Comparative Study of Alcohol Blends on Biocatalyzed Hydrolysis and Transesterification of Cottonseed Oil

A Dissertation

Submitted in partial fulfillment of the requirement

For the award of degree of

Masters of technology

in

Environmental Science and Technology

Submitted by

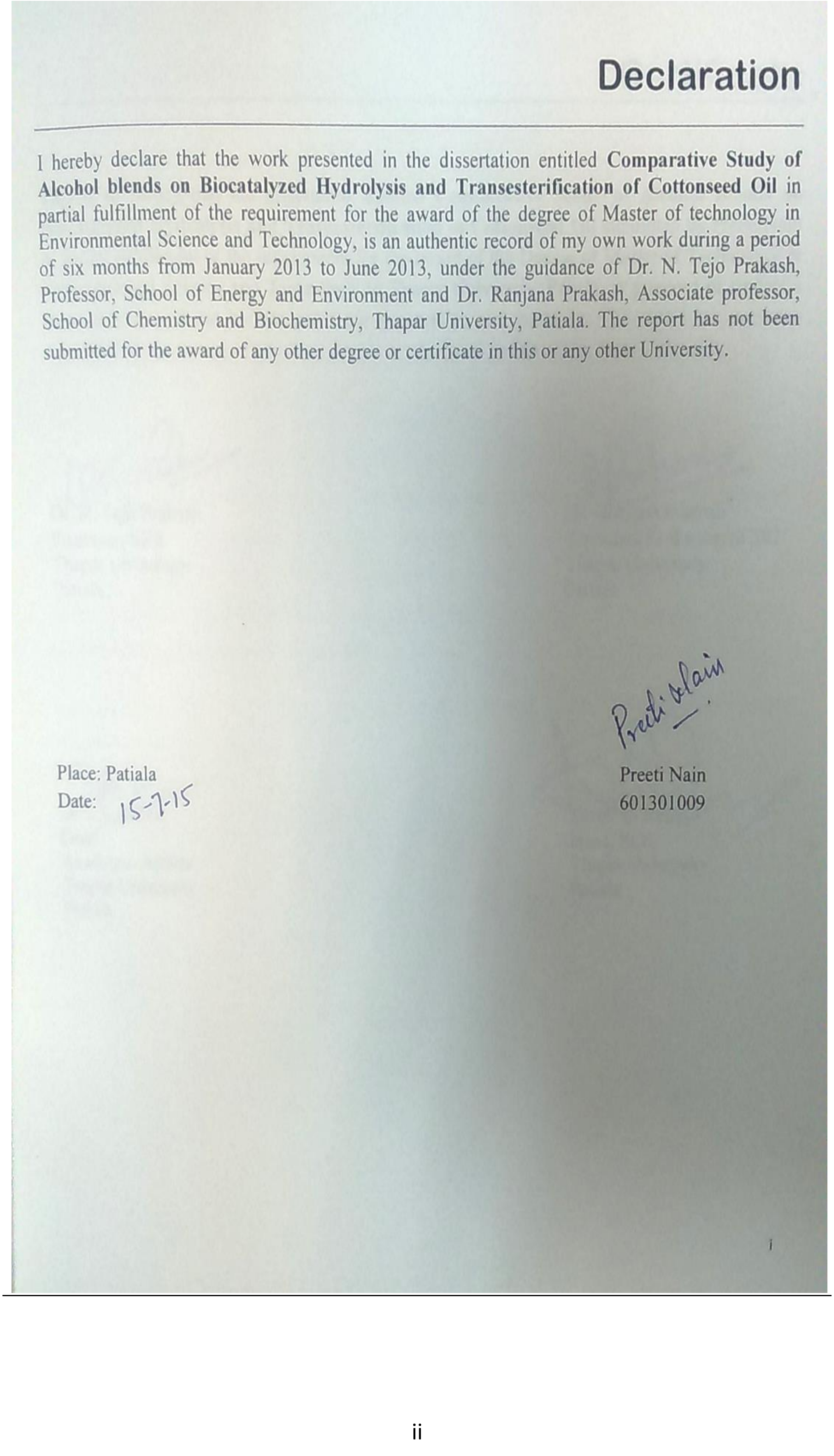
Preeti Nain
(601301009)

Under the supervision of

Dr. N. Tejo Prakash
Professor
School of Energy & Environment

Dr. Ranjana Prakash
Associate Professor
School of Chemistry & Biochemistry

THAPAR UNIVERSITY
PATIALA-147004
INDIA
July, 2015



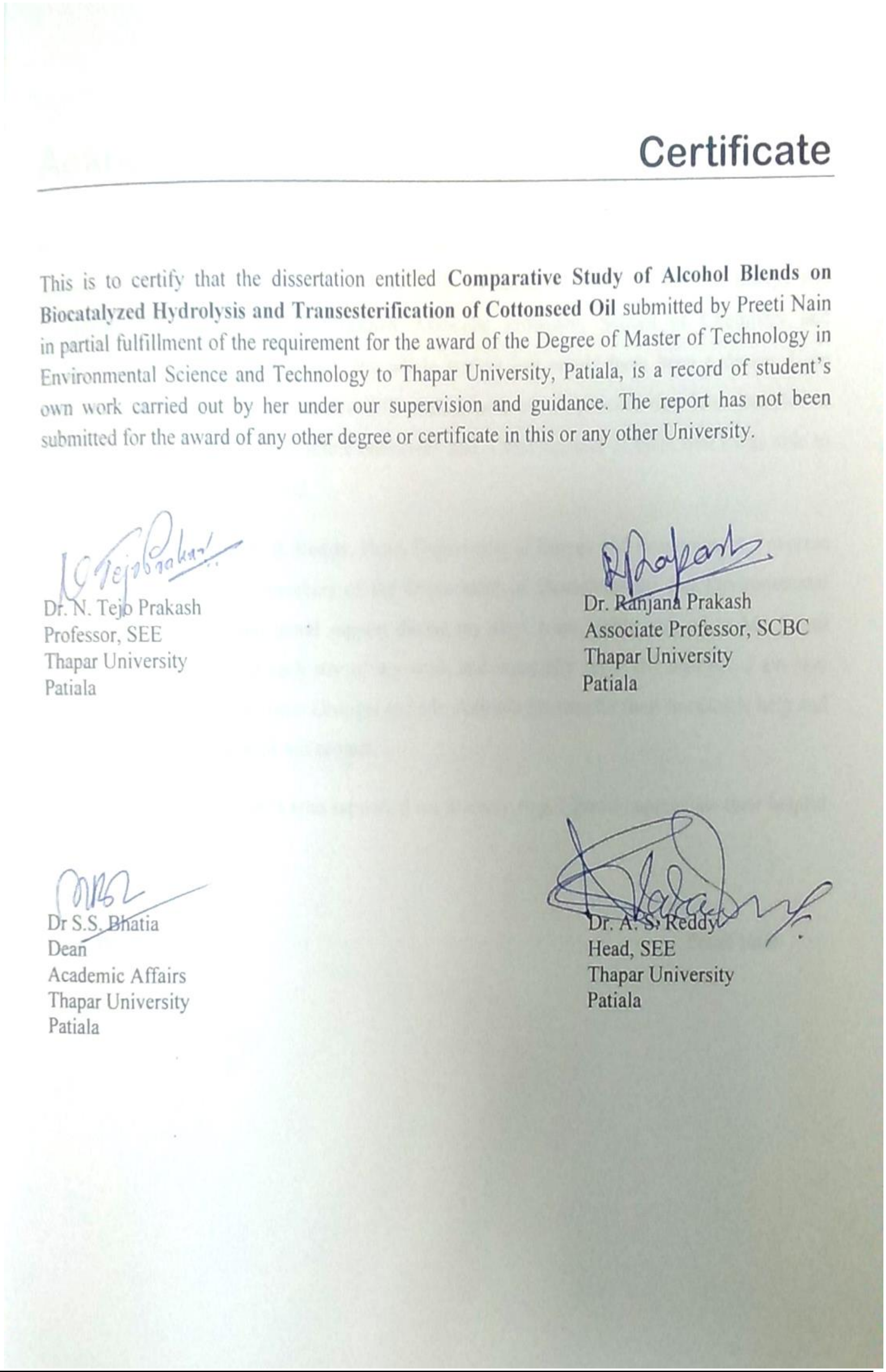
Declaration

I hereby declare that the work presented in the dissertation entitled **Comparative Study of Alcohol blends on Biocatalyzed Hydrolysis and Transesterification of Cottonseed Oil** in partial fulfillment of the requirement for the award of the degree of Master of technology in Environmental Science and Technology, is an authentic record of my own work during a period of six months from January 2013 to June 2013, under the guidance of Dr. N. Tejo Prakash, Professor, School of Energy and Environment and Dr. Ranjana Prakash, Associate professor, School of Chemistry and Biochemistry, Thapar University, Patiala. The report has not been submitted for the award of any other degree or certificate in this or any other University.

Preeti Nain

Place: Patiala
Date: 15-7-15

Preeti Nain
601301009



Certificate

This is to certify that the dissertation entitled **Comparative Study of Alcohol Blends on Biocatalyzed Hydrolysis and Transesterification of Cottonseed Oil** submitted by Preeti Nain in partial fulfillment of the requirement for the award of the Degree of Master of Technology in Environmental Science and Technology to Thapar University, Patiala, is a record of student's own work carried out by her under our supervision and guidance. The report has not been submitted for the award of any other degree or certificate in this or any other University.

N Tejo Prakash

Dr. N. Tejo Prakash
Professor, SEE
Thapar University
Patiala

RPrakash

Dr. Ranjana Prakash
Associate Professor, SCBC
Thapar University
Patiala

Dr S.S. Bhatia
Dean
Academic Affairs
Thapar University
Patiala

Dr. A. S. Reddy
Head, SEE
Thapar University
Patiala

Acknowledgement

It's said that life is a carnival of experience and a journey with various goals. I take this opportunity to thank my supervisors,**Dr. N. Tejo Prakash**, Professor,School of Energy and Environment and **Dr. Ranjana Prakash**Associate Professor, School of Chemistry and Biochemistry without their presence, the whole project just would have been a dream. I am extremely indebted to them for the scientific attitude and utmost patience they have installed in me which definitely stand in all future endeavors and it was because of them that I was able to learn so much in this short period.

My sincere thanks to **Dr. A. S. Reddy**, Head, Department of Energy and Environment. I express my regards to all faculty members of the Department of Biotechnology and Environmental Sciences for their help and moral support during my stay.I am highly obliged to Mr.SumitJaiswal for his guidance at each step of my work and especially for NMR analysis. I am also highly thankful toMs. NoorpreetDhangal and Mr.Anirudh Sharma for their invaluable help and guidance during the course of this project.

I am grateful to all my friendswhosupported me at every step. I greatly appreciate their helpful suggestions andcorrections.

Place: Patiala Preeti Nain

Abstract

The need to search for alternative sources of energy which are safe, renewable and non-polluting has gained highest priority across the globe due to uncertain supplies and high price hikes in fossil fuels. One of the promising alternatives is Biodiesel, a renewable fuel which can be defined as methyl or ethyl ester of fatty acid made from vegetable oils (both edible and non-edible) or animal fat. The present study outlines the observations on the fatty acid alkyl ester generation by transesterification reaction of cottonseed oil using *Aspergillus* species as whole-cell biocatalysts. Work also represents difference in ester yield on addition of alcohol blends with two different molar ratio (1:4 or 1:6). No significant difference was observed in transesterification ester yield with both the molar ratio of alcohols. The transesterification of cottonseed oil yielded 91% and 87% of alkyl esters using molar ratio 1:4 and and 1:6 molar ratio (oil to alcohol) respectively. As per ASTM 6751 the properties like density, viscosity, gross calorific value, flash point, cloud point and pour point for accessing the fuel quality of cottonseed oil ethyl-butyl and ethyl-propyl blend ester showed significant similarities with petroleum based diesel. The study thus demonstrates the use of whole cell as biocatalyst for transesterification of cottonseed oil to alkyl ester can be potentially used as biodiesel.

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1. Introduction

There is rapid increase in the demand of petroleum-based fuels due to transportation and industrialization of the world. These finite, petroleum-based fuel reserves are highly concentrated in some regions of the world resulting in energy crisis in those countries which do not have these resources. Thus, development of alternative fuels that can be produced from resources which are already available within a country such as vegetable oils, alcohol, etc. Using biodiesel is one of those initiatives that is showing greater promise as an alternative to diesel fuel (Sharma et al., 2013).

Biodiesel can be defined as methyl or ethyl ester of fatty acid made from vegetable oils (both edible and non-edible) or animal fat by the process of transesterification. Biodiesel is a fuel derived from triglycerides or free fatty acids by the process of transesterification or esterification with short chain alcohols. Non-edible oils obtained from plant species is main resource for biodiesel production. This can be used in its pure form or as blends with diesel. One of the most obvious advantages of biodiesel is that it operates in compression ignition (diesel) engine and can be used with or withoutengine modifications as it has similar type of properties to that of mineral diesel. There is substantial reduction in emission of unburned hydrocarbons, carbon monoxide and particulate when biodiesel is used in conventional diesel engines.

Biodiesel benefits include renewable source of energy, non-toxic, sulphur free (< 0.001 %) and 60% less net carbon dioxide emissions. Also, it has high flash point (greater than 160ºC) which is helpful during transportation and storage. One of the important quality of biodiesel is that it decomposes more easily on exposure to environment and can be produced more easily as compared to diesel and petrol. Another advantage of using biodiesel is that compounds like polycyclic aromatic hydrocarbons (PAH) and nitrated PAH which causes cancer in humans get eliminated. Biodiesel has higher lubricity as compared to mineral diesel which increases the engine life. There is lesser emissions of carbon dioxide (CO_2), hydrocarbon (HC) and particulate matter (PM), as compared with diesel. The only limitation in the case of biodiesel is release of higher NOx emission which needs to be reduced. Many researchers are in process to reduce the NOx emission during and after the combustion process (Prabu et al., 2012).

Biodiesel is produced through catalysed hydrolysis of oils followed by esterification to esters. Transesterification is the displacement of an alcohol from an ester by another alcohol in a process similar to hydrolysis, except that an alcohol is employed instead of water. Alcohols include methanol, ethanol, propanol, butanol, and amyl alcohol. Methanol and ethanol are utilized most frequently because of their low cost and physical and chemical advantages. This process reduces the viscosity of triglycerides, which further enhances the physical properties of renewable fuels to improve engine performance (Fukuda et al., 2001).

Biodiesel can be produced following chemical and biological approach (Du et al., 2008). Chemical ways has been used in many countries with success. It includes use of two kinds of catalysts: alkali catalysts and acid catalysts. Although chemical transesterification has facilitated the industrialization of biodiesel production in many countries and gives high conversions of triglycerides to their corresponding methyl esters in a short reaction time, the reaction has several drawbacks. It is energy intensive; glycerol recovery is difficult; the acidic or alkaline catalyst has to be removed from the products; presence of free fatty acid interfere the reaction.

Bio-catalyzed transesterification can be done using both extracellular and intracellular lipases. In both the cases, the enzyme is immobilized and used for eliminating downstream operations like separation and recycling. Either immobilized (extracellular) enzymes or immobilized whole cells (intracellular enzymes) are used to carry out catalysis. Both the processes are reported to be highly efficient compared to using free enzymes (Ranganathan et al., 2009). The major limitation with lipase as catalyst is the cost of pure enzyme. As an alternative, the use of whole cell biocatalyst has been studied extensively since recent past. Various fungal species i.e. *Aspergillus species* and *Rhizopus species* have been used to carry out whole cell catalysed transesterification reaction. In addition, whole cell immobilization is also very efficient way to carry out the same for biodiesel production.

In addition to biodiesel being used in its pure form, the blends of biodiesel with the conventional diesel have been extensively promoted and used as alternatives to current fuels. Physical characteristics of these blends are the most important parameter to determine the quality. Presently the most common form of biodiesel is blending the conventional diesel fuel with esters of vegetable oils. The most common ratio used is 80% conventional diesel fuel and 20% vegetable oil ester. It is termed as “B20” indicating the 20% level of biodiesel. Advantage of biodiesel blends is its simplicity of fuel preparation which requires mixing of the components only.

Biodiesel produced from different feedstocks have different fuel properties depending upon the fatty acid composition, presence of the contaminants and minor components. Important properties of biodiesel that are directly influenced include exhaust emissions, low-temperature operability, cetane number, oxidative and storage stability, kinematic viscosity and energy content. Minor components are the naturally occurring species found in vegetable oils and animal fats; and may include phospholipids, sterylglucosid, chlorophyll, fat soluble vitamins (Moser et al., 2009).

However there are several problems related to the economics and properties of biodiesel which impaired the widespread use of biodiesel. For example, virgin vegetable oils reported to cause engine deposits. Furthermore, the problems related with the use of biodiesel are very complex and no satisfactory solution has yet been achieved despite the efforts of many researchers around the world.

Cold flow properties of biodiesel are one of the important concern today. In cold climates, it is a challenge for automobiles running with biodiesel as this tends to gel (freeze) at lower temperatures. A diesel fuel’s cold-weather characteristics are measured by the cloud point (CP) and the pour point (PP). Fossil fuels have broader range of temperatures (≈20° C) among the CP and the PP. The actual temperature at which biodiesel freezes depends on the type of oil or fat and acyl acceptor from which it is made. Unlike diesel fuel, flow properties of fatty acid methyl ester and ethyl ester (FAME /FAEE) at low temperatures are characteristics of concern inhibiting their use in cold climatic conditions. Fatty acid esters have reasonably high CP and PP.

However, when compares to FAME/FAEE, fatty acid butyl esters shows improved cold flow properties (Moser 2009).

The present study isfocused on the use of alcohol blends for carrying out the transesterification of cottonseed oil with whole cell biocatalysis and studying the effect of different molar ratios of alcohols on the efficiency of reaction. The fuel properties of the alkyl esters, thus obtained, have also been studied.

2. Literature Review

The limited amount of fossil fuels, increasing prices of crude oil, and environmental concerns with time have been the reasons for exploring alternative fuels. However, they cannot be used directly because of their viscous nature and low ignition point. Methyl/ethyl esters of fatty acids of such oils have proved promising enough to be called biodiesel. They can be used without any engine modification and produce much lower levels of most of the pollutants. Biodiesel is a non-petroleum-based fuel that mainly consists of fatty acid methyl esters (FAME) or fatty acid ethyl esters (FAEE), derived from the transesterification of triglycerides (TG) with methanol or ethanol, respectively (Vicente et al., 2006).

Biodiesel is defined by ASTM International as a fuel composed of monoalkyl esters of long-chain fatty acids derived from renewable vegetable oils or animal fats meeting the requirements of ASTM D6751. Biodiesel is a biofuel of biological ingredients rather than fossil fuels. Important substrates available for producing biodiesel are agricultural co-products and byproducts such as soybean oil, rapeseed oil, jatropha oil, karanj oil, cottonseed oil and other natural oils; feedstocks including soapstocks, acid oils, used cooking oils, waste restaurant grease, animal fats (tallow), non-edible oils obtained from trees and microorganisms (Moser, 2009). It is a clean fuel, non-renewable resource of energy, less toxic and biodegradable. Biodiesel is also used in blends with diesel (Fukuda et al., 2001), biodiesel blends are denoted as “BXX” where “XX” represents the percentage of biodiesel contained in the blend (i.e. B20 is 20% biodiesel). Biodiesel offers various advantages over diesel such as sustainability, reduction in the emission of greenhouse gases, agricultural development, and security of supply and reduction in the level of pollutants (Reijiders, 2006).

2.1 Biofuels

India’s energy security would remain safe only when there is availability of alternative fuels to replace the produced renewable feedstocks. Biofuels provide a ray of hope in providing that required energy security. They are environment friendly and their utilization would address global concerns about containment of carbon emissions. The transportation sector is the major polluting sector. Therefore, use of biofuels became compelling in view of the tightening automotive vehicle emission standards to reduce air pollution (Reijnders, 2006).

Biofuels are derived from renewable bio-mass resources and promote sustainable development and supplement conventional energy to meet the increasing demand of energy for transportation with high economic growth, as well as to meet the energy needs of India's vast rural population. Biofuels reduce the dependence on import of fossil fuels and satisfies energy needs in an environmentally benign and cost effective manner. The growth of biofuels around the globe is spurred largely by energy security and environmental concerns and a wide range of market mechanisms, incentives and subsidies have been developed to facilitate their growth. For developing countries, biofuels are potential means to stimulate rural development and create employment opportunities (Crabble et al., 2001). The Indian approach to biofuels is different to the current international approaches that lead to conflict with food security. It is based on non-food feedstocks raised on degraded or wastelands that are not suitable for agriculture, thus settling the conflict between fuels vs. food security (Giampietro et al., 2011).

There are various types of biofuels which comes in different forms and meet energy needs. At this point, there are two types of biofuels i.e. First generation and Second generation biofuels.First generation biofuels are produced from sugar, vegetable oil, animal fats, plants or algal material. They would have large impact on food supply if used in large quantity. They consist of a majority of portion of biofuels. Biodiesel and biogas are the examples of first generation biofuels. Second generation biofuels are made from sustainable feedstock such as waste vegetable oil and inedible plant matter. Most of the second generation fuels are underdevelopment and not widely available for use.Biohydrogen and syngas are the examples of second generation biofuels (http://biofuel.org.uk/types-of-biofuels.html).

