suitable for use as biodiesel including *Azadirachta indica*, *Calophyllum inophyllum*, *Jatropha curcus* and *Pongamia pinnata*.

In 2005, Ramadhas *et al.* [28] studied biodiesel production from high FFA rubber seed oil. The major components of rubber oil were oleic, linoleic and linolenic acid. The FFA contents of rubber seed oil was about 17 %. The rubber seed oil was converted to methyl esters by 2-step process. The important properties of biodiesel such as specific gravity, flash point, cloud point and pour point were found within the biodiesel standard. This study supports the production of biodiesel from unrefined rubber seed oil as a viable alternative to diesel fuel.

In 2006, Holser *et al*. [29] synthesized the biodiesel fuel from milkweed (*Asclepias*) seed oil. The milkweed ester was compared to soybean esters in laboratory tests to determine biodiesel fuel performance properties. The properties of biodiesel fuel are influenced by the structure and distribution of the component fatty acid esters which vary depending on the source. The milkweed esters contain more unsaturated fatty structures than the soybean esters and would be expected to oxidize more rapidly if the oxidation rates of the individual fatty esters were the same. The milkweed esters have higher viscosities and lubricity values than soybean esters.

In 2006, Meher *et al.* [30] studied transesterification of *Pongamia pinnata* oil with methanol for the production of biodiesel. The reaction parameters such as catalysts concentration, alcohol to oil molar ratio, temperature, and rate of mixing were optimized for production of methyl esters. From the results, the reaction conditions were optimized and found to be 2 h reaction time, the stirring speed of 360 rpm, 1% KOH as catalyst, the methanol to oil molar ratio = 9:1, reaction temperature 65 °C. The maximum yield reached 97-98% of methyl esters.

In 2007, Berchmans *et al*. [31] synthesized biodiesel from crude *Jatropha curcas* L. seed oil having high free fatty acids (15% FFA). The FFA level of crude oil was reduced to less than 1% by a two- step pretreatment process. The first step was carried out with 0.6 w/w methanol to oil and 1% w/w H_2SO_4 as an acid catalyst at 50 °C in 1 h. The second step was transesterified using 0.24 w/w methanol to oil, 1.4% w/w NaOH to oil as alkaline catalyst and reaction temperature at 65 C° in 2 h. The two-step process gave 90 % methyl ester.

In 2007, Yong *et al*. [32] synthesized biodiesel from waste cooking oils which contain large contents of FFAs from restaurants by uses two-step catalyzed process. The FFAs of waste cooking oils was esterified with methanol catalyzed via ferric sulfate in the first step and the triglycerides were transesterified with methanol catalyzed via potassium hydroxide. The conversion of FFA reached 97.22% when the parameters were as follows: amount of ferric sulfate 2 wt%, molar ratio of methanol to triglycerides 10:1 and reacted at 95 ^{o}C for 4 h. The remained triglyceride in transesterification stage was performed at 65 ^{o}C for 1 h in a presence of 1 wt% of potassium hydroxide and 6:1 molar ratio of methanol to triglycerides. The final product gave 97.02% of biodiesel after the two-step catalyzed process. The new process had many advantage compared with the acid or alkaline catalyzed process, such as high efficiency, no acidic waste water and easy recovery of the catalyst.

CHAPTER III

MATERIALS AND METHODS

3.1 Materials and equipments

3.1.1 Raw material as biodiesel production

All raw materials used in this experiment are listed in Table 3.1.

Table 3.1 Oilseeds and sources

Oilseeds	Sources
Acacia auricalifomis	Department of Forestry, Bangkok; Thailand
Acacia mangium	Department of Forestry, Bangkok; Thailand
Annona squamosa Linn.	Jatujak market, Bangkok; Thailand
Azadirachta indica Valeton	Sumpeng market, Bangkok; Thailand
Calophyllum inophyllum	Kasetsart university, Bangkok; Thailand
Canarium subulatum	Sumpeng market, Bangkok; Thailand
Carica papaya L.	Jatujak market, Bangkok; Thailand
Ceiba pentandra Gaertn.	Jatujak market, Bangkok; Thailand
Croton tiglium L.	Sumpeng market, Bangkok; Thailand
Eucalyptus camaldulensis	Department of Forestry, Bangkok; Thailand
Gmelina arborea Roxb	Department of Forestry, Bangkok; Thailand
Hevea brasiliensis	Nakornsritommarat Province; Thailand
Hydnocarpus ilicifolius	Sumpeng market, Bangkok; Thailand
Hyptis suaveolens (L.) Poit	Lansak district, Uthaithanee Province; Thailand
Irvingia malayana	Lansak district, Uthaithanee Province; Thailand
Litsea glutinosa Robins	Lansak district, Uthaithanee Province; Thailand
Melia azadirachta L.	Department of Forestry, Bangkok; Thailand
Parinari anamense Hance	Sumpeng market, Bangkok; Thailand
Pinus kesiya	Department of Forestry, Bangkok; Thailand
Pinus merkusii	Department of Forestry, Bangkok; Thailand
Sapindus emarginatus	Sumpeng market, Bangkok; Thailand
Sterculia foetida L.	Jatujak market, Bangkok; Thailand
Terminalia bellirica Roxb	Department of Forestry, Bangkok; Thailand
Wrightia tomentosa	Department of Forestry, Bangkok; Thailand
Xylia kerrii	Department of Forestry, Bangkok; Thailand

3.1.2 Chemicals

Anhydrous sodium sulfate: analytical grade; Carlo Erba
Carbon tetrachloride;analytica grade; Mallinckrodt
Chloroform-D: NMR spectroscopy grade; Merck
Dichloromethane: analytical grade; Lab-Scan
Ethanol: analytical grade; Merck
Glacial acetic acid: analytical grade; Merck
Heptane: analytical grade; Merck
Hexane: analytical grade; Lab-Scan
Hydrochloric acid: analytical grade; Merck
Methanol: analytical grade; Merck
Methyl heptadecanoate: analytical grade; Fluka
Methyl stearate; analytical grade; Merck
Potassium hydroxide: analytical grade; Lab-Scan
Potassium dichromate: analytical grade; Lab-Scan
Potassium iodide: analytical grade; Lab-Scan
Potassium hydrogen phthalate
Sodium hydrogen carbonate: analytical grade; Carlo Erba
Sodium hydroxide: analytical grade; ACS
Sodium thiosulfate: analytical grade; Lab-Scan
Sulfuric acid: analytical grade; Carlo Erba
Toluene: analytical grade; Merck
Wijs solution: analytical grade; Merck
37 Component FAME standard; Supleco
2-Propanol: analytical grade; Merck

3.1.3 Equipments

Fourier-Transform NMR Spectrometer: Mercury (400MHz); Varian
Fourier-Transform Infrared Spectrophotometer: Model Impact410; Nicolet
Gas-liquid Chromatography; Model 3800; Varian
Cannon Automatic Viscometer: Model CAV-3; Cannon

Density meters: Model DMA 4500 ; Anton parr
Mini Flash: FLA; Grabner instruments
Rotary evaporator: Model ; Buchi

3.2 Methods

3.2.1 Solvent extraction of oilseed plants

500 ml of hexane was added to 500 g of the crushed kernel in 2000 ml of Erlenmeyer flask and allowed to shake overnight. The organic extract was filtered and evaporated to dry by rotary evaporator. The percentage of oil was determined. The physical and chemical properties of oils were determined according to 3.2.2.

3.2.2 Determination of physical and chemical properties of crude oil from oilseed plants as biodiesel production

3.2.2.1 Determination of free fatty acids contained in crude oils

The free fatty acid was determined according to ASTM D 5555; Standard test method for determination of free fatty acids contained in animal, marine, and vegetable fats and oils used in fat liquors and stuffing compounds.

To the 250 ml of Erlenmeyer flask, oil sample (7.05 g), ethanol (75 ml) and 2 ml of 1% phenolphthalein were added. The mixture was titrated with 0.25 N sodium hydroxide solutions until the pink color occurred. The volume of alkali solution used was recorded.

The percentage of free fatty acid was calculated as follows:

$$\%\ \text{free fatty acids} = (\text{ml of alkali} \times N \times 28.2)\ /\ \text{weight of sample}$$

N = normality of alkaline solution

ml of alkali = ml of sodium hydroxide solution

3.2.2.2 Determination of the saponification value of crude oils

The saponification value was determined according to ASTM D 5558; Standard test method for determination of the saponification value of fats and oils.

To the 250 ml of Erlenmeyer flask, oil sample (2 g), alcoholic potassium hydroxide (50 ml) and 1 ml of 1% phenolphthalein were added. The mixture was titrated with 0.5 N of hydrochloric acid until the pink color disappeared. The volume of acid solution used was recorded.

The saponification value was calculated as follows:

saponification value = 28.05($A – B$) / weight of sample
A = titration of blank
B = titration of sample
Alcoholic KOH = 40 g of potassium hydroxide dissolved in 1 L of ethanol

3.2.2.3 Determination of the iodine value of crude oils

The iodine value was determined according to ASTM D 5554; Standard test method for determination of the iodine value of fats and oils.

To the 500 ml of glass-stopper flask, oil sample (0.1 g), carbon tetra chloride (20 ml) and Wijs solution (25 ml) were added. Store the flasks in a dark place for 30 min. From storage, removed the flasks and add 20 ml of KI solution followed by 100 ml of distilled water. The mixture was titrated with 0.1 N of sodium thiosulfate until the yellow color has almost disappeared. Add 2 ml of starch indicator solution, and continued the titration until the blue color has just disappeared. The volume of sodium thiosulfate used was recorded.

The iodine value was calculated as follows:

Iodine value = $(B - S) \times N \times 12.69$ / weight of sample
B = titration of blank
S = titration of sample
N = normality of $Na_2S_2O_3$ solution

3.2.2.4 Determination of the fatty acid composition of crude oils

The fatty acid compositions of the oils from oilseed plants were analyzed by gas chromatography (GC).

Sample (250 mg) was dissolved in heptadecanoic solution (5 ml) (500 mg heptadecanoate was dissolved in heptane (50 ml)) and 1 µl of solution was injected into using 1:100 split ratios.

The condition of GC used was as follows:

Column: ZB-Wax plus; Zebron, 30m, 0.25mm ID, 0.25µm
Injector temperature: 240 °C
Detector: Flame ionization
Detector temperature: 250 °C
Column Oven: 50 °C (hold 2 min), rate of 4 °C/min to 220 °C (hold 15 min)
Carrier gas: N_2
Flow rate: 1.2 ml/min
Volume injected: 1 µl

3.2.3 Biodiesel production

3.2.3.1 Optimization for base catalyzed process

- Effect of free fatty acid to product yield of palm oil methyl ester.

The synthesis of biodiesel was carried out using palm oil with various amount of free fatty acid (1, 2, 3 and 4 % wt). The reaction condition was used as conventional one as 1:6 molar ratios of oil to methanol, 1% NaOH (1% by wt. of oil), reaction temperature at 65 °C and reaction time for 1 hour.

20 g of each sample were added into 100 ml of two-neck round bottom flask equipped with condenser. After the oil was heated to 65 °C, the solution of sodium hydroxide (0.2 g) in methanol (5.79 ml) was added and then the mixture was heated to 65^{o}C for 1 h. The mixture was transferred to a separatory funnel, allowed glycerol to separate for 2 h. The methyl ester layer (upper layer) was separated, and washed with hot water (5 x 100 ml), dried over Na_2SO_4 and filtrated. The product yield (%wt) was calculated by:

$$\text{Product yield (\% wt)} = \frac{\text{weight of palm oil methyl ester}}{\text{weight of palm oil}} \times 100$$

- Effect of methanol to oil molar ratio and reaction time

The palm oil methyl ester synthesized following the method described in 3.2.3.1, by using molar ratios of methanol to oil equal 3:1, 6:1, 9:1 and 12:1, the reaction times equal 0.25, 0.5, 1, 1.5 and 2 h. The methyl ester (5 mg) was subjected to ^{1}H-NMR analysis. The calculation conversion of product was shown in Appendix C.

3.2.3.2 Optimization for two-step catalyzed process (Acid – base catalyzed)

- Effect of reaction time and amount of free fatty acid.

The experiment plan involved five levels of reaction time 0, 0.5, 1, 1.5, 2 h; four levels of amount of free fatty acid 10, 15, 20, 25 (% wt). The reaction condition was used as conventional one as 1:10 molar ratios of oil to methanol, the reactions at 60 °C and 1% H_2SO_4 (1% by wt. of oil).

20 g of each sample were added into100 ml of two-neck round bottom flask equipped with condenser. After the oil was heated to 60 °C for esterification, the solution of sulfuric acid (0.11 ml) in methanol (9.65 ml) was added and then the mixture was heated to 60 ^{o}C for 1 h. The mixture was transferred to a separatory funnel, allowed glycerol to separate for 2 h. The methyl ester and unreacted triglyceride that was subjected to the calculate amount of FFA in lower oil layer according to ASTM D 5555.

3.2.3.3 Synthesis of methyl ester from oilseed plants via base-catalyzed process (FFA< 3%)

The fatty acid methyl ester was synthesized following the method described in 3.2.3.1, using oils of 13 species with less than 3 % FFA. The product was characterized by FT-IR, ^{1}H-NMR and GC techniques.

3.2.3.4 Synthesis of methyl ester from oilseed plants via 2-step catalyzed process (FFA> 3%)

This process involved an esterification in first step and transesterification in second step. At first step, the fatty acid methyl ester was synthesized following the method described in 3.2.3.2, using 12 species of oils with more than 3 % FFA. The lower layer was methyl ester and unreacted triglyceride was used in the second step following the method described in 3.2.3.1. The product was characterized by FT-IR and ^{1}H-NMR techniques.

3.2.4 Characterization and determination of the biodiesels

3.2.4.1 Characterization of the biodiesels

The biodiesels were characterized by:

3.2.4.1.1. Fourier-Transform Infrared Spectrophotometer (FT-IR)

FT-IR spectra of biodiesels were recorded from 4000-600 cm^{-1} on NaCl cell.

3.2.4.1.2. Fourier-transform NMR spectrometer (NMR)

^1H NMR spectra were recorded in $CDCl_3$ using $CHCl_3$ as an internal standard.

3.2.4.2 % Ester contents

The %ester content was determined by ^1HNMR and GC.

3.2.4.2.1. Fourier-transform NMR spectrometer (NMR)

The conversion of methyl esters from oilseed plants was determined using ^1H NMR. The conversion of methyl esters was calculated by comparing the peak area of methoxy and methylene protons using the following equation:

$$\boxed{\%\ \text{conversion} = [(2I_{Me}) / (3I_{CH2})] \times 100}$$

3.2.4.2.2. Gas-liquid chromatography (GC)

The fatty acid compositions and methyl esters content of the oil from oilseed plants were also determined by GC.

The GC condition for the determination of methyl ester was set as 3.2.2.4. Methyl heptadecanoate was used as an internal standard. The methyl ester content of biodiesel was calculated by the following equation:

$$C = \left(\frac{\sum A - A_i}{A_i} \right) \times \left(\frac{C_i \times V_i}{m} \right) \times 100$$

C = Methyl ester content

∑A = Total area of fatty acid methyl esters

Ai = Area of methyl heptadecanoate

Ci = Concentration of methyl heptadecanoate solution

Vi = Volume of methyl heptadecanoate solution

m = Mass of the sample

The results were shown in appendix A.

3.2.4.3 Determination of the properties of biodiesel

The physical properties of biodiesel were determined according to the test methods shown in Table 3.2.

Table 3.2 Test method of biodiesel fuels

Property	Method
Viscosity at 40 °C (cSt)	ASTM D445
Flash point (°C)	ASTM D93
Density at 15 °C (kg/cm^3)	ASTM D4052
Acid number (mg KOH/g)	ASTM D974
Ester content (%wt)	EN 14103

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Solvent extraction of oilseed plants

The oilseed plants were collected from different parts of Thailand. The oils were extracted from the kernel or seed using hexane as a solvent. The percent of oils obtained were shown in the Table 4.1.

From Table 4.1 the oil content of 25 species was obtained in the range of 10-76 %wt. More than 50 % of oil content can be extracted from *Irvingia malayana* (IM), *Calophyllum inophyllum* (CI), *Litsea glutinosa* (LG), *Hydnocarpus ilicifolius* (HI) and *Gmelina arborea* (GA), respectively. In contrast, oil content in *Eucalyptus camaldulensis* (EA) is the lowest one and its oil content is only 10 %wt. In addition, some oilseed plants in this study contain a high percentage of oil content which may be a new source of raw materials for biodiesel production.

4.2 Physical and chemical properties of crude oil from oilseed plants for biodiesel production

The physical and chemical properties of crude oil from oilseed plants were listed in Table 4.1. These properties include % free fatty acid, saponification value, iodine value and fatty acid profiles.

Table 4.1 Summary of physical and chemical properties of crude oils from oilseed plants

Oilseed plants	Family	Codes	% Oil	% FFA	SN*	IV*	Fatty acid composition (%)
Acacia auriculiformis	Leguminosae	AA	20	1.65	179.59	121.31	C16:0(7.02), C18:0(1.73), C18:1n9c(12.09), C18:1n9t(0.37), C18:2n6t (69.12), C18:3n3(0.47),C20:0(0.99), C20:1(0.55), C20:2(0.19), C20:3n6 (0.11), C22:0(4.76), C22:1(0.16), C23:0(0.20), C24:0(0.89), Unk (1.33)
Acacia mangium	Leguminosae	AM	25	6.33	185.60	129.29	C16:0(8.91), C16:1(0.17), C18:0(1.04), C18:1n9c(10.33), C18:1n9t(1.14), C18:2n6c(71.4), C18:3n3(0.5), C20:0(0.64), C20:1(0.53), C20:2(0.2), C22:0(4.03), C22:1(0.2), Unk (0.9)
Annona squamosa Linn.	Annonaceae	AS	32	1.38	178.30	74.89	C16:0(13.83), C16:1(0.1), C18:0(12.35), C18:1n9t(49.67), C18:2n6c(21.7), C18:3n6(0.15), C18:3n3(0.78), C20:0(0.86), C20:1(0.27), C22:0(0.09), C24:0(0.09), Unk (0.11)
Azadirachta indica Valeton	Meliaceae	AI	27	13.05	176.48	100.56	C14:0(0.74), C16:0(22.36), C18:0(19.08), C18:1n9c(38.85), 18:2n6c (16.63), C18:2n6t(0.54), C18:3n3(0.61), C20:0(1.19)
Calophyllum inophyllum	Guttiferae	CI	65	18.66	176.46	93.45	C12:0(0.07),C14:0(0.07),C16:1(0.22),C18:0(14.53),C18:1n9c(38.86), C18:1n9t(0.25),C18:2n6c(28.65),C18:3n6(0.1),C18:3n3(0.25),C20:0(0.76), C20:1(0.19),C22:0(0.17),C24:1n9(0.26),Unk (1.13)
Canarium subulatum	Burseraceae	CS	53	1.67	184.98	116.24	C16:0(7.52), C18:0(5.97), C18:1n9c(21.6), C18:1n9t(0.21), C18:2n6c (63.42), C18:3n6(0.46), C20:0(0.36), C20:1(0.47)
Carica papaya L.	Caricaceae	CAP	33	0.35	178.41	75.29	C14:0(0.13), C16:0(16.24), C16:1(0.17), C18:0(4.35), C18:1n9c(74.07), C18:1n9t(0.27), C18:2n6c(2.96), C18:3n3(0.09), C20:0(0.34), C20:1(0.42), C20:4n6(0.87), C22:0(0.11)
Ceiba pentandra Gaertn.	Bombacaceae	CP	20	12.69	182.35	102.77	C14:0(0.15), C16:0(21.75), C16:1(0.24), C17:1(0.73),C18:0(4.26), C18:1n9c(27.61),C18:1n9t(0.54),C18:2n6c(41.04), C18:3n6(1.95), C18:3n3(0.14), C20:0(0.59), C22:0(0.35), Unk(0.65)
Croton tiglium L.	Euphorbiaceae	CT	45	25.23	203.92	113.17	C10:0(1.17), C12:0(0.87), C14:0(3.36), C16:0(5.38), C16:1(0.34), C18:0 (2.33), C18:1n9c(17.91),C18:1n9t (0.22), C18:2n6c (46.7), C18:3n6 (2.38), C18:3n3(0.56), C20:0(3.64), C20:1(11.79), C20:2(1.23), C22:0(0.23), C22:1(0.57), Unk (1.3)

SN* = Saponification values
IV* = Iodine values

Table 4.1 Summary of physical and chemical properties of crude oils from oilseed plants *(continued)*

Oilseed plants	Family	Codes	% Oil	% FFA	SN*	IV*	Fatty acid composition (%)
Eucalyptus camaldulensis Dehn	Myrtaceaea	EA	10	14.21	172.41	127.29	C16:0(6.58), C18:0(2.93),C18:1n9c(11.68), C18:1n9t(0.33), C18:2n6t (77.34), C18:3n6(0.11), c20:0(0.53),C20:1(0.22)
Gmelina arborea Roxb	Verbenaceae	GA	56	4.38	175.55	103.75	C16:0(7.08), C18:0(6.43), C18:1n9c(32.28), C18:1n9t(0.23), C18:2n6c (38.04), C20:0(2.26), C20:1(7.51), C20:2(0.35), C22:0(4.13), C22:1(0.87), C24:0(0.82)
Hevea brasiliensis Muell.Arg.	Euphorbiaceae	HB	52	9.25	182.05	117.29	C14:0(0.13), C16:0(10.69), C16:1(0.16), C18:0(10.11), C18:1n9c (23.26) , C18:1n9t(0.49), C18:2n6c(35.8), C18:3n6(0.05), C18:3n3(18.68), C20:0 (0.35), C20:1(0.18), C22:0(0.07)
Hydnocarpus ilicifolius	Flacourtiaceae	HI	60	14.47	213.68	72.29	C14:0(0.23), C16:0(10.09), C16:1(0.23), C18:0(0.99), C18:1n9c (4.77), C18:2n6c(0.87), C18:3n6(0.2), C20:5(0.39), Hydnocapic acid[1](60.35), Chaulmoogric acid[2](18.94)
Hyptis suaveolens Poit	Lamiaceae	HS	15	1.45	171.81	135.83	C16:0(9.6), C18:0(2.36), C18:1n9c(5.9), C18:1n9t(0.68), C18:2n6c (79.75), C18:3n3(0.38), C20:0(0.24), Unk(1.1)
Irvingia malayana	Irvingiaceae	IM	76	1.03	206.97	5.16	C8:0(0.07), C10:0(2.99), C11:0(0.08), C12:0(49.65), C14:0(39.35), C16:0 (3.61), C16:1(0.5), C18:0(0.31), C18:1n9c(2.23), C18:1n9t (0.38), C18:2n6c(0.41), C18:3n6(0.09), C18:3n3(0.04), C22:1 (0.04), C24:1(0.05)
Litsea glutinosa Robins	Lauraceae	LG	65	1	240.38	15.28	C10:0(3.55), C12:0(91.05), C14:0(1.76), C16:0(0.62), C18:0(0.06), C18:1n9c (2.04), C18:2n6c(0.64), C18:3n6(0.08), C18:3n3(0.08), C20:1(0.12)
Melia azedarach L.	Meliaceae	MA	40	7.76	179.76	124.45	C12:0(0.17), C14:0(0.19), C16:0(8.6), C16:1(0.22), C18:0(4.75), C18:1n9c (16.91), C18:1n9t(0.33), C18:2n6c(67.11), C18:3n3(1.06), C20:0(0.29), C20:1(0.34)

SN* = Saponification values
IV* = Iodine values
X[1] = Analysed by GC-MS
X[2] = Analysed by GC-MS

Table 4.1 Summary of physical and chemical properties of crude oils from oilseed plants *(continued)*

Oilseed plants	Family	Codes	% Oil	% FFA	SN*	IV*	Fatty acid composition (%)
Parinari anamense Hance	Chrysobalanaceae	PA	54	2.45	188.61	140.97	C16:0(11.29), C18:0(8.34), C18:1n9c(19.73), C18:1n9t(0.69), C18:2n6c(9.94), C18:3n6(0.31), C18:3n9t(45.99)[3], C20:0(0.43), C20:1(0.47), C22:1(1.39), C24:1n9(1.41)
Pinus kesiya	Pinaceae	PK	21	5.06	183.50	143.20	C16:0(4.77), C16:1(0.19), C18:0(1.39), C18:1n9c(17.98), C18:1n9t (0.39), C18:2n6c(44.49), C18:3n3(0.35), C20:0(0.25), C20:1(0.76), C20:2(0.98), C20:3n6(0.44), C20:4n6(0.26), Unk(27.74)
Pinus merkusii	Pinaceae	PM	26	2.10	210.16	147.40	C16:0(4.63), C18:0(3.44), C18:1n9c(12.38), C18:2n6t(56.64), C18:3n6(11.09), C18:3n3(2.59), C20:0(0.28), C20:1(0.73), C20:2(1.63), C20:3n6(0.15), Unk(6.43)
Sapindus emarginatus	Sapindaceae	SE	35	4.50	183.56	91.48	C12:0(0.08), C14:0(0.11), C16:0(5.0), C16:1(0.21), C18:0(1.67), C18:1n9c (51.73), C18:1n9t(0.5), C18:2n6c(6.59), C18:3n6(0.09) , C18:3n3 (1.47), C20:0 (7.41), C20:1(22.49) ,C20:2(0.09) ,C22:0 (1.39), C22:1(0.87), C24:0(0.3)
Sterculia foetida L.	Sterculiaceae	SF	50	1.71	168.00	76..46	C14:0(0.16), C16:0(16.76), C16:1(0.17), C18:0(1.61), C18:1n9c (6.67), C18:1n9t(1.4), C18:2n6c(9.41), C18:3n6(0.25), C18:3n3 (0.67), Sterculic acid[4](62.89)
Terminalia bellirica Roxb	Combretaceae	TB	40	1.11	167.65	81.36	C16:0(27.67), C16:1(0.49), C18:0(11.41), C18:1n9c(37.79), C18:1n9t(0.51), C18:2n6c(21.28), C20:0(0.54), C22:1(0.3)
Wrightia tomentosa	Apocynaceae	WT	30	1.01	160.84	126.22	C16:0(6.04), C18:0(2.77), C18:1n9c(7.53),C18:1n9t(0.83), C18:2n6c (7.26), C18:3n6(0.11), C18:3n3(0.79), C20:0(0.34), C20:1 (0.44), C20:2(0.08), C22:0(0.15), C23:0(0.29), C24:0(0.13), C24:1(72.6), Unk(0.65)
Xylia kerrii	Apocynaceae	XK	20	1.34	179.49	97.67	C16:0(5.07), C18:0(4.16), C18:1n9c(21.15), C18:1n9t(0.27), C18:2n6c(44.1), C20:0(2.48), C20:1(0.95), C20:4n6(0.53), C22:0(13.7), C22:1(0.57), C23:0(0.28), C24:0(4.39), Unk(2.35)

SN* = Saponification values
IV* = Iodine values
X^{3} = Analysed by GC-MS
X^{4} = Analysed by GC-MS

4.2.1 % Free fatty acids contained in crude oils

From Table 4.1, the free fatty acid content is in the range of 0.35-25.23 %wt. The highest free fatty acid content was belonged to *Croton tiglium* (CT) oil which has an average value of 25.23 %wt. The lowest free fatty acid content was belonged to the *Carica papaya* (CAP) oil which has an average value of 0.35% wt. The value of free fatty acid content could be concerned with type of plants and duration of oil storage.

4.2.2 Saponification value of crude oils

The saponification values of various crude oils are in the range of 160.84 - 240.38 mg KOH/g of oil as shown in Table 4.1. The *Litsea glutinosa* (LG) oil had the highest saponification value, followed by the *Hydnocarpus ilicifolius* (HI), *Pinus merkusii* (PM), *Irvingia malayana* (IM) oils, etc. The lowest saponification value is belonged to the *Wrightia tomentosa* (WT) oil. The saponification number is defined as the number of milligrams of potassium hydroxide needed to saponify completely one gram of a fat or an oil [33]. One mole of oil requires three moles of potassium hydroxide for complete saponification. If a oil contains fatty acids of relatively high molecular weights, then one gram of the oil will consist of fewer moles. Thus, oils having greater percentages of high molecular weight fatty acids will have lower saponification numbers than oils having greater percentages of lower molecular weight fatty acids. Thus, *Litsea glutinosa* (LG) oil, which contained 91 percent of lauric acid, has high saponification value of 240.38 mg KOH/g. *Wrightia tomentosa* (WT) oil, which contains mostly 24-carbon fatty acids, has low saponification value of 160 mg KOH/g.

4.2.3 Iodine value of crude oils

The iodine values of these oilseed plants are in the range of 5.16-147.4 mg I_2/g of oil as shown in Table 4.1.The highest iodine value was that of the *Pinus mercusii* (PM) oil, followed by the PK, PA, HS, AM, WT, EA, MA, and AA oils, which has iodine values more than 120. The lowest iodine value is belonged to the *Irvingia malayana* (IM) oil, which has iodine value of 5.16. Iodine value is a measure of the total amount of unsaturated fatty acids in the oil and is a measure of the number of grams of iodine which will combine with 100 grams of the oil [25]. Iodine absorption occurs at double bond positions, thus a higher iodine value indicates a higher quantity of double bonds in the oil. From the results, shown that PM, PK, PA, HS, AM, WT, EA, MA and AA seed oil contains mostly unsaturated fatty acids. In contrast, IM seed oil contains mostly saturated fatty acids.

As the iodine value was set to the maximum value of 120 mg I_2/g according to ASTM standard. Therefore the oil of 16 species such as AI, AS, CAP, CI, CP, CS, CT, GA, HB, HI, IM, LG, SE, SF, TB, XK oils could be used to produce biodiesel.

4.2.4 Fatty acid composition of crude oils

Fatty acid profiles of these oilseed plants were determined by the comparison of 37 FAMEs standard. The results of these fatty acid profiles were shown in table 4.1. The GC chromatogram of these standards and these fatty acid profiles were shown in Figure A101-126.

From table 4.1, the fatty acid compositions of these oilseed plants could be classified into 3 groups: 1. C12-C14 included IM and LG oils, 2. C16-C18 included AA, AI, AM, AS, CAP, CI, CP, CS, EA, HB, HS, MA, PM and TB oils 3. > C20 included CT, GA, HI, PA, PK, SE, SF, WT and XK. Apart from 37 FAMEs standard from Supleco, there were three methyl ester components showed in HI, PM and SF oils.

In HI oil, two methyl ester components at retention time of 39.639 minutes (60.35% by weight) and 43.832 minutes (18.94% by weight) (Figure A114) were identified by GC-MS and the results showed that the component at retention time of 39.639 minutes was hydnocarpic acid methyl ester (Figure A114.1) and 43.832 minutes was chaulmoogric acid methyl ester (Figure A114.2)[6,24,34].

In PA oil, one methyl ester components at retention time of 48.056 minutes (45.99% by weight) (Figure A119) was identified by GC-MS and the results showed that the component at retention time of 48.056 minutes was 9,12,15- Octadecatrienoic acid methyl ester (Figure A119.1).

In SF oil, one methyl ester components at retention time of 43.606 minutes (62.89% by weight) (Figure A123) was identified by GC-MS and the results showed that the component at retention time of 43.606 minutes was sterculic acid methyl ester (Figure A123.1) [6,24,35].

4.3 Biodiesel production

4.3.1. Optimization for base catalyzed process

- **Effect of free fatty acid to product yield of palm oil methyl ester**.

The synthesis of biodiesel from palm oil, with vary free fatty acids content (1, 2, 3 and 4 % wt) were investigated. The reactions were carried out using the conditions as follows at 65 °C, 1:6 molar ratios of oil to methanol and 1%wt. of sodium hydroxide. The results were shown in Table 4.2.

Table 4.2 Effect of free fatty acid to product yield of palm oil methyl ester

No.	% Free fatty acid		% Product
	Set	Real	
1	0	0.14	95
2	1	1.1	93
3	2	2.1	91
4	3	3.3	89
5	4	4.2	-

The effect of the amount of free fatty acid in palm oil was shown in Figure 4.1, which product yield decreased as amount of free fatty acid increased. The highest yield (95 %wt) was given to palm oil (0.14% FFA), as well as 89% yield was given to palm oil (3.3% FFA). Therefore, the oil with 3% FFA (maximum) could be used to produce biodiesel using base catalyzed process [36].

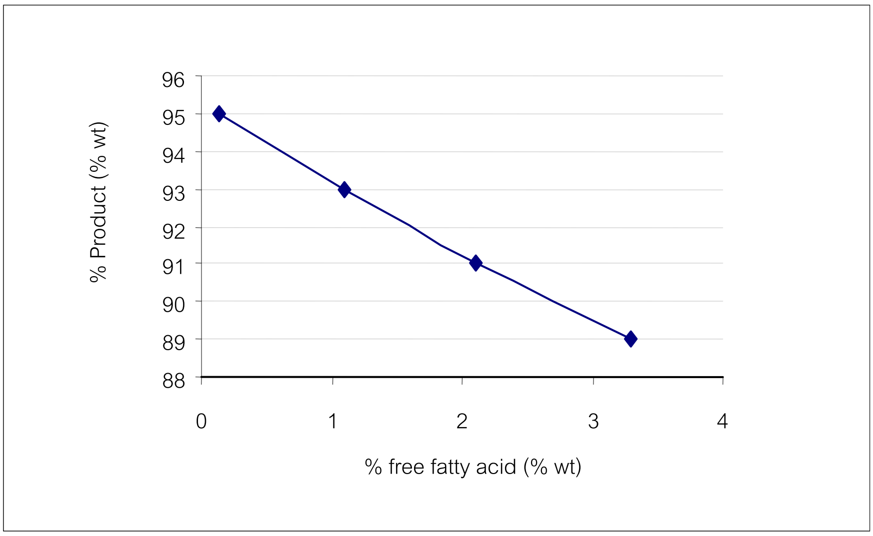


Figure 4.1 Effect of amount of free fatty acid to product yield (%wt) of palm oil methyl ester.

Therefore in table 4.1, oilseed plants such as CAP, LG, WT, IM, TB, XK, AS, HS, AA, CS, SF, PM and PA could be used to synthesis of biodiesel by base catalyzed process. For oils with high FFA more than 3% such as CI, CT, HI, EA, AI, CP, HB, MA, AM, PK, GA and SE, the suitable process for biodiesel production was two-step process [37].

4.3.2. Optimization for two-step catalyzed process (Acid – base catalyzed)

- Effect of reaction time and amount of free fatty acid.

The synthesis of biodiesel from palm oil, with various amount of free fatty acid (10, 15, 20 and 25 %wt) and reaction times (0, 30, 60, 90 and 120 min) were investigated. The reaction conditions were used as 10:1 molar ratio of methanol to oil, the reaction time at 60 °C and 1% of conc.sulfuric acid by weight of oil. The results were shown in Figure 4.2.

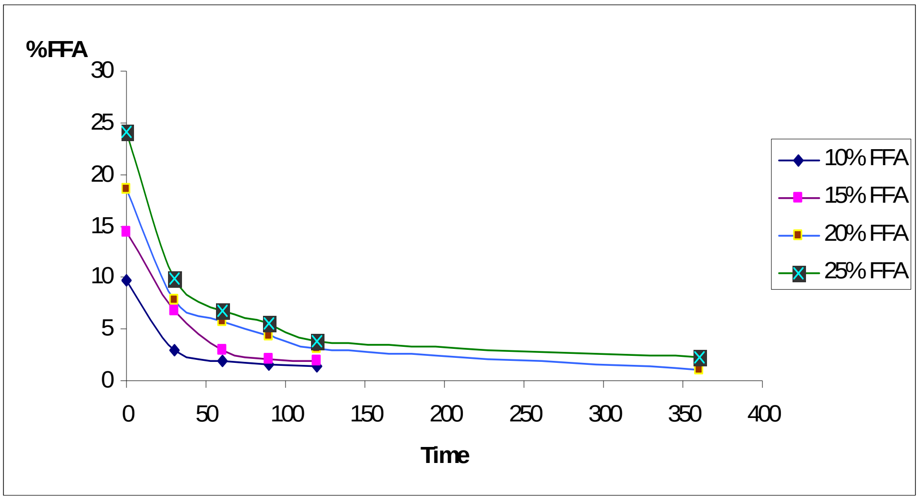


Figure 4.2 Effect of reaction time to amount of %FFA.

As seen in the Figure 4.2, the free fatty acid content of palm oil methyl ester decreased as a reaction time increased.

From the results, the free fatty acid contents up to 15% could be reduced to about 2% with in 90 minutes. For free fatty acid contents 20% and 25%, the reaction time will take up to 360 minutes to reduced %FFA to about 2%. Therefore, biodiesel of oils of HI, EA, AI, CP HB, MA, AM, PK, SE and GA were synthesized using reaction time for 90 minutes, and 360 minutes for CI and CT.

4.3.3 Synthesis of methyl ester from oilseed plants via base-catalyzed process (FFA< 3%)

By using the method and conditions as described in 3.2.3.1, the methyl ester of oilseed plants with free fatty acid contents less than 3 % (Table 4.1) could be easily prepared.

As discussed in 4.3.1, the percentage yield was effected by % free fatty acid contents. Therefore, oilseed plants with free fatty acid contents less than 3% was chosen to synthesize biodiesel via base catalyzed process [38]. The results of % product yield and characteristic property were shown in Table 4.3.

Table 4.3 Summary of synthetic results of methyl ester of oilseed plants

Oilseed plants	Codes	% FFA	% Product yield	Characteristic property
Parinari anamense Hance	PA	2.45	87	Yellow liquid
Pinus merkusii	PM	2.10	90	Red-orange liquid
Sterculia foetida L.	SF	1.71	91	Yellow liquid
Canarium subulatum	CS	1.67	91	Yellow liquid
Acacia auriculifomis	AA	1.65	90	Brown liquid
Hyptis suaveolens Poit	HS	1.45	90	Dark green liquid
Annona squamosa Linn.	AS	1.38	91	Light yellow liquid
Xylia kerrii	XK	1.34	90	Yellow liquid
Terminalia bellirica Roxb	TB	1.11	91	Red-orange liquid
Irvingia malayana	IM	1.03	93	Yellow liquid
Wrightia tomentosa	WT	1.01	93	Brown liquid
Litsea glutinosa Robins	LG	1	94	Yellow liquid
Carica papaya L.	CAP	0.35	93	Yellow liquid

The percentage yield of these methyl esters were in the range of 87-94% and the calculation of the percentage yield were shown in Appendix C.

4.3.4 Synthesis of methyl ester from oilseed plants via 2-step catalyzed process (FFA>3%)

By using the method and conditions as described in 3.2.3.2, the methyl ester of oilseed plants with free fatty acid content more than 3% (Table 4.1), could be easily prepared.

As discussed in 4.3.1 and 4.3.2, the percentage yield was effected by % free fatty acid contents and reaction time. Therefore, oilseed plants with free fatty acid contents more than 3% was chosen to synthesize biodiesel via two-step catalyzed process [39]. The results of % product yield and characteristic property were shown in Table 4.4.

Table 4.4 Summary of methyl ester of oilseed plant from synthesize with 2-step catalyzed process (FFA>3%)

Oilseed plants	**Codes**	**% FFA**	**% Product yield**	**Characteristic property**
Croton tiglium L.[1]	CT	25.23	82	Black brown liquid
Calophyllum inophyllum[1]	CI	18.66	85	Yellow liquid
Hydnocarpus ilicifolius[2]	HI	14.47	88	Yellow liquid
Eucalyptus camaldulensis Dehn[2]	EA	14.21	89	Yellow liquid
Azadirachta indica Valeton[2]	AI	13.05	87	Brown liquid
Ceiba pentandra Gaertn.[2]	CP	12.69	89	Jadegreen liquid
Hevea brasiliensis Muell.Arg.[2]	HB	9.25	91	Yellow liquid
Melia azedarach L.[2]	MA	7.76	90	Yellow liquid
Acacia mangium[2]	AM	6.33	91	Brown liquid
Pinus kesiya[2]	PK	5.06	91	Red-orange liquid
Sapindus emarginatus[2]	SE	4.50	92	Yellow liquid
Gmelina arborea Roxb[2]	GA	4.38	92	Yellow liquid

X^1= Reaction time : 1 hour
X^2= Reaction time : 6 hours

The percentage yield of these methyl esters were in the range of 82-92% and the calculation of the percentage yield were shown in Appendix C.

Finally, the fatty acid methyl esters from 25 species of oilseed plants could be synthesized and % product yields were in the range of 82-94%.

4.4 Characterization of the biodiesels.

Fatty acid methyl esters of various oilseed plants were characterized by FT-IR and ^{1}H-NMR.

4.4.1 FT-IR (Fourier Transform Infrared Spectroscopy)

The IR spectra of crude oils and fatty acid methyl esters of oils of 25 species were shown in Figure A1-A50. The important absorption bands of crude oil and fatty acid methyl esters of *Canarium subulatum* (CS) oil, which are representative oil, were shown in Table 4.5. The IR spectra of crude CS oil and CS oil methyl ester were shown in Figures 4.3 and 4.4, respectively

Table 4.5 The absorption assignments of CS crude oil and CS oil methyl ester.

Wave number (cm^{-1})		Assignments
CS crude oil	CS oil methyl ester	
3004	3005	=C-H Stretching
2857	2849	C-H Stretching, Aliphatic
1745	1742	C=O Stretching
1652	1654	C=C Stretching
1459	1461	C-H Bending, Aliphatic
1167	1167	C-O Stretching

According to their absorption bands, in Table 4.5 and Figure 4.3 and Figure 4.4, both oils could not be distinguished. Therefore, crude oil with low FFA such as CS oil etc., could not be characterized by IR technique. For crude oil with high free fatty acid such as CP, HI and CT oils, it could be clearly seen that IR spectra of CP, HI and CT oils showed COOH absorption bands from 2500-3600 cm^{-1}, and COOH absorption bands was disappeared in CP, HI and CT oil methyl esters. There fore IR technique could be used distinguished between crude oil with high FFA and fatty acid methyl ester.

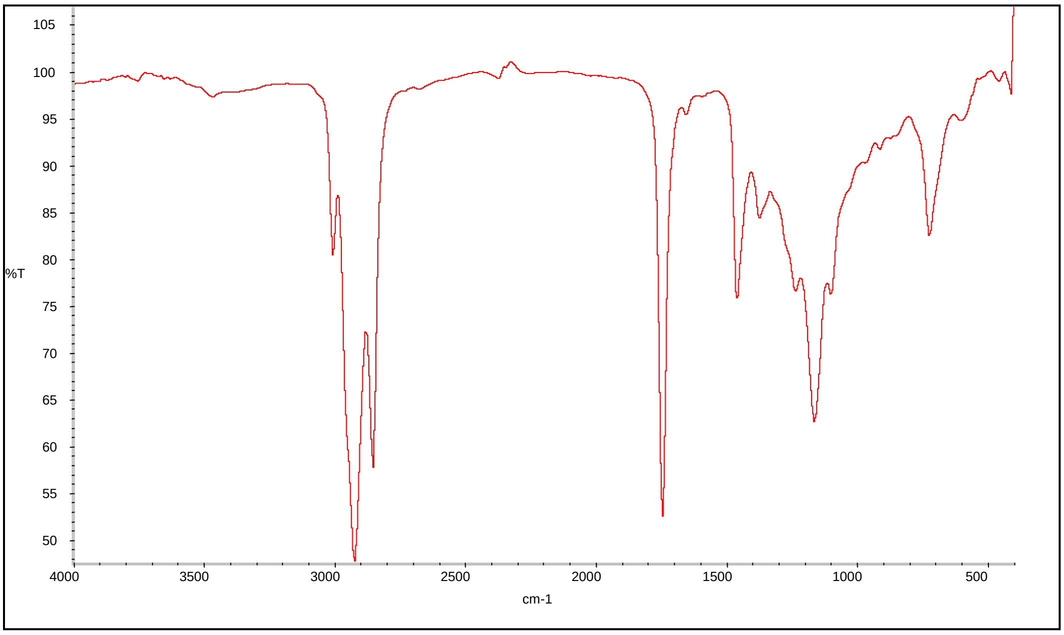


Figure 4.3 IR spectrum of crude CS oil.

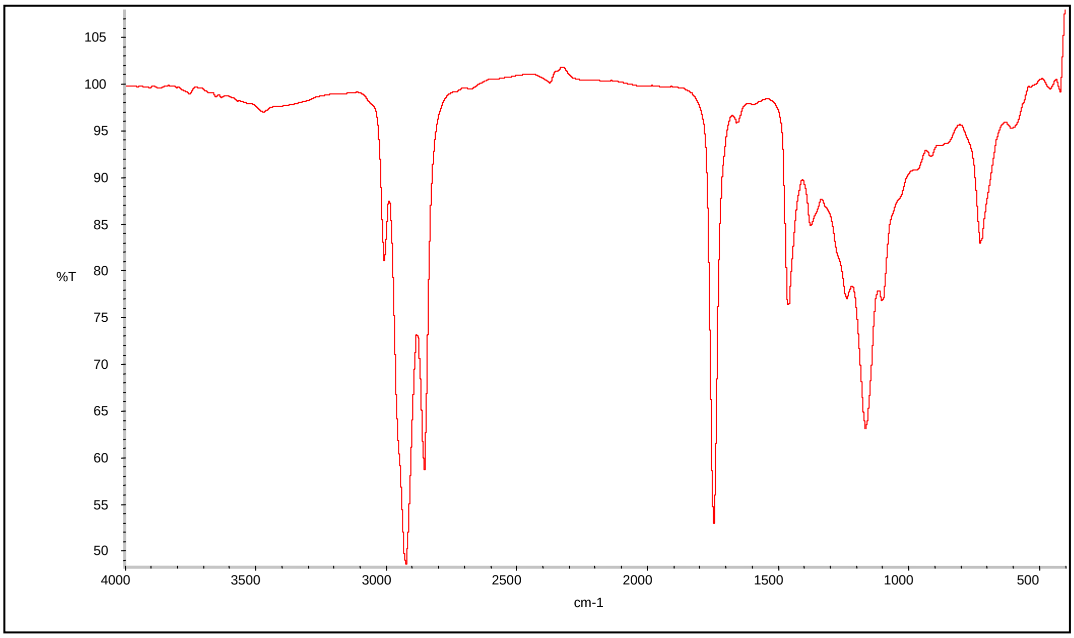


Figure 4.4 IR spectrum of CS oil methyl ester.

4.4.2 ^{1}H NMR (Nuclear magnetic resonance)

The ^{1}H NMR spectra of crude oils and fatty acid methyl esters of oils of 25 species were shown in Figure A51-A100. For the representative example, the ^{1}H-NMR spectra of crude CS oil and CS oil methyl ester were shown in Figures 4.5 and 4.6, respectively. The important signals of crude CS oil and CS oil methyl ester were shown in Table 4.6.

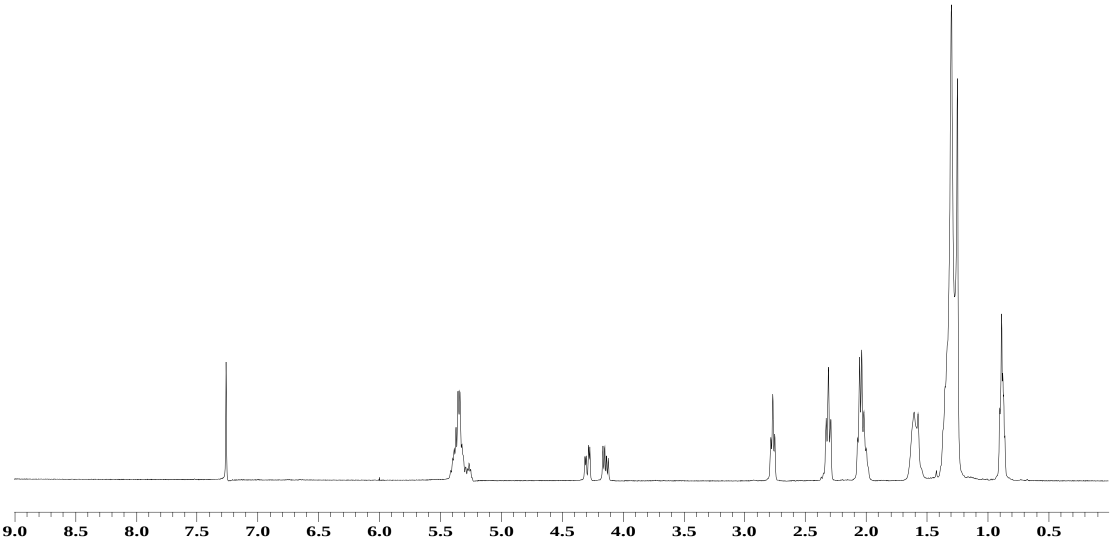


Figure 4.5 ^{1}H NMR spectrum of crude CS oil.

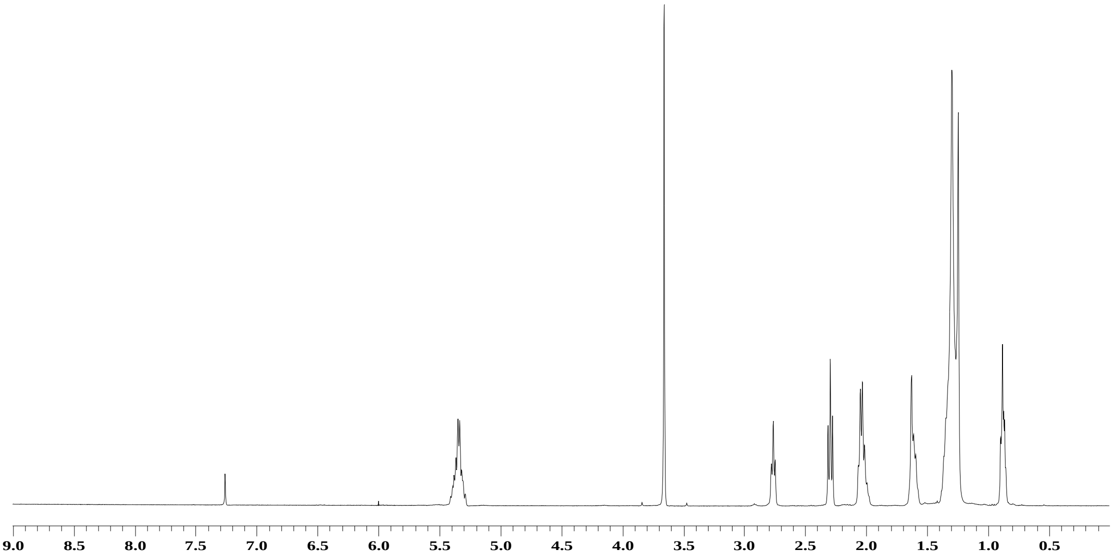
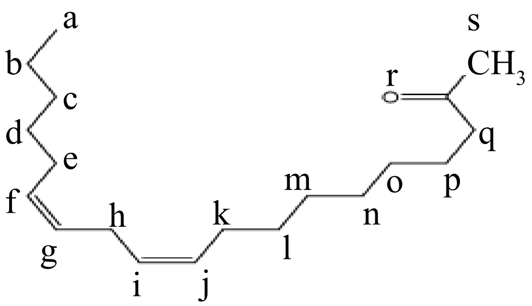


Figure 4.6 ^{1}H NMR spectrum of CS oil methyl ester.

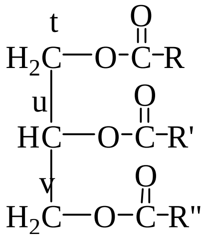
Table 4.6 The assignments of ^{1}H-NMR spectra of crude CS oil and CS oil methyl ester.

Position of Proton	Multiplicity	Chemical Shift (δ,ppm)	
		Crude CS oil	CS oil methyl ester
a	m	0.81-0.93	0.82-0.92
b,c,d,e,k,l,m,n,o,p	m	1.21-2.12	1.21-2.10
q	t	2.24-2.36	2.25-2.35
h	m	2.69-2.81	2.72-2.82
s	s	-	3.61-3.72
t,v	m	4.05-4.35	-
u	m	5.22-5.29	-
f,g,i,j	m	5.30-5.43	5.30-5.44

From the ^{1}H-NMR spectra of CS oil methyl ester (Figure 4.6) and crude CS oil (Figure 4.5), the characteristic peaks of crude CS oil are the signals at δ_H 4.05-4.35 ppm which are belonged to the protons of glycerol moiety and the characteristic peak of CS oil methyl ester is the signal at δ_H 3.7 ppm which is belonged to protons of methoxy group.



CS oil methyl ester



Crude CS oil

Therefore, ^{1}H NMR could be used to analyze both oils as proton signal at δ_H 4.05- 4.35 ppm in Figure 4.5 was disappeared in Figure 4.6. In the other hand, signal of methoxy group at δ_H 3.7 ppm in Figure 4.6 was not shown in Figure 4.5.

4.5 Properties of biodiesel

The some important properties (i.e. viscosity, flash point, acid value, density and ester content) were determined. The standard values of these properties were shown in Table 4.7, as well as, the values of these properties of methyl esters of these oilseed plants were shown in Table 4.8.

Table 4.7 Specification for quality of biodiesel

Property	Value	Method
Viscosity at 40 °C	3-5 cSt	ASTM D445
Flash point	>120 °C	ASTM D93
Density at 15 °C	860-900 kg/cm^3	ASTM D4052
Acid number	< 0.5 mg KOH/g	ASTM D974
Ester content	> 96.5 %wt	EN 14103

Table 4.8 Summary of properties of biodiesel from various oilseed plants

Oilseed plants	**Codes**	**Viscosity (cSt)**	**Flash point (°C)**	**Density (kg/cm^3)**	**Acid number (mg KOH/g)**	**conversion (^{1}H NMR)**	**Ester content (GC)**
Croton tiglium L.	CT	6.3	130	897.19	0.4870	87.74	86.65
Calophyllum inophyllum	CI	5.0	133	888.20	0.1885	93.92	92.64
Hydnocarpus ilicifolius	HI	5.3	>150	909.29	0.1038	52.17^a	95.12
Eucalyptus camaldulensis Dehn	EA	4.3	>150	886.02	0.0549	90.84	95.01
Azadirachta indica Valeton	AI	7.8	>150	900.94	0.2192	88.77	84.58
Ceiba pentandra Gaertn.	CP	4.3	>150	880.48	0.0798	92.64	94.18
Hevea brasiliensis Muell.Arg.	HB	5.7	>150	882.54	0.1077	92.55	92.33
Melia azadirachta L.	MA	7.1	>150	908.70	0.0825	91.39	90.33
Acacia mangium	AM	4.3	>150	886.32	0.0791	92.19	94.61
Pinus kesiya	PK	4.6	>150	890.58	0.0817	90.73	92.92
Sapindus emarginatus	SE	5.0	>150	876.30	0.1089	93.36	95.40
Gmelina arborea Roxb	GA	4.6	>150	880.41	0.0547	92.63	92.81

- X^a = *peaks overlab*

Table 4.8 Summary of properties of biodiesel from various oilseed plants *(continued)*

Oilseed plants	**Codes**	**Viscosity (cSt)**	**Flash point (°C)**	**Density (kg/cm^3)**	**Acid number (mg KOH/g)**	**Conversion (^{1}H NMR)**	**Ester content (GC)**
Parinari anamense Hance	PA	9.3	>150	913.27	0.0778	100^{b}	77.71
Pinus merkusii	PM	4.1	>150	886.08	0.0131	91.17	89.37
Sterculia foetida L.	SF	4.9	>150	886.78	0.0816	90.97	88.20
Canarium subulatum	CS	3.7	>150	878.05	0.1033	92.26	91.21
Acacia auricalifomis	AA	4.3	>150	885.69	0.0519	92.32	94.62
Hyptis suaveolens Poit	HS	4.0	>150	885.02	0.17229	91.98	92.87
Annona squamosa Linn.	AS	4.9	>150	879.76	0.1638	92.21	93.10
Xylia kerrii	XK	5.2	>150	898.02	0.0541	93.03	92.05
Terminalia bellirica Roxb	TB	5.8	>150	880.68	0.1026	90.34	92.55
Irvingia malayana	IM	3.0	130	872.52	0.0268	92.37	92.29
Wrightia tomentosa	WT	16.4	>150	923.02	0.1647	90.13^{c}	84.07
Litsea glutinosa Robins	LG	3.0	120	877.46	0.0824	93.19	92.28
Carica papaya L.	CAP	4.7	>150	877.85	0.0273	93.10	94.00

- X^{b}, X^{c} = *peaks overlab*

4.5.1 Viscosity

The viscosity is a very important property of biodiesel. From Table 4.8, the viscosity of methyl esters of oilseed plants were in the range of 3-16.4 cSt. The viscosity of methyl esters of 16 species of oilseed plants (including AA, AM, AS, CAP, CI, CP, CS, EA, GA, HS, IM, LG, PK, PM, SE and SF) were within standard values(3-5cSt), and values of 9 species (including AI, CT, HB, HI, MA, PA, TB, WT and XK) were above the standard values.

In addition, it could be clearly seen that the viscosity values were depended on chain length of fatty acids degree of unsaturation of fatty acid and unreacted triglycerides (including mono- and di-glycerides). For example, the viscosity value of WT was 16.4 cSt and the fatty acid compositions were in the range of C16-C24 atoms with C24:1 was the main composition (72.6%). While the viscosity values of CS was 3.7 cSt and the fatty acid compositions were in the range of C16-C20 atoms, with C18:2 was the main composition (63.42%). It could be concluded that oil with long chain carbon should have high viscosity.

4.5.2 Density

Density is another important property of biodiesel. The density of methyl esters of 20 species of oilseed plants (including AA, AM, AS, CAP, CI, CP, CS, CT, EA, GA, HS, HB, IM, LG, PK, PM, SE, SF, TB and XK) were within standard values(3-5cSt), and values of 5 species (including AI, HI, MA, PA, and WT) were above the standard values.

According to the relationship between viscosity and density, the equation between viscosity and density for biodiesel is:

$VS = -16.155DN + 930.78$ [40]

Where: VS = viscosity

DN = density,

Therefore, density is proportioned with viscosity. It could be concluded that densities of biodiesels increase with viscosity, and the same reasons for increasing viscosity is applied to density.

4.5.3 Flash point

Flash point is another important property of biodiesel. It could be seen in Table 4.8 that, the flash point of all methyl esters of 25 species of oilseed plants were higher than the specification value (120 °C). According to the correlation of viscosity and flash point, in flash point is also proportioned with the viscosity, as flash point increase with viscosity. In addition, all of seed oils have the length of free fatty acid chain from C12-C24 atoms, which are higher than diesel fuel (C9-C16 atoms).

4.5.4 Acid number

Acid number is another important property of biodiesel. It could be seen in Table 4.8 that the acid number of all methyl ester of 25 species of oilseed plants were in the range of 0.01-0.49 mg KOH/g, which were lower than the specification value (0.5 mg KOH/g). It should be concluded that the most of oils and free fatty acid contents were essentially converted to methyl esters.

4.5.5 Methyl ester content

Ester content is another important property of biodiesel. It could be shown to the purity of biodiesel. From the Table 4.8, the methyl ester contents of 25 species of oilseed plants were in the range of 84.07-95.4 % (GC) and 87.74-100 % (^{1}H NMR). Both techniques gave a similar values of ester contents incase of there are no overlapping peaks at δ 2.3 ppm and δ 3.7 ppm. In addition, there were some different values for HI, PA and WT oils, which have different between the values from ^{1}H NMR and GC.

In HI oil methyl ester, it was different between the value from ^{1}H NMR (52.17%) and GC (95.12%). From ^{1}H NMR spectrum (Figure A76) of HI oil methyl ester, at δ 2.3 ppm was overlapped by other peaks. This caused the area of $COCH_2$- higher than unoverlapped area making the % ester contents of this oil lower than expected value as compared to the value from the GC.

