Compositional Analysis of Mixtures of Oleate Esters of Short Chain Alcohols (C1-C4) by Quantitative Proton Nuclear Magnetic Resonance Spectroscopy (qPNMR)

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Abstract Quantitative nuclear magnetic resonance spectroscopy (qNMR) is a technique used to determine the concentration of one or more analyte within a mixture. Although NMR spectroscopy is typically used to qualitatively determine molecular structure, the quantitative application of NMR extends to concentration determinations and purity assessments. Described herein is an experiment designed to increase awareness of both the qualitative and quantitative applications of NMR spectroscopy that could be integrated into undergraduate analytical and instrumental chemistry laboratory course curriculums. The experiment entails the quantitative analysis of binary long-chain monounsaturated fatty acid mixtures ranging from 0% to 100% in 20% intervals of methyl oleate (MeOl), ethyl oleate (EtOl), propyl oleate (PrOl) and butyl oleate (BuOl) using proton NMR. The goal of the experiment is to determine the structure and weight percent composition of both analytes in each of the mixtures. The results show a strong, linear correlation between the gravimetric compositions and the weight percent compositions found using proton NMR. The experiment supports qNMR as a tool for determining weight percent compositions of mixtures and can be incorporated at the undergraduate chemistry laboratory level.

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1. Introduction

Although qualitative and quantitative analyses of organic mixtures using proton nuclear magnetic resonance spectroscopy have been performed [1-5], the application of qNMR for educational purposes is limited [6-14]. Accordingly, this experiment has been designed to implement qNMR methodology and analysis into undergraduate chemistry laboratory curriculums. This experiment determines the weight percent composition of reagents in a binary mixture of short-chain fatty acid oleate esters. The application of qNMR is effective because the integration value of a peak is directly proportional to the number of protons producing a signal at that chemical shift value. Chemical shift and integration values were obtained by running all of the samples neat, without an internal reference standard. The results of the NMR spectra were qualitatively analyzed using multiplicity, spin-spin coupling and integration values to determine which proton(s) produced each individual peak. Binary mixtures ranging from 0% to 100% in 20% intervals of methyl oleate (MeOl), ethyl oleate (EtOl), propyl oleate (PrOl) and butyl oleate (BuOl) were made.

1.1. Learning Objectives

1. To learn how to properly prepare binary mixtures using volumetric and gravimetric techniques.
2. To understand the principles of proton NMR and the functions of the JEOL Delta software.
3. To relate the chemical shift (δ), multiplicity and integration values of the peaks to the structures of the components of the binary mixtures.
4. To establish correlation curves between the integrated NMR peak areas and the calculated weight % compositions for a binary mixture.

2. Materials and Methods

2.1. Student Procedure

Each student will aliquot mixtures of approximately 20%, 40%, 60%, and 80% by volume of a mixture of either MeOl-EtOl, MeOl-PrOl, MeOl-BuOl, or EtOl-PrOl. 7 mL sample vials will be weighed before and after each addition of reagent to the vial to determine weight % composition. Students will run proton NMR on their samples and

develop a calibration curve of calculated weight % of the component of interest and the weight % determined by NMR. The students will then use the curve to determine the weight % of an unknown sample created by the instructor.

2.2. Experimental Materials

Methyl, ethyl, propyl and butyl oleate were all purchased from various vendors as anhydrous liquids with greater than 90% purity. All reagents were used without purification. The NMR tubes used in this study were Wilmad Pyrex glass 5 mm x 7" thin wall tubes.

2.2.1. Proton NMR

The proton NMR spectra were obtained using a 400 MHz JEOL model ECS-400 NMR spectrometer. The JEOL Delta NMR control and process software version 5.0.2 (Windows) was used to analyze the spectra. Each sample was run neat as a single pulse, 1D proton NMR with a 0.25 Hz resolution and a relaxation time ranging from 8 to 10 seconds. The experimental analysis is not limited to this specific NMR hardware and software.

2.2.2. Experimental Procedure

Sixteen 5.0 mL binary mixtures of methyl oleate (MeOl), ethyl oleate (EtOl), propyl oleate (PrOl) and butyl oleate (BuOl) were prepared as shown in Table 1. Each reagent was added using a Gilson classic model P1000 pipette and 1 mL was added to NMR tubes for qNMR analysis. All of the 7 mL vials and NMR tubes were labeled with the volumetric ratio of reagents in the mixture. Following each addition, the mass of the vial was recorded using an analytical balance having a precision of 0.1 mg. The weight percent composition of the mixtures were determined using these masses. All mixtures were analyzed without an internal reference standard.

