

# Analytical Monitoring of base Catalyzed Methanolysis of Jatropha Curcus Oil by Reversed-Phase High-Performance Liquid Chromatography Assisted by Ultrasonication

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**Abstract:** Gradient elution reversed phase high performance liquid chromatography (RP-HPLC) was used to monitor the progress of reaction during the base catalyzed transesterification reaction of Jatropha Curcus oil with methanol assisted by ultrasonication. A high performance liquid chromatographic method for the simultaneous determination of triacylglycerol, diacylglycerol, monoacylglycerol as well as free fatty acids and their corresponding methyl esters has been developed. The determination of these components was carried out using a 40 min combined linear gradient with aqueous-organic and non-aqueous mobile phase steps: 90% methanol + 10% water in 10 min, 100% methanol in 0 min, 60% methanol + 15% n-hexane + 25% propan-2-ol in 30 min, followed by isocratic elution with 90% methanol + 10% water for last 10 min was used for fast monitoring of conversion of Jatropha curcus oil to fatty acid methyl esters. Individual calibration curves obtained with UV detection at 215 nm were found to be use for quantitative analysis of methyl esters and acylglycerol. The use of the RP-HPLC method in the elution of the mechanism of base catalyzed transesterification in biodiesel production was described.

**Keywords:** Reversed-phase HPLC, Biodiesel, Base Catalyst, Ultrasonication, Jatropha Curcus oil

## 1. Introduction

Besides mono alkyl esters, glycerol, alcohol, catalyst, free fatty acids, tri-, di- and monoglycerides compose the final mixture of biodiesel production process. The quality control of biodiesel is greatly significant to the success of its commercialization and market acceptance. Some important issues on the biodiesel quality control involve the monitoring of transesterification reaction, the quantification of mono alkyl esters, free and bounded glycerol as well as determination of residual catalyst and alcohol. Moreover, the determination of blend levels is another key aspect of biodiesel analyses. Chromatography and spectroscopy are the analytical methods most used for the biodiesel characterization [1]. Lipids analysis by liquid chromatography has become a favourite tool for plant researchers. Triacylglycerols (TGs) and free fatty acids (FFAs) are the principal components of natural lipids in Jatropha curcus oil. The plant oil are complex mixtures containing triacylglycerols (TGs) for various fatty acids, such as oleic (O), linoleic (L), linolenic (Ln), Steric (S), Palmitic (P), differing in the acyl chain lengths and their position sn-1, 2 or 3 on the glycerol skeleton, and in the number and position of the double bonds in the acyl chain. The standard notation of TGs employs the initial of fatty acid names, arranged in the order of their position on the glycerol skeleton. Adulteration of fuels is also a practice in other countries. In India, the adulteration of petroleum byproduct has been a serious problem, particularly of diesel oil. Groups of researchers are developing new analytical techniques to detect these frauds which make use of adulterants such as kerosene and cyclohexane, among others [2] The transesterification reaction

of TGs with methanol gives rise to fatty acid esters commonly known as biodiesel and 1, 2, 3- propanetriol (glycerol) [3]. Current specification only use the term biodiesel to define mixtures of compounds containing no more than 0.3% of alcohol, 0.8% of monoacylglycerols [MGs], 0.4% of diacylglycerols [DGs], 0.4% of triacylglycerol, 0.02% of free glycerol and 0.25% of total glycerol [4]. The transesterification is an equilibrium reaction that can be achieved with an alkaline, acid or enzymatic catalyst [5, 6]. The system that has met with the greatest success used methanol and KOH as the alkaline catalyst [3, 7-10].

The present work, the transesterification of Jatropha curcus oil with methanol in the presence of catalyst KOH producing biodiesel and glycerol using ultrasonic irradiation [11] was investigated. The applications of sonochemistry have been developed in virtually all areas of chemistry and related chemical technologies. Ultrasound is the process of propagation of the compression waves with frequencies above the range of human hearing. Ultrasound energy may activate various mechanisms to promote the effects but the mechanisms involved are not always known. So far, most investigators agree that there are three phenomena attributed to the effect of ultrasonic irradiation. First, a rapid movement of fluids caused by a variation of sonic pressure causes solvent compression and rarefaction. The second one, by far the most important, is cavitations. Most investigators accept that the formation and collapse of micro bubbles are responsible for most of the significant chemical effects observed [12]. Ultrasounds help improve the liquid-liquid interfacial area through emulsification, which is important for viscous films

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containing gas-filled bubbles and cavitations bubbles [13]. Gas-filled bubbles within the films, oscillating because of ultrasound and mobilized by acoustic streaming, entrain sonic of the film. Simultaneously, cavitation bubbles spray solvent on the film that covers the pulsing gas bubble. The pulsing action of the gas bubble is therefore disrupted and the liquid is scattered on its surface, leading to highly dispersed emulsions. Very fine ultrasonic emulsions, which are much smaller in size and more stable than those obtained conventionally, greatly improve the interfacial area available for reaction, increase the effective local concentration of reactive species, and enhance the mass transfer in interfacial region [14]. Therefore it leads to a remarkable increase in reaction rate under phase-transfer conditions. Furthermore, cavitations during sonication produces extreme local conditions and a micro environment with high temperature and high pressure, which may also create active intermediates that, permit the reaction to proceed instantaneously [15].

Numerous analytical methods were developed for characterizing a mixture composed of reactants and products for transesterification [16-18]. The method that has been most successful is the one recommended in the ASTM standard D 6584 and its European counterpart, EN. 14105 [4], which are provide specification on the use of gas chromatography and a flame ionization detector. This analytical method is not very convenient for identifying non-volatile triacylglycerols, however [8, 19, 20]: these compounds need to be derivatized and analyzed at high temperatures (approximately 350°C), with a consequent fallout on the life of the column. To avoid the derivatization step and enable even triacylglycerol with a high molecular mass to be recorded, high performance liquid chromatography (HPLC) methods have been developed that allow for the sample to be kept in the liquid state, without any pretreatment. Various detection techniques including UV detection [21, 22], refractive index detection [23, 24], flame ionization detection [25, 26], evaporative light scattering detection (ELSD) [21, 24], and mass spectrometric (MS) detection [21, 27] have been employed along with HPLC for the determination of acylglycerols. The quantification of the acylglycerol can be achieved using UV at 205-210 nm, ELSD and MS detection [21, 26]. Unlike ELS and MS detections, UV is the most widely available detector. However, as mentioned above, UV has limited use with the solvents having absorbance at wavelengths where acylglycerols are detected.