In PA oil methyl ester, it was different between the value from ^{1}H NMR (100%) and GC (77.71%). These may caused by unsaponification matter containing in oil, as could be seen that ester content value of PA (100%) from ^{1}H NMR.

In WT oil methyl ester, it was different between the value from ^{1}H NMR (90.13%) and GC (84.07%). From ^{1}H NMR spectrum (Figure A98) of WT oil methyl ester, at δ 3.7 ppm was overlapped by other peaks. This caused the area of CH_3O- higher than unoverlapped area making the % ester contents of this oil higher than expected value as compared to the value from the GC.

It could be concluded that both ^{1}H NMR and GC could be used to measured ester contents if there are no overlapping peaks.

CHAPTER V

CONCLUSION AND SUGGESTION

5.1 Conclusion

In this study, seed oils of 25 species were investigated as an alternative source for biodiesel production. All properties and other data of oil of these plants are as follow:

1) Oil content: it was varied from 10 to 76 %wt.
2) Free fatty acid content: it was varied from 0.35-25.23% wt
3) Saponification value: it was varied from 160.84-240.38 mg KOH/g
4) Iodine value: it was varied from 5.16-147.4 mg I_2/g

From these data, AA, AM, AS, AI, CI, CS, CAP, CP, CT, GA, HB, HI, IM, LG, MA, PA, PK, PM, SE, SF, TB, WT, and XK oils gave oil content more than 20 %wt. The biodiesel of oils of these 25 species could be conveniently synthesized by either base catalyzed process (transesterification reaction) or 2-step catalyzed process (esterification and transesterification reactions).

In one step process, the biodiesel of oils of PA, PM, SF, CS, AA, (HS), AS, XK, TB, IM, WT, LG and CAP oils were synthesized using 6:1 molar ratio of methanol to oil, sodium hydroxide 1 % by weight of oil and reaction temperature at 65 °C for 1 h.

In two step process, oils of HI, (EA), AI, CP, HB, MA, AM, PK, SE, and GA were subjected to synthesize using 10:1 molar ratio of methanol to oil, sulfuric acid 1 % by weight of oil and reaction temperature at 60 °C for 1 h, except oils of CI and CT were used reaction time for 6 h, for esterification reaction, and then the resulted oils were subjected to transesterification reaction using the same condition as described in one step process. The product yields of biodiesel from oils of these plants from both processes were in the range of 82-94 %wt.

For fatty acid compositions, oils of these plants were classified into 3 groups as C12-C14 (IM and LG), C16-C18 (AA, AI, AM, AS, CAP, CI, CP, CS, EA, HB, HS, MA, PM and TB) and > C20 (CT, GA, HI, PA, PK, SE, SF, WT and XK), respectively.

For ester content both ^{1}H NMR and GC gave ester content in biodiesel from oils of these plants in the range of 84.07-95.4 %. Except for HI, PA and WT oils, there was a different between the values from ^{1}H NMR and GC.

For the properties of biodiesel, from oils of these plants, the values of viscosity, density, flash point and acid number were mostly in the range of standard values (viscosity = 3-5 cSt, density 860-900 g/cm^3, flash point >120 °C and acid number < 0.5 mg KOH/g) except for AI (viscosity = 7.8 cSt, density = 900.94 g/cm^3), CT (viscosity = 6.3 cSt), HI (viscosity = 5.3 cSt, density = 909.29 g/cm^3), HB (viscosity = 5.7), MA (viscosity = 7.1 cSt, density = 908.7 g/cm^3), PA (viscosity = 9.3 cSt, density = 913.27 g/cm^3), WT (viscosity = 16.4 cSt, density = 923.02 g/cm^3), XK (viscosity = 5.2 cSt) and TB (viscosity = 5.8 cSt).

Finally the methyl esters of oils of 16 species (AA, AM, AS, CI, CS, CAP, CP, EA, GA, HS, IM, LG, PK, PM, SE and SF) were found to have great potential of biodiesel and they met the most specification of biodiesel standards.

5.2 Suggestions

For further study,

1. New sources of oilseed plants which have oil content over 30% should be investigated.
2. The *Hevea brasiliensis* should be scale-up in the industry.

REFERENCES

[1] Tickell, J. From the Fryer to the Fuel Tank. 3th ed. New Orleans: Jo shua Tickell Publication, 2003: 55-78.

[2] Barnwal BK, Sharma MP. Prospects of biodiesel production from vegetables oils in India. Renew and Sustain Energy Rev 9 (2005): 363–378.

[3] Ma F., Hanna MA. Biodiesel production: a review. Bioresour. Technol. 70 (1999): 1–15.

[4] Demirbas, A. Importance of biodiesel as transportation fuel. Energy Policy 35 (2007): 4661-4670.

[5] Korbitz W. Biodiesel production in Europe and North America, an encouraging prospect. Renew Energy 16 (1999): 1708-183.

[6] Mohibbe A.M., Waris A, Nahar Nm. Prospects and potential of fatty acid methyl esters of some non-traditional seed oils for use as biodiesel in India. Biomass Bioenerg. 29 (2005): 203-302.

[7] Elsevier Science Publishers, B.V. The petroleum handbook. Elsevier Science Publishing Company Inc 6 (1983): 386-416.

[8] Agarwal A.K. Biofuels (alcohols and biodiesel) applications as fuels for internal combustion engines. Progr. Energ. Combust. Sci. 33 (2007): 233-271.

[9] Srivastava A., Prasad R. Triglycerides-based diesel fuels. Renew Sustain Energy Rev 4 (2000): 111–133.

[10] Phulkerd P. Preparation of 1,3-Dioxolane derivatives from glycerol in biodiesel production. Master’s Thesis, Department of Chemistry, Faculty of Science, Graduate School, Chulalongkorn University, 2006.

[11] Pramanik K. Properties and use of *Jatropha curcas* oil and diesel fuel blends in compression ignition engine. Renew Energy 28 (2003): 239–248.

[12] Schuchardta U, Serchelia R, Vargas RM. Transesterification of vegetable oils: a review. J. Braz. Chem. Soc. 9 (1998): 199–210.

[13] Knothe, G., Sharp, C.A. and Ryan, T.W. Exhaust emissions of biodiesel, petrodiesel, neat methyl esters, and alkanes in a new technology engine. Energy & Fuels 20 (2006): 403–408.

[14] Freedman B., Butterfield R.O. and Pryde EH. Transesterification kinetics of soybean oil. J. Am. Oil. Chem. Soc. 63 (1986): 1375–1380.

[15] Gerpen, J.V. Biodiesel processing and production. Fuel. Proces. Technol. 86 (2005): 1097-1107.

[16] Meher, L.C., Vidya Sagar, D. and Naik, S.N. Technical aspects of biodiesel production by transesterification: a review. Renew and Sustain Energy Rev 2000: 10248–10268.

[17] Dorado, M.P., Ballesteros, E., Almeida, J.A., Schellet, C., Lohrlein, H.P. and Krause, R. An alkali-catalyzed transesterification process for high free fatty acid oils. Trans ASAE 45 (2002): 525–529.

[18] Canakci, M. and Gerpen, J.V. Biodiesel production from oils and fats with high free fatty acid. Trans ASAE 44 (2001): 1429–1436.

[19] Freedman, B., Pryde, E.H. and Mounts, T.L. Variables affecting the yields of fatty esters from transesterified vegetable oils. JAOCS 61 (1984): 1638–1643.

[20] Ma, F., Clements, L.D. and Hanna, M.A. The effect of mixing on transesterification of beef tallow. Bioresour. Technol. 69 (1999): 289–93.

[21] Knothe, G., Matheaus, A.C. and Ryan, T.W. Cetane numbers of branched and straight-chain fatty esters determined in an ignition quality tester. Fuel 82 (2003): 971-975.

[22] Demirbas, M.F. and Balat, M. Recent advances on the production and utilization trends of bio-fuels: a global perspective. Energy Convers Manage 47 (2006): 2371–2381.

[23] Saka, S. and Kusdiana, D. Biodiesel fuel from rapeseed oil as prepared in supercritical methanol. Fuel 80 (2001): 225–231.

[24] Kaimal, T.N. and Lakshminarayana, G. Fatty acid composition of lipids isolated from different parts of Ceiba pentandra, Sterculia foetida and Hydnocarpus wightiana. Phytochemistry 9 (1970): 2225-2229.

[25] Antolín, G., Tinaut, F. V., Castaño, V., Pérez , C. and Ramírez, A. I. Optimisation of biodiesel production by sunflower oil transesterification. Bioresour. Technol. 83 (2002): 111-114.

[26] Vicente, G., Martínez, M., Aracil, J. Integrated biodiesel production: a comparison of different homogeneous catalysts systems. Bioresour. Technol. 92 (2004): 297-305.

[27] Ghade, S.V. and Raheman, H. Biodiesel production from mahua (*Madhuca indica*) oil having high free fatty acids. Biomass Bioenerg 28 (2005): 601-605.

[28] Ramadhas, A.S. and Jayaraj, S. Muraleedharan, C. Biodiesel production from high FFA rubber seed oil. Fuel 84 (2005): 335-340.

[29] Holser, R.A. and Harry O'Kuru R. Transesterified milkweed (*Asclepias*) seed oil as a biodiesel fuel. Fuel 85 (2006): 2106-2110.

[30] Meher, L.C., Dharmagadda V. and Naik S.N. Optimization of alkali-catalyzed transesterification of Pongamia pinnata oil for production of biodiesel. Bioresour. Technol. 97 (2006): 1392-1397.

[31] Berchmans, H.J, Hirata, S. Biodiesel production from crude *Jatropha curcas* L. seed oil with a high content of free fatty acids. Bioresour. Technol. 99 (2008): 1716-1721.

[32] Yong, W., Shiyi, O., Pengzhan, L. and Zhisen, Z. Preparation of biodiesel from waste cooking oil via two-step catalyzed process. Energ. Convers. Manage 48 (2007): 184-188.

[33] Dmytryshyn, S.L., Dalai, A.K., Chaudhari, S.T., Mishra, H.K. and Reaney,M.J. Synthesis and characterization of vegetable oil derived esters:evaluation for their diesel additive properties. Bioresour. Technol. 92 (2004): 55-64.

[34] Shriner, R.L. Cyclopentyl and cyclopentenyl acids [online]. (n.d.). Available from: http://www.cyberlipid.org/ fa/acid002.html [2008,January 8]

[35] Eisele, T.A., Libbey, L.M., Pawlowski, N.E., Nixon, J.E. and Sinnhuber, R.O. Mass spectrometry of the silver nitrate derivatives of cyclopropenoid compounds. Department of Food Science and Technology, Oregon State University 1974.

[36] Crabbe, E., Nolasco-Hipolito, C.N., Kobayashi, G., Sonomato, K. and Ishizaki, A. Biodiesel production from crude palm oil and evaluation of butanol extraction and fuel properties. Process Biochemistry 37 (2001): 65-71.

[37] Encinar, J.M., Gonzalez, J.F. and Rodriguez-Reinares, A. Ethanolysis of used frying oil. Biodiesel preparation and characterization, Fuel. Process. Tech. 88 (2007): 513-522.

[38] Berrious, M., Siles, J., Martin, M.A. and Martin, A. A kinetic study of the esterification of free fatty acids (FFA) in sunflower oil. Fuel 86 (2007): 2383-2388.

[39] Demirbas, A. Comparison of transesterification methods for production of biodiesel from vegetable oils and fats. Energ. Convers. Manage 49 (2007): 125-130.

[40] Demirbas, A. Relationships drived from physical properties of vegetable oil and biodiesel fuels. Fuel 8 (2008): 1743-1748.

APPENDICES

APPENDIX A

^{1}H NMR SPECTRA, IR SPECTRA and GC CHROMATOGRAMS OF CRUDE OILS AND METHYL ESTERS

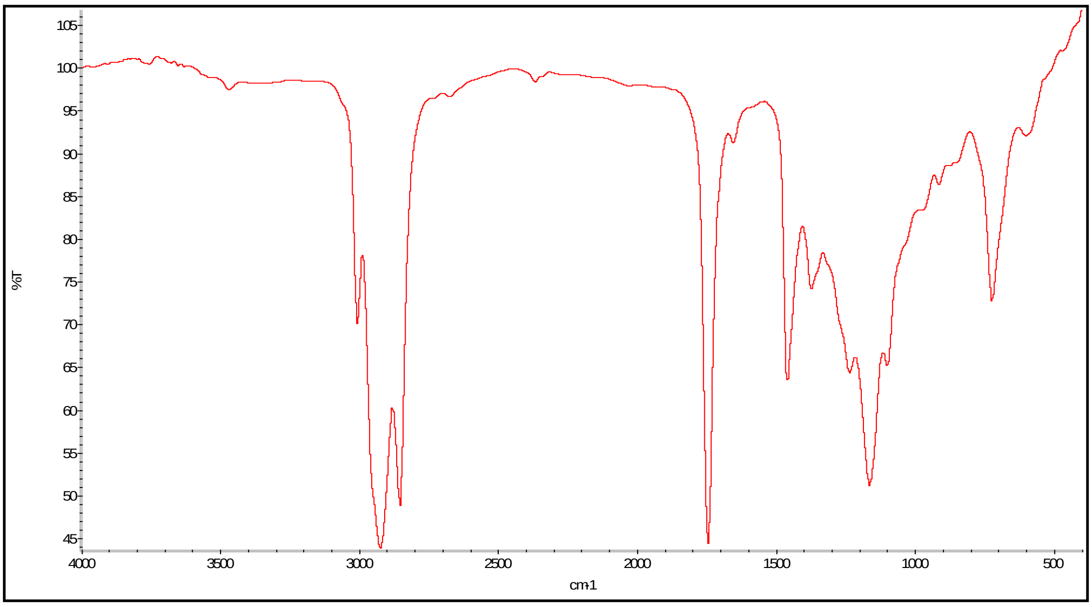


Figure A1 IR spectrum of crude AA oil.

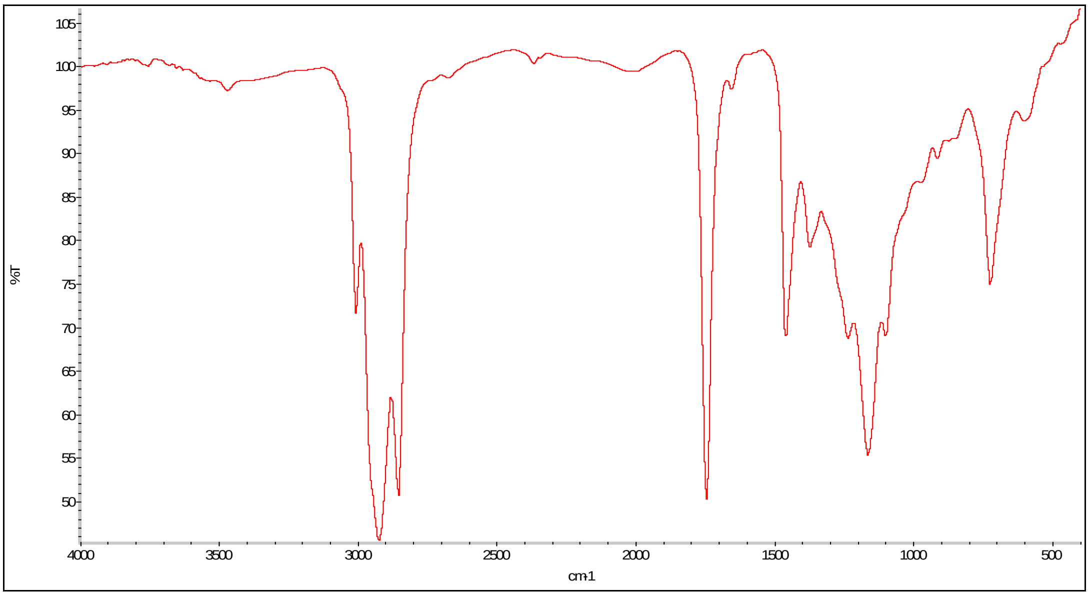


Figure A2 IR spectrum of AA oil methyl ester.

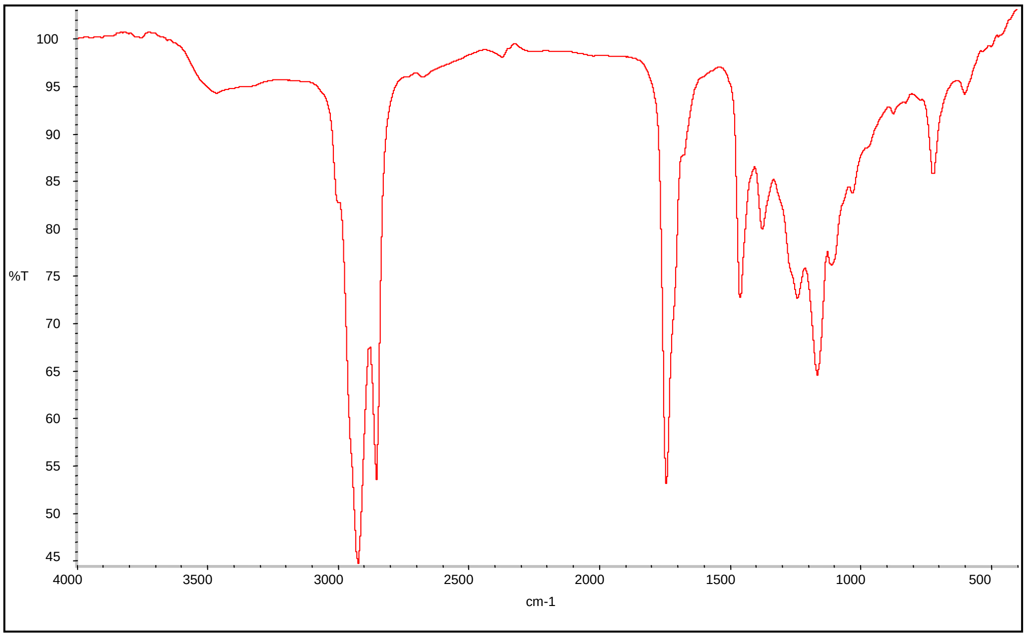


Figure A3 IR spectrum of crude AI oil.

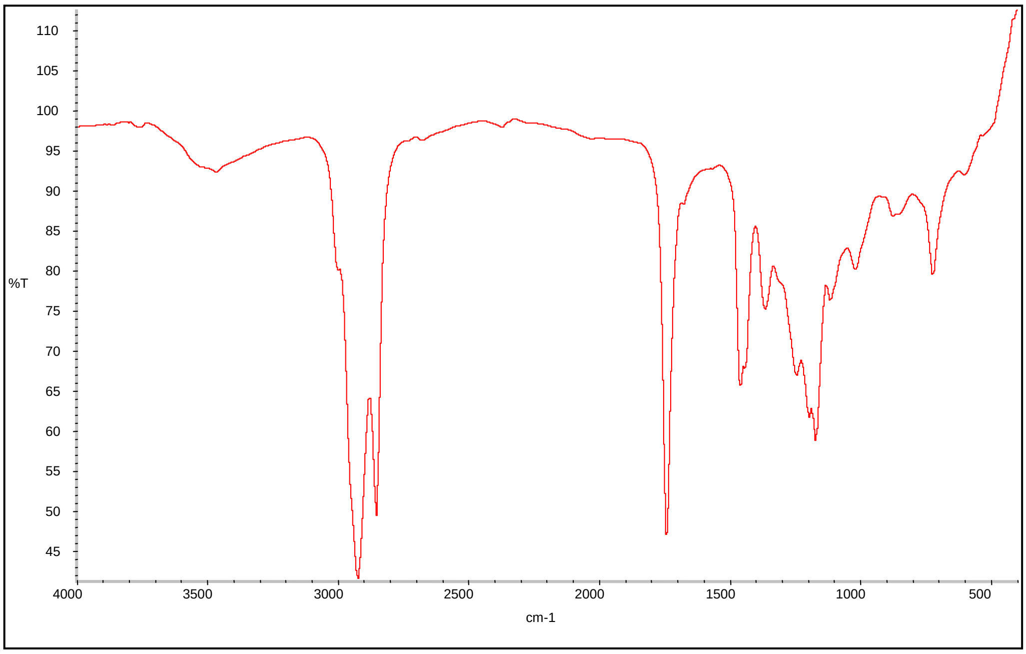


Figure A4 IR spectrum of AI oil methyl ester.

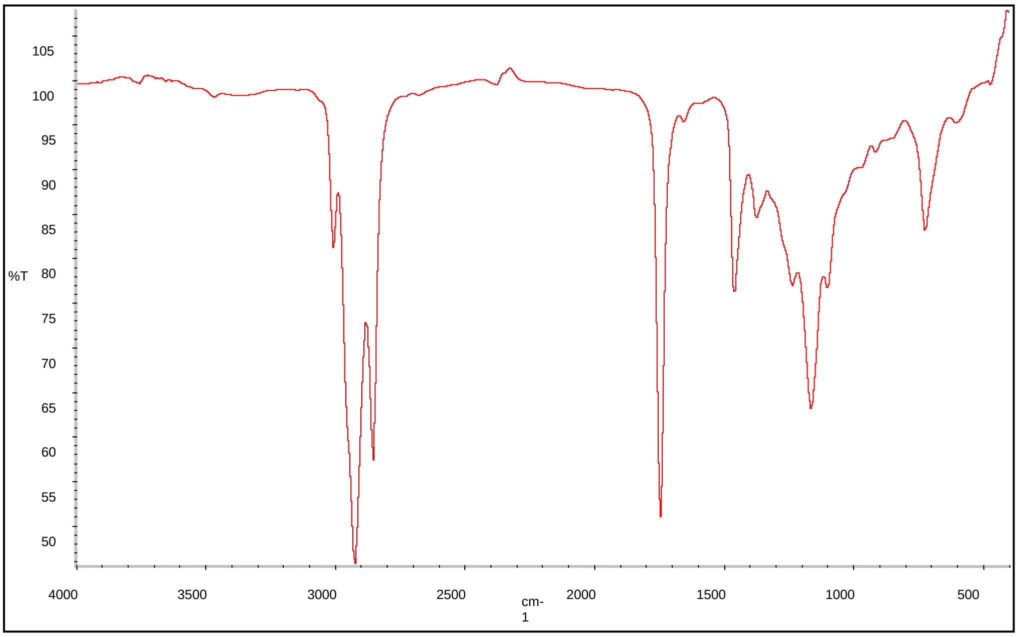


Figure A5 IR spectrum of crude AM oil.

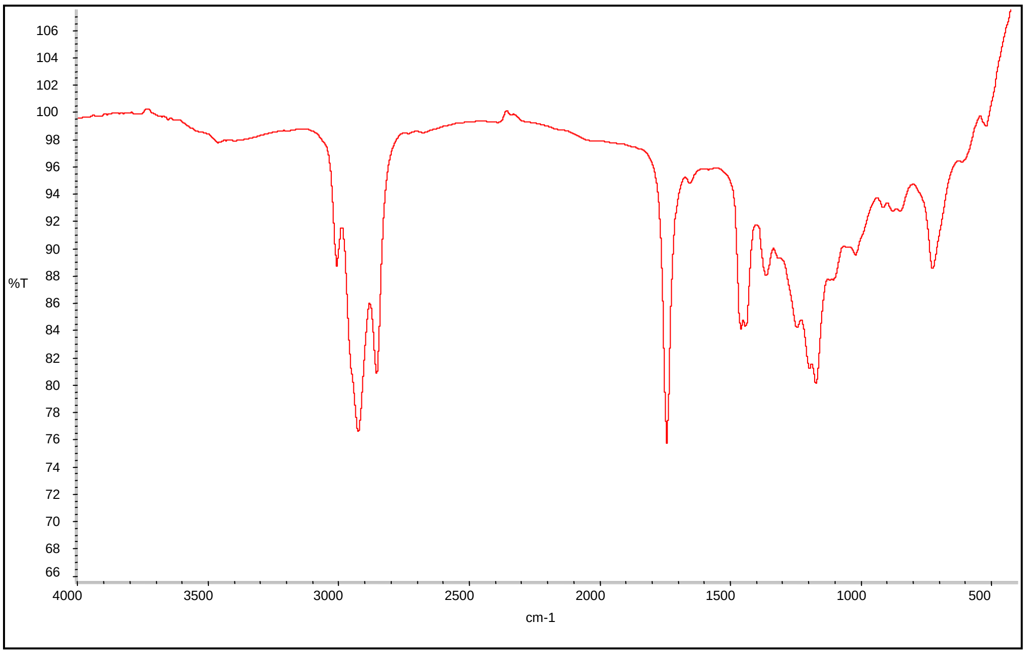


Figure A6 IR spectrum of AM oil methyl ester.

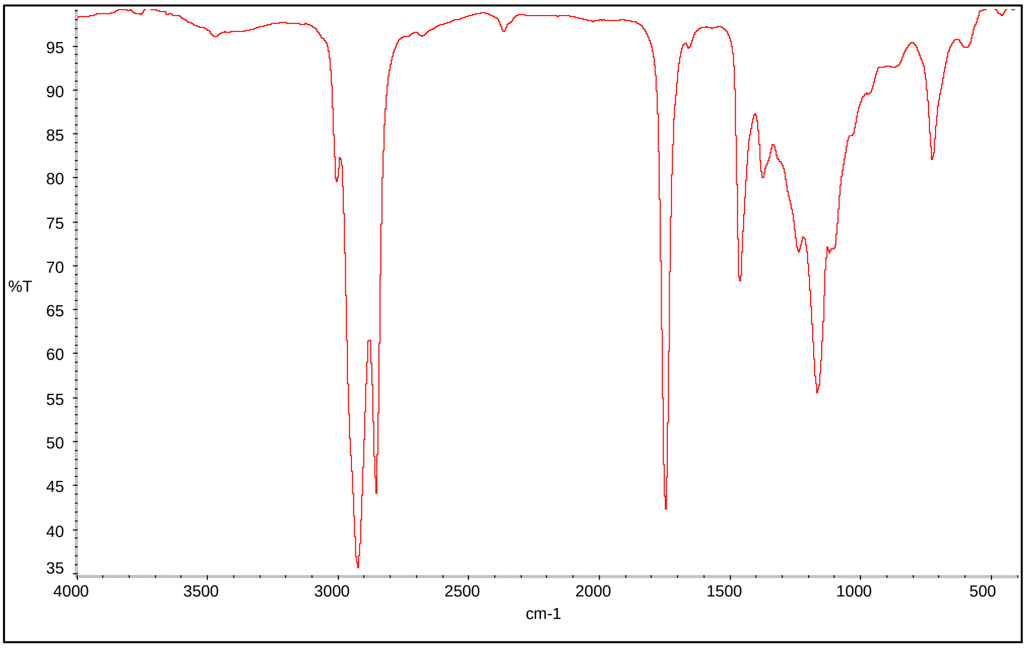


Figure A7 IR spectrum of crude AS oil.

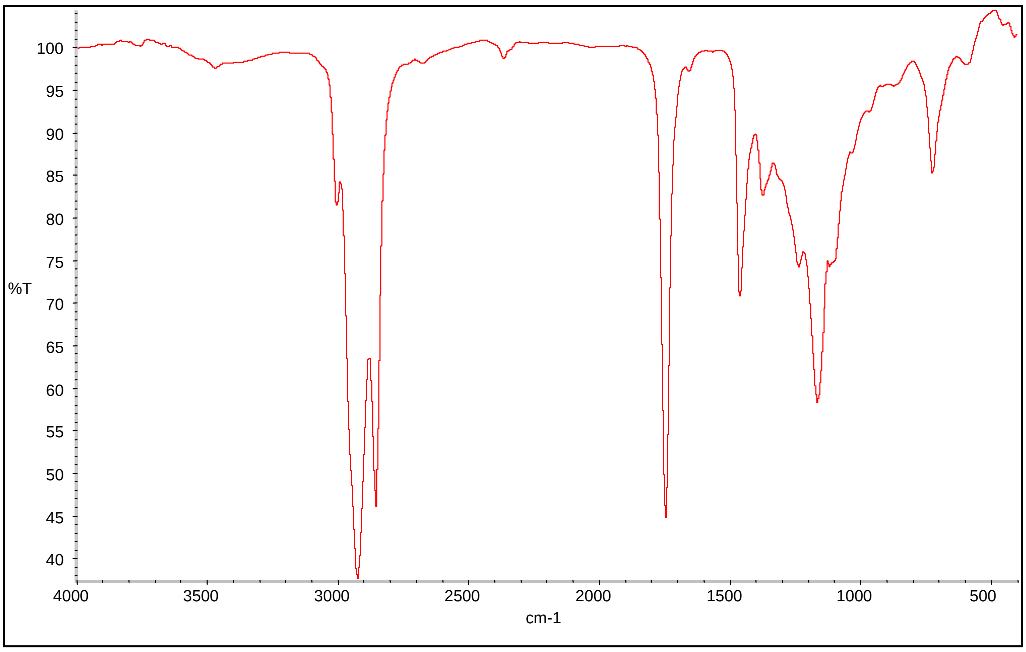


Figure A8 IR spectrum of AS oil methyl ester.

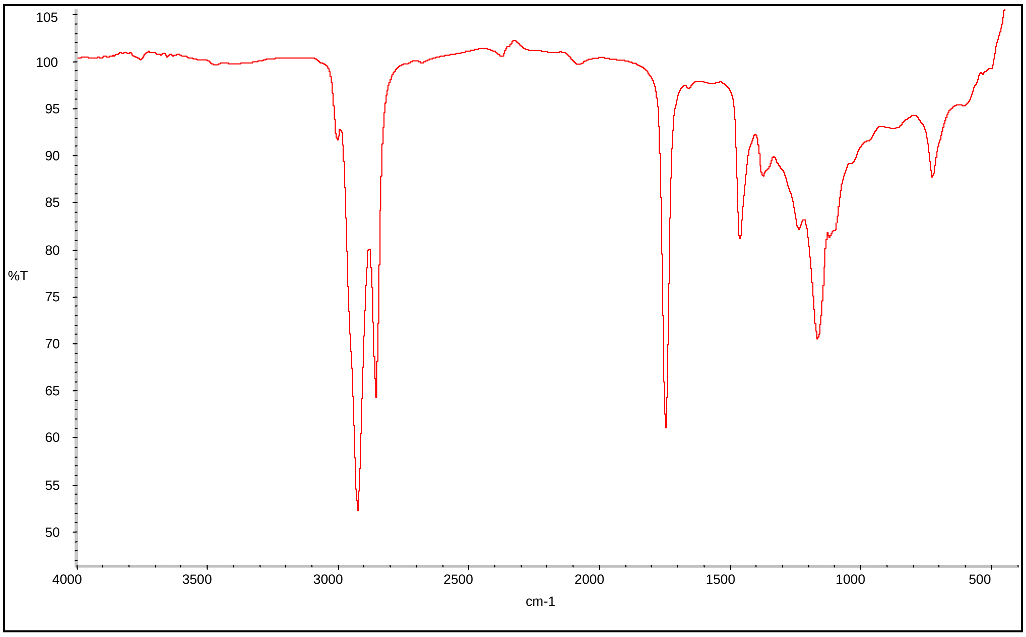


Figure A9 IR spectrum of crude CAP oil.

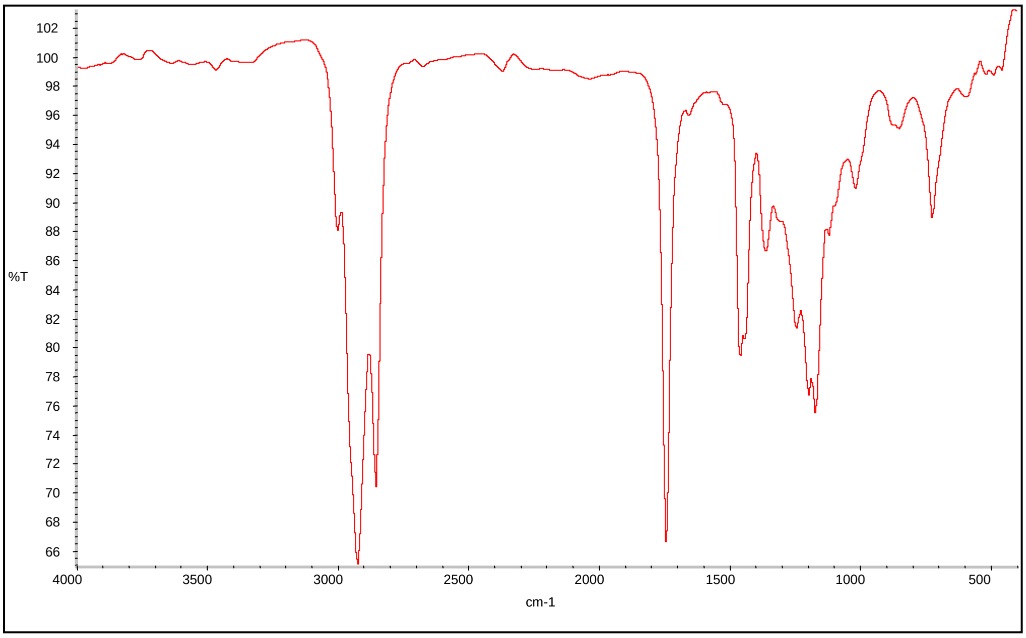


Figure A10 IR spectrum of CAP oil methyl ester.

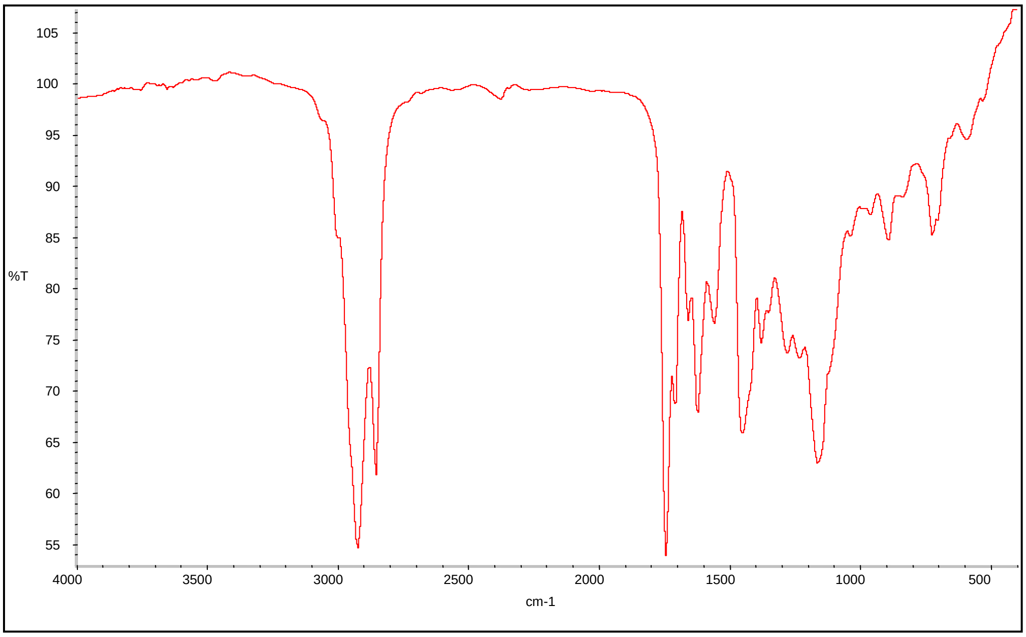


Figure A11 IR spectrum of crude CI oil.

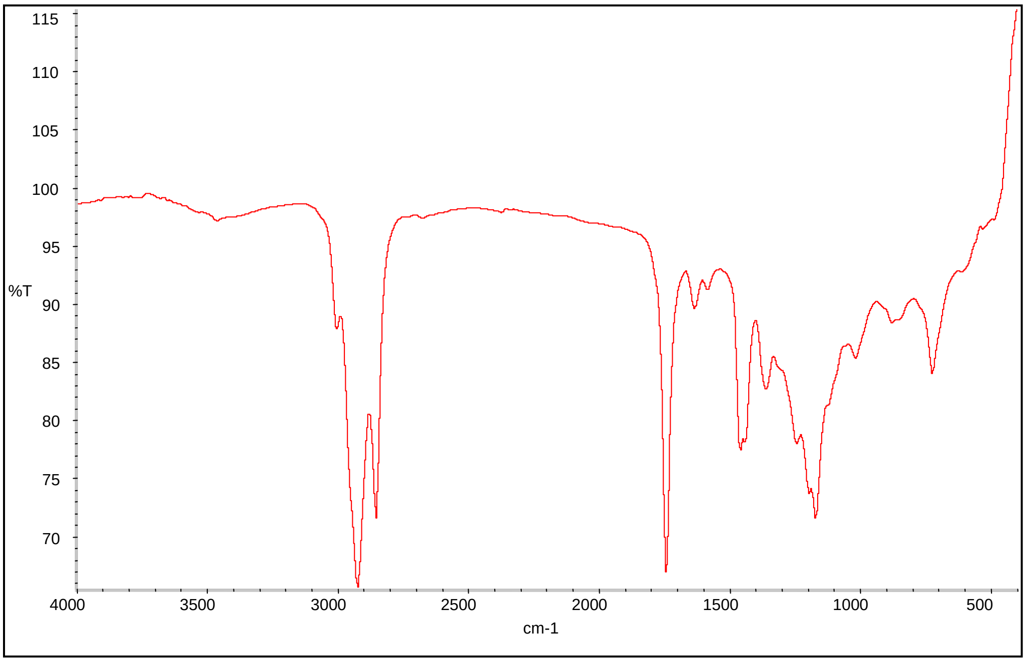


Figure A12 IR spectrum of CI oil methyl ester.

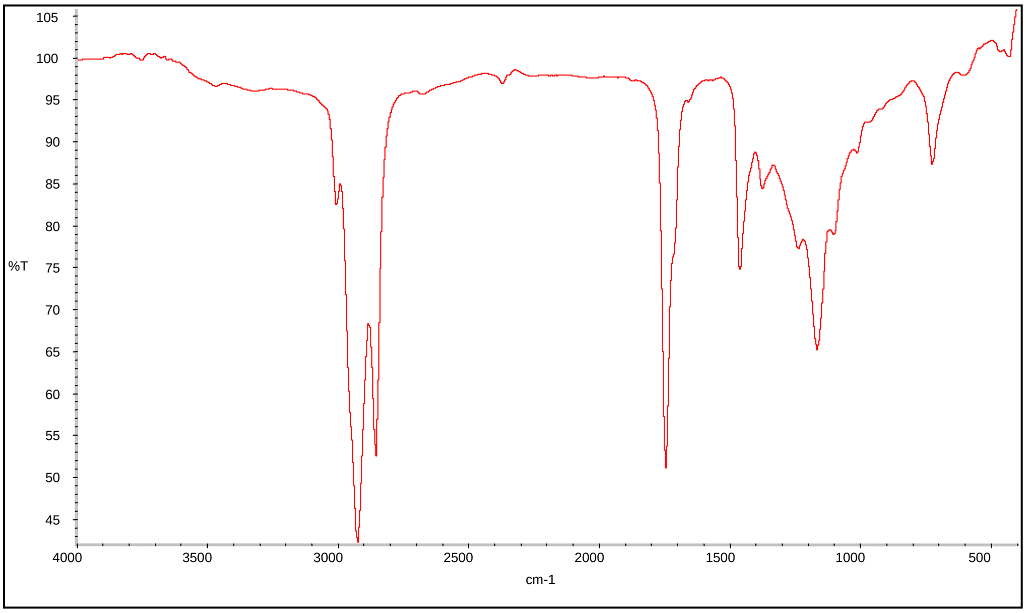


Figure A13 IR spectrum of crude CP oil.

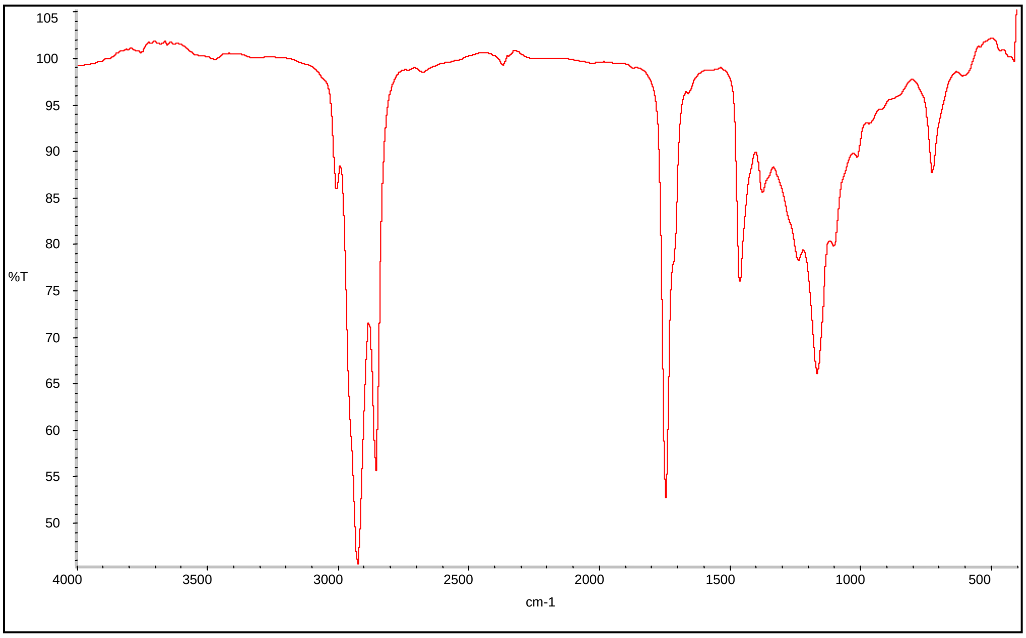


Figure A14 IR spectrum of CP oil methyl ester.

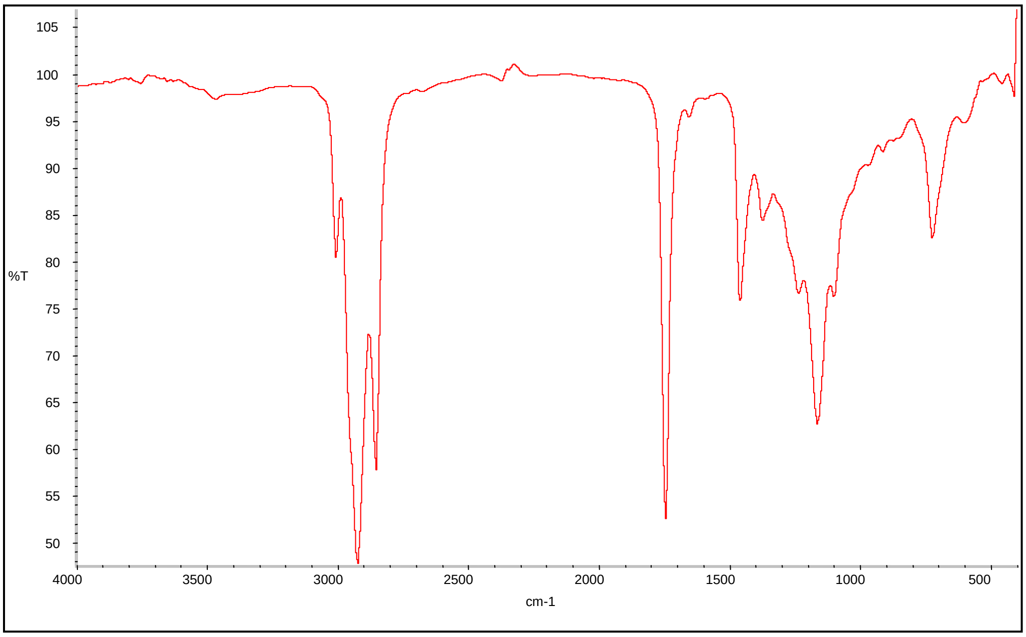


Figure A15 IR spectrum of crude CS oil.

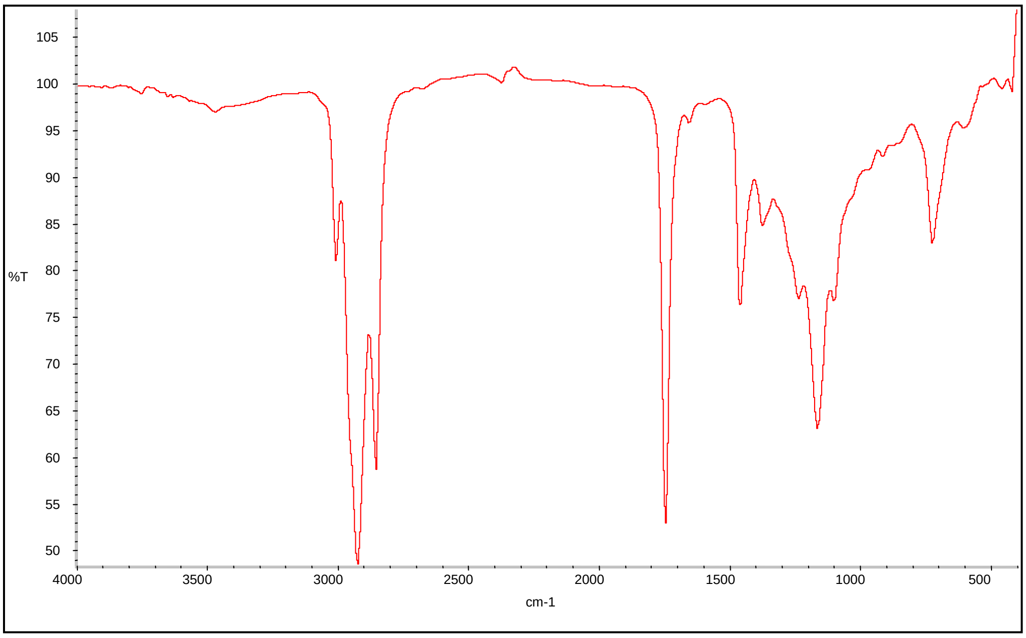


Figure A16 IR spectrum of CS oil methyl ester.

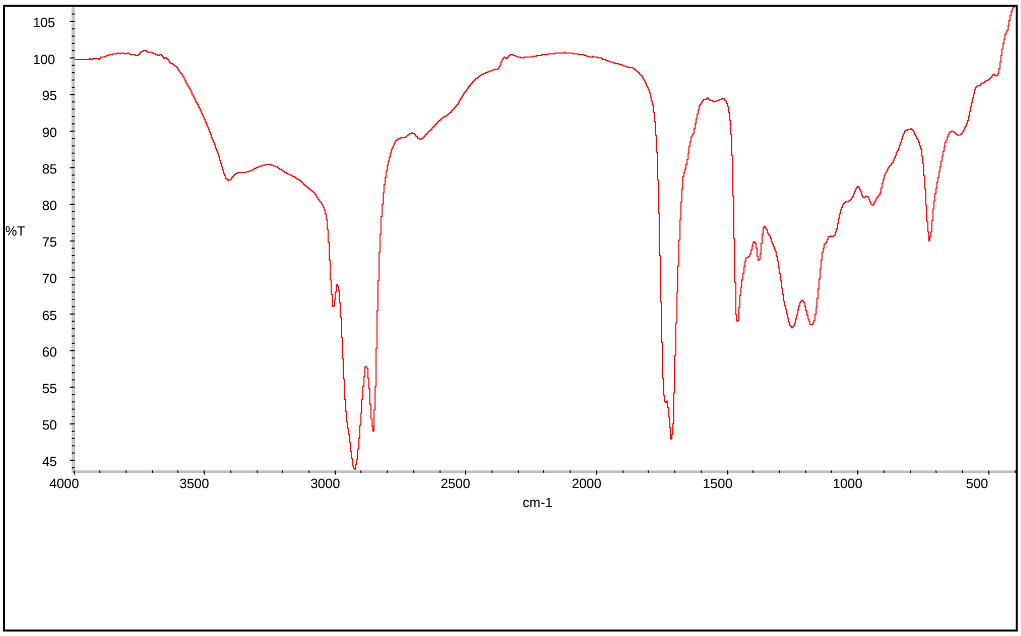


Figure A17 IR spectrum of crude CT oil.

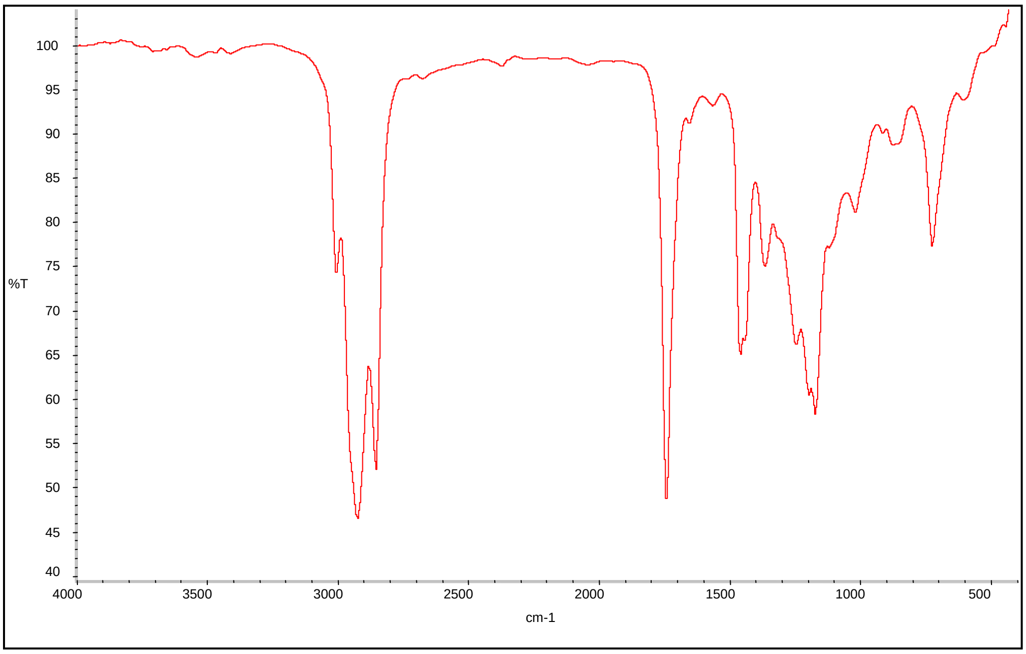


Figure A18 IR spectrum of CT oil methyl ester.

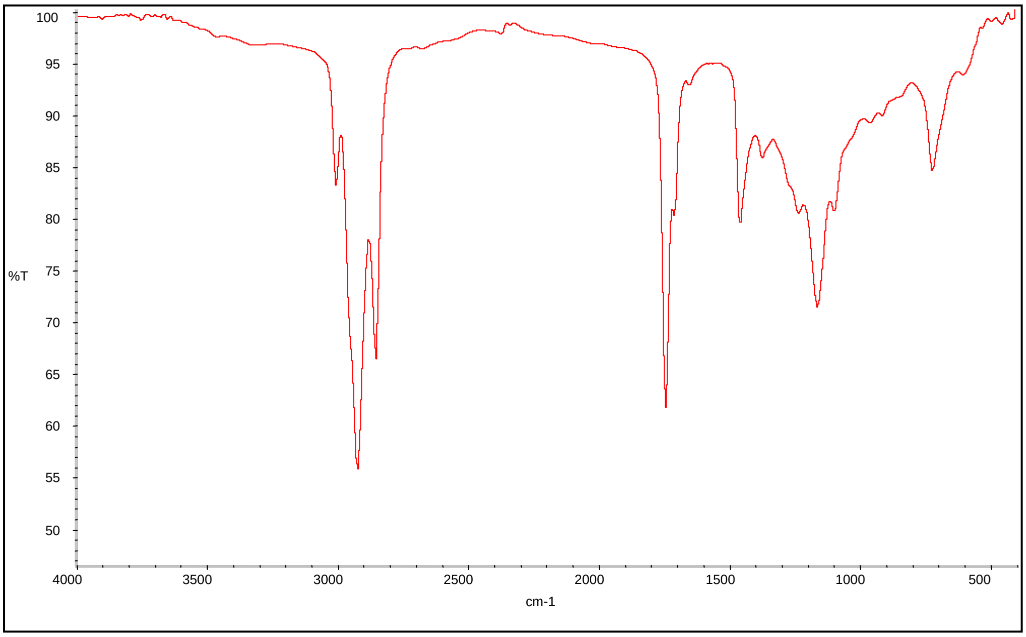


Figure A19 IR spectrum of crude EA oil.

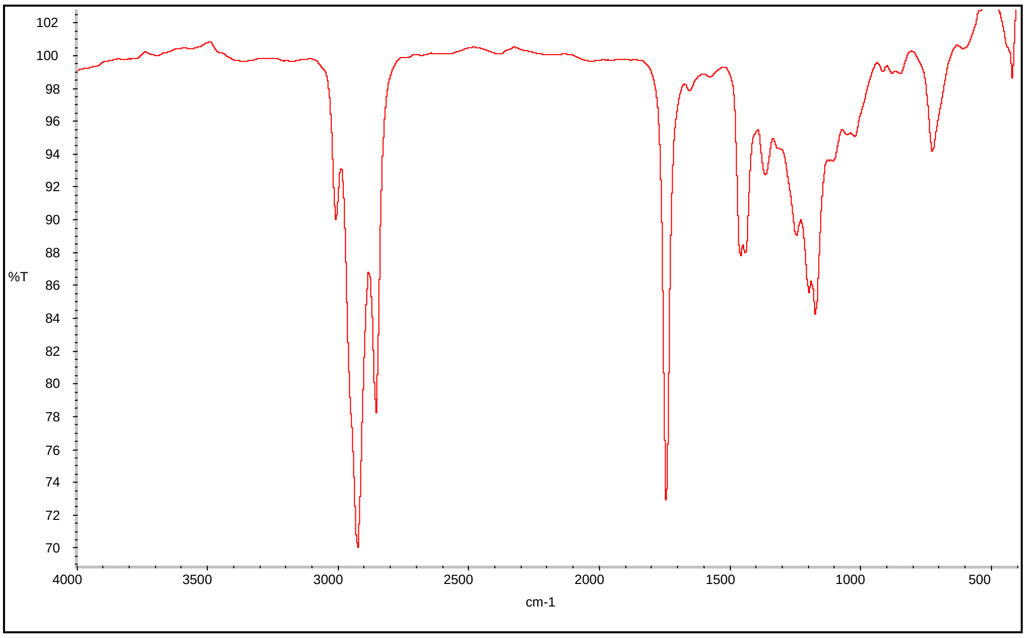


Figure A20 IR spectrum of EA oil methyl ester.

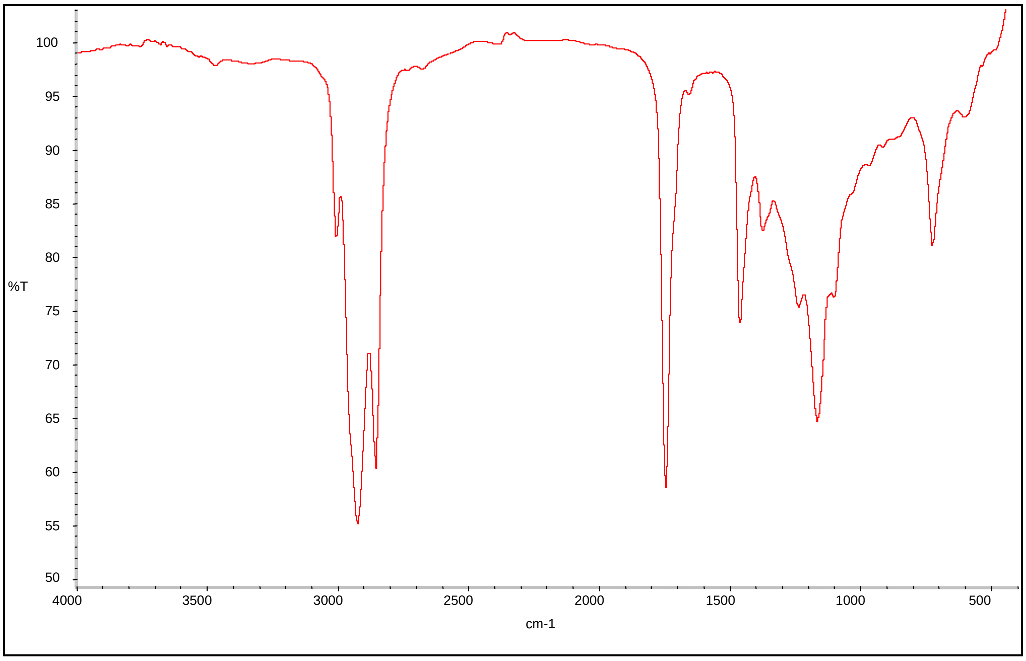


Figure A21 IR spectrum of crude GA oil.

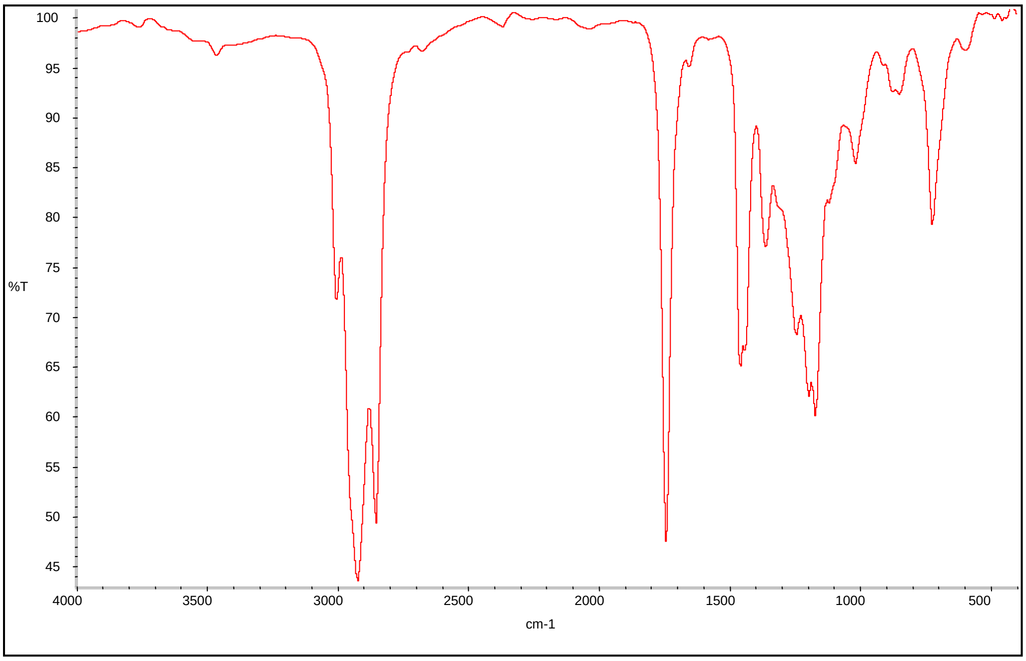


Figure A22 IR spectrum of GA oil methyl ester.