Table 1. Summary of Aliquoted Binary Mixtures

Volume ratio	
Methyl Oleate: Ethyl Oleate	Experimental weight % Methyl Oleate
0ml:5ml MeOl:EtOl	0
1ml:4ml MeOl:EtOl	19.714
2ml:3ml MeOl:EtOl	41.471
3ml:2ml MeOl:EtOl	60.907
4ml:1ml MeOl:EtOl	81.356
5ml:0ml MeOl:EtOl	100
Methyl Oleate: Propyl Oleate	Experimental weight % Methyl Oleate
0ml:5ml MeOl:PrOl	0
1ml:4ml MeOl:PrOl	20.013
2ml:3ml MeOl:PrOl	41.017
3ml:2ml MeOl:PrOl	61.316
4ml:1ml MeOl:PrOl	79.872
5ml:0ml MeOl:PrOl	100
Methyl Oleate: Butyl Oleate	Experimental weight % Methyl Oleate
0ml:5ml MeOl:BuOl	0
1ml:4ml MeOl:BuOl	19.737
2ml:3ml MeOl:BuOl	39.826
3ml:2ml MeOl:BuOl	61.946
4ml:1ml MeOl:BuOl	82.517
5ml:0ml MeOl:BuOl	100
Ethyl Oleate: Propyl Oleate	Experimental weight % Ethyl Oleate
0ml:5ml EtOl:PrOl	0
1ml:4ml EtOl:PrOl	20.466
2ml:3ml EtOl:PrOl	39.635
3ml:2ml EtOl:PrOl	60.342
4ml:1ml EtOl:PrOl	79.483
5ml:0ml EtOl:PrOl	100

2.2.3. Hazards

Methyl oleate (CAS# 112-62-9), ethyl oleate (CAS# 111-62-6), propyl oleate (CAS#111-59-1) and butyl oleate (CAS# 142-77-8) are consistently used as pharmaceutical solvents and in commercial products such as lotions as they do not pose any significant health risks. Goggles and gloves are nonetheless mandatory in order to avoid exposure to the eyes and skin. Waste solutions should be disposed of according to EPA and local guidelines. Students with metallic implants should not be in the same room as the NMR machine at any time because the magnetic fields from the NMR may interfere with the implants.

3. Results and Discussion

Figure 1 through Figure 4 depict the NMR spectra of 100% methyl oleate, ethyl oleate, propyl oleate and butyl oleate respectively. Table 2 summarizes the chemical shifts, multiplicities, and normalized integration values for each specifically identified, unique peak in the neat samples.

All reagents had two de-shielded protons around 4.6 ppm. This is due to the electron withdrawing effects of the carbon-carbon double bond. The result is the protons off the carbons in the double bond are de-shielded from the external magnetic field caused by the NMR, shifting them more downfield than expected. Each reagent also had a peak around 1.6 ppm and this corresponds to the methylene group adjacent to the carbon in the ester functional group. These protons are more de-shielded due to their proximity to the ester, as the oxygen atom is inductively electron withdrawing, however, they are not as downfield as the hydrogens off the carbon-carbon double bond or the hydrogens off the carbon adjacent to the oxygen atom of the ester. The main differences between MeOl, EtOl, PrOl, and BuOl are the hydrogens off the carbon adjacent to the oxygen atom in the ester, which is labelled protons A, and the hydrogens off the next adjacent carbon labelled protons B. In MeOl, protons A are singlets because the terminal methyl protons have no proton neighbors, thus there would be no spin-spin coupling resulting, and the peak has an integration value of 3 which refers to the 3 hydrogen atoms in the methyl group. The chemical shift value of these protons is around 2.8 ppm, which is less than that of the methylene protons labelled A in EtOl, PrOl, and BuOl because these protons are more de-shielded due to the presence of an additional R group. Accordingly, in EtOl, PrOl, and BuOl, the A methylene protons have normalized integration values of 2 and chemical shift values near 3.4 ppm. In EtOl, the peak is a quartet due to the adjacent methyl group with 3 hydrogens, whereas in PrOl and BuOl, the peak is a triplet due to the adjacent methylene group with 2 hydrogens.

Protons B refer to the hydrogens off the carbon 2 away from the oxygen of the ester group. MeOl does not have any protons labelled B because there is only a methyl group bonded to the ester oxygen. For EtOl, protons B have a normalized integration value of 3 and refer to the 3 hydrogen atoms in the methyl group. The peak is a triplet because there is one adjacent methylene group with 2 hydrogens. The chemical shift value of these protons is