Out of these, the most commonly used biofuel is biodiesel and biogas due to easy availability of feedstocks. Biodiesel is refined from vegetable oil and occasionally animal fats. It is generally recognized that biodiesel has lower emissions, with the exception of nitrogen oxides (NO_x), than conventional petroleum-based diesel fuel. The lower emissions have caused biodiesel to be used in urban bus fleets and to make it especially suitable for other niche markets such as mining and marine engines. Biodiesel provides 9% less energy than petro-diesel. Besides environmental and health reasons with accompanying Government regulations, focusing on the use of biodiesel in niche markets is rendered additionally attractive because not enough vegetable oil is produced to supply the whole diesel market with biodiesel (Scott et al., 2012).

2.2 Diesel and Biodiesel

Diesel and biodiesel, both comes from different sources yet performing the same function i.e. used as a fuel in diesel vehicle. Diesel fuel (DF) is a mixture of hydrocarbons which is obtained from the distillation of crude oil. Diesel is obtained as by-product of petroleum distillation which is a non-renewable fossil fuel whereas biodiesel is produced from vegetable oils or animal fats by various enzymatic or chemical processes. Conventional DF is similar to gasoline, obtained from cracking of petroleum. It is a fraction which is found boiling at an initial distillation temperature of 160°C (90% range of 290-360°C) (Lane et.al.,1980), also known as middle distillates as its boiling range is in the mid-range of cracking products. Ignition quality is the ability of a fuel to ignite when it is injected into the compressed-air charge in a diesel cylinder and it is reported as the cetane number (CN). Shorter the ignition delay time, the higher the CN. ASTM D613 measures the ignition quality of DF (Gerhard et al., 2013).

The term biodiesel stands for vegetable oils used as well as methyl esters prepared from vegetable oils or animal fats and blends of conventional diesel fuel with vegetable oils or methyl esters. With increasing focus on the use of esters, however, the term “biodiesel” generally refers to alkyl esters of vegetable oils or animal fats and not the neat oils or fats themselves. In an article on proposed ASTM standards, biodiesel was defined as “the mono alkyl esters of long chain fatty acids derived from renewable lipid feedstock, such as vegetable oils or animal fats, for use in compression ignition (diesel) engines (Biodiesel Report; National Biodiesel Board., 2006).

2.2.1 Advantages of the use of biodiesel

- Renewable fuel, obtained from vegetable oils or animal fats.
- Lower environmental effects as it degrades more rapidly than diesel fuel.
- Lower emissions of contaminants: carbon monoxide, particulate matter aromatic hydrocarbons.
- Lower health risk, due to reduced emissions of carcinogenic substances.
- No sulfur dioxide (SO_2) emissions.
- Low toxicity as compared to diesel fuel and excellent lubricant.
- High flash point (100°C minimum).
- Can be blended with diesel fuel at any proportion.
- Can be used in a conventional diesel engine, without any engine modifications.
- Used cooking oils and fat residues from meat processing can be used as raw materials.

2.2.2 Disadvantages of the Use of Biodiesel

- Slightly higher fuel consumption due to the lower calorific value of biodiesel.
- Higher freezing point than diesel fuel.
- Slightly higher nitrous oxide (NOx) emissions than diesel fuel.
- Less stable than diesel fuel, and therefore long-term storage is not recommended.

2.2.3 Economics and environmental issues

Petroleum-based DF is many times cheaper than biodiesel due to cost of feedstock. The feedstock used for the production of biodiesel is more expensive than conventional DF. For example, the cost of a gallon of soybean oil is approximately two to three times as much as the cost of a gallon of conventional DF. However, during the conversion of vegetable oils or fats to their esters, the resulting co-product glycerol, which has a potential market of its own, may offset some of the input cost. Emissions produced by biodiesel are lower than those generated by fossil fuels. Biodiesel does not generate SO_2 emissions because of its higher oxygen content, the resulting combustion is more effective and also, particulate matter and CO emissions are lower (Scragg et al., 2003).

2.3Transesterification

Vegetable oils and animal fats are composed of triacylglycerols (TAG) consisting of long chain fatty acids which are chemically bound to a glycerol (1,2,3- propanetriol) backbone. The chemical process by which biodiesel is prepared is known as the transesterification reaction, involving a TAG reaction with a short-chain monohydric alcohol in the presence of a catalyst at elevated temperature to form fatty acid alkyl esters (FAAE) and glycerol (Fig. 1). The TAG is converted to biodiesel in a stepwise process whereby the alcohol initially reacts with TAG as the alkoxide anion to form FAAE and diacylglycerols, which further reacts with alcohol (alkoxide) to give another molecule of FAAE and generate monoacylglyerols. Lastly, MAG undergoes alcoholysis to produce glycerol and FAAE with the combined FAAE collectively known as biodiesel. Every mole of TAG produces three moles of biodiesel and one mole of glycerol after complete conversion. The transesterification reaction is reversible, although the reverse reaction is negligible because FAAE, especially fatty acid methyl esters (FAME) are immiscible with glycerol when methanol is used as alcohol component (Moser, 2009).

During the process of transesterification, two liquid phases are formed. The lower phase consists of glycerol, catalyst, intermediate products, and may contain water and soap and the upper phase mainly contains methyl ester, which after removing an excess of methanol and washing with water is used as biodiesel. Glycerol as a byproduct of the transesterification reaction has a number of applications in the pharmaceutical, cosmetics, plastic and food industries but it requires extensive washing and purification from the trace compounds which is tedious (Lingfeng et al., 2007).

2.3.1 Transesterification reaction

Transesterification is the chemical reaction in which one form of ester is transformed into a different ester. Basically, it is a process in which the alkoxy group of an ester is exchanged with that of an alcohol. Catalyst used for the reaction is mainly alkalis and acids. For biodiesel synthesis, the predominantly alcohol used is methanol. Due to this, the transesterification reaction is also known as transmethylation. Since the reaction involves the cleavage of an ester group by an alcohol, it is also known as alcoholysis.

As shown in Figure 1, Triglycerides (TG) are molecules that consist of a glycerol backbone to which three free fatty acids are attached, where x, y and z are long alkyl or alkenyl chains. These glycerol esters are the major components of the vegetable oils and animal fats.

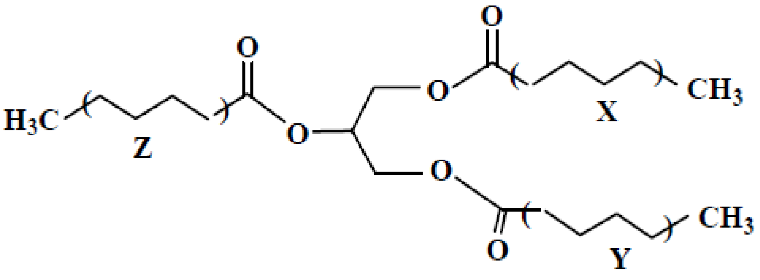

Figure1:Structure of triglyceride

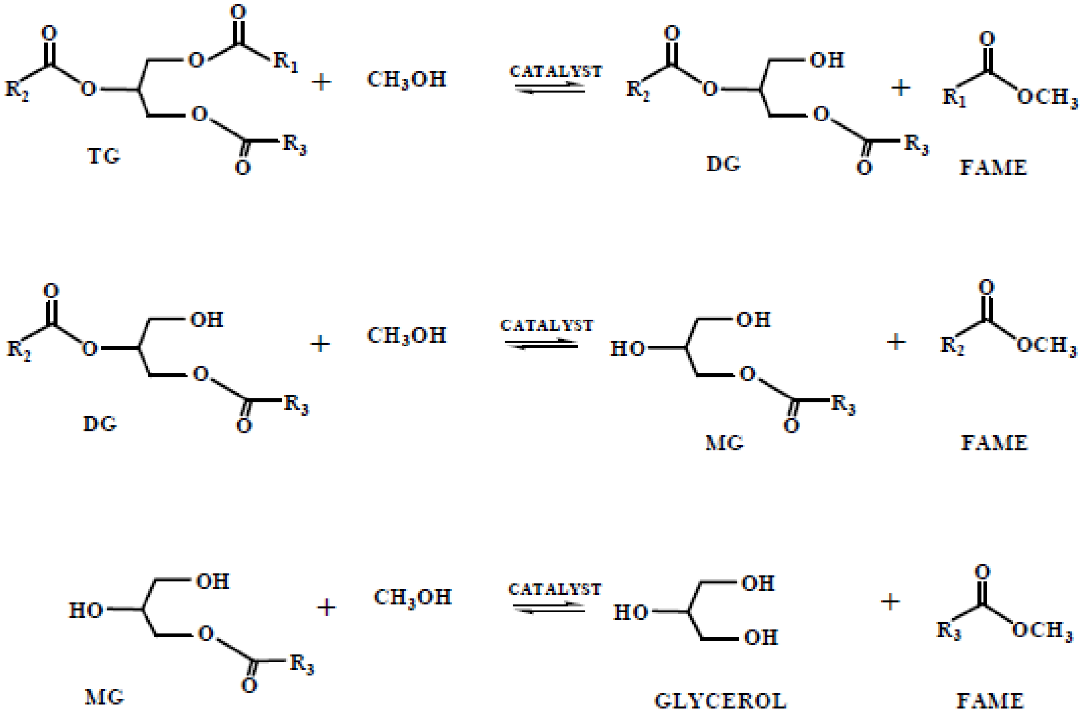

Figure 2: Step wise reaction involved in transesterification of TG

As shown in the Figure 2, Transesterification of TG is a stepwise process where the TG reacts first with the alcohol to produce a diglyceride (DG) and a mono-alkyl ester molecule, then the DG reacts with another alcohol to produce a MG monoglyceride (MG) and a second molecule of mono-alkyl ester and finally the MG reacts with another alcohol to produce glycerol and a third molecule of mono-alkyl ester (Freedman et al., 1986).

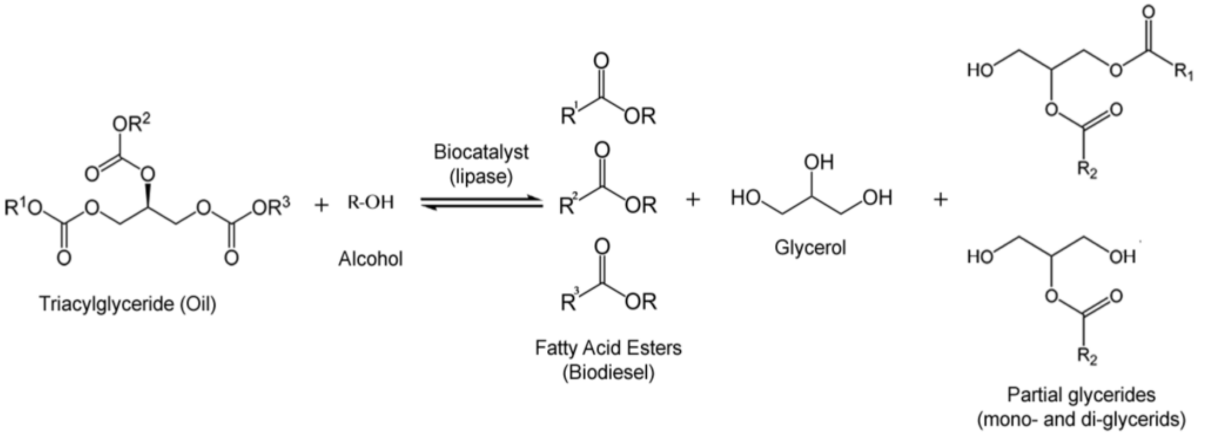

Figure 3: Overall reaction of TG in to FAME

Figure 3 shows the overall transesterification reaction in which one mole of TG reacts with three moles of alcohol (methanol) to give three moles of FAME and glycerol as a byproduct. In the transesterification process, intermediates such as MG and DG are formed, which may remain in the final biodiesel and may also contaminate the unreacted TG. These contaminated glycerides may cause problems at the engine injectors.

2.3.2 Chemical Transesterification

The transesterification reaction with alcohol represented by the general equation shown in Fig.2 consists of a number of consecutive, reversible reactions. The first step is the conversion of triglycerides to diglycerides, which is followed by the conversion of diglycerides to monoglycerides and then monoglycerides to glycerol, yielding one methyl ester molecule from each glyceride at each step.

2.3.2.1 Acid catalysed transesterification

Acids used for transesterification process include sulfuric, phosphoric, hydrochloric, and organic sulfonic acids. Transesterification by acid catalysis is slower than the alkali catalysis, as acid-catalyzed transesterification is more suitable for glycerides that have relatively high free fatty acid contents and more water. Transesterification can be done under an acidic condition when the oil component was a low grade material such as sulphur olive oil. In general, the ethyl esters of monounsaturated or short-chain fatty acids with 2% sulfuric acid make good alternative fuels (Aksoyet al., 2012). In *situ* transesterification is different from the conventional reaction. During in *situ* transesterification, the oil-bearing material contacts acidified alcohol directly instead of reacting with purified oil and alcohol. Extraction and transesterification proceed within the same process, alcohol acts as both extraction solvent and an esterification reagent (Guan et al., 2009).

2.3.2.2 Alkali-catalyzed transesterification

In the alkali process, sodium hydroxide (NaOH) or potassium hydroxide (KOH) is used as a catalyst along with methanol or ethanol. Initially, alcoxy is formed due to the reaction of the catalyst with alcohol and the alcoxy reacts with vegetable oil to form biodiesel and glycerol (Barnwal and Sharma, 2005; Srivastava and Prasad, 2000). As glycerol is denser, it settles at the bottom and biodiesel can be decanted from the top. The reaction rate of this process is reasonably high even at a low temperature of 60°C and is the most efficient and least corrosive of all the processes. There may be risk of contamination due to free acid or water and there is possibility of soap formation which makes the separation process difficult. Alkali-catalyzed transesterification is most often used commercially as it proceeds approximately 4000 times faster than that catalyzed by the same amount of an acidic catalyst (Hass, 2004).

2.3.3 Biocatalyzedtransesterification

Although chemical transesterification using an alkali-catalysis process gives high conversion levels of triglycerides to their corresponding methyl esters in short reaction times, it has several drawbacks: it is energy intensive, recovery of glycerol is difficult, purification of the product, alkaline wastewater requires treatment, and free fatty acids and water lower the reaction product (Fukuda et al., 2001). Both extracellular and intracellular lipases can effectively catalyze the transesteritication of triglycerides in both aqueous and non-aqueous system.

Lipases (EC 3.1.1.3) act as catalysts in splitting the oils and fats with successive release of glycerols and free fatty acids. They play an efficient role in diverse reactions of aminolysis, transesterification and esterification. Sources of these enzymes are microorganisms, animals and plants (Harding et al., 2007). Among lipases from these organisms, lipases of microbial origin are the most important because of their vast application. This is because microbes can be easily cultivated with short generation times as compared to other organisms, and their lipases can catalyze a wide range of hydrolytic and synthetic reactions. The most important thing is that the by-product, glycerol, can be easily recovered without any complex process, and also that free fatty acids present in the waste oils and fats can be completely converted to methyl esters. On the other hand, the production cost of a lipase catalyst is significantly greater than that of an alkaline one (Crabble et al., 2001; Watanabe et al., 2000).

2.3.3.1 Extracellular lipase as biocatalyst

Mittel et al. (1990) reported transesterification of sunflower oil with primary alcohols like methanol, ethanol and butanol. Species such as *M. miehei* and *C. antarctica* (Novozym 435) are used in the presence and absence of the solvent, petroleum ether. Methanol was found to produce traces of methyl esters without the solvent but yields obtained for ethanol and butanol were high even without the solvent.

Nelson et al. (1996) conducted batch experiments and found that *M. miehei* was efficient for primary short chain alcohols (95% conversion) like methanol, ethanol, propanol and butanol in the presence of hexane as a solvent and *C. antarctica* was suitable for secondary alcohols (80% conversion) like iso-propanol and 2-butanol. However, methanol was the least efficient with a methyl ester yield of 19.4% in the presence of solvent. The low yield was mainly due to the inhibitory effects on the immobilized enzyme caused by methanol. This was further confirmed by Abigor et al. (2000) who reported the conversion of palm kernel oil using methanol and ethanol as 15% and 72%, respectively.

Noureddini et al. (2001) reported the transesterification of soyabean oil with immobilized enzyme obtained from *Pseudomonas flourescens* and reported the conversions 67% and 65% for methanol and ethanol respectively. Iso et al. (2001) reported 90% conversion of vegetable oil using *P. fluorescens* enzyme with butanol as the acyl acceptor. Similarly, usage of other alcohols as alternate acyl acceptors instead of methanol have been used and conversions around 90% were constantly obtained.

2.3.3.2 Intracellular lipase as a whole cell biocatalyst

Intracellular lipase (whole cell immobilization) can also be used in the place extracellular lipase with high efficiency. There is considerable reduction in cost with the use of intracellular lipase. Matsumoto et al. (2001) developed the whole cell biocatalyst by immobilizing *R. oryzae* cells and permeabilizing them by air drying. Then, methanolysis of plant oil in solvent free and water containing system is done to produce methyl esters. After a 165h reaction at 37 ˚C, the methyl ester content in the reaction mixture was reported as 71 wt% with stepwise addition of methanol.

Ban et al. (2001) reported the transesterification of vegetable oils by using immobilized whole cell *R. oryzae* and investigated the cell pretreatment, culture conditions and effect of water content on the production process. Several substrate related compounds were added to the medium to enhance the methanolysis activity of the immobilized cells, out of which oleic acid and olive oil were found to be effective. A high conversion yield of 90% was reported with stepwise addition of methanol and with 15% water.

2.4 National and International standards for biodiesel

Besides favourable economics and health benefits, the environment aspect should also be taken in to consideration. There is development of reliable standards, which will instil confidence with biodiesel users, engine manufacturers, and other parties. Standardisation is the process of establishing a technical standard which brings benefits among competing entities in a market, without hurting competition. Different organisations establish different standards on different levels, i.e. national, European and international standards.

The national standards institutes are responsible for standardisation on a national level (Knothe et al., 1993). EPA (Environmental Protection Agency) is working with several standard-setting organizations, both nationally and internationally, and other government organizations to harmonize standards for biofuels produced from a variety of feedstocks and blended in petroleum-based fuels at various concentrations. ASTM D 6751 was first published in 2002 (ASTM D 6751-02). Since then it has been periodically revised and updated. EPA's works to register biodiesel that meets the standards specified in ASTM D 6751 in effect at the time of registration.

Table 1: ASTM, EN and Indian Standards

Property	**IS 15607 : 2005 standards**	**ASTM D6751 Standards**	**En 14214 – 2003 standards**
Composition	HC (C10- C12)	HC (C10- C12)	Not mentioned
Viscosity at 40°C, mm^2 s-1	2.5-6.0	1.9-6.0	3.5-5.0
Density Kg / m^3	860 - 900	870 - 900	860 – 900
Specific gravity	0.87	0.88	0.88

Flash point °C	120	100-170	Above 101
Cloud point °C	0-15	-3 to 12	0-15
Pour point °C	<10	-15 to 16	<10
Water	500 mg / Kg	0.050% by vol.	500 mg/Kg
Acid number, mg KOH/g	0.70 max.	0.80 max.	0.50 max.
Carbon, wt%	0.05	0.77	0.3
Sulphur, mg/Kg	50	50	10
Sulfated ash content, % by mass	0.20	0.020	0.02
Cetane number, min	51	48-60	51
Ester content	96.5	96.5	96.5

In India, the fuel specifications of diesel have been aligned with the corresponding European Fuel Specifications for meeting the Euro II, Euro III and Euro IV emission norms. Indian Standards are formulated in a transparent manner through a consensus process by the Technical Committees comprising of experts from all concerned areas such as Consumers, Producers, Manufacturers, R&D Centres, NGOs, Regulatory Bodies etc. Adoption of Indian Standards is generally voluntary in nature and their implementation depends on adoption by concerned parties. An Indian Standard becomes binding if it is stipulated in a contract, referred to in legislation or made mandatory by specific orders by the Central or State Governments. Ministry of Petroleum and Natural Gas has adopted Indian Standards on diesel in the country through a Govt. Gazette notification (Tyagi et al., 2010).

2.5 Alcohol for Transesterification

In the commercial production of biodiesel, the most commonly used alcohol is methanol as it is less expensive than other alcohols, but ethanol prevails in regions such as Brazil where methanol is more expensive. Alcohols other than methanol and ethanol are also of interest for the production of biodiesel because FAAE produced from higher alcohols may have different fuel properties in comparison to methyl or ethyl esters (Knothe, 2008).

Alcohols used in catalyzed transesterification process are methanol, ethanol, propanol, butanol and amyl alcohol. Methanol and ethanol are used most frequently in both laboratory research and the biodiesel industry (Fukuda et al., 2001). As already mentioned, due to the low cost of methanol, it is the first choice for the transesterification reaction. It prevents soap formation, reactivity is high and its recovery is easier, as it does not form zoetrope (Chongkhong et al., 2007). Ethanol is derived from renewable sources (agriculture products) and biologically less objectionable to the environment than methanol. Thus, ethanol is considered as the ideal candidate for the production of biodiesel (Demirbas, 2005).