The silver ion HPLC in normal-phase systems is a widespread technique for the separation of lipids, such as TGs, based on selective interactions of silver ions with species differing in the number and positions of double bonds [28-32]. The selectivity is usually poor for TGs differing only in the lengths of alkyl chains; therefore non-aqueous reversed-phase high-performance liquid chromatography (NARP-HPLC) usually provides a better resolution of complex TGs mixtures such as plant oils. Adlof et al. [32] used a series of four silica gel columns modified with silver ions to separate the stereochemical isomers of conjugated linoleic acid enriched TGs. Since acylglycerols do not absorb the UV detection above 220 nm, the UV detection is possible only at very low

wavelength and necessitates the highest purity HPLC solvents. The UV detection at 205 nm or 210 nm can provide linear calibration curves and very good sensitivity even with gradient elution technique [33]. But one of the most common and valuable HPLC techniques [21] is based on a linear ternary gradient consisting of two steps, the first aqueous-organic, the second non-aqueous. The proposed analysis consists of the following: 70% acetonitrile + 30% water at 0 min, 100% acetonitrile at 10 min, 50% acetonitrile + 50% isopropanol n-hexane (5:4, V/V) from 20 to 25 min, UV detection is used at 205 nm. Using the recommended settings, MGs, fatty acid methyl esters (FAMEs), DGs and TGs are accurately distinguished and some of the main individual TGs can also be separated with the Equivalent Carbon Number (ECN). The ECN is defined as  $ECN = CN - 2DB$ , where CN is the number of carbon atoms and BD the number of double bonds. The main objective of this study was to develop an HPLC method to monitor qualitatively and quantitatively progress of reaction during the alkaline catalyzed biodiesel production from *Jatropha curcus* oil. For this purpose, RP-HPLC procedures with UV detection at 215 nm were proposed and used in the analysis of the samples taken at certain time intervals from the transesterification reactions of *Jatropha curcus* oil with methanol catalyzed by KOH. The individual compounds occurring during the transesterification reactions were determined by HPLC with the relative retention time of the reference compounds. Using individual calibration curves obtained with UV detection at 215 nm, the quantitative analyses of double bond containing compounds (methyl esters) and the intermediates (mono and diacylglycerols) were found to be feasible.

## 2. Experimental

### 2.1. Materials

Methanol, hexane, 2-propanol and water were purchased from Merck (Darmstadt, Germany), all the solvents were HPLC grade filtered through a 0.2  $\mu$ m filter and were used without purification. All the standards were purchased from sigma-aldrich (st. Louis, Mo, USA). Commercial grades *Jatropha curcus* oil was procured from local market purchase, methanol (purity 98%) and KOH (purity 85%) were used from M/S Ranboxy.

### 2.2. Ester preparation

An ultrasonic procedure UP 200S from Hielscher ultrasonic GmbH was used to perform the transesterification reaction. The ultrasonic processes operate at 200W and 24 kHz frequency. The amplitude and cycle for the reaction were adjustable from 20-100 % and from 0.1 to 1 cycle per tone, respectively. The titanium sonotrode S<sub>7</sub> with a diameter of 7 mm and length of 100mm was used to transmit the ultrasound into the liquid. All the experiments were carried out Erlenmeyer type flask having 50 ml volume [34]. The procedure of biodiesel (ethyl ester) from *Jatropha curcus* oil involves the base catalyzed transesterification with ethanol to

give ethyl esters. Glycerin is a byproduct in the transesterification reaction.

The reaction condition was molar ratio oil to methanol 1:6, Catalyst (KOH) 0.75 wt% of oil, amplitude 60% and cycle 0.3. The sample was taken after every 2 min interval, crude glycerol separated by gravity and catalyst removed by hot water washing. The complete removal of the catalyst was checked by phenolphthalein indicator. Traces of moisture and unreacted methanol were removed by vacuum distillation. The distillation was continued until the loss in weight of ester was constant thus confirming the complete removal of moisture and unreacted methanol. Finally esters were dried over anhydrous sodium sulphate and analysed by HPLC. The conversion of the component present in the *Jatropha curcus* biodiesel at different time interval is given in *Table 1*. The best result of *Jatropha curcus* ethyl ester was observed with in 6 min after that the conversion rate was slower and finally reached steady state.

### 2.3. HPLC analysis

HPLC was used to monitor the progress of reaction, analyse the purity, conversion and FAME composition of the biodiesel esters sample. The Reverse phase high performance liquid chromatography (RP-HPLC) is separate different components of biodiesel on the bases of their polarity. The chromatographic apparatus consisted of a model waters 600 pump with waters 600 controller, waters 2996 photodiode array detector, a nova-pack®, 3.9 X 150 mm column with guard column of dimension 3.9 X 20 mm, both packed with C18 particle with diameter 4 µm. (all from waters, Milford MA, USA).

HPLC condition: RP-HPLC method flow rate of 1ml/min, an injection volume of 5µl, a column temperature of 45°C, the UV detection at 215 nm and a 40 min gradient mobile phase 90% CH<sub>3</sub>OH + 10% H<sub>2</sub>O in 10 min, 100% CH<sub>3</sub>OH in 0 min, 60% CH<sub>3</sub>OH + 15% hexane + 25% propane-2-ol in 30 min and for the last 10 min system back to initial state 90% CH<sub>3</sub>OH + 10% H<sub>2</sub>O were used for the separation and identification of the compound produced during the methanolysis of *Jatropha curcus* oil in all the experiments.

### 2.4. HPLC/mass spectrometry analysis

HPLC/MS analysis were conducted with an HPLC/MSD ion trap (waters) using RP-HPLC Zorbax XDB C18 (150mm×4.6mm i.d., 5µm) column with atomic pressure chemical ionization probe connected in series to a diode array UV detector. The flow-rate was 1.0 mL/min and the column temperature was about 15°C. The mass spectrometric data were acquired in the range *m/z* 35–1000 with the following setting of tuning parameters: alternating ion-polarity mode, pressure of the nebulizing gas 80 psi, drying gas flow-rate 5 mL/min, the temperatures of the drying gas and APCI heater were 380 and 420°C, respectively. The retention time of all the compounds identified by APCI-MS are given in *Table 2*.

