

Application of Quantitative Proton Nuclear Magnetic Resonance Spectroscopy for the Compositional Analysis of Short-Chain Fatty Acid Ethyl Ester Mixtures

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Abstract Nuclear magnetic resonance spectroscopy (NMR) is a widely used, powerful, and perhaps one of the most important instrumental techniques to qualitatively determine the molecular structure of an analyte. Using proton NMR in quantitative applications, also known as qNMR, is, however, uncommon, particularly in quantifying analytes within a mixture. To increase exposure to both qualitative and quantitative aspects of NMR in an undergraduate chemistry laboratory curriculum, we have developed a straightforward qNMR experiment suitable for adaptation into analytical and instrumental chemistry courses. The objective of this experiment is to determine the weight percent composition of a binary mixture containing short-chain fatty acid ethyl esters. We report on the methodologies used to determine the weight percent composition of ethyl acetate (EtAc), ethyl propionate (EtPr), and ethyl butyrate (EtBu) with mixtures ranging from 0% to 100%. The results demonstrate a strong, linear correlation of the weight percent composition of a selected component in a binary mixture found using proton qNMR with the theoretical compositions calculated gravimetrically. The experiment demonstrates the quantitative utility of proton NMR and serves as an educational tool for the undergraduate chemical laboratory.

Keywords: quantitative analysis, nuclear magnetic resonance, proton NMR, undergraduate laboratory experiment, hands-on learning, ethyl esters, ethyl ester mixtures

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1. Introduction

Although proton nuclear magnetic resonance (^1H NMR) has been used for both qualitative and quantitative analyses of organic mixtures [1-6], quantitative experiments for educational instruction are limited [7-12]. A simple solution is to develop more quantitative NMR (qNMR) experiments; hence, this straightforward experiment was designed for simple implementation of the qNMR methodology into undergraduate chemistry laboratory curriculums. Quantitative analyses using qNMR is possible due to each peak's integration value being directly proportional to the proton count. This experiment determines the weight percent (wt. %) composition of a two-component mixture of short-chain fatty acid ethyl esters. Chemical shift and integration values were obtained by analyzing the neat ethyl ester samples without an internal reference standard; afterward, peak assignments were qualitatively interpreted based on their multiplicity, spin-spin coupling, and integration values. A series of binary mixtures containing either ethyl acetate (EtAc), ethyl propionate (EtPr), or ethyl

butyrate (EtBu) were made, each ranging from 20% to 100%.

1.1. Learning Objectives

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

2. Materials and Methods

2.1. Student Procedure

The instructor will assign each student to prepare mixtures of approximately 20%, 40%, 60%, and 80% by

volume of a mixture of either EtAc-EtPr, EtAc-EtBu, or EtPr-EtBu. 20-mL sample vials will be weighed before the addition of a reagent. The vial will be weighed again before and after adding the second reagent. All recorded weights will be used to compute wt. % of each component. Students will run proton NMR on their samples, where they will construct a calibration curve of the calculated wt. % of the component of interest and the wt. % determined via NMR. Afterward, the students will receive an unknown sample prepared by the instructor, where they must determine the wt. % composition of a chosen component.

2.2. Experimental Materials

Ethyl acetate, ethyl propionate, and ethyl butyrate were purchased from Sigma-Aldrich as anhydrous liquids with greater than 99% purity. All reagents were used without purification. The NMR tubes were Wilmad Pyrex glass 5mm x 7" thin wall tubes.

2.2.1. Proton NMR

^1H 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 between 8 and 10 seconds. Any vendors of NMR hardware and software can be used to perform this experiment.

2.2.2. Experimental Procedure

Twelve, 5.0-mL binary mixtures and three pure samples of ethyl acetate (EtAc), ethyl propionate (EtPr), and ethyl butyrate (EtBu) were prepared as shown in Table 1. All 20-mL vials and NMR tubes were labeled based on the volumetric ratio in the mixture. Each reagent was added using a Gilson classic model P1000 pipette. After each addition of a reagent into a vial, their masses were recorded using an analytical balance with a 0.1-mg precision. These masses were used to calculate theoretical wt. % composition of the mixtures. 1 mL of each sample mixture was added to their respective NMR tubes for qNMR analysis. All mixtures were analyzed without an internal reference standard.

2.2.3. Hazards

Ethyl acetate (CAS# 141-78-6), ethyl propionate (CAS# 105-37-3), and ethyl butyrate (CAS# 105-54-3) are all flammable liquids; they can cause serious eye irritations and are harmful if swallowed or inhaled. Safety glasses are mandatory, and the use of hoods is advisable. Students are required to wear protective gloves during the experiment. Waste solutions containing flammable liquids must be collected for waste disposal according to EPA and local guidelines. NMR magnets may interfere with electronic and metallic implants; students with these should avoid proximity to the NMR instrument.