Working with high molecular weight alcohols, such as butanol or pentanol brings about interesting advantages. For example, butanol has better miscibility with the lipid feedstock than smaller alcohols. Also, the higher boiling points of larger alcohols enable the reaction system to be operated at higher temperatures along with maintaining the moderate pressures. This is an advantage for the acid catalyzed transesterification as higher reaction temperatures is required to achieve high reaction rates. However, the influence of alcohol characteristics on transesterification process has been addressed by a limited number of researchers. Nye et al. (1983) compared the linear alcohols from methanol to butanol to find the most suitable alcohol for the transesterification of waste frying oil. All the reactions were carried out using 0.1% sulfuric acid at reflux temperatures while keeping the other conditions constant. Highest reaction rates and conversion were observed in butanol followed by 1-propanol and ethanol. Even though alkali-catalyzed reactions carried out with methanol proved to be the fastest, the reverse was true for the acid-catalyzed process.

2.6 Combination of alkyl esters as biodiesel

There are very limited studies on blending of various alkyl esters to obtain desirable properties of biodiesel. Joshi et al. (2011) studied the transesterification of refined cottonseed oil (CSO) with methanol, ethanol, 1-butanol, and various mixtures of these alcohols to produce biodiesel. Formation of methyl esters was *favoured* over ethyl and butyl esters in the mixed alcohol transesterification. Fuel properties were determined with respect to the ester head group. Specifically, lubricity, cold flow properties, and energy content improved in the order: butyl esters, ethyl esters, methyl esters. Alcohol esters with larger ester head groups were observed with higher kinematic viscosities as well as lower iodine values. Blends of methyl esters, ethyl esters, butyl esters exhibited properties intermediate to the neat esters.

All ester samples fulfil the prescribed limits in ASTM D6751 and EN 14214 for cetane number, acid value, glycerol (free and total) content, sulfur, and phosphorous. All blends showed the improved cold flow properties versus unblended alkyl esters. Also examined was the influence of blending alkyl esters with petrodiesel. There is enhancement in lubricity after blending. Finally, all blends were within the limits specified in ASTM D975 and D7467 for acid value, cetane number and sulphur.

2.7 Blending of biodiesel and diesel

Presently, blending esters of vegetable oils with conventional DF is the most common form of biodiesel. The most common ratio used is 80% conventional diesel fuel and 20% vegetable oil ester (also called as “B20”, indicating the 20% amount of biodiesel). It has been reported that these blends lead to significant emission reductions. Ester blends have been reported as most stable. Goodrum et al.(1982) reported that a blend of 20% peanut oil with 80% DF did not separate at room temperature over a period of 3 months. Mittalbach et al. (1985) also reported that stability was also found for 50:50 blends of peanut oil with DF.

When blends of B20 were used as fuel in conventional urban bus, no engine problems were found. There is slightly 2-5% higher consumption than that of conventional DF with fuel economy comparable to other conventional DF blends. Another important advantage of biodiesel blends is the simplicity of fuel preparation which only requires mixing of the components at room temperature. Zhang et al.(1996) observed that irregularities are found as compared to other ester blends when isopropyl ester of soybean oil is blended with conventional DF. Deposits were formed on the injector tips.

Zhao et al.(2014) studied the blended alcohols consisting of methanol and ethanol as acyl acceptors for biodiesel synthesis from soybean oil by lipase-catalyzed transesterification. Novozym 435 *from Candida antarctica* was used to carry out the tranesterfication reaction. Reaction was optimized by using different molar proportion of methanol in the blended alcohol. Effect of temperature, and enzyme loading were also investigated. Also, the relative consumption rates of methanol and ethanol were also studied during the reaction. During experiment, the six proportions selected, 0 (100 mol% ethanol), 20, 40, and 60 mol% methanol in the blended alcohols exhibited high yields of biodiesel. Yield of 95 wt% biodiesel was obtained at an enzyme loading of 5–10 wt% at a temperature of 30ºC.

In the early 1980s, research was done on blending of oil and fat with fossil fuel in various proportions. Physical and chemical properties of the vegetable oil as a fuel were improved on blending oils and fats with petroleum diesel by lower viscosity, higher flash point and higher volatility. Shivakumar et al. (2013) have reported that dilution agent prepared by blending of vegetable oil with diesel or ethanol can be used to lower viscosity and enhance volatility. The blends of different proportions, from 5% to 90% diesel, have been tested in both direct and indirect diesel engines. Short-term performance test gave satisfactory results but the long-term use of blends of the oils leads to decrease in power output and thermal efficiency by carbon deposit and lubricating oil fouling. As a result, based on research done up to till date, the means of blending is not considered practical in modern diesel engines.

2.8 Alternative feedstock for biodiesel production

There is a rapid increase in biodiesel production and governmental mandates for alternative fuel usage around the world in last several years. This has necessitated the development of alternative biodiesel feedstocks, as the traditional sources of biodiesel (soybeans, rapeseed/canola, palm and used cooking oils) are not enough to meet the increasing need. Governmental mandates for alternative fuel usage and increased global production capacity lead to the development of alternative sources of biodiesel fuel due to the continued demand for energy sustainability and independence among energy-consuming countries.

The desirable characteristics of an alternative oilseed feedstocks for biodiesel production consists of adaptability to local growing environment (rainfall, soil type, latitude, etc.), easy availability, high oil content, favourable fatty acid composition, low agricultural inputs (water, fertilizer, pesticides), definable growth season, compatibility with existing farm infrastructure, potential markets for agricultural by-products, uniform seed maturation rates, and the ability to grow in agriculturally undesirable lands. Biodiesel fuels prepared from feedstocks that meet at least a majority of the above criteria can be the most promise alternative to petrodiesel. Generally, there are four major biodiesel feedstock categories: algae, oilseeds, animal fats, and various low-value materials such as used cooking oils, and soapstocks.

Non edible jatropha oil, karanj oil can be used as a substrate for biodiesel production. Tamalampudi et al. (2008) carried out research utilizing whole-cell catalyzed alcoholysis of the non-edible jatropha oil with *Rhizopusoryzae* cells that had been immobilized into biomass support particles. Algae, oilseeds, animal fats (tallow) and various low-value materials such as used cooking oils, soapstocks and greases are the major feed stocks for biodiesel (Moser, 2009).

2.9Lacunae

Enzymatic and whole cell catalysis is a time consuming process as compared to acid and alkali catalysis. This process requires 72-96 h for significant transesterification reaction. The efficiency of biodiesel production from cottonseed oil is 80-90%.

Keeping in view, the existing limitations of chemically and biologically catalysed transesterification processes, limited literature on the biocatalyzed transesterification with reference to presence of blends of alcohols for transesterification process especially with fungal system (biocatalyst), the proposed study was envisaged to increase the efficiency of the biocatalyzed process by using various mixtures of alcohols and optimising reaction condition.

3. Materials and Methods

3.1 Materials and Reagents

Refined cottonseed oil was obtained from retail market. Culture media i.e. potato dextrose broth (PDB), mycological peptone and bushnallhass broth (BHB) was purchased from HiMedia, India. Other chemicals such as hexane, ethyl acetate, silica gel (G) for TLC, diammonium hydrogen ortho-phosphate ($(NH_4)_2HPO_4$), potassium hydroxide (KOH) and phenolphthalein indicator were purchased from SD Fine-Chem limited, India. Alcohols such methanol, ethanol, propanol, butanol, pentanol, hexanol, hetpanol, octanol, nonanol, decanol were purchased from SpectrochemPvt. Ltd. Mumbai, India. All the reagents used were of analytical grade.

3.2Cultivation of test organisms

Fungus strain*viz.*, *Aspergillus sp.*, (RBD-01) used as a test species, was obtained from bio-contaminated butter and was reported by our group to exhibit significant oil tolerance and transesterification potential (Aulakh and Prakash, 2010; Prakash and Aulakh, 2011).

The culture of RBD-01 was cultivated on PDB and the spores from active plates were inoculated aseptically in 500 ml Erlenmeyer flask containing 250 ml of sterile PDB separately and incubated at 30°C, 150 rpm for 72 h. The active culture biomass obtained from PDB was further used for experimentation.The minimal media BHB containing $MgSO_4$ (0.2 g/l), $CaCl_2$ (0.02 g/l), KH_2PO_4 (1.0 g/l), K_2HPO_4 (1.0 g/l) and $FeCl_3$ (0.05 g/l) was supplemented with mycological peptone (0.5% w/v), $(NH_4)_2HPO_4$ (0.5% w/v) and virgin cottonseed oil was used as growth medium. The growth medium prepared contains 70 ml cottonseed oil and 30 ml distilled water. It is autoclaved and used as growth medium. Nitrogen was supplemented by mycological peptone and $(NH_4)_2HPO_4$ and cottonseed oil was used as main carbon source for fungal growth.

3.3Hydrolysis of oils to fatty acids

Active culture was inoculated in 250 ml Erlenmeyer flask containing 100 ml of sterile growth medium supplemented with cottonseed oil (70 % oil and 30% media) and incubated at 30˚C at 150 rpm for 120 h for carrying out the reaction. The hydrolytic reaction was constantly monitored by thin layer chromatography for a period of 72 hrs (Samukawa et al., 2000). Samples were collected at periodic intervals of 12 h and subjected to thin layer chromatography (TLC) with silica gel as stationary phase and hexane:ethyl acetate (9:1) as a mobile phase. The chromatogram was developed in the iodine chamber. Further, the samples collected at periodic interval of 12h were analysed by proton nuclear magnetic resonance spectroscopy (^{1}H NMR) (400 MHz; JEOL JNM-ECS 400).

3.4Transesterification Reaction

Subsequent to the hydrolytic reaction,molar ratio of oil to alcohol was maintained at 1:4 and 1:6. With reference to alcohols, ethanol and other alcohols were taken in equal proportions with ethanol being kept constant in all fractions i.e., 50% ethanol and 50% other alcohol.

All the transesterification reactions were done in duplicate. The varying additions were carried out to understand the effect of different amount of alcohol on extent of transesterification and to compare the alcohol ratio required for best conversion efficiency in the reaction. Followed by alcohol addition, samples were collected at an interval of 12 h. Progress of the reaction was monitored at regular intervals by TLC followed by quantification by ^{1}H NMR. After complete transesterification reaction, remaining alcohol in reaction was evaporated using rota evaporator.The ester was quantified using ^{1}H NMR (400 MHz; Jeol JNM-ECS 400). $CDCl_3$ (deuterated chloroform) was used as solvent and tetra methyl silane as internal standard. ^{1}H NMR spectra were recorded with pulse duration of 2.18 sec with a relaxation delay of 4 sec and 16 scans.

Ethyl ester quantification by ^{1}H NMR spectroscopy is complex, due to a superimposition of the glyceryl methylenic hydrogens in oil and the $-OCH_2$ from ethyl ester where partial conversion was obtained (Ghesti et al.,2007). However, where the peak due to glyceryl methylenic hydrogens in oil at 4.25-4.35 ppm completely disappeared, the process of oil conversion was considered to be nearly complete.

3.5 Determination of fuel properties of fatty acid esters relevant to biodiesel

Fuel properties, viz., calorific value (IS:1350 (P-II), 1970) flash point (IS:1448 (P-21), 1992), pour point (IS:1448 (P-10), 1970), kinematic viscosity (IS:1448 (P-25), 1976), ash (IS:1448 (P-4)), density (IS:1448 (P-16), 1990), FFA as oleic acid (SP:18 (P-13), 1984), sulphur content (IS:1448 (P-33), 1991), sediment (IS:1448 (P-30), 1970) and cloud point (IS:1448 (P-10), 1970) were carried out using standard protocols.

4. Results and Discussion

The present study was aimed at optimizing the molar ratio of different alcohols with ethanol as a common component of the blend so as to obtain enhanced transesterification using whole cell catalyst. Alkyl ester blends were prepared by using different combinations of alcohols (in the equal ratio) simultaneously during transesterification reaction. In the present study, two molar ratios, 1:4 and 1:6 (oil to alcohol) have been used to compare the extent of transesterification. In the blend of two alcohols, ethanol was kept constant whereas the other alcohol was varied from methanol to octanol. Ethanol and other alcohols were taken in equimolar ratio. The mixture of two alcohols were added after complete hydrolysis of oil, in four consecutive steps at 12 h interval.

4.1 Hydrolysis and extent of transesterification catalyzed by RBD-01

The hydrolysis of cottonseed oil using RBD-01 (*Aspergillus species*) resulted in FFA yield up to 89.3% after 72 h. (Figure 4).

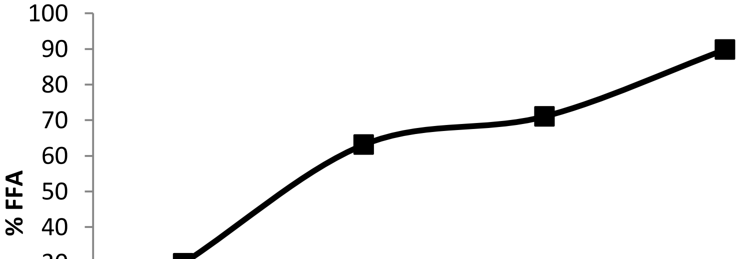

Figure 4.Time dependent variations in hydrolysis of oil by RBD01 as a whole cell catalyst

The progress of hydrolysis was monitored through TLC (figure 5). In thin layer chromatogram, Lane 1 represents pure oil and lane 2 represents hydrolysis product (FFA) of oil after 72 h. After confirmation of complete hydrolysis by thin layer chromatography (TLC), alcohol was added.

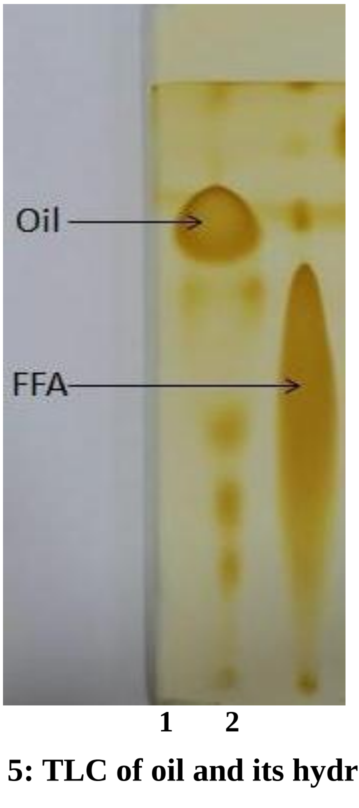

Figure 5: TLC of oil and its hydrolysis to FFA

4.2 Effect of oil: alcohol molar ratio

The stoichiometric ratio of oil to alcohol is 1:3, which means that one mole of oil after hydrolysis requires 3 moles of alcohol during esterification(Georgogianni et al., 2008). However, transesterification is an equilibrium reaction in which slightly excess of alcohol than stoichiometric ratio is required to drive the reaction towards the products (Meher et al., 2004).In the present study,we have used 1:4 and 1:6 molar ratio of oil to alcohol keeping the ethanol constant in all blends.Ethanol and other alcohols were taken in equal proportions i.e., 50% ethanol and 50% other alcohol.

In general practice, chemical catalyst is used for the production of biodiesel which require higher oil to alcohol molar ratio to obtain maximum ester yield. According to Kumar et al.(2012), 98% yield have been achieved using 1:9 Jatropha oilto methanol molar ratio with Na/SiO_2 as a heterogeneous catalyst. Similarly, Vyas et al., (2011) showed the maximum conversion with oil to methanol molar ratio of 1:12. The previous studies suggest that high oil to alcohol molar ratio is required for higher conversion which adds to the production cost. Unlike chemical catalyst, biological catalysts like lipases or lipase containing cells can facilitate ester yield up to 94% with lower molar ratio (1:4 to 1:6) of alcohol during transesterification (Dantas et al., 2013; Kumari et al., 2009). However, the optimum level of molar ratio depends on the alcohol, lipase and feedstock used (Robles et al., 2009).

In the present study, total ester yield up to 91%was obtained using 1:4 and 1:6 molar ratios. No significant difference in the ester yields was observed with variation in molar ratio from 1:4 to 1:6. Therefore, 1:4 molar ratio was taken as optimum for maximum ester yield. This is economical as compared to higher oil to alcohol molar ratio required for chemical transesterification. In the present study, enzyme as whole cell was used rather than purified form, which is also a cost effective factor.

4.3 Effect of increase in carbon chain

Across the combination of ester obtained, ethyl ester formed after transesterification was found in between the range of 25% - 32% in all reactions except in the case of methanol. In case of methanol, ethyl ester was observed to be favoured over the methyl ester. Generally, methanol and ethanol are used as an acyl acceptor during transesterification but these alcohols inactivate the lipase enzyme and poorly mix with oil or fat (Salis et al., 2005). Solubility of methanol and ethanol in oil is approximately 50% and 75% respectively (Shimada et al., 2002). Whereas the solubility of propanol and other higher alcohols is better as they are completely miscible with vegetable oils and animal fats because of their low polarity over methanol and ethanol (Boocock et al.,1996).

Consequently, transesterification reactions with higher alcohols having carbon atoms >3 are monophasic throughout (Zhou and Boocock 2006 a,b). The monophasic nature of reactions influences the rate and extent of the reaction. There are no mass transfer limitations in the case of higher alcohols, since all reactants are in a single (oil) phase. Alcohols other than ethanol facilitate only marginal increase in ester yield with increase in carbon chain with no significant variation from propanol to octanol.

4.4 Quantification of different alkyl esters by ^{1}H NMR

In ^{1}H NMR spectrum, amplitude of a proton peak is proportional to the number of hydrogen nuclei contained in the molecule and each peak indicates different environments of hydrogen atoms in a molecule. Figure 6 showing a typical ^{1}H NMR spectrum of ethyl-butyl ester blend. After ethanolysis, if sample contains oil, methylene hydrogen of glycerol ($-C\underline{H}_2OCOR$) in unreacted oil and ethoxy hydrogen ($-OCOC\underline{H}_2CH_3$)of ethyl ester are superimposed at the region of 4.09-4.15 ppm as a quadrate. Unlike ethoxy hydrogen (quadrate), signal of alcoxy hydrogen ($-OCOC\underline{H}_2CH_2CH_2-$) of other alcoxy ester (propyl onwards) appears at lower δ value (4.00-4.09 ppm) as a triplet. Similarly, in the case of methyl-ethyl ester blend, signal of methoxy hydrogen ($-OCOC\underline{H}_3$) appears at 3.66 ppm as a singlet (figure. 7).

Whereas, signal of α-acyl methylenic hydrogen ($-OCOC\underline{H}_2-$) of all esters and unreacted FFA and oil appears in between 2.22-2.24 ppm. For the quantification of ester yield, integral values ofalcoxy hydrogen of individual ester and α-acyl methylenichydrogenhas been taken in the consideration.The ester percentage conversion C, was calculated using the following formula given by Silva et al.(2005).

$$\%C = 100 \times \left(\frac{I_{TAG+AE} - I_{TAG}}{I_{\alpha CH_2}} \right)$$

The notations of which are (i) C = conversion of oil into biodiesel (ii) I_{TAG} = integration of glyceryl methylenic hydrogen (-C$\underline{H}_2$OCOR) of oil at 4.28-4.32 ppm; (iii) $I_{\mathrm{TAG+AE}}$ = joint integration of glyceryl methylenic hydrogen of oil and ethoxy hydrogen of ethyl ester at 4.09-4.15 ppm (quadrate) oralcoxy hydrogen of other alkyl esters in between 4.00-4.09 (triplet) ; and (iv) $I_{\alpha\mathrm{CH2}}$ = integration of α-acyl methylenic hydrogen (-OCOCH$_2$-) in oil and ethyl esters in between 2.2-2.4 ppm (figure 6).

The methyl ester was quantified using following formula given by Gelbard et al.(1997).

$$\%C = 100 \times \frac{2I_{ME}}{3I_{\alpha CH_2}}$$

The notations of which are (i) C = percentage conversion of triglycerides to corresponding methyl ester; (ii) $2I_{\mathrm{ME}}$= integration value of the methoxy protons of the methyl esters at 3.66 ppm; and (iii) $3I_{\alpha\mathrm{CH2}}$= integration value of α-methylene protons in between 2.2-2.4 ppm.

Table 2 outlines the observations on ester yield (n=2) across two different blends of various alcohols.The longer chain alcohol was preferred over smaller chain as evident by increasing yield of ester in methanol-ethanol blend as well as blends of ethanol-long chain alcohols.