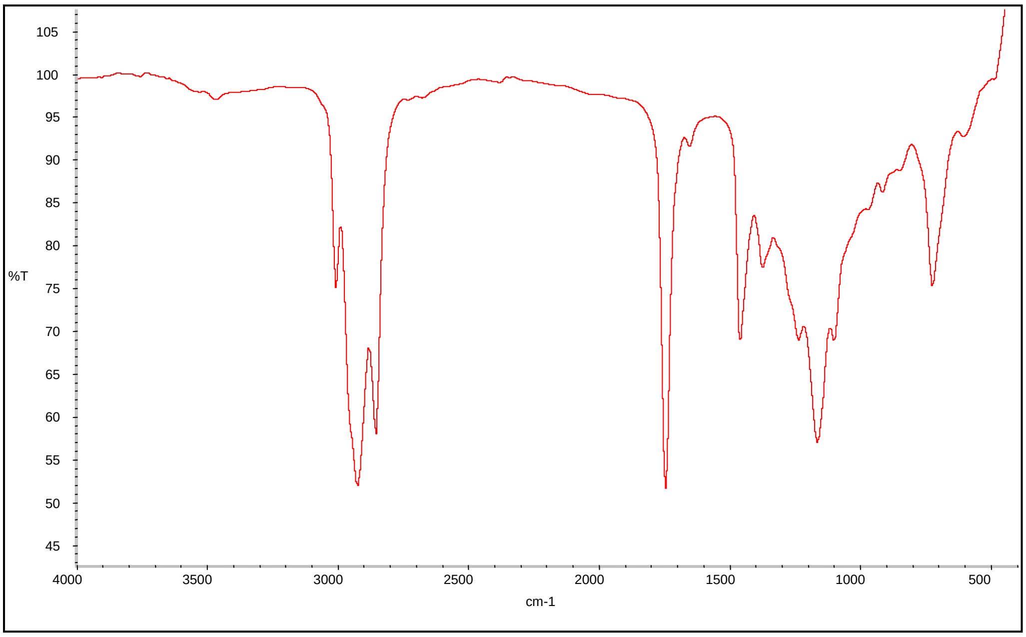


Figure A23 IR spectrum of crude HB oil.

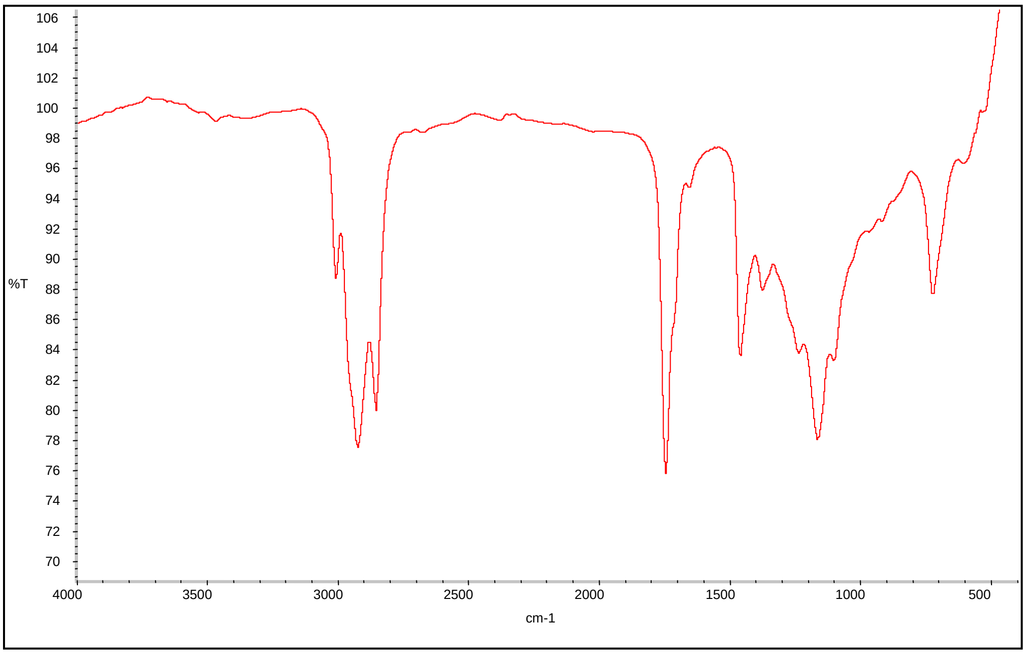


Figure A24 IR spectrum of HB oil methyl ester.

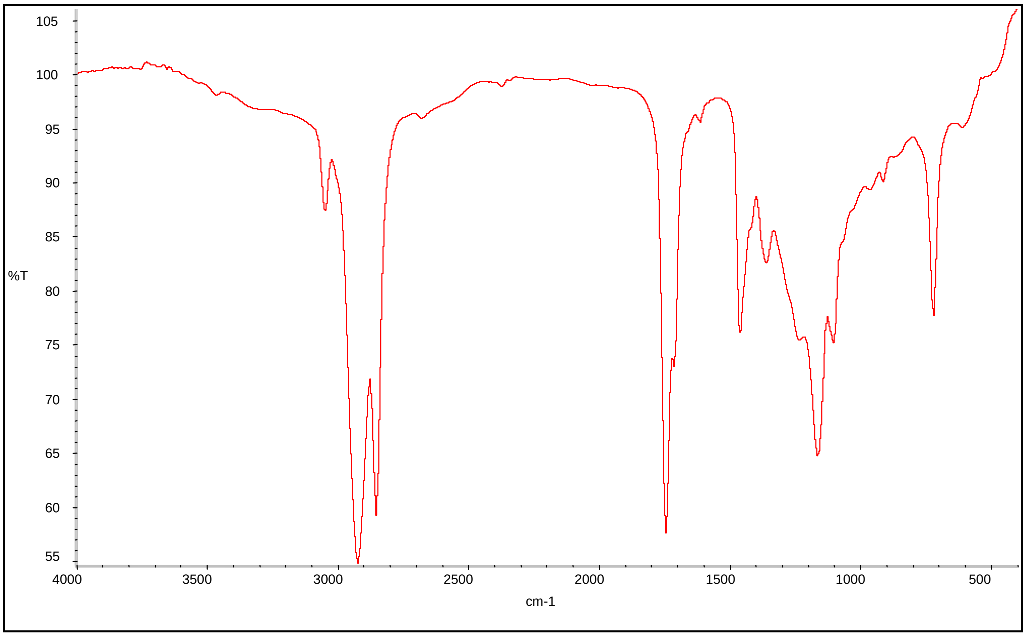


Figure A25 IR spectrum of crude HI oil.

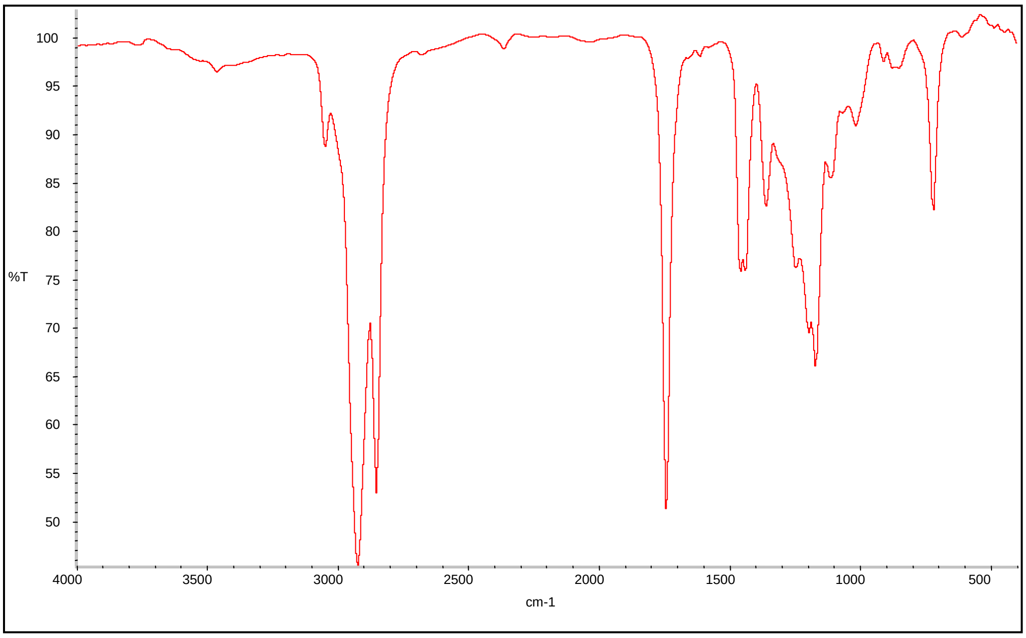


Figure A26 IR spectrum of HI oil methyl ester.

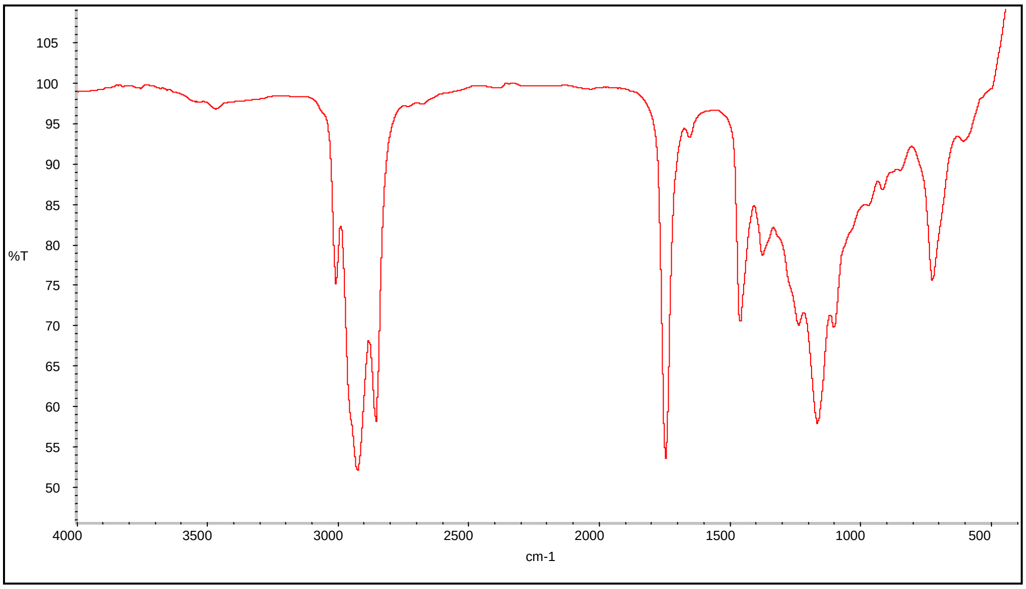


Figure A27 IR spectrum of crude HS oil.

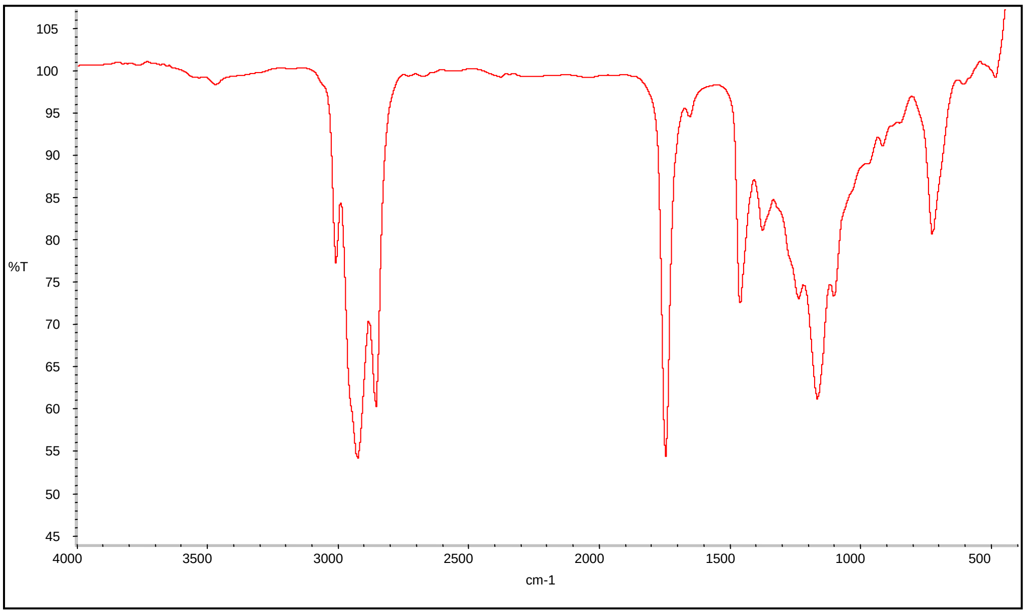


Figure A28 IR spectrum of HS oil methyl ester.

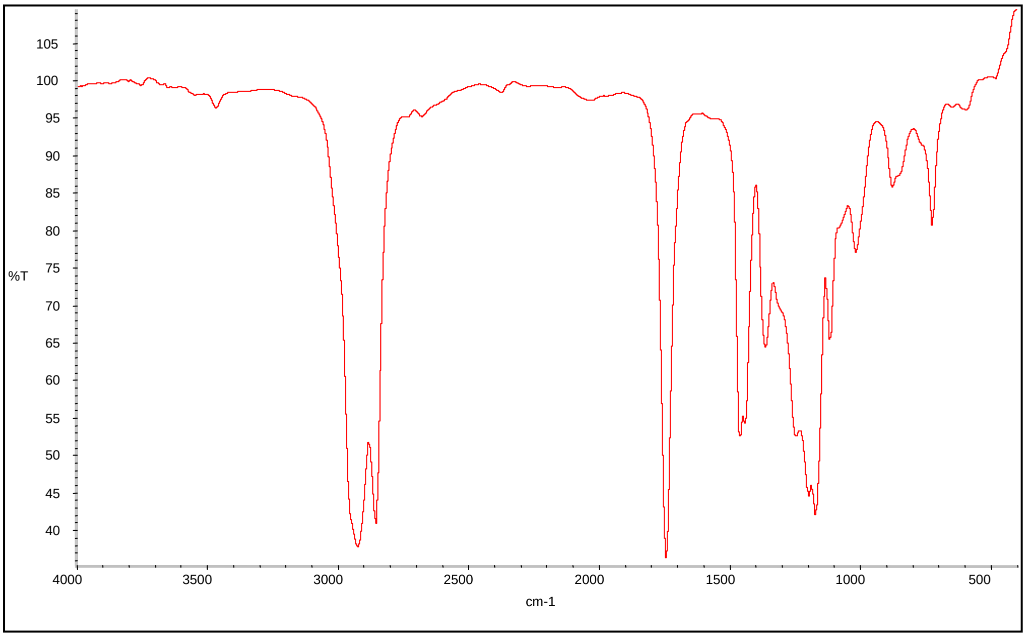


Figure A29 IR spectrum of crude IM oil.

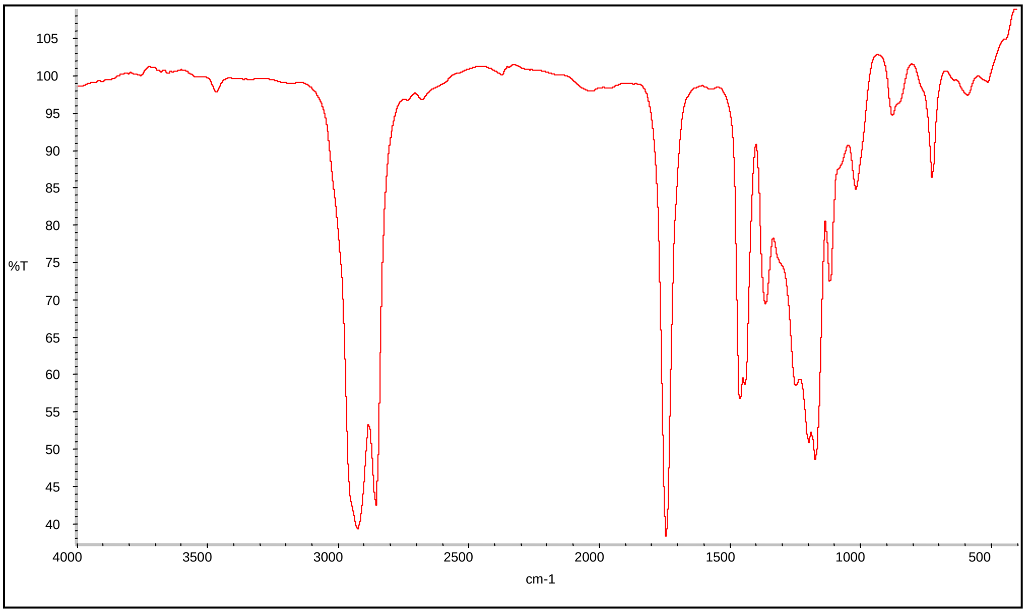


Figure A30 IR spectrum of IM oil methyl ester.

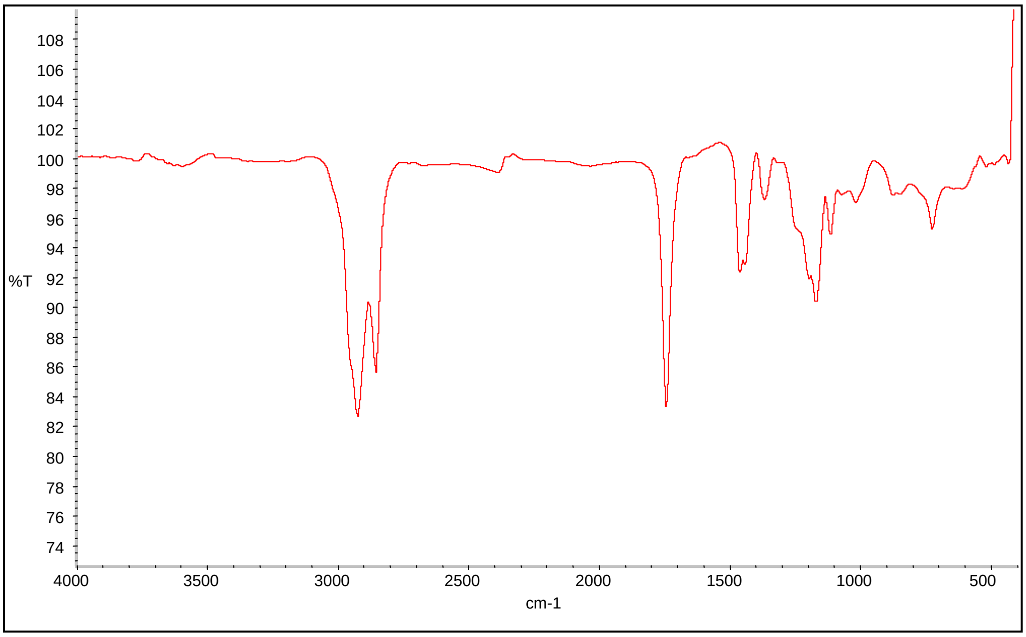


Figure A31 IR spectrum of crude LG oil.

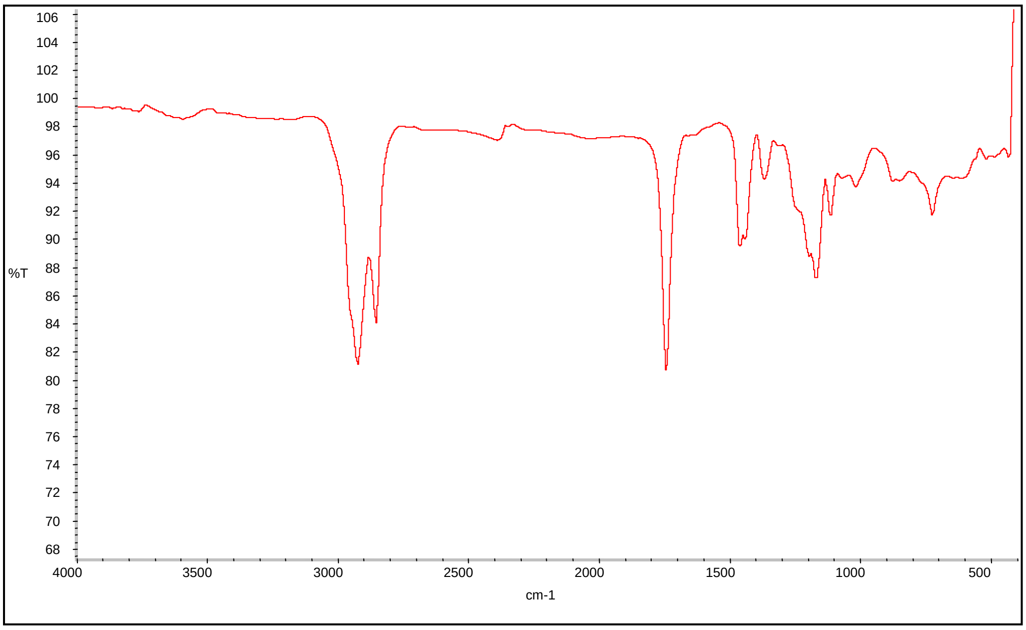


Figure A32 IR spectrum of LG oil methyl ester.

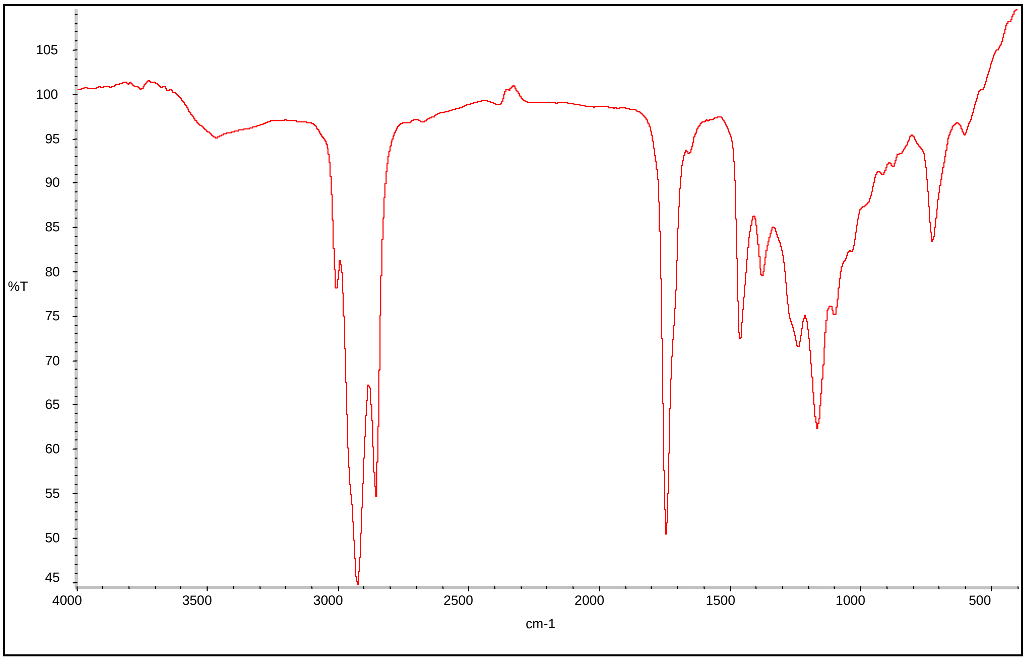


Figure A33 IR spectrum of crude MA oil.

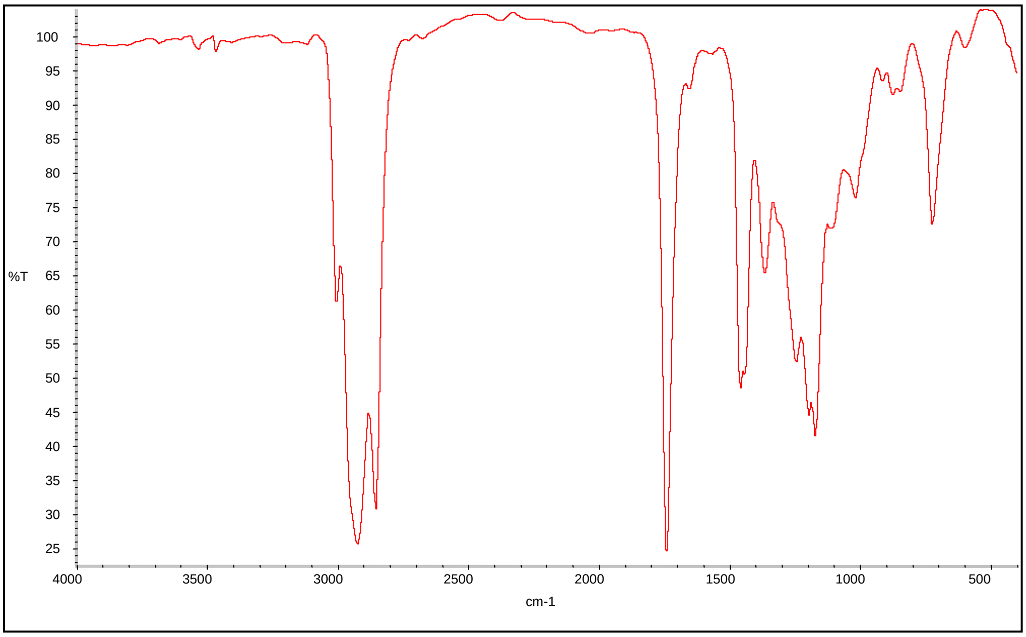


Figure A34 IR spectrum of MA oil methyl ester.

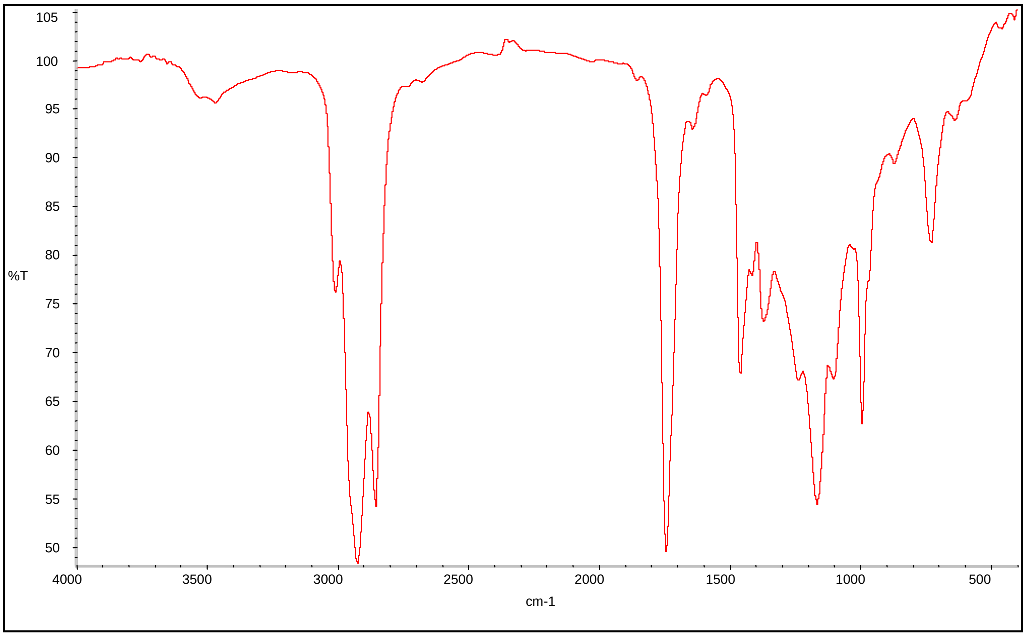


Figure A35 IR spectrum of crude PA oil.

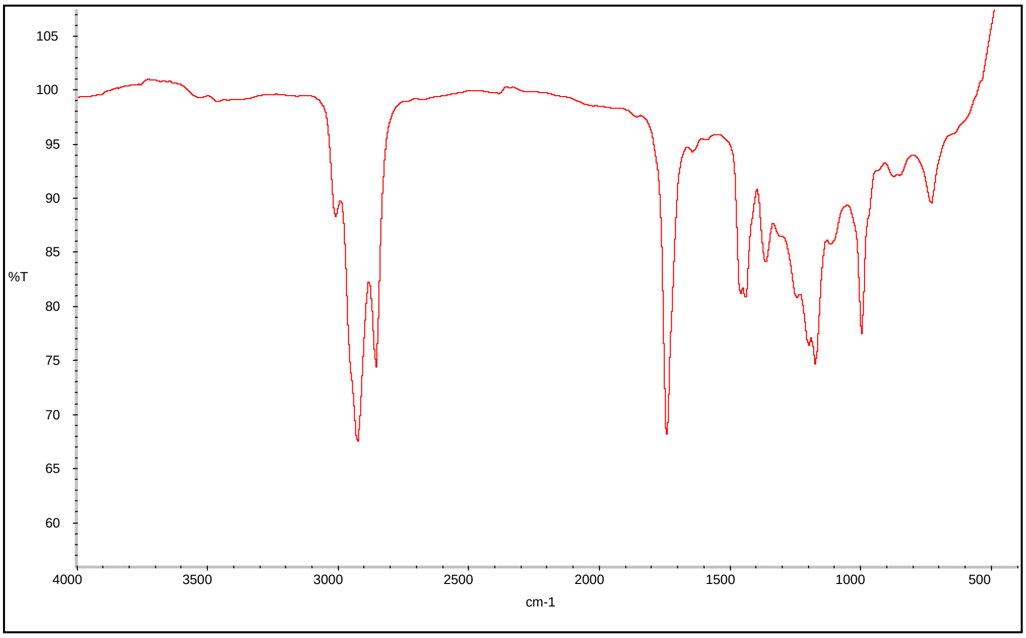


Figure A36 IR spectrum of PA oil methyl ester.

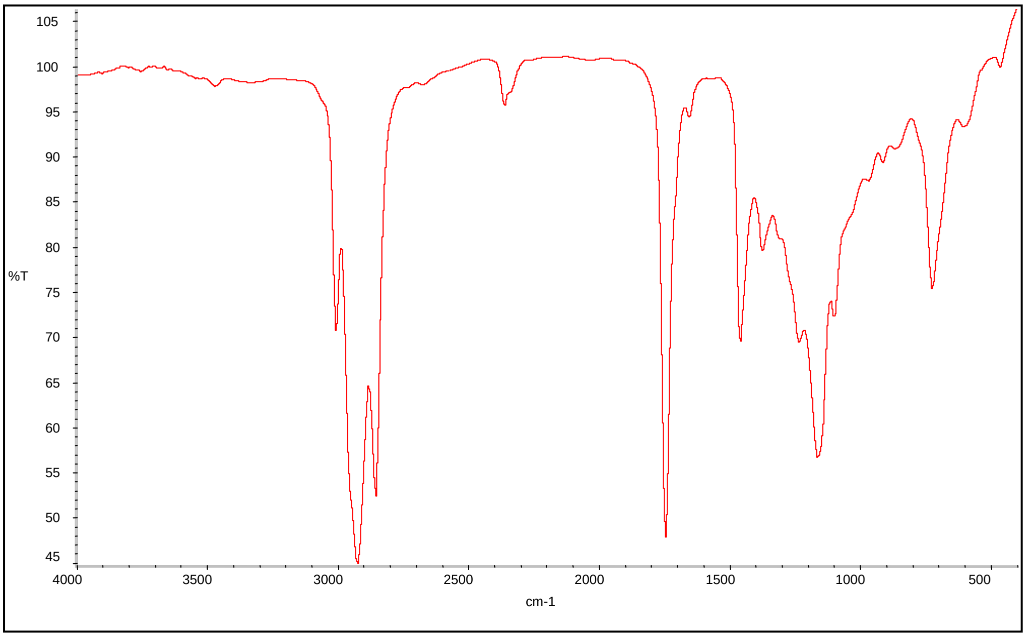


Figure A37 IR spectrum of crude PK oil.

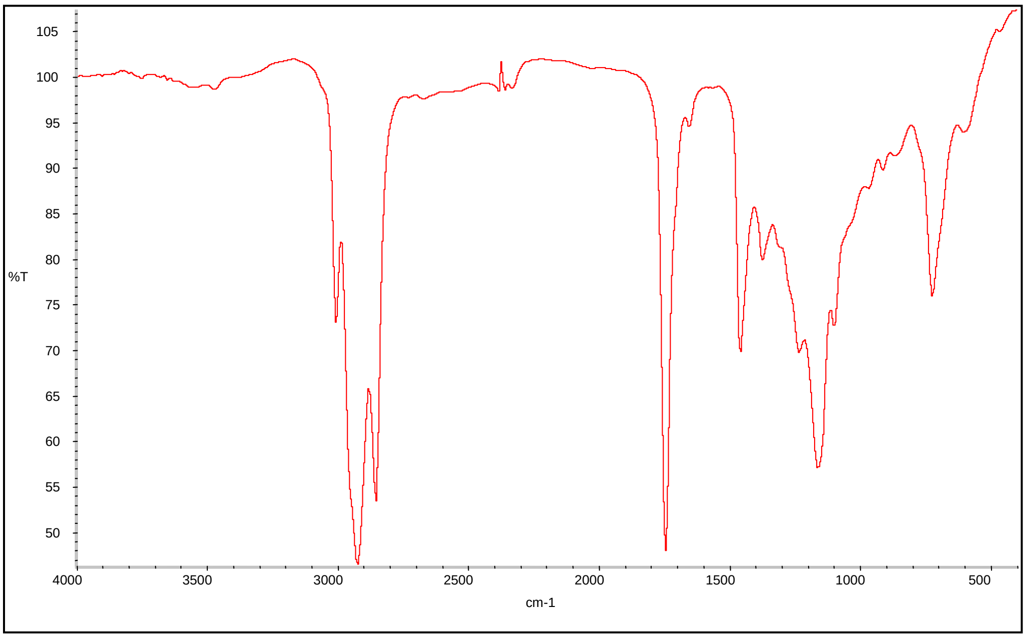


Figure A38 IR spectrum of PK oil methyl ester.

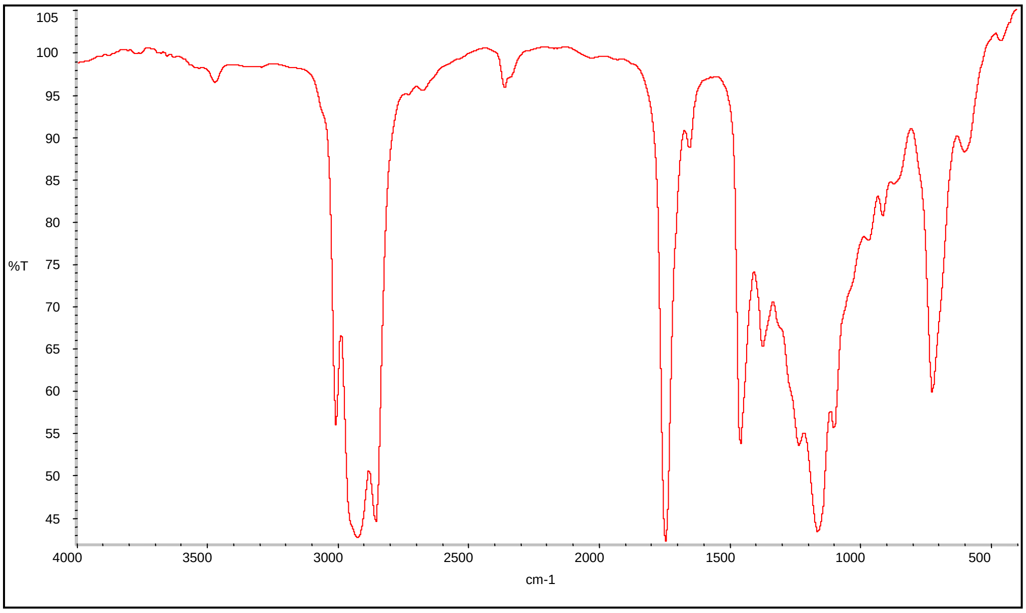


Figure A39 IR spectrum of crude PM oil.

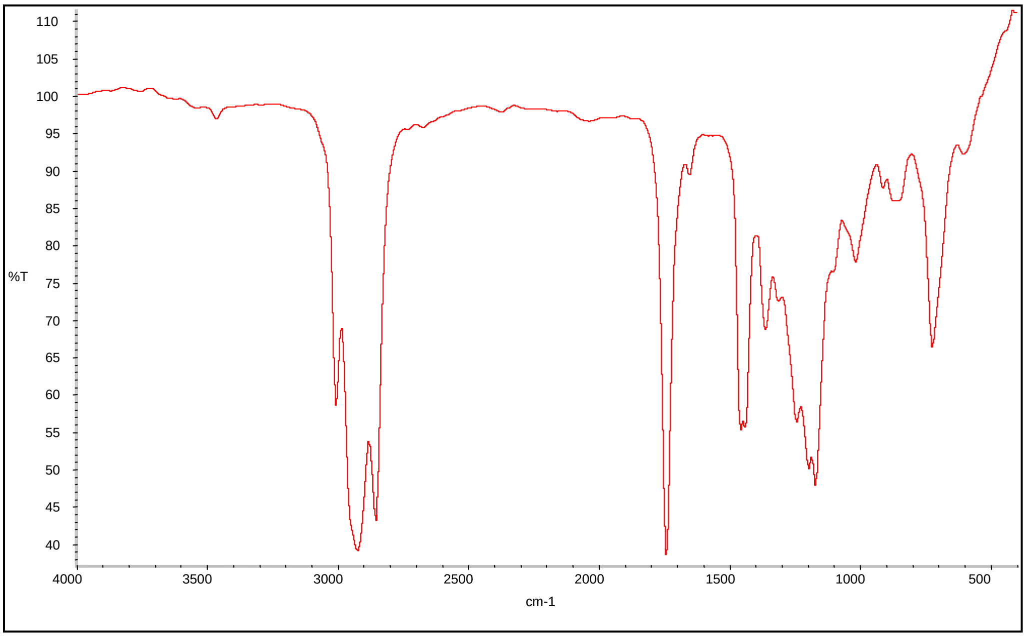


Figure A40 IR spectrum of PM oil methyl ester.

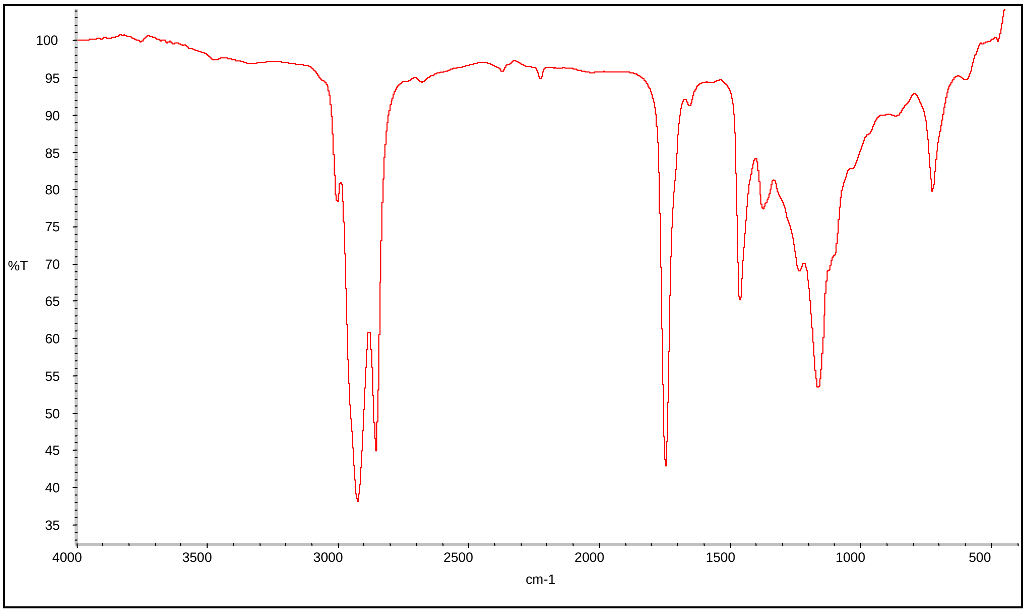


Figure A41 IR spectrum of crude SE oil.

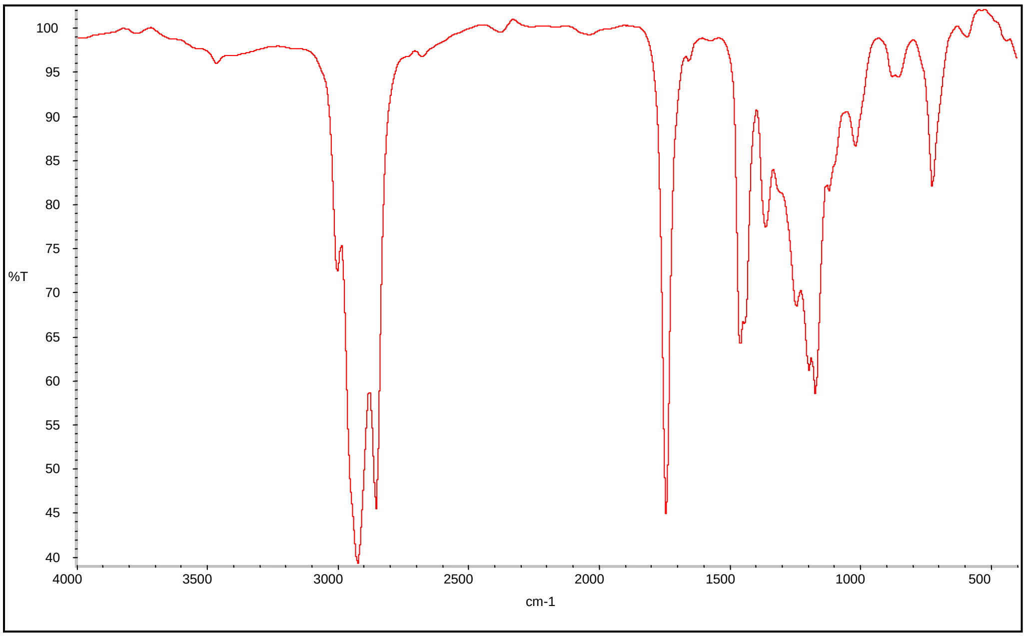


Figure A42 IR spectrum of SE oil methyl ester.

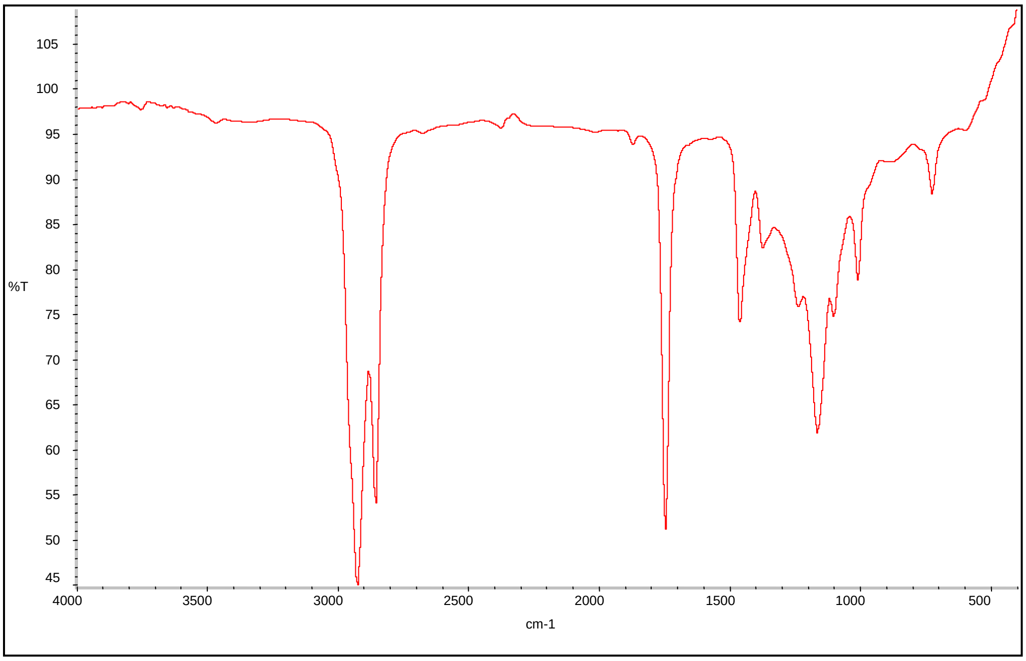


Figure A43 IR spectrum of crude SF oil.

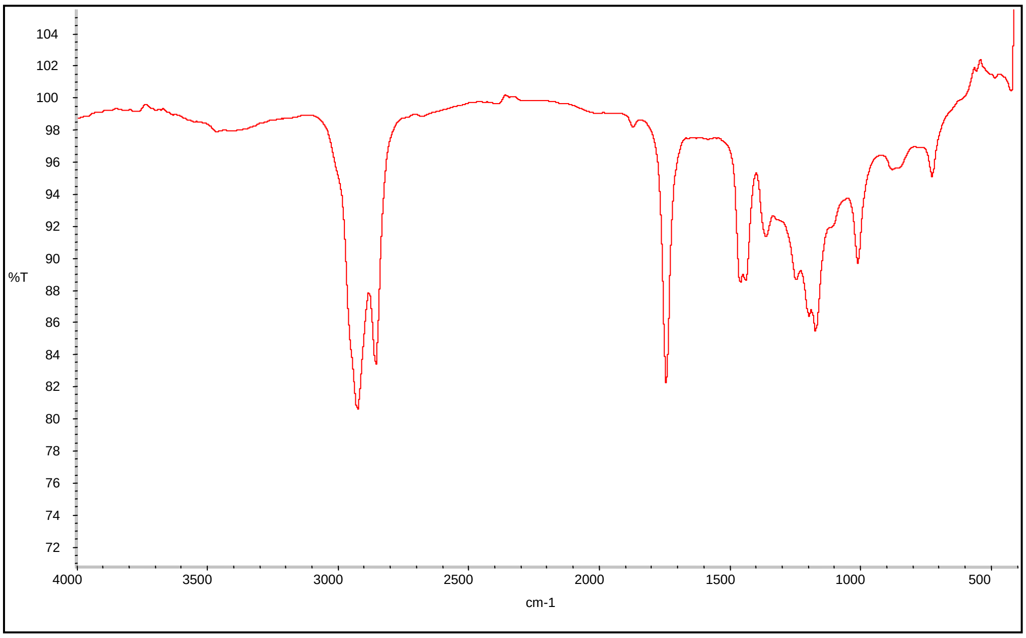


Figure A44 IR spectrum of SF oil methyl ester.

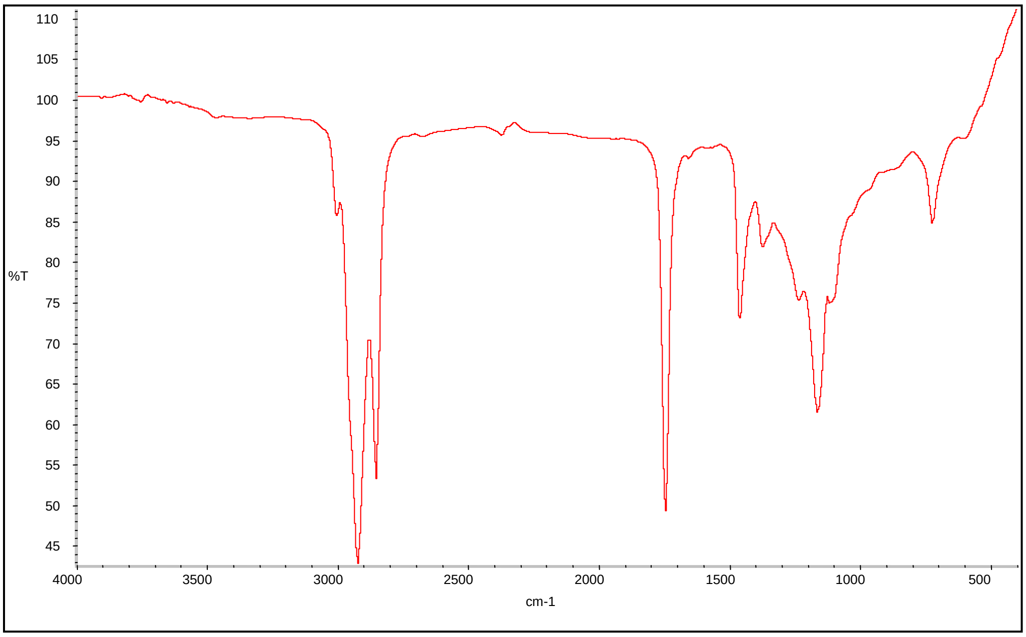


Figure A45 IR spectrum of crude TB oil.

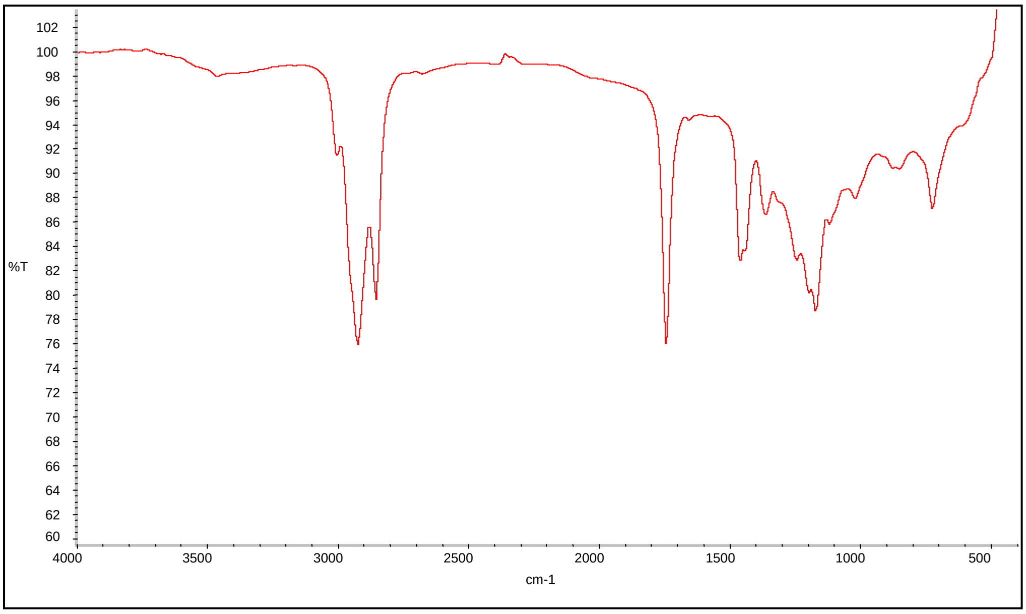


Figure A46 IR spectrum of TB oil methyl ester.

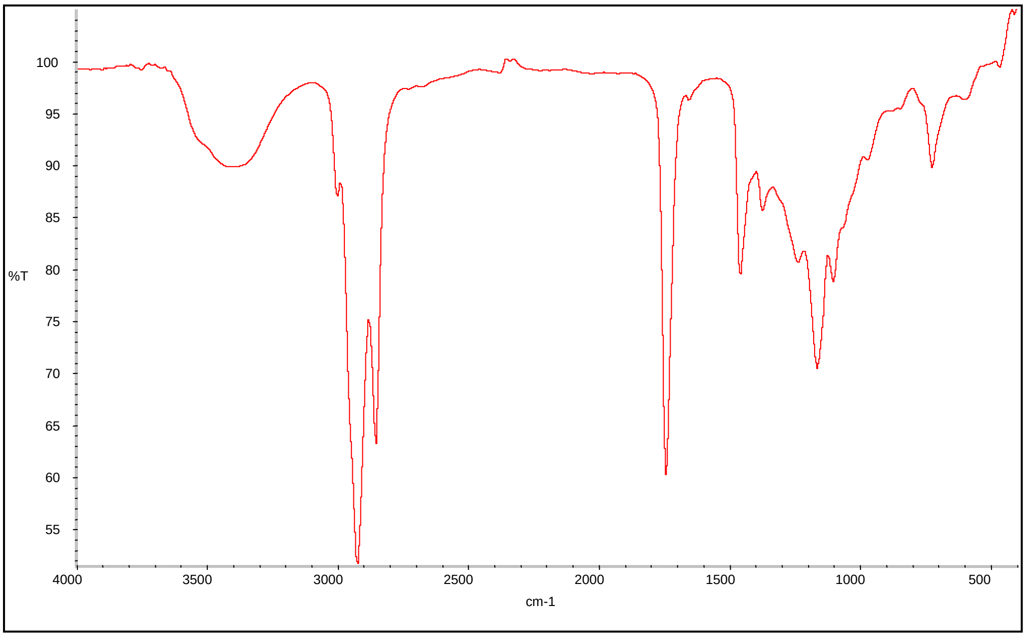


Figure A47 IR spectrum of crude TB oil.

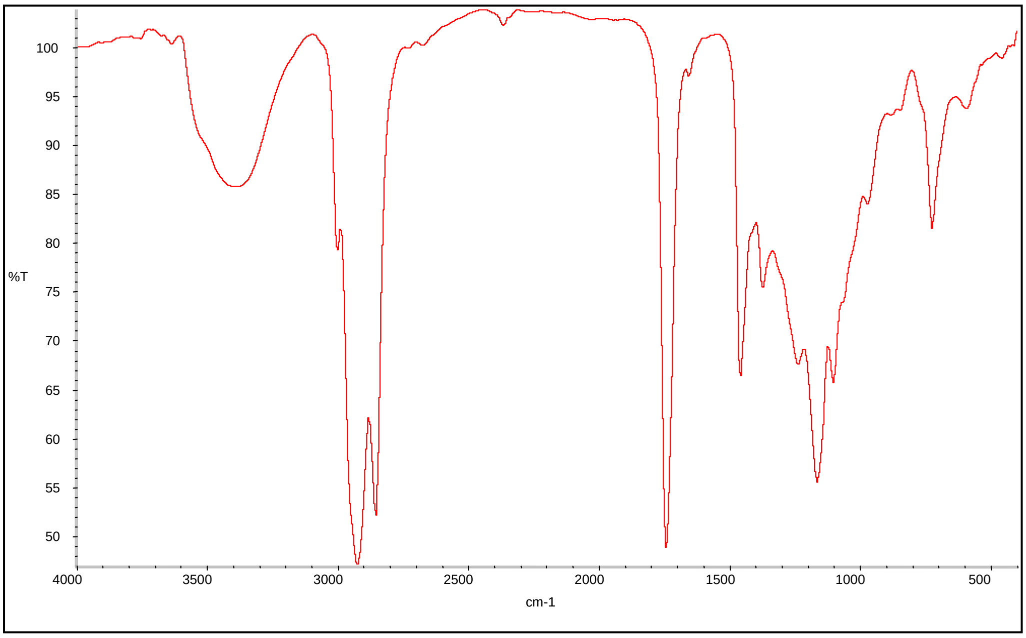


Figure A48 IR spectrum of TB oil methyl ester.

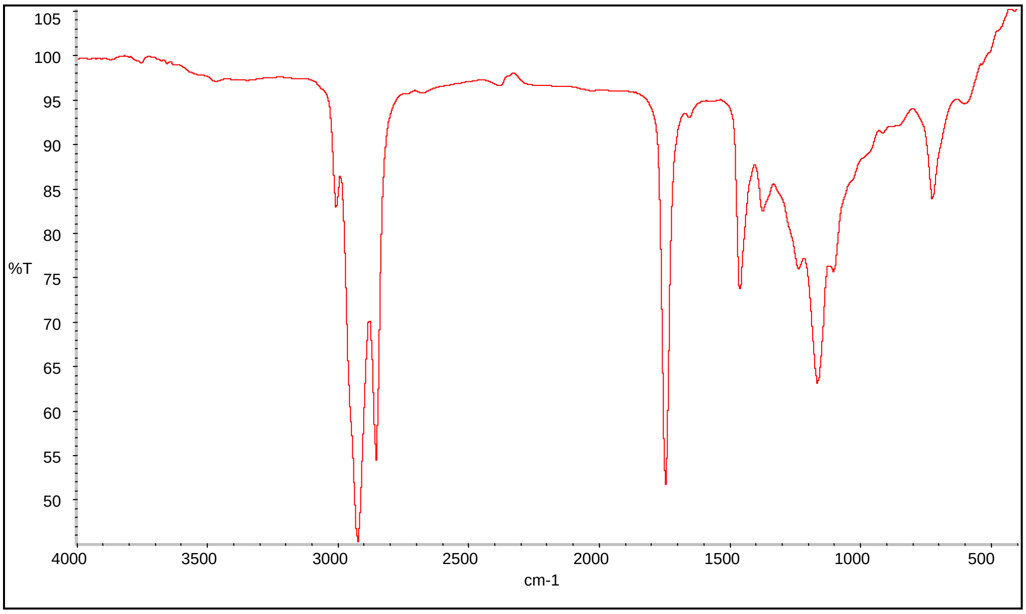


Figure A49 IR spectrum of crude XK oil.

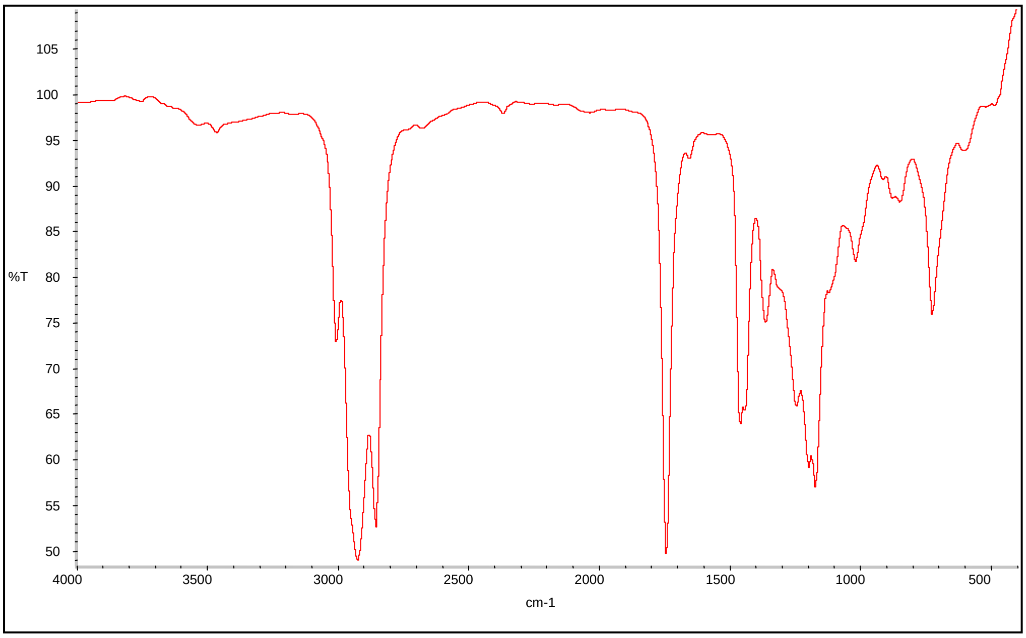


Figure A50 IR spectrum of XK oil methyl ester.

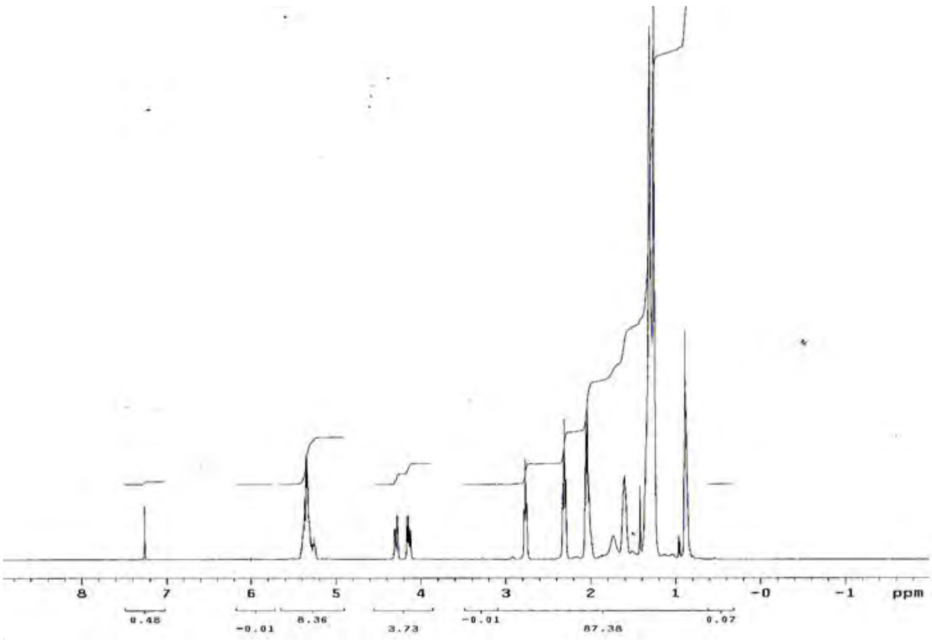


Figure A51 ^{1}H-NMR spectrum of crude AA oil.