## 3. Results and Discussion

### 3.1. HPLC separation of compounds occurring during base catalyzed transesterification reaction

Reversed phase high performance liquid chromatography (RP-HPLC) with UV detection at 215 nm was proposed for the separation and identification of compounds occurring during the base catalyzed methanolysis of *Jatropha curcus* oil. *Fig 2* illustrates the separation of component present in *Jatropha curcus* oil and *Fig 3* illustrates the separation of mixture of FAMES and nearly completes resolution of all C<sub>18</sub> free fatty acids with different number of double bonds and their mono-, di-, and triacylglycerols under these conditions. The retention increases in the order: FFA < MGs < FAMES < DGs < TGs. The MGs, DGs and TGs are eluted in the order of increasing Equivalent carbon number (ECN). The position isomers of DGs are partially resolved, 1, 3-DGs are eluted before 1, 2-DGs because of the steric shielding of the central hydroxyl group in the 1, 3-isomer. The separation of the components in the reaction mixture after the transesterification of *Jatropha curcus* oil is accomplished with in 40 min. An example of the chromatogram of a real sample of biodiesel prepared by the transesterification of *Jatropha curcus* oil is shown in *fig 3*

Holcolpek et al. [21] described the deconvolution of overlapping APCI-MS peaks of some DGs and TGs. *Fig 3* illustrates the reconstruction of the selected ion chromatogram of ions *m/z* 601 and *m/z* 599, as an example, from the total ion chromatogram in the positive-ion mode. Furthermore, the selected ion chromatogram of the ion that gave good signal in the negative-ion mode such as ions produced from the OOO and LLL were constructed from the total ion chromatogram obtained in the negative-ion mode. By this way, the exact retention times of coeluting compounds were determined.

### 3.2 Quantitative Analysis

The calibration curves were measured in two series of experiments. Each data point was obtained from at least two repeated measurements. Stock solutions of the standards MeL, MeO, 1-L, 1-O, 1, 3-LL, 1, 3-OO, LLL and OOO were prepared as about 5 mg/ml in hexane, and then serial dilution were made as required. The calibration curves were plotted relative integrated peak areas versus amount of the methyl esters standard. The constant of calibration curves (relative peak areas versus amount of compound) of the standards determined by linear regression analysis are given in *table 3*.

Quantitative determination of the reaction products and the intermediates is essential for the studies of kinetic of transesterification reactions. In this study, we also tried to find the suitability of the UV detection at 215 nm for the determination of the concentrations of the methyl ester products and the mono- and diacylglycerol intermediates even through hexane used in the gradient of non aqueous mobile phase has some absorbance at 215 nm. The construction of the calibration curves for each compound would not be practical because there are many products and the

intermediates present in the reaction mixture. Therefore, only several examples are presented here. Fig-8 shows the calibration curves of methyl linoleate and methyl oleate standards as a function of relative integrated peak area versus amount of standard are linear. The correlation coefficients of both are 0.999 (see table-3) indicating good linearity. The saturated fatty acids do not have significant absorbance at 215 nm. Similar calibration curves were obtained with 1-L, 1-O, 1, 3-LL, 1, 3-OO, LLL and OOO standards. The constants of calibration curves (relative peak areas versus amount of compound) of MeL, MeO, 1-L, 1-O, 1, 3-LL, 1, 3-OO, LLL and OOO standards determined by linear regression analysis are given in table-3. As can be seen, the response factors are very similar for all of them.

### 3.3 Identification of the peak with APCI – MS

The molecular mass of individual compounds and, therefore, the structure of acylglycerols can be readily determined by atmospheric pressure chemical ionization-mass spectrometry (APCI-MS) [21, 35-36]. The APCI-MS fragmentation pattern of the fatty acid methyl esters studied in the present work. Characteristic fragment ion are formed by the loss of one acid molecule from the  $[M+H]^+$  ions of TGs (ion A in fig. 1) and DGs (ion B in fig. 1) or of a molecule of water from the  $[M+H]^+$  ion of DGs (ion A fig. 1) and of MGs (ion B in fig. 1, base peak). Another abundant fragment ion in the mass spectra of TGs is formed by subsequent losses of an acid molecule and an acyl  $R_iCO$  (ion B in fig. 1). Further, acyl ions (C in fig. 1) and dehydrated acyl ions (D in fig. 1) are observed in the mass spectra of MGs and MEs. The masses of all important ions of TGs, DGs, MGs and MEs are containing oleic, linoleic, palmitic and stearic acids. Representative mass spectra of several compounds of each class are in fig-4-7. Note the peak of the ion A with  $m/z$  577 in the spectrum of OOO in fig. 4 corresponding to TGs with one or two palmitic acyls, coeluted with OOO. The base peak in the mass spectra of TGs with five and more double bonds corresponds to the protonated molecule. The mass spectra of TGs with three or two different acyls exhibit three or two  $[M+H-R_iCOOH]^+$  ions differing by two units, in the spectra of TGs with three identical alkyls only one  $[M+H+R_iCOOH]^+$  ion appears. The APCI mass spectra of TGs show similar fragmentation patterns as the EI mass spectra [37]. In the mass spectra of DGs with two and one double bonds, the base peak corresponds to the protonated molecules. Isomeric 1, 2-DGs can be distinguished from 1, 3-DGs and the bases of the ratio of protonated molecules or of the ion A to the ion B (fig. 5). This ratio is always higher for 1, 3-DG than the 1, 2-DG isomers. The rule can be applied for the identification of unknown 1, 2-DG and 1, 3-DG isomers.

The base peak in the mass spectra of MGs (fig. 6) corresponds to the loss of water, the protonated molecule is also apparent. Other important ion are  $[R_iCO]^+$  and  $[R_iCO-H_2O]^+$ . In the mass region below  $m/z$  200, the mass attributed to the aliphatic series formed by the fragmentation of alkyl chains are observed like in the EI mass spectra of MGs.

The APCI mass spectra of MEs (fig. 7) are very close to their EI mass spectra with characteristic aliphatic ion, but the relative abundances of the high mass ions (ion  $[M+H]^+$ , C and D) are higher. The base peak in the APCI mass spectra corresponds either to the ion C (e.g., MeO) or to the  $[M+H]^+$  ion (e.g., MeL).

In contrast to all other studied compounds free fatty acids give no response in position-ion APCI-MS, but good signals are obtained in negative-ion APCI-MS, where  $[M-H]^-$  ion are observed. The abundances of the fragment ions in these spectra are very low.