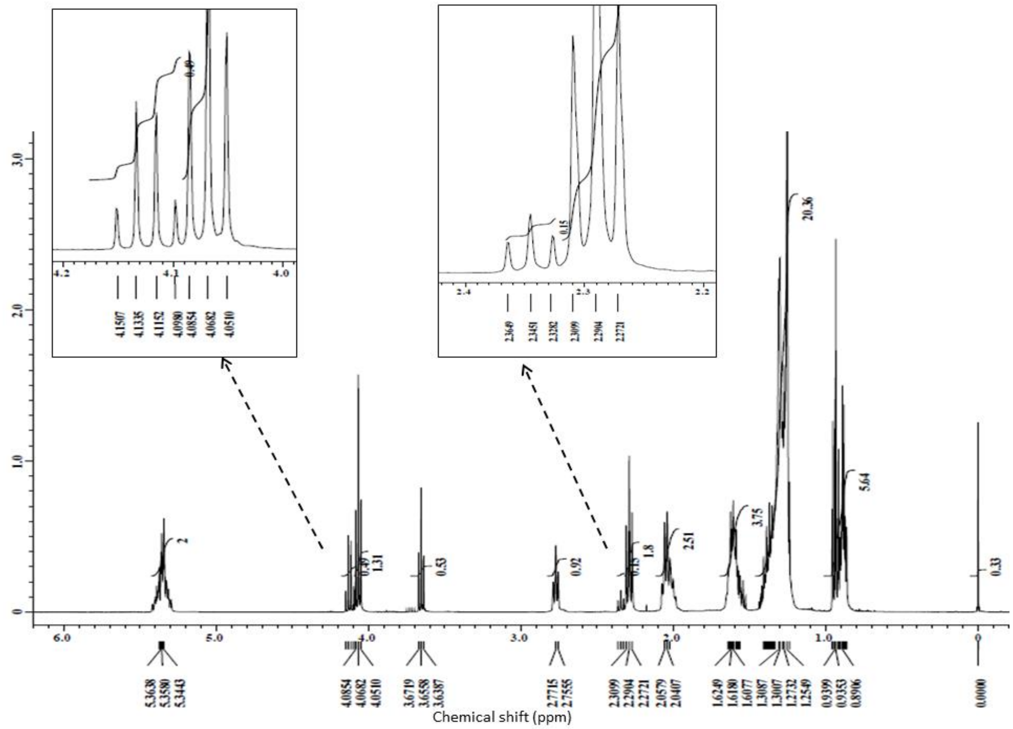

Figure 6: ^{1}H NMR spectra of ethyl-butyl ester

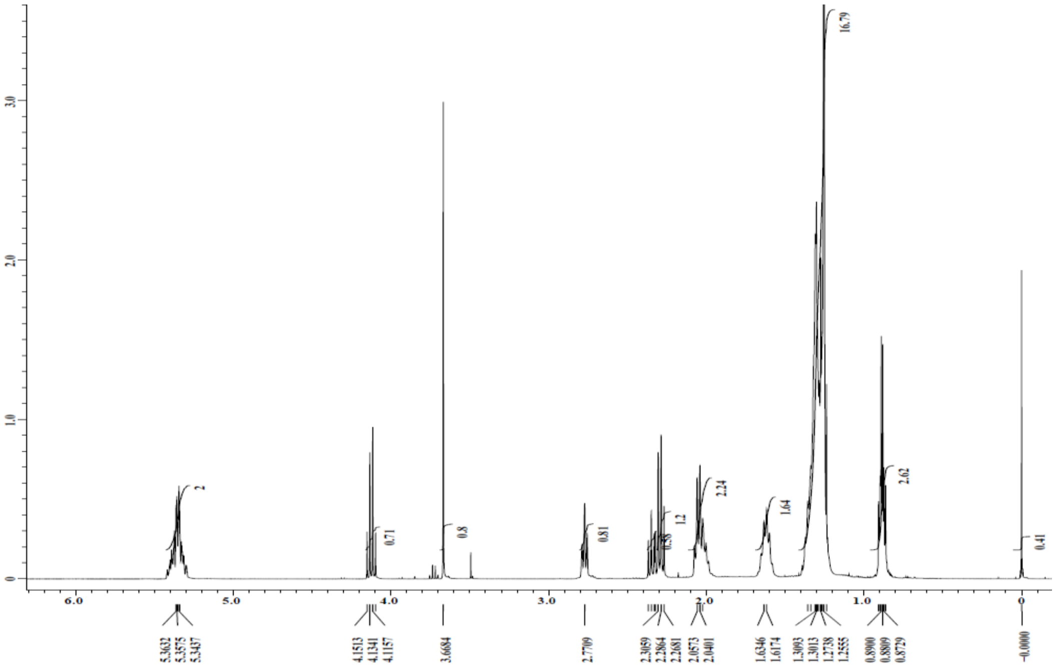

Figure 7: ^{1}H NMR spectra of mthyl-ethyl ester

Table 2: Percentage of ethyl ester and corresponding ester in different molar ratio

Type of Alcohol	Total ester (%)		% Ethyl ester		% of Other corresponding ester	
	Alcohol molar ratio					
	1:4	1:6	1:4	1:6	1:4	1:6
Methanol	77.00±7.1	76.85±0.1	46.67±4.2	45.50±0.7	30.33±3.5	31.35±0.1
Propanol	91.25±0.4	87.15±2.0	32.70±0.4	31.45±2.2	58.50±0.7	55.85±2.0
Butanol	90.00±3.3	83.60±3.5	25.20±0.2	29.75±3.5	64.80±3.4	53.85±3.5
Pentanol	82.53±1.4	80.15±4.3	32.13±5.6	27.80±1.1	50.40±4.2	52.25±4.3
Hexanol	84.35±2.6	74.50±0.8	29.05±2.6	28.80±0.6	55.30±0.0	45.70±0.8
Heptanol	87.30±1.8	80.70±4.0	31.65±0.5	29.55±0.6	55.65±2.3	51.15±4.0
Octanol	87.75±6.7	80.65±8.5	24.70±0.4	27.50±2.1	63.10±7.2	53.00±8.5

4.4.1 A comparative analysis of trends between molar ratios and between different types of alcohols

Statistical analysis (t-test) was carried out compare the yields of ethyl ester over the yields of esters from other alcohols at a given molar ratio; and over different molar ratios. The results show that there was no significant difference (p=ns) between the yield of ethyl esters (EE) as well as other esters (other) in the blends across both the molar ratios (figure 8).The observations, thus, indicate that the increase in the concentrations of the alcohols in terms of molar ratio does not result any significant variation in the yield of esters.

In addition the yield of ethyl ester was higher than methyl ester when methanol was blended with ethanol as acyl acceptor, whereas in case of other blends the yield of other esters was higher than ethyl ester. This clearly indicates that the alcohols with longer carbon chain are preferred over the shorter carbon chain (figure 9), during the transesterification process, an observation that is notable in the present study. There was a significant ($p<0.005$) variation between the yields of ethyl ester over the yield of other esters in either of the molar ratios.

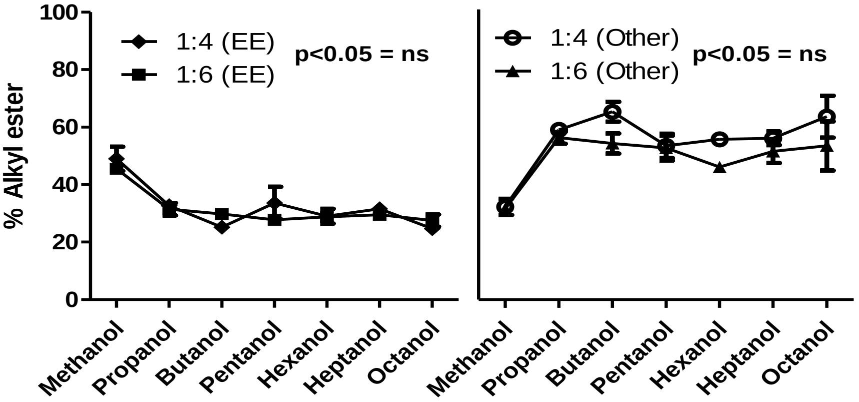

Figure 8: Trends in the yields of ethyl and other esters across 1:4 and 1:6 molar ratios

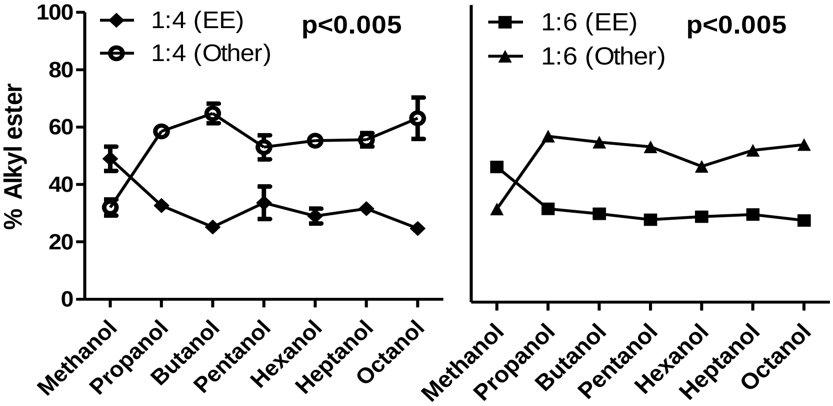

Figure 9: Variations in the yields of ethyl ester and other esters in both 1:4 and 1:6 molar ratios

4.5 Effect of blends on fuel properties of ethyl-propyl and ethyl-butyl esters

In general practice, alcohol-biodiesel blends are used to overcome the disadvantage of higher viscosity and poor cold flow behaviour for biodiesel and the lower cetane number for alcohols; whereas the blendsshows better fuel properties compared to biodiesel fuel (Choi et al., 1997).

Table 3: Different parameters of propyl and butyl ester produced from cottonseed oil

Parameters	Unit	Propyl ester	Butyl ester	ASTM D6751 standard for biodiesel
Gross calorific Value (GCV)	Kcal/kg	9382	8866	7870
Flash Point	°C (minimum)	104	66	130
Ash	% w/w	ND	ND	NA
Viscosity @ 40°C	centipoise	8.0223	8.1129	4.0 - 6.0
Sediment (%)	% w/w	ND	ND	0.05
Sulphur (%)	% w/w (maximum)	ND	ND	0.05
Density @15°C	gm/cc	0.8728	0.8669	0.8780
Cloud point	°C	-5	-6	NA
Pour point	°C	-20	-26	-15.0 to 10.0
Ester Content	%	90.07	93.21	NA

NA: Not available, ND: Not detected

After transesterification for selected ratio of alcohols, samples with high ester yield viz., ethyl-propyl and ethyl-butyl was subjected to various tests to determine physio-chemical properties relevant to biodiesel. The resulting ethyl-propyl and ethyl-butyl esters were examined for gross calorific value (GCV), flash point, ash, kinematic viscosity, sediment, sulphur content, density, cloud point (CP) and pour point (PP). The results indicated that the characteristics of ethyl-propyl as well as ethyl-butyl blends were approximately similar to as per the standards specified in Table 3.

4.5.1. Effect on cloud point and pour point

Pour points (PP) and cloud points (CP) have been used to estimate the behaviour of diesel fuels in cold weather. According to the ASTM standard, the PP is the "lowest temperature at which movement of the oil is observed", whereas the CP is the highest temperature at which crystals formed within the oil become visible in form of cloudiness(Edith et al., 2009).

Ester head group has prominent effect on fuel properties of biodiesel.Esters with short, unsaturated and branched carbon chains possess very good cold flowproperties. Due to the high temperature of crystallization exhibited by the saturated fatty acids, biodiesel with high composition of saturated fatty acid esters exhibits inferior cold flow properties(Edith et al., 2011).The cloud point of soybean oil methyl, ethyl, isopropyl, and 2-butyl esters is −2, −2, −9 and −12°C, respectively (Lee et al., 1995) whereas cloud point of beef tallow methyl, ethyl, propyl, and butyl esters is 17°C, 15°C, 12°C, and 9°C, respectively (Fogliaet al.,1997). Soybean oil contains 14% saturated fatty acids and beef tallow contains 25% saturated fatty acids.Therefore low-temperature properties of biodiesel are influenced by double bond content of esters and type of alcohol used. Even though, cottonseed oil contains 26% saturated fatty acids yet the cloud point of propyl (-5) and butyl ester (-6) found to be better than soybean oil and beef tallow.

Similarly, PP of biodiesel also depends upon the degree of saturation. For example, PP of palm oil methyl esters is 14°C due to presence of 41% of saturated fatty acids (Ali et al., 2013). PP of ethyl-propyl and ethyl-butyl esters came to be -20°C and -26°C which is better in spite of presence of 25% saturated fatty acids.

4.5.2. Effect on flash point

The flash point of a fuel is the lowest temperature at which the fuel can form an ignitable mixture with air. A higher flash indicates that biodiesel is less flammable than petroleum diesel; hence, biodiesel is safer to handle.Fuel with higher flash point ensures greater safety in the storage and transportation. The flash points of ethyl-propyl and ethyl-butyl esters are 104°C and 66°C respectively which are higher than that of diesel fuel with 60°C.Since these blends exhibit higher flash points than diesel, they are safer fuels than the latter(Laza et al., 2011).

4.5.3. Effect on viscosity and density

Viscosity affects the atomization of a fuel upon injection into the combustion chamber and thereby higher the viscosity, the greater the tendency of the fuel to cause the formation of engine deposits (Knothe, 2004).Several structuralfeatures influence the kinematic viscosities of FAAE, such as chain length, degree of unsaturation, double bond orientation, and type of ester head group.

Factors such as longer chain length and larger ester head group result in increases in kinematic viscosity. For example, methyl esters of lauric, myristic, palmitic, and stearic acids have kinematic viscosities of 2.43, 3.30, 4.38, and 5.85cp respectively. Furthermore, methyl, ethyl, and butyl esters of stearic acid exhibit kinematic viscosities of 5.85, 5.92, and 7.59cp respectively (Moser, 2009).In present study, ethyl-propyl and ethyl-butyl ester blends shows viscosity of 8.0223cp and 8.1129cp respectively at 40°C. This is much lower than that of the feedstock oil i.e. 30cp and is an advantage over the refined cottonseed oil. But it is higher than viscosity of standard biodiesel (4.0-6.0cp).

Biodiesel has densities between 0.860gm/cc and 0.897gm/cc at 15°C which is higher than that of petroleum diesel (Bhale et al., 2009). The density of ethyl-propyl and ethyl-butyl ester blends came to be 0.8728gm/cc and 0.8669gm/cc which full fill the prescribed limits. This high density can be said to make up for their lower volumetric energy content.

4.5.4. Effect on gross calorific value

Heating Value of a fuel is the thermal energy released per unit quantity of fuel when the fuel is burned completely. The Gross Heat of Combustion or Higher Heating Value (GHC or HHV) is obtained by oxygen – bomb calorimeter method as the latent heat of moisture in the combustion products is recovered (Demirbas et al., 2003). Factors that influence the energy content of biodiesel include the oxygen content and carbon to hydrogen ratio. The energy content of FAAE is directly proportional to chain length (Knothe, 2008; Moser, 2009b) Biodiesel fuels with larger ester head groups (such as ethyl, propyl, or butyl) are expected to have greater energy content as a result of their greater carbon to oxygen ratios.The GCV of ethyl-propyl and ethyl-butyl ester blends are 9382 and 8866 Kcal/kg respectively which is higher than standard biodiesel(7870 Kcal/kg). But it doesn't full fill the condition of carbon to hydrogen ratio which can be due to experimental error.

The present study, thus, demonstrates that higher ester yield can also be achieved using a molar ratio of 1:4 or 1:6 (oil: alcohol) and whole cell catalyst for the production of fatty acid alkyl esters. By the use of lower molar ratio and whole cell catalyst, there is significant reduction in the cost of production of biodiesel. Also, biodiesel shows better cold flow properties with increase in carbon chain of alcohol used. Alcohols other than ethanol facilitate only marginal increase in ester yield with increase in carbon chain with no significant variation from propanol to octanol.

Conclusion

The salient findings of the study are as follows:

- Total ester yield up to 91% and 87% was obtained using 1:4 and 1:6 molar ratios. No significant difference in the ester yields was observed with variation in molar ratio from 1:4 to 1:6 (oil: alcohol).
- Alcohols other than ethanol facilitate only marginal increase in ester yield with increase in carbon chain with no significant variation from propanol to octanol. Also, longer chain alcohol was preferred over shorter chain as evident by increasing yield of ester in methanol-ethanol blend as well as blends of ethanol-long chain alcohols.
- Cold flow properties of ethyl-alkyl ester blends were observed to be better than properties of standard biodiesel. Also, flash point was improved using alcohol blends than standard biodiesel flash point.

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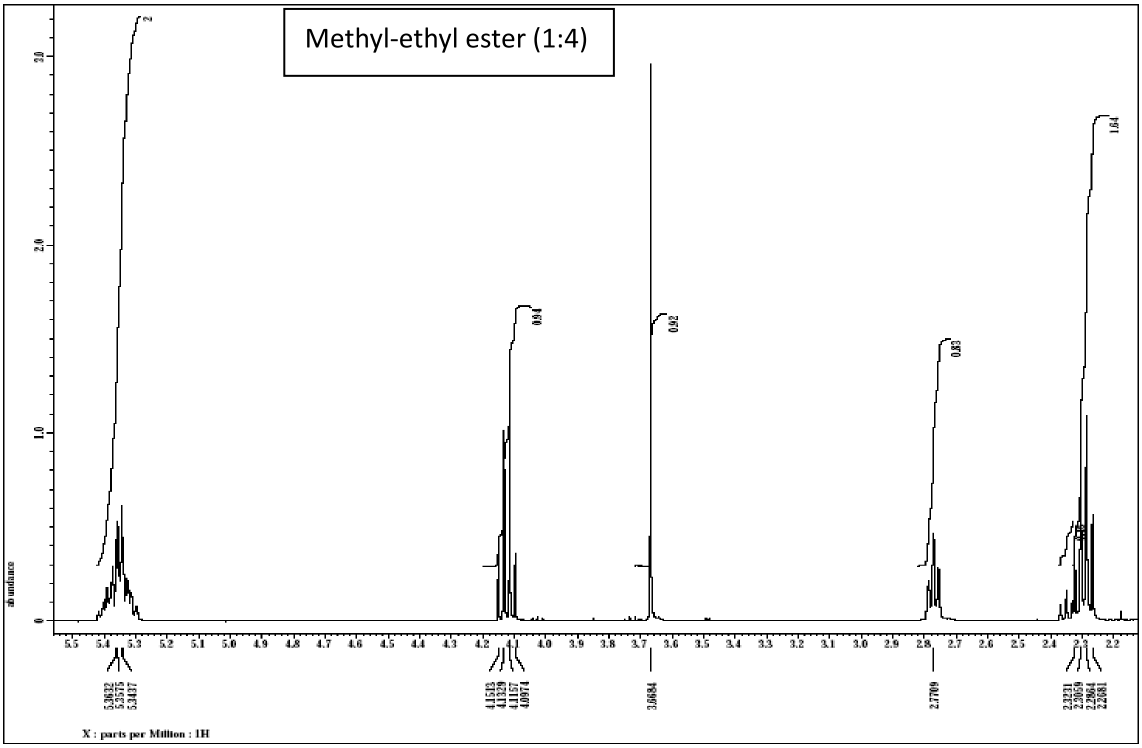
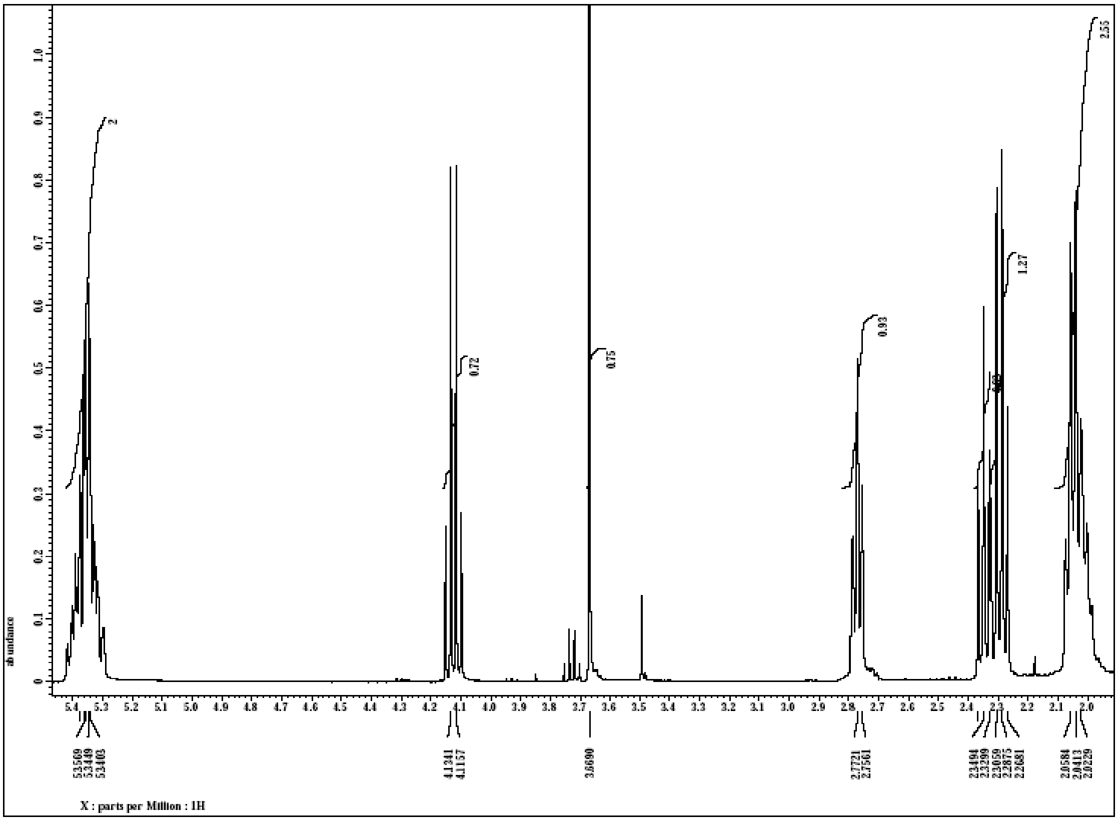
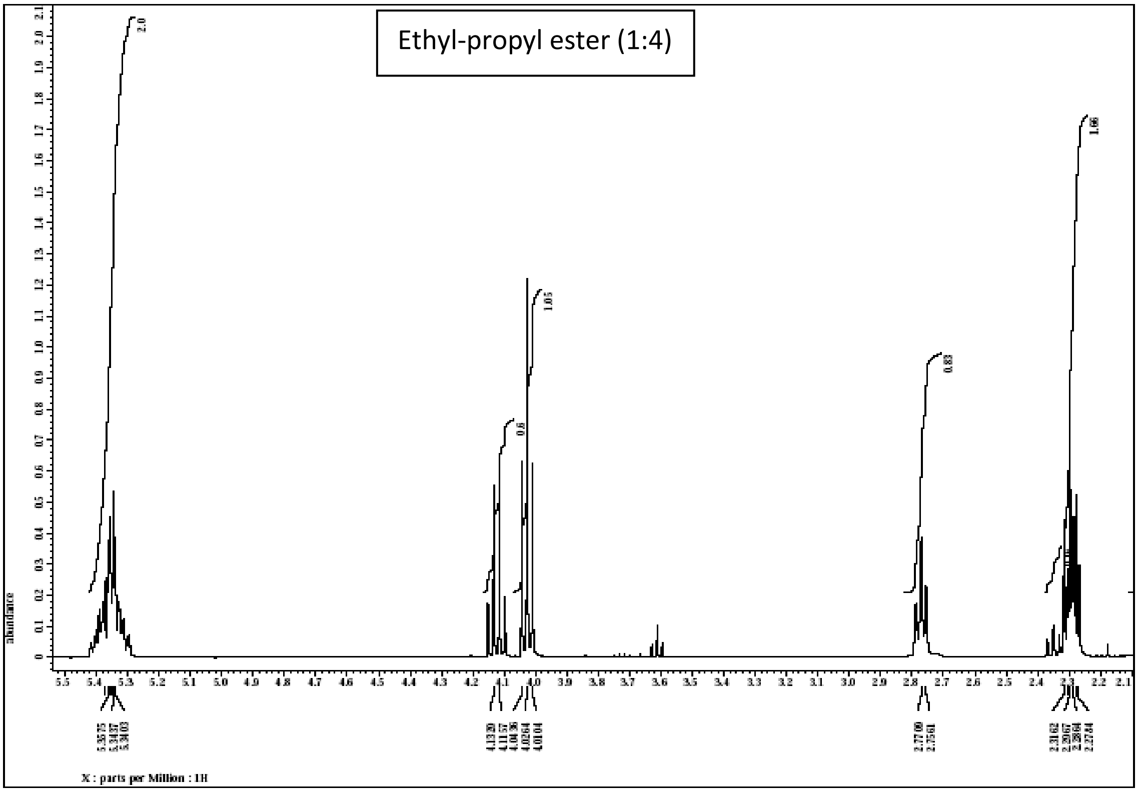
Annexure