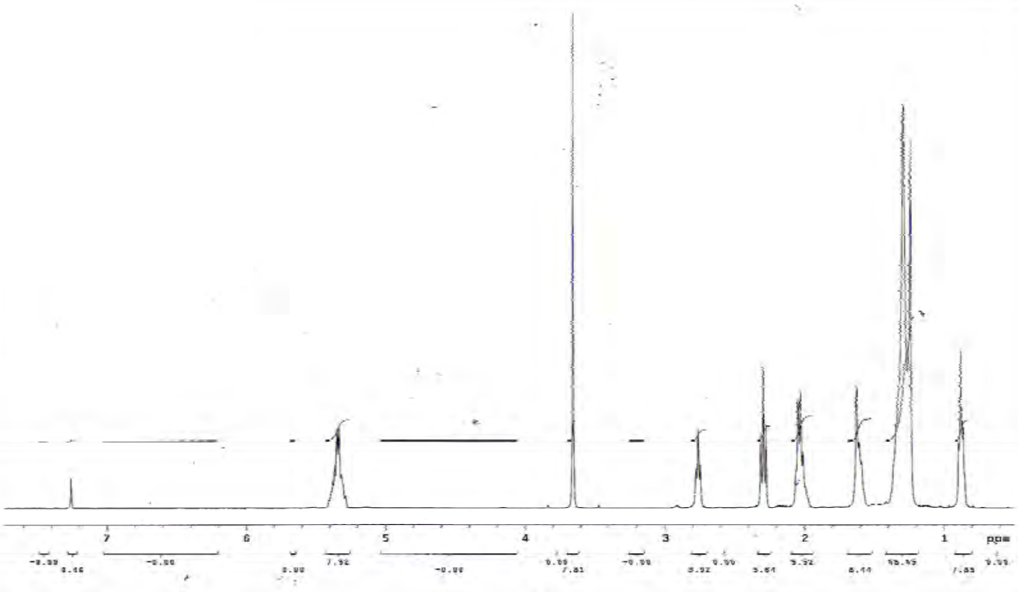


Figure A52 ^{1}H-NMR spectrum of AA oil methyl ester.

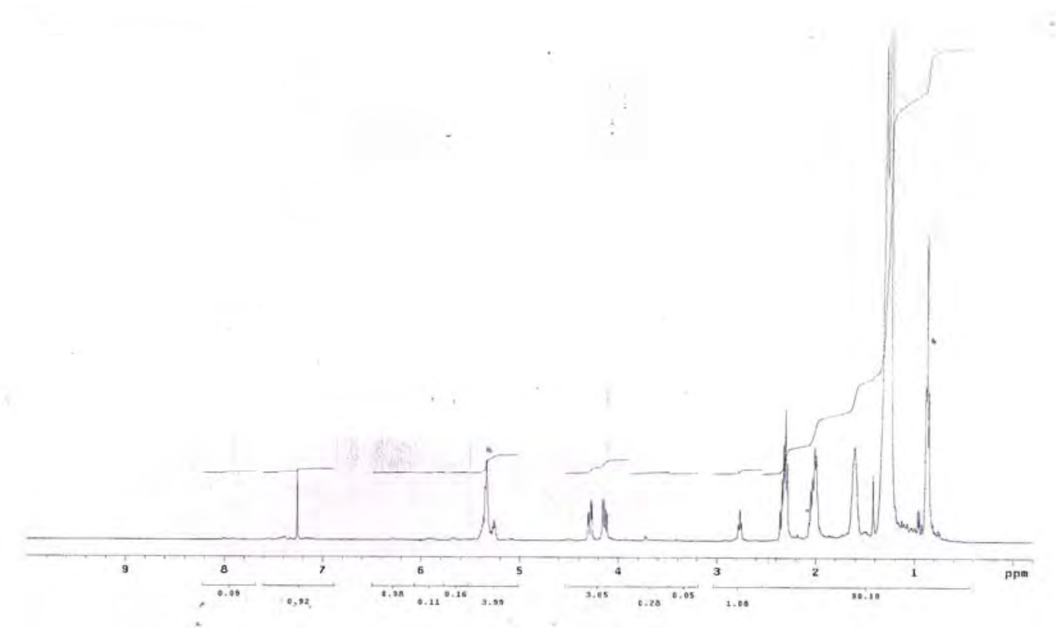


Figure A53 ^{1}H-NMR spectrum of crude AI oil.

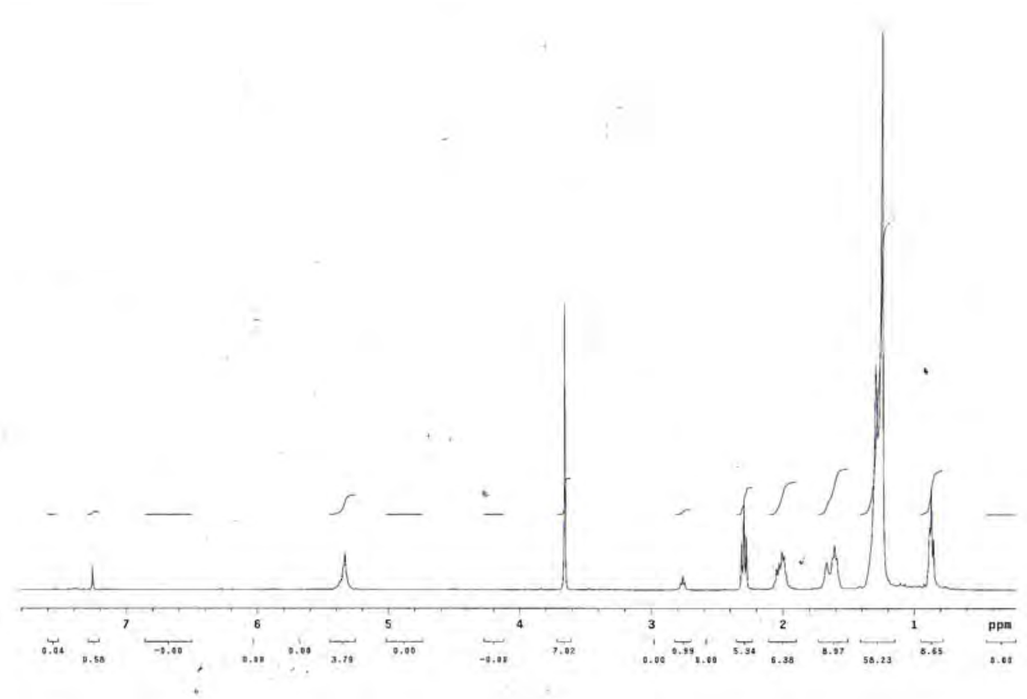


Figure A54 ^{1}H-NMR spectrum of AI oil methyl ester.

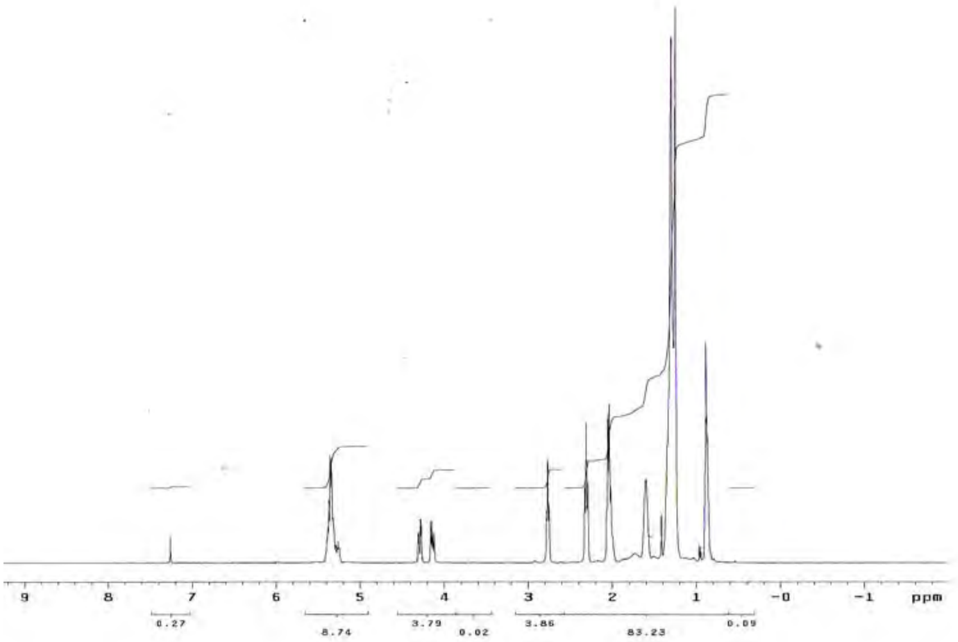


Figure A55 ^{1}H-NMR spectrum of crude AM oil.

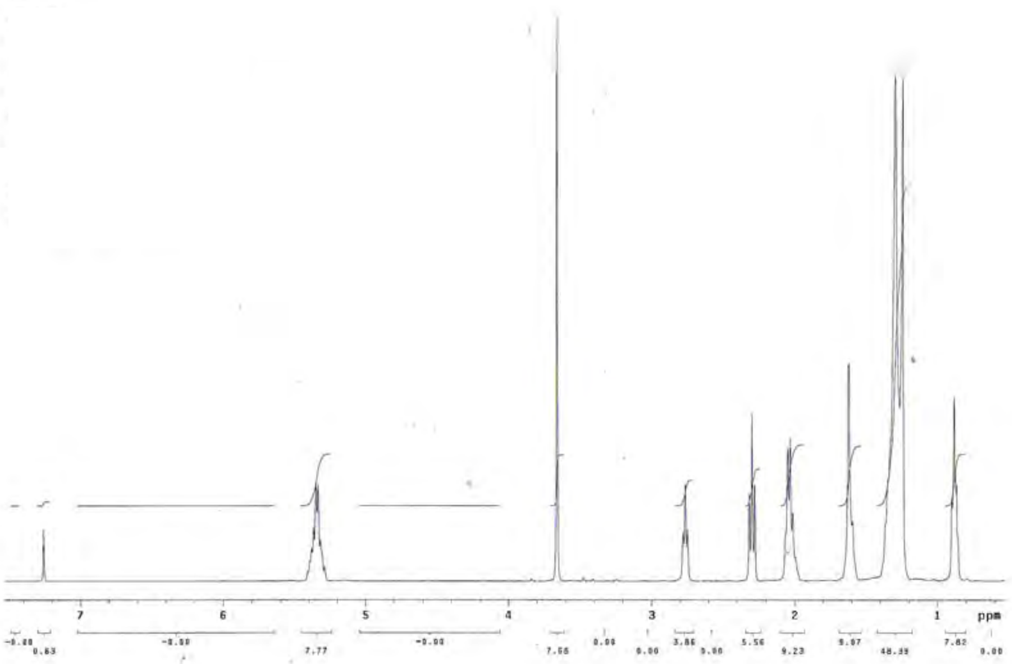


Figure A56 ^{1}H-NMR spectrum of AM oil methyl ester.

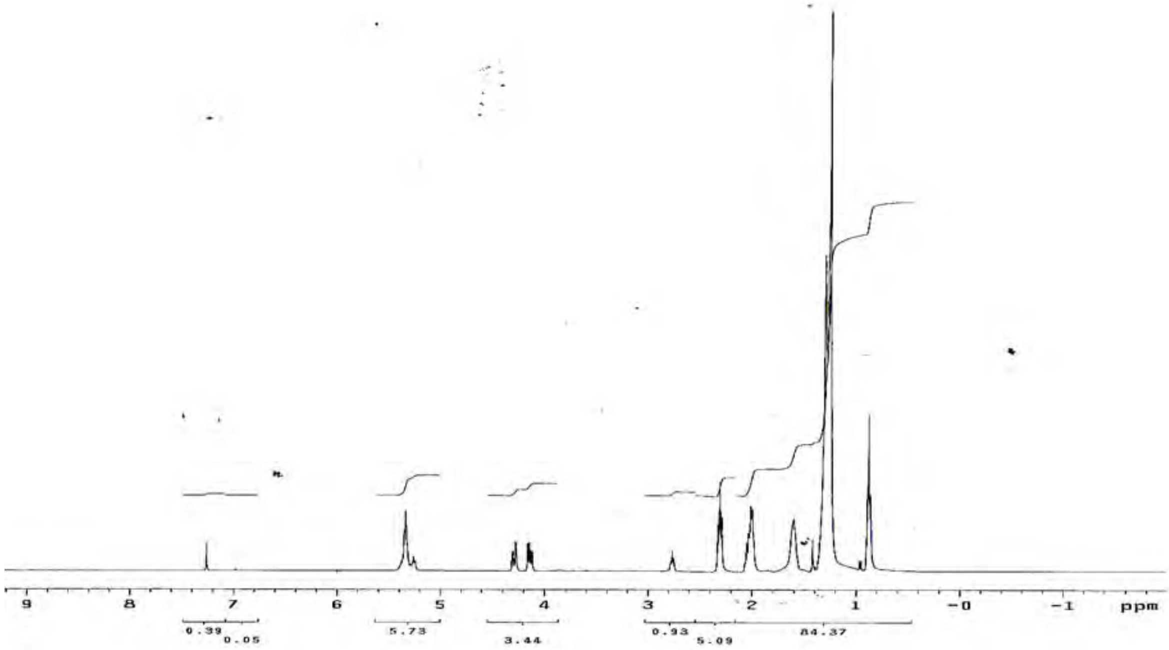


Figure A57 ^{1}H-NMR spectrum of crude AS oil.

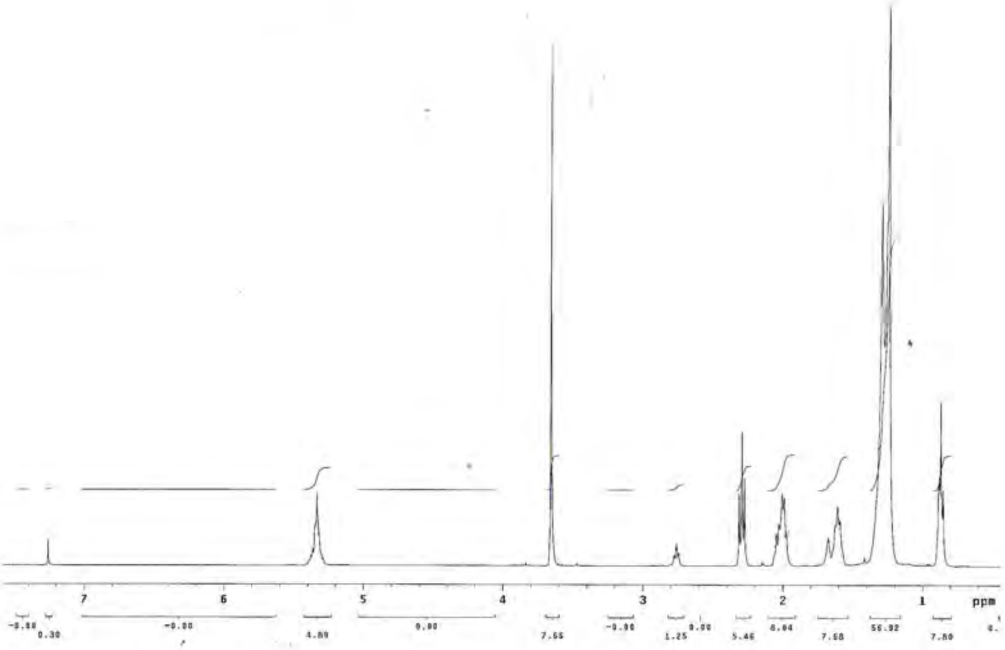


Figure A58 ^{1}H-NMR spectrum of AS oil methyl ester.

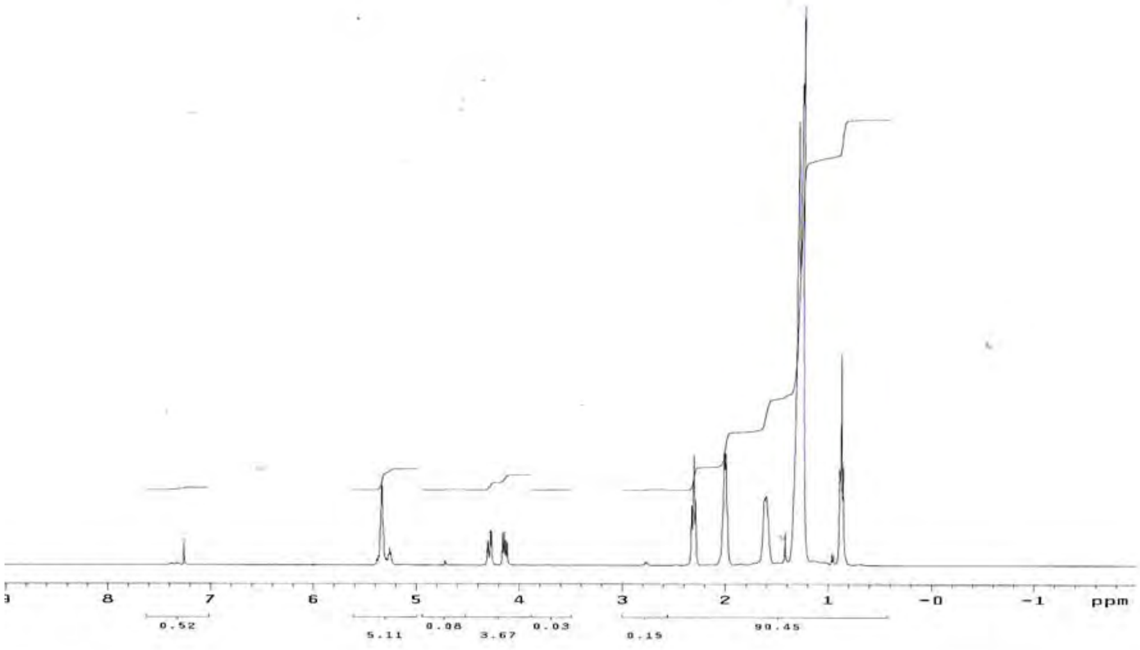


Figure A59 ^{1}H-NMR spectrum of crude CAP oil.

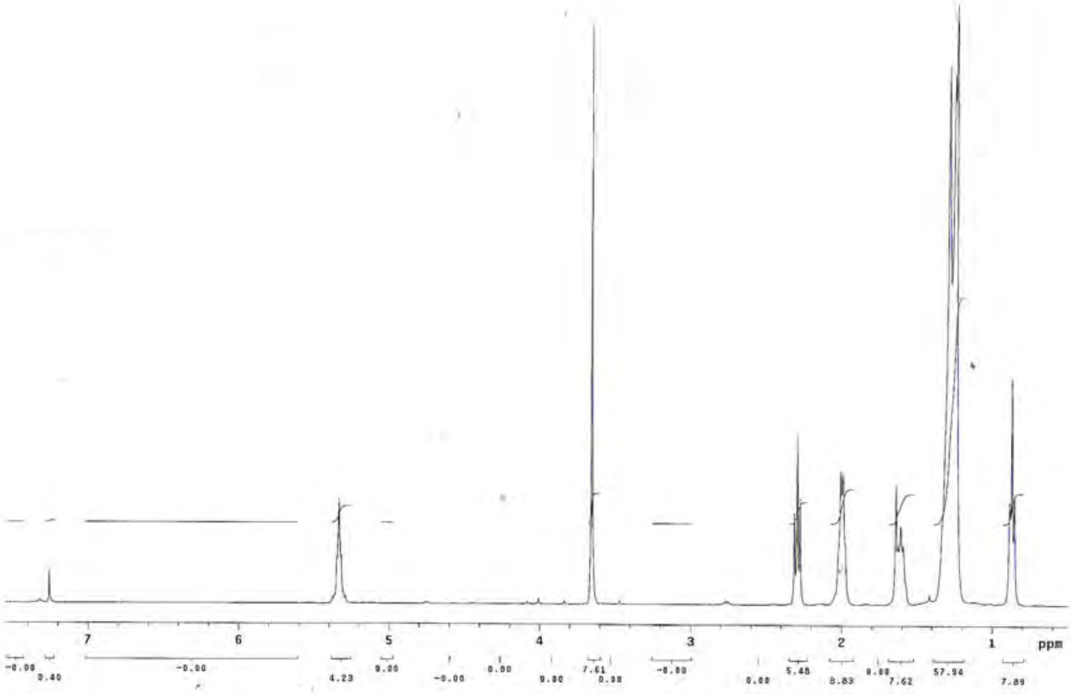


Figure A60 ^{1}H-NMR spectrum of CAP oil methyl ester.

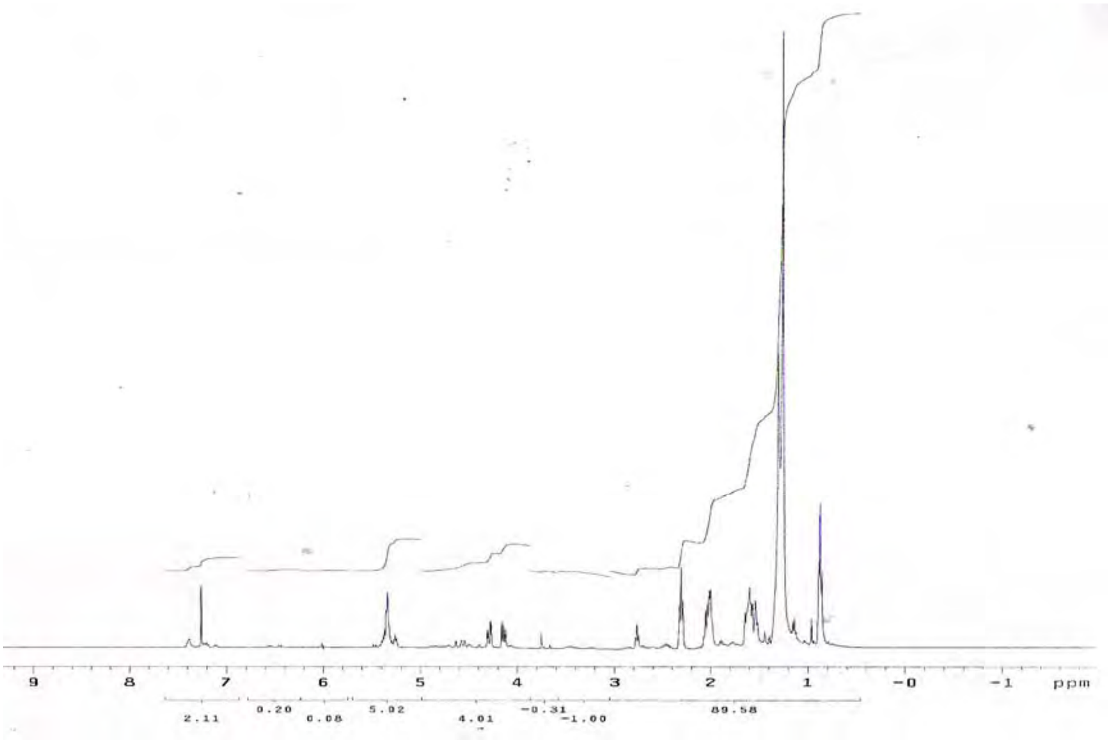


Figure A61 ^{1}H-NMR spectrum of crude CI oil.

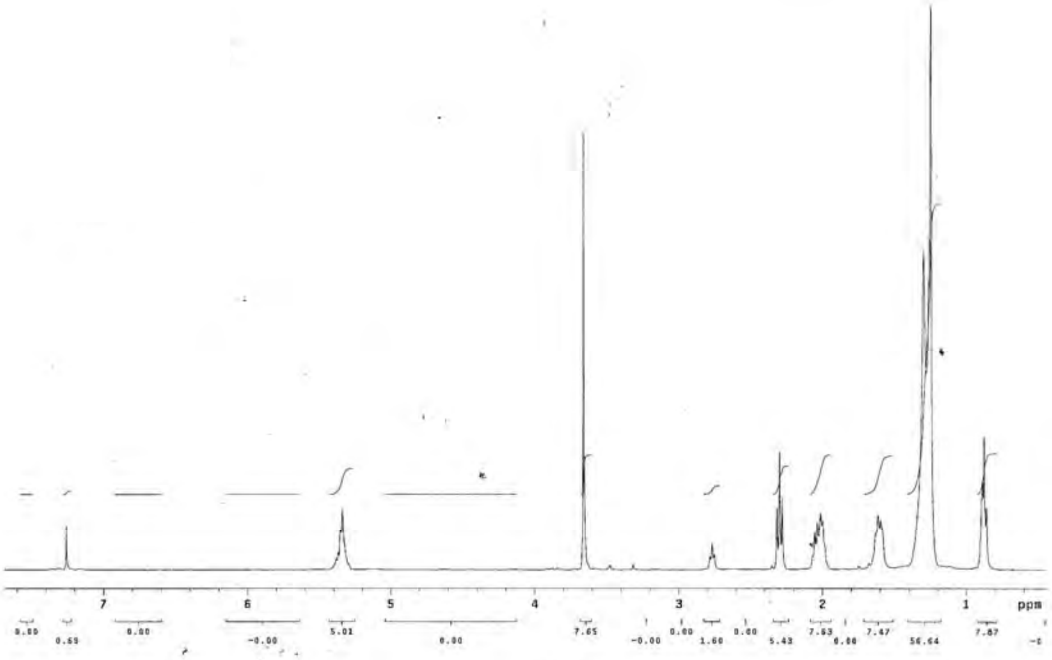


Figure A62 ^{1}H-NMR spectrum of CI oil methyl ester.

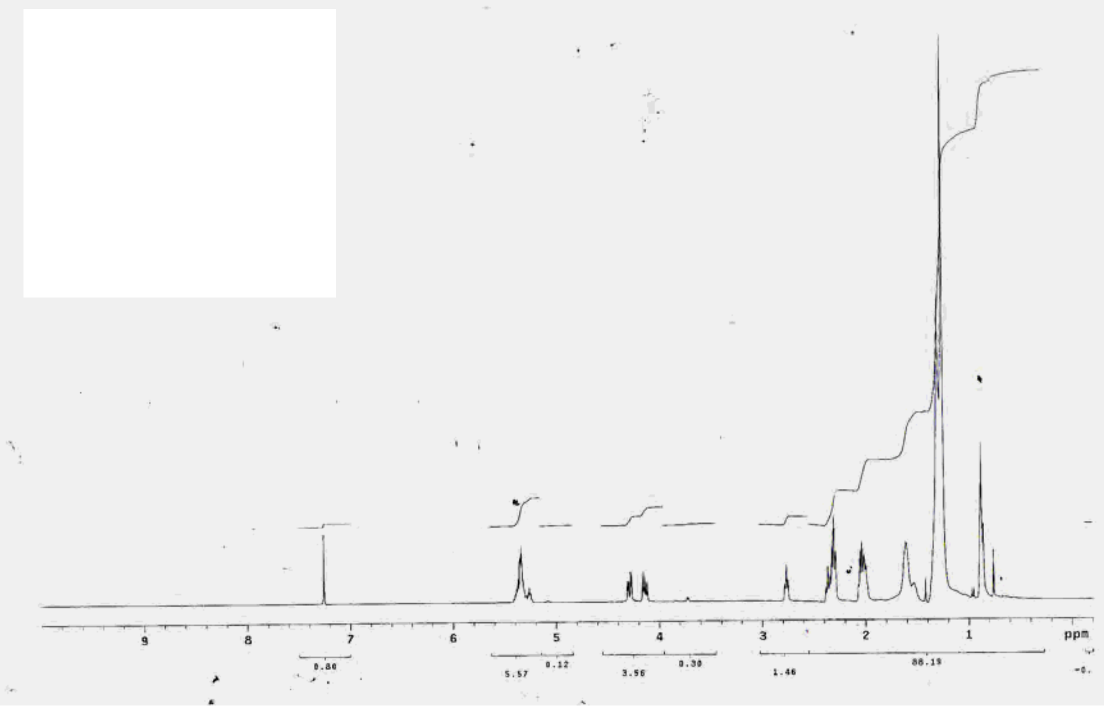


Figure A63 ^{1}H-NMR spectrum of crude CP oil.

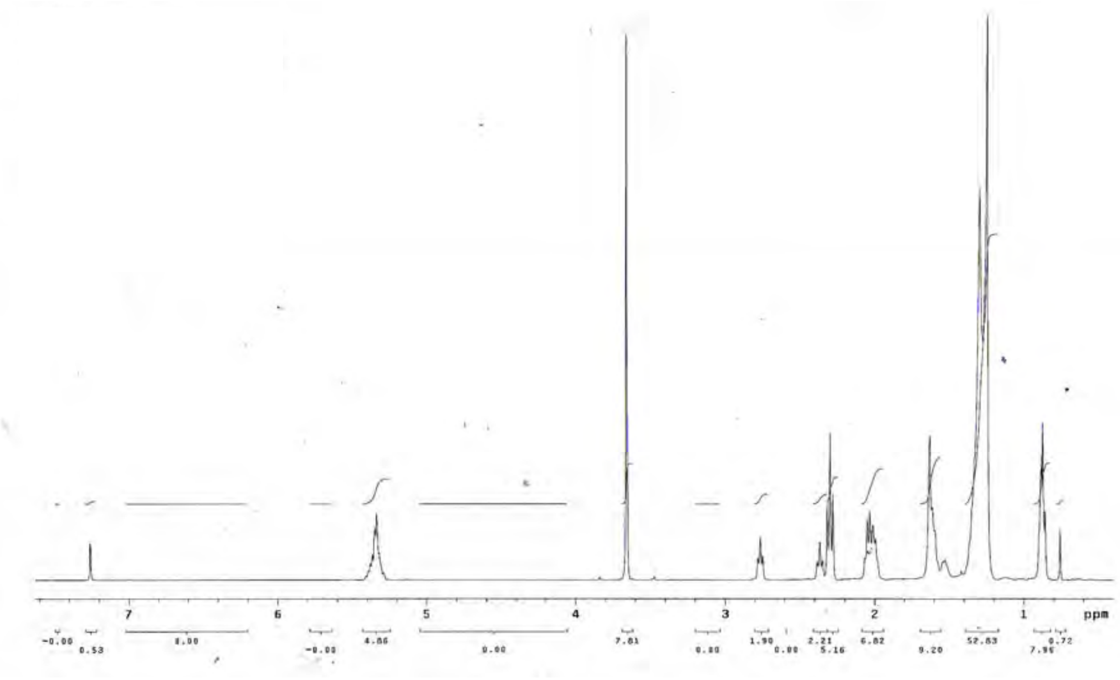


Figure A64 ^{1}H-NMR spectrum of CP oil methyl ester.

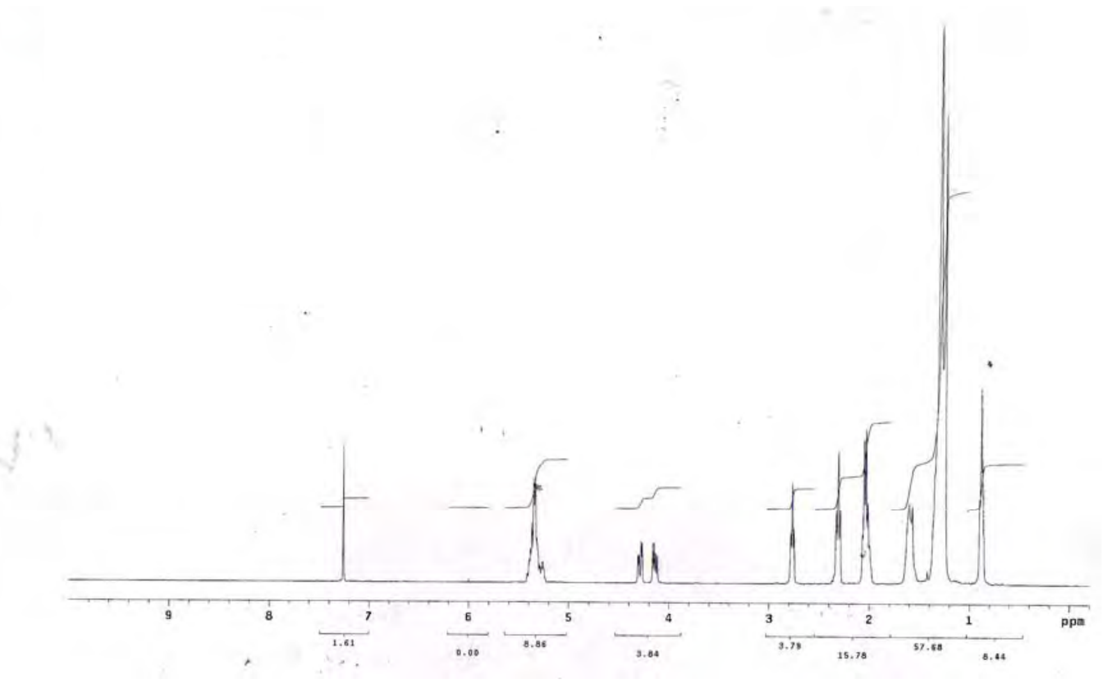


Figure A65 ^{1}H-NMR spectrum of crude CS oil.

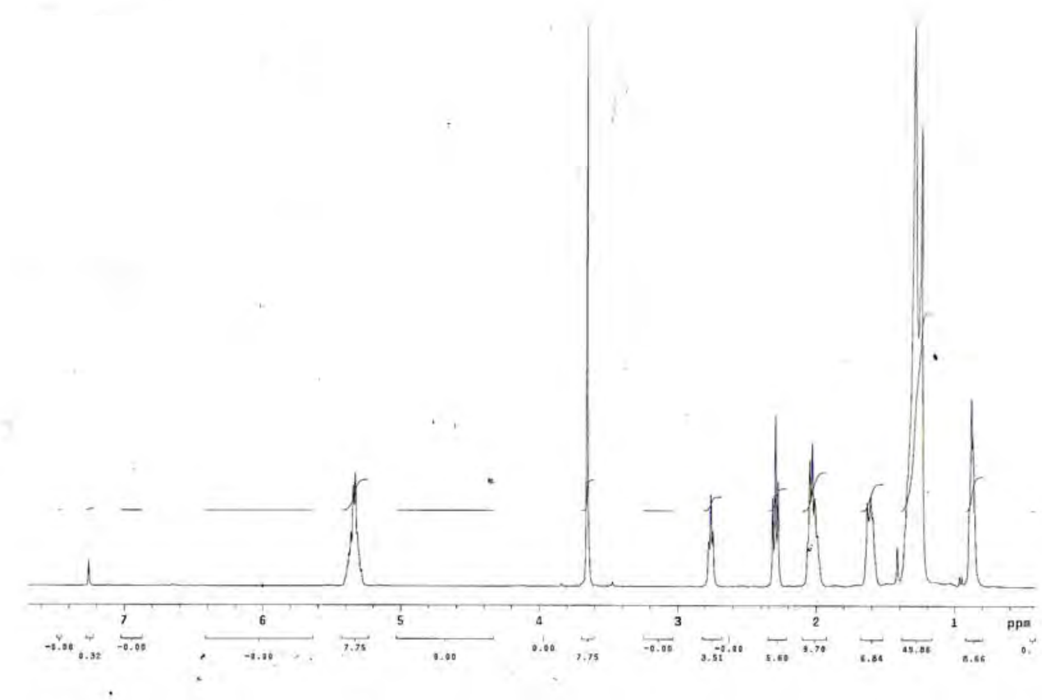


Figure A66 ^{1}H-NMR spectrum of CS oil methyl ester.

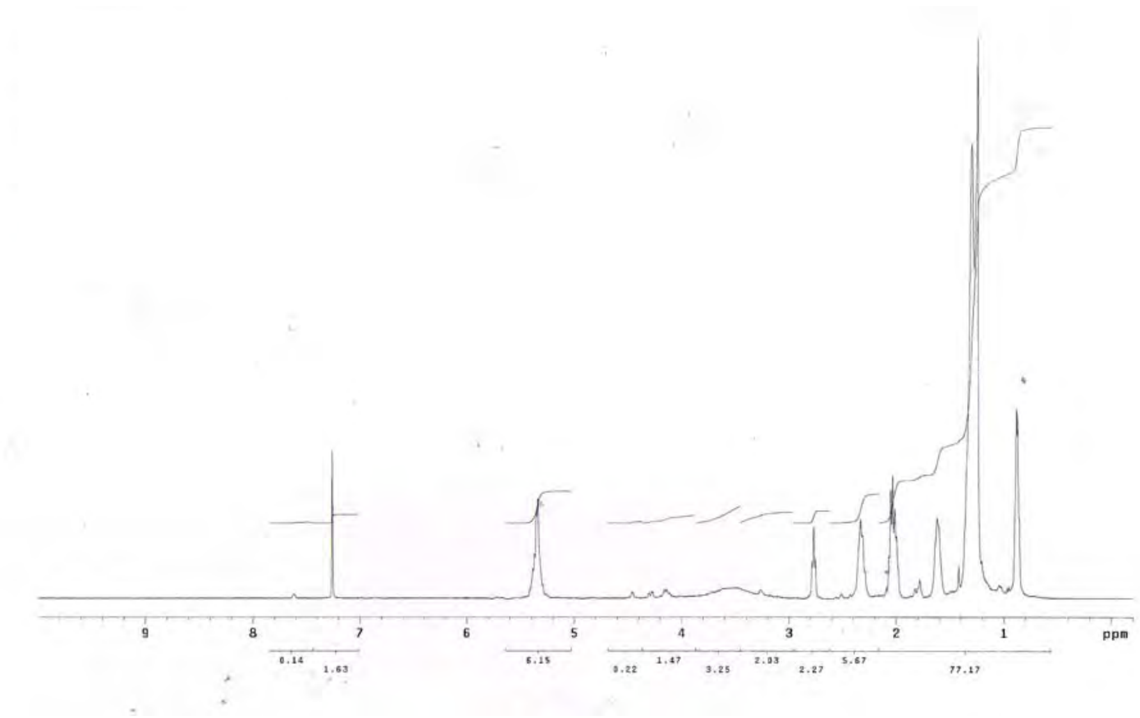


Figure A67 ^{1}H-NMR spectrum of crude CT oil.

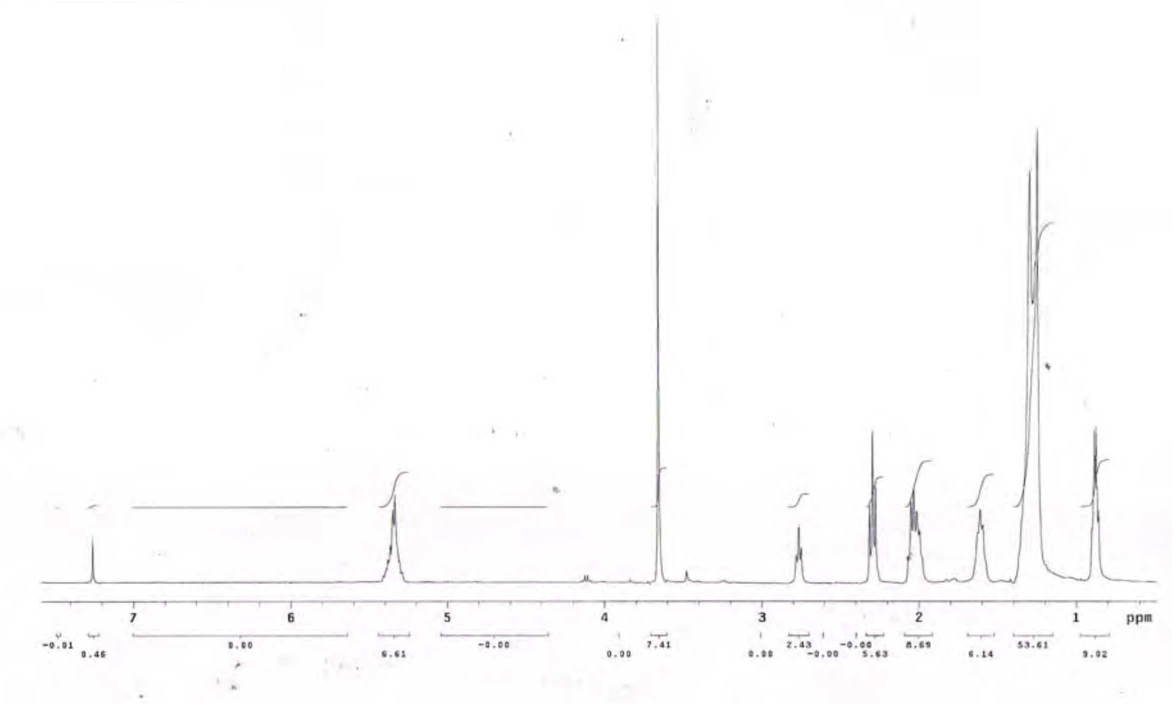


Figure A68 ^{1}H-NMR spectrum of CT oil methyl ester.

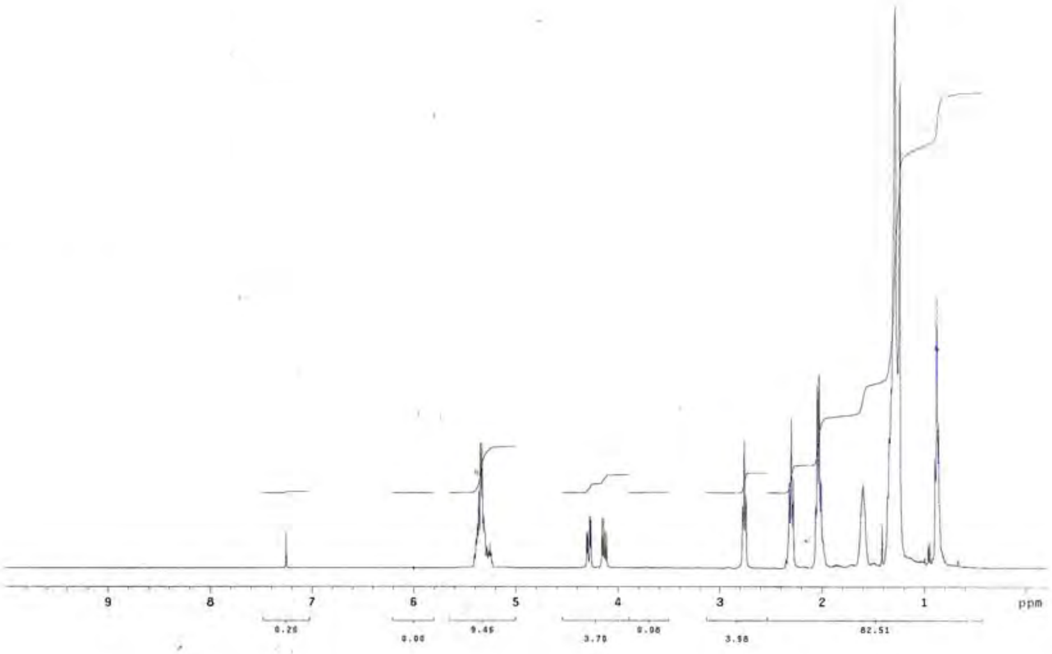


Figure A69 ^{1}H-NMR spectrum of crude EA oil.

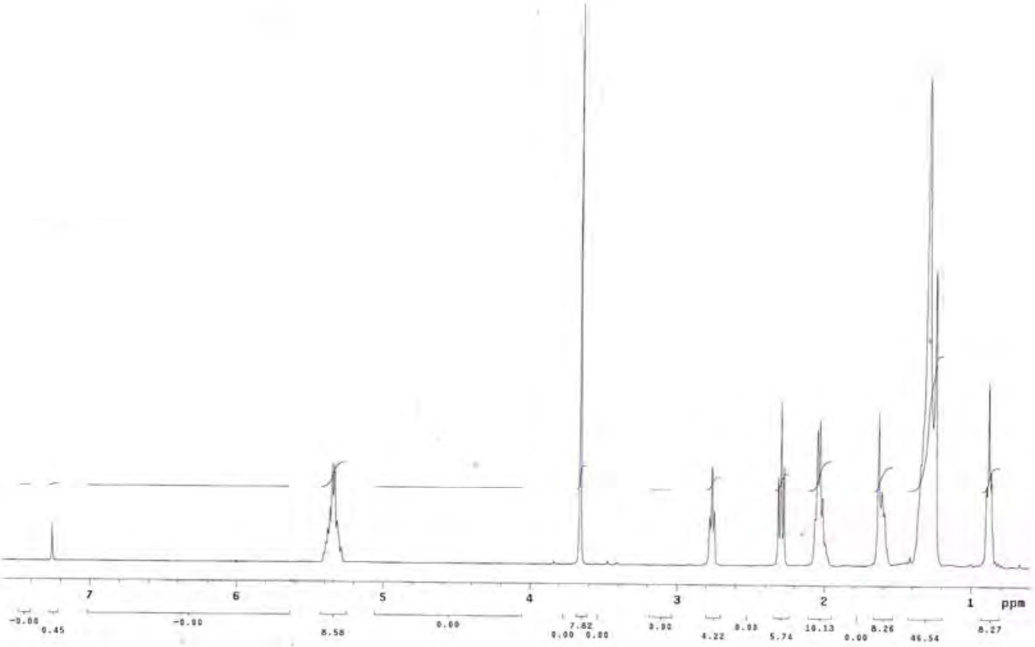


Figure A70 ^{1}H-NMR spectrum of EA oil methyl ester.

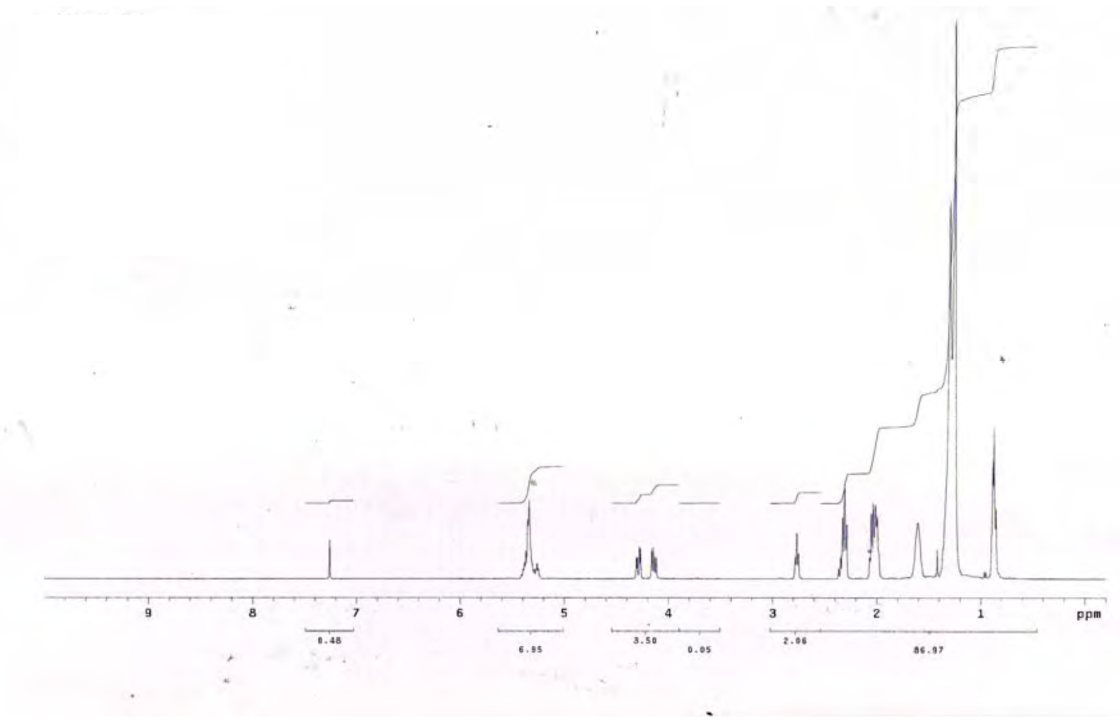


Figure A71 ^{1}H-NMR spectrum of crude GA oil.

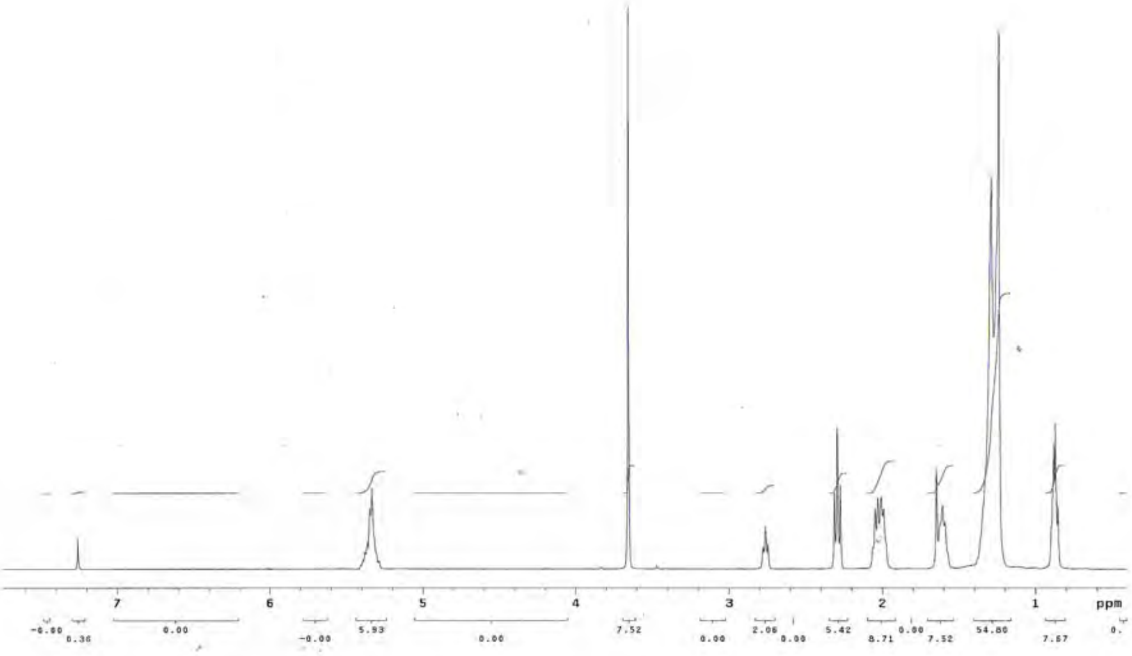


Figure A72 ^{1}H-NMR spectrum of GA oil methyl ester.

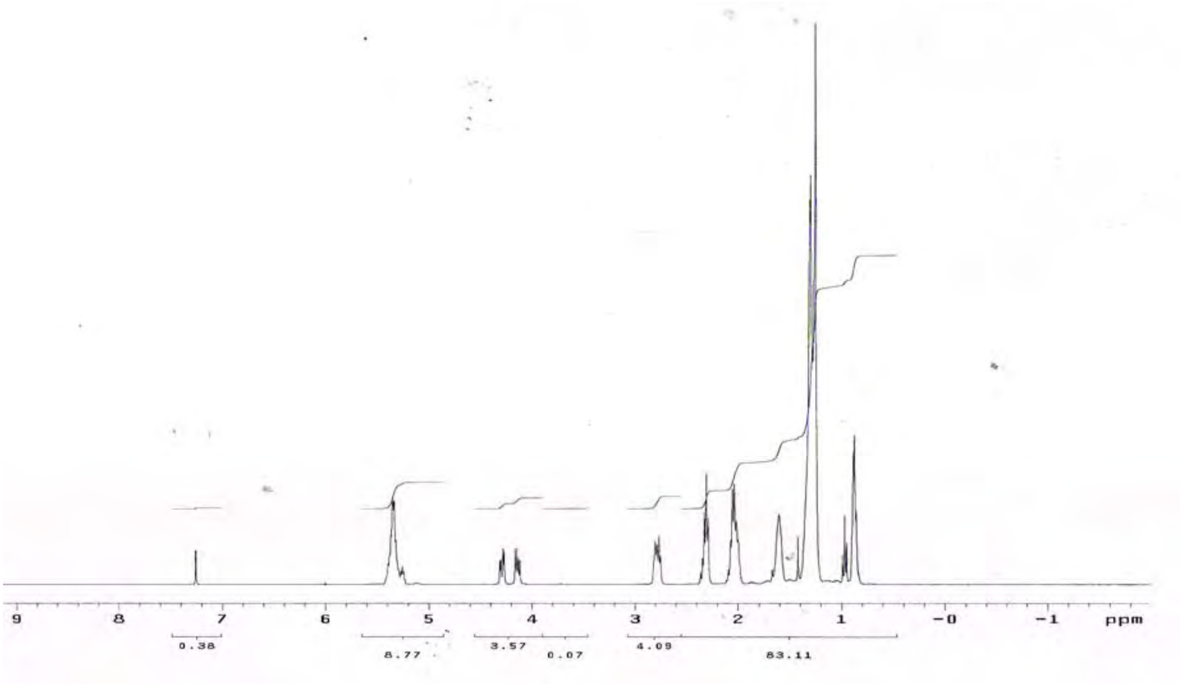


Figure A73 ^{1}H-NMR spectrum of crude HB oil.

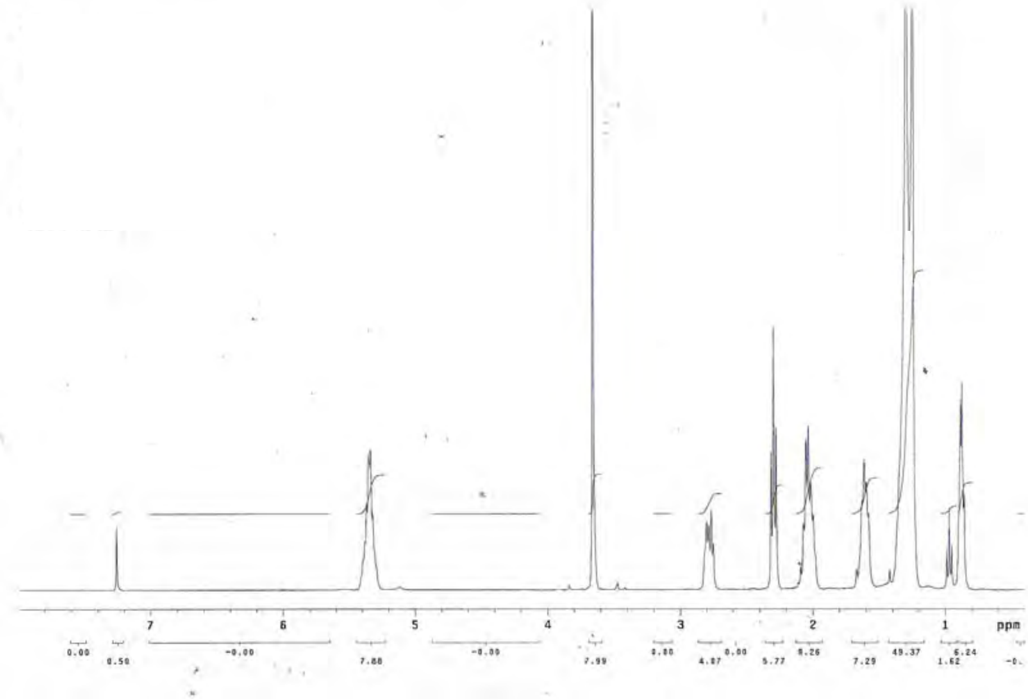


Figure A74 ^{1}H-NMR spectrum of HB oil methyl ester.

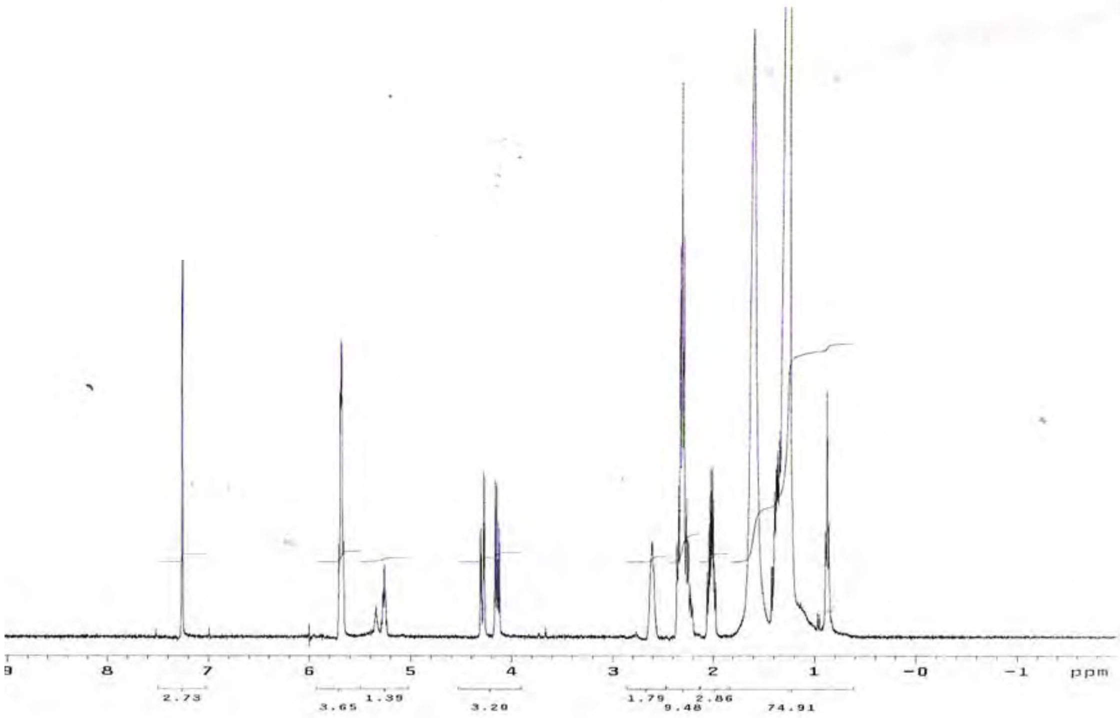


Figure A75 ^{1}H-NMR spectrum of crude HI oil.

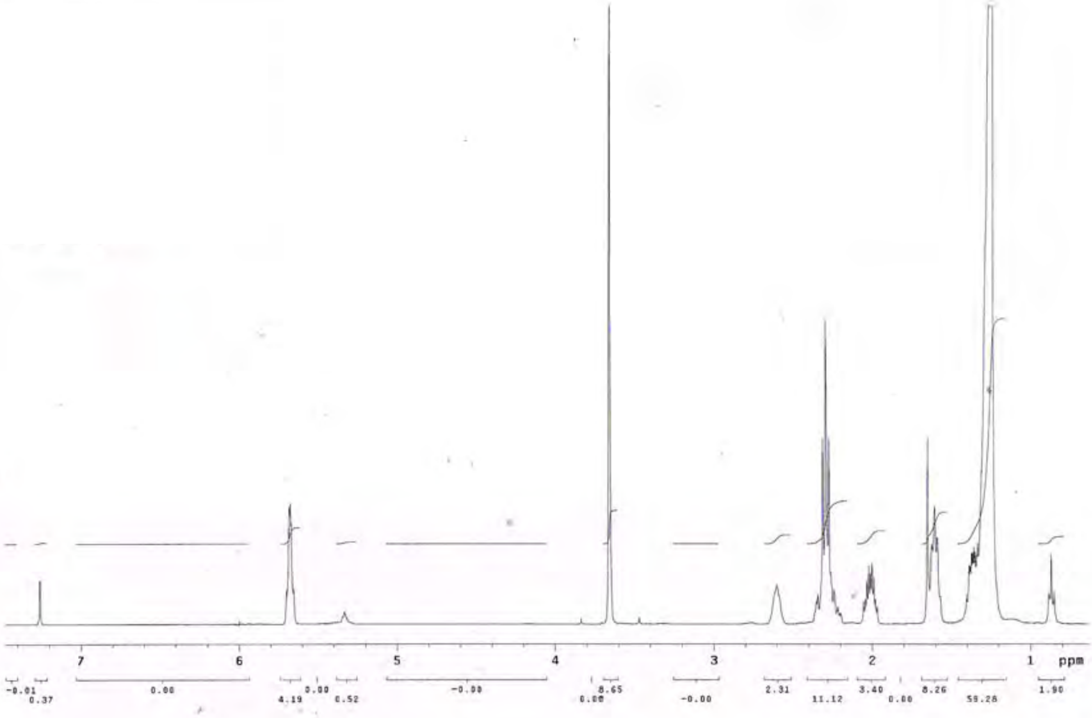


Figure A76 ^{1}H-NMR spectrum of HI oil methyl ester.