## 4. Conclusion

In this study we showed that NARP-HPLC procedure with universal UV detection at 215 nm proposed here is efficient in the separation and the determination of the compound occurring during the base catalysed methanolysis of *Jatropha curcus* oil with methanol. Using a combined aqueous-organic and non-aqueous gradient elution, the resolution is improved, so separation of all free fatty acids, methyl esters, mono-, di- and triacylglycerols differing in their ECNs is possible in a single run in approximately 40 min. the identification of the individual compounds can be done by APCI-MS in the positive ion modes. Using individual calibration curves, the quantitative analyses of double bond containing products (methyl esters) and the intermediates (mono- and diacylglycerols) can be performed. The sensitivity of UV detection is also different for the individual TGs. APCI-MS is the best suited detection mode for the analyses of *Jatropha curcus* oil and of biodiesel. It elucidates additional structural information on the acylglycerols present in natural oils and in the synthetic products of their transesterification. As the retention time is increases with increasing equivalent carbon number. The contribution of two methylene groups to the increase in the retention in the reversed-phase systems employed in this work is slightly higher than the contribution of one double bond to the decrease in the retention time.

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**Table 1:** Conversion of oil (Triglycerides) to Fatty acid methyl esters (Biodiesel) every five Minuit interval

Time	Palmitate	Linoleate	Oleate	Sterate
Min	C16	C18:2	C18:1	C18
00	0.0	0.0	0.0	0.0
02	9.2	19.4	10.7	1.2
04	12.8	28.5	38.5	2.9
06	15.8	35.6	43.4	3.8
08	15.8	35.6	43.3	3.8
10	15.6	35.6	43.4	3.7

**Table 2:** Retention time ( $t_R$ ) and molecular mass of the components identified by APCI-MS during the transesterification of jatropha curcus oil with methanol catalyzed by KOH

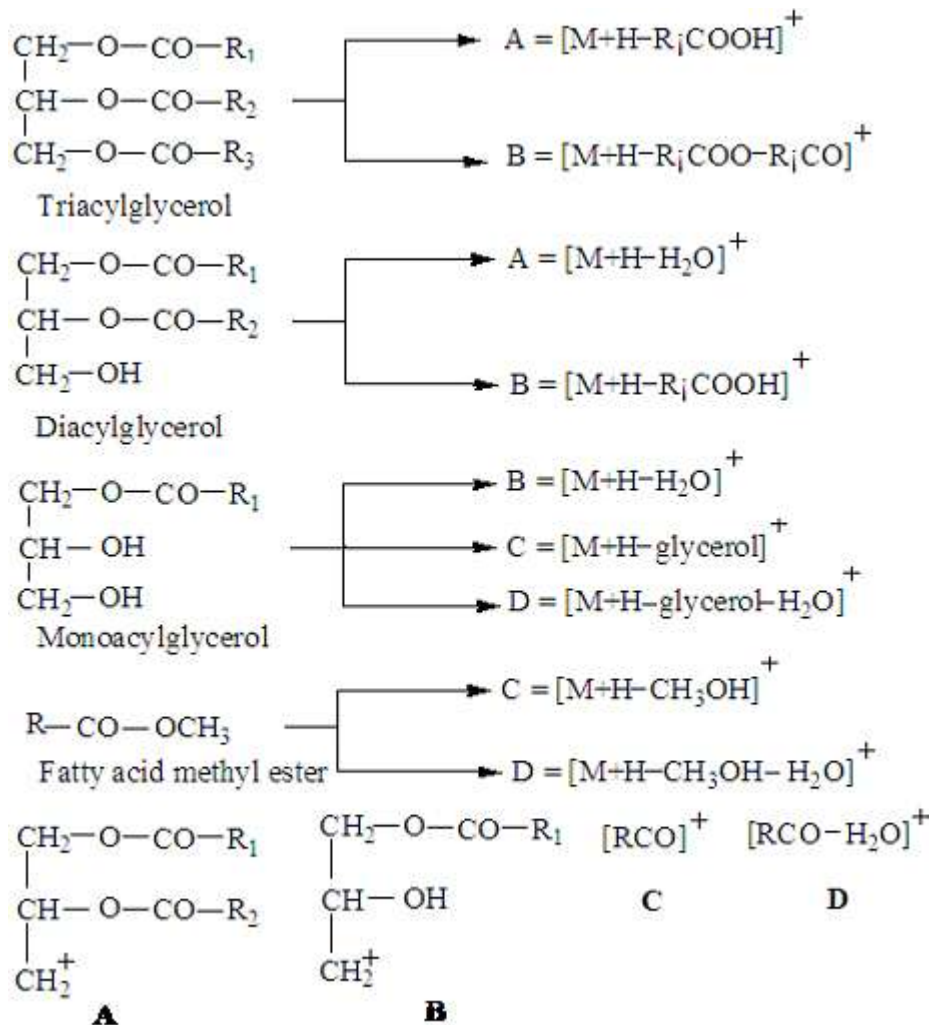
$t_R$	Name	Notation	ECN <sup>b</sup>	Molecular mass
<i>Fatty acid</i>				
2.01	Linolic acid	L acid	-	280.3
2.87	Oleic acid	O acid	-	282.3
3.25	Palmitic acid	P acid	-	256.3
3.18	Steric acid	S acid	-	284.3
<i>Monoacylglycerol</i>				
4.92	2-Monolinolein	2-L	14	354.3
5.32	1-Monolinolein	1-L	14	354.3
6.15	2-Monoolein	2-O	14	356.3
6.89	1-Monoolein	1-O	16	356.3
7.65	1-Monopalmitin	1-P	16	330.3
8.72	1-Monostearin	1-S	18	358.3
<i>Methyl esters of fatty acids</i>				
9.61	Methyl ester of linoleic acid	MeL	-	294.3
11.58	Methyl ester of oleic acid	MeO	-	296.3
13.65	Methyl ester of palmitic acid	MeP	-	270.3
15.92	Methyl ester of stearic acid	MeS	-	298.3
<i>Diacylglycerol</i>				
16.85	1,3-Dilimolein	1,3-LL	28	616.5
17.95	1,2-Dilimolein	1,2-LL	28	616.5
18.78	1,3-Oleoyl-limoleoyl-glycerol	1,3-OL	30	618.5
19.56	1,2-Oleoyl-limoleoyl-glycerol	1,2-OL	30	618.5
20.86	1,3-Palmitoyl-limoleoyl-glycerol	1,3-PL	30	592.5
20.92	1,2-Palmitoyl-limoleoyl-glycerol	1,2-PL	30	592.5
21.78	1,3-Diolein	1,3-OO	32	620.5
21.65	1,2-Diolein	1,2-OO	32	620.5
22.69	1,3-Stearoyl-limoleoyl-glycerol	1,3-SL	32	620.5
22.99	1,3-Palmitoyl-oleoyl-glycerol	1,3-PO	32	564.5
23.48	1,2-Stearoyl-limoleoyl-glycerol	1,2-SL	32	620.5
23.63	1,2-Palmitoyl-oleoyl-glycerol	1,2-PO	32	594.5
24.35	1,3-Stearoyl-oleoyl-glycerol	1,3-SO	34	622.6
24.73	1,2-Stearoyl-oleoyl-glycerol	1,2-SO	34	622.6
<i>Triacylglycerols</i>				
25.65	Trilimolein	LLL	42	878.7
26.53	Oleoyl-dilimoleoyl-glycerol	OLL	44	880.7
27.43	Palmitoyl-dilimoleoyl-glycerol	PLL	44	854.7
27.62	Dioleoyl-limoleoyl-glycerol	OOL	46	882.7
28.15	Stearoyl-dilimoleoyl-glycerol	SLL	46	882.7
28.21	Triolein	OOO	48	884.7
29.05	Palmitoyl-oleoyl-limoleoyl-glycerol	POL	46	856.7
29.25	Tripalmitin	PPP	48	806.7
30.11	Tristearin	SSS	54	891.7