around 0.6 ppm, which is less than that of the methylene protons labelled B in PrOl and BuOl because those protons are more de-shielded due to the presence of an additional R group. Similar to how the A protons in MeOl have lower chemical shift values than the other oleates because these protons are off a terminal carbon, the B protons in EtOl have lower chemical shifts than the other oleates because these are also bonded to a terminal carbon. For PrOl and BuOl, proton B refers to the 2 hydrogens in the methylene group and has a normalized integration value of 2. In PrOl, the peak is a sextet because there is 1 adjacent methyl group and 1 adjacent methylene group with 3 and 2 hydrogens respectively. In BuOl, the peak is a quintet because there are 2 adjacent methylene groups, each with 2 hydrogens. The chemical shift values of these protons in both reagents are around 1.6 ppm because they are both bonded to the oxygen of an ester group and an additional R group, which results in similar electron withdrawing and de-shielding effects. The A protons have a higher chemical shift value than the B protons because they are more de-shielded by the applied magnetic field of the ester oxygens. As the distance between a specified proton and the oxygens of the ester group increases, the chemical shift value decreases as the protons are less de-shielded by the electron withdrawing ester oxygens. The multiplicities of each of the protons was determined by the number of neighboring protons off adjacent carbons, as per the n+1 multiplicity rule of one-dimensional proton NMR.

Figure 5 – Figure 8 are proton NMR spectra of 4:1 mixtures of MeOl-EtOl, MeOl-PrOl, MeOl-BuOl, or EtOl-PrOl. The percent compositions of these mixtures were calculated by analyzing the 2.92 ppm singlet peak to quantify the presence of MeOl, the 3.39 quartet peak to quantify the presence of EtOl, the 3.35 triplet peak to quantify the presence of PrOl, and the 3.37 triplet to quantify the presence of BuOl. The formula to calculate the percent composition of a component is as follows, where X and Y are the components in the binary mixture and A is the integration value (number of protons):

$$\% \; composition = \frac{A_X}{A_X + A_Y} * 100\%$$

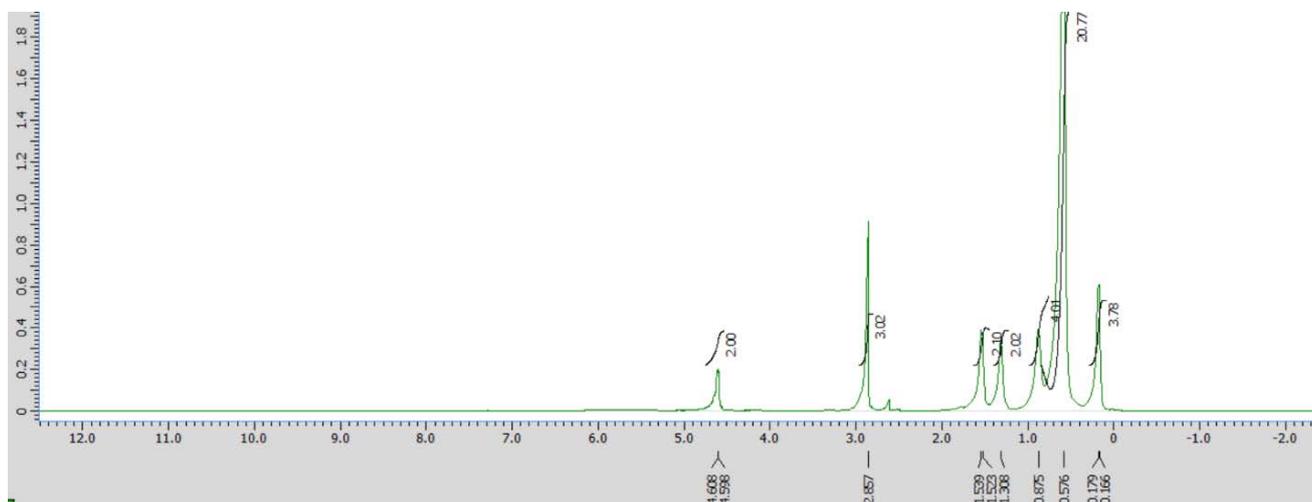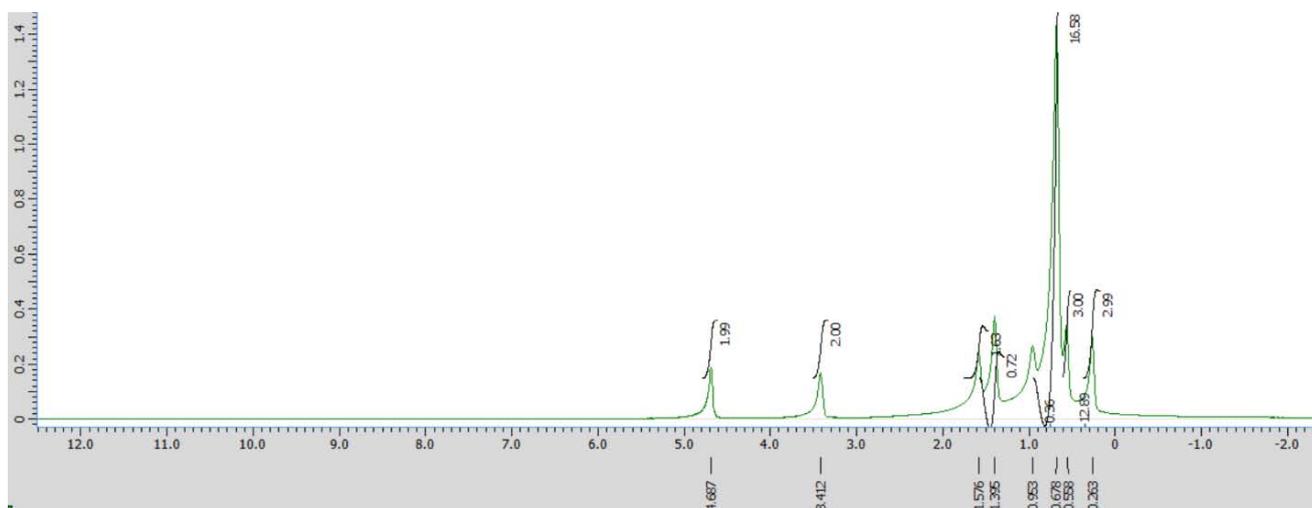**Figure 1.** NMR spectrum of >90% anhydrous methyl oleate