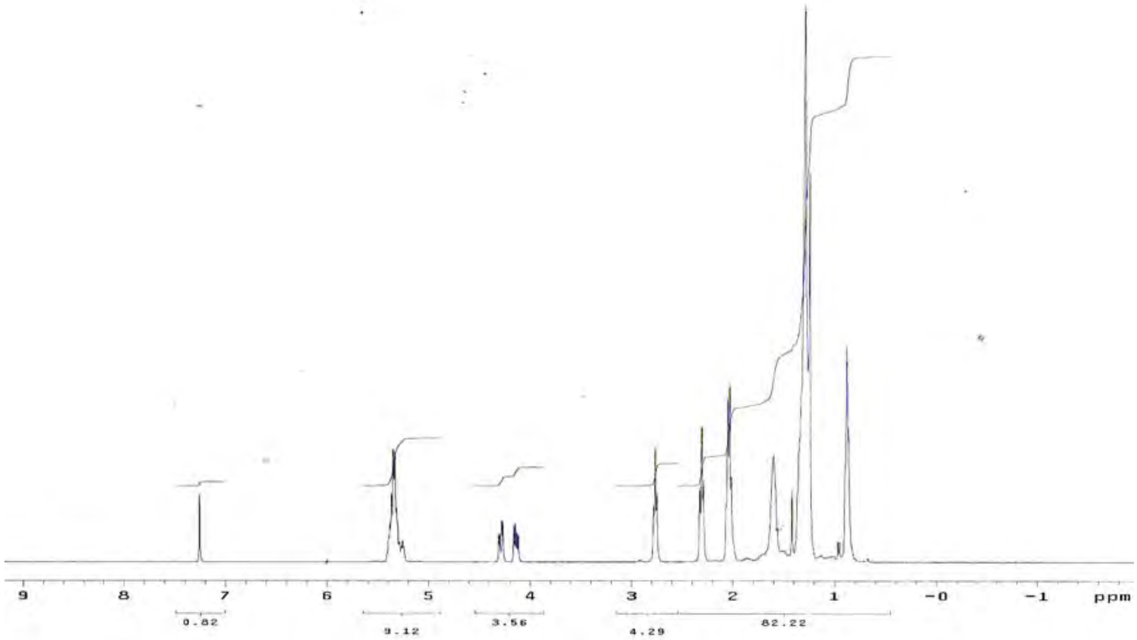


Figure A77 ^{1}H-NMR spectrum of crude HS oil.

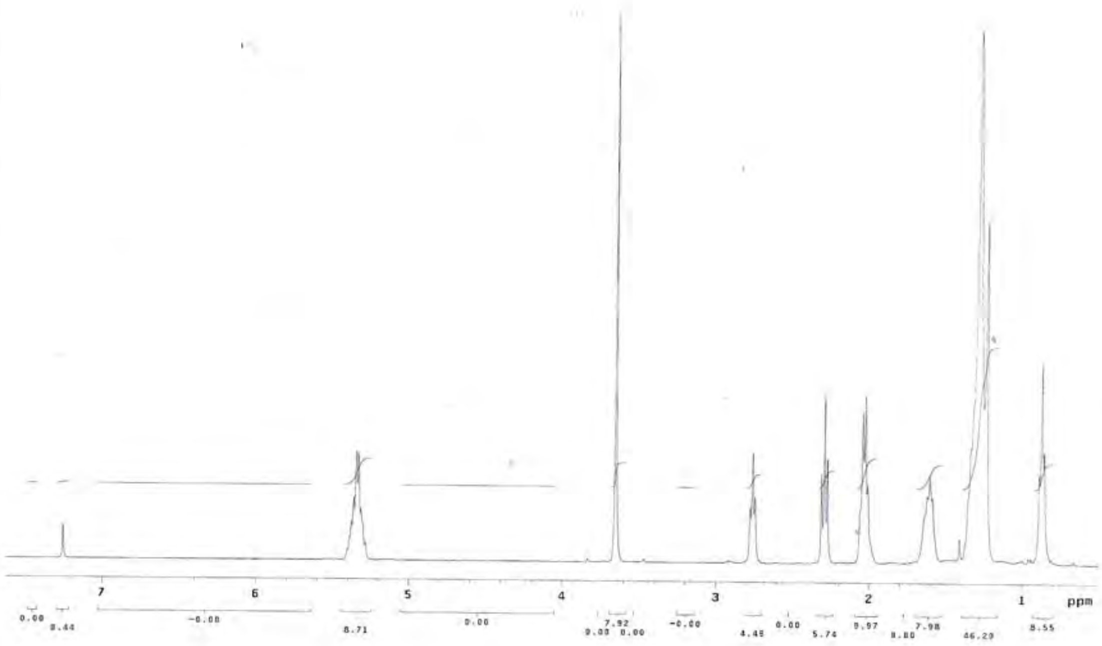


Figure A78 ^{1}H-NMR spectrum of HS oil methyl ester.

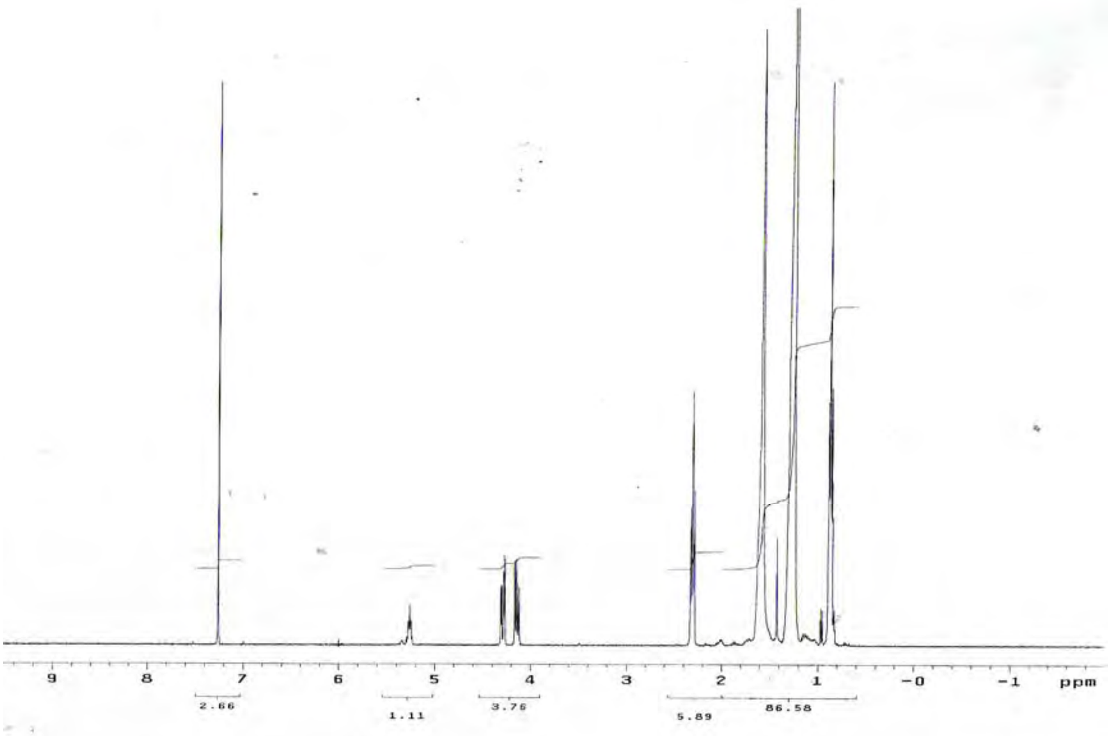


Figure A79 ^{1}H-NMR spectrum of crude IM oil.

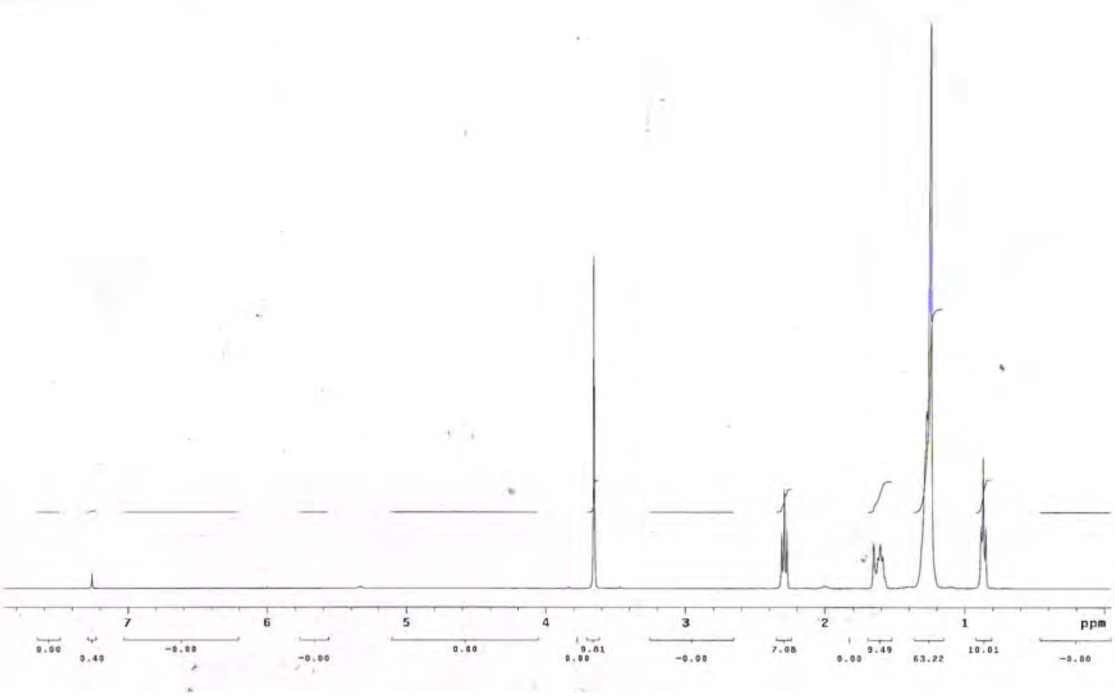


Figure A80 ^{1}H-NMR spectrum of IM oil methyl ester.

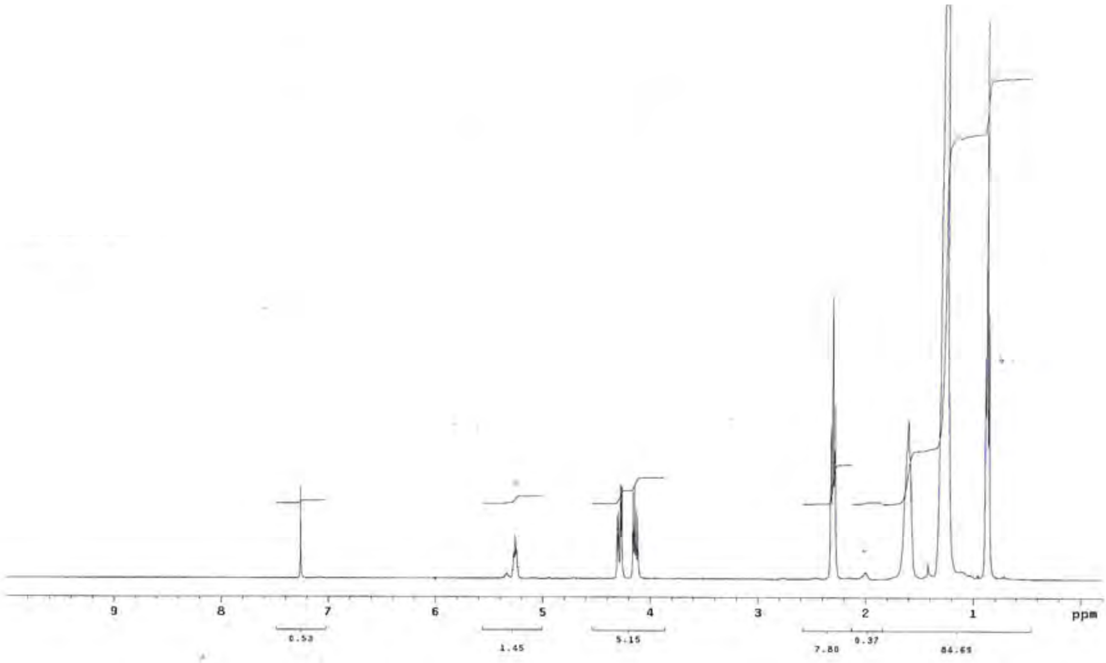


Figure A81 ^{1}H-NMR spectrum of crude LG oil.

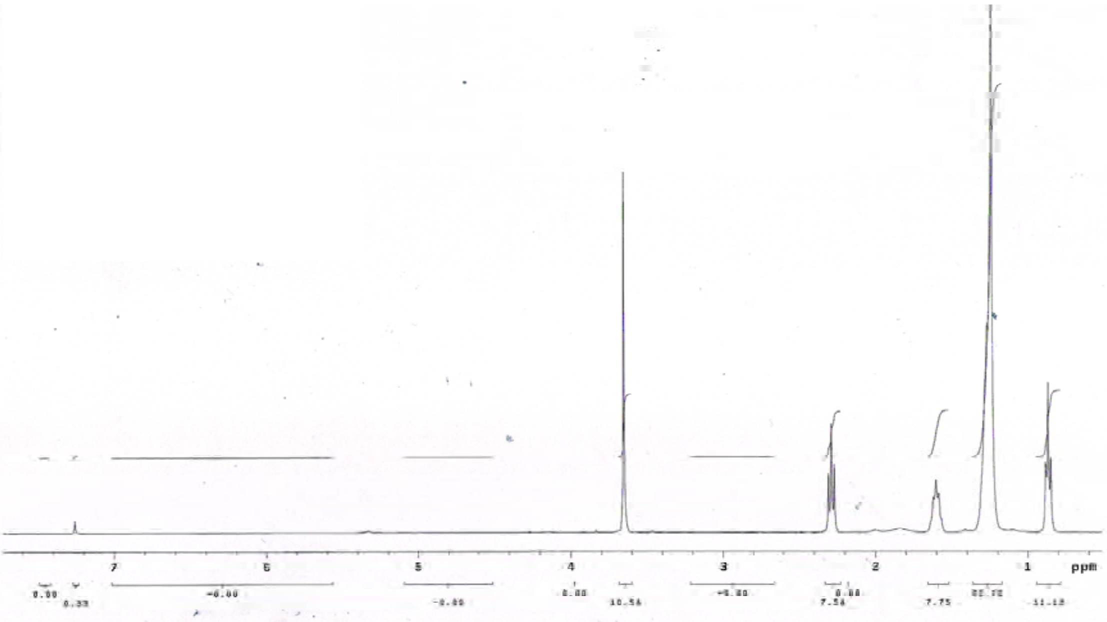


Figure A82 ^{1}H-NMR spectrum of LG oil methyl ester.

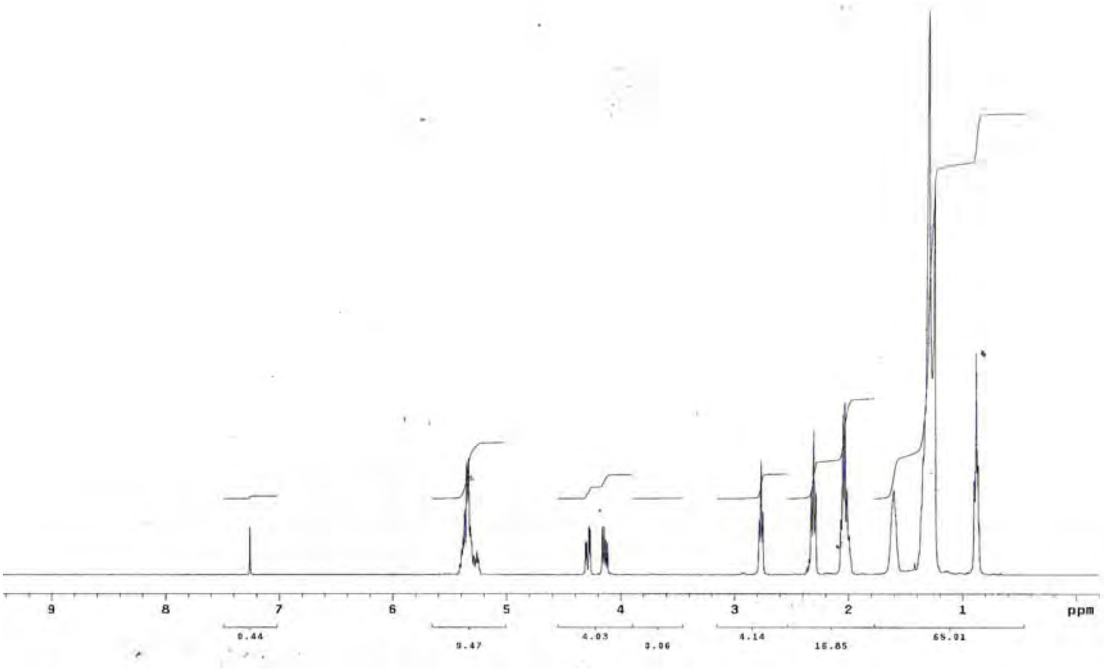


Figure A83 ^{1}H-NMR spectrum of crude MA oil.

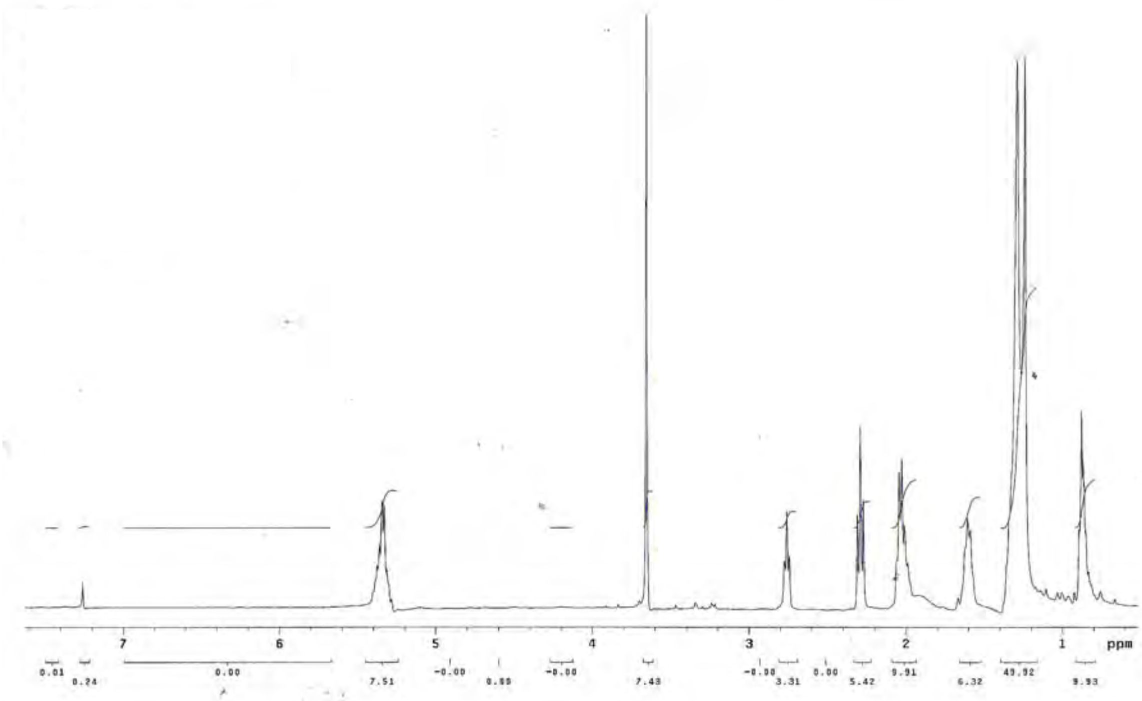


Figure A84 ^{1}H-NMR spectrum of MA oil methyl ester.

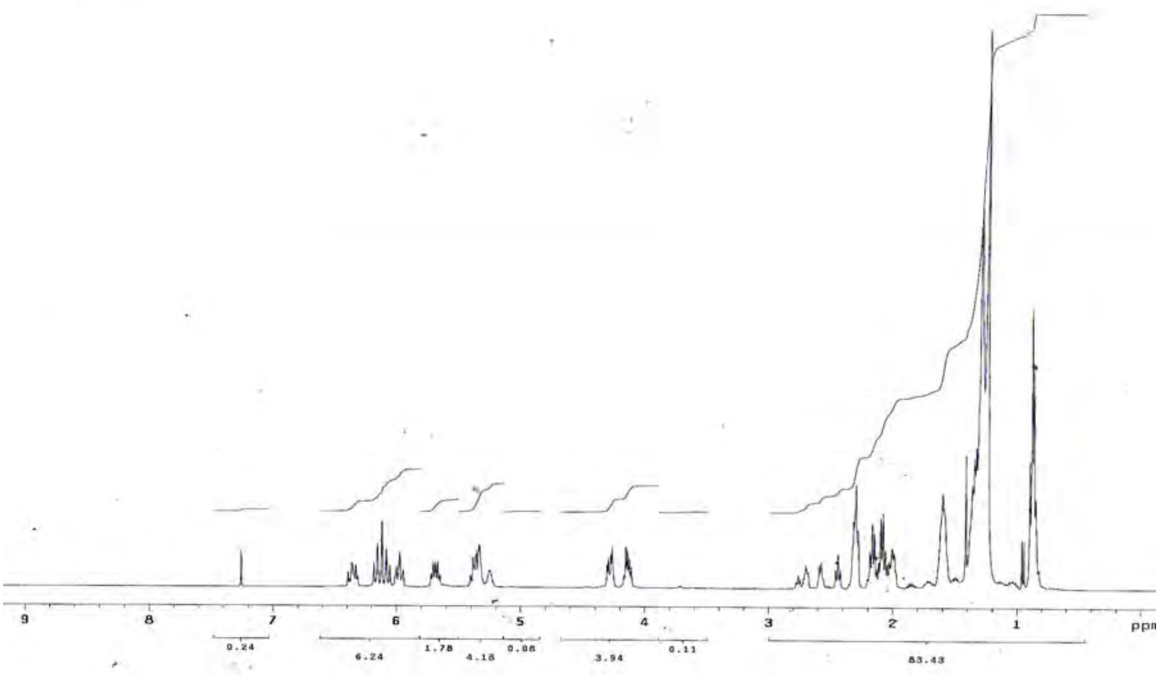


Figure A85 ^{1}H-NMR spectrum of crude PA oil.

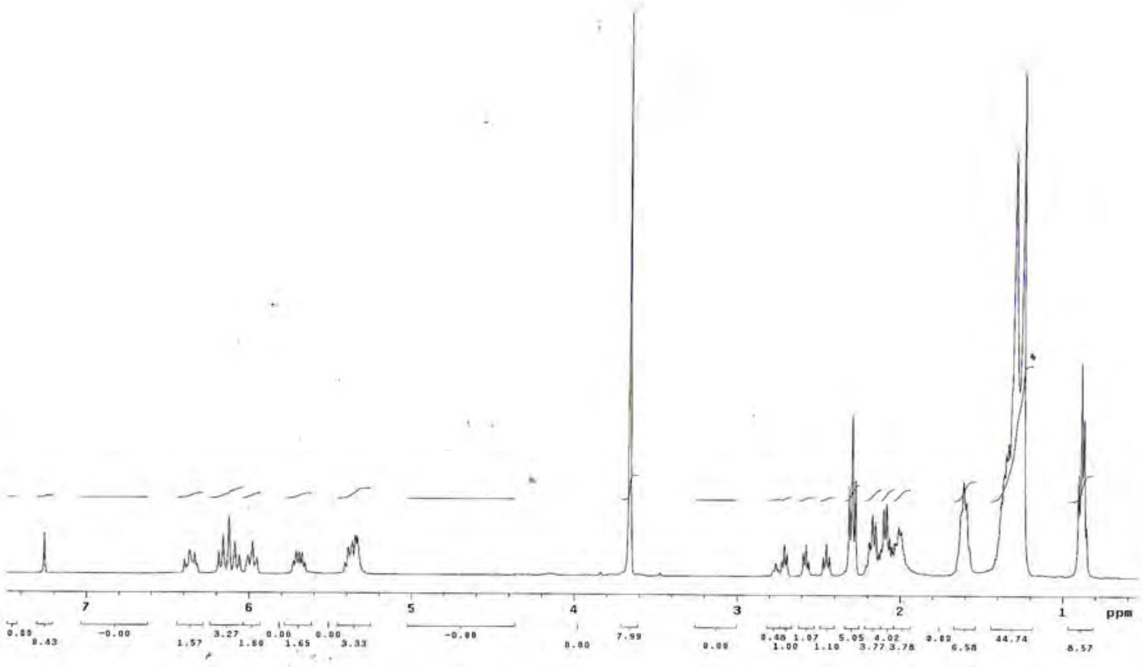


Figure A86 ^{1}H-NMR spectrum of PA oil methyl ester.

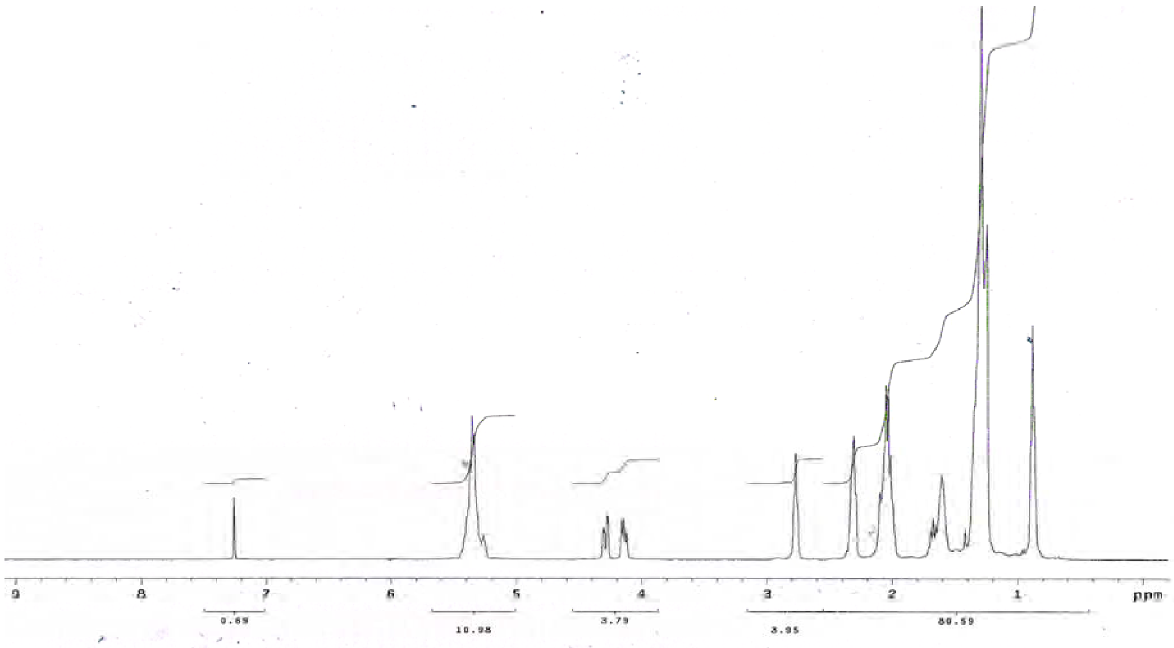


Figure A87 ^{1}H-NMR spectrum of crude PM oil.

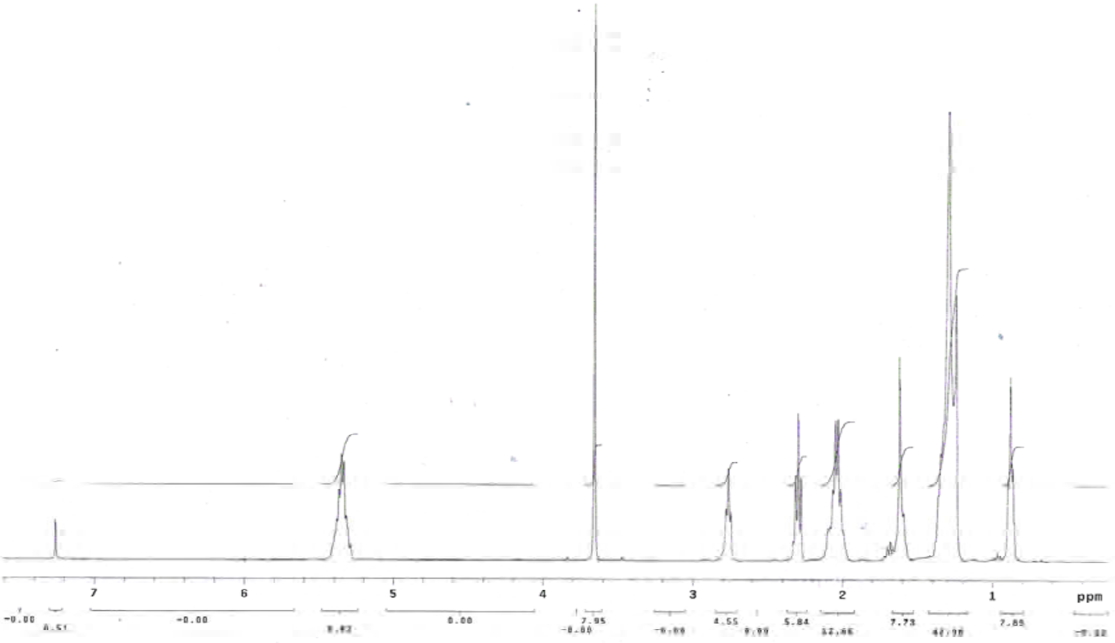


Figure A88 ^{1}H-NMR spectrum of PM oil methyl ester.

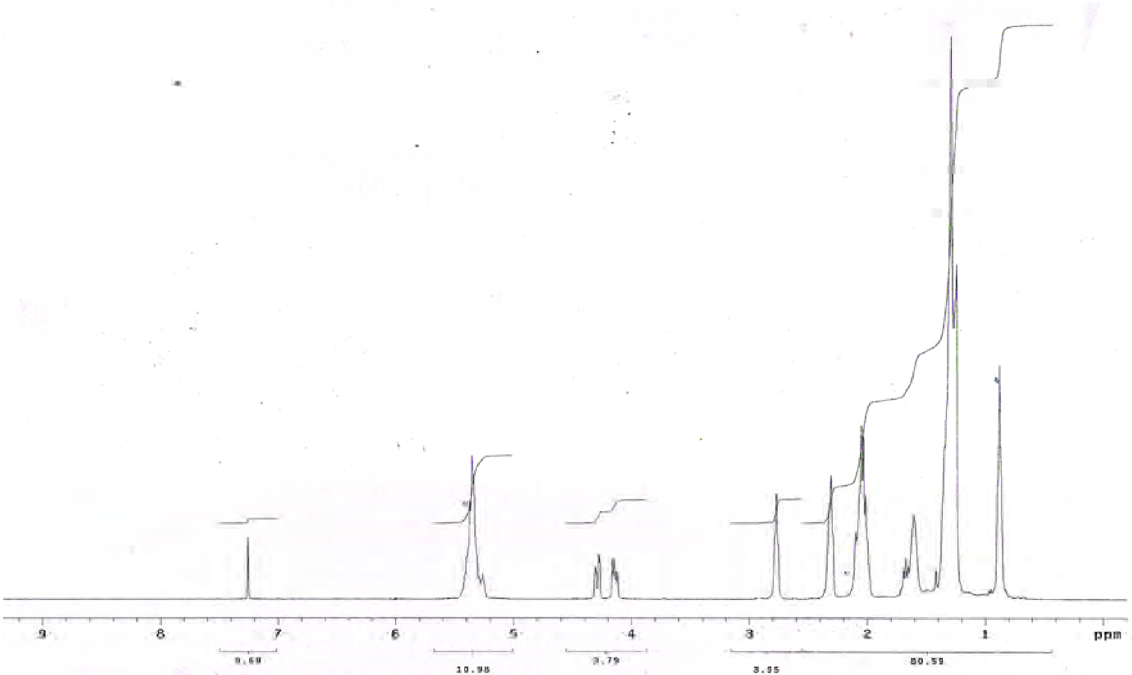


Figure A89 ^{1}H-NMR spectrum of crude PK oil.

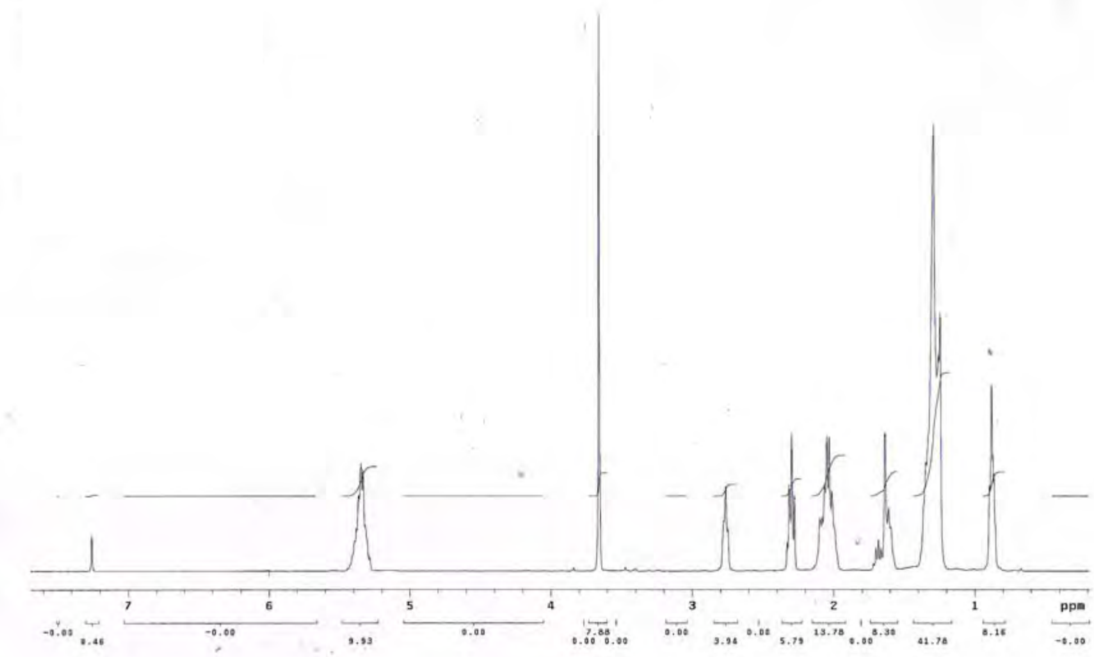


Figure A90 ^{1}H-NMR spectrum of PK oil methyl ester.

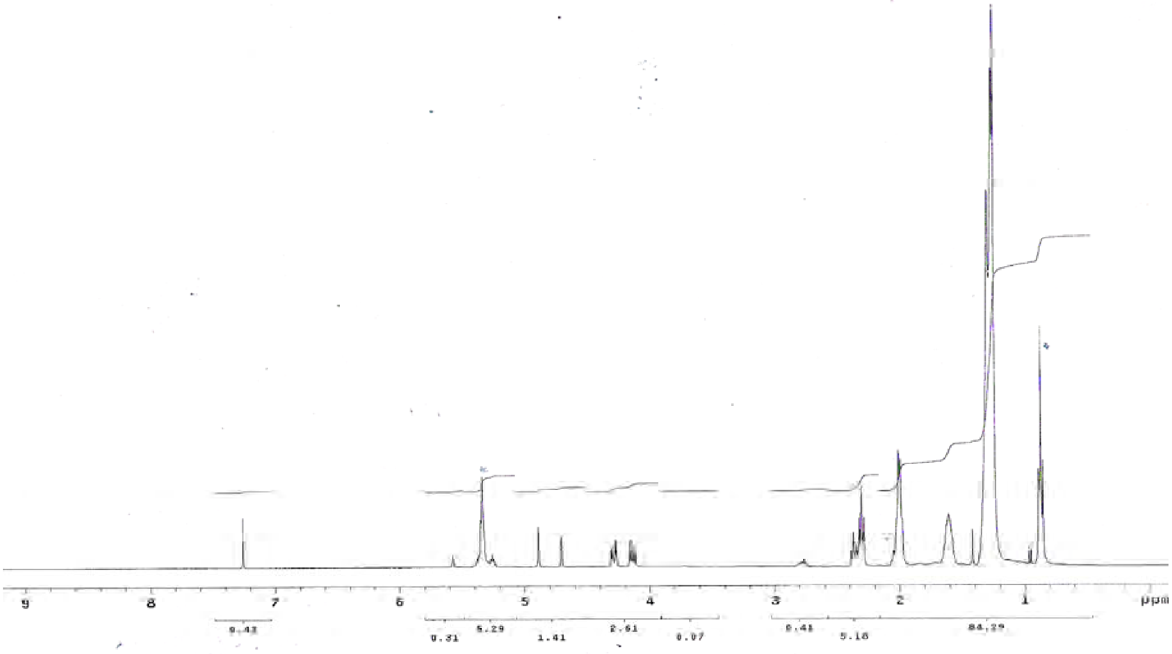


Figure A91 ^{1}H-NMR spectrum of crude SE oil.

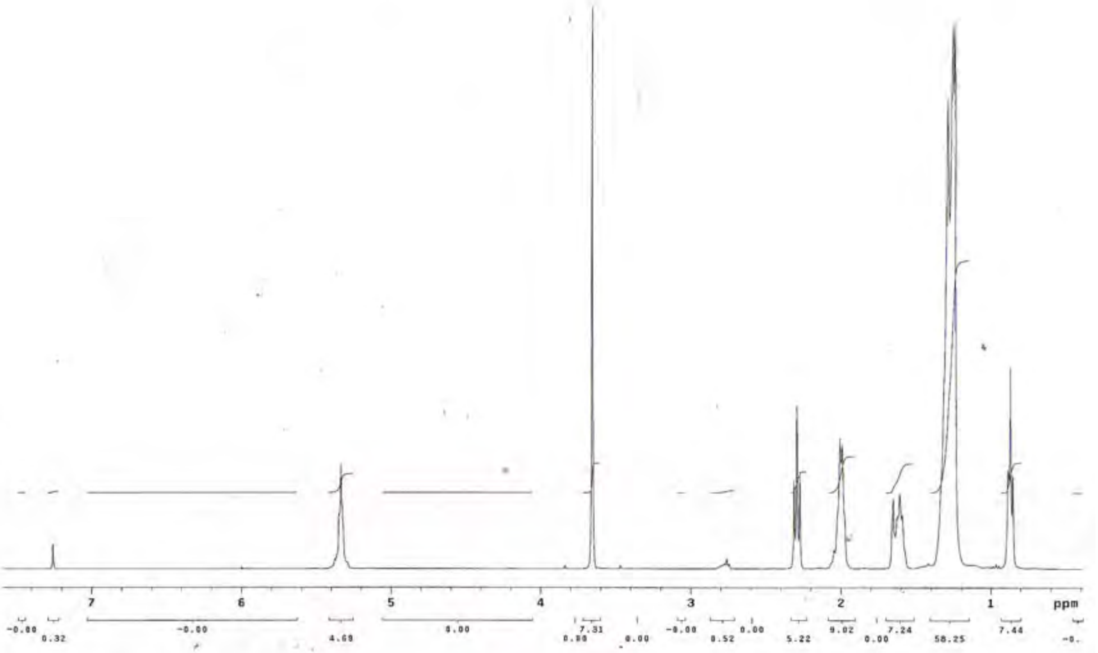


Figure A92 ^{1}H-NMR spectrum of SE oil methyl ester.

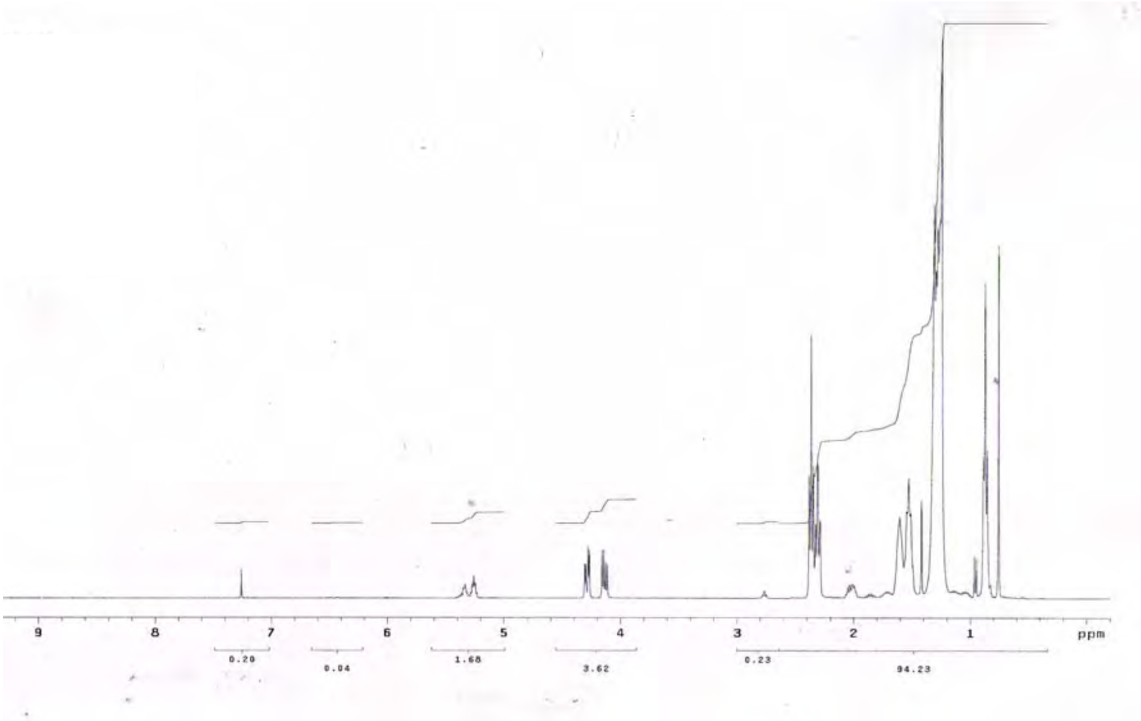


Figure A93 ^{1}H-NMR spectrum of crude SF oil.

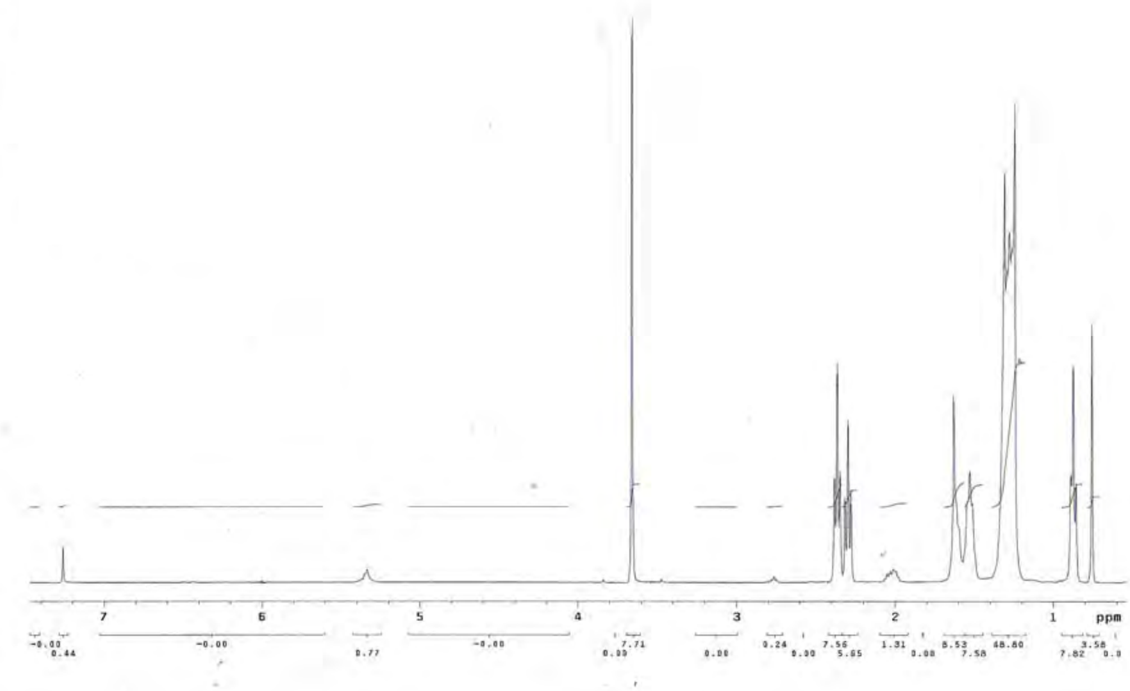


Figure A94 ^{1}H-NMR spectrum of SF oil methyl ester.

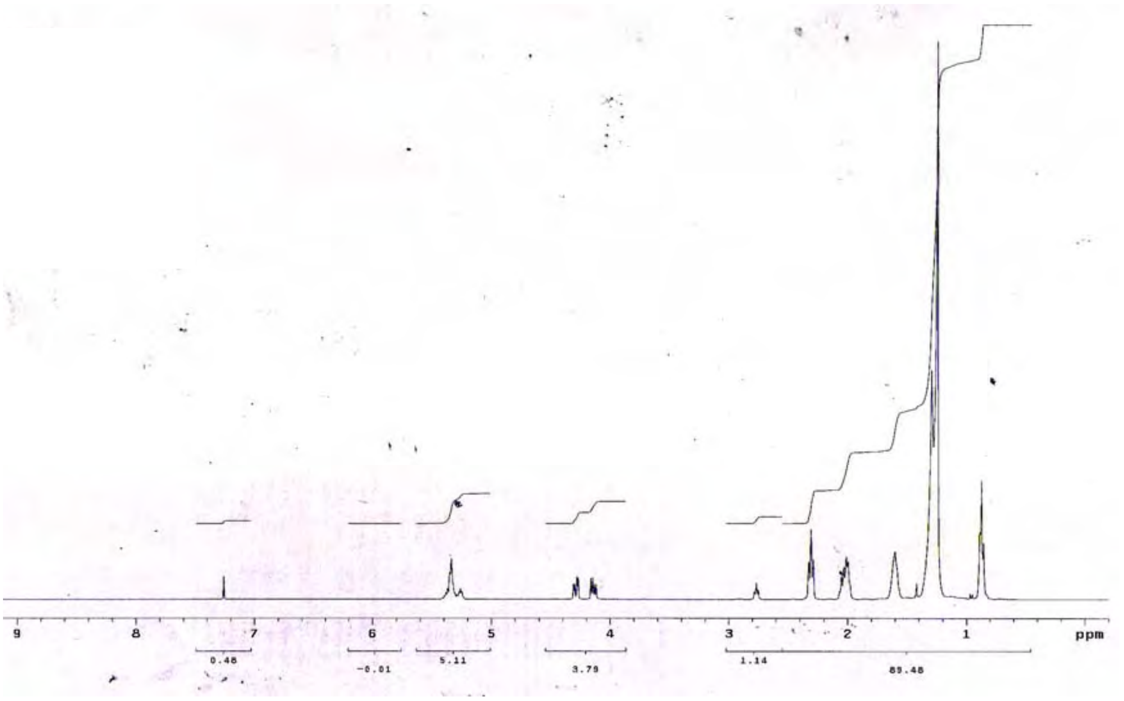


Figure A95 ^{1}H-NMR spectrum of crude TB oil.

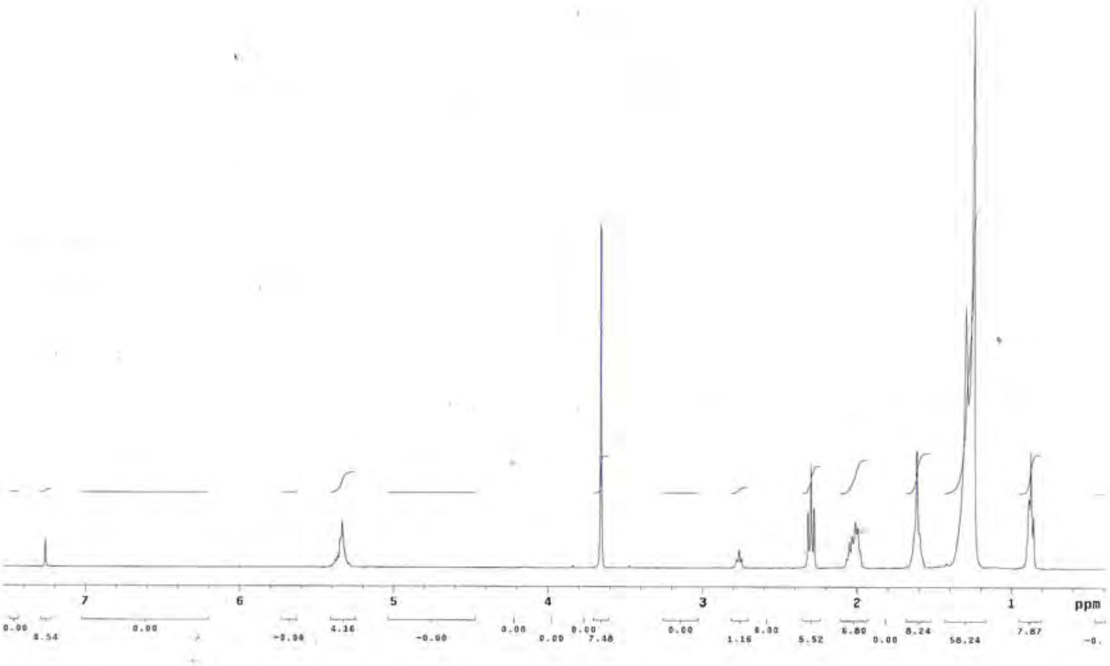


Figure A96 ^{1}H-NMR spectrum of TB oil methyl ester.

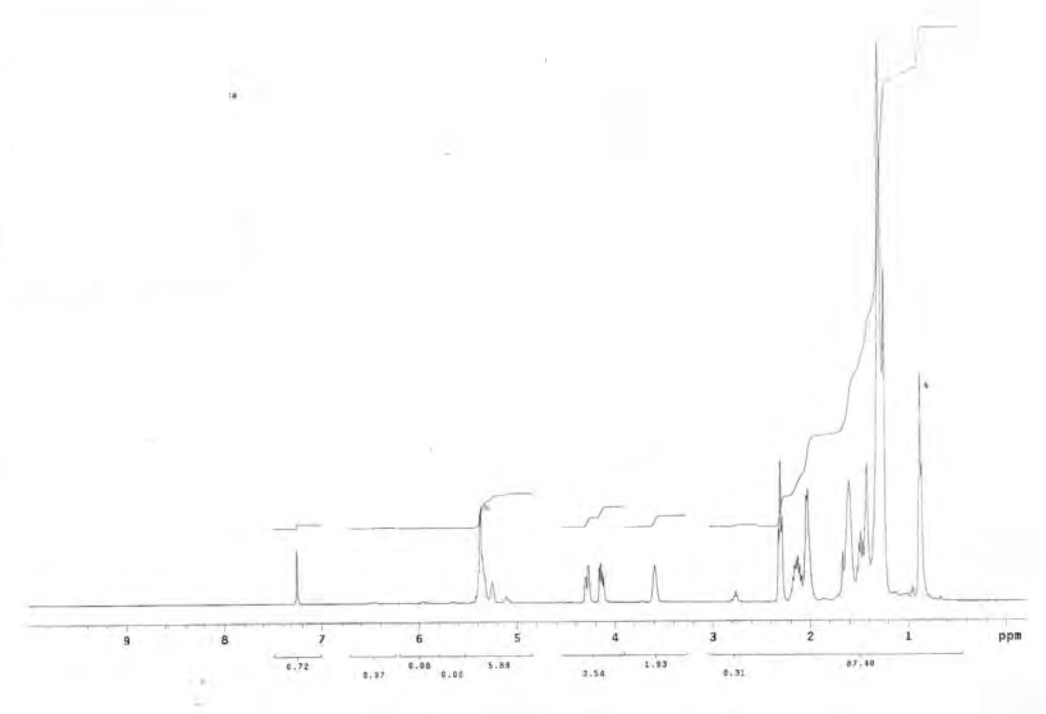


Figure A97 ^{1}H-NMR spectrum of crude WT oil.

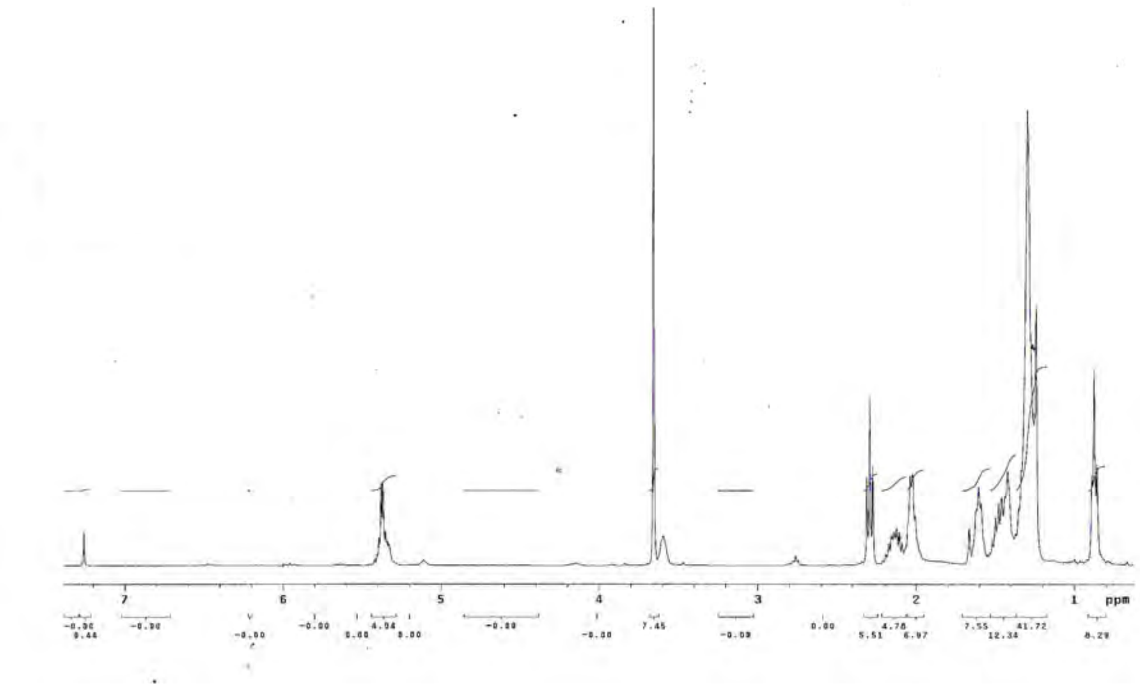


Figure A98 ^{1}H-NMR spectrum of WT oil methyl ester.

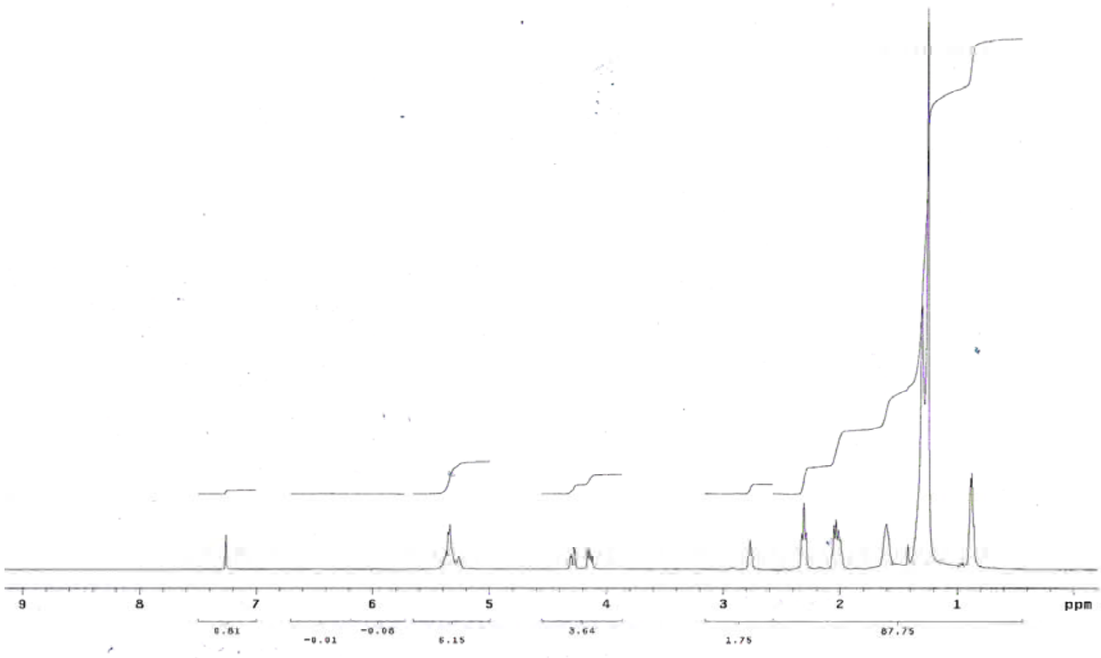


Figure A99 ^{1}H-NMR spectrum of crude XK oil.