Methyl-ethyl ester (1:4)

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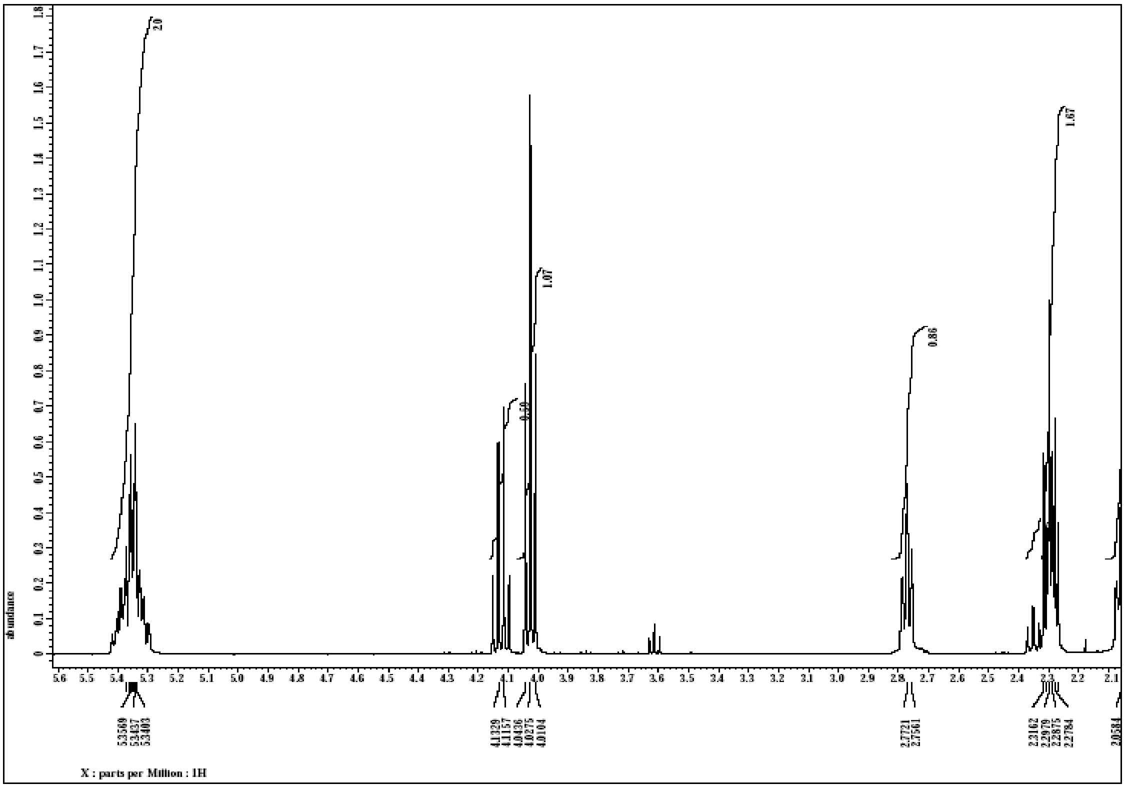
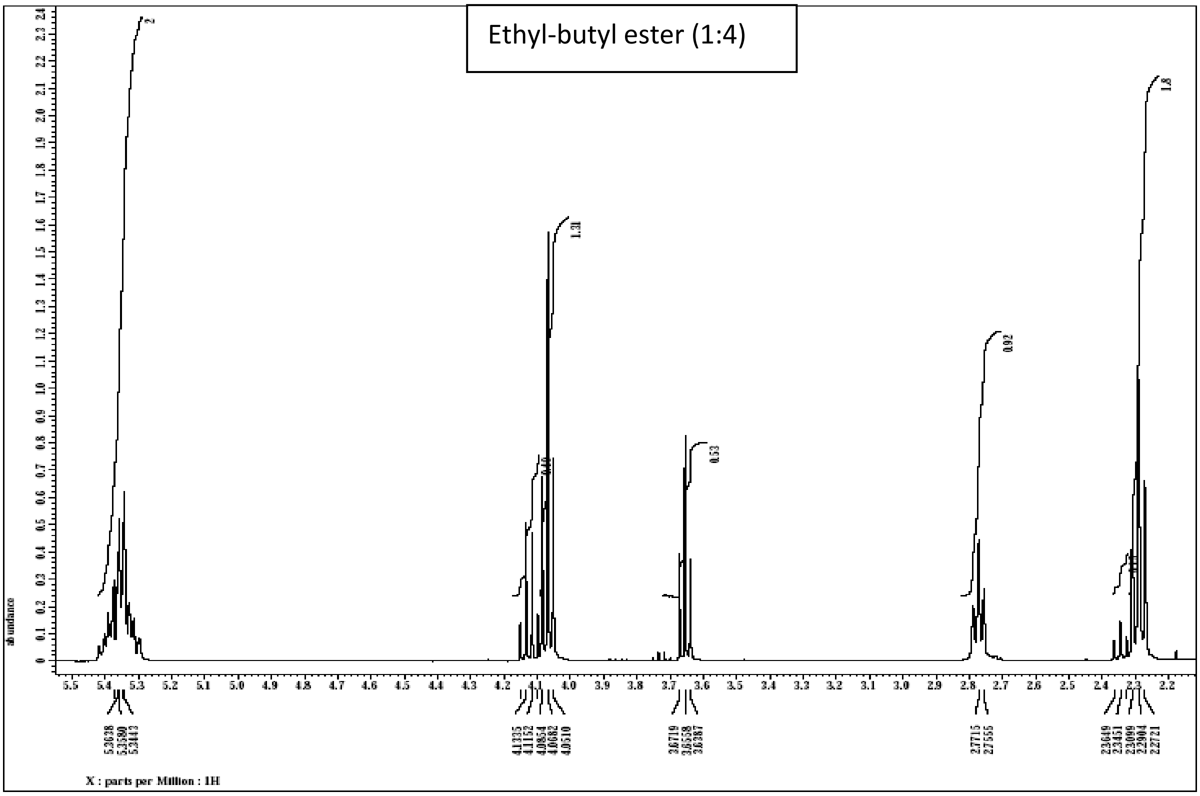
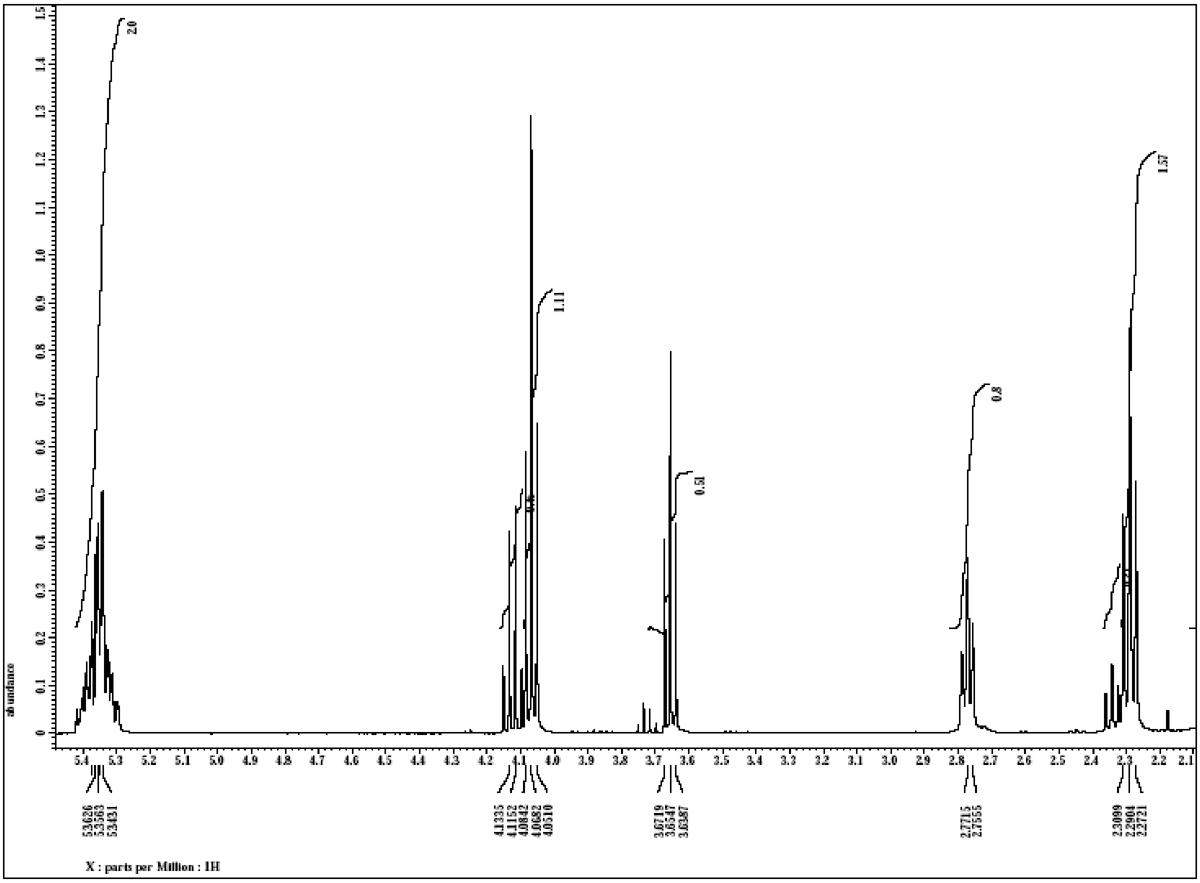
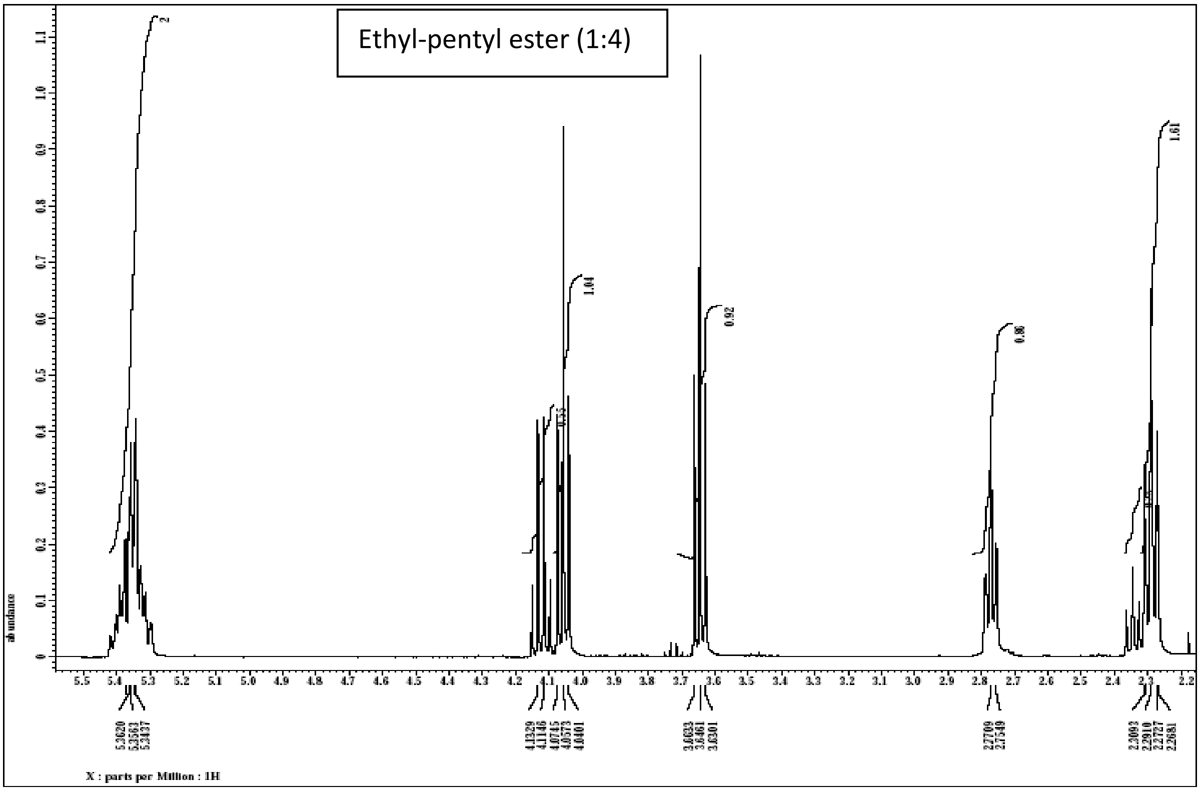
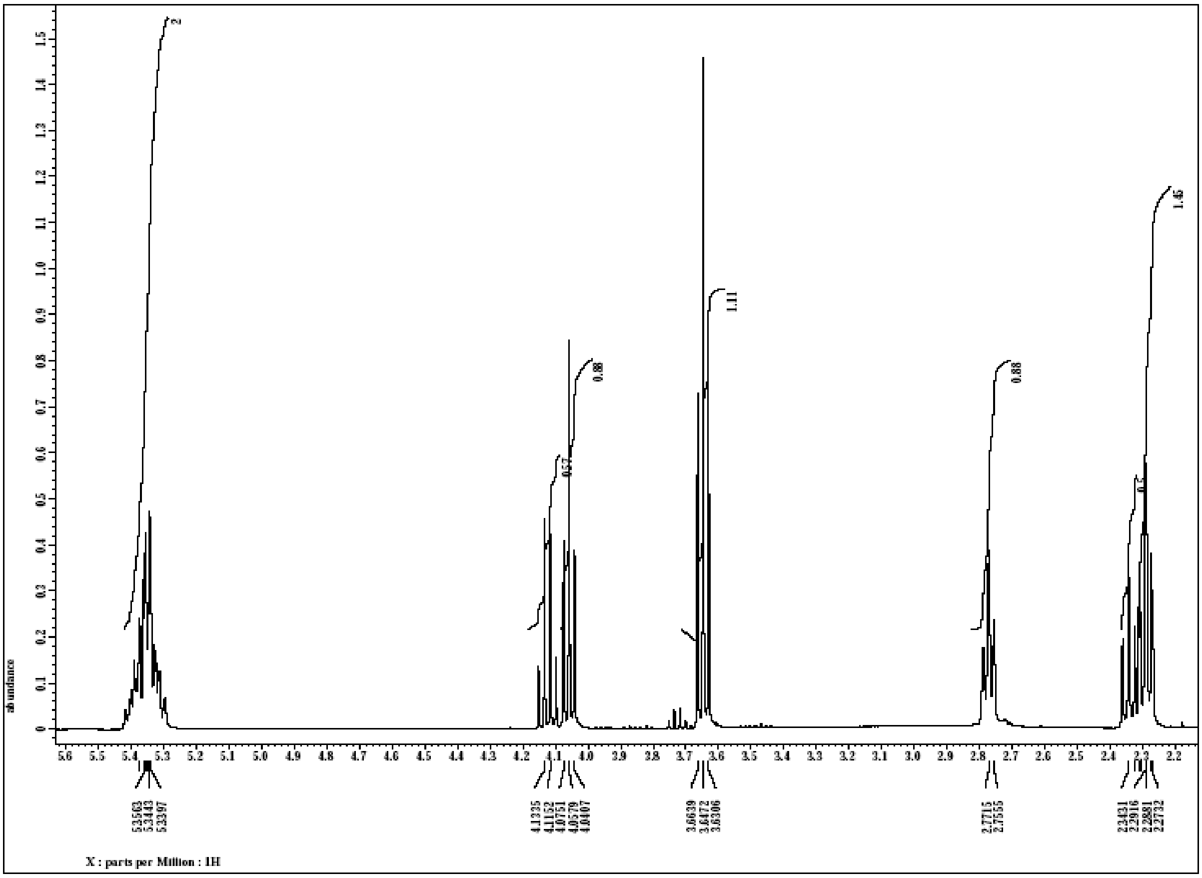