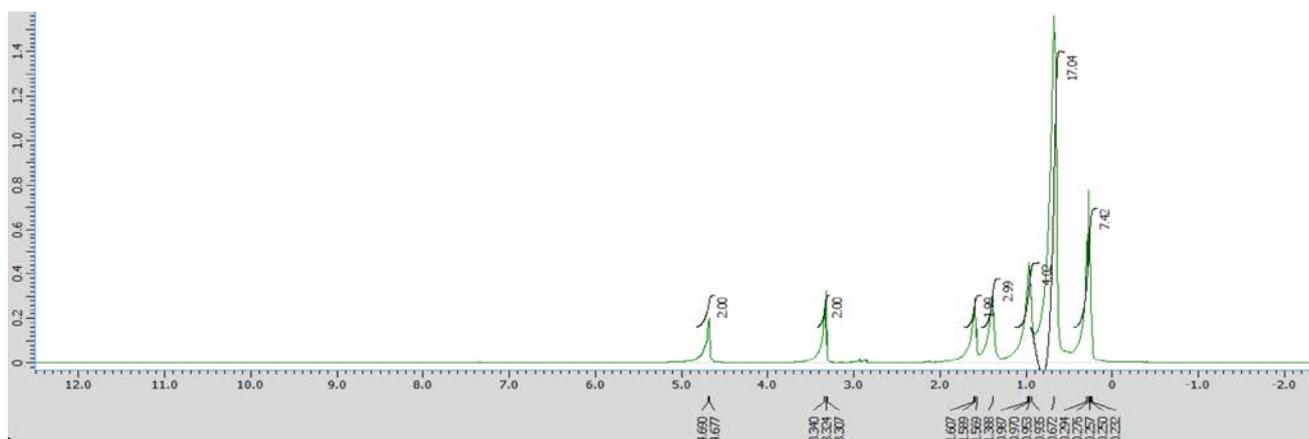**Figure 2.** NMR spectrum of >90% anhydrous ethyl oleate

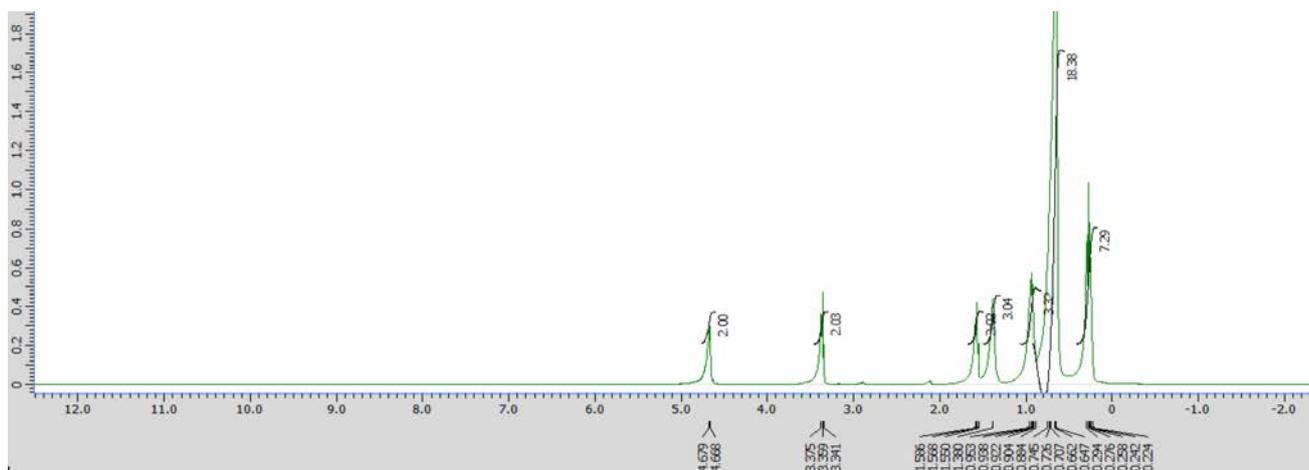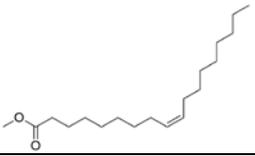**Figure 3.** NMR spectrum of >90% anhydrous propyl oleate
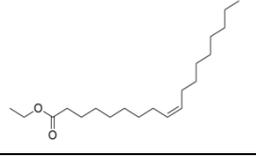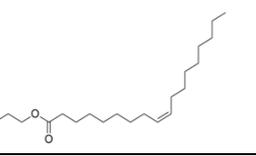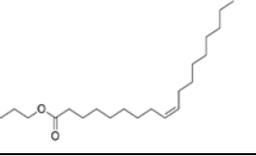