ECN<sup>b</sup> is the equivalent carbon number, ECN = CN-2DB, where CN is the carbon atoms in all acyls and DB is the number of double bond.

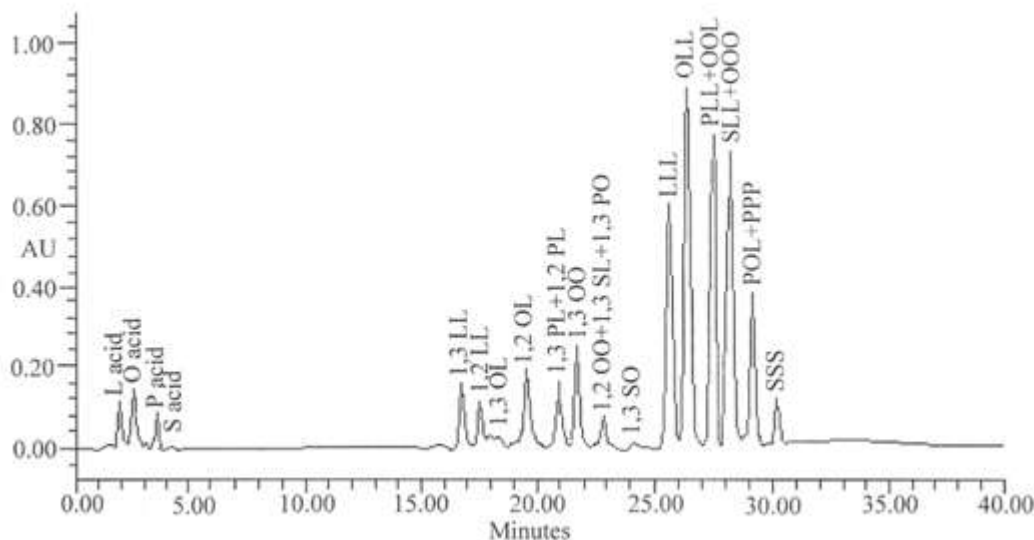
**Table 3:** The components of calibration curves (relative peak area vs. amount of compound) of several methyl esters, mono-, di- and triacylglycerols standards determined by linear regression analysis.

Compound	Slop	Intercept	correlation	RSD for	RSD for
Coefficient (R <sup>2</sup> )	retention time (%)	peak area (%)			
MeL	6x10 <sup>6</sup>	37924	0.999	0.103	1.47
MeO	17068	65980	0.999	0.118	1.21
1-L	8658	11265	0.998	0.192	1.37
1-O	9284	9887	0.999	0.092	1.65
1,3-LL	46482	15184	0.999	0.188	1.32
1,3-OO	28153	10352	0.999	0.128	1.34
LLL	16438	7524	0.998	0.114	1.90
OOO	10768	5962	0.999	0.089	1.88

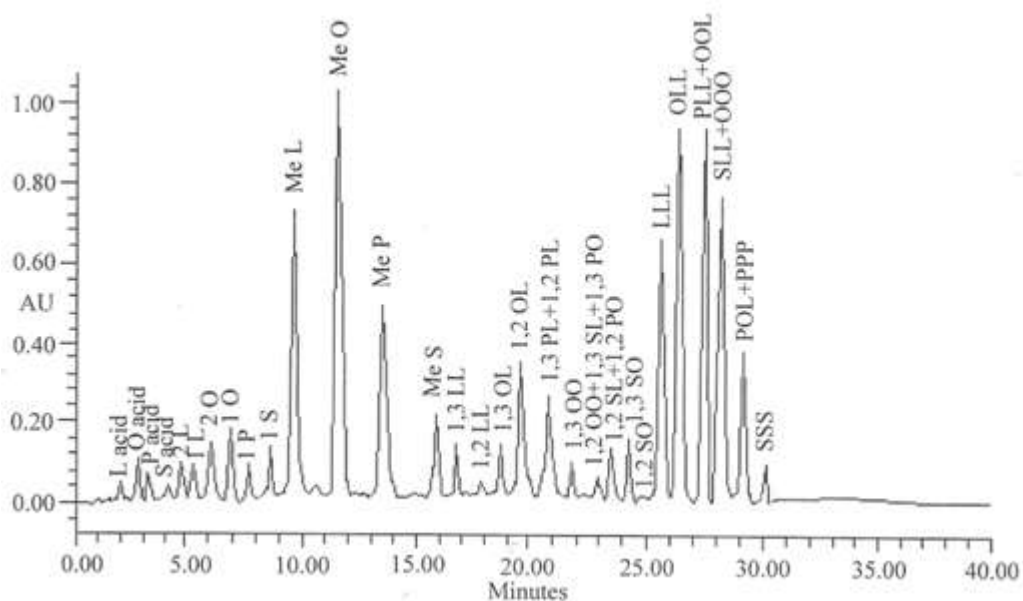
HPLC Condition as described in section 2.3.