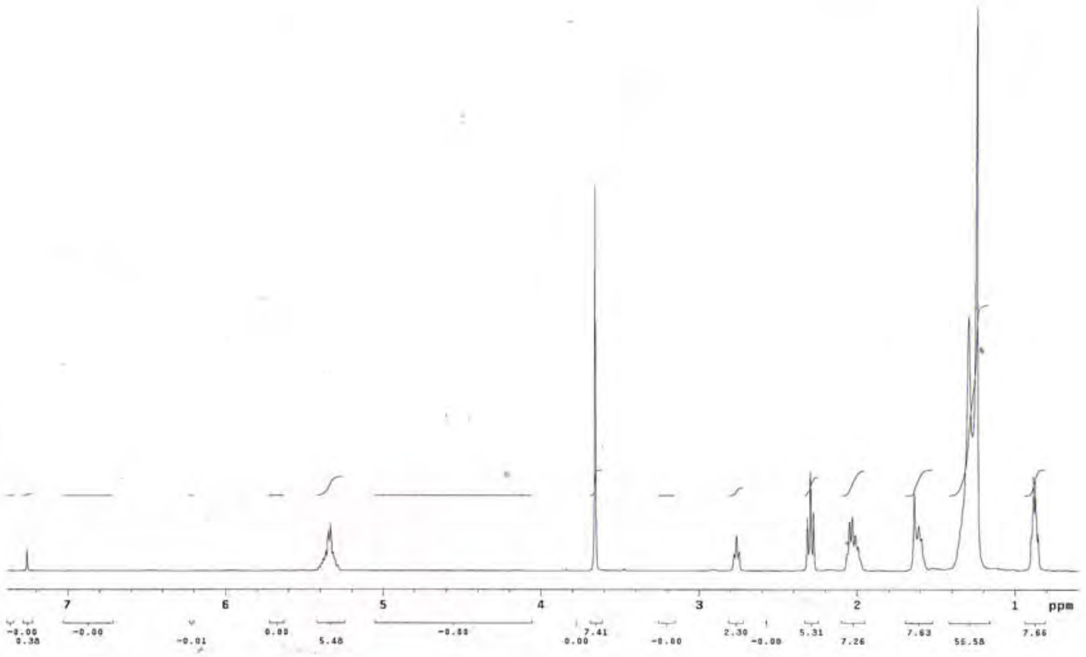
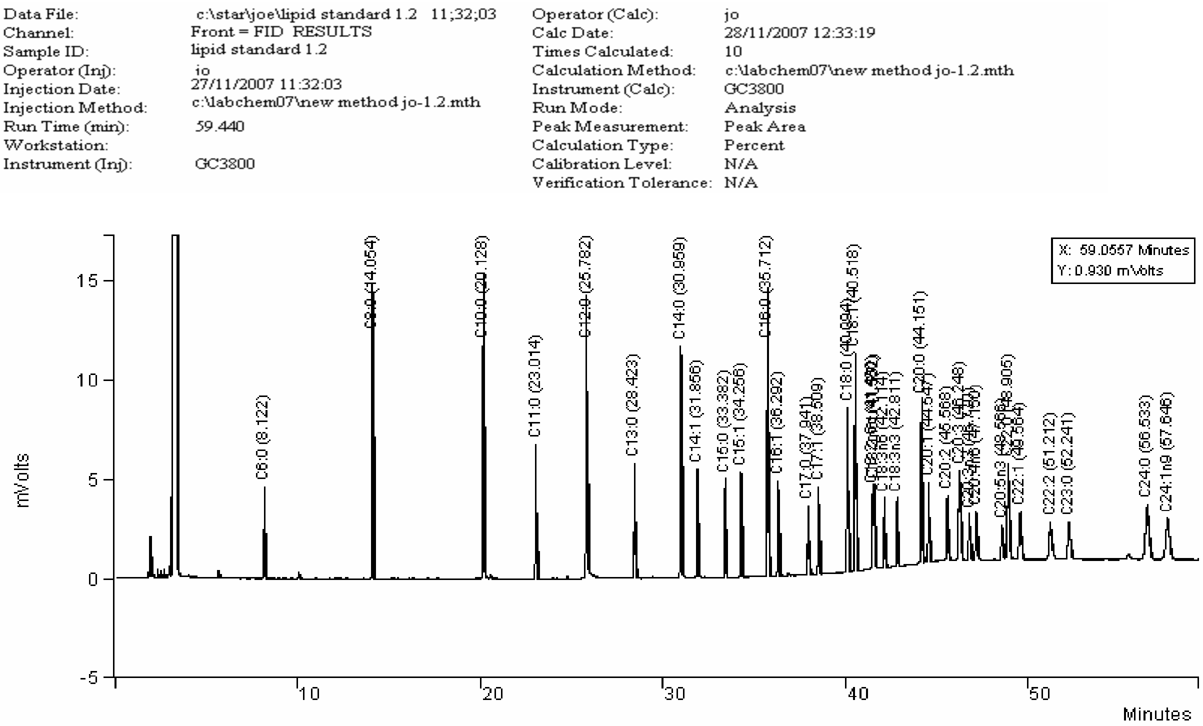


Figure A100 ^{1}H-NMR spectrum of XK oil methyl ester.



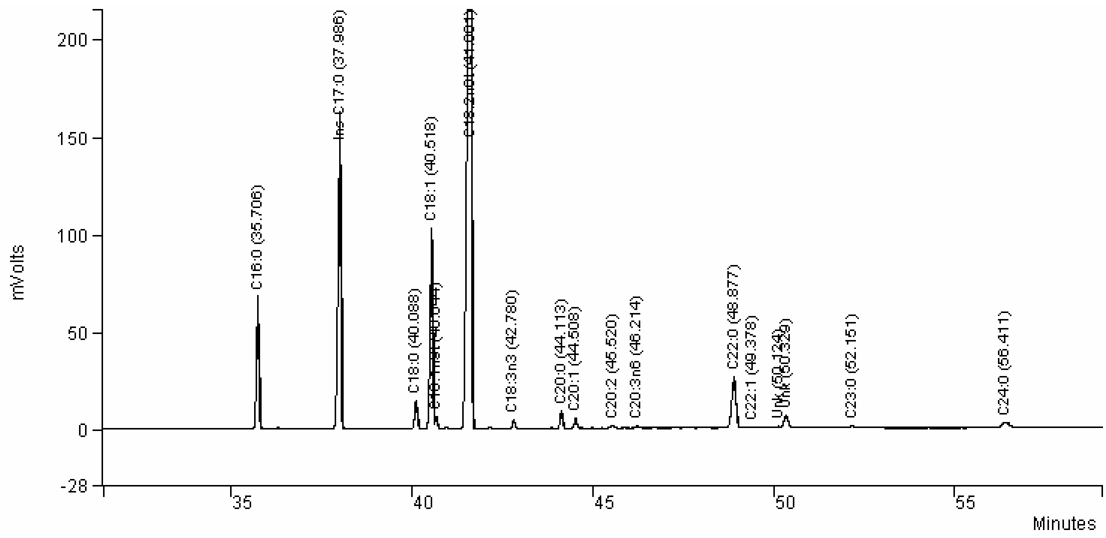
Data File: c:\star\joe\lipid standard 1.2 11;32;03
Channel: Front = FID RESULTS
Sample ID: lipid standard 1.2
Operator (Inj): io
Injection Date: 27/11/2007 11:32:03
Injection Method: c:\labchem07\new method jo-1.2.mth
Run Time (min): 59.440
Workstation:
Instrument (Inj): GC3800

Operator (Calc): jo
Calc Date: 28/11/2007 12:33:19
Times Calculated: 10
Calculation Method: c:\labchem07\new method jo-1.2.mth
Instrument (Calc): GC3800
Run Mode: Analysis
Peak Measurement: Peak Area
Calculation Type: Percent
Calibration Level: N/A
Verification Tolerance: N/A

Identification (Peak Name)	Retention time (min)
C6:0 (Caproic)	8.122
C8:0 (Caprylic)	14.054
C10:0 (Capric)	20.128
C11:0 (Undecanoic)	23.014
C12:0 (Lauric)	25.782
C13:0 (Tridecanoic)	28.423
C14:0 (Myristic)	30.959
C14:1 (Myristoleic)	31.856
C15:0 (Pentadecanoic)	33.382
C15:1 (cis-10-Pentadecenoic)	34.256
C16:0 (Palmitic)	35.712
C16:1 (Palmitoleic)	36.292
C17:0 (Heptadecanoic) (Internal standard)	37.941
C17:1 (cis-10-Heptadecenoic)	38.509
C18:0 (Stearic)	40.094
C18:1n9c (Oleic)	40.518
C18:1n9t (Elaidic)	40.612
C18:2n6c (Linoleic)	41.480
C18:2n6t (Linolelaidic)	41.552
C18:3n6 (γ-Linolenic)	42.114
C18:3n3 (α-Linolenic)	42.811
C20:0 (Arachidic)	44.151
C20:1n9 (cis-11-Eicosenoic)	44.547
C20:2 (cis-11,14-Eicosadienoic)	45.568
C20:3n6 (cis-8,11,14-Eicosatrienoic)	46.248
C20:3n3 (cis-11,14,17-Eicosatrienoic)	46.770
C20:4n6 (Arachidonic)	47.150
C20:5n3 (cis-5,8,11,14,17-Eicosapentaenoic)	48.566
C22:0 (Behenic)	48.905
C22:1n9 (Erucic)	49.564
C22:2 (cis-13,16-Docosadienoic)	51.212
C23:0 (Tricosanoic)	52.241
C24:0 (Lignoceric)	56.533
C24:1n9 (Nervonic)	57.646

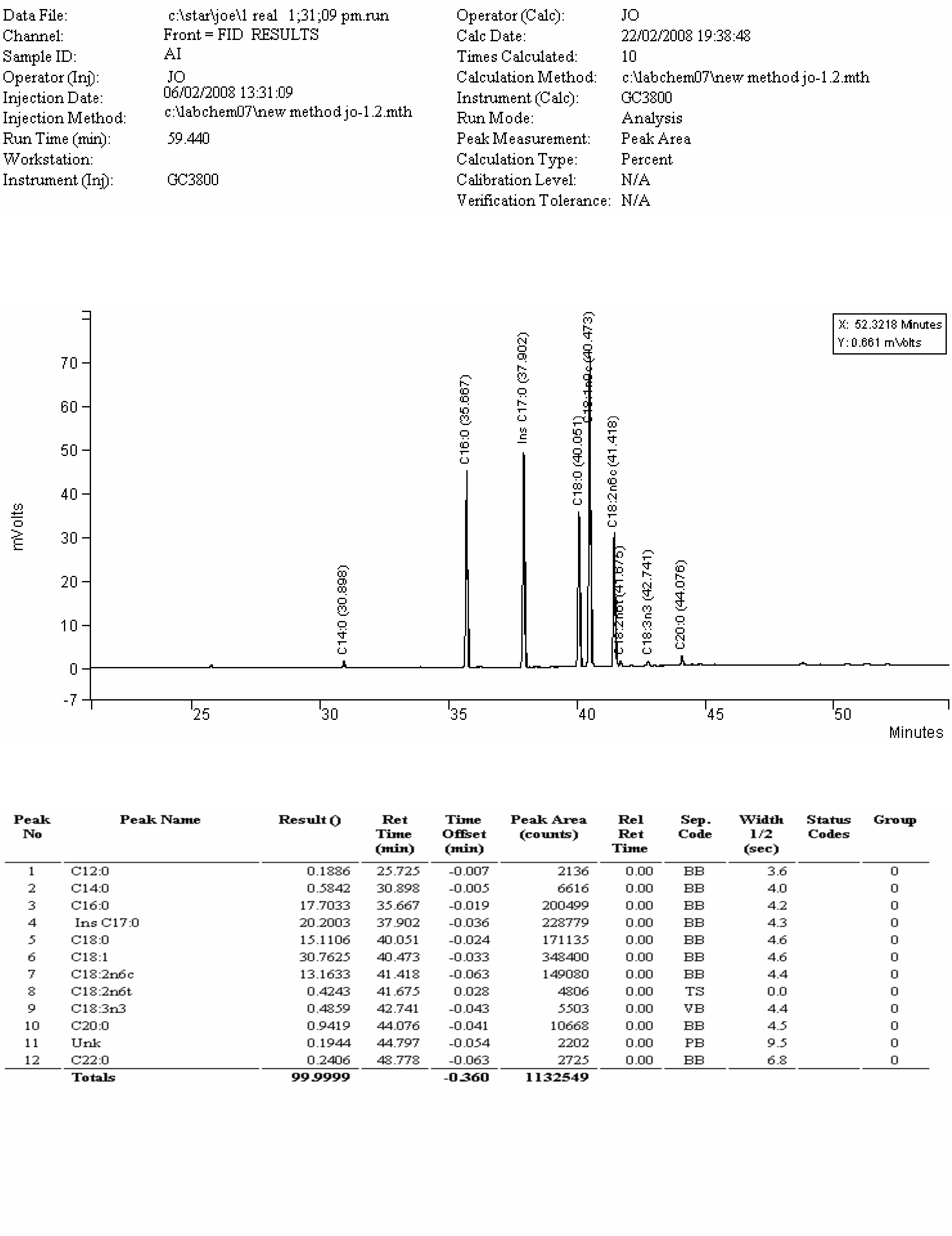
Figure A101 GC chromatogram of 37 FAMEs standard.

Data File:	c:\star\joe\me(aa) 8;28;48 pm.run	Operator (Calc):	jo
Channel:	Front = FID RESULTS	Calc Date:	24/02/2008 01:16:34
Sample ID:	ME(AA)	Times Calculated:	5
Operator (Inj):	jo	Calculation Method:	c:\labchem07\new method jo-1.2.mth
Injection Date:	28/12/2007 20:28:48	Instrument (Calc):	GC3800
Injection Method:	c:\labchem07\new method jo-1.2.mth	Run Mode:	Analysis
Run Time (min):	59.440	Peak Measurement:	Peak Area
Workstation:		Calculation Type:	Percent
Instrument (Inj):	GC3800	Calibration Level:	N/A
		Verification Tolerance:	N/A



Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C16:0	5.8736	35.706	0.020	311179	0.00	BB	4.3		0
2	Ins C17:0	16.3650	37.986	0.048	867009	0.00	VB	5.1		0
3	C18:0	1.4494	40.088	0.013	76790	0.00	BP	4.9		0
4	C18:1	10.1109	40.518	0.012	535672	0.00	PB	4.7		0
5	C18:1n9t	0.3077	40.644	0.000	16304	0.00	TS	0.0		0
6	C18:2n6t	57.8111	41.601	-0.047	3062802	0.00	BP	6.9		0
7	C18:3n3	0.3916	42.780	-0.004	20745	0.00	VB	0.0		0
8	C20:0	0.8337	44.113	-0.004	44170	0.00	PP	4.5		0
9	C20:1	0.4558	44.508	-0.010	24148	0.00	PB	4.5		0
10	C20:2	0.1620	45.520	-0.007	8585	0.00	BB	5.3		0
11	C20:3n6	0.0955	46.214	0.087	5060	0.00	BB	5.5		0
12	C22:0	3.9830	48.877	0.036	211015	0.00	BP	7.4		0
13	C22:1	0.1351	49.378	-0.119	7159	0.00	PB	0.0		0
14	Unk	0.0985	50.124	0.001	5220	0.00	BV	8.2		0
15	Unk	1.0105	50.329	0.016	53537	0.00	VB	7.9		0
16	C23:0	0.1699	52.151	0.000	9002	0.00	BB	10.0		0
17	C24:0	0.7465	56.411	0.013	39547	0.00	BB	12.4		0
	Totals	**99.9998**		**0.055**	**5297944**					

Figure A102 GC chromatogram of AA oil methyl ester.

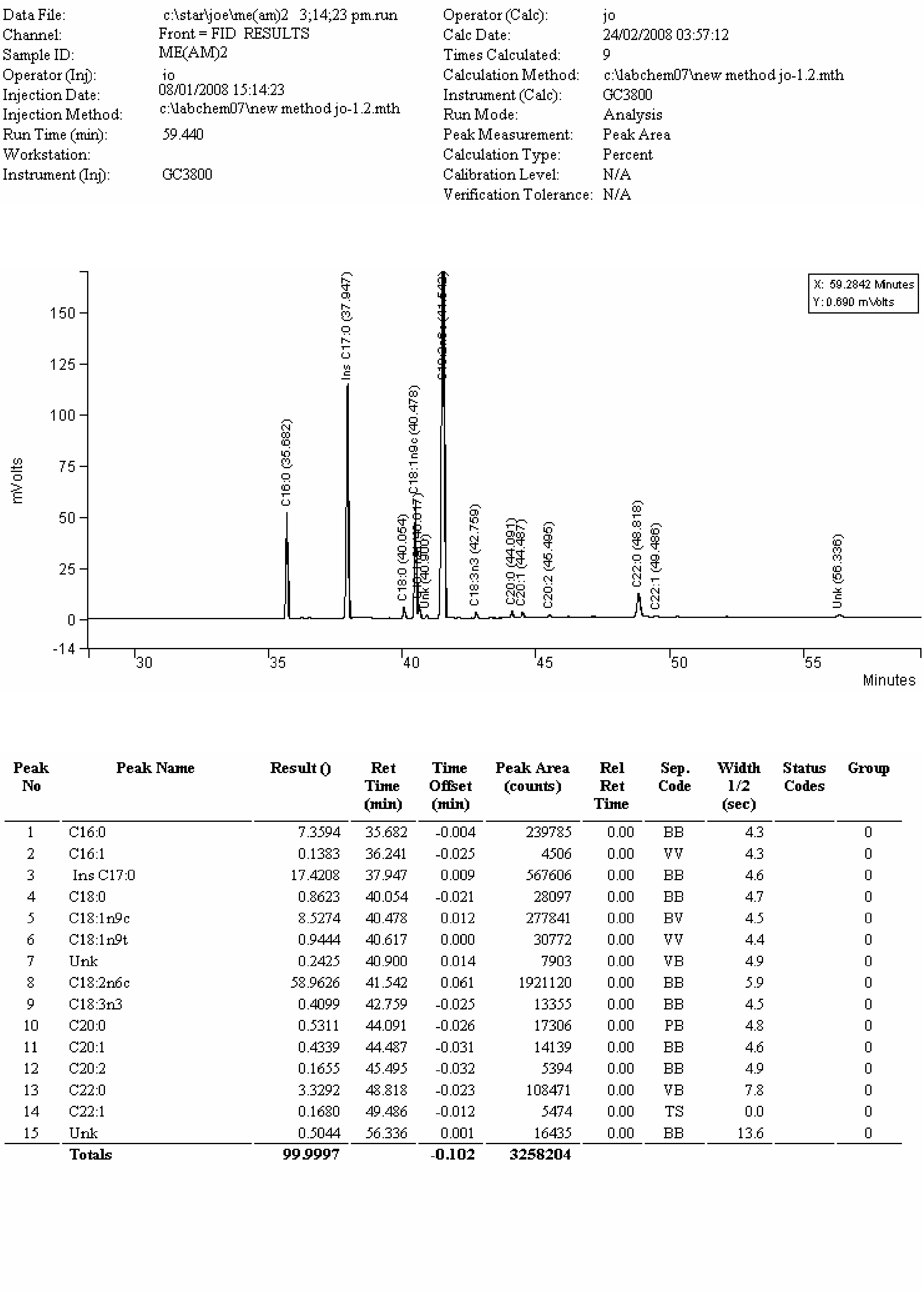


Data File: c:\star\joe\1 real 1;31;09 pm.run
Channel: Front = FID RESULTS
Sample ID: AI
Operator (Inj): JO
Injection Date: 06/02/2008 13:31:09
Injection Method: c:\labchem07\new method jo-1.2.mth
Run Time (min): 59.440
Workstation:
Instrument (Inj): GC3800

Operator (Calc): JO
Calc Date: 22/02/2008 19:38:48
Times Calculated: 10
Calculation Method: c:\labchem07\new method jo-1.2.mth
Instrument (Calc): GC3800
Run Mode: Analysis
Peak Measurement: Peak Area
Calculation Type: Percent
Calibration Level: N/A
Verification Tolerance: N/A

Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C12:0	0.1886	25.725	-0.007	2136	0.00	BB	3.6		0
2	C14:0	0.5842	30.898	-0.005	6616	0.00	BB	4.0		0
3	C16:0	17.7033	35.667	-0.019	200499	0.00	BB	4.2		0
4	Ins C17:0	20.2003	37.902	-0.036	228779	0.00	BB	4.3		0
5	C18:0	15.1106	40.051	-0.024	171135	0.00	BB	4.6		0
6	C18:1	30.7625	40.473	-0.033	348400	0.00	BB	4.6		0
7	C18:2n6c	13.1633	41.418	-0.063	149080	0.00	BB	4.4		0
8	C18:2n6t	0.4243	41.675	0.028	4806	0.00	TS	0.0		0
9	C18:3n3	0.4859	42.741	-0.043	5503	0.00	VB	4.4		0
10	C20:0	0.9419	44.076	-0.041	10668	0.00	BB	4.5		0
11	Unk	0.1944	44.797	-0.054	2202	0.00	PB	9.5		0
12	C22:0	0.2406	48.778	-0.063	2725	0.00	BB	6.8		0
	Totals	**99.9999**		**-0.360**	**1132549**					

Figure A103 GC chromatogram of AI oil methyl ester.



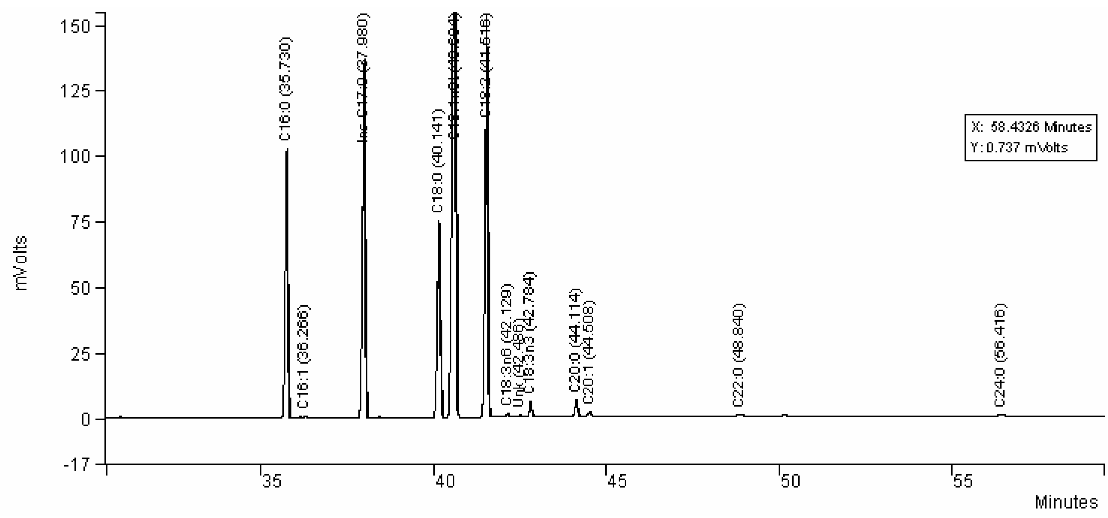
Data File: c:\star\joe\me(am)2 3;14;23 pm.run
Channel: Front = FID RESULTS
Sample ID: ME(AM)2
Operator (Inj): io
Injection Date: 08/01/2008 15:14:23
Injection Method: c:\labchem07\new method jo-1.2.mth
Run Time (min): 59.440
Workstation:
Instrument (Inj): GC3800

Operator (Calc): jo
Calc Date: 24/02/2008 03:57:12
Times Calculated: 9
Calculation Method: c:\labchem07\new method jo-1.2.mth
Instrument (Calc): GC3800
Run Mode: Analysis
Peak Measurement: Peak Area
Calculation Type: Percent
Calibration Level: N/A
Verification Tolerance: N/A

Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C16:0	7.3594	35.682	-0.004	239785	0.00	BB	4.3		0
2	C16:1	0.1383	36.241	-0.025	4506	0.00	VV	4.3		0
3	Ins C17:0	17.4208	37.947	0.009	567606	0.00	BB	4.6		0
4	C18:0	0.8623	40.054	-0.021	28097	0.00	BB	4.7		0
5	C18:1n9c	8.5274	40.478	0.012	277841	0.00	BV	4.5		0
6	C18:1n9t	0.9444	40.617	0.000	30772	0.00	VV	4.4		0
7	Unk	0.2425	40.900	0.014	7903	0.00	VB	4.9		0
8	C18:2n6c	58.9626	41.542	0.061	1921120	0.00	BB	5.9		0
9	C18:3n3	0.4099	42.759	-0.025	13355	0.00	BB	4.5		0
10	C20:0	0.5311	44.091	-0.026	17306	0.00	PB	4.8		0
11	C20:1	0.4339	44.487	-0.031	14139	0.00	BB	4.6		0
12	C20:2	0.1655	45.495	-0.032	5394	0.00	BB	4.9		0
13	C22:0	3.3292	48.818	-0.023	108471	0.00	VB	7.8		0
14	C22:1	0.1680	49.486	-0.012	5474	0.00	TS	0.0		0
15	Unk	0.5044	56.336	0.001	16435	0.00	BB	13.6		0
	Totals	**99.9997**		**-0.102**	**3258204**					

Figure A104 GC chromatogram of AM oil methyl ester.

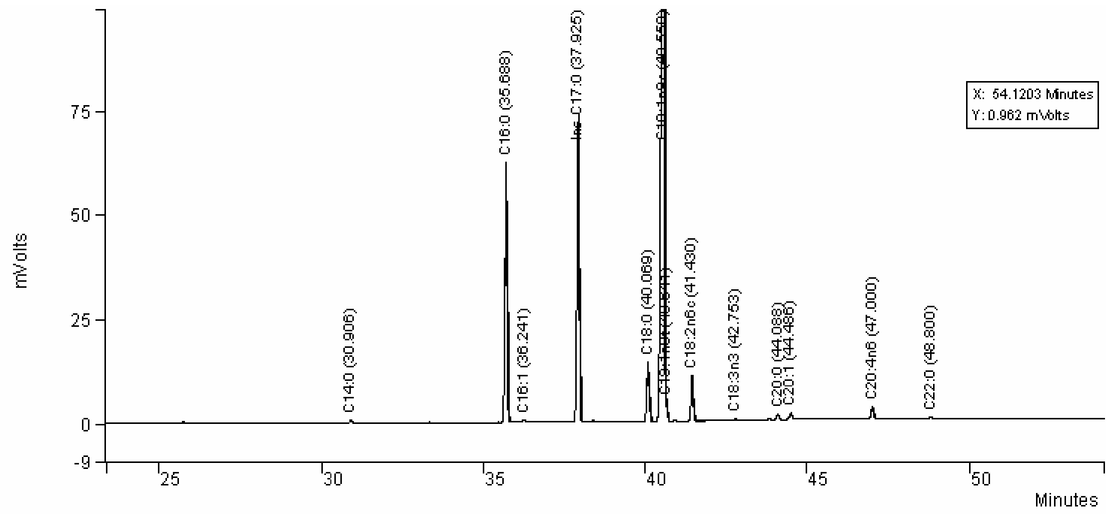
Data File:	c:\star\joe\me(as) 1;56;46 pm.run	Operator (Calc):	jo
Channel:	Front = FID RESULTS	Calc Date:	25/12/2007 17:23:12
Sample ID:	ME(AS)	Times Calculated:	8
Operator (Inj):	io	Calculation Method:	c:\labchem07\new method jo-1.2.mth
Injection Date:	25/12/2007 13:56:46	Instrument (Calc):	GC3800
Injection Method:	c:\labchem07\new method jo-1.2.mth	Run Mode:	Analysis
Run Time (min):	59.440	Peak Measurement:	Peak Area
Workstation:		Calculation Type:	Percent
Instrument (Inj):	GC3800	Calibration Level:	N/A
		Verification Tolerance:	N/A



Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C16:0	11.5353	35.730	0.044	495611	0.00	BB	4.6		0
2	C16:1	0.0863	36.266	0.000	3709	0.00	VB	4.2		0
3	Ins C17:0	16.6215	37.980	0.042	714136	0.00	BB	5.0		0
4	C18:0	10.2966	40.141	0.066	442389	0.00	BP	5.5		0
5	C18:1n9t	41.4163	40.604	-0.031	1779437	0.00	PB	5.8		0
6	C18:2	18.0960	41.516	0.035	777487	0.00	BB	4.7		0
7	C18:3n6	0.1273	42.129	0.000	5469	0.00	BB	4.5		0
8	Unk	0.0895	42.486	-0.000	3844	0.00	BV	4.5		0
9	C18:3n3	0.6465	42.784	-0.000	27777	0.00	VB	4.4		0
10	C20:0	0.7170	44.114	-0.003	30805	0.00	BP	4.4		0
11	C20:1	0.2212	44.508	-0.010	9505	0.00	PB	6.2		0
12	C22:0	0.0745	48.840	-0.000	3203	0.00	BB	6.6		0
13	C24:0	0.0720	56.416	0.018	3093	0.00	BB	11.7		0
	Totals	**100.0000**		**0.161**	**4296465**					

Figure A105 GC chromatogram of AS oil methyl ester.

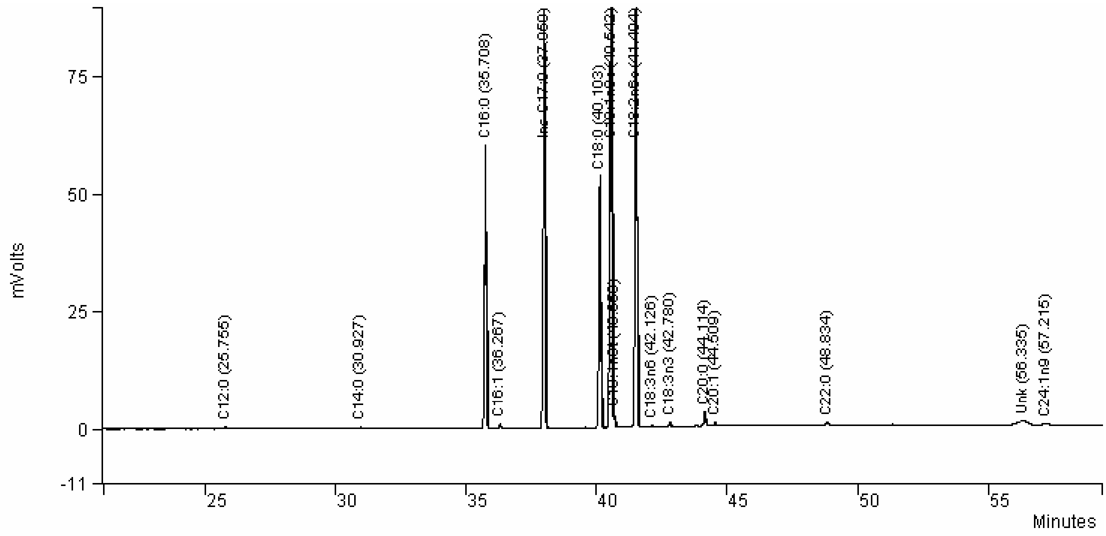
Data File:	c:\star\joe\me(cap) 11;12;12 am.run	Operator (Calc):	jo
Channel:	Front = FID RESULTS	Calc Date:	24/02/2008 01:29:38
Sample ID:	ME(CAP)	Times Calculated:	11
Operator (Inj):	io	Calculation Method:	c:\labchem07\new method jo-1.2.mth
Injection Date:	04/01/2008 11:12:12	Instrument (Calc):	GC3800
Injection Method:	c:\labchem07\new method jo-1.2.mth	Run Mode:	Analysis
Run Time (min):	59.440	Peak Measurement:	Peak Area
Workstation:		Calculation Type:	Percent
Instrument (Inj):	GC3800	Calibration Level:	N/A
		Verification Tolerance:	N/A



Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C14:0	0.1065	30.906	0.003	2239	0.00	BB	4.1		0
2	C16:0	13.5153	35.688	0.002	284077	0.00	BB	4.3		0
3	C16:1	0.1396	36.241	-0.025	2934	0.00	VB	4.2		0
4	Ins C17:0	16.7548	37.925	-0.013	352168	0.00	BB	4.5		0
5	C18:0	3.6193	40.069	-0.006	76074	0.00	BP	5.0		0
6	C18:1n9c	61.6570	40.550	-0.006	1295965	0.00	PB	5.5		0
7	C18:1n9t	0.2271	40.641	-0.000	4774	0.00	TS	0.0		0
8	C18:2n6c	2.4627	41.430	-0.051	51764	0.00	BB	4.4		0
9	C18:3n3	0.0762	42.753	-0.031	1602	0.00	BB	4.5		0
10	C20:0	0.2792	44.088	-0.029	5869	0.00	PB	4.7		0
11	C20:1	0.3481	44.486	-0.032	7317	0.00	BB	4.8		0
12	C20:4n6	0.7210	47.000	-0.000	15154	0.00	BB	4.8		0
13	C22:0	0.0932	48.800	-0.041	1958	0.00	BB	6.2		0
	Totals	**100.0000**		**-0.229**	**2101895**					

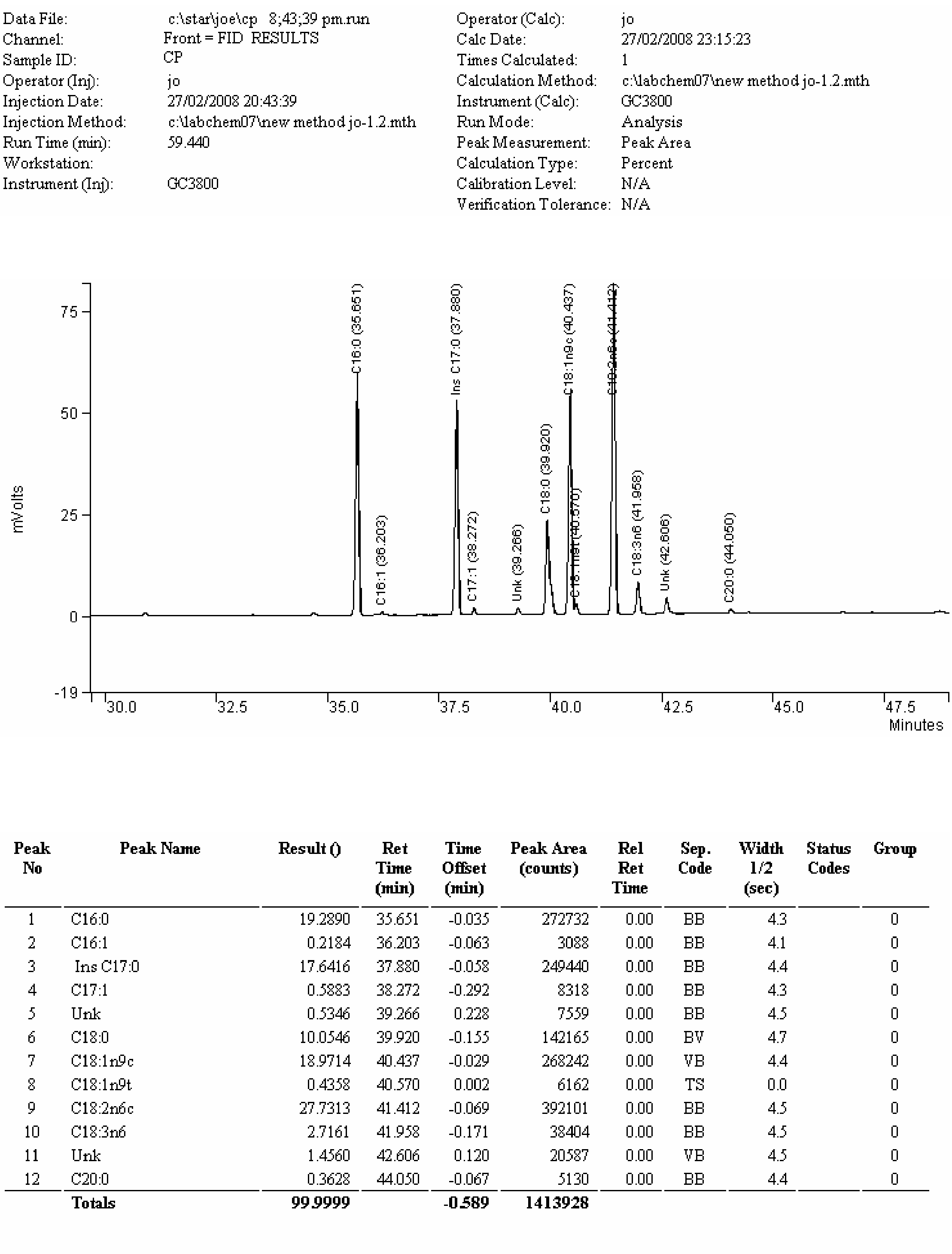
Figure A106 GC chromatogram of CAP oil methyl ester.

Data File:	c:\star\joe\me\me(ci) 2;56;51 pm.run	Operator (Calc):	jo
Channel:	Front = FID RESULTS	Calc Date:	24/02/2008 01:01:15
Sample ID:	ME(CI)	Times Calculated:	20
Operator (Inj):	jo	Calculation Method:	c:\labchem07\new method jo-1.2.mth
Injection Date:	19/12/2007 14:56:51	Instrument (Calc):	GC3800
Injection Method:	c:\labchem07\new method jo-1.2.mth	Run Mode:	Analysis
Run Time (min):	59.440	Peak Measurement:	Peak Area
Workstation:		Calculation Type:	Percent
Instrument (Inj):	GC3800	Calibration Level:	N/A
		Verification Tolerance:	N/A



Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C12:0	0.0604	25.755	0.015	1391	0.00	BB	3.4		0
2	C14:0	0.0540	30.927	0.014	1244	0.00	BB	4.0		0
3	C16:0	11.8454	35.708	0.022	272616	0.00	BB	4.2		0
4	C16:1	0.1780	36.267	0.001	4097	0.00	BB	4.1		0
5	Ins C17:0	18.3446	37.959	0.021	422190	0.00	BB	4.4		0
6	C18:0	11.8625	40.103	0.028	273010	0.00	BV	4.8		0
7	C18:1n9c	31.7320	40.542	0.007	730295	0.00	VB	4.7		0
8	C18:1n9t	0.2022	40.650	-0.006	4654	0.00	TS	0.0		0
9	C18:2n6c	23.3938	41.494	0.013	538395	0.00	BB	4.6		0
10	C18:3n6	0.0814	42.126	-0.003	1874	0.00	BB	4.4		0
11	C18:3n3	0.2081	42.780	-0.004	4789	0.00	BB	4.4		0
12	C20:0	0.6235	44.114	-0.003	14350	0.00	BB	4.7		0
13	C20:1	0.1511	44.509	-0.009	3477	0.00	BB	4.5		0
14	C22:0	0.1349	48.834	-0.007	3105	0.00	BB	6.6		0
15	Unk	0.9196	56.335	-0.000	21163	0.00	BB	24.6		0
16	C24:1n9	0.2084	57.215	-0.351	4796	0.00	BB	13.5		0
	Totals	**99.9999**		**-0.262**	**2301446**					

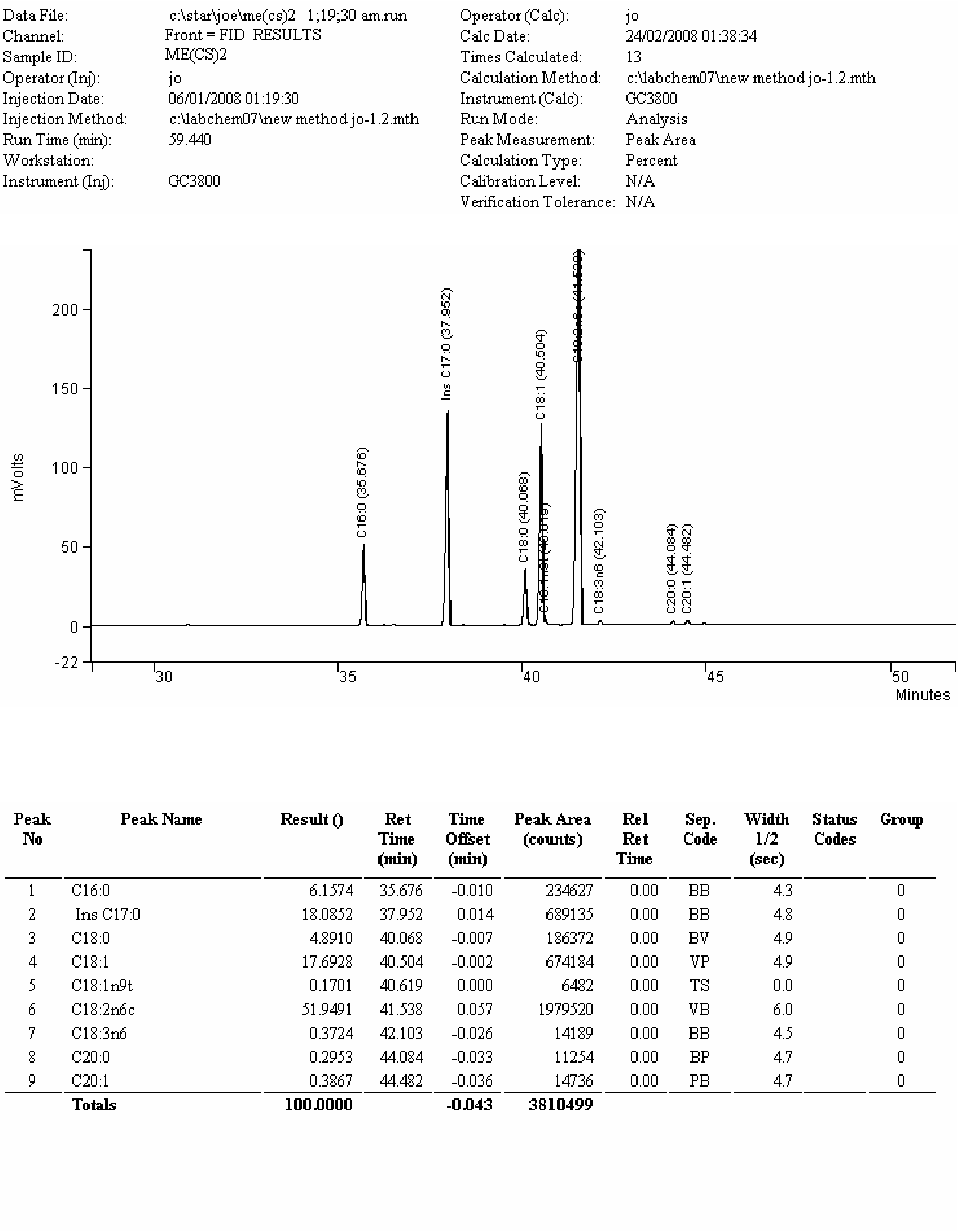
Figure A107 GC chromatogram of CI oil methyl ester.



Data File:	c:\star\joe\cp 8;43;39 pm.run	Operator (Calc):	jo
Channel:	Front = FID RESULTS	Calc Date:	27/02/2008 23:15:23
Sample ID:	CP	Times Calculated:	1
Operator (Inj):	jo	Calculation Method:	c:\labchem07\new method jo-1.2.mth
Injection Date:	27/02/2008 20:43:39	Instrument (Calc):	GC3800
Injection Method:	c:\labchem07\new method jo-1.2.mth	Run Mode:	Analysis
Run Time (min):	59.440	Peak Measurement:	Peak Area
Workstation:		Calculation Type:	Percent
Instrument (Inj):	GC3800	Calibration Level:	N/A
		Verification Tolerance:	N/A

Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C16:0	19.2890	35.651	-0.035	272732	0.00	BB	4.3		0
2	C16:1	0.2184	36.203	-0.063	3088	0.00	BB	4.1		0
3	Ins C17:0	17.6416	37.880	-0.058	249440	0.00	BB	4.4		0
4	C17:1	0.5883	38.272	-0.292	8318	0.00	BB	4.3		0
5	Unk	0.5346	39.266	0.228	7559	0.00	BB	4.5		0
6	C18:0	10.0546	39.920	-0.155	142165	0.00	BV	4.7		0
7	C18:1n9c	18.9714	40.437	-0.029	268242	0.00	VB	4.4		0
8	C18:1n9t	0.4358	40.570	0.002	6162	0.00	TS	0.0		0
9	C18:2n6c	27.7313	41.412	-0.069	392101	0.00	BB	4.5		0
10	C18:3n6	2.7161	41.958	-0.171	38404	0.00	BB	4.5		0
11	Unk	1.4560	42.606	0.120	20587	0.00	VB	4.5		0
12	C20:0	0.3628	44.050	-0.067	5130	0.00	BB	4.4		0
	Totals	**99.9999**		**-0.589**	**1413928**					

Figure A108 GC chromatogram of CP oil methyl ester.



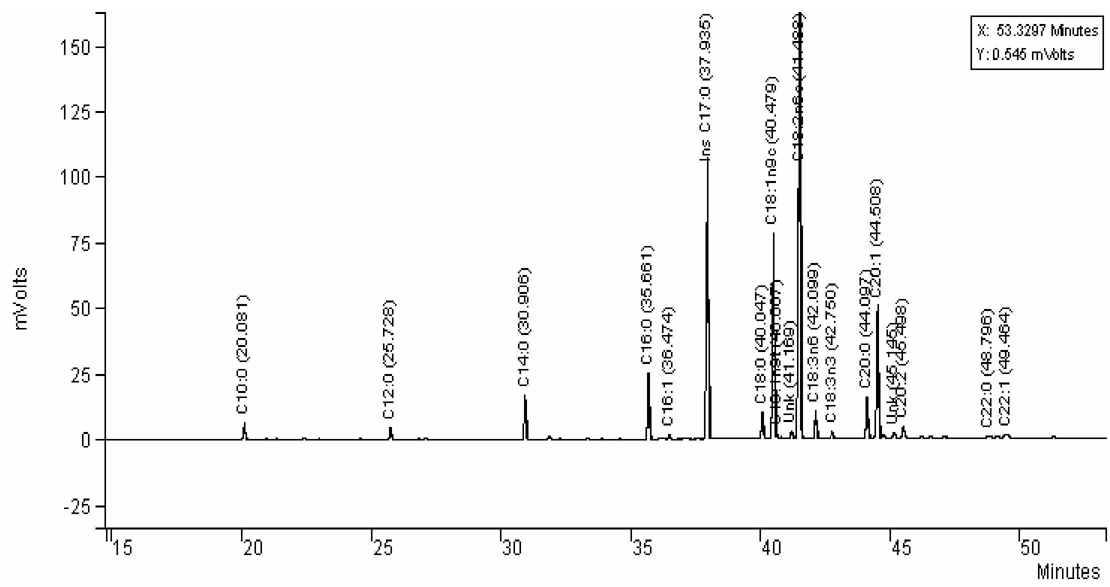
Data File: c:\star\joe\me(cs)2 1;19;30 am.run
Channel: Front = FID RESULTS
Sample ID: ME(CS)2
Operator (Inj): jo
Injection Date: 06/01/2008 01:19:30
Injection Method: c:\labchem07\new method jo-1.2.mth
Run Time (min): 59.440
Workstation:
Instrument (Inj): GC3800

Operator (Calc): jo
Calc Date: 24/02/2008 01:38:34
Times Calculated: 13
Calculation Method: c:\labchem07\new method jo-1.2.mth
Instrument (Calc): GC3800
Run Mode: Analysis
Peak Measurement: Peak Area
Calculation Type: Percent
Calibration Level: N/A
Verification Tolerance: N/A

Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C16:0	6.1574	35.676	-0.010	234627	0.00	BB	4.3		0
2	Ins C17:0	18.0852	37.952	0.014	689135	0.00	BB	4.8		0
3	C18:0	4.8910	40.068	-0.007	186372	0.00	BV	4.9		0
4	C18:1	17.6928	40.504	-0.002	674184	0.00	VP	4.9		0
5	C18:1n9t	0.1701	40.619	0.000	6482	0.00	TS	0.0		0
6	C18:2n6c	51.9491	41.538	0.057	1979520	0.00	VB	6.0		0
7	C18:3n6	0.3724	42.103	-0.026	14189	0.00	BB	4.5		0
8	C20:0	0.2953	44.084	-0.033	11254	0.00	BP	4.7		0
9	C20:1	0.3867	44.482	-0.036	14736	0.00	PB	4.7		0
	Totals	**100.0000**		**-0.043**	**3810499**					

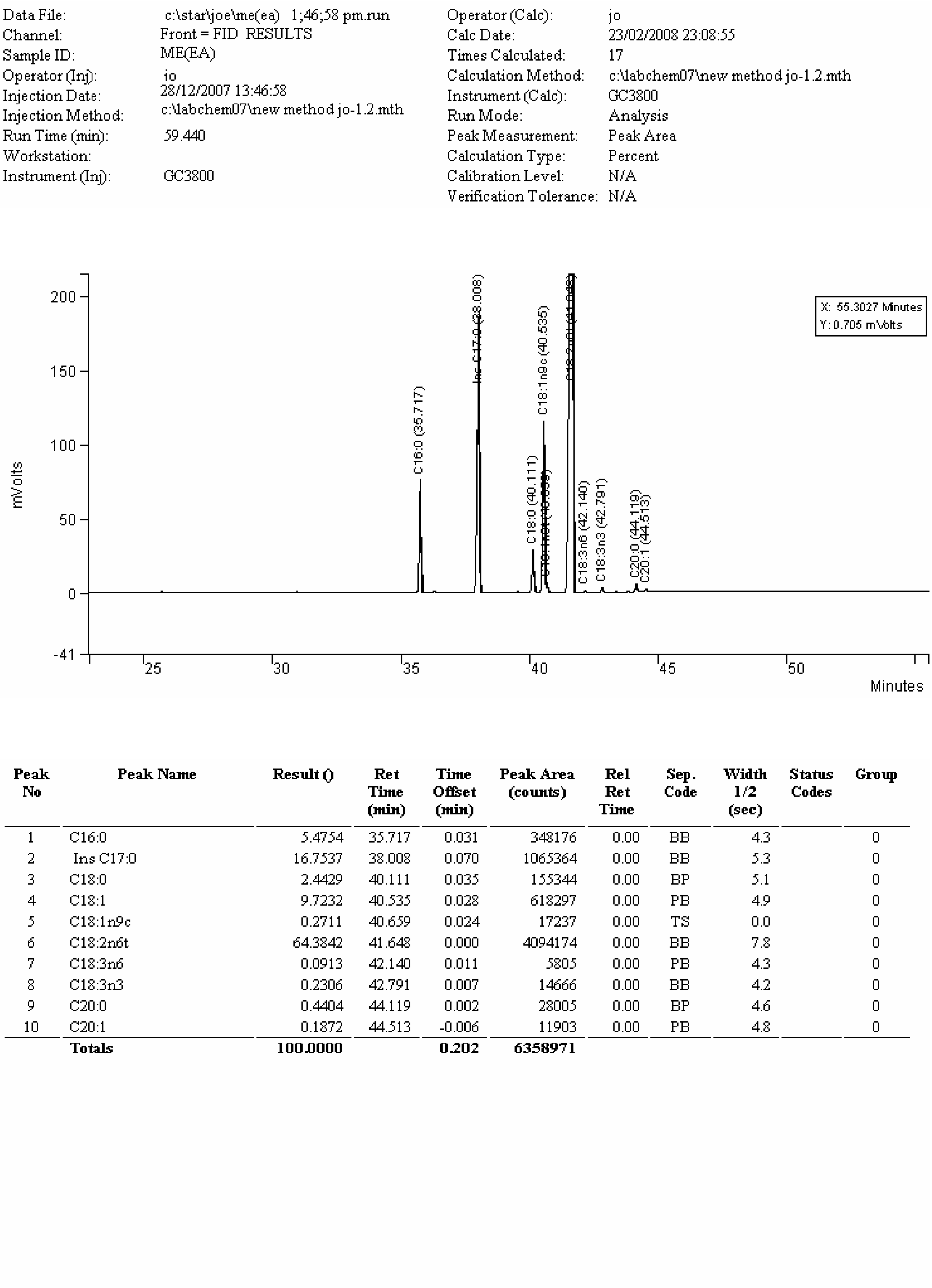
Figure A109 GC chromatogram of CS oil methyl ester.

Data File:	c:\star\joe\me(ct) 11;59;36 am.run	Operator (Calc):	jo
Channel:	Front = FID RESULTS	Calc Date:	24/02/2008 04:27:36
Sample ID:	ME(CT)	Times Calculated:	9
Operator (Inj):	jo	Calculation Method:	c:\labchem07\new method jo-1.2.mth
Injection Date:	09/01/2008 11:59:36	Instrument (Calc):	GC3800
Injection Method:	c:\labchem07\new method jo-1.2.mth	Run Mode:	Analysis
Run Time (min):	59.440	Peak Measurement:	Peak Area
Workstation:		Calculation Type:	Percent
Instrument (Inj):	GC3800	Calibration Level:	N/A
		Verification Tolerance:	N/A



Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C10:0	0.9354	20.081	-0.042	24611	0.00	BB	3.7		0
2	C12:0	0.7008	25.728	-0.004	18437	0.00	BB	3.8		0
3	C14:0	2.6984	30.906	0.003	70993	0.00	BB	4.1		0
4	C16:0	4.3149	35.661	-0.025	113521	0.00	BB	4.3		0
5	C16:1	0.2768	36.474	0.208	7283	0.00	BB	4.3		0
6	Ins C17:0	19.7586	37.935	-0.003	519834	0.00	BB	4.5		0
7	C18:0	1.8707	40.047	-0.028	49216	0.00	BB	4.5		0
8	C18:1n9c	14.3720	40.479	0.013	378117	0.00	BP	4.4		0
9	C18:1n9t	0.1817	40.607	-0.000	4781	0.00	TS	0.0		0
10	Unk	0.6224	41.169	-0.012	16374	0.00	PV	0.0		0
11	C18:2n6c	37.4745	41.488	0.007	985928	0.00	VB	5.0		0
12	C18:3n6	1.9115	42.099	-0.030	50290	0.00	BB	4.4		0
13	C18:3n3	0.4479	42.750	-0.034	11785	0.00	BB	4.3		0
14	C20:0	2.9201	44.097	-0.020	76826	0.00	BV	4.7		0
15	C20:1	9.4642	44.508	-0.010	248996	0.00	VB	4.6		0
16	Unk	0.4200	45.145	0.006	11049	0.00	BB	4.9		0
17	C20:2	0.9920	45.498	-0.029	26100	0.00	BB	5.8		0
18	C22:0	0.1820	48.796	-0.045	4788	0.00	BV	7.1		0
19	C22:1	0.4562	49.464	-0.033	12001	0.00	VB	8.3		0
	Totals	**100.0001**		**-0.078**	**2630930**					

Figure A110 GC chromatogram of CT oil methyl ester.

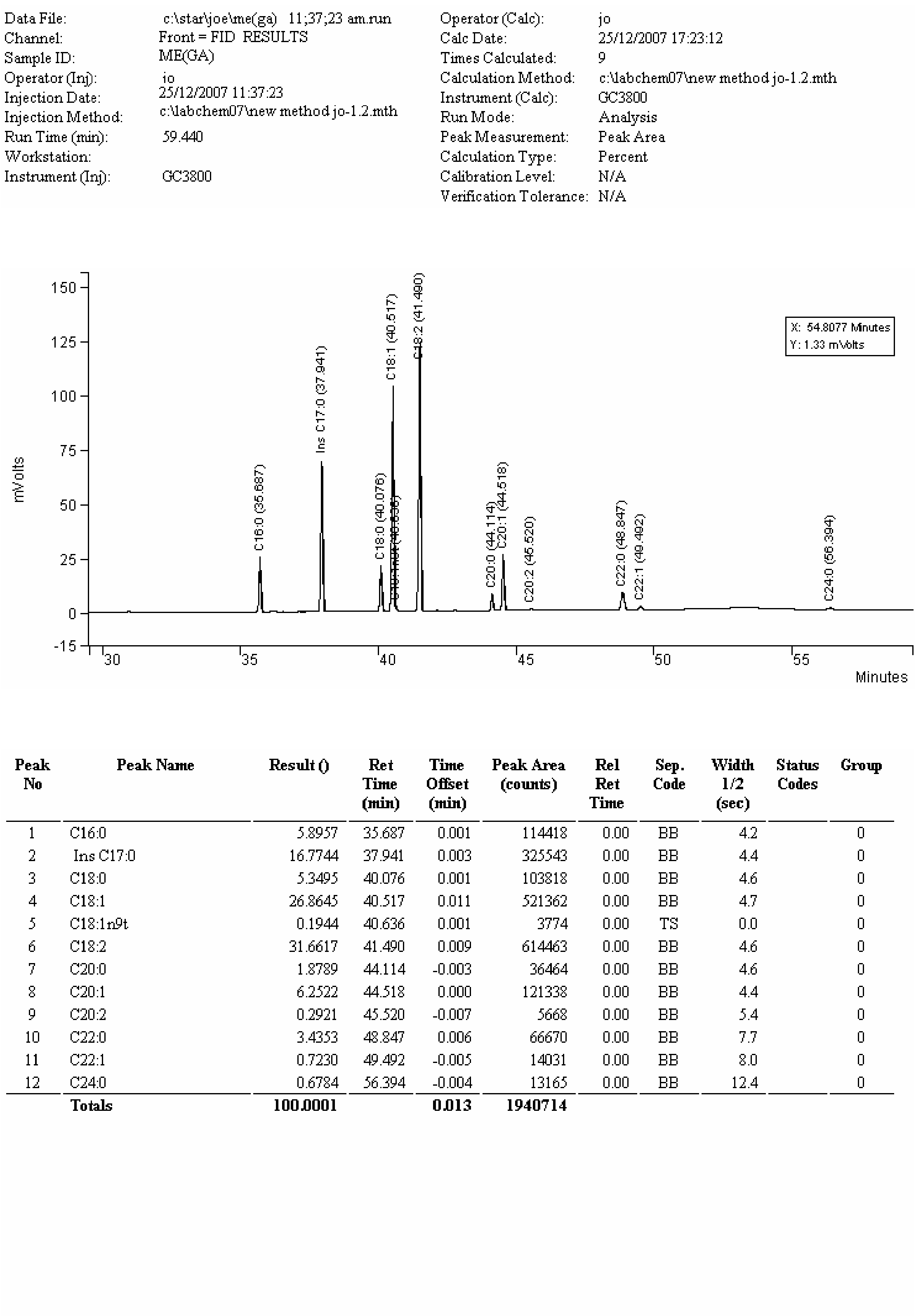


Data File: c:\star\joe\me(ea) 1;46;58 pm.run
Channel: Front = FID RESULTS
Sample ID: ME(EA)
Operator (Inj): jo
Injection Date: 28/12/2007 13:46:58
Injection Method: c:\labchem07\new method jo-1.2.mth
Run Time (min): 59.440
Workstation:
Instrument (Inj): GC3800

Operator (Calc): jo
Calc Date: 23/02/2008 23:08:55
Times Calculated: 17
Calculation Method: c:\labchem07\new method jo-1.2.mth
Instrument (Calc): GC3800
Run Mode: Analysis
Peak Measurement: Peak Area
Calculation Type: Percent
Calibration Level: N/A
Verification Tolerance: N/A

Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C16:0	5.4754	35.717	0.031	348176	0.00	BB	4.3		0
2	Ins C17:0	16.7537	38.008	0.070	1065364	0.00	BB	5.3		0
3	C18:0	2.4429	40.111	0.035	155344	0.00	BP	5.1		0
4	C18:1	9.7232	40.535	0.028	618297	0.00	PB	4.9		0
5	C18:1n9c	0.2711	40.659	0.024	17237	0.00	TS	0.0		0
6	C18:2n6t	64.3842	41.648	0.000	4094174	0.00	BB	7.8		0
7	C18:3n6	0.0913	42.140	0.011	5805	0.00	PB	4.3		0
8	C18:3n3	0.2306	42.791	0.007	14666	0.00	BB	4.2		0
9	C20:0	0.4404	44.119	0.002	28005	0.00	BP	4.6		0
10	C20:1	0.1872	44.513	-0.006	11903	0.00	PB	4.8		0
	Totals	**100.0000**		**0.202**	**6358971**					

Figure A111 GC chromatogram of EA oil methyl ester.