Figure 4. NMR spectrum of >90% anhydrous butyl oleate

Table 2. Summary of reagent chemical shift values, multiplicities, and normalized integration values

Methyl Oleate (MeOl)	Ethyl Oleate (EtOl)	Propyl Oleate (PrOl)	Butyl Oleate (BuOl)

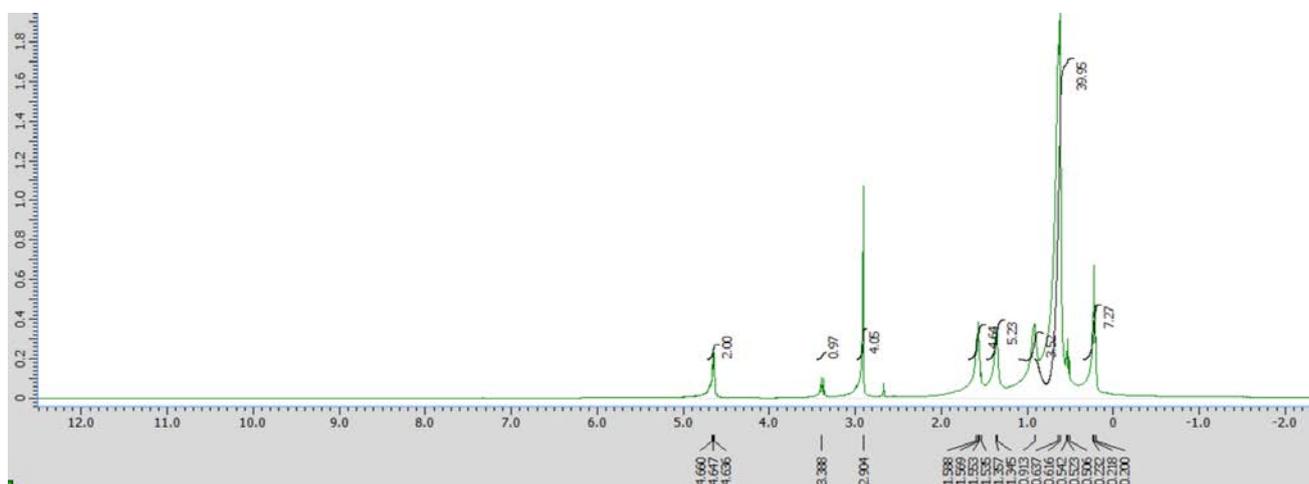| Proton	Shift (δ) ppm	Multiplicity	Integration	Proton	Shift (δ) ppm	Multiplicity	Integration	Proton	Shift (δ) ppm	Multiplicity	Integration	Proton	Shift (δ) ppm	Multiplicity	Integration
A	2.587	singlet	3.02	A	3.412	quartet	2.00	A	3.324	triplet	2.00	A	3.359	triplet	2.03
				B	0.558	triplet	3.00	B	1.589	sextet	1.99	B	1.568	quintet	2.00