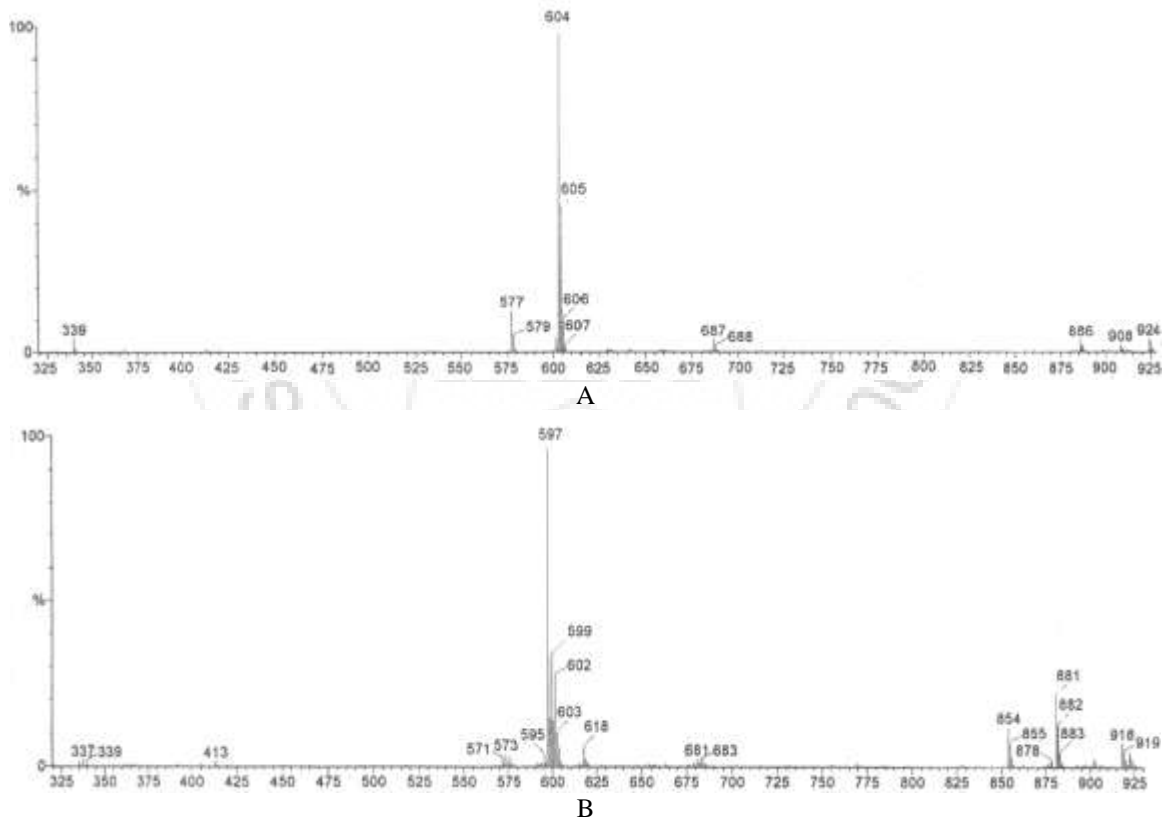
**Figure 1:** Fragmentation ion of triacylglycerol (TGs), diacylglycerol (DGs), monoacylglycerol (MGs) and fatty acid methyl esters using positive-ion APCI-MS. A, B, C and D are the most important fragment ion.



**Figure 2:** HPLC separation of Jatropa Curcus oil. HPLC condition are given in section 2.3. Notation of compounds as in Table 2.

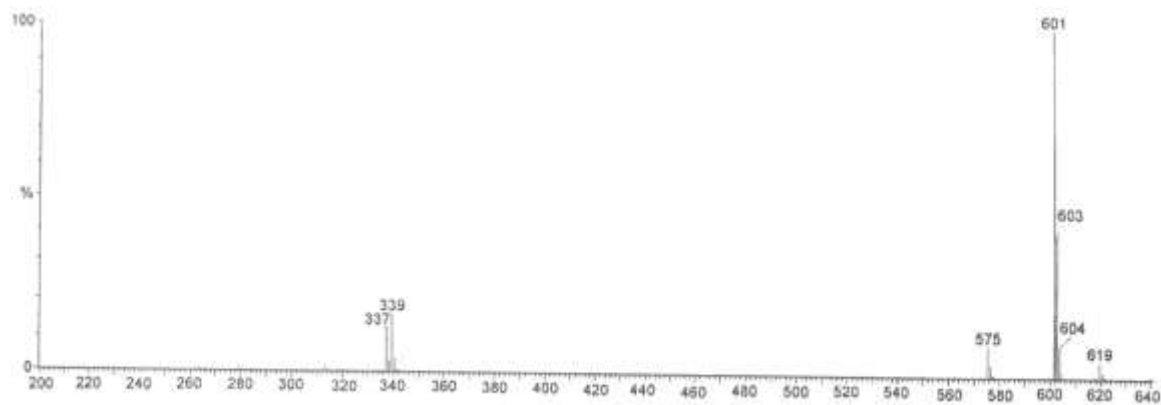


**Figure 3:** HPLC separation of reaction mixture Jatropa Curcus oil methanolysis. HPLC condition are given in section 2.3. Notation of compounds as in Table 2.

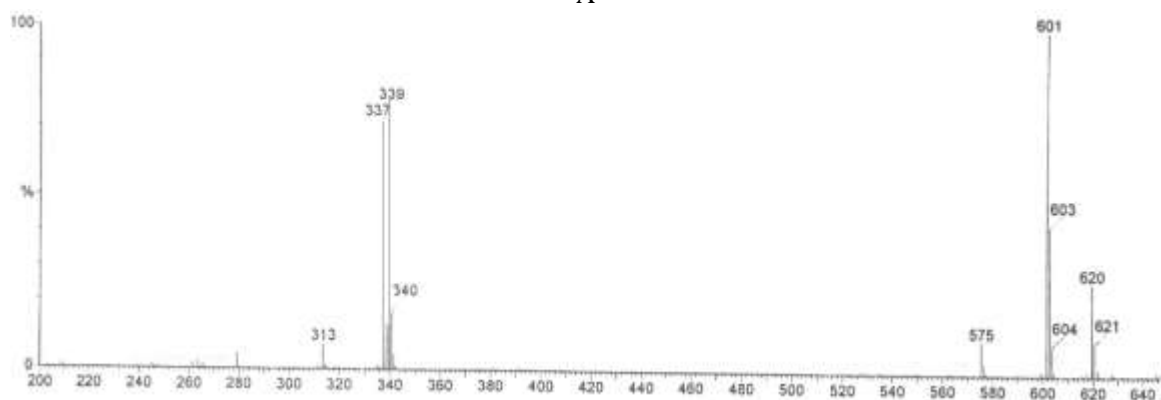


**Figure 4:** Positive-ion APCI mass spectra of [A] triolein (OOO), [B] trilinolein (LLL), masses of ions are given in Table 2.