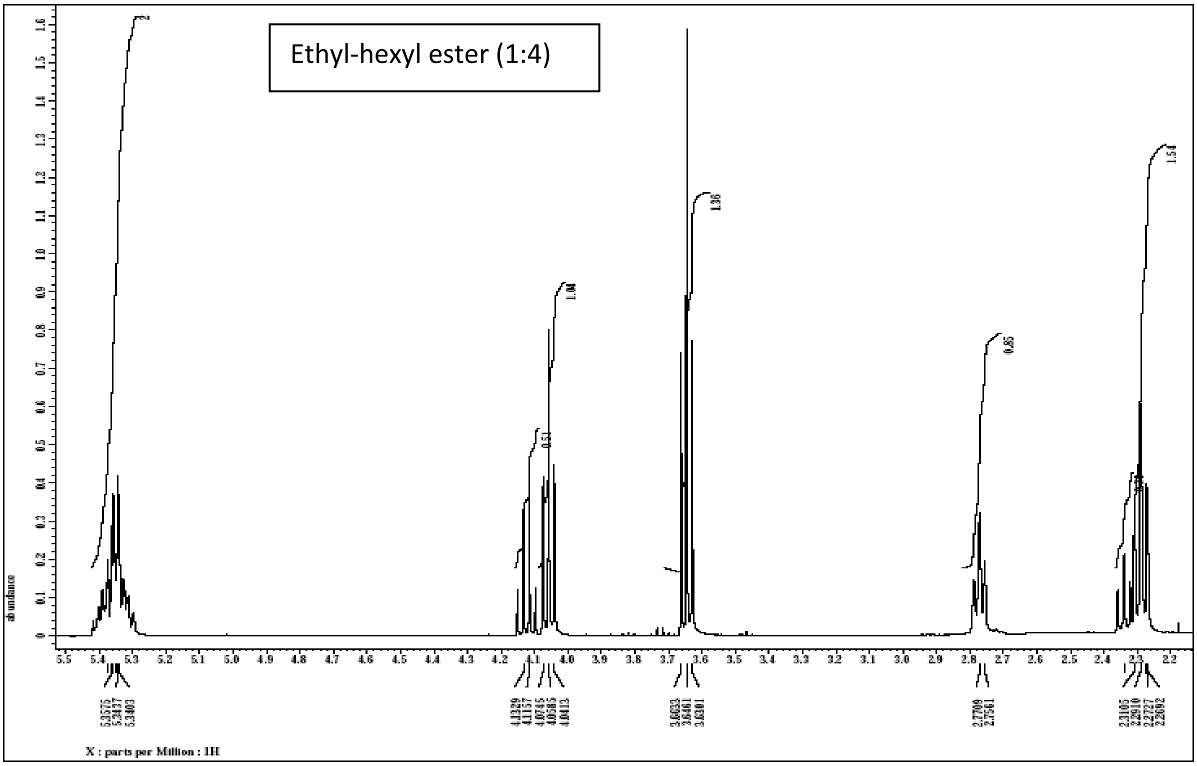


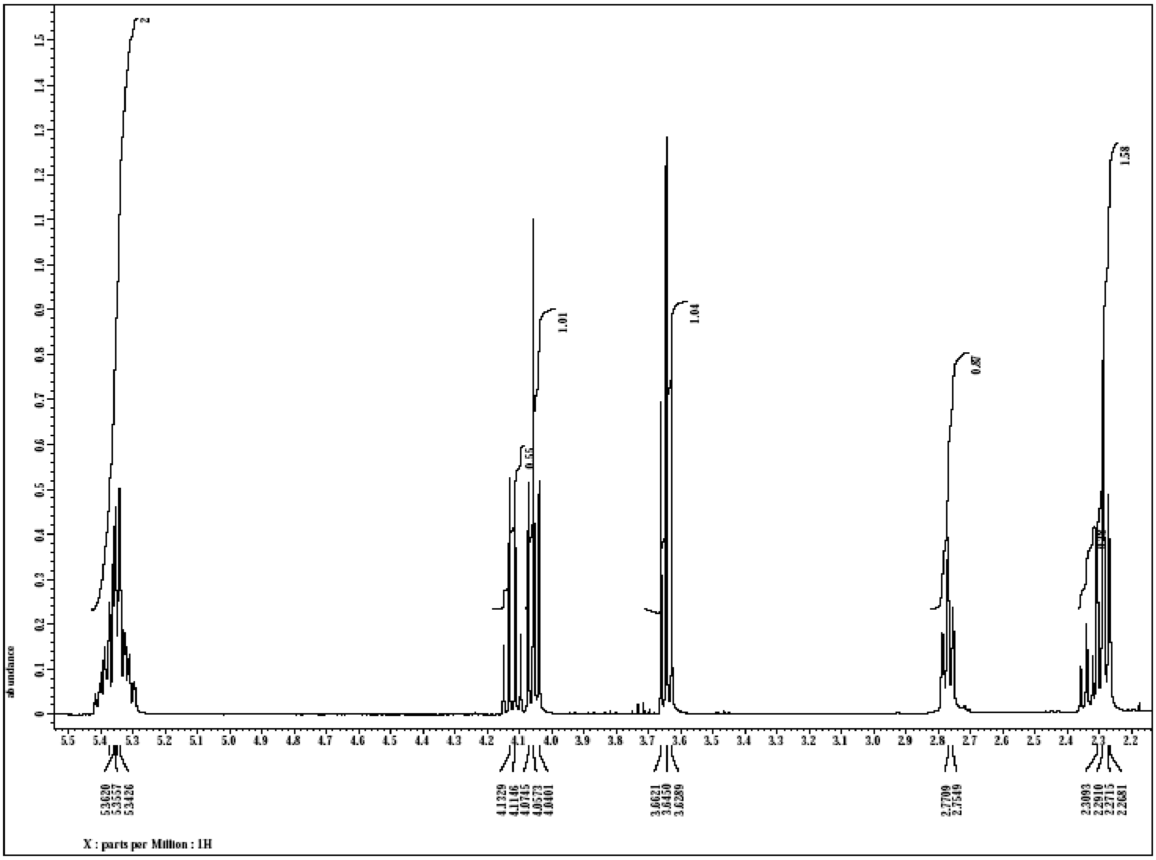
Ethyl-butyl ester (1:4)




Ethyl-pentyl ester (1:4)
abundance
X : parts per Million : 1H
2
1.04
0.92
0.80
1.61
5.3620
5.3563
5.3437
4.1329
4.1146
4.0745
4.0573
4.0401
3.6633
3.6461
3.6301
2.7709
2.7549
2.3093
2.2910
2.2727
2.2681


abundance
X : parts per Million : 1H
2
0.88
1.11
0.88
1.45
5.3563
5.3443
5.3397
4.1335
4.1152
4.0751
4.0579
4.0407
3.6639
3.6472
3.6306
2.7715
2.7555
2.3431
2.2916
2.2881
2.2732


Ethyl-hexyl ester (1:4)
abundance
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
1.2
1.3
1.4
1.5
1.6
2
0.51
1.04
1.36
0.85
1.54
5.5
5.4
5.3
5.2
5.1
5.0
4.9
4.8
4.7
4.6
4.5
4.4
4.3
4.2
4.1
4.0
3.9
3.8
3.7
3.6
3.5
3.4
3.3
3.2
3.1
3.0
2.9
2.8
2.7
2.6
2.5
2.4
2.3
2.2
5.3575
5.3437
5.3403
4.1329
4.1157
4.0745
4.0585
4.0413
3.6633
3.6461
3.6301
2.7709
2.7561
2.3105
2.2910
2.2727
2.2692
X : parts per Million : 1H


abundance
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
1.2
1.3
1.4
1.5
2
0.55
1.01
1.04
0.80
1.58
5.5
5.4
5.3
5.2
5.1
5.0
4.9
4.8
4.7
4.6
4.5
4.4
4.3
4.2
4.1
4.0
3.9
3.8
3.7
3.6
3.5
3.4
3.3
3.2
3.1
3.0
2.9
2.8
2.7
2.6
2.5
2.4
2.3
2.2
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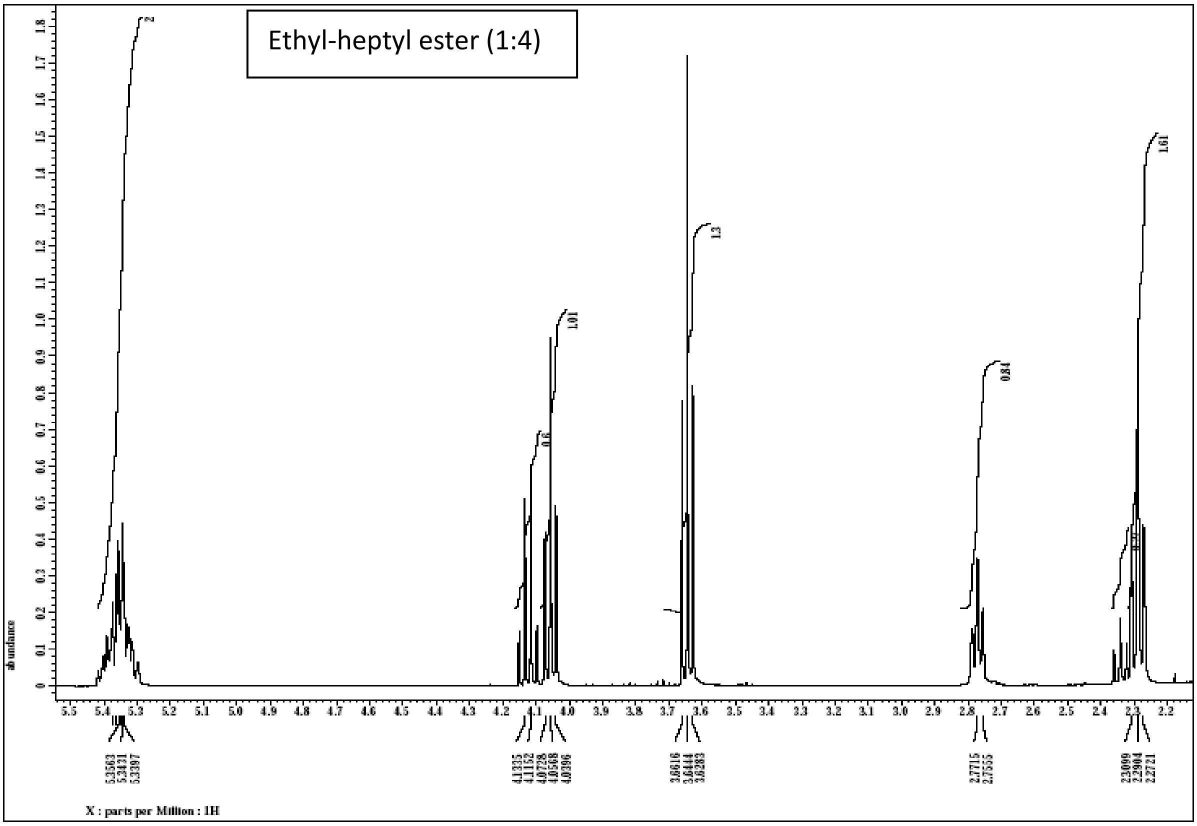
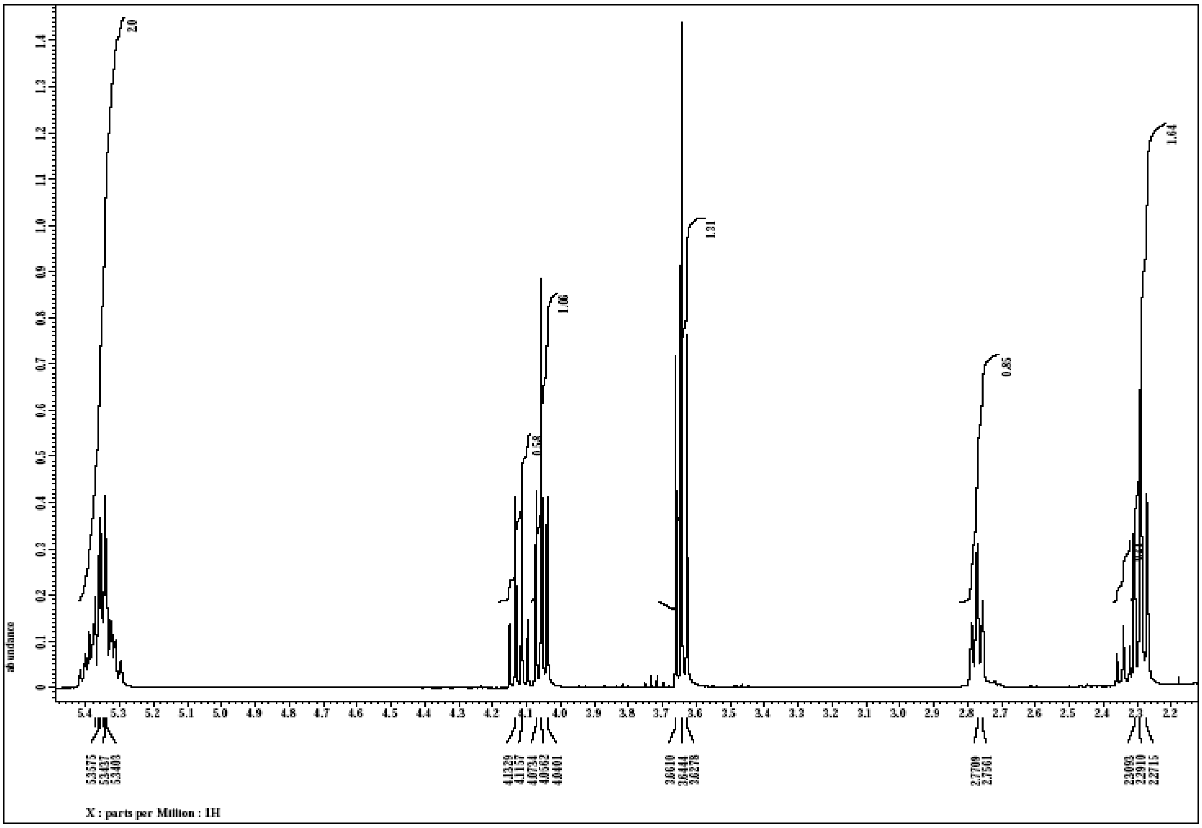
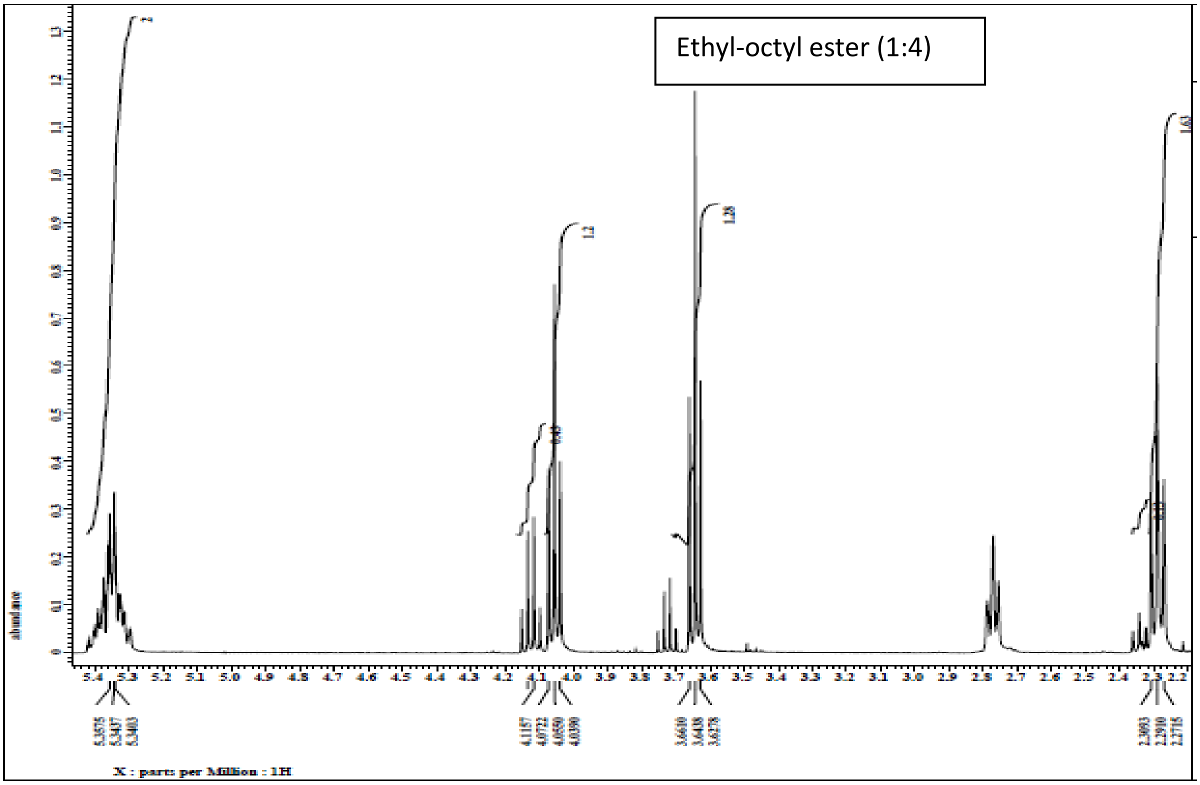
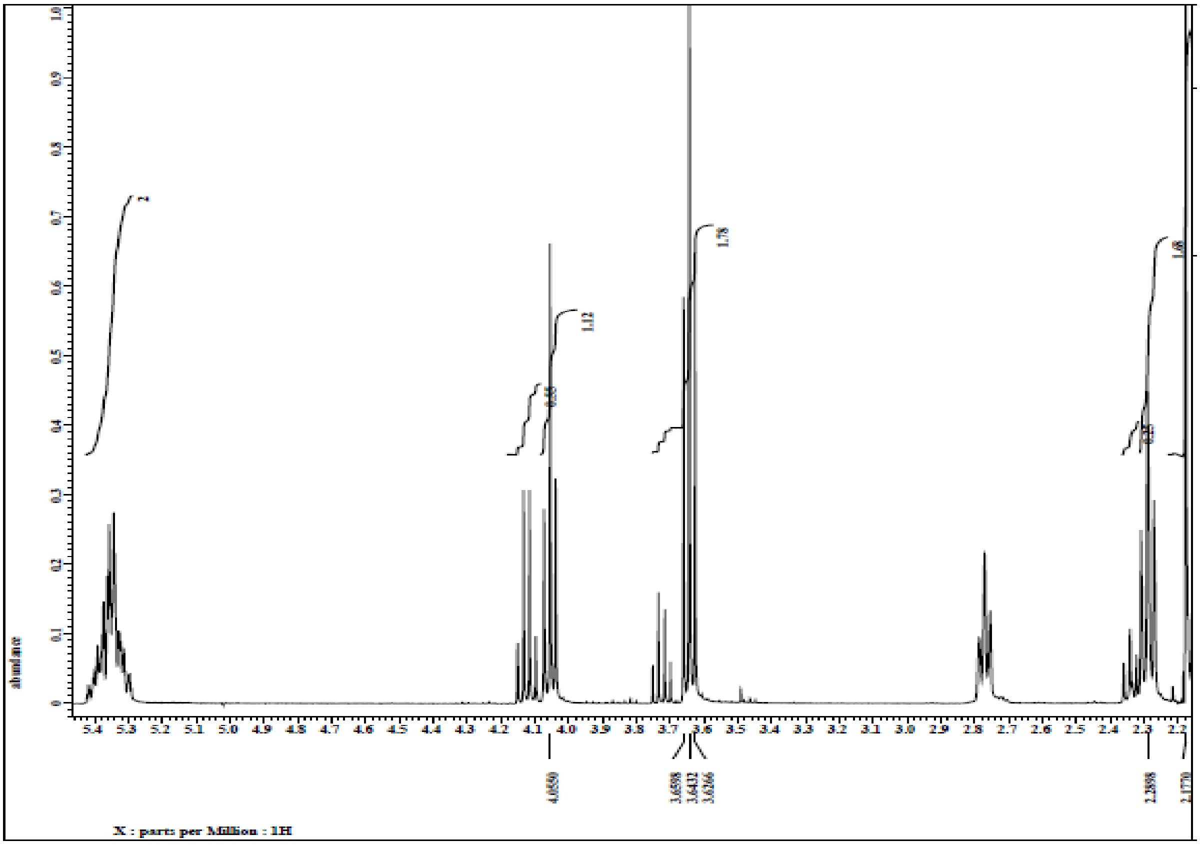
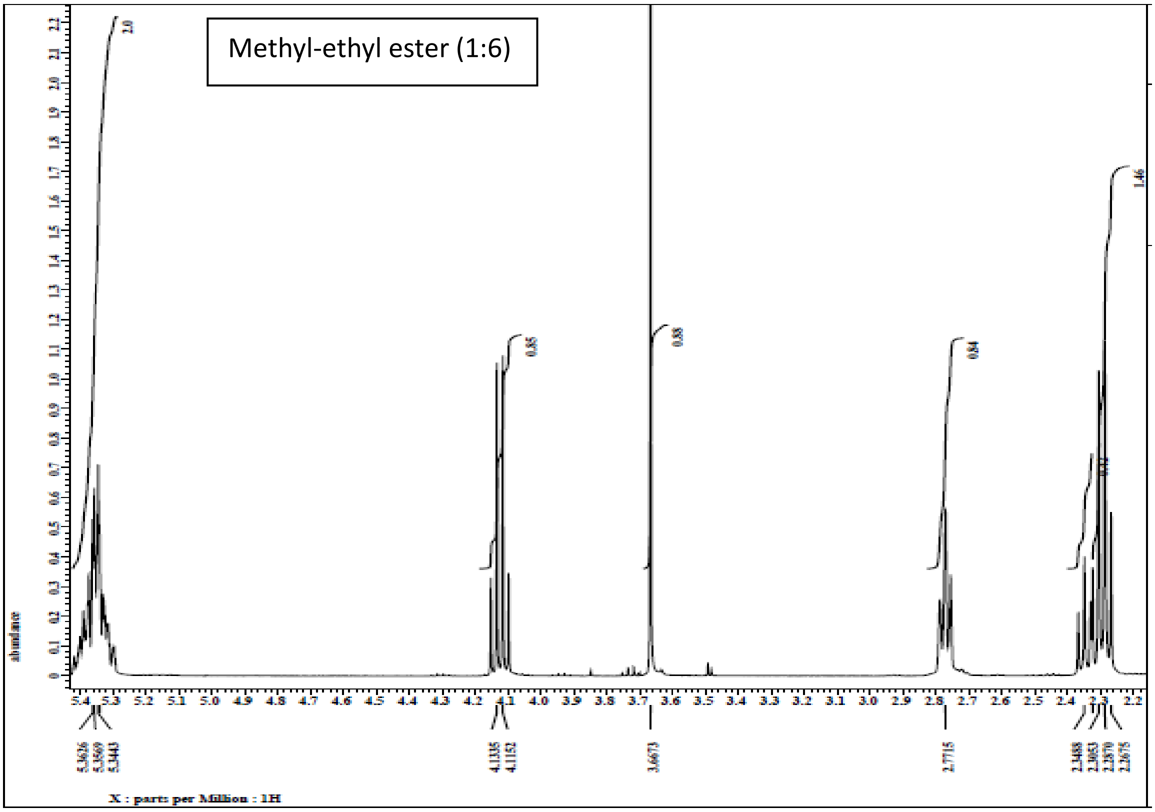
2.2910
2.2715
2.2681
X : parts per Million : 1H