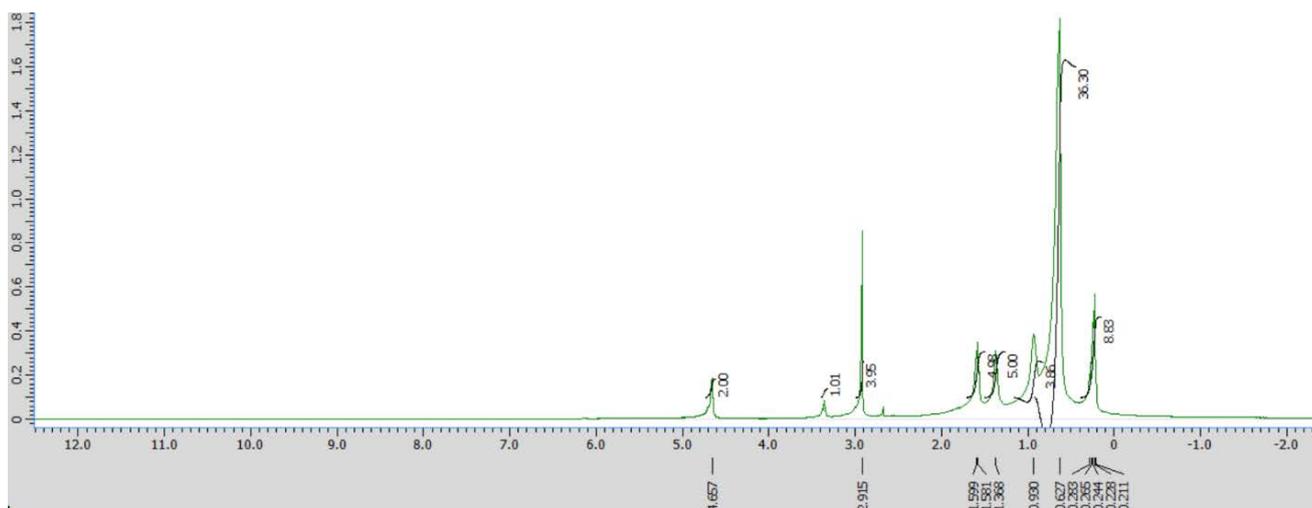**Figure 5.** Proton NMR of a 4:1 mixture of MeOl: EtOl

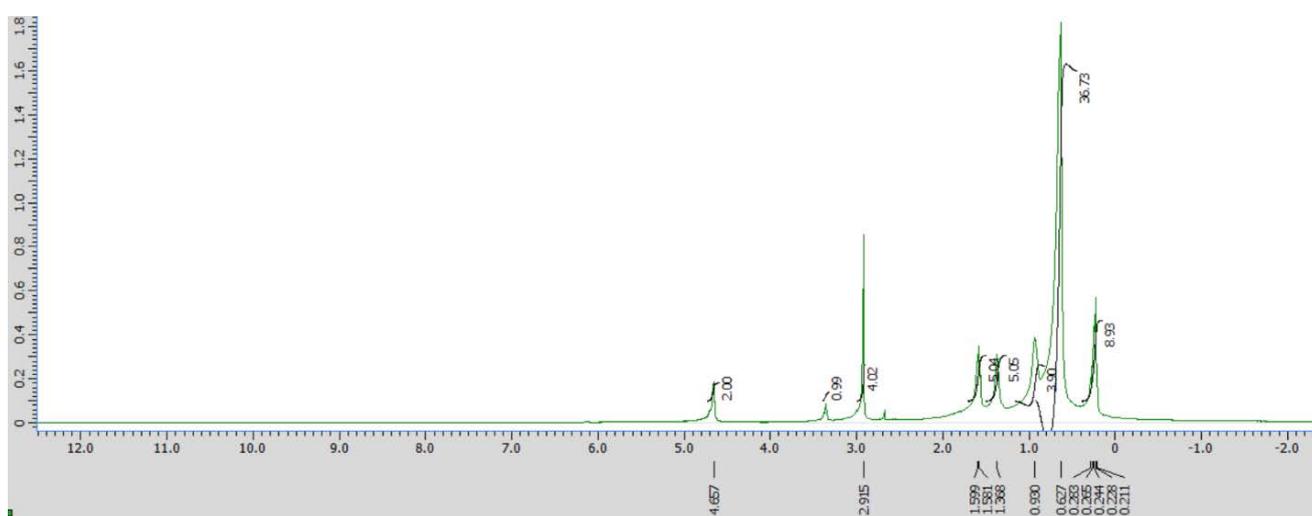**Figure 6.** Proton NMR of a 4:1 mixture of MeOl: PrOl