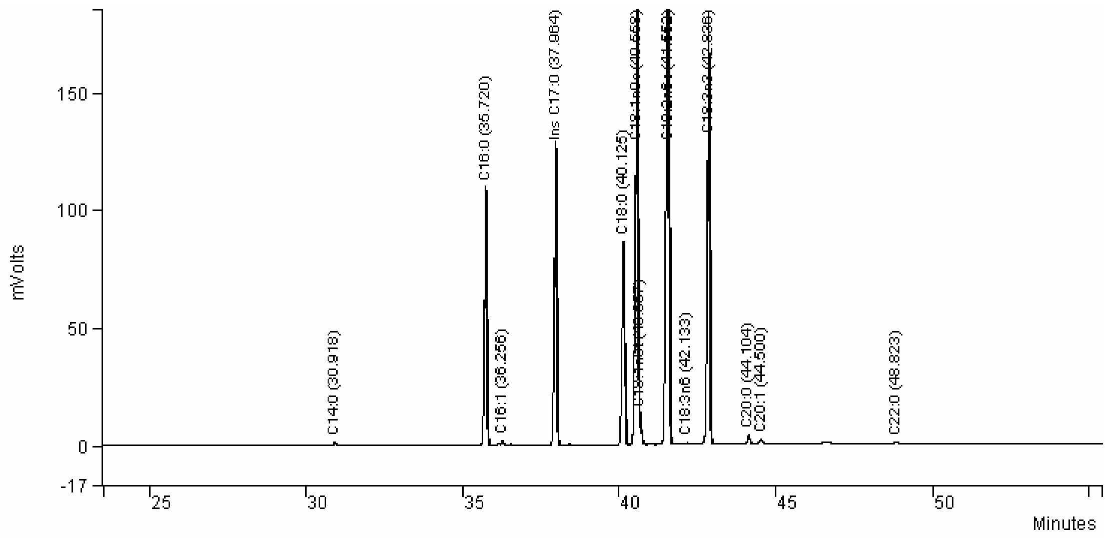
Data File:	c:\star\joe\me(ga) 11;37;23 am.run	Operator (Calc):	jo
Channel:	Front = FID RESULTS	Calc Date:	25/12/2007 17:23:12
Sample ID:	ME(GA)	Times Calculated:	9
Operator (Inj):	jo	Calculation Method:	c:\labchem07\new method jo-1.2.mth
Injection Date:	25/12/2007 11:37:23	Instrument (Calc):	GC3800
Injection Method:	c:\labchem07\new method jo-1.2.mth	Run Mode:	Analysis
Run Time (min):	59.440	Peak Measurement:	Peak Area
Workstation:		Calculation Type:	Percent
Instrument (Inj):	GC3800	Calibration Level:	N/A
		Verification Tolerance:	N/A

Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C16:0	5.8957	35.687	0.001	114418	0.00	BB	4.2		0
2	Ins C17:0	16.7744	37.941	0.003	325543	0.00	BB	4.4		0
3	C18:0	5.3495	40.076	0.001	103818	0.00	BB	4.6		0
4	C18:1	26.8645	40.517	0.011	521362	0.00	BB	4.7		0
5	C18:1n9t	0.1944	40.636	0.001	3774	0.00	TS	0.0		0
6	C18:2	31.6617	41.490	0.009	614463	0.00	BB	4.6		0
7	C20:0	1.8789	44.114	-0.003	36464	0.00	BB	4.6		0
8	C20:1	6.2522	44.518	0.000	121338	0.00	BB	4.4		0
9	C20:2	0.2921	45.520	-0.007	5668	0.00	BB	5.4		0
10	C22:0	3.4353	48.847	0.006	66670	0.00	BB	7.7		0
11	C22:1	0.7230	49.492	-0.005	14031	0.00	BB	8.0		0
12	C24:0	0.6784	56.394	-0.004	13165	0.00	BB	12.4		0
	Totals	**100.0001**		**0.013**	**1940714**					

Figure A112 GC chromatogram of GA oil methyl ester.

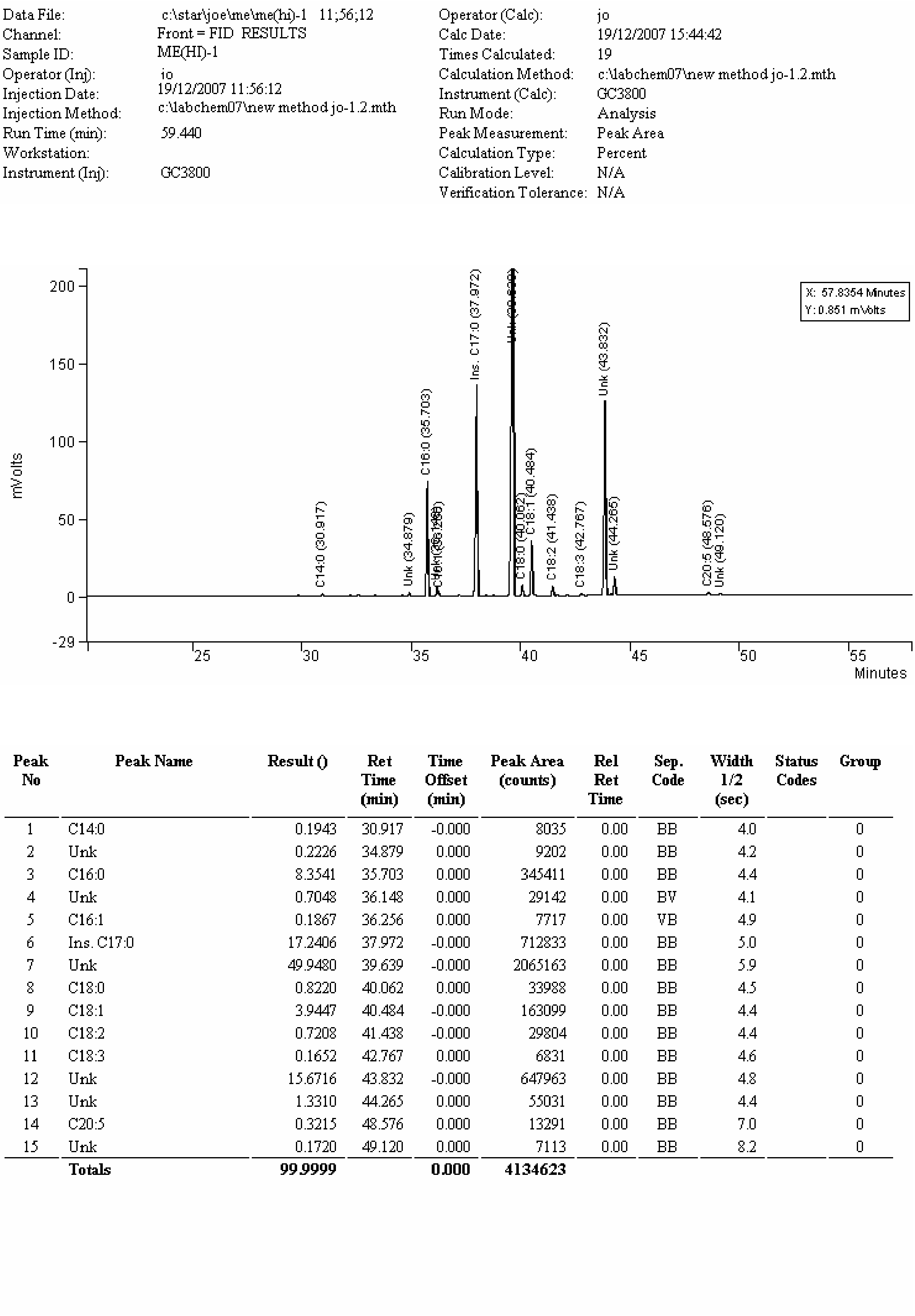
Data File: c:\star\joe\me(hb)2 5;47;42 pm.run
Channel: Front = FID RESULTS
Sample ID: ME(HB)2
Operator (Inj): jo
Injection Date: 27/12/2007 17:47:42
Injection Method: c:\labchem07\new method jo-1.2.mth
Run Time (min): 59.440
Workstation:
Instrument (Inj): GC3800

Operator (Calc): jo
Calc Date: 23/02/2008 23:51:42
Times Calculated: 6
Calculation Method: c:\labchem07\new method jo-1.2.mth
Instrument (Calc): GC3800
Run Mode: Analysis
Peak Measurement: Peak Area
Calculation Type: Percent
Calibration Level: N/A
Verification Tolerance: N/A



Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C14:0	0.1176	30.918	0.005	6608	0.00	BB	4.1		0
2	C16:0	9.4338	35.720	0.034	530272	0.00	BB	4.6		0
3	C16:1	0.1429	36.256	-0.010	8032	0.00	BB	4.0		0
4	Ins C17:0	11.8181	37.964	0.026	664297	0.00	BB	4.8		0
5	C18:0	8.9158	40.125	0.049	501159	0.00	BP	5.5		0
6	C18:1n9c	20.5130	40.558	0.000	1153037	0.00	PB	5.3		0
7	C18:1n9t	0.4354	40.657	-0.000	24475	0.00	TS	0.0		0
8	C18:2n6c	31.5722	41.552	0.071	1774676	0.00	BB	5.7		0
9	C18:3n6	0.0484	42.133	0.004	2721	0.00	BB	4.7		0
10	C18:3n3	16.4723	42.836	0.052	925912	0.00	BB	4.8		0
11	C20:0	0.3085	44.104	-0.013	17342	0.00	BB	4.5		0
12	C20:1	0.1599	44.500	-0.018	8986	0.00	BB	5.3		0
13	C22:0	0.0621	48.823	-0.018	3493	0.00	BB	6.7		0
	Totals	**100.0000**		**0.182**	**5621010**					

Figure A113 GC chromatogram of HB oil methyl ester.

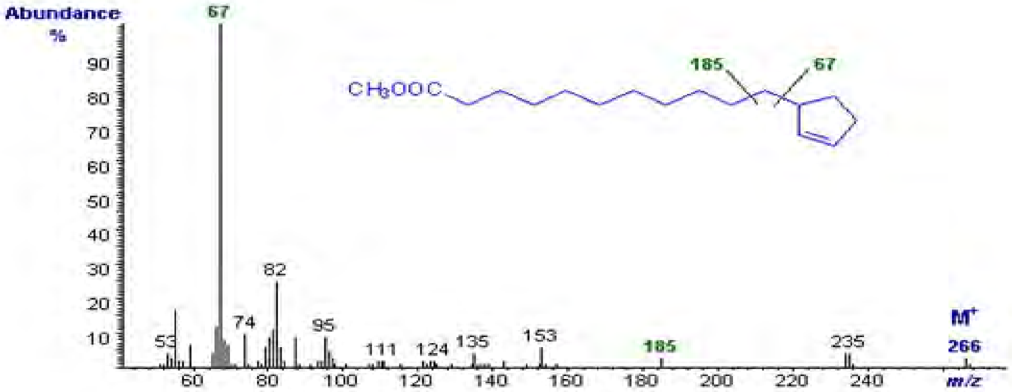


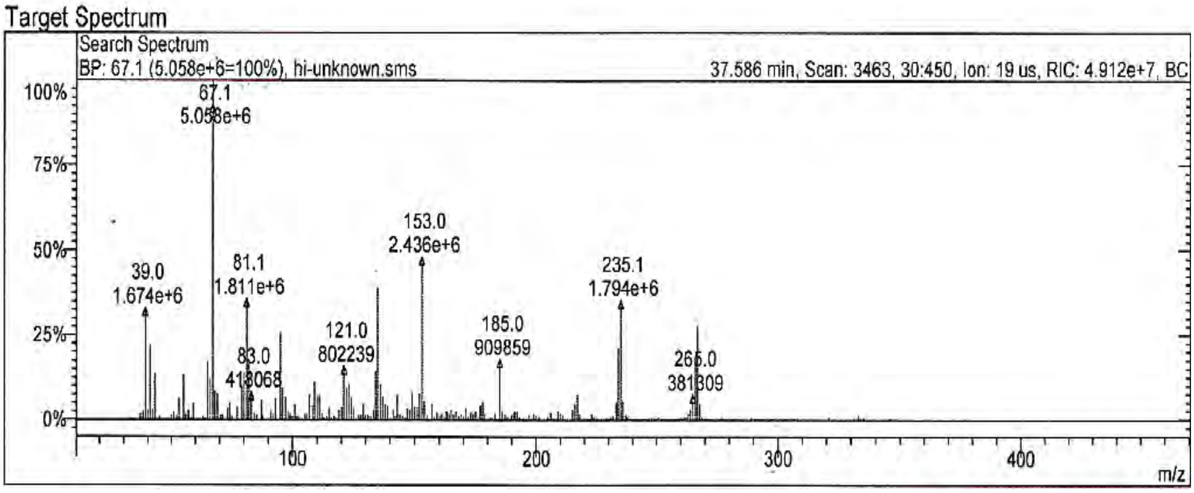
Data File: c:\star\joe\me\me(hi)-1 11;56;12
Channel: Front = FID RESULTS
Sample ID: ME(HI)-1
Operator (Inj): io
Injection Date: 19/12/2007 11:56:12
Injection Method: c:\labchem07\new method jo-1.2.mth
Run Time (min): 59.440
Workstation:
Instrument (Inj): GC3800

Operator (Calc): jo
Calc Date: 19/12/2007 15:44:42
Times Calculated: 19
Calculation Method: c:\labchem07\new method jo-1.2.mth
Instrument (Calc): GC3800
Run Mode: Analysis
Peak Measurement: Peak Area
Calculation Type: Percent
Calibration Level: N/A
Verification Tolerance: N/A

Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C14:0	0.1943	30.917	-0.000	8035	0.00	BB	4.0		0
2	Unk	0.2226	34.879	0.000	9202	0.00	BB	4.2		0
3	C16:0	8.3541	35.703	0.000	345411	0.00	BB	4.4		0
4	Unk	0.7048	36.148	0.000	29142	0.00	BV	4.1		0
5	C16:1	0.1867	36.256	0.000	7717	0.00	VB	4.9		0
6	Ins. C17:0	17.2406	37.972	-0.000	712833	0.00	BB	5.0		0
7	Unk	49.9480	39.639	-0.000	2065163	0.00	BB	5.9		0
8	C18:0	0.8220	40.062	0.000	33988	0.00	BB	4.5		0
9	C18:1	3.9447	40.484	-0.000	163099	0.00	BB	4.4		0
10	C18:2	0.7208	41.438	-0.000	29804	0.00	BB	4.4		0
11	C18:3	0.1652	42.767	0.000	6831	0.00	BB	4.6		0
12	Unk	15.6716	43.832	-0.000	647963	0.00	BB	4.8		0
13	Unk	1.3310	44.265	0.000	55031	0.00	BB	4.4		0
14	C20:5	0.3215	48.576	0.000	13291	0.00	BB	7.0		0
15	Unk	0.1720	49.120	0.000	7113	0.00	BB	8.2		0
	Totals	**99.9999**		**0.000**	**4134623**					

Figure A114 GC chromatogram of HI oil methyl ester.





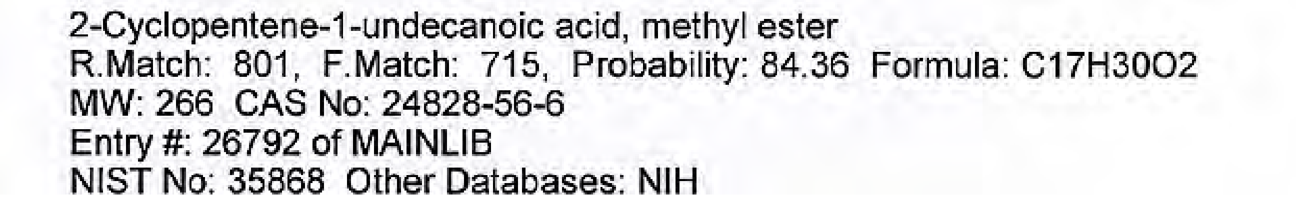
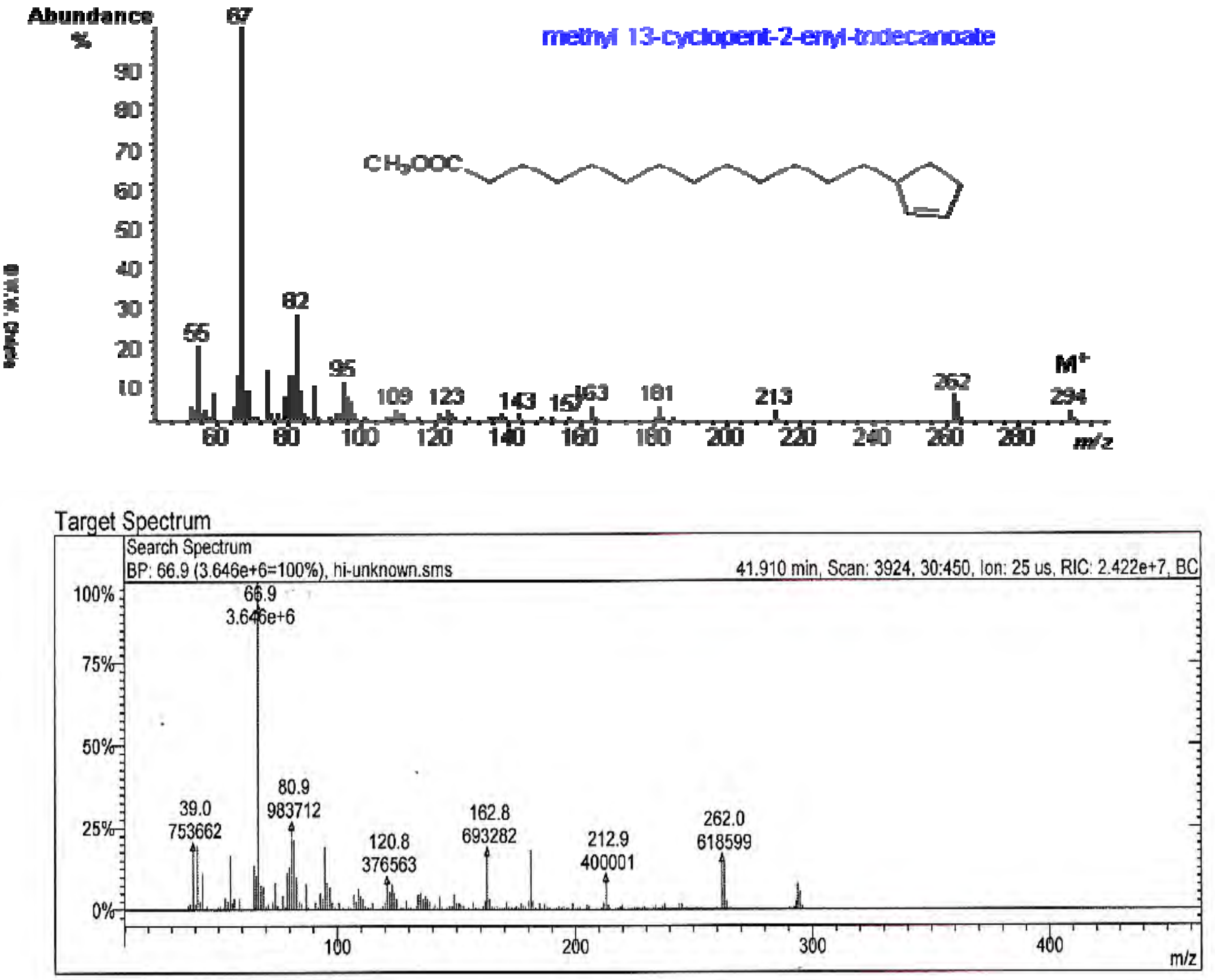
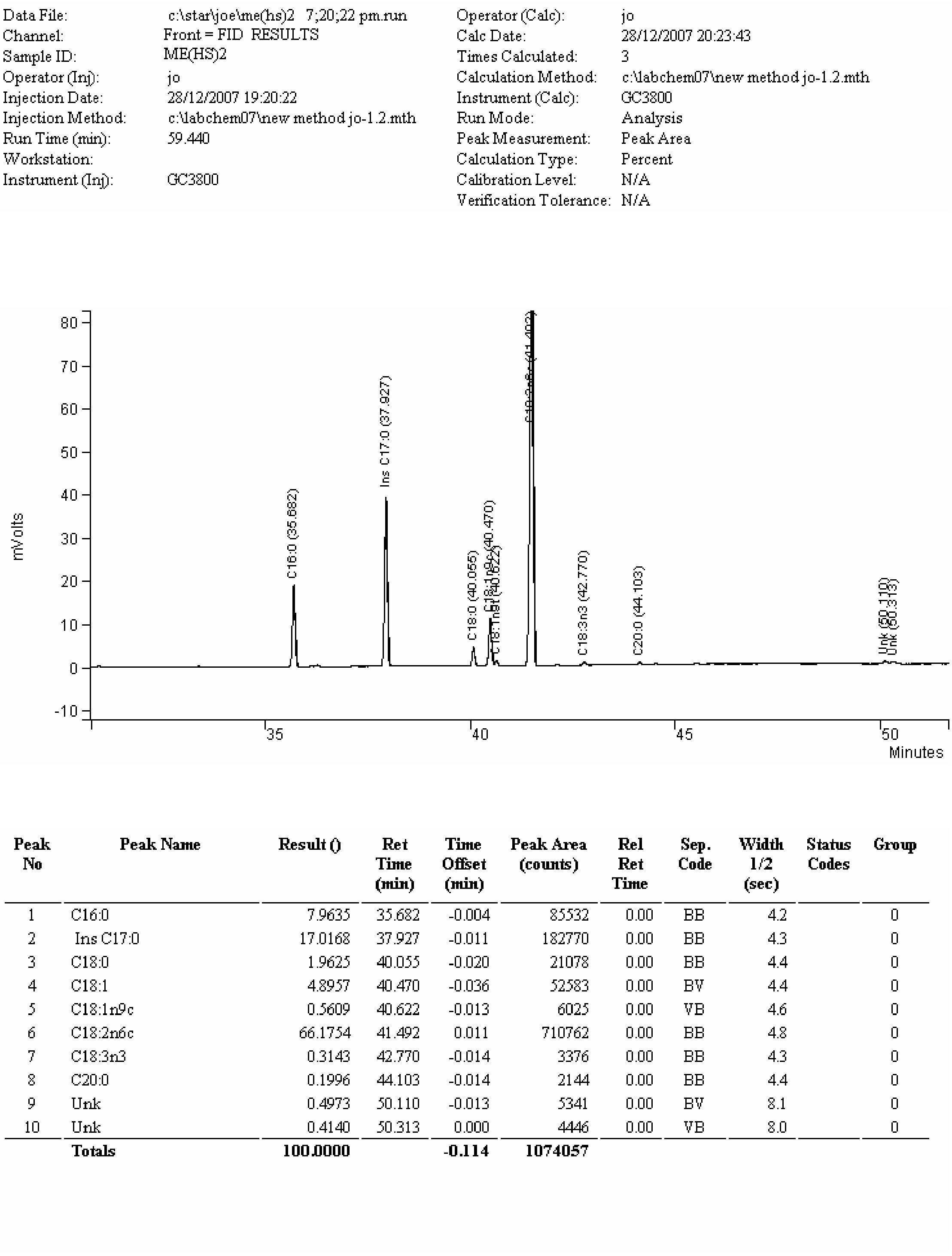

2-Cyclopentene-1-undecanoic acid, methyl ester
R.Match: 801, F.Match: 715, Probability: 84.36 Formula: C17H30O2
MW: 266 CAS No: 24828-56-6
Entry #: 26792 of MAINLIB
NIST No: 35868 Other Databases: NIH

Figure A114.1 GC-MS chromatogram of HI oil methyl ester at retention time of 39.639 minutes.



2-Cyclopentene-1-tridecanoic acid, methyl ester, (S)-
R.Match: 853, F.Match: 835, Probability: 81.81 Formula: $C_{19}H_{34}O_2$
MW: 294 CAS No: 24828-59-9
Entry #: 27153 of MAINLIB
NIST No: 14067 Other Databases: HODOC NIH EINECS

Figure A114.2 GC-MS chromatogram of HI oil methyl ester at retention time of 43.832 minutes.



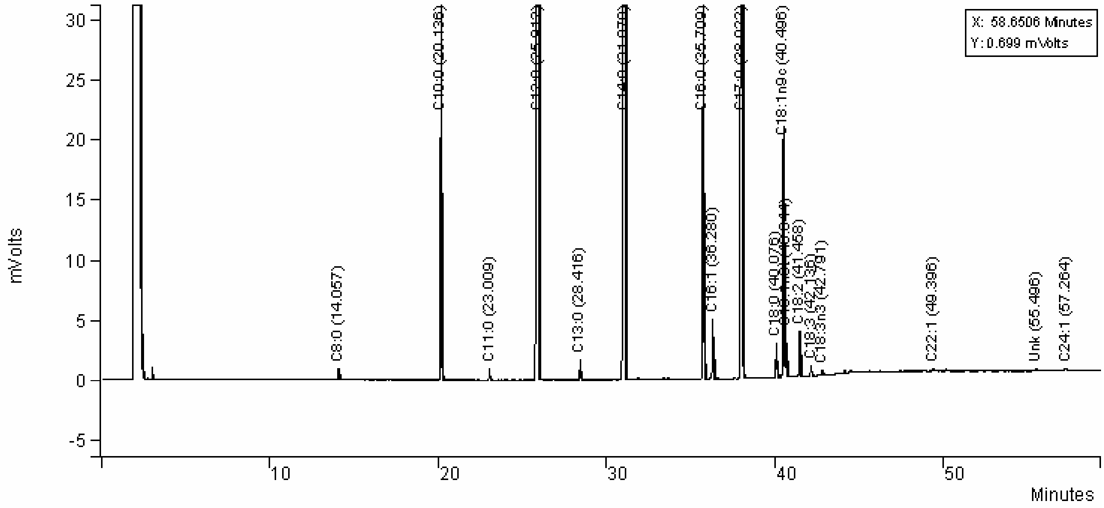
Data File:	c:\star\joe\me(hs)2 7;20;22 pm.run	Operator (Calc):	jo
Channel:	Front = FID RESULTS	Calc Date:	28/12/2007 20:23:43
Sample ID:	ME(HS)2	Times Calculated:	3
Operator (Inj):	jo	Calculation Method:	c:\labchem07\new method jo-1.2.mth
Injection Date:	28/12/2007 19:20:22	Instrument (Calc):	GC3800
Injection Method:	c:\labchem07\new method jo-1.2.mth	Run Mode:	Analysis
Run Time (min):	59.440	Peak Measurement:	Peak Area
Workstation:		Calculation Type:	Percent
Instrument (Inj):	GC3800	Calibration Level:	N/A
		Verification Tolerance:	N/A

Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C16:0	7.9635	35.682	-0.004	85532	0.00	BB	4.2		0
2	Ins C17:0	17.0168	37.927	-0.011	182770	0.00	BB	4.3		0
3	C18:0	1.9625	40.055	-0.020	21078	0.00	BB	4.4		0
4	C18:1	4.8957	40.470	-0.036	52583	0.00	BV	4.4		0
5	C18:1n9c	0.5609	40.622	-0.013	6025	0.00	VB	4.6		0
6	C18:2n6c	66.1754	41.492	0.011	710762	0.00	BB	4.8		0
7	C18:3n3	0.3143	42.770	-0.014	3376	0.00	BB	4.3		0
8	C20:0	0.1996	44.103	-0.014	2144	0.00	BB	4.4		0
9	Unk	0.4973	50.110	-0.013	5341	0.00	BV	8.1		0
10	Unk	0.4140	50.313	0.000	4446	0.00	VB	8.0		0
	Totals	**100.0000**		**-0.114**	**1074057**					

Figure A115 GC chromatogram of HS oil methyl ester.

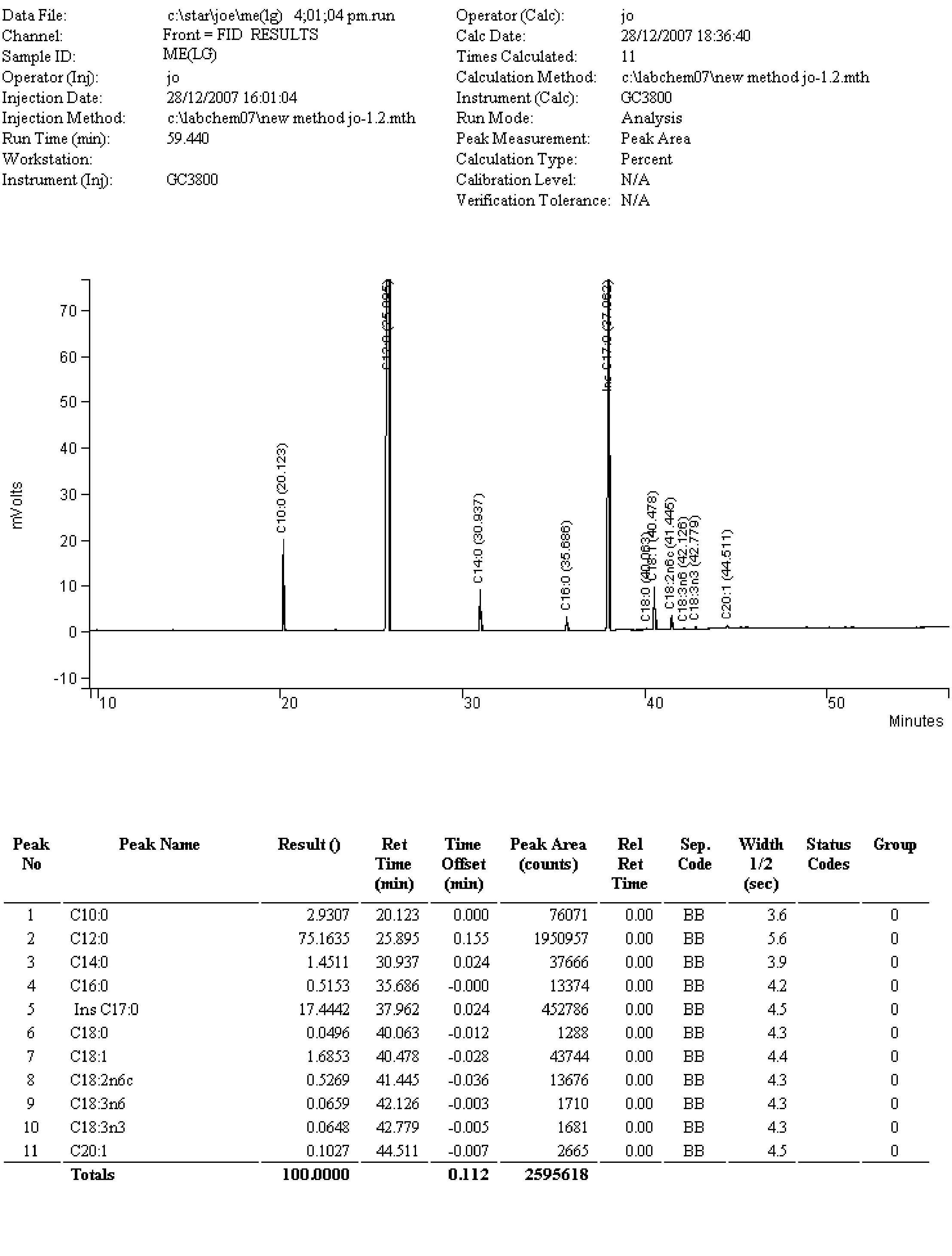
Data File: c:\star\joe\me\im-1 249.68 2;00;46
Channel: Front = FID RESULTS
Sample ID: im-1 249.68
Operator (Inj): io
Injection Date: 14/12/2007 14:00:46
Injection Method: c:\labchem07\new method jo-1.2.mth
Run Time (min): 59.440
Workstation:
Instrument (Inj): GC3800

Operator (Calc): jo
Calc Date: 19/12/2007 17:25:48
Times Calculated: 17
Calculation Method: c:\labchem07\new method jo-1.2.mth
Instrument (Calc): GC3800
Run Mode: Analysis
Peak Measurement: Peak Area
Calculation Type: Percent
Calibration Level: N/A
Verification Tolerance: N/A



Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C8:0	0.0567	14.057	0.000	3068	0.00	BB	3.3		0
2	C10:0	2.4118	20.136	0.000	130548	0.00	BB	3.5		0
3	C11:0	0.0658	23.009	0.000	3562	0.00	BB	3.6		0
4	C12:0	40.0942	25.912	-0.000	2170235	0.00	BB	5.9		0
5	C13:0	0.1278	28.416	-0.000	6917	0.00	BB	4.0		0
6	C14:0	31.7753	31.070	0.000	1719943	0.00	BB	5.8		0
7	C16:0	2.9186	35.709	-0.000	157978	0.00	BB	4.3		0
8	C16:1	0.4017	36.280	0.000	21744	0.00	BB	4.1		0
9	C17:0	19.2476	38.022	-0.000	1041841	0.00	BB	5.2		0
10	C18:0	0.2509	40.076	-0.000	13580	0.00	BB	4.4		0
11	C18:1n9c	1.8045	40.496	0.000	97676	0.00	BV	4.4		0
12	C18:1n9t	0.3086	40.644	0.000	16705	0.00	VB	4.4		0
13	C18:2	0.3286	41.458	-0.000	17785	0.00	BB	4.4		0
14	C18:3	0.0752	42.136	-0.000	4072	0.00	BB	4.5		0
15	C18:3n3	0.0323	42.791	0.000	1747	0.00	BB	4.5		0
16	C22:1	0.0345	49.396	0.000	1867	0.00	BB	0.0		0
17	Unk	0.0242	55.496	0.000	1309	0.00	BB	10.8		0
18	C24:1	0.0417	57.264	-0.000	2257	0.00	BB	13.5		0
	Totals	**100.0000**		**0.000**	**5412834**					

Figure A116 GC chromatogram of IM oil methyl ester.

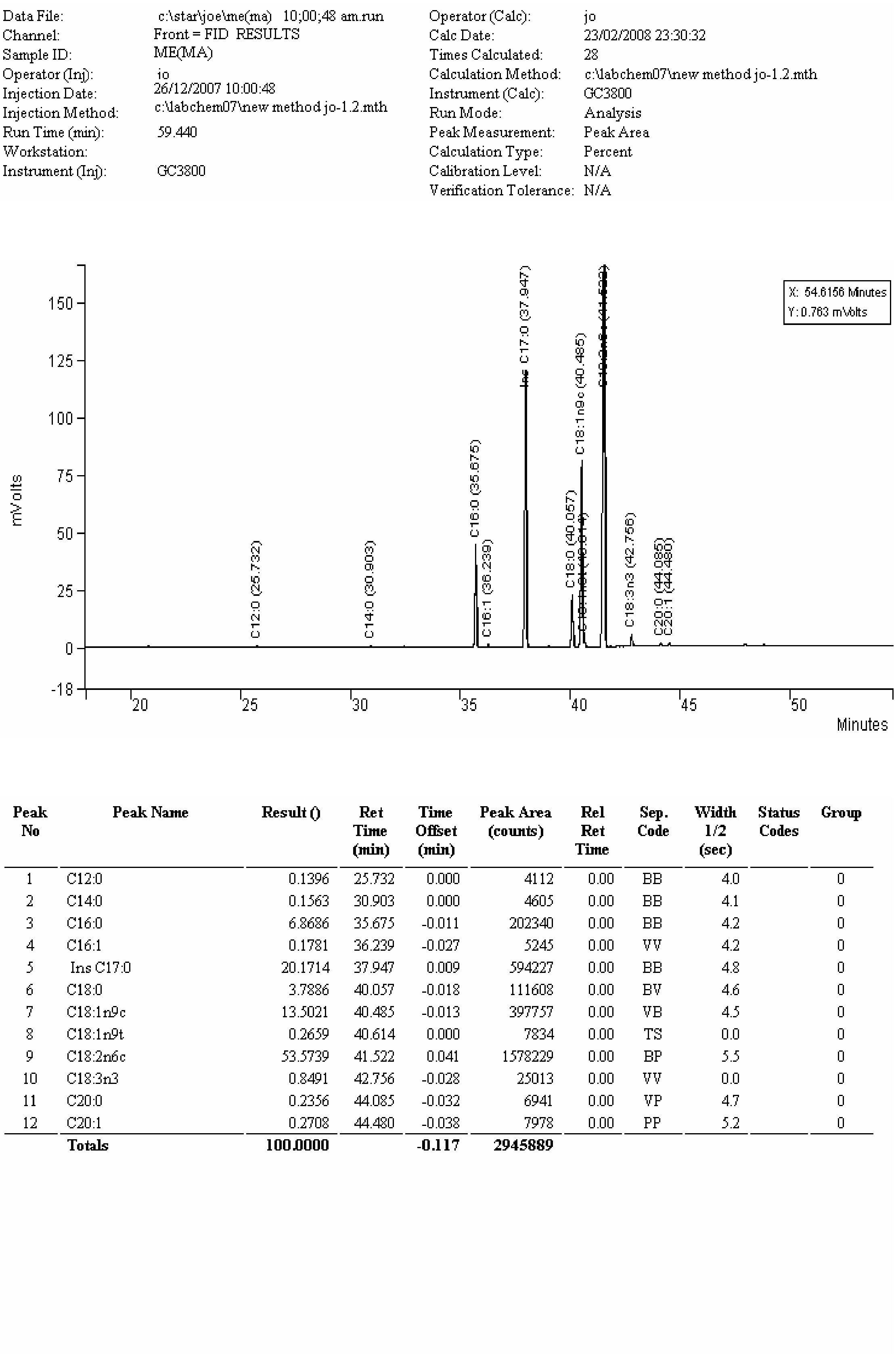


Data File: c:\star\joe\me(lg) 4;01;04 pm.run
Channel: Front = FID RESULTS
Sample ID: ME(LG)
Operator (Inj): jo
Injection Date: 28/12/2007 16:01:04
Injection Method: c:\labchem07\new method jo-1.2.mth
Run Time (min): 59.440
Workstation:
Instrument (Inj): GC3800

Operator (Calc): jo
Calc Date: 28/12/2007 18:36:40
Times Calculated: 11
Calculation Method: c:\labchem07\new method jo-1.2.mth
Instrument (Calc): GC3800
Run Mode: Analysis
Peak Measurement: Peak Area
Calculation Type: Percent
Calibration Level: N/A
Verification Tolerance: N/A

Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C10:0	2.9307	20.123	0.000	76071	0.00	BB	3.6		0
2	C12:0	75.1635	25.895	0.155	1950957	0.00	BB	5.6		0
3	C14:0	1.4511	30.937	0.024	37666	0.00	BB	3.9		0
4	C16:0	0.5153	35.686	-0.000	13374	0.00	BB	4.2		0
5	Ins C17:0	17.4442	37.962	0.024	452786	0.00	BB	4.5		0
6	C18:0	0.0496	40.063	-0.012	1288	0.00	BB	4.3		0
7	C18:1	1.6853	40.478	-0.028	43744	0.00	BB	4.4		0
8	C18:2n6c	0.5269	41.445	-0.036	13676	0.00	BB	4.3		0
9	C18:3n6	0.0659	42.126	-0.003	1710	0.00	BB	4.3		0
10	C18:3n3	0.0648	42.779	-0.005	1681	0.00	BB	4.3		0
11	C20:1	0.1027	44.511	-0.007	2665	0.00	BB	4.5		0
	Totals	**100.0000**		**0.112**	**2595618**					

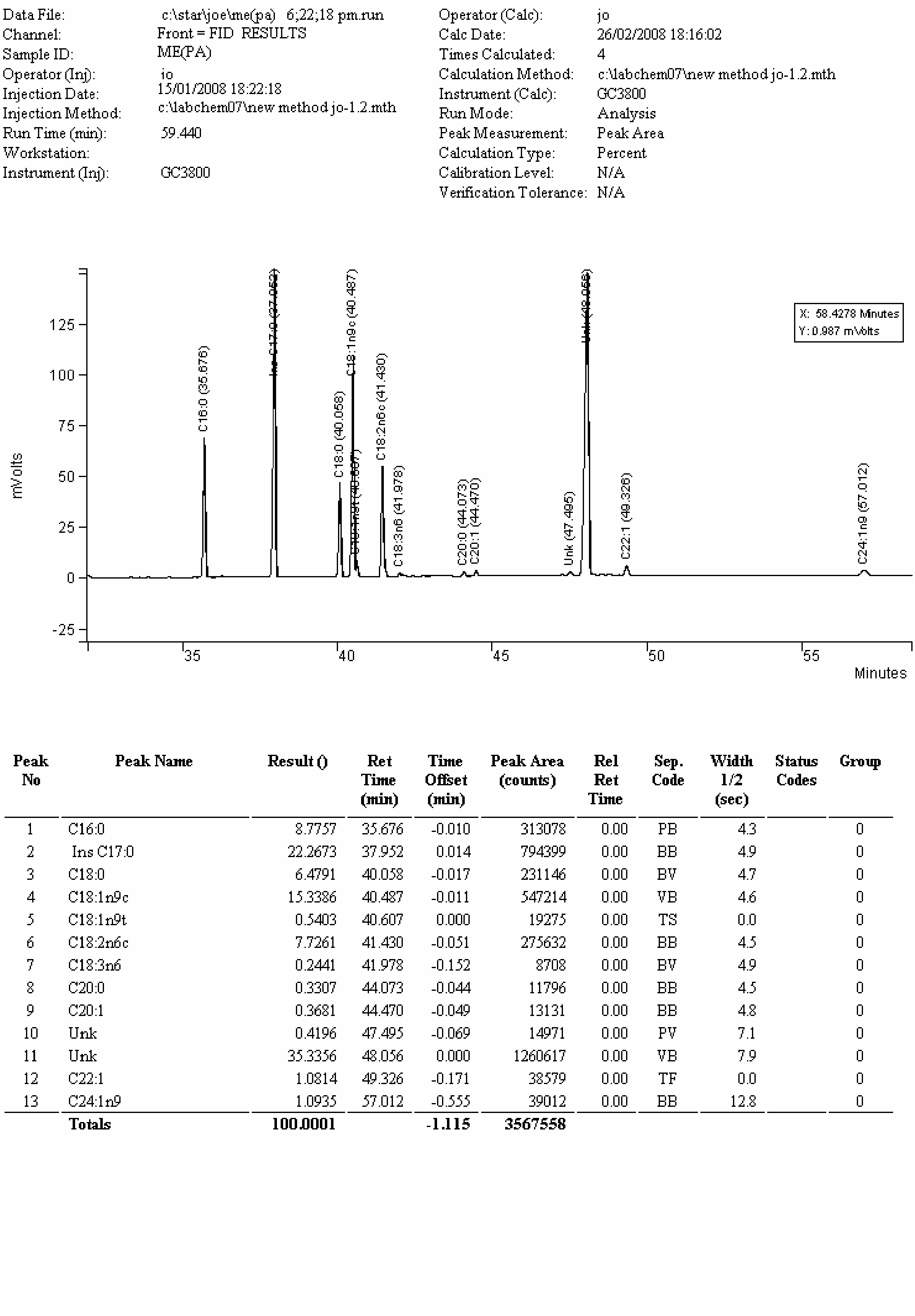
Figure A117 GC chromatogram of LG oil methyl ester.



Data File:	c:\star\joe\me(ma) 10;00;48 am.run	Operator (Calc):	jo
Channel:	Front = FID RESULTS	Calc Date:	23/02/2008 23:30:32
Sample ID:	ME(MA)	Times Calculated:	28
Operator (Inj):	jo	Calculation Method:	c:\labchem07\new method jo-1.2.mth
Injection Date:	26/12/2007 10:00:48	Instrument (Calc):	GC3800
Injection Method:	c:\labchem07\new method jo-1.2.mth	Run Mode:	Analysis
Run Time (min):	59.440	Peak Measurement:	Peak Area
Workstation:		Calculation Type:	Percent
Instrument (Inj):	GC3800	Calibration Level:	N/A
		Verification Tolerance:	N/A

Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C12:0	0.1396	25.732	0.000	4112	0.00	BB	4.0		0
2	C14:0	0.1563	30.903	0.000	4605	0.00	BB	4.1		0
3	C16:0	6.8686	35.675	-0.011	202340	0.00	BB	4.2		0
4	C16:1	0.1781	36.239	-0.027	5245	0.00	VV	4.2		0
5	Ins C17:0	20.1714	37.947	0.009	594227	0.00	BB	4.8		0
6	C18:0	3.7886	40.057	-0.018	111608	0.00	BV	4.6		0
7	C18:1n9c	13.5021	40.485	-0.013	397757	0.00	VB	4.5		0
8	C18:1n9t	0.2659	40.614	0.000	7834	0.00	TS	0.0		0
9	C18:2n6c	53.5739	41.522	0.041	1578229	0.00	BP	5.5		0
10	C18:3n3	0.8491	42.756	-0.028	25013	0.00	VV	0.0		0
11	C20:0	0.2356	44.085	-0.032	6941	0.00	VP	4.7		0
12	C20:1	0.2708	44.480	-0.038	7978	0.00	PP	5.2		0
	Totals	**100.0000**		**-0.117**	**2945889**					

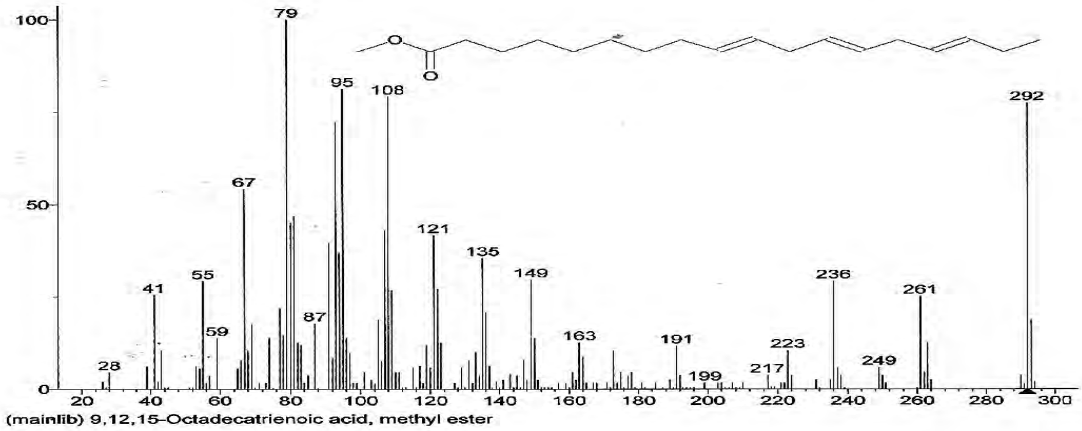
Figure A118 GC chromatogram of MA oil methyl ester.

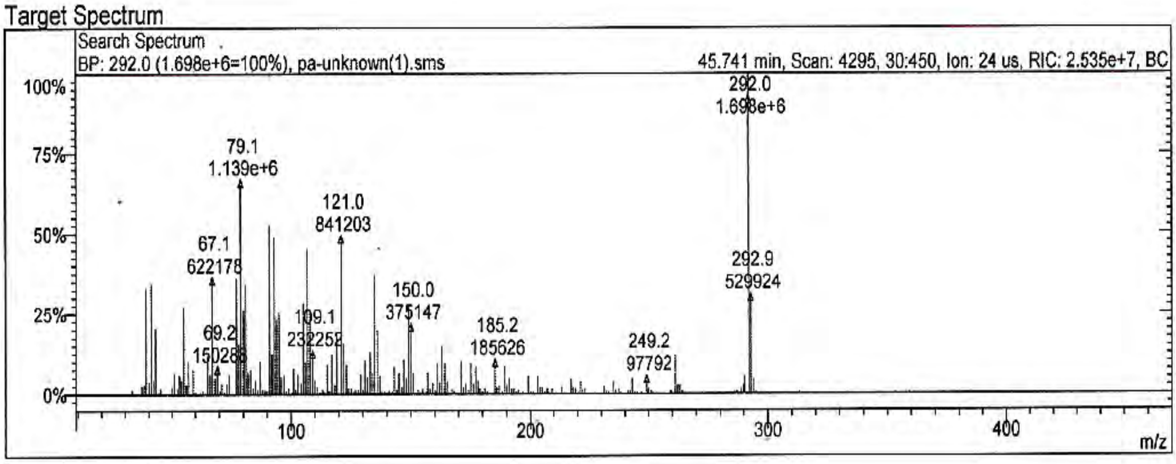


Data File:	c:\star\joe\me(pa) 6;22;18 pm.run	Operator (Calc):	jo
Channel:	Front = FID RESULTS	Calc Date:	26/02/2008 18:16:02
Sample ID:	ME(PA)	Times Calculated:	4
Operator (Inj):	io	Calculation Method:	c:\labchem07\new method jo-1.2.mth
Injection Date:	15/01/2008 18:22:18	Instrument (Calc):	GC3800
Injection Method:	c:\labchem07\new method jo-1.2.mth	Run Mode:	Analysis
Run Time (min):	59.440	Peak Measurement:	Peak Area
Workstation:		Calculation Type:	Percent
Instrument (Inj):	GC3800	Calibration Level:	N/A
		Verification Tolerance:	N/A

Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C16:0	8.7757	35.676	-0.010	313078	0.00	PB	4.3		0
2	Ins C17:0	22.2673	37.952	0.014	794399	0.00	BB	4.9		0
3	C18:0	6.4791	40.058	-0.017	231146	0.00	BV	4.7		0
4	C18:1n9c	15.3386	40.487	-0.011	547214	0.00	VB	4.6		0
5	C18:1n9t	0.5403	40.607	0.000	19275	0.00	TS	0.0		0
6	C18:2n6c	7.7261	41.430	-0.051	275632	0.00	BB	4.5		0
7	C18:3n6	0.2441	41.978	-0.152	8708	0.00	BV	4.9		0
8	C20:0	0.3307	44.073	-0.044	11796	0.00	BB	4.5		0
9	C20:1	0.3681	44.470	-0.049	13131	0.00	BB	4.8		0
10	Unk	0.4196	47.495	-0.069	14971	0.00	PV	7.1		0
11	Unk	35.3356	48.056	0.000	1260617	0.00	VB	7.9		0
12	C22:1	1.0814	49.326	-0.171	38579	0.00	TF	0.0		0
13	C24:1n9	1.0935	57.012	-0.555	39012	0.00	BB	12.8		0
	Totals	**100.0001**		**-1.115**	**3567558**					

Figure A119 GC chromatogram of PA oil methyl ester.

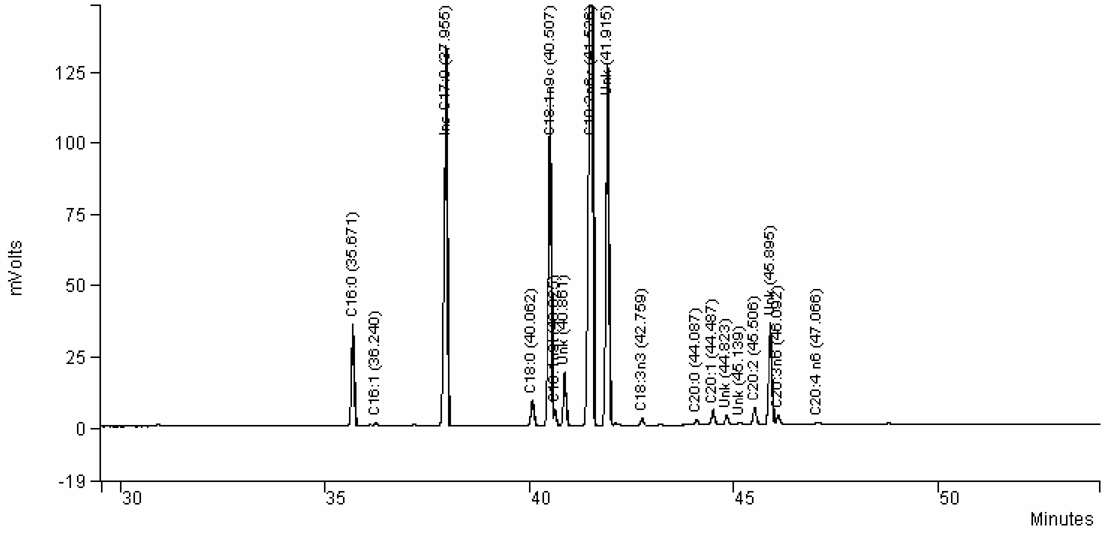




9,12,15-Octadecatrienoic acid, methyl ester
R.Match: 798, F.Match: 792, Probability: 75.28 Formula: C19H32O2
MW: 292 CAS No: 7361-80-0
Entry #: 38780 of MAINLIB
NIST No: 13998 Other Databases: HODOC

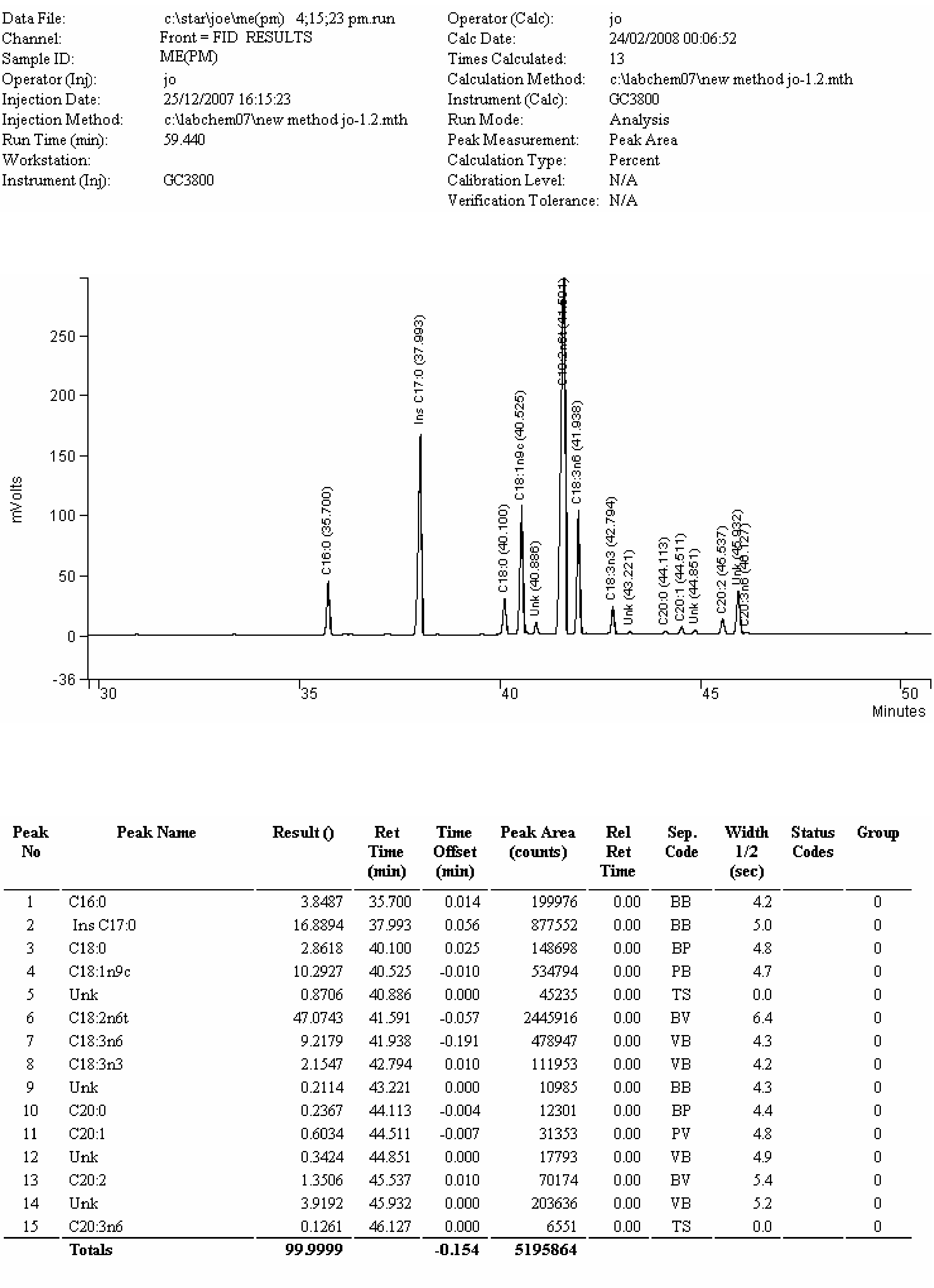
Figure A119.1 GC-MS chromatogram of PA oil methyl ester at retention time of 48.056 minutes.

Data File:	c:\star\joe\me(pk) 9;41;01 pm.run	Operator (Calc):	jo
Channel:	Front = FID RESULTS	Calc Date:	24/02/2008 02:58:41
Sample ID:	ME(PK)	Times Calculated:	22
Operator (Inj):	jo	Calculation Method:	c:\labchem07\new method jo-1.2.mth
Injection Date:	05/01/2008 21:41:01	Instrument (Calc):	GC3800
Injection Method:	c:\labchem07\new method jo-1.2.mth	Run Mode:	Analysis
Run Time (min):	59.440	Peak Measurement:	Peak Area
Workstation:		Calculation Type:	Percent
Instrument (Inj):	GC3800	Calibration Level:	N/A
		Verification Tolerance:	N/A



Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C16:0	3.9827	35.671	-0.015	161910	0.00	BB	4.2		0
2	C16:1	0.1618	36.240	-0.026	6577	0.00	VB	4.3		0
3	Ins C17:0	16.5304	37.955	0.017	672010	0.00	BB	4.7		0
4	C18:0	1.1636	40.062	-0.013	47305	0.00	BB	4.9		0
5	C18:1n9c	15.0063	40.507	0.008	610054	0.00	BB	4.7		0
6	C18:1n9t	0.3239	40.625	-0.000	13167	0.00	TS	0.0		0
7	Unk	2.1304	40.861	-0.025	86609	0.00	VB	4.2		0
8	C18:2n6c	37.1387	41.526	0.045	1509804	0.00	BV	5.5		0
9	Unk	15.1805	41.915	0.180	617133	0.00	VP	4.5		0
10	C18:3n3	0.2918	42.759	-0.025	11864	0.00	VB	0.0		0
11	C20:0	0.2101	44.087	-0.030	8542	0.00	BP	4.5		0
12	C20:1	0.6361	44.487	-0.031	25859	0.00	PV	4.5		0
13	Unk	0.4579	44.823	-0.028	18614	0.00	VV	5.0		0
14	Unk	0.2146	45.139	-0.000	8724	0.00	VV	21.1		0
15	C20:2	0.8154	45.506	-0.021	33148	0.00	VV	5.2		0
16	Unk	5.1735	45.895	-0.037	210317	0.00	VB	5.4		0
17	C20:3n6	0.3646	46.092	-0.035	14822	0.00	TS	0.0		0
18	C20:4n6	0.2177	47.066	0.066	8848	0.00	BV	13.3		0
	Totals	**100.0000**		**0.030**	**4065307**					

Figure A120 GC chromatogram of PK oil methyl ester.



Data File: c:\star\joe\me(pm) 4;15;23 pm.run
Channel: Front = FID RESULTS
Sample ID: ME(PM)
Operator (Inj): jo
Injection Date: 25/12/2007 16:15:23
Injection Method: c:\labchem07\new method jo-1.2.mth
Run Time (min): 59.440
Workstation:
Instrument (Inj): GC3800

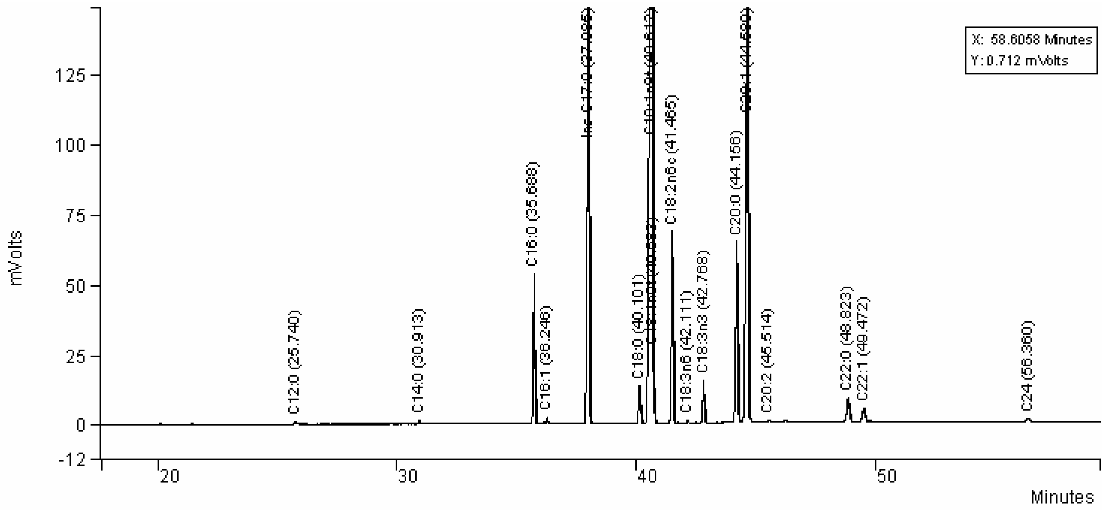
Operator (Calc): jo
Calc Date: 24/02/2008 00:06:52
Times Calculated: 13
Calculation Method: c:\labchem07\new method jo-1.2.mth
Instrument (Calc): GC3800
Run Mode: Analysis
Peak Measurement: Peak Area
Calculation Type: Percent
Calibration Level: N/A
Verification Tolerance: N/A

Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C16:0	3.8487	35.700	0.014	199976	0.00	BB	4.2		0
2	Ins C17:0	16.8894	37.993	0.056	877552	0.00	BB	5.0		0
3	C18:0	2.8618	40.100	0.025	148698	0.00	BP	4.8		0
4	C18:1n9c	10.2927	40.525	-0.010	534794	0.00	PB	4.7		0
5	Unk	0.8706	40.886	0.000	45235	0.00	TS	0.0		0
6	C18:2n6t	47.0743	41.591	-0.057	2445916	0.00	BV	6.4		0
7	C18:3n6	9.2179	41.938	-0.191	478947	0.00	VB	4.3		0
8	C18:3n3	2.1547	42.794	0.010	111953	0.00	VB	4.2		0
9	Unk	0.2114	43.221	0.000	10985	0.00	BB	4.3		0
10	C20:0	0.2367	44.113	-0.004	12301	0.00	BP	4.4		0
11	C20:1	0.6034	44.511	-0.007	31353	0.00	PV	4.8		0
12	Unk	0.3424	44.851	0.000	17793	0.00	VB	4.9		0
13	C20:2	1.3506	45.537	0.010	70174	0.00	BV	5.4		0
14	Unk	3.9192	45.932	0.000	203636	0.00	VB	5.2		0
15	C20:3n6	0.1261	46.127	0.000	6551	0.00	TS	0.0		0
	Totals	**99.9999**		**-0.154**	**5195864**					

Figure A121 GC chromatogram of PM oil methyl ester.