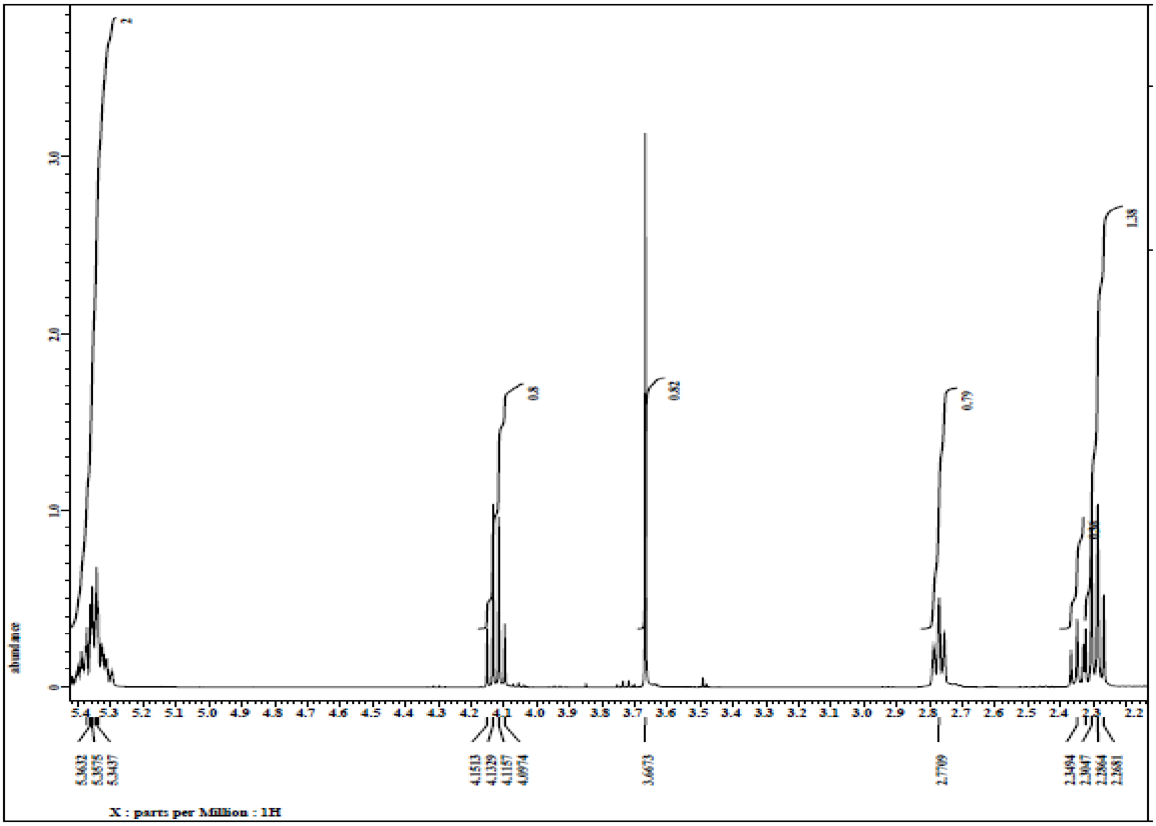

Ethyl-heptyl ester (1:4)

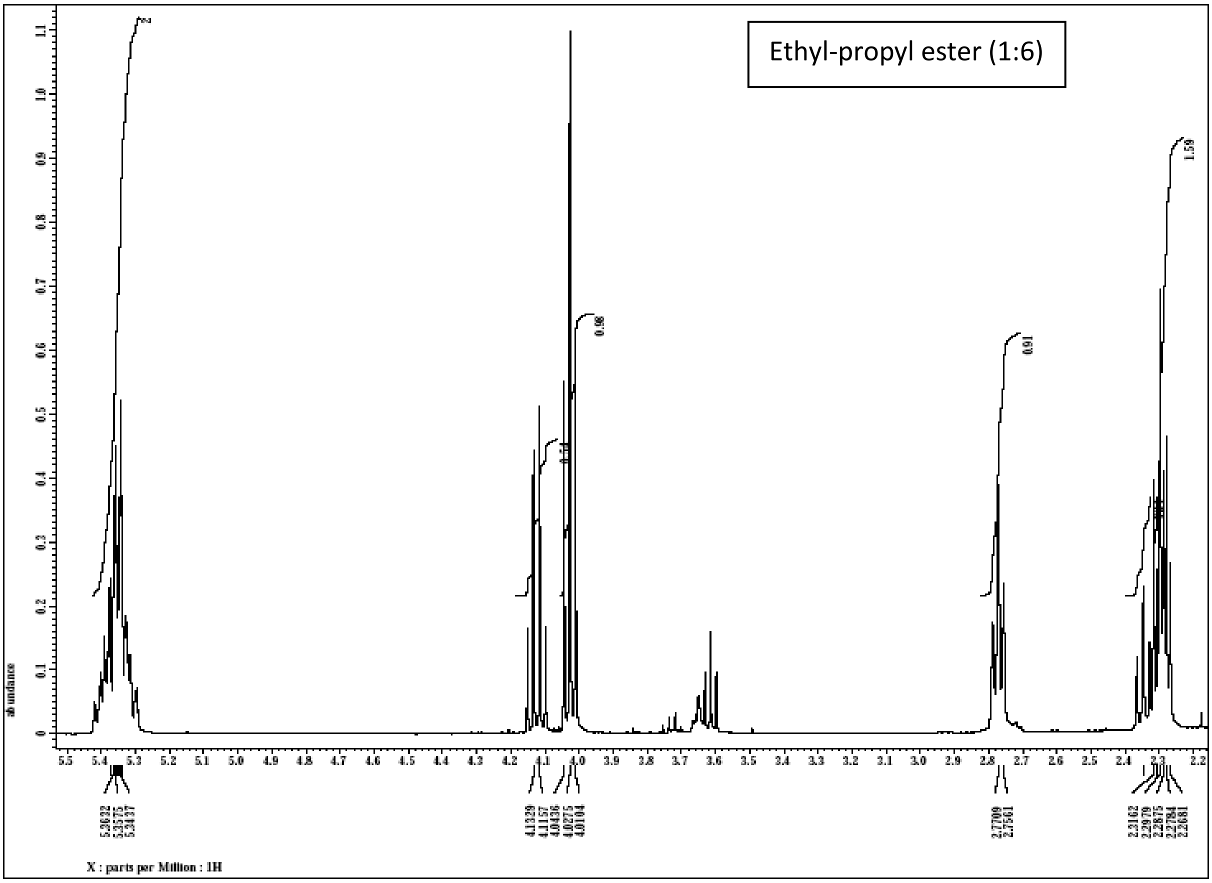



Ethyl-octyl ester (1:4)
abundance
X : parts per Million : 1H
2
1.2
0.43
1.28
1.63
5.3575
5.3437
5.3403
4.1157
4.0722
4.0550
4.0390
3.6610
3.6438
3.6278
2.3093
2.2910
2.2718

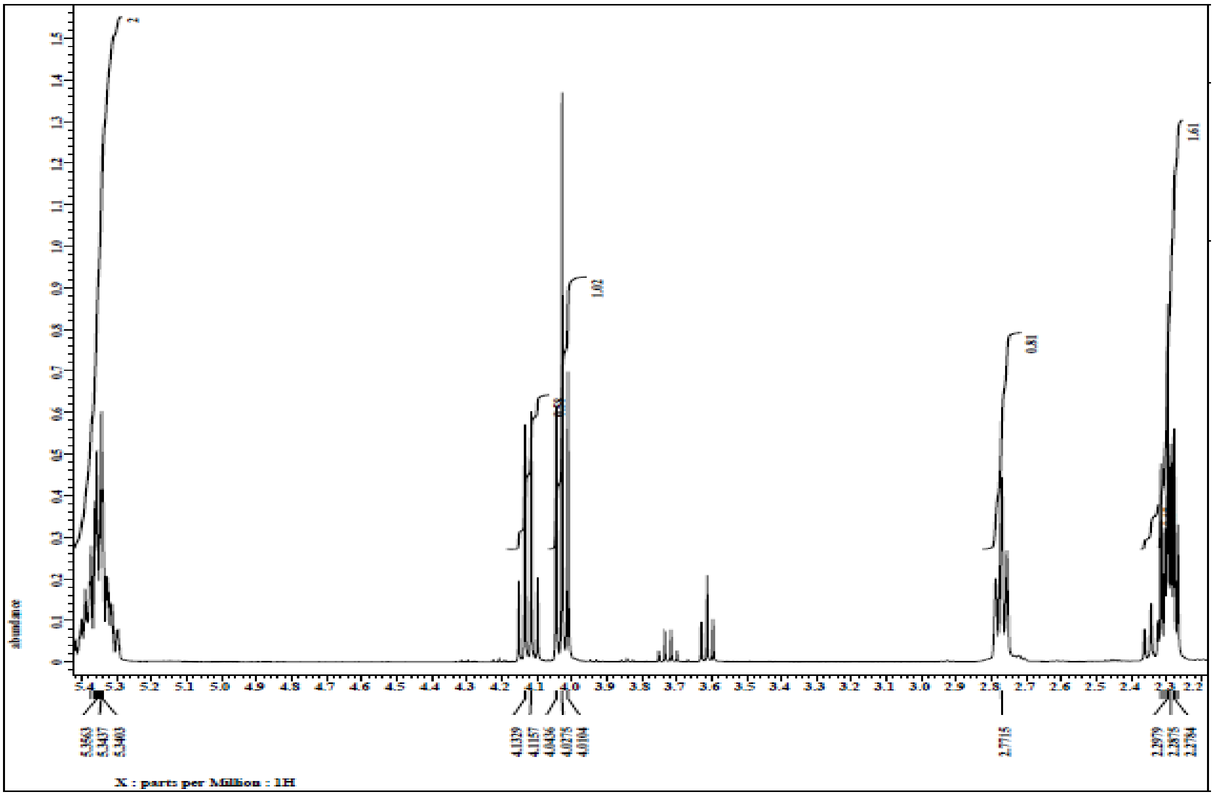

abundance
X : parts per Million : 1H
2
1.12
0.55
1.78
1.66
4.0550
3.6598
3.6432
3.6266
2.2898
2.1770

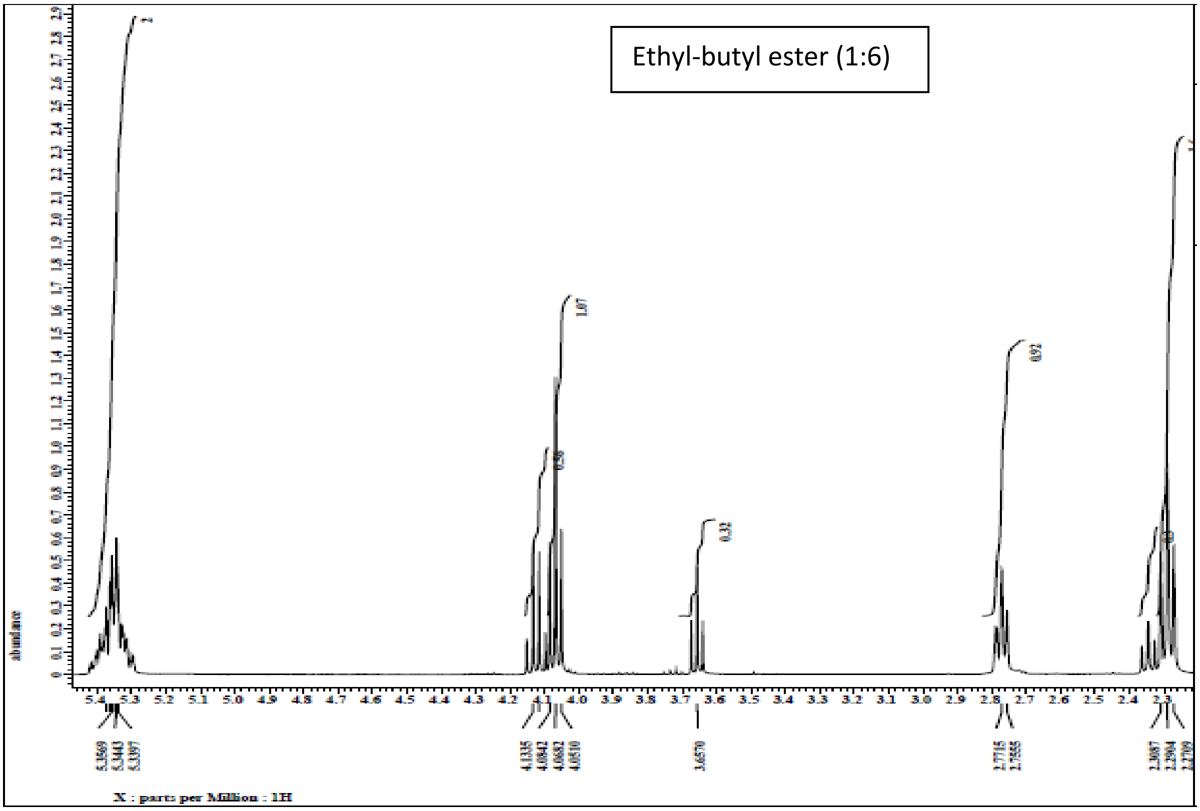

Methyl-ethyl ester (1:6)

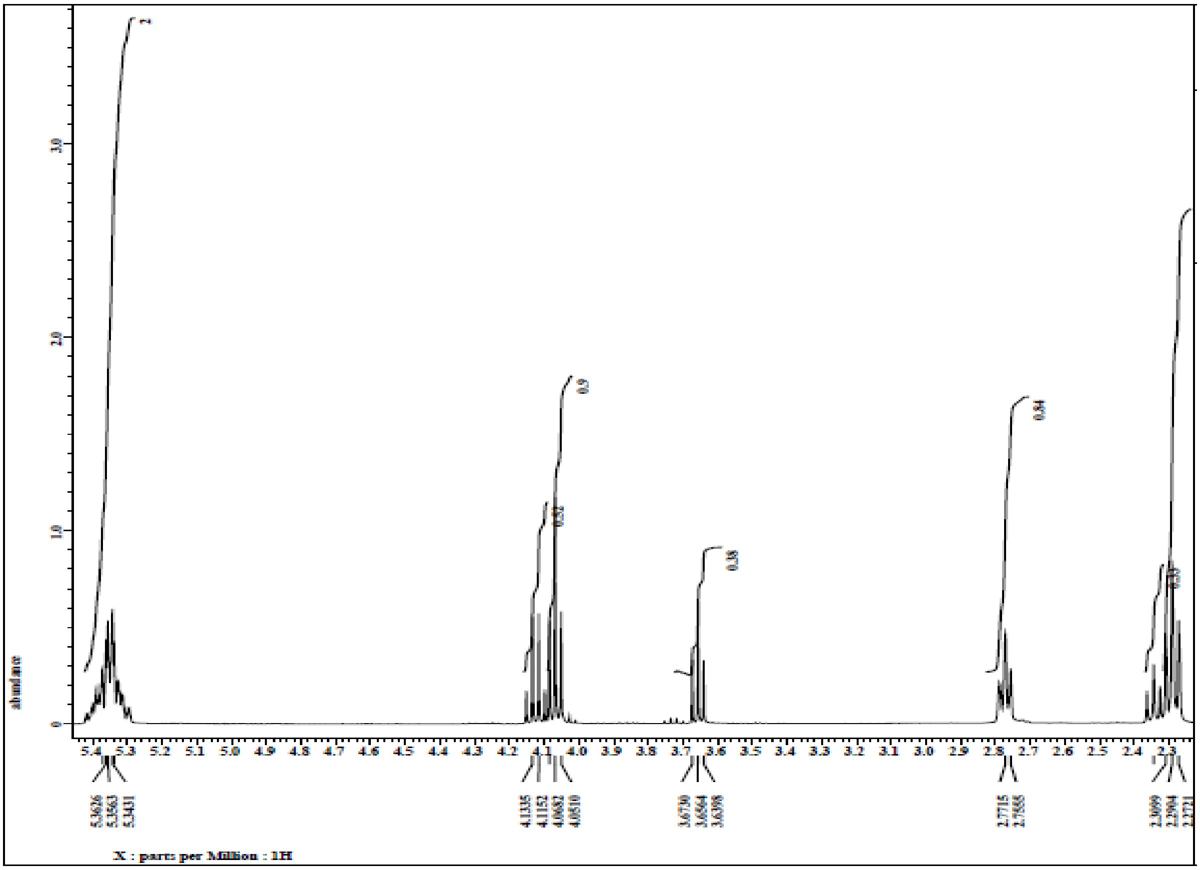




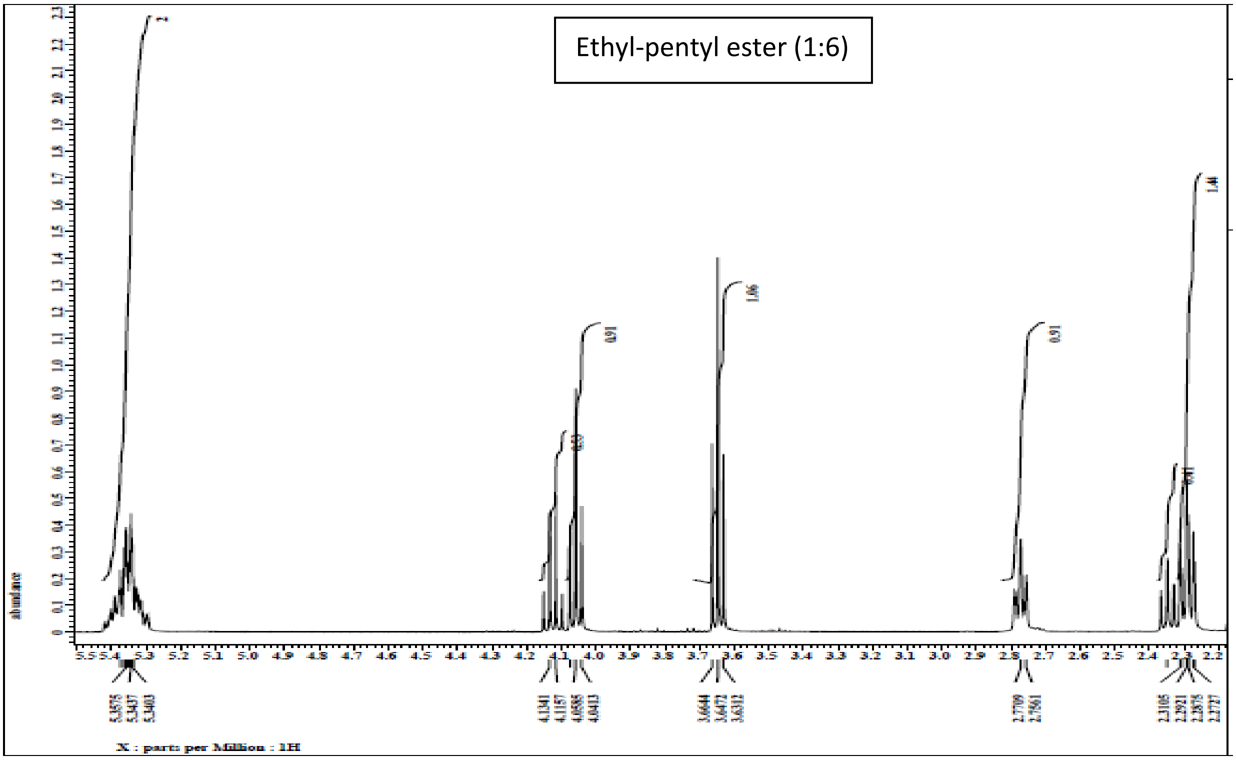
Ethyl-propyl ester (1:6)