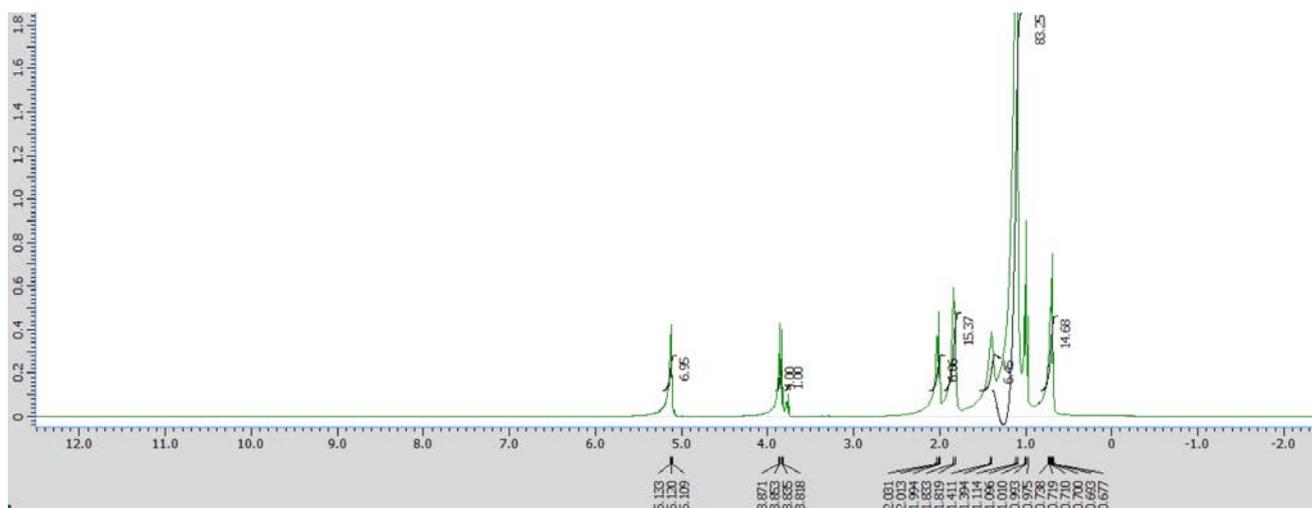**Figure 7.** Proton NMR of a 4:1 mixture of MeOl: BuOl

Figure 8. Proton NMR of a 4:1 mixture of EtOl: PrOl

Figure 9 through Figure 12 show the strong, linear correlation between the NMR calculated weight % and the gravimetrically determined weight %. In all three cases, the experimental weight % obtained via gravimetric analysis and the qNMR weight % align in a one-to-one relationship evidenced by the slopes in all cases being very close to 1. The qNMR methodology is shown to be very accurate, as all calibration curves have an R^2 value of greater than 0.99.
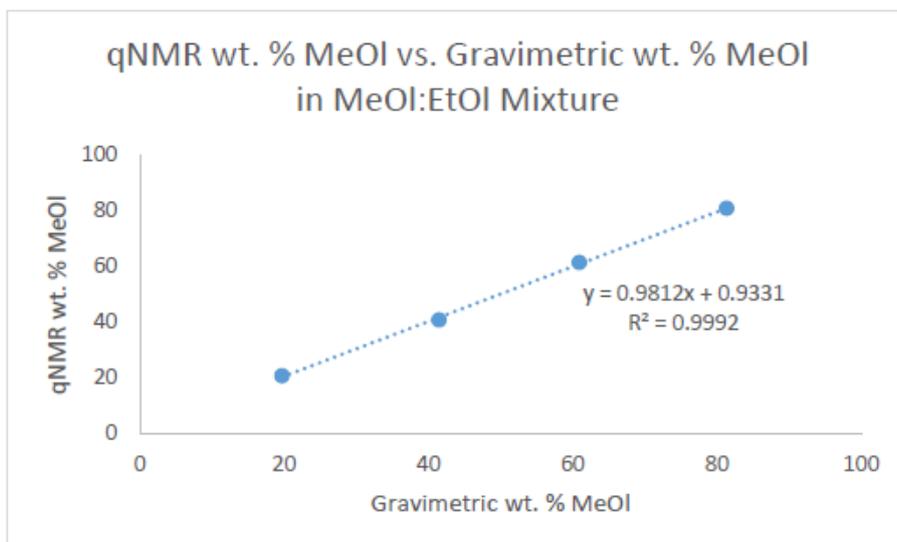
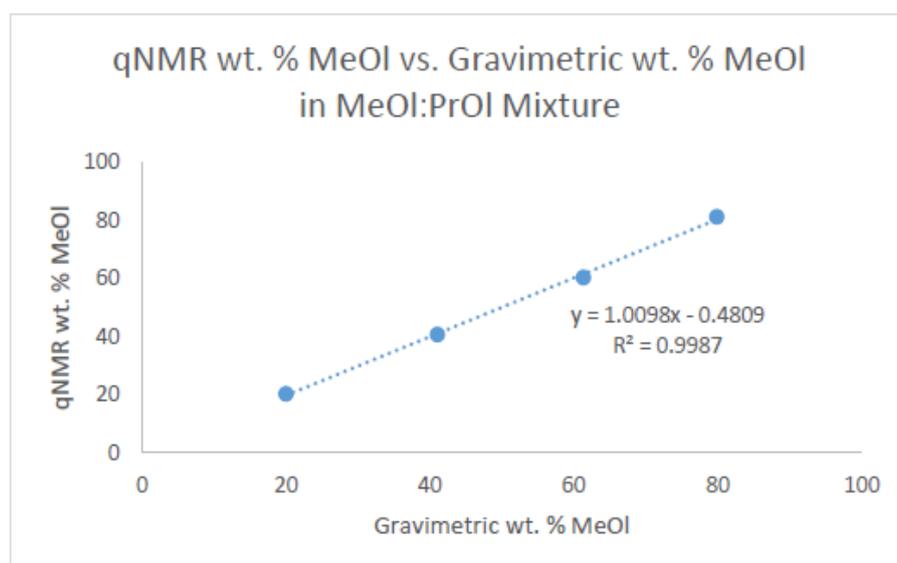**Figure 9.** qNMR wt. % MeOl vs. gravimetric wt. % MeOl in MeOl:EtOl mixture