Data File: c:\star\joe\me(se)3 12;16;03 pm.run
Channel: Front = FID RESULTS
Sample ID: ME(SE)3
Operator (Inj): jo
Injection Date: 26/12/2007 12:16:03
Injection Method: c:\labchem07\new method jo-1.2.mth
Run Time (min): 59.438
Workstation:
Instrument (Inj): GC3800

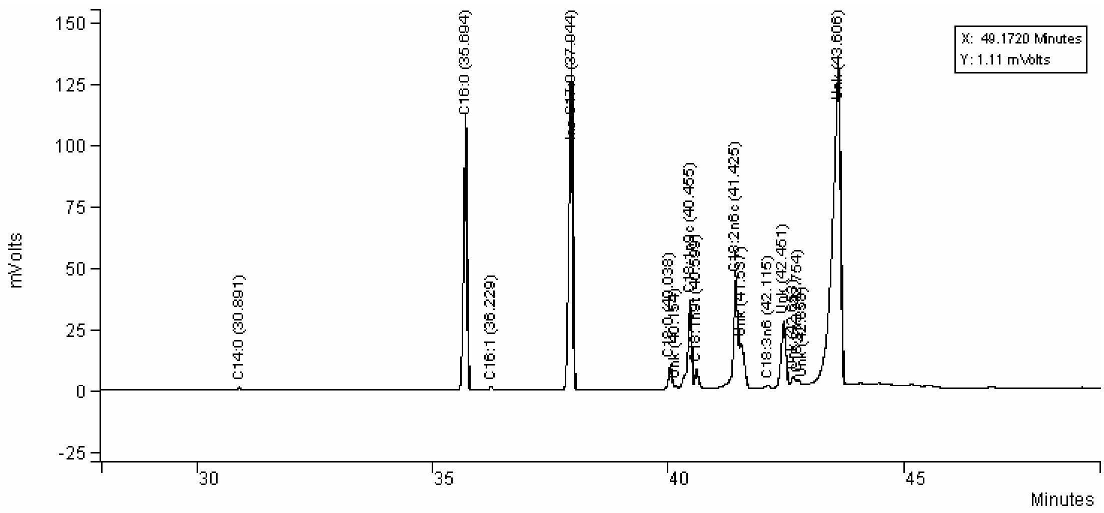
Operator (Calc): jo
Calc Date: 24/02/2008 00:49:04
Times Calculated: 7
Calculation Method: c:\labchem07\new method jo-1.2.mth
Instrument (Calc): GC3800
Run Mode: Analysis
Peak Measurement: Peak Area
Calculation Type: Percent
Calibration Level: N/A
Verification Tolerance: N/A



Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C12:0	0.0629	25.740	-0.000	3702	0.00	BB	3.9		0
2	C14:0	0.0944	30.913	-0.000	5558	0.00	BB	4.1		0
3	C16:0	4.1666	35.688	0.002	245333	0.00	BB	4.3		0
4	C16:1	0.1786	36.246	-0.020	10514	0.00	VB	4.3		0
5	Ins C17:0	16.7237	37.985	0.047	984713	0.00	BB	5.1		0
6	C18:0	1.3912	40.101	0.026	81916	0.00	BP	5.8		0
7	C18:1n9t	43.0747	40.612	-0.002	2536288	0.00	PB	6.9		0
8	C18:1n9t	0.4165	40.683	0.000	24522	0.00	TS	0.0		0
9	C18:2n6c	5.4913	41.465	-0.016	323332	0.00	BB	4.4		0
10	C18:3n6	0.0754	42.111	-0.018	4438	0.00	BB	4.5		0
11	C18:3n3	1.2223	42.768	-0.016	71970	0.00	VB	4.4		0
12	C20:0	6.1704	44.156	0.039	363321	0.00	BP	5.3		0
13	C20:1	18.7270	44.589	0.071	1102669	0.00	PB	5.7		0
14	C20:2	0.0720	45.514	-0.013	4241	0.00	BB	5.4		0
15	C22:0	1.1581	48.823	-0.018	68190	0.00	BB	7.4		0
16	C22:1	0.7232	49.472	-0.025	42583	0.00	BB	7.7		0
17	C24:0	0.2517	56.360	-0.038	14822	0.00	BB	12.5		0
	Totals	**100.0000**		**0.019**	**5888112**					

Figure A122 GC chromatogram of SE oil methyl ester.

Data File:	c:\star\joe\me(sf-2)4.5.51 3;08;33	Operator (Calc):	jo
Channel:	Front = FID RESULTS	Calc Date:	26/02/2008 18:28:57
Sample ID:	ME(SF-2)4.5.51	Times Calculated:	9
Operator (Inj):	jo	Calculation Method:	c:\labchem07\new method jo-1.2.mth
Injection Date:	05/02/2008 15:08:33	Instrument (Calc):	GC3800
Injection Method:	c:\labchem07\new method jo-1.2.mth	Run Mode:	Analysis
Run Time (min):	59.440	Peak Measurement:	Peak Area
Workstation:		Calculation Type:	Percent
Instrument (Inj):	GC3800	Calibration Level:	N/A
		Verification Tolerance:	N/A



Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C14:0	0.1314	30.891	-0.012	5203	0.00	BB	4.1		0
2	C16:0	13.6154	35.694	0.008	539286	0.00	BB	4.4		0
3	C16:1	0.1374	36.229	-0.037	5443	0.00	VB	4.3		0
4	Ins C17:0	18.7661	37.944	0.006	743297	0.00	BB	4.8		0
5	C18:0	1.3052	40.038	-0.037	51698	0.00	PV	4.6		0
6	Unk	0.2069	40.154	-0.000	8194	0.00	VV	6.0		0
7	C18:1n9c	5.4176	40.455	-0.011	214584	0.00	VV	4.7		0
8	C18:1n9t	1.1411	40.599	-0.000	45199	0.00	VV	4.7		0
9	C18:2n6c	7.6478	41.425	-0.056	302918	0.00	VV	5.5		0
10	Unk	3.1362	41.537	0.000	124219	0.00	VP	10.8		0
11	C18:3n6	0.2029	42.115	-0.014	8038	0.00	PV	0.0		0
12	Unk	4.9422	42.451	-0.000	195753	0.00	VV	6.8		0
13	Unk	0.6822	42.653	0.000	27021	0.00	VV	7.4		0
14	C18:3n3	0.5467	42.754	-0.030	21656	0.00	VV	0.0		0
15	Unk	0.1791	42.858	0.000	7095	0.00	VV	0.0		0
16	Unk	41.9417	43.606	-0.000	1661249	0.00	VB	10.9		0
	Totals	**99.9999**		**-0.183**	**3960853**					

Figure A123 GC chromatogram of SF oil methyl ester.

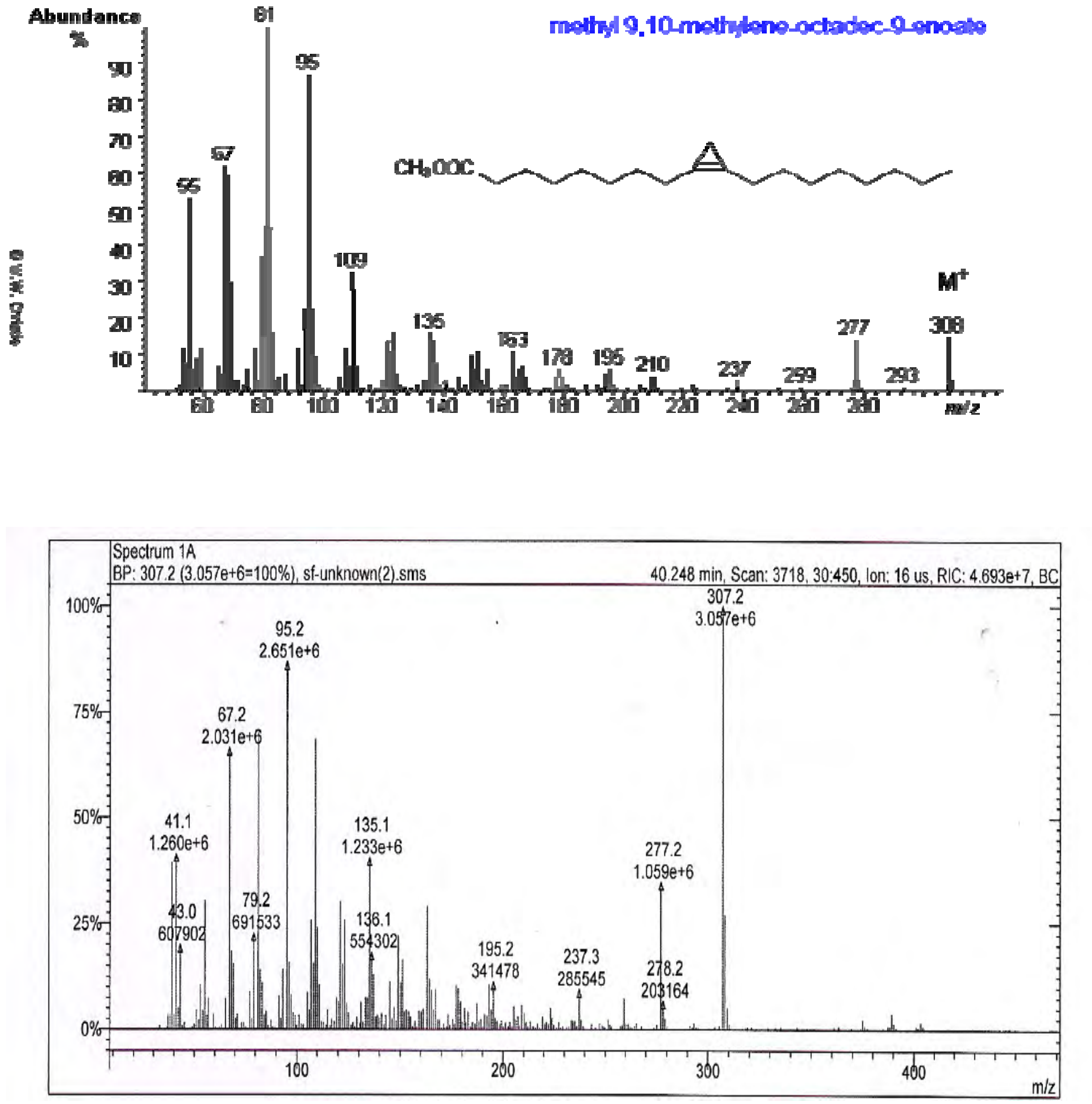
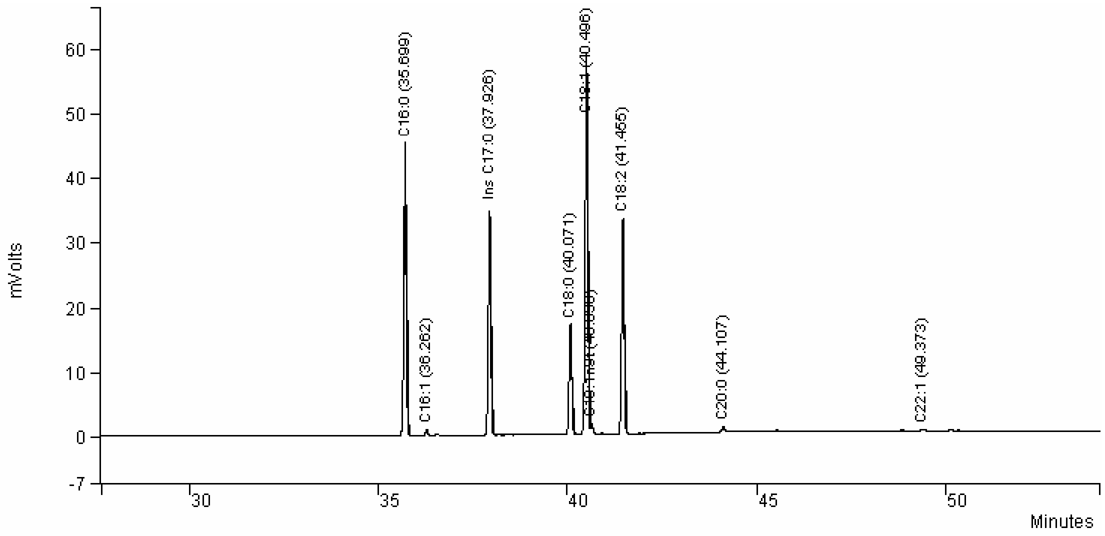


Figure A123.1 GC-MS chromatogram of SF oil methyl ester at retention time of 43.606 minutes.

Data File:	c:\star\joe\me(tb) 7;01;30 pm.run	Operator (Calc):	jo
Channel:	Front = FID RESULTS	Calc Date:	25/12/2007 21:21:14
Sample ID:	ME(TB)	Times Calculated:	4
Operator (Inj):	jo	Calculation Method:	c:\labchem07\new method jo-1.2.mth
Injection Date:	25/12/2007 19:01:30	Instrument (Calc):	GC3800
Injection Method:	c:\labchem07\new method jo-1.2.mth	Run Mode:	Analysis
Run Time (min):	59.440	Peak Measurement:	Peak Area
Workstation:		Calculation Type:	Percent
Instrument (Inj):	GC3800	Calibration Level:	N/A
		Verification Tolerance:	N/A

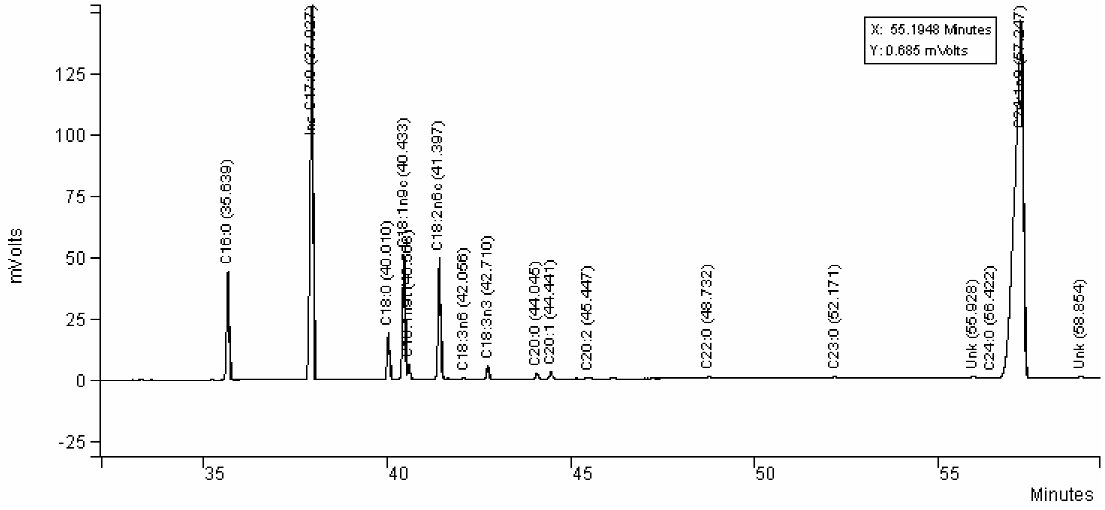


Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C16:0	22.7455	35.699	0.013	202642	0.00	BB	4.2		0
2	C16:1	0.4097	36.262	-0.004	3650	0.00	BB	4.2		0
3	Ins C17:0	17.7949	37.926	-0.012	158537	0.00	BB	4.3		0
4	C18:0	9.3826	40.071	-0.004	83590	0.00	BB	4.6		0
5	C18:1	31.0724	40.496	-0.010	276828	0.00	BB	4.4		0
6	C18:1n9t	0.4156	40.630	-0.005	3702	0.00	TS	0.0		0
7	C18:2	17.4923	41.455	-0.026	155841	0.00	BB	4.4		0
8	C20:0	0.4417	44.107	-0.010	3936	0.00	BB	4.5		0
9	C22:1	0.2454	49.373	-0.124	2186	0.00	BB	7.2		0
	Totals	**100.0001**		**-0.182**	**890912**					

Figure A124 GC chromatogram of TB oil methyl ester.

Data File: c:\star\joe\me wt-7 5;59;56 am.run
Channel: Front = FID RESULTS
Sample ID: ME WT-7
Operator (Inj): io
Injection Date: 11/02/2008 05:59:56
Injection Method: c:\labchem07\new method jo-1.2.mth
Run Time (min): 59.440
Workstation:
Instrument (Inj): GC3800

Operator (Calc): jo
Calc Date: 24/02/2008 06:38:56
Times Calculated: 4
Calculation Method: c:\labchem07\new method jo-1.2.mth
Instrument (Calc): GC3800
Run Mode: Analysis
Peak Measurement: Peak Area
Calculation Type: Percent
Calibration Level: N/A
Verification Tolerance: N/A

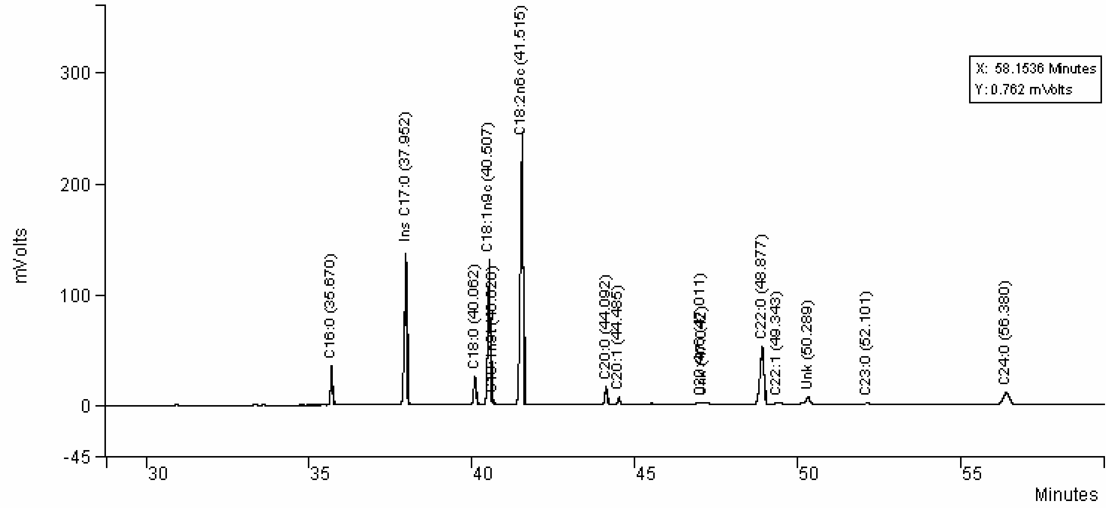


Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C16:0	4.8008	35.639	-0.047	197753	0.00	BB	4.2		0
2	Ins C17:0	20.4541	37.927	-0.011	842541	0.00	BB	5.0		0
3	C18:0	2.2055	40.010	-0.065	90848	0.00	BB	4.4		0
4	C18:1n9c	5.9889	40.433	-0.033	246692	0.00	BV	4.5		0
5	C18:1n9t	0.6572	40.568	-0.000	27071	0.00	VB	4.3		0
6	C18:2n6c	5.7761	41.397	-0.084	237926	0.00	BB	4.4		0
7	C18:3n6	0.0888	42.056	-0.073	3659	0.00	BB	4.6		0
8	C18:3n3	0.6307	42.710	-0.074	25979	0.00	VB	4.3		0
9	C20:0	0.2696	44.045	-0.072	11105	0.00	BB	4.5		0
10	C20:1	0.3466	44.441	-0.077	14278	0.00	BB	4.6		0
11	C20:2	0.0639	45.447	-0.080	2631	0.00	BB	5.6		0
12	C22:0	0.1223	48.732	-0.109	5037	0.00	BB	7.0		0
13	C23:0	0.2269	52.171	0.021	9348	0.00	BB	9.9		0
14	Unk	0.2740	55.928	-0.096	11288	0.00	BV	14.6		0
15	C24:0	0.1064	56.422	0.024	4382	0.00	VV	21.4		0
16	C24:1n9	57.7464	57.247	-0.319	2378678	0.00	VB	15.4		0
17	Unk	0.2418	58.854	-0.126	9962	0.00	VB	13.3		0
	Totals	**100.0000**		**-1.221**	**4119178**					

Figure A125 GC chromatogram of WT oil methyl ester.

Data File: c:\star\joe\me(xk) 6;38;07 pm.run
Channel: Front = FID RESULTS
Sample ID: ME(XK)
Operator (Inj): io
Injection Date: 05/01/2008 18:38:07
Injection Method: c:\labchem07\new method jo-1.2.mth
Run Time (min): 59.440
Workstation:
Instrument (Inj): GC3800

Operator (Calc): jo
Calc Date: 24/02/2008 03:34:14
Times Calculated: 13
Calculation Method: c:\labchem07\new method jo-1.2.mth
Instrument (Calc): GC3800
Run Mode: Analysis
Peak Measurement: Peak Area
Calculation Type: Percent
Calibration Level: N/A
Verification Tolerance: N/A



Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C16:0	4.1759	35.670	-0.016	161940	0.00	BB	4.2		0
2	Ins C17:0	17.5604	37.952	0.014	680986	0.00	BB	4.6		0
3	C18:0	3.4321	40.062	-0.013	133094	0.00	BB	4.8		0
4	C18:1n9c	17.4384	40.507	0.009	676254	0.00	BB	4.7		0
5	C18:1n9t	0.2236	40.620	0.000	8673	0.00	TS	0.0		0
6	C18:2n6c	36.3522	41.515	0.034	1409729	0.00	BB	5.4		0
7	C20:0	2.0463	44.092	-0.025	79356	0.00	BB	4.5		0
8	C20:1	0.7806	44.485	-0.033	30273	0.00	BB	4.6		0
9	C20:4n6	0.4363	47.011	0.011	16920	0.00	BV	14.6		0
10	Unk	0.5370	47.042	-0.000	20826	0.00	VB	18.3		0
11	C22:0	11.2965	48.877	0.036	438074	0.00	BP	7.9		0
12	C22:1	0.4721	49.343	-0.154	18308	0.00	PB	0.0		0
13	Unk	1.3981	50.289	-0.024	54218	0.00	VB	7.8		0
14	C23:0	0.2306	52.101	-0.049	8942	0.00	BB	9.8		0
15	C24:0	3.6199	56.380	-0.018	140378	0.00	BB	12.5		0
	Totals	**100.0000**		**-0.228**	**3877971**					

Figure A126 GC chromatogram of XK oil methyl ester.

APPENDIX B

VALUES OF FREE FATTY ACID, SAPONIFICATION NUMBER, IODINE VALUE, ACID VALUE, VISCOSITY and METHYL ESTER CONTENT

Table B1 Values of Free Fatty Acids contain in crude oils

Sample	Weight of sample (g)	Volume of titrant (ml)	Concentration of NaOH (N)	% FFA
Acacia auriculiformis (AA oil)	2.0103	0.8	0.25	1.65
	2.0084	0.75	0.25	
Acacia mangium (AM oil)	2.5012	2.25	0.25	6.33
	2.5083	2.25	0.25	
Annona squamosa Linn. (AS oil)	2.0045	0.39	0.25	1.38
	2.0015	0.39	0.25	
Azadirachta indica Valeton (AI oil)	2.0038	3.71	0.25	13.05
	2.0054	3.71	0.25	
Calophyllum inophyllum (CI oil)	2.0600	5.66	0.25	18.66
	2.0540	5.22	0.25	
Canarium subulatum (CS oil)	2.0056	0.5	0.25	1.67
	2.0070	0.45	0.25	
Carica papaya L.(CAP oil)	2.0030	0.25	0.1	0.35
	2.0026	0.25	0.1	
Ceiba pentandra Gaertn. (CP oil)	2.0500	3.69	0.25	12.69
	2.0080	3.61	0.25	
Croton tiglium L.(CT oil)	2.0065	7.18	0.25	25.23
	2.0150	7.21	0.25	
Eucalyptus camaldulensis (EA oil)	2.050	4.13	0.25	14.21
	2.0520	4.14	0.25	
Gmelina arborea Roxb (GA oil)	2.0230	1.26	0.25	4.38
	2.0044	1.25	0.25	
Hevea brasiliensis (HB oil)	2.1316	2.79	0.25	9.25
	2.0594	2.7	0.25	
Hydnocarpus ilicifolius (HI oil)	2.5038	5.0	0.25	14.47
	2.5124	5.3	0.25	
Hyptis suaveolens (L.) Poit (HS oil)	1.9380	0.39	0.25	1.45
	2.0100	0.41	0.25	

Table B1 Values of Free Fatty Acids contain in crude oils *(continued)*

Sample	Weight of sample (g)	Volume of titrant (ml)	Concentration of NaOH (N)	% FFA
Irvingia malayana (IM oil)	2.0400	0.3	0.25	1.03
	2.0560	0.3	0.25	
Litsea glutinosa Robins (LG oil)	1.9860	0.28	0.25	1
	1.9940	0.28	0.25	
Melia azadirachta L. (MA oil)	2.0572	2.26	0.25	7.76
	2.0900	2.3	0.25	
Parinari anamense (PA oil)	2.0182	0.7	0.25	2.45
	2.0165	0.7	0.25	
Pinus kesiya (PK oil)	2.5083	1.8	0.25	5.06
	2.5036	1.8	0.25	
Pinus merkusii (PM oil)	2.0182	0.6	0.25	2.1
	2.0120	0.6	0.25	
Sapindus emarginatus (SE oil)	2.0730	1.32	0.25	4.5
	2.1588	1.38	0.25	
Sterculia foetida L. (SF oil)	2.0652	0.5	0.25	1.71
	2.0820	0.5	0.25	
Terminalia bellirica (TB oil)	2.5122	0.4	0.25	1.11
	2.5031	0.39	0.25	
Wrightia tomentosa (WT oil)	2.4664	0.36	0.25	1.01
	2.5065	0.36	0.25	
Xylia kerrii (XK oil)	2.0412	0.39	0.25	1.34
	2.1902	0.42	0.25	

Table B2 Saponification value of crude oils

Sample	Weight of sample (g)	Volume of titrant (ml)	Volume of blank (ml)	SN
Acacia auriculiformis (AA oil)	2.0024	18.4	30.9	179.59^a
	2.0121	18.2	30.9	
Acacia mangium (AM oil)	2.0186	17.8	30.9	185.60^a
	2.0202	17.8	30.9	
Annona squamosa Linn. (AS oil)	2.0100	18.4	31.4	178.30^b
	2.0600	18	31.4	
Azadirachta indica Valeton (AI oil)	2.0360	18.4	30.9	176.48^a
	2.0331	18.3	30.9	
Calophyllum inophyllum (CI oil)	2.0065	18.8	31.3	176.46^a
	2.0473	18.8	31.3	
Canarium subulatum (CS oil)	2.0101	17.9	30.9	184.98^a
	2.0111	17.9	30.9	
Carica papaya L.(CAP oil)	2.0200	18.2	31.4	178.41^b
	2.0400	18.25	31.4	
Ceiba pentandra Gaertn. (CP oil)	2.0200	18.1	31.4	182.35^b
	2.0500	17.7	31.4	
Croton tiglium L.(CT oil)	2.0350	16.4	30.9	203.92^a
	2.0338	16.4	30.9	
Eucalyptus camaldulensis (EA oil)	2.0500	18.6	31.4	172.41^b
	2.0000	18.8	31.4	
Gmelina arborea Roxb (GA oil)	2.0111	14.6	30.9	175.55^a
	2.0047	16.2	30.9	
Hevea brasiliensis (HB oil)	2.2800	16.1	29.1	182.05^b
	2.0800	14	29.1	
Hydnocarpus ilicifolius (HI oil)	2.0111	16.7	31.3	213.68^a
	2.0045	16.85	31.3	
Hyptis suaveolens (L.) Poit (HS oil)	2.0800	16.1	29.1	171.81^b
	2.0900	16.05	29.1	

****** *X^a was titrate with 0.51 N HCL*
X^b was titrate with 0.49 N HCL

Table B2 Saponification value of crude oils *(continued)*

Sample	Weight of sample (g)	Volume of titrant (ml)	Volume of blank (ml)	SN
Irvingia malayana (IM oil)	2.0111	16.70	31.3	206.97[a]
	2.0045	16.85	31.3	
Litsea glutinosa Robins (LG oil)	2.0100	13.7	31.4	240.38[b]
	2.0500	13.6	31.4	
Melia azadirachta L. (MA oil)	2.0117	18.3	30.9	179.76[a]
	2.0150	18.2	30.9	
Parinari anamense (PA oil)	2.0210	17.5	30.9	188.61[a]
	2.0291	17.6	30.9	
Pinus kesiya (PK oil)	2.0892	17.8	31.3	183.50[a]
	2.0910	17.9	31.3	
Pinus merkusii (PM oil)	2.0095	16.2	30.9	210.16[a]
	2.0064	16.1	30.9	
Sapindus emarginatus (SE oil)	2.0262	17.9	30.9	183.56[a]
	2.0106	18	30.9	
Sterculia foetida L. (SF oil)	2.0388	18.9	30.9	168[a]
	2.0312	19	30.9	
Terminalia bellirica (TB oil)	2.0315	19	30.9	167.65[a]
	2.0140	19.2	30.9	
Wrightia tomentosa (WT oil)	2.0253	19.8	31.3	160.84[a]
	2.0395	19.95	31.3	
Xylia kerrii (XK oil)	2.0350	18.5	31.3	179.49[a]
	2.0295	18.6	31.3	

****** *X^a was titrate with 0.51 N HCL*
X^b was titrate with 0.49 N HCL

Table B3 Iodine value of crude oils

Sample	Weight of sample (g)	Volume of titrant (ml)	Volume of blank (ml)	IV
Acacia auriculiformis (AA oil)	0.1322	29.1	41.3	121.31^{a}
	0.1238	28	41.3	
Acacia mangium (AM oil)	0.1133	29.1	41.3	129.29^{a}
	0.1157	29.2	41.3	
Annona squamosa Linn. (AS oil)	0.1346	30.1	38.1	74.89^{c}
	0.1341	30.4	38.1	
Azadirachta indica Valeton (AI oil)	0.1213	33.1	41.3	100.56^{a}
	0.1210	32.9	41.3	
Calophyllum inophyllum (CI oil)	0.1318	30	41.3	93.45^{a}
	0.1297	30	41.3	
Canarium subulatum (CS oil)	0.1356	30.8	43.3	116.24^{b}
	0.1300	31.22	43.3	
Carica papaya L.(CAP oil)	0.1200	42.1	49.6	75.29^{d}
	0.1298	42.3	49.6	
Ceiba pentandra Gaertn. (CP oil)	0.1490	37.65	49.6	102.77^{d}
	0.1333	38.7	49.6	
Croton tiglium L.(CT oil)	0.1172	30	41.3	113.17^{a}
	0.1133	33.2	41.3	
Eucalyptus camaldulensis (EA oil)	0.1304	24.4	38.1	127.29^{c}
	0.1401	25	38.1	
Gmelina arborea Roxb (GA oil)	0.1187	33.5	43.3	103.75^{b}
	0.1271	32.8	43.3	
Hevea brasiliensis (HB oil)	0.1140	32.9	43.3	117.29^{b}
	0.1137	32.88	43.3	
Hydnocarpus ilicifolius (HI oil*)*	0.1108	34.8	41.3	72.29^{a}
	0.1183	34.2	41.3	
Hyptis suaveolens (L.) Poit (HS oil)	0.1150	37.8	49.6	135.83^{a}
	0.1183	37.78	49.6	

****** *X^a was titrate with 0.096 N $Na_2S_2O_3$*
X^b was titrate with 0.099 N $Na_2S_2O_3$
X^c was titrate with 0.101 N $Na_2S_2O_3$
X^d was titrate with 0.102 N $Na_2S_2O_3$
X^e was titrate with 0.105 N $Na_2S_2O_3$

Table B3 Iodine value of crude oils *(continued)*

Sample	Weight of sample (g)	Volume of titrant (ml)	Volume of blank (ml)	IV
Irvingia malayana (IM oil)	0.1031	40.3	40.7	5.16e
	0.1035	40.3	40.7	
Litsea glutinosa Robins (LG oil)	0.1511	47.85	49.6	15.28d
	0.1120	48.2	49.6	
Melia azadirachta L. (MA oil)	0.1295	30.5	43.3	124.45b
	0.1259	30.8	43.3	
Parinari anamense (PA oil)	0.1236	27.6	40.7	140.97a
	0.1231	27.7	40.7	
Pinus kesiya (PK oil)	0.1133	28.1	41.3	143.20a
	0.1130	28.1	41.3	
Pinus merkusii (PM oil)	0.1501	34.2	49.6	134.93d
	0.1103	37.5	49.6	
Sapindus emarginatus (SE oil)	0.1174	34.2	41.3	91.48a
	0.1130	34.5	41.3	
Sterculia foetida L. (SF oil)	0.1529	31.8	40.7	76.46e
	0.1556	31.9	40.7	
Terminalia bellirica (TB oil)	0.1218	33	41.3	81.36a
	0.1284	32.9	41.3	
Wrightia tomentosa (WT oil)	0.1053	32.7	43.3	126.22b
	0.1087	32.4	43.3	
Xylia kerrii (XK oil)	0.1763	27.8	40.7	97.67a
	0.1743	27.9	40.7	

****** *X^a was titrate with 0.096 N $Na_2S_2O_3$*
X^b was titrate with 0.099 N $Na_2S_2O_3$
X^c was titrate with 0.101 N $Na_2S_2O_3$
X^d was titrate with 0.102 N $Na_2S_2O_3$
X^e was titrate with 0.105 N $Na_2S_2O_3$

Table B4 Acid value of biodiesels

Sample	Weight of sample (g)	Volume of titrant (ml)	Volume of blank (ml)	AV
Acacia auriculiformis (AA oil)	2.1184	0.04	0.02	0.0519
	2.1174	0.04	0.02	
Acacia mangium (AM oil)	2.1202	0.05	0.02	0.0791
	2.1205	0.05	0.02	
Annona squamosa Linn. (AS oil)	2.0133	0.08	0.02	0.1638
	2.0125	0.08	0.02	
Azadirachta indica Valeton (AI oil)	2.0064	0.1	0.02	0.2192
	2.0056	0.1	0.02	
Calophyllum inophyllum (CI oil)	2.0413	0.08	0.02	0.1885
	2.0302	0.09	0.02	
Canarium subulatum (CS oil)	2.1706	0.06	0.02	0.1034
	2.1692	0.06	0.02	
Carica papaya L.(CAP oil)	2.0133	0.08	0.02	0.1638
	2.0128	0.08	0.02	
Ceiba pentandra Gaertn. (CP oil)	2.0678	0.05	0.02	0.0798
	2.0674	0.05	0.02	
Croton tiglium L.(CT oil)	2.0366	0.2	0.02	0.4870
	2.0267	0.2	0.02	
Eucalyptus camaldulensis (EA oil)	2.0026	0.04	0.02	0.0549
	2.0020	0.04	0.02	
Gmelina arborea Roxb (GA oil)	2.0086	0.04	0.02	0.0547
	2.0082	0.04	0.02	
Hevea brasiliensis (HB oil)	2.0598	0.06	0.02	0.1077
	2.0259	0.06	0.02	
Hydnocarpus ilicifolius (HI oil)	2.1178	0.06	0.02	0.1038
	2.1112	0.06	0.02	
Hyptis suaveolens (L.) Poit (HS oil)	1.9146	0.09	0.03	0.1723
	1.9122	0.09	0.03	

Table B4 **Acid value of biodiesels** *(continued)*

Sample	Weight of sample (g)	Volume of titrant (ml)	Volume of blank (ml)	IV
Irvingia malayana (IM oil)	2.0476	0.03	0.02	0.0268
	2.0468	0.03	0.02	
Litsea glutinosa Robins (LG oil)	2.0026	0.05	0.02	0.0824
	2.0008	0.05	0.02	
Melia azadirachta L. (MA oil)	2.0145	0.05	0.02	0.0825
	2.0150	0.05	0.02	
Parinari anamense (PA oil)	2.1202	0.05	0.02	0.0778
	2.1210	0.05	0.02	
Pinus kesiya (PK oil)	2.0194	0.05	0.02	0.0817
	2.0200	0.05	0.02	
Pinus merkusii (PM oil)	2.0950	0.025	0.02	0.0131
	2.0964	0.026	0.02	
Sapindus emarginatus (SE oil)	2.0177	0.06	0.02	0.1089
	2.0188	0.06	0.02	
Sterculia foetida L. (SF oil)	2.0213	0.04	0.02	0.0816
	2.0220	0.04	0.02	
Terminalia bellirica (TB oil)	1.0721	0.04	0.02	0.1026
	1.0740	0.04	0.02	
Wrightia tomentosa (WT oil)	2.0026	0.08	0.02	0.1647
	2.0020	0.08	0.02	
Xylia kerrii (XK oil)	2.0322	0.04	0.02	0.0541
	2.0318	0.04	0.02	

Table B5 Viscosity of biodiesels

Sample	Time 1 (s)	Time 2 (s)	Constants of tube	Viscosiy (cSt)
Acacia auriculiformis (AA oil)	303.56	303.04	0.01434[a]	4.3
Acacia mangium (AM oil)	302.28	302.84	0.01434[a]	4.3
Annona squamosa Linn. (AS oil)	347.41	347.32	0.01434[a]	4.9
Azadirachta indica Valeton (AI oil)	543.95	544.1	0.01434[a]	7.8
Calophyllum inophyllum (CI oil)	350.65	350	0.01434[a]	5
Canarium subulatum (CS oil)	258.5	258.37	0.01434[a]	3.7
Carica papaya L.(CAP oil)	331.09	331.19	0.01434[a]	4.7
Ceiba pentandra Gaertn. (CP oil)	301.13	300.88	0.01434[a]	4.3
Croton tiglium L.(CT oil)	830.47	829.75	0.00757[b]	6.3
Eucalyptus camaldulensis (EA oil)	298.1	297.56	0.01434[a]	4.3
Gmelina arborea Roxb (GA oil)	620.72	620.41	0.00757[b]	4.6
Hevea brasiliensis (HB oil)	400.6	400.72	0.01434[a]	5.7
Hydnocarpus ilicifolius (HI oil)	369.94	370.03	0.01434[a]	5.3
Hyptis suaveolens (L.) Poit (HS oil)	280.59	281.06	0.01434[a]	4

****** *X^a = constant values of no.100 of viscometer tube*
X^b = constant values of no.150 of viscometer tube
X^c = constant values of no.75 of viscometer tube

Table B5 Viscosity of biodiesels *(continued)*

Sample	Time 1 (s)	Time 2 (s)	Constants of tube	Viscosity (cSt)
Irvingia malayana (IM oil)	401.08	401.45	0.00757[b]	3
Litsea glutinosa Robins (LG oil)	393.4	393.38	0.00757[b]	3
Melia azadirachta L. (MA oil)	938.38	938.1	0.00757[b]	7.1
Parinari anamense (PA oil)	646.62	646.59	0.01434[a]	9.3
Pinus kesiya (PK oil)	614.5	613.99	0.00757[b]	4.6
Pinus merkusii (PM oil)	286.44	285.1	0.01434[a]	4.1
Sapindus emarginatus (SE oil)	354.94	354.97	0.01434[a]	5
Sterculia foetida L. (SF oil)	346.09	345.84	0.01434[a]	4.9
Terminalia bellirica (TB oil)	774.12	774.78	0.00757[b]	5.8
Wrightia tomentosa (WT oil)	486.41	486.25	0.0337[c]	16.4
Xylia kerrii (XK oil)	365.78	365.75	0.01434[a]	5.2

****** X^a *= constant values of no.100 of viscometer tube*
X^b *= constant values of no.150 of viscometer tube*
X^c *= constant values of no.75 of viscometer tube*

Table B6 Methyl ester content of biodiesels

Codes	C17:0 (mg)	Oil (mg)	Area FAME	Area Ins.	%Ester
AA	501.97	270.45	5287321	867009	94.628
AI	521.86	243.75	1132549	228779	84.576
AM	491.04	245.28	3249980	567606	94.607
AS	515.25	277.35	4292621	714136	93.091
CAP	501.97	265.31	2101895	352168	94.003
CI	521.86	250.75	2301446	422190	92.638
CP	501.97	248.82	1413928	249440	94.180
CS	501.97	249.3	3810499	689135	91.200
CT	521.86	244.57	2630930	519834	86.655
EA	501.97	262.51	6358971	1065364	95.013
GA	515.25	275.45	1940714	325543	92.807
HB	521.86	84.35	5621010	664297	92.327[a]
HI	521.86	263.14	4134623	712833	95.199
HS	501.97	263.57	1074057	182770	92.874
IM	515.25	234.24	5412834	1041841	92.285
LG	501.97	257.44	2595618	452786	92.277
MA	515.25	225.74	2945889	594227	90.329
PA	521.86	234.42	3567558	794399	77.713
PK	501.97	270.46	4036394	672010	92.919
PM	515.25	265.32	4916024	877552	89.370
SE	515.25	268.94	5888112	984713	95.400
SF	515.25	252.89	3960853	743297	88.196
TB	515.25	257.19	890912	158537	92.547
WT	501.97	250.3	4119178	842541	77.992
XK	501.97	255.99	3877971	680986	92.057

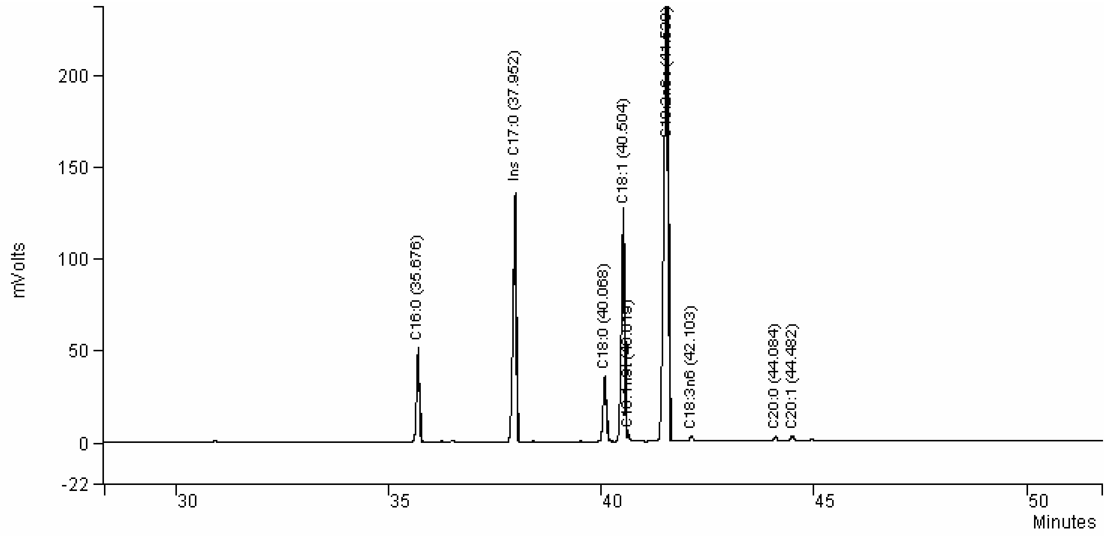
******X[a] = used C17 solution 1 ml dissolved oil(84.35 mg)

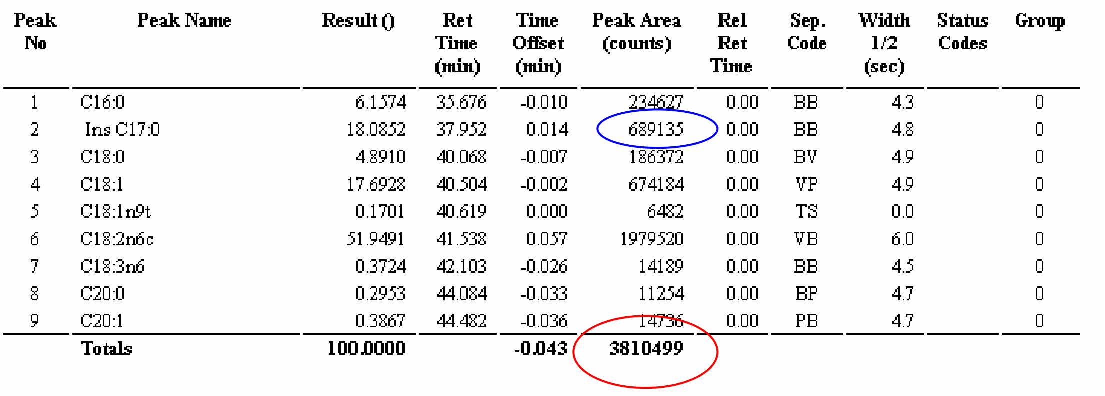
APPENDIX C

CALCULATIONS

A. Calculated % Methyl ester content from GC

% Methyl ester content from GC of CS oil methyl ester was calculated as follow:





Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	C16:0	6.1574	35.676	-0.010	234627	0.00	BB	4.3		0
2	Ins C17:0	18.0852	37.952	0.014	689135	0.00	BB	4.8		0
3	C18:0	4.8910	40.068	-0.007	186372	0.00	BV	4.9		0
4	C18:1	17.6928	40.504	-0.002	674184	0.00	VP	4.9		0
5	C18:1n9t	0.1701	40.619	0.000	6482	0.00	TS	0.0		0
6	C18:2n6c	51.9491	41.538	0.057	1979520	0.00	VB	6.0		0
7	C18:3n6	0.3724	42.103	-0.026	14189	0.00	BB	4.5		0
8	C20:0	0.2953	44.084	-0.033	11254	0.00	BP	4.7		0
9	C20:1	0.3867	44.482	-0.036	14736	0.00	PB	4.7		0
	Totals	**100.0000**		**-0.043**	**3810499**					

$$C = \frac{(\sum A - Ai)}{Ai} \times \frac{(Ci \times Vi)}{m} \times 100$$

C = Methyl ester content

∑A = Total area of fatty acid methyl esters

Ai = Area of methyl heptadecanoate

Ci = Concentration of methyl heptadecanoate solution

Vi = Volume of methyl heptadecanoate solution

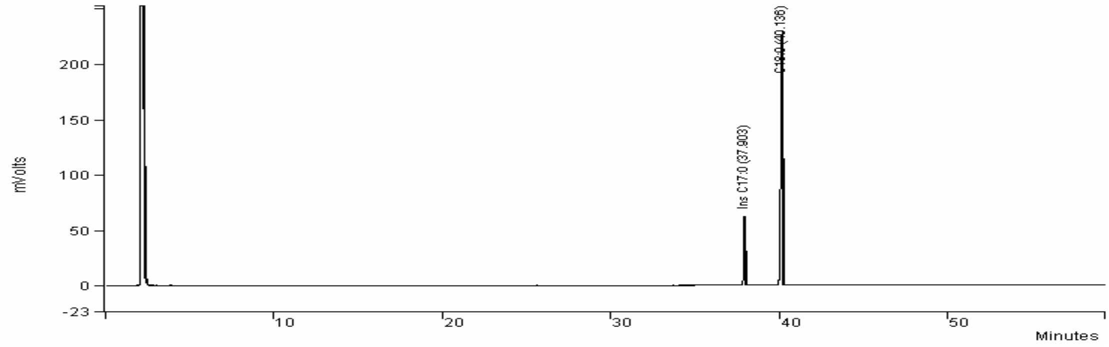
m = Mass of the sample

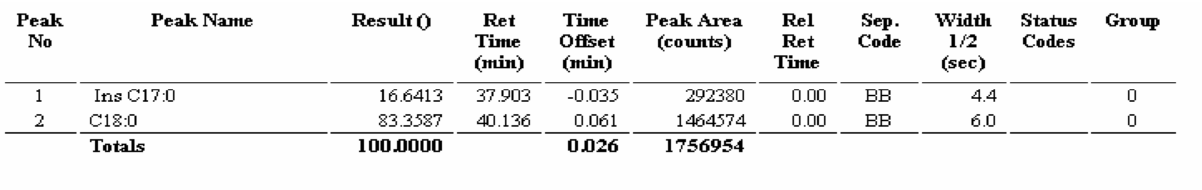
$$\text{C of CS oil} = \frac{(3810499 - 689135)}{689135} \times \frac{(10.0394 \times 5)}{249.25} \times 100$$

$$= 91.21\%$$

B. ***% precision from GC***

Methyl sterate (250 mg) was dissolved in heptadecanoic solution (5 ml) (500 mg heptadecanoate dissolved in heptane (50 ml)) and 1 µl of solution was injected in to GC using 1:100 split ratios. The GC condition for the determination of methyl ester was set as 3.2.2.4. Methyl heptadecanoate was used as an internal standard. The methyl ester content of biodiesel was calculated by the following equation:





Peak No	Peak Name	Result ()	Ret Time (min)	Time Offset (min)	Peak Area (counts)	Rel Ret Time	Sep. Code	Width 1/2 (sec)	Status Codes	Group
1	Ins C17:0	16.6413	37.903	-0.035	292380	0.00	BB	4.4		0
2	C18:0	83.3587	40.136	0.061	1464574	0.00	BB	6.0		0
	Totals	**100.0000**		**0.026**	**1756954**					

$$\text{C of Methyl Stearate} = \frac{(\sum A - Ai)}{Ai} \times \frac{(Ci \times Vi)}{m} \times 100$$

$$= \frac{(1756954-292380)}{292380} \times \frac{(10.0346 \times 5)}{254.45} \times 100$$

$$= 98.77\ \%$$

% precision = 98.77 × purity of C18 (99.5%)

= 99.26 %

C. *Calculated % methyl ester of transesterification from ^{1}H-NMR spectrum*

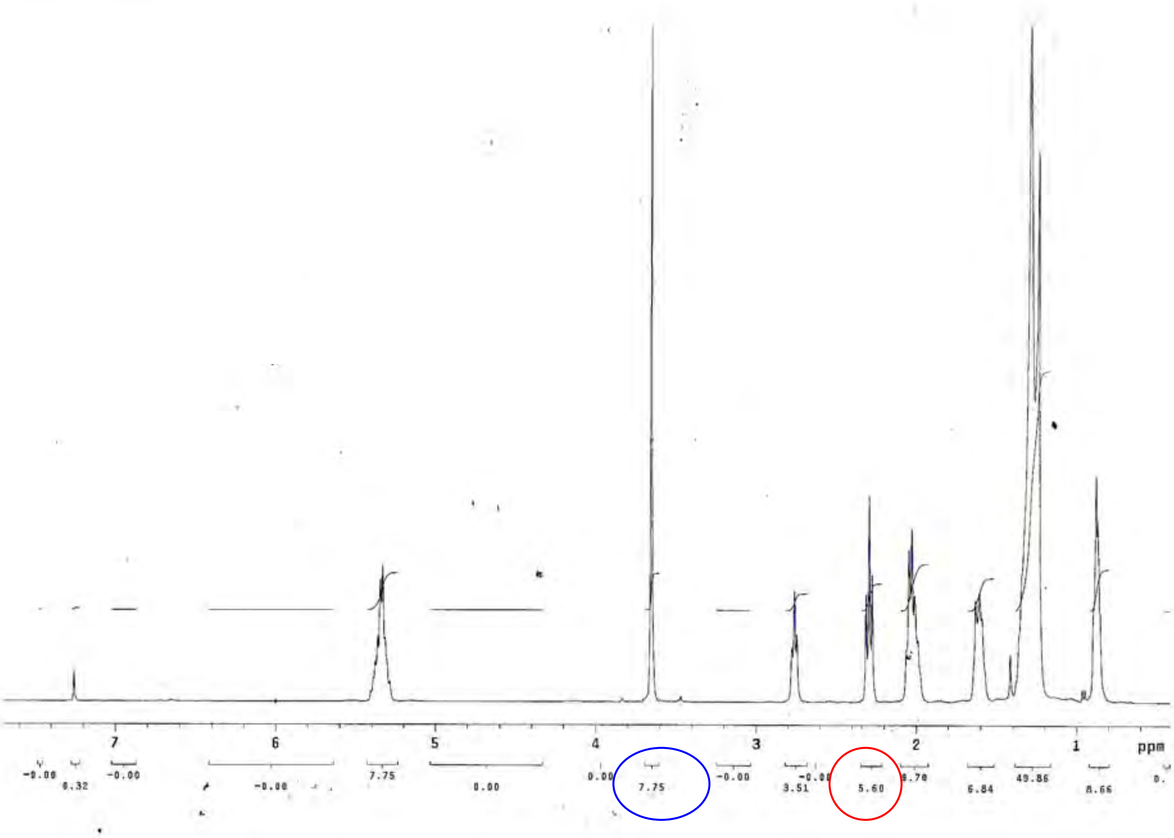
The % methyl ester of transesterification was calculated in following:

$$\boxed{\% \text{ Methyl ester} = [(2I_{Me}) / (3I_{CH2})] \times 100}$$

I_{Me} = Integration value of the protons of the methyl esters (the strong singlet), appear at δ 3.7 ppm

I_{CH2} = Integration value of the methylene protons, appear at δ 2.3 ppm

Sample: CS oil methyl ester



$$\% \text{ Methyl ester of CS oil} = [(2\times 7.75) / (3\times 5.6)] \times 100$$

$$= 92.26\ \%$$

D. Determine the % free fatty acid (ASTM D5555)

Reagent

1. Ethanol
2. Phenolphthalein
3. 0.25 N NaOH

To the 250 ml of Erlenmeyer flask, oil sample (2 g), ethanol (75 ml) and 2ml of 1% phenolphthalein were added. The mixture was subject to titrate with 0.25 N sodium hydroxide solutions until the pink color was occurred. The ml of alkali solution used was recorded.

The percentage of free fatty acids was calculated as follows:

% free fatty acids = (ml of alkali x N x 28.2) / weight of sample
N = normality of alkaline solution
ml of alkali = ml of sodium hydroxide solution

Example:

$$\% \text{ FFA of CS oil} = \frac{0.5 \times 0.25 \times 28.2}{2.0056}$$

$$= 1.75\ \%$$

E. Determination of the Saponification value (ASTM D5558)

Reagent

1. Phenolphthalein
2. Alcoholic KOH solution
3. 0.5 N HCl

To the 250 ml of Erlenmeyer flask, oil sample (2 g), alcoholic potassium hydroxide (50 ml) and 1 ml of 1% phenolphthalein were added. The mixture was subject to titrate with 0.5 N of hydrochloric acid until the pink color has disappeared. Prepare a blank determination and carried out same with the sample. The ml of acid solution used was recorded.

The saponification value was calculated as follows:

saponification value = 28.05 × (A – B) / weight of sample

A = titration of blank

B = titration of sample

Alcoholic KOH = 40 g of potassium hydroxide dissolved in 1 L of ethanol

Example:

$$\text{SN of CS oil} = \frac{28.05 \times (30.9-17.9)}{2.0101}$$

$$= 181.41 \text{ mg KOH/g of oil}$$

F. Determination of the Iodine value (ASTM D5554)

Reagent

1. KOH solution
2. Wijs solution
3. CCl_4
4. 0.1 N $Na_2S_2O_3$
5. Starch

To the 500 ml of Glass-Stopper flask, oil sample (0.1 g), carbon tetra chloride (20 ml) and Wijs solution (25 ml) were added. Store the flasks in a dark place for 30 min. From storage, removed the flasks and add 20 mL of KI solution followed by 100 ml of distilled water. The mixture was subject to titrate with 0.1 N of sodium thiosulfate until the yellow color has almost disappeared, add 2 ml of starch indicator solution, and continued the titration until the blue color has just disappeared. Prepare a blank determination and carried out same with the sample. The ml of sodium thiosulfate used was recorded.

The iodine value was calculated as follows:

Iodine value = $(B - S) \times N \times 12.69$ / weight of sample

B = titration of blank

S = titration of sample

N = normality of Na2S2O3 solution

Example:

$$\text{IV of CS oil} = \frac{(43.3\text{-}30.8) \times 0.099 \times 12.69}{0.1356}$$

$$= 115.81 \text{ mg } I_2 \text{ /g of oil}$$

G. Determination of the acid value (ASTM D974)

Reagent

1. p-naphtholbenzein indicator solution
2. 0.1 M Alcoholic KOH solution
3. Titration solvent (250 ml toluene + 250 ml isopropyl alcohol)

To the 250 ml of Erlenmeyer flask, oil sample (2 g), titration solvent (25 ml) and 0.125 ml of 1% p-naphtholbenzein indicator solution were added. The mixture was subject to titrate with 0.1 M of alcoholic KOH until the green color was occured. Prepare a blank determination and carried out same with the sample. The ml of acid solution used was recorded.

The acid value was calculated as follows:

Acid value = $[(A - B) \times M \times 56.1]$ / weight of sample
A = titration of sample
B = titration of blank
Alcoholic KOH = 0.6 g of potassium hydroxide dissolved in 100 ml of isopropyl alcohol

Example:

$$\text{Acid value of CS oil methyl ester} = \frac{[(0.06\text{-}0.02) \times 0.1 \times 56.1]}{2.1706}$$

$$= 0.1033 \text{ mg KOH/g of oil}$$

H. Determination of viscosity (ASTM D445)

Viscosity is the unit specifying the resistance to flow; therefore viscosity is normally a specific value.

7 ml of each sample were added into the Viscometer tube, and insert the viscometer into the bath. After insertion, allow the viscometer to reach bath temperature, Use suction to adjust the head level of the test sample to a position in the capillary arm of the instrument about 7 mm above the first timing mark. With the sample flowing freely, measure, in seconds to within 0.1 s, the time required for the meniscus to pass from the first to the second timing mark. The time of sample used was recorded.

The viscosity was calculated as follows:

viscosity = Ct

C = Constant of viscometer tube (mm^2/s^2)

time = measured flow times for $t1$ and $t2$, respectively(s)

Example:

$$\text{Viscosity of CS oil methyl ester} = 0.01434 \times \frac{(258.5 + 258.37)}{2}$$

$$= 3.7\ \text{cSt}$$

VITA

Mr. Kittipong Makchay was born on November 16, 1982 in Uthaitanee, Thailand. He graduated with Bachelor Degree of Science, from Department of Social Forestry, Faculty of Forestry, Kasetsart University in 2005. He was admitted to the Master degree of Science in Petrochemistry and Polymer Science, Faculty of Science, Chulalongkorn University in 2005.

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