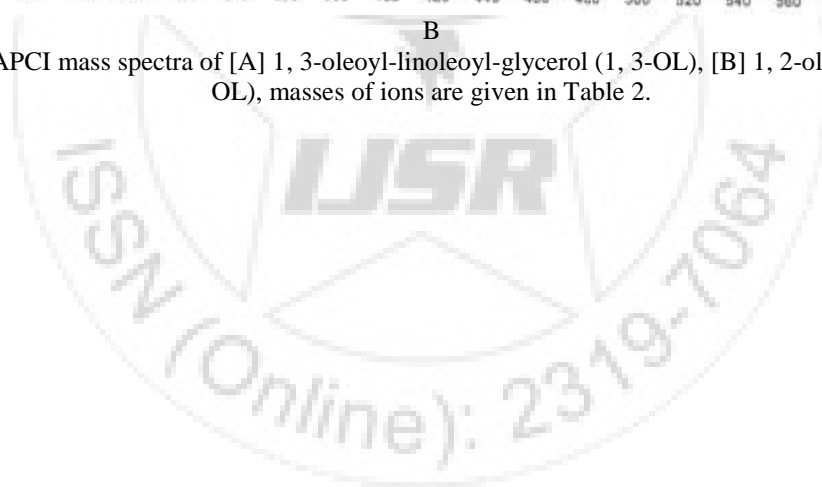


A



B

**Figure 5:** Positive-ion APCI mass spectra of [A] 1, 3-oleoyl-linoleoyl-glycerol (1, 3-OL), [B] 1, 2-oleyl-linoleoyl-glycerol (1, 2-OL), masses of ions are given in Table 2.