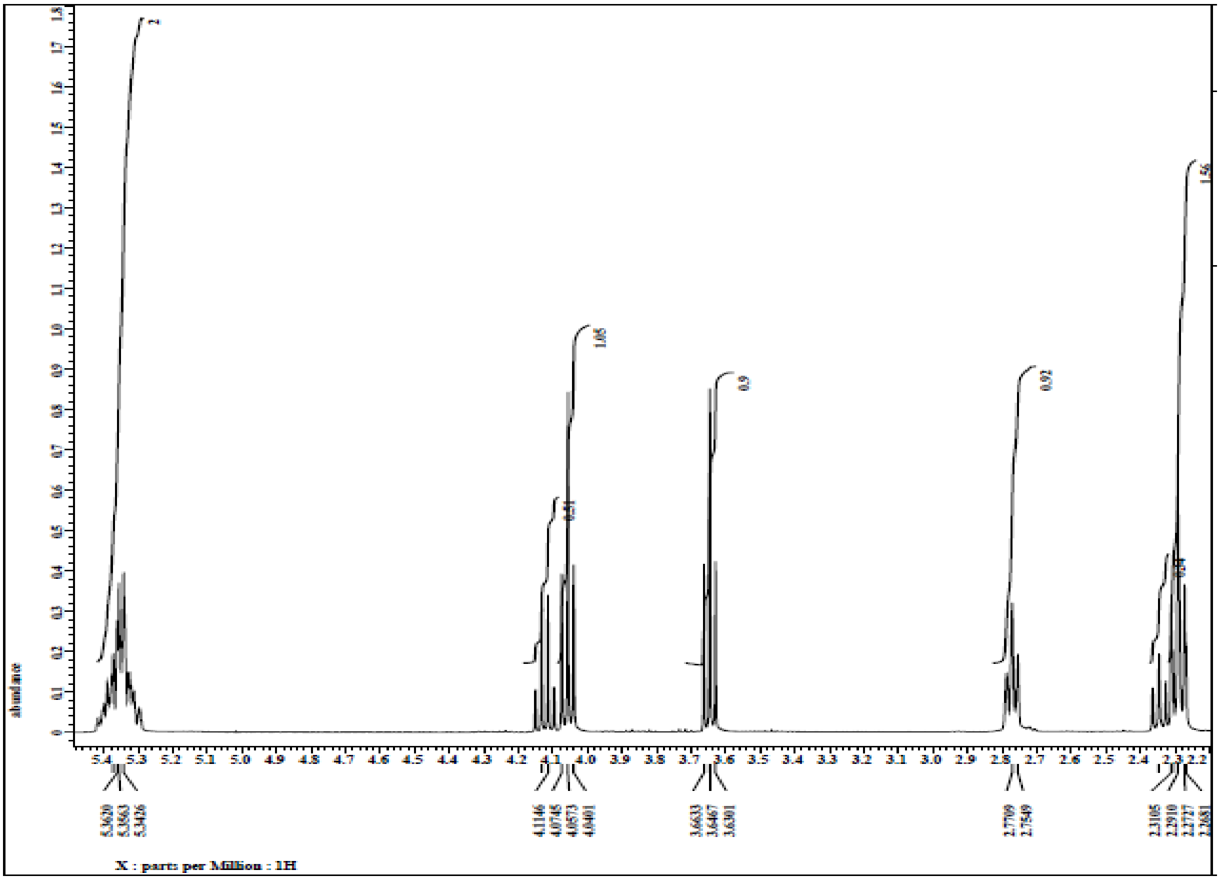

Ethyl-butyl ester (1:6)
abundance
X : parts per Million : 1H
2
0.56
1.07
0.32
0.92
5.3569
5.3443
5.3397
4.1335
4.0842
4.0682
4.0510
3.6570
2.7715
2.7555
2.3087
2.2904
2.2709

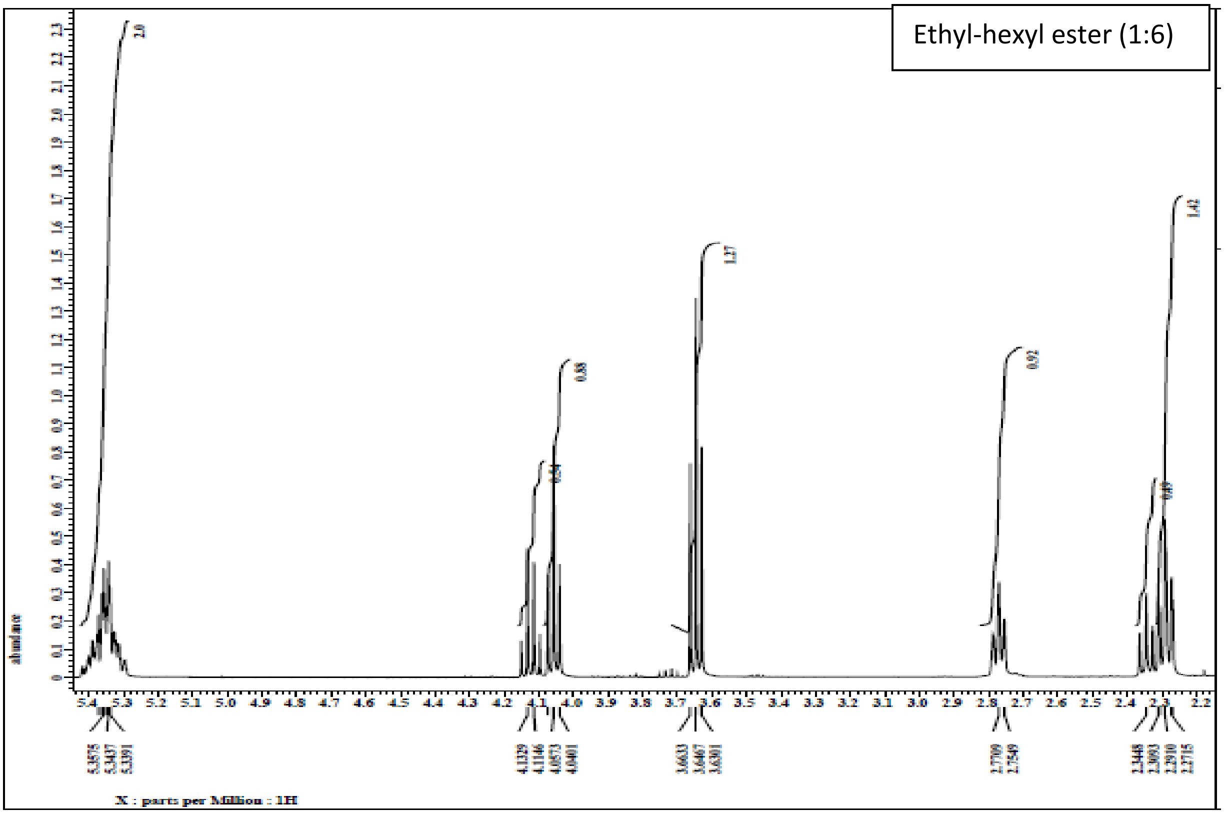

abundance
X : parts per Million : 1H
2
0.52
0.9
0.38
0.84
0.53
5.3626
5.3563
5.3431
4.1335
4.1152
4.0682
4.0510
3.6730
3.6564
3.6398
2.7715
2.7555
2.3099
2.2904
2.2721

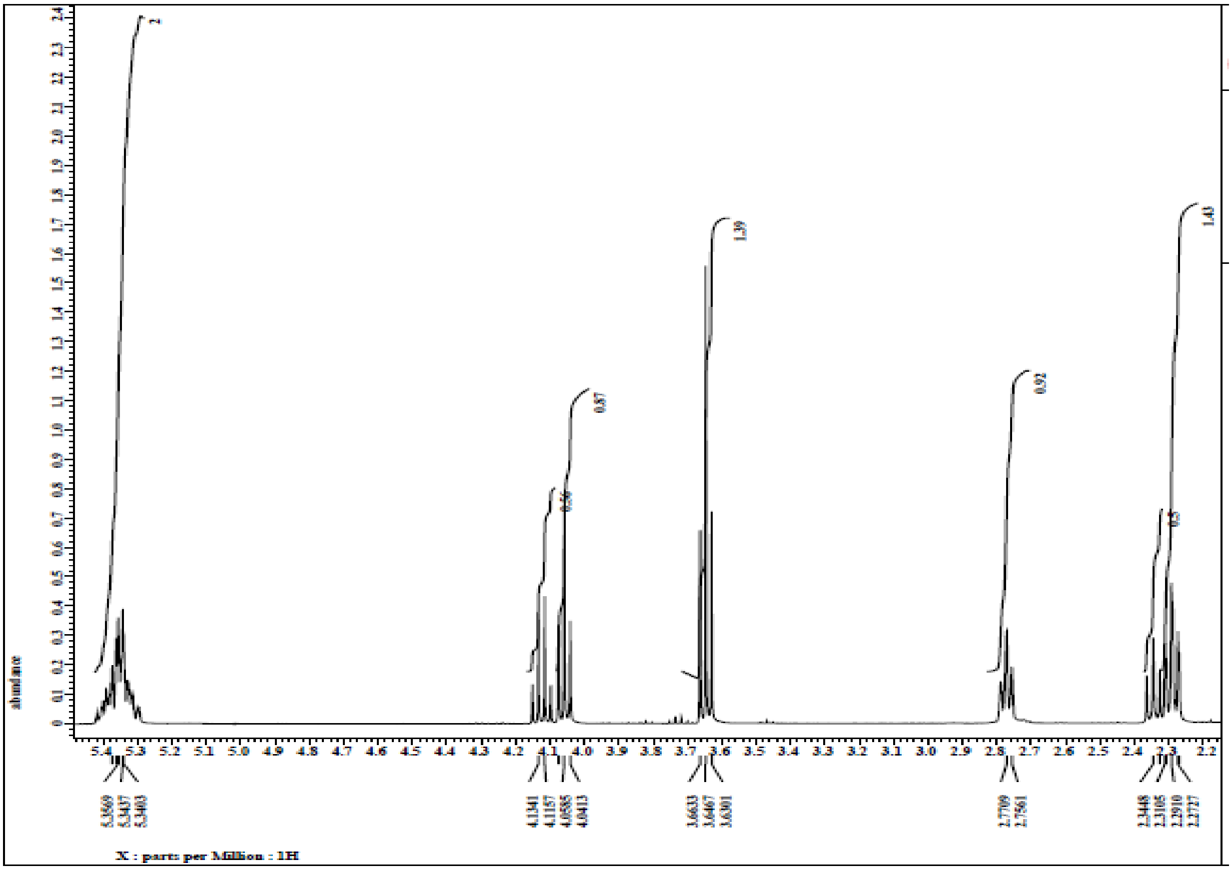
x

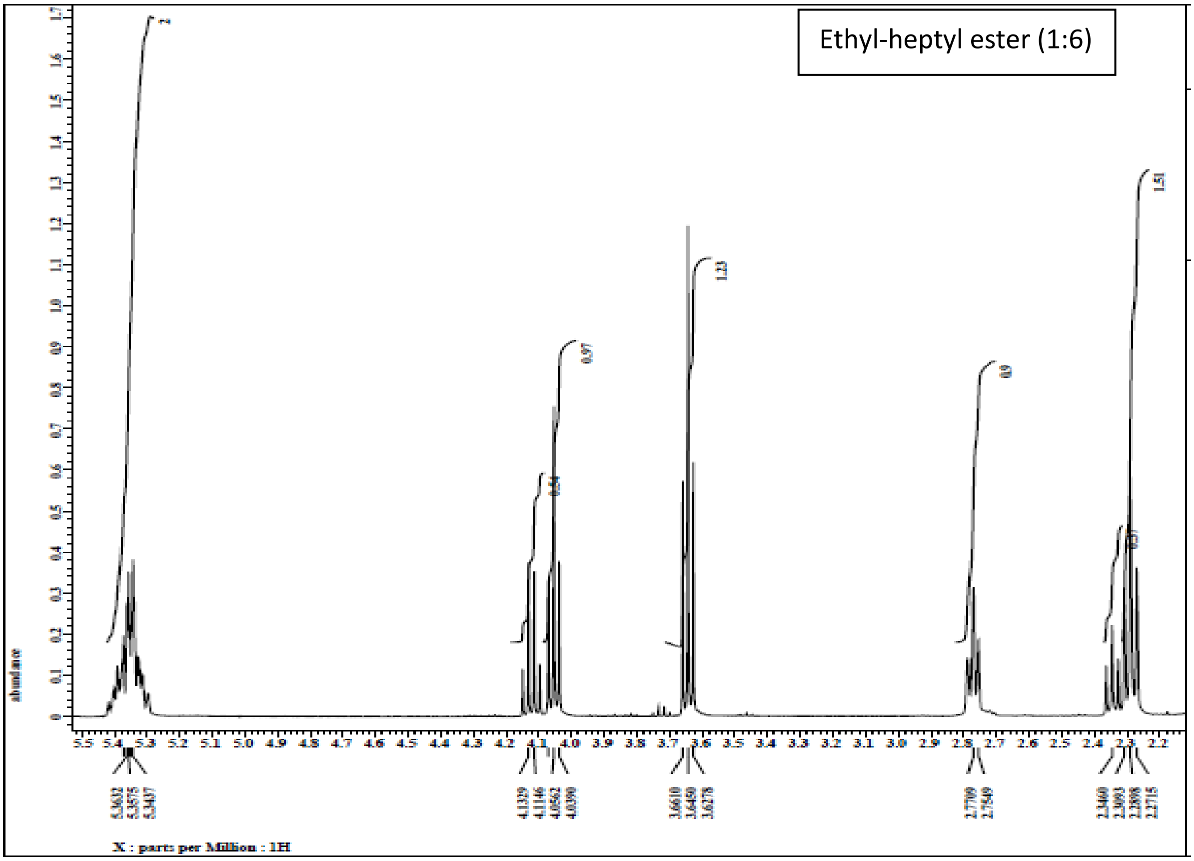


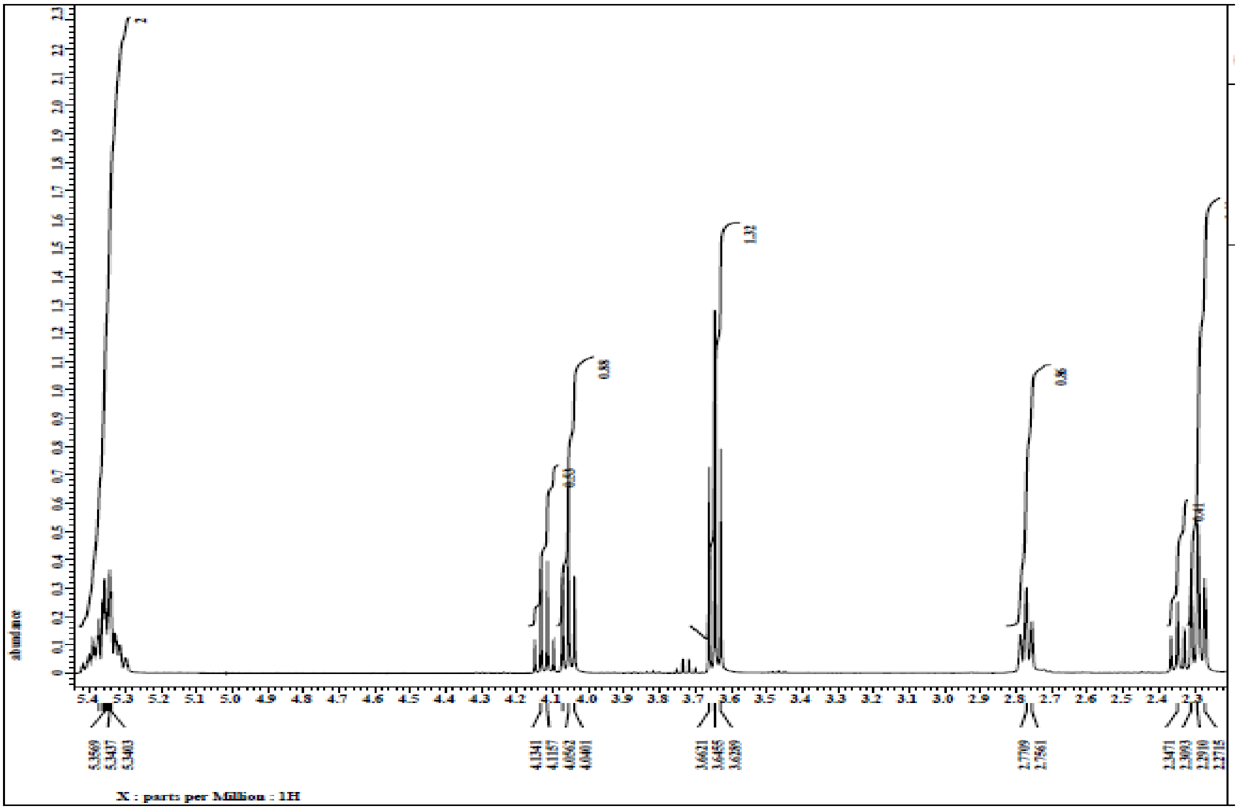
Ethyl-pentyl ester (1:6)

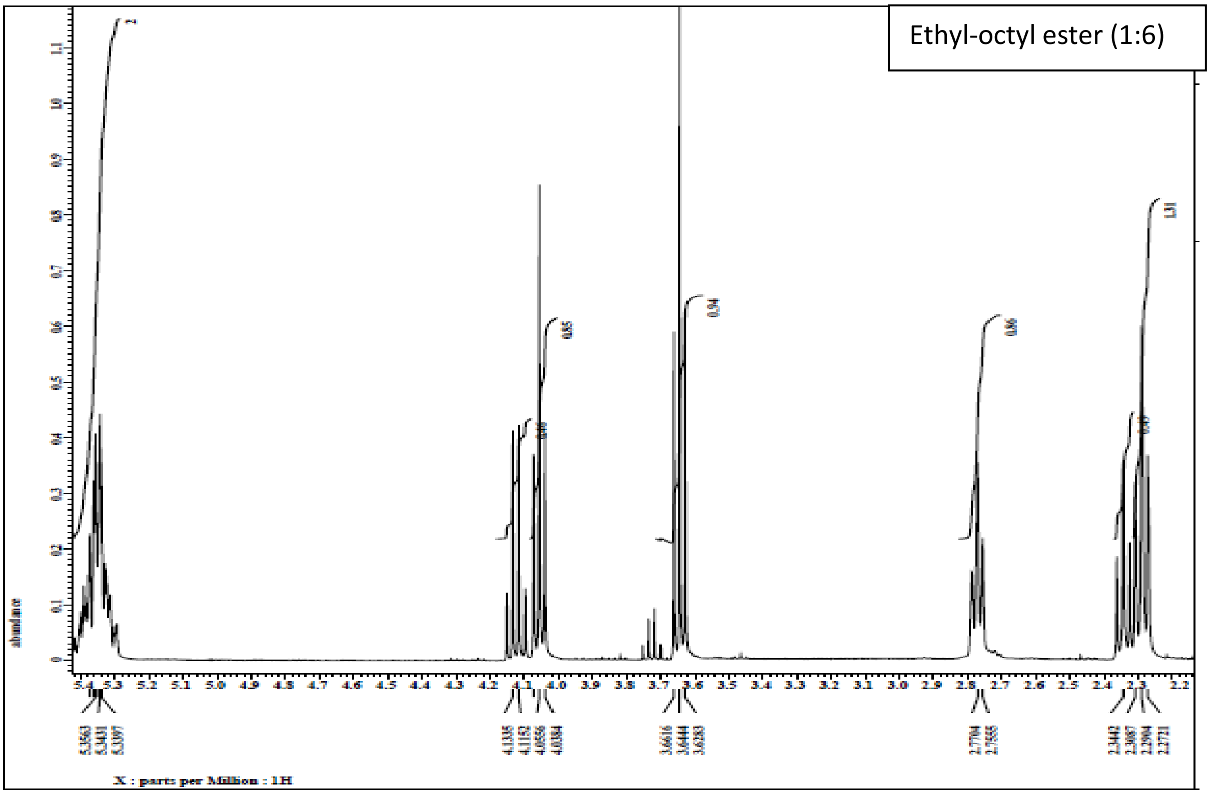



Ethyl-hexyl ester (1:6)
abundance
0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0 1.1 1.2 1.3 1.4 1.5 1.6 1.7 1.8 1.9 2.0 2.1 2.2 2.3
2.0
0.84
0.88
1.27
0.92
0.69
1.42
5.4 5.3 5.2 5.1 5.0 4.9 4.8 4.7 4.6 4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2 3.1 3.0 2.9 2.8 2.7 2.6 2.5 2.4 2.3 2.2
5.3575
5.3437
5.3391
4.1329
4.1146
4.0573
4.0401
3.6633
3.6467
3.6301
2.7709
2.7549
2.3448
2.3093
2.2910
2.2718
X : parts per Million : 1H


abundance
0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0 1.1 1.2 1.3 1.4 1.5 1.6 1.7 1.8 1.9 2.0 2.1 2.2 2.3 2.4
2
0.86
0.87
1.39
0.92
0.5
1.43
5.4 5.3 5.2 5.1 5.0 4.9 4.8 4.7 4.6 4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4 3.3 3.2 3.1 3.0 2.9 2.8 2.7 2.6 2.5 2.4 2.3 2.2
5.3569
5.3437
5.3403
4.1341
4.1157
4.0585
4.0413
3.6633
3.6467
3.6301
2.7709
2.7561
2.3448
2.3105
2.2910
2.2727
X : parts per Million : 1H


Ethyl-heptyl ester (1:6)
abundance
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
1.2
1.3
1.4
1.5
1.6
1.7
2
0.97
0.54
1.23
0.9
1.51
0.37
5.5
5.4
5.3
5.2
5.1
5.0
4.9
4.8
4.7
4.6
4.5
4.4
4.3
4.2
4.1
4.0
3.9
3.8
3.7
3.6
3.5
3.4
3.3
3.2
3.1
3.0
2.9
2.8
2.7
2.6
2.5
2.4
2.3
2.2
5.3632
5.3575
5.3437
4.1329
4.1146
4.0562
4.0390
3.6610
3.6450
3.6278
2.7709
2.7549
2.3460
2.3093
2.2898
2.2715
X : parts per Million : 1H


abundance
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
1.2
1.3
1.4
1.5
1.6
1.7
1.8
1.9
2.0
2.1
2.2
2.3
2
0.88
0.53
1.32
0.86
0.41
5.4
5.3
5.2
5.1
5.0
4.9
4.8
4.7
4.6
4.5
4.4
4.3
4.2
4.1
4.0
3.9
3.8
3.7
3.6
3.5
3.4
3.3
3.2
3.1
3.0
2.9
2.8
2.7
2.6
2.5
2.4
2.3
5.3569
5.3437
5.3403
4.1341
4.1157
4.0562
4.0401
3.6621
3.6455
3.6289
2.7709
2.7561
2.3471
2.3093
2.2910
2.2715
X : parts per Million : 1H



Ethyl-octyl ester (1:6)