Table 1. Summary of Aliquoted Binary Mixtures and Pure Samples

Volume Ratio	
Ethyl acetate: Ethyl propionate	Gravimetric wt. % Ethyl acetate
0 ml:5 ml EtAc:EtPr	0.00
1 ml:4 ml EtAc:EtPr	19.64
2 ml:3 ml EtAc:EtPr	40.49
3 ml:2 ml EtAc:EtPr	60.90
4 ml:1 ml EtAc:EtPr	81.04
5 ml:0 ml EtAc:EtPr	100.00
Ethyl acetate: Ethyl butyrate	Gravimetric wt. % Ethyl acetate
0 ml:5 ml EtAc:EtBu	0.00
1 ml:4 ml EtAc:EtBu	20.00
2 ml:3 ml EtAc:EtBu	40.82
3 ml:2 ml EtAc:EtBu	61.82
4 ml:1 ml EtAc:EtBu	81.50
5 ml:0 ml EtAc:EtBu	100.00
Ethyl propionate: Ethyl Butyrate	Gravimetric wt. % Ethyl propionate
0 ml:5 ml EtPr:EtBu	0.00
1 ml:4 ml EtPr:EtBu	20.00
2 ml:3 ml EtPr:EtBu	40.65
3 ml:2 ml EtPr:EtBu	61.16
4 ml:1 ml EtPr:EtBu	80.67
5 ml:0 ml EtPr:EtBu	100.00

3. Results and Discussion

Figure 1 - Figure 3 show the proton NMR spectra of 100% ethyl acetate, ethyl propionate, and ethyl butyrate, respectively. Table 2 summarizes the chemical shifts, multiplicities, and normalized integration values for each peak in the neat samples.

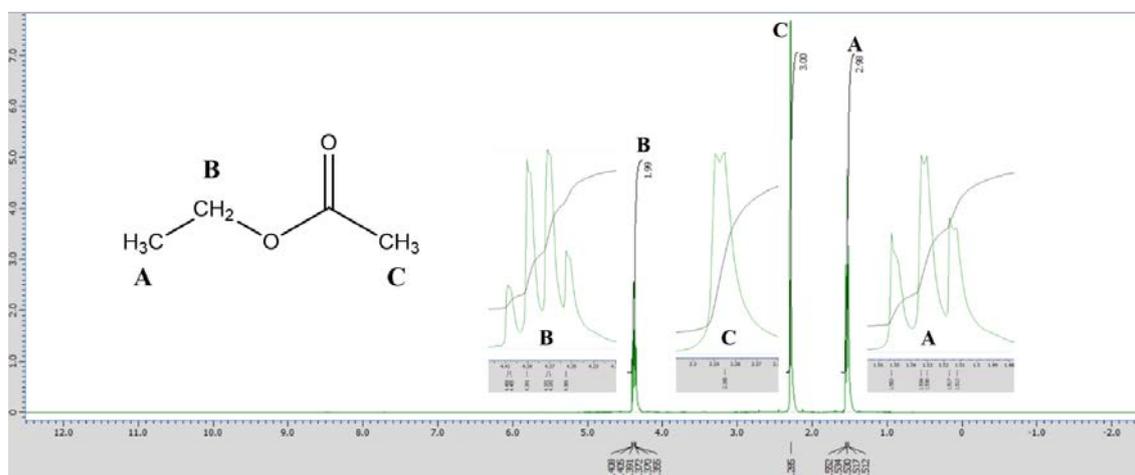


Figure 1. ^1H NMR spectrum of 100% ethyl acetate, neat

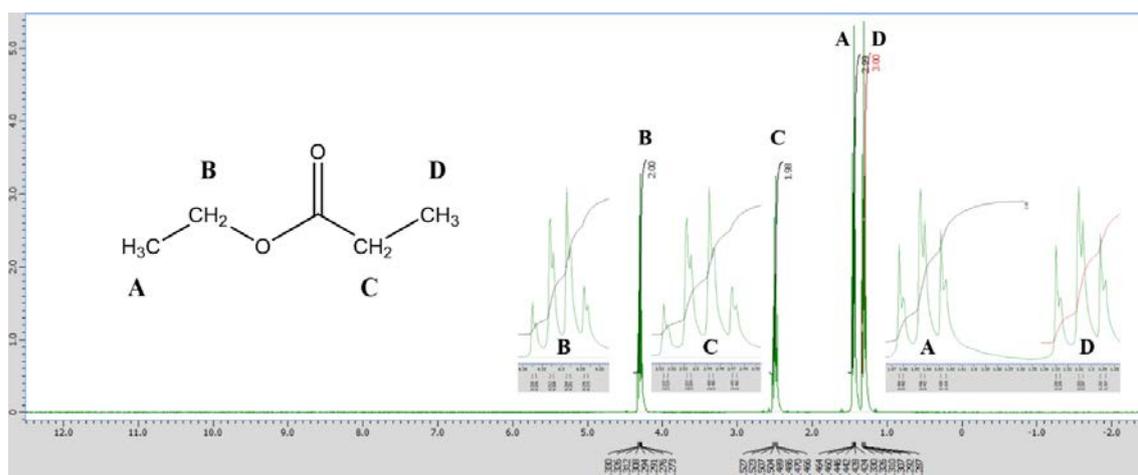


Figure 2. ^1H NMR spectrum of 100% ethyl propionate, neat