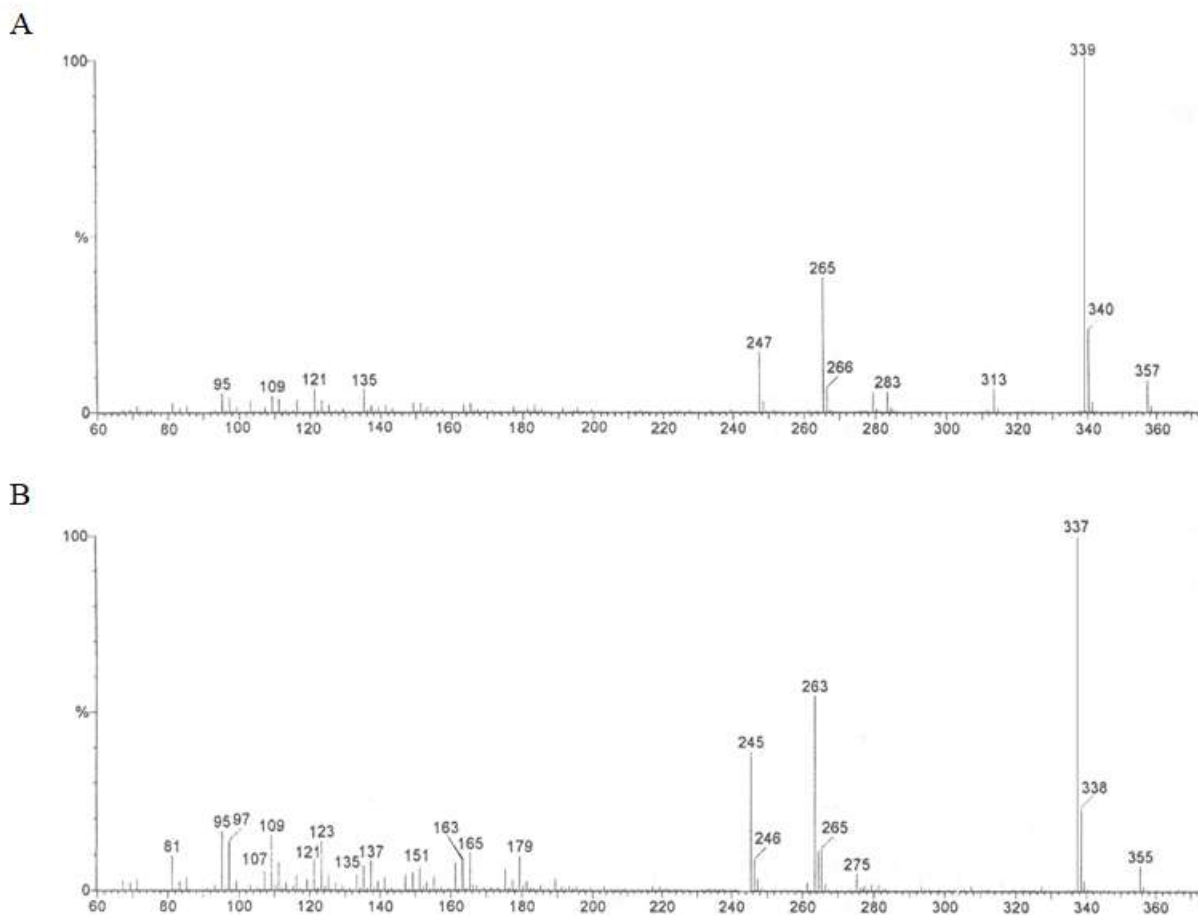
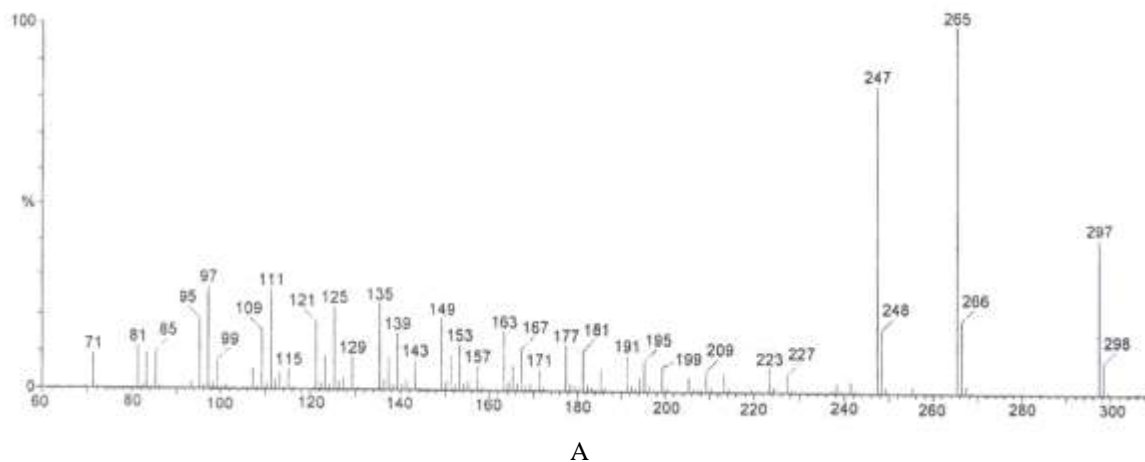
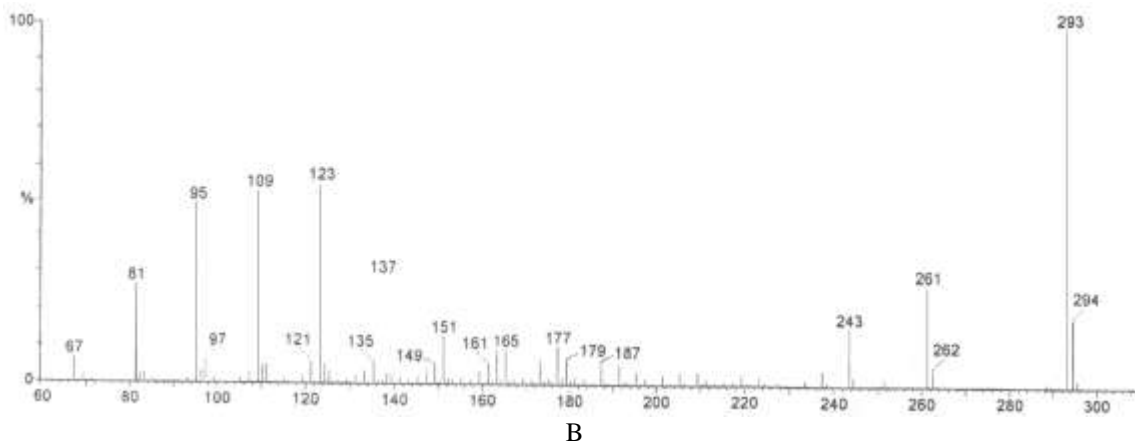
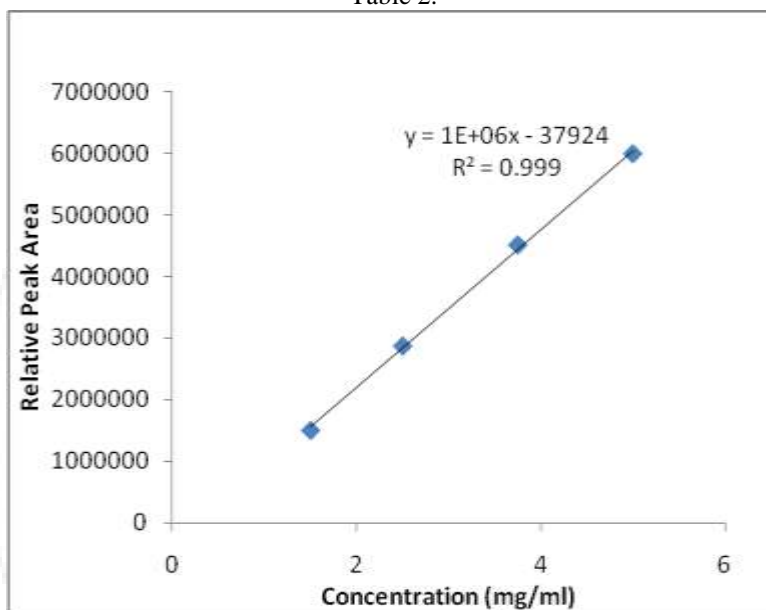


Figure 6: Positive-ion APCI mass spectra of [A] 1-monoolein (1-O), [B] 1-monolinolein (1-L), masses of ions are given in Table 2.

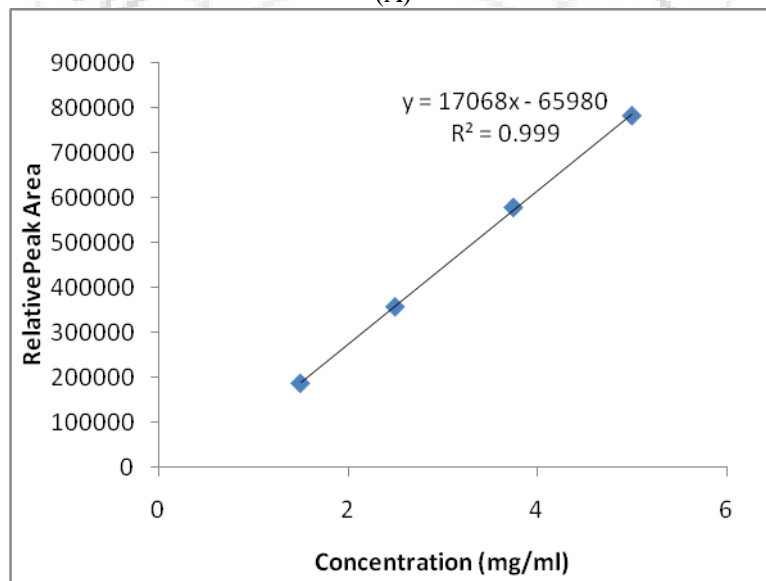




**Figure 7:** Positive-ion APCI mass spectra of [A] methyl oleate (MeO), [B] methyl linoleate (MeL), masses of ions are given in Table 2.



(A)



(B)

**Figure 8:** Calibration curve of pure standards of (A) MeL and (B) MeO. HPLC condition: UV detection at 215 nm, injection volumes 5  $\mu$ L. Other condition as determined in section 3.2.