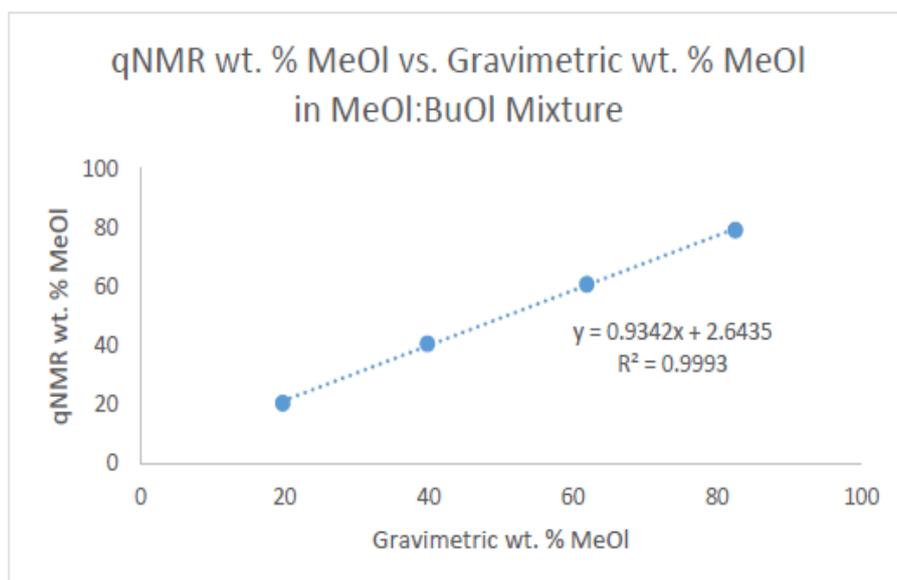**Figure 10.** qNMR wt. % MeOl vs. gravimetric wt. % MeOl in MeOl:PrOl mixture

Figure 11. qNMR wt. % MeOl vs. gravimetric wt. % MeOl in MeOl:BuOl mixture
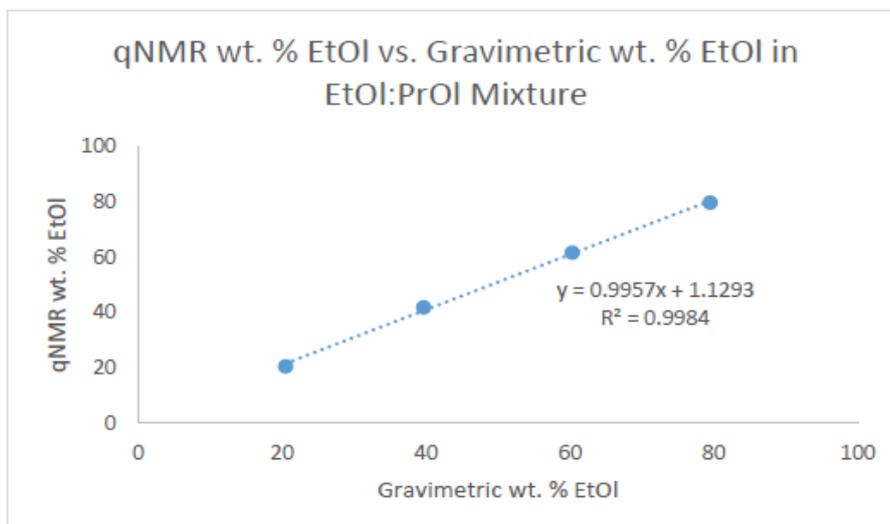
Figure 12. qNMR wt. % MeOl vs. gravimetric wt. % MeOl in MeOl:PrOl mixture

4. Conclusions

There is a strong, linear correlation between the gravimetric compositions and the weight percent compositions found using proton NMR. The calibration curves can be used to accurately determine the % composition of an analyte in binary mixtures involving oleate esters. The experiment corroborates the quantitative use of proton NMR to determine the composition of binary mixtures as performed in previous experiments [5]. The experiment serves as an excellent tool for the undergraduate chemistry laboratory as the methodology can be readily adopted for similar experiments with different reagents.

Acknowledgments

List of Abbreviations

qNMR - Quantitative NMR
NMR - Nuclear Magnetic Resonance Spectroscopy
BuOl- Butyl Oleate
EtOl- Ethyl Oleate
MeOl – Methyl Oleate
PrOl- Propyl Oleate

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