

Proton NMR based method for the quantification of epoxidized methyl oleate

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Abstract

Epoxidized methyl esters (EMO) with their high oxirane ring reactivity, acts as a raw material in the synthesis of various industrial chemicals including polymers, stabilizers, plasticizers, glycols, polyols, carbonyl compounds, biolubricants etc. EMO has been generally quantified by the gas chromatography (GC) and high performance liquid chromatography (HPLC) techniques. Taking into the account of the limitations of these techniques, two qHNMR based equations have been proposed for the quantification of EMO in the mixture of EMO and methyl esters (MO). The validity of the proposed method was determined using standard mixtures of MO and EMO having different molar concentrations. The developed equations have been applied on the samples of EMO prepared from oleic acid in two step process viz., esterification followed by epoxidation. The qHNMR based EMO quantification showed acceptable agreement with the results obtained from HPLC analysis.

Abstract

Epoxidized methyl esters (EMO) with their high oxirane ring reactivity, acts as a raw material in the synthesis of various industrial chemicals including polymers, stabilizers, plasticizers, glycols, polyols, carbonyl compounds, biolubricants etc. EMO has been generally quantified by the gas chromatography (GC) and high performance liquid chromatography (HPLC) techniques. Taking into the account of the limitations of these techniques, two qHNMR based equations have been proposed for the quantification of EMO in the mixture of EMO and methylesters (MO). The validity of the proposed method was determined using standard mixtures of MO and EMO having different molar concentrations. The developed equations have been applied on the samples of EMO prepared from oleic acid in two step process viz ., esterification followed by epoxidation. The qHNMR based EMO quantification showed acceptable agreement with the results obtained from HPLC analysis.

Key words: Oleic acid, Methyl oleate, Epoxidized methyl oleate,¹H NMR Quantification, Epoxidation, Fatty acid methyl esters.

Introduction

¹H NMR spectroscopy is one of the most commonly used spectroscopy technique for the structure elucidation of the synthetic and natural organic compounds (Elyashberg 2015). ¹H NMR has conjointly been applied as a quantitative analytical tool for the quantification owing to the fact that the signal intensity in ¹H NMR corresponds directly to the number of proton nuclei responsible for that signal (Rizzo & Pinciroli 2005). Quantitative ¹H NMR (qHNMR) has emerged as an effective analytical tool for quality analysis in various fields such as natural drugs (Yan *et al.* 2016), food and beverages (Cao *et al.* 2014), PLGA based microspheres (Zhang *et al.* 2017), medicinal components (Göger *et al.* 1999; Hollis 1963) and dietary supplements (Phansalkar *et al.* 2017). Apart from qHNMR, HPLC and GC are widely used chromatographic techniques for the quantification of the molecules (Gelbard *et al.* 1995). GC with its destructive nature

required complex operational procedures including handling of explosive H_2 gas, volatile substances and mass spectra for product conformation (Monteiro *et al.* 2008). On the other hand in case of HPLC, requirement of reference standards, HPLC grade solvents, specific detector for the compound of interest and equilibration of the columns has made the technique costly and time consuming (Sun *et al.* 2017). The limitations of both the chromatographic techniques can be overcome by qHNMR as it is rapid technique with no specific requirement of reference standard and offers recovery of the analyte after analysis (Cerceanu *et al.* 2016). Moreover the amount of solvent (deuterated $CDCl_3$, DMSO, D_2O) required for each qHNMR analysis is minimum (~ 0.5 ml) as compared to that for HPLC and GC methods.

In the past decade, fatty acid methyl esters (FAMES) popularly known as biodiesel (BD) has gained the attention in the automobile industry worldwide as a sustainable, non-toxic and biodegradable substitute for the diesel fuel (Su & Guo 2014). However, a high degree of unsaturation in the fatty acid chain of BD has led to a decrease in its oxidative stability thus limiting its applicability as bio-lubricants (Kumar & Ali 2012). Epoxidation of the double bond of the unsaturated fatty acid chain to form epoxidized fatty acid methyl esters (EFAMES) or epoxidized methyl oleate (EMO) is one of the alternatives to improve the oxidative stability of biodiesel. This approach has opened avenues for exploring the use of formed oleochemicals as lubricants (Suarez *et al.* 2009). Beside acting as lubricants, EFAMES such as epoxidized methyl oleate (EMO) have been utilized as a building blocks for the synthesis of various products such as stabilizers in resins, substitutes to phthalates as plasticizer (Di Serio *et al.* 2012), surfactants (Doll & Erhan 2006), asphalt additives and transformer fluids (Milchert *et al.* 2015) as well as antifoaming compounds (Tiozzo *et al.* 2013), in cosmetics and pharmaceutical industry (Kumar & Ali 2012).

In the literature, the prepared epoxides have generally been quantified by the evaluation of oxirane number of the modified product (Di Serio *et al.* 2012) or by GC technique (Gorla *et al.* 2013). HPLC has also been used for the quantification (Orellana-Coca *et al.* 2005) but requirement of sample derivatization has made this technique a tedious one (Dupard-Julien *et al.* 2007). As an alternative, 1H NMR has also been utilized for the quantification of the epoxides. Although the NMR technique exhibit expensive instrumentation and requirement of expertise for carrying out the analysis but in comparison to HPLC it is less time consuming and require little of the solvent for carrying out one single analysis. Considering the above advantages, 1H NMR based derivation for the quantification of mono and di-epoxides from sunflower oil was reported by Aerts and Jacobs (2004). However, the authors proposed an extended formula utilizing peaks at 2.01 ppm and 2.90 ppm along with a separate peak at 0.88 ppm as internal standard. Moreover, the results obtained are not consistent with the recent studies on epoxides (Xia *et al.* 2016). Williamson and Hatzakis (2019) has also utilized the NMR characterization described by Xie *et al.* for the analysis of the epoxidised product formed from the extracted coffee oil which are potential candidates as bioplastic precursors. Considering the importance of the epoxides in the industrial field it is important to find out a simple reliable method for easy quantification of the epoxides in the reaction mixture.

To the best of our knowledge, qHNMR technique has not been further explored for the EMO quantification. To make the quantification process simple, quick and easy, we herein propose a qHNMR based derivation for the determination of the epoxidized methyl oleate (EMO) derived from oleic acid (OA).

2. Experimental

2.1 Materials and Instrumental

2.1.1 Materials

Oleic acid ($C_{18}H_{34}O_2$), sodium hydroxide (NaOH), hydrogen peroxide (H_2O_2), and glacial acetic acid (CH_3COOH) were purchased from Loba Chemie, INDIA. Formic acid ($HCOOH$) and deuterated chloroform ($CDCl_3$) were purchased from Sigma-Aldrich, USA. HPLC grade hexane and isopropanol high purity solvents and sulfuric acid (H_2SO_4) were purchased from Spectrochem, INDIA.

2.1.2 Instrumentation

Shimadzu Analytical (Shimadzu, ATX224) electronic balance has been used for weighing purpose and 400

MHz NMR spectrometer JEOL JNM-ECS has been used for the characterization of the epoxides. ^1H NMR (JEOL JNM-ECS) spectra were recorded on 400 MHz on TH5 probe and are reported in parts per million (δ) downfield relative to Me_4Si as internal standard and ^{13}C NMR spectra were recorded at 100 MHz and assigned in parts per million (δ) relative to internal internal standard Me_4Si .

2.2 Experimental methods

EMO and MO required for preparing the standard mixtures for the ^1H NMR quantification were prepared taking OA as starting material. First, MO was prepared by acid catalyzed esterification of the OA (**Scheme 1**). In second step, the obtained fatty ester was epoxidized resulting in the production of EMO as final product (**Scheme 1**). Both the MO and EMO obtained from the esterification and epoxidation reaction respectively were purified using column chromatography prior to the preparation of standard EMO and MO mixtures.

2.2.1 Synthesis of Methyl oleate

Esterification reaction of OA with methanol to obtain MO was performed in the presence of H_2SO_4 (catalyst) (Aranda *et al.* 2008). In a typical reaction, OA (35.401 mmol, 10g), CH_3OH (318.61 mmol, 12.90 ml) and H_2SO_4 (0.193 ml, 3.540 mmol) were added to the 50 ml double necked round bottomed flask equipped with an oil bath, reflux condenser and a magnetic stirrer. The reaction mixture was stirred for 2 h at 80 $^\circ\text{C}$ and the progress of the reaction was monitored with the help of thin layer chromatography (TLC) taking hexane:ethylacetate (90:10) as mobile phase. After completion, the reaction was quenched with saturated sodium bicarbonate solution. The organic phase was extracted with ethyl acetate (3 x 30 mL), washed with brine, dried over anhydrous Na_2SO_4 , concentrated in *vacuo*, to get methyl oleate (MO) as colourless liquid. Esterification reaction of OA is a clean reaction and MO is obtained as an exclusive product which is further confirmed from the ^1H NMR spectra (**Fig. 1**).

2.2.2 Synthesis of Epoxy methyl oleate

The obtained methyl oleate (MO) was epoxidized with the help of in-situ generated performic acid using toluene as a solvent (Campanella *et al.* 2008). A solution of MO (5 g) in toluene (25 ml) was taken in a 25 ml two neck round bottomed flask kept at 0 $^\circ\text{C}$. To the above solution, H_2O_2 (6.20 ml, 202.364 mmol) and formic acid (1.90 ml, 50.59 mmol) were added sequentially as catalyst. Initially, the reaction mixture was stirred at 0 $^\circ\text{C}$ as the reaction is exothermic in nature. After 15 min, the reaction mixture was stirred at 80 $^\circ\text{C}$ for 8 h. After completion (as monitored by TLC), the reaction mixture was cooled to room temperature and quenched with 5% (w/w) NaHCO_3 to neutralize the acid. The organic layer (containing EMO) was extracted using ethylacetate (3 x 30 mL) from above biphasic mixture (toluene and water), collected, dried over anhydrous Na_2SO_4 and concentrated in *vacuo*, further the obtained residue was purified by flash chromatography (EtOAc/hexane 0.5: 9.5) to afford epoxy methyl oleate (~ 97% pure) as colourless liquid. [R_f = 0.7, EtOAc/hexane 0.5 : 9.5 v/v].

2.2.3 Standard sample preparation of MO and EMO

Different sample mixtures having known quantities of MO and EMO in various molar ratios were prepared to check the continuity and reproducibility of qHNMR technique as shown in **Table 1**. A known amount (12 mg) from these prepared mixtures was weighed and diluted with 0.5 ml of CDCl_3 and was subjected to ^1H NMR analysis.

2.2.4. ^1H -NMR analysis

Prepared MO and EMO were characterized by ^1H NMR technique using 5 mm NMR tube. ^1H NMR spectra were recorded in CDCl_3 employing TMS as an internal chemical shift reference with the following acquisition parameters: 45 $^\circ$ pulse angle of 4.87 μs pulse width; transmitter frequency offset (O1P) of 5 ppm; relaxation delay (d1) time 4 s; acquisition time 2.18 s; spectral width 8.75 ppm, 64 scans; spectral acquisition temperature 291–298 K. All spectra were analyzed by the MestReNova package (6.0.2-5475). To integrate the

proton signals, the methoxy protons (-OCH₃) signal of the MO appearing at 3.63 ppm has been assigned to 3 protons. In other words, the MO peak at 3.63 ppm is acting as an internal standard.

2.2.5 HPLC analysis

For HPLC analysis, a series of mixture of the MO/EMO having varying molar ratios were prepared as shown in **Table 1**. To prepare the solutions of 0.01 M concentrations, all the samples were dissolved in a hexane:isopropanol (90:10, v/v) solvent mixture. All samples were syringe filtered prior to the injection and were analyzed by using HPLC (Agilent infinity 1200) equipped with a RI detector. The RX-SIL normal phase column (4.6 mm inner diameter × 250 mm, 5 μm particles) at 35 °C was employed as stationary phase and a hexane:isopropanol (90:10, v/v) as mobile phase at a flow rate of 0.4 mL min⁻¹ with injection volume of 20 μL. In the HPLC chromatogram, the content of MO and EMO in the mixture was determined by calculating the peak area percentage corresponding to their respective peaks with respect to the total area of the chromatogram as shown in **Fig 2s** (supporting information)

3. Results and Discussion

3.1 Characterization of MO and EMO with ¹H-NMR

¹H NMR spectra of OA, MO and its epoxide (EMO) are shown in **Fig. 1**. Esterification of OA leads to the formation of MO which is supported by the appearance of a signal at 3.63 ppm (**Fig. 1**) corresponding to methoxy group (-OCH₃) in MO. The same signal was not originally present in the proton NMR spectra of OA. The signal appearing at 5.3 ppm is due to the presence of the unsaturation (-CH=CH-) in the hydrocarbon chain of OA as well as MO. Accordingly, the formation of EMO from MO was confirmed by the appearance of signal at 2.9 ppm corresponding to the epoxy protons and disappearance of signal at 5.3 ppm due to the unsaturated protons (**Fig. 1**).

¹H-¹H COSY spectrum of epoxy methyl oleate and coupling pattern also shows the formation of EMO. Methoxy protons do not show any coupling and appear as a singlet at δ3.57 ppm. Further, Epoxide ring protons shows coupling with the adjacent protons, therefore, appeared as a multiplet at δ~ 2.81 ppm (**Fig. 1s**, supporting information).

Protons *α* to the carbonyl group shows coupling with protons *β* to carbonyl group and appear as a triplet at δ 2.23-2.19 ppm. Terminal methyl group also shows coupling with adjacent -CH₂- (methylene protons) and found resonating as a triplet at δ 0.84-0.79 ppm.

All these couplings show confirmation of epoxy methyl oleate structure and splitting pattern.

Characterization of methyl oleate by ¹H NMR (400 MHz, CDCl₃) δ (ppm) : 5.39 - 5.30 (m, 2H, -CH=CH-), 3.66 (s, 3H -OCH₃), 2.32-2.28 (t, 3H -CH₂-CO-), 2.07-1.98 (m, 4H, -CH₂CH=CHCH₂-), 1.63-1.58 (m, 2H, -CH₂-CH₂-CO-), 1.35- 1.25 (m, 20H, (CH₂)_n-), 0.89-0.86 (t, 3H, -CH₂-CH₃) as shown in

Fig. 1b.

Characterization of epoxy methyl oleate by ¹H NMR (400 MHz, CDCl₃) δ (ppm) : 3.57 (s, 3H, -OCH₃), 2.85-2.75 (m, 2H, -CH OCH-), 2.23-2.19 (t, 3H -CH₂-CO-), 1.62-1.13(m, 26 H, -CH₂-CHOCH-CH₂,-CH₂-CH₂-CO, -(CH₂)_n-), 0.84-0.79 (m, -CH₂-CH₃) as shown in

Fig. 1a.

3.2 ¹³C NMR analysis of MO and EMO

In ¹³C NMR spectrum, formation of EMO was confirmed through the appearance of signal at δ 57.1 ppm corresponding to oxirane ring carbon and simultaneously disappearance of signals due to olefinic carbons of MO at δ 125 - 130 ppm (**Fig. 2**).

Characterization of methyl oleate by ¹³C NMR (100 MHz, CDCl₃) δ (ppm) : 174 (-CH₂C O-), 130.1 (-CH=C H-(CH₂)₇CO-), 130 (-CH₂-C H=CH-), 57.1 (-C HOC H-), 51.4 (-OC H₃), 34.0 (-C H₂CO-), 31.8

(-CH₃CH₂C H₂-), 29.6 (-(C H₃)₂(CH₂)₂CHOCH(CH₂)₂(C H₂)₃), 27.8 (-(C H₃)₂(CH₂)₂CHOCH(CH₂)₂(C H₂)₃), 27.2 (-C H₂CHOCHC H₂), 26.6 (-C H₂CH₂CHOCHCH₂C H₂-), 24.9 (-C H₂CH₂CO-), 22.6 (-C H₂CH₃), 14.1 (-C H₃) as shown in **Fig. 2a**.

Characterization of epoxymethyl oleate by ¹³C NMR (100 MHz, CDCl₃) δ (ppm) : 174 (-CH₂C O-), 57.1 (-C HOC H-), 51.4 (-OC H₃), 34.0 (-C H₂CO-), 31.8 (-CH₃CH₂C H₂-), 29.6 (-(C H₂)₃(CH₂)₂CHOCH(CH₂)₂(CH₂)₃), 27.8 (-(C H₂)₃(CH₂)₂CHOCH(CH₂)₂(CH₂)₃), 27.2 (-C H₂CHOCHC H₂), 26.6 (-C H₂CH₂CHOCHCH₂C H₂-), 24.9 (-C H₂CH₂CO-), 22.6 (-C H₂CH₃), 14.1 (-C H₃) as shown in **Fig. 2b**.

3.3 Assignment of the signals for the quantification by qHNMR

For deriving ¹H NMR based quantification some prerequisite conditions have to be met such as complete solubility of the analyte, proper relaxation delay within the scans, no overlapping of the peaks and no side reactions in the mixture (Bharti & Roy 2012), which also happen to be the limitations of this technique. In our case, with reference to the overlapping of the peaks, there was no overlap of the peaks of OA, MO and EMO that are selected for the quantification purpose and all are completely soluble in the CDCl₃ with no side reaction. The ¹H NMR equation has been derived keeping in the view of the basic principle of qHNMR that integrated peak area value directly corresponds to the number of protons responsible for that peak which in turn will provide the molar concentration of the corresponding molecule (Bharti & Roy 2012). It has been observed that with the progress of the epoxidation reaction, the double bond present in the MO gets converted into the oxirane ring resulting in the appearance of new signal **c** at 2.87 ppm and disappearance of signal **c**₁ at 5.3 ppm. However, the methoxy signal, **a** remained intact in both MO and EMO as shown in **Fig. 3**. Thus the formation of EMO in the reaction mixture could be quantified either by following the peak area corresponding to the disappearing alkene proton (*I*_{c₁}) as given in **equation 1** or by following the peak area corresponding to the appearing oxirane proton (*I*_c) as given in **equation 2**.

$$\% \text{ EMO} = 100 - \frac{3 \times I_{c_1}}{2 \times I_a} \times 100$$

equation 1

$$\% \text{ EMO} = \frac{3 \times I_c}{2 \times I_a} \times 100$$

equation 2

Where % EMO = molar percentage of epoxy methyl oleate in the mixture; *I*_{c₁} = integration of signal corresponding to alkene protons at 5.33 ppm (**equation 1**); *I*_a = integration of signal corresponding to methoxy protons at 3.63 ppm (**equation 1 & 2**); *I*_c = integration of signal corresponding to oxirane ring protons at 2.88 ppm (**equation 2**).

3.4 Method Validation

3.4.1 Accuracy:

Accuracy refers to how close the measured values are in agreement with that of the true values. A comparison of the actual EMO molar concentrations taken to those of predicted by **equation 1** and **2** (**Table 1**) shows an acceptable agreement with the standard deviation value of up to ± 6 % (**equation 1**) and ± 2 % (**equation 2**). As **equation 2** showed better acceptable results, it could be employed to quantify the EMO in MO and EMO mixture.

3.4.2 Reproducibility and Linearity:

A linearity curve for both the derived equations was also evaluated (**Fig. 4**) which showed the correlation coefficient of > 0.999. To test the reproducibility and linearity of the method, three different technical hands have followed the identical experimental procedure to quantify the EMO in the mixture of MO/EMO and employed **equation 1** and **2**. The observed results showed good correlation with the true concentrations confirming the excellent reproducibility of the method (**Table 1a**, supporting information). As shown in **Table 1**, as the EMO concentration in the mixture increases beyond 70%, both the equations were found to

be consistent with the actual values. Nevertheless, **equation 2** was found to be more consistent with actual concentration as well as with HPLC analysis at all concentration levels.

3.4.3 Comparison with HPLC results

In order to compare the results obtained from the qHNMR technique, the prepared standard samples with different MO and EMO molar ratios were analyzed by an HPLC technique. In the HPLC chromatogram, the retention time of MO and EMO were found to be 7.92 and 8.38 minutes, respectively. In the HPLC chromatogram of standard mixtures of MO/EMO, percentage peak area values were calculated (**Table 1**) and was found to be directly proportional to the percentage molar concentration of the compounds (**Fig 2s – 5s**, supporting information). The results of EMO molar concentration obtained from HPLC when correlated with that obtained from the qHNMR technique showed acceptable agreements affirming the validation of the qHNMR method (**Fig. 5**).

3.4.4. Applicability

In order to demonstrate the applicability of the proposed **equation 2**, MO was epoxidized by performic acid to obtain EMO following the reported procedure (26). The quantification analysis by the same method supports the formation of 94.5 % EMO which was very similar to the value 89.0% obtained by HPLC technique (**Fig. 5s**, supporting information).

4. Conclusion:

In this study, qHNMR was used to quantify epoxy methyl oleate prepared from oleic acid. Two different ^1H NMR equations have been proposed for the quantification of EMO in EMO and MO mixture. The EMO quantification values obtained from **equation 2** was found to demonstrate better correlation (0.999) with that of EMO present in the standard mixture of EMO and MO. The results obtained from the ^1H NMR technique are also in accordance with that of HPLC ($R^2 = 0.996$). The developed **equation 2** has also been applied on the quantification of the real EMO sample obtained from the epoxidation of methyl oleate. The linearity, reproducibility and validity of the qHNMR technique suggest that qHNMR could be used as a reliable and quick method for the quantification of epoxy methyl oleate (EMO).

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Conflict of Interest

The authors declare that they have no conflict of interest.

References

- Aerts, H. A. J., & Jacobs, P. A. (2004). Epoxide Yield Determination of Oils and Fatty Acid Methyl Esters using ^1H NMR. *Journal of the American Oil Chemists' Society*, **81** (9), 841–846. <https://doi.org/10.1007/s11746-004-0989-1>.
- Aranda, D. A. G., Santos, R. T. P., Tapanes, N. C. O., Ramos, A. L. D., & Antunes, O. A. C. (2008). Acid-Catalyzed Homogeneous Esterification Reaction for Biodiesel Production from Palm Fatty Acids. *Catalysis Letters*, **122** (1–2), 20–25. <https://doi.org/10.1007/s10562-007-9318-z>.
- Bharti, S. K., & Roy, R. (2012). Quantitative ^1H NMR Spectroscopy. *Trends in Analytical Chemistry*, **35**, 5–26. <https://doi.org/10.1016/j.trac.2012.02.007>.
- Campanella, A., Fontanini, C., & Baltanás, M. A. (2008). High Yield Epoxidation of Fatty Acid Methyl Esters with Performic Acid Generated In Situ. *Chemical Engineering Journal*, **144** (3), 466–475. <https://doi.org/10.1016/j.cej.2008.07.016>.

- Cao, R., Komura, F., Nonaka, A., Kato, T., Fukumashi, J., & Matsui, T. (2014). Quantitative Analysis of D-(+)-Glucose in Fruit Juices Using Diffusion Ordered- ^1H Nuclear Magnetic Resonance Spectroscopy. *Analytical Sciences* , **30** (3), 383–388. doi: 10.2116/analsci.30.383.
- Cerceau, C. I., Barbosa, L. C. A., Filomeno, C. A., Alvarenga, E. S., Demuner, A. J., & Fidencio, P. H. (2016). An Optimized and Validated ^1H NMR Method for the Quantification of α -Pinene in Essentials Oils. *Talanta* , **150** , 97–103. doi: 10.1016/j.talanta.2015.10.087.
- Di Serio, M., Turco, R., Pernice, P., Aronne, A., Sannino, F., & Santacesaria, E. (2012). Valuation of $\text{Nb}_2\text{O}_5\text{-SiO}_2$ Catalysts in Soybean Oil Epoxidation. *Catalysis Today* , **192** (1), 112–116. <https://doi.org/10.1016/j.cattod.2012.03.069>.
- Doll, K. M., & Erhan, S. Z. (2006). Synthesis and Performance of Surfactants Based on Epoxidized Methyl Oleate and Glycerol. *Journal of Surfactants and Detergents* , **9** , 377–383. <https://doi.org/10.1007/s11743-006-5016-x>.
- Dupard-Julien, C. L., Kandlakunta, B., & Uppu, R. M. (2007). Determination of Epoxides by High-Performance Liquid Chromatography Following Derivatization with N,N-Diethyldithiocarbamate. *Analytical and Bioanalytical Chemistry* , **387** (3), 1027–1032. doi:10.1007/s00216-006-1003-3.
- Elyashberg, M. (2015). Identification and Structure Elucidation by NMR Spectroscopy. *Trends in Analytical Chemistry* , **69** , 88–97. <https://doi.org/10.1016/j.trac.2015.02.014>.
- Gelbard, G., Brès, O., Vargas, R. M., Vielfaure, F., & Schuchardt, U. F. (1995). ^1H Nuclear Magnetic Resonance Determination of the Yield of the Transesterification of Rapeseed Oil with Methanol. *Journal of the American Oil Chemists' Society* , **72** (10), 1239–1241. <https://doi.org/10.1007/BF02540998>.
- Göger, N. G., Parlatan, H. K., Basan, H., Berkkan, A., & Özden, T. (1999). Quantitative Determination of Azathioprine in Tablets by ^1H NMR Spectroscopy. *Journal of Pharmaceutical and Biomedical Analysis* , **21** (3), 685–689. doi:10.1016/s0731-7085(99)00156-9.
- Gorla, G., Kour, S. M., Padmaja, K. V., Karuna, M. S. L., & Prasad, R. B. N. (2013). Preparation and Properties of Lubricant Base Stocks from Epoxidized Karanja Oil and its Alkyl Esters. *Industrial and Engineering Chemistry Research* , **52** (47), 16598–16605. <https://doi.org/10.1021/ie4024325>.
- Hollis, D. P. (1963). Quantitative Analysis of Aspirin, Phenacetin, and Caffeine Mixtures by Nuclear Magnetic Resonance Spectrometry. *Analytical Chemistry* , **35** (11), 1682–1684. <https://doi.org/10.1021/ac60204a043>.
- Kumar, D., & Ali, A. (2012). Ti/SiO₂ as a Nanosized Solid Catalyst for the Epoxidation of Fatty Acid Methyl Esters and Triglycerides. *Energy and Fuels* , **26** (5), 2953–2961. <https://doi.org/10.1021/ef300127c>.
- Milchert, E., Malarczyk, K., Kłós, M., & Novak, L. (2015). Technological Aspects of Chemoenzymatic Epoxidation of Fatty Acids, Fatty Acid Esters and Vegetable Oils: A Review. *Molecules* , **20** (12), 21481–21493. doi: 10.3390/molecules201219778.
- Monteiro, M. R., Ambrozín, A. R. P., Lião, L. M., & Ferreira, A. G. (2008). Critical Review on Analytical Methods for Biodiesel Characterization. *Talanta* , **77** (2), 593–605. <https://doi.org/10.1016/j.talanta.2008.07.001>.
- Orellana-Coca, C., Adlercreutz, D., Andersson, M. M., Mattiasson, B., & Hatti-Kaul, R. (2005). Analysis of Fatty Acid Epoxidation by High Performance Liquid Chromatography Coupled with Evaporative Light Scattering Detection and Mass Spectrometry. *Chemistry and Physics of Lipids* , **135** (2), 189–199. <https://doi.org/10.1016/j.chemphyslip.2005.02.014>.
- Phansalkar, R. S., Simmler, C., Bisson, J., Chen, S.-N., Lankin, D.C., Mcalpine, J.B., Nietnitz, M., & Pauli, G. F. (2017). Evolution of Quantitative Measures in NMR: Quantum Mechanical qHNMR Advances Chemical Standardization of a Red Clover (*Trifolium pratense*) Extract. *Journal of Natural Products* , **80** (3), 634–647. <https://doi.org/10.1021/acs.jnatprod.6b00923>.

Rizzo, V., & Pincioli, V. (2005). Quantitative NMR in Synthetic and Combinatorial Chemistry. *Journal of Pharmaceutical and Biomedical Analysis* , **38** (5 SPEC. ISS.), 851–857. <https://doi.org/10.1016/j.jpba.2005.01.045>.

Su, F., & Guo, Y. (2014). Advancements in Solid Acid Catalysts for Biodiesel Production. *Green Chemistry* , **16** (6), 2934–2957. doi:10.1039/c3gc42333f.

Suarez, P. A. Z., Pereira, M. S. C., Doll, K. M., Sharma, B. K., & Erhan, S. Z. (2009). Epoxidation of Methyl Oleate using Heterogeneous Catalyst. *Industrial and Engineering Chemistry Research* ,**48** (7), 3268–3270. doi: 10.1021/ie801635b.

Sun, S., Jin, M., Zhou, X., Ni, J., Jin, X., Liu, H., & Wang, Y. (2017). The Application of Quantitative ¹H-NMR for the Determination of Orlistat in Tablets. *Molecules* , **22** (9), 1–10. doi: 10.3390/molecules22091517.

Tiozzo, C., Bisio, C., Carniato, F., Marchese, L., Gallo, A., Ravasio, N., Psaro, R., & Guidotti, M. (2013). Epoxidation with Hydrogen Peroxide of Unsaturated Fatty acid Methyl Esters over Nb(V)-Silica Catalysts. *European Journal of Lipid Science and Technology* ,**115** (1), 86–93. doi: 10.1002/ejlt.201200217.

Williamson, K., & Hatzakis, E. (2019). NMR Analysis of Roasted Coffee Lipids and Development of a Spent Ground Coffee Application for the Production of Bioplastic Precursors. *Food Research International*,**119**, 683–692. <https://doi.org/10.1016/j.foodres.2018.10.046>.

Xia, W., Budge, S. M., & Lumsden, M. D. (2016). ¹H-NMR Characterization of Epoxides Derived from Polyunsaturated Fatty Acids. *Journal of the American Oil Chemists' Society* , **93** (4), 467–478. <https://doi.org/10.1007/s11746-016-2800-2>.

Yan, K. J., Chu, Y., Huang, J. H., Jiang, M.-M., Li, W., Wang, Y.-F., Huang, H.-Y., Qin, Y.-H., Ma, X.-H., Zhou, S.-P., Sun, H., & Wang, W. (2016). Qualitative and Quantitative Analyses of Compound Danshen Extract Based on ¹H NMR Method and its Application for Quality Control. *Journal of Pharmaceutical and Biomedical Analysis* , **131** , 183–187. <https://doi.org/10.1016/j.jpba.2016.08.017>.

Zhang, Q., Guo, N., Sun, Y., Li, X., & Yang, H. (2017). Absolute Quantification of Poly(DL-lactide-glycolide) in Microspheres Using Quantitative ¹H NMR Spectroscopy. *Journal of Pharmaceutical and Biomedical Analysis* , **146** , 273–278. doi: 10.1016/j.jpba.2017.08.046.

Table 1 The actual epoxy methyl oleate concentrations *versus* those predicted by the qHNMR and HPLC techniques.

S.No	Molar % taken MO	Molar % taken EMO	Predicted % C _{EMO} by NMR Equation 1 (± S.D.)	Predicted % C _{EMO} by NMR Equation 2 (± S.D.)	Predicted % C _{EMO}
1	90	10	16.56 ± 6.5	10.7 ± 0.6	8.76
2	80	20	25.94 ± 6	20.7 ± 0.6	22.66
3	70	30	35.62 ± 6	31.0 ± 0.5	31.68
4	60	40	44.75 ± 5	41.0 ± 0.5	43.80
5	50	50	54.34 ± 4	51.3 ± 1	47.86
6	40	60	64.36 ± 4	61.2 ± 1	60.20
7	30	70	72.66 ± 3	71.2 ± 1	70.98
8	20	80	82.93 ± 3	81.0 ± 1	79.67
9	10	90	91.48 ± 1	91.8 ± 2	87.11

Above values observed by the qHNMR technique are average of three measurements, S.D. = Standard Deviation, MO = Methyl oleate, EMO = Epoxy methyl oleate, C_{EMO} = % Molar concentration of epoxy methyl oleate.

Table 2 Tabular description of the peaks obtained in the ¹H NMR spectra of OA, MO and EMO

S.No	H- Atom Description	Oleic Acid (OA)	Methyl Oleate (MO)	Epoxy Methyl Oleate (EMO)
		¹ H-Shift in ppm	¹ H-Shift in ppm	¹ H-Shift in ppm
I	Alkene protons, a	5.39-5.30 (m, 2H)	5.39-5.30 (m, 2H)	-
II	Methoxy protons, b	-	3.66 (s, 3H)	3.57 (s, 3H)
III	Epoxide ring protons, h	-	-	2.85-2.79 (m, 2H)
IV	Protons <i>a</i> to carbonyl, c	2.36-2.33 (t, 2H)	2.32-2.28 (t, 2H)	2.23-2.19 (t, 3H)
V	Protons <i>a</i> to double bond, d	2.07-1.98 (m, 4H)	2.07-1.98 (m, 4H)	-
VI	Protons β to carbonyl group, e	1.66-1.59 (m, 2H)	1.63-1.58 (m, 2H)	-
VII	Chain protons, g	1.39-1.25 (m, 20H)	1.35-1.25 (m, 20H)	-
VIII	Terminal Methyl group protons, f	0.89-0.86 (t, 3H)	0.89-0.86 (t, 3H)	0.84-0.79 (t, 3H)
IX	Protons, d, e, g in case of E.M.O	-	-	1.62-1.13 (m, 26 H)

Table 3 Tabular description of the peaks obtained in the ¹³C NMR spectra of OA, MO and EMO

S.No	C- Atom Description (Comparison of characteristic peaks)	Methyl Oleate (M.O)	Epoxy Methyl Oleate (E.M.O)
		¹³ C-Shift in ppm	¹³ C-Shift in ppm
I	-C=O-, i	173.99	174.240
II	-CH=CH-, a	130.00, 130.09	-
III	-CHOCH-, h	-	57.231
IV	OCH ₃ , b	50.976	51.433
V	-CH ₂ -CO-OCH ₃ , c	33.631	34.034
VI	-CH ₂ -CH ₂ -CO-OCH ₃ , e	24.494	24.899
VII	-CH ₂ -CH ₃ , f	22.403	22.689
VIII	-CH ₂ -CH ₃ , g	13.669	14.118
Rest chain carbons were found resonating at their respective positions.	Rest chain carbons were found resonating at their respective positions.	Rest chain carbons were found resonating at their respective positions.	Rest chain carbons were found resonating at their respective positions.

Scheme 1 Schematic representation of the two step synthesis of epoxy methyl oleate from oleic acid.

Fig. 1 Comparison of ¹H NMR spectra of (a) epoxy methyl oleate; (b) methyl oleate and (c) oleic acid.

Fig. 2 ¹³C NMR spectra of (a) epoxy methyl oleate and (b) methyl oleate.

Fig. 3 ¹H NMR spectra of (a) 80:20 (m/m) mixture of EMO/MO; b) 60:40 (m/m) mixture of EMO/MO; c) 40:60 (m/m) mixture of EMO/MO; d) 20:80 (m/m) mixture of EMO/ MO.

Fig. 4 A correlation line between the actual molar concentrations of epoxy methyl oleate (EMO) *versus* those predicted by the proposed equations.

Fig. 5 A correlation line between the actual concentrations of epoxy methyl oleate (EMO) *versus* those predicted by the HPLC and qHNMR techniques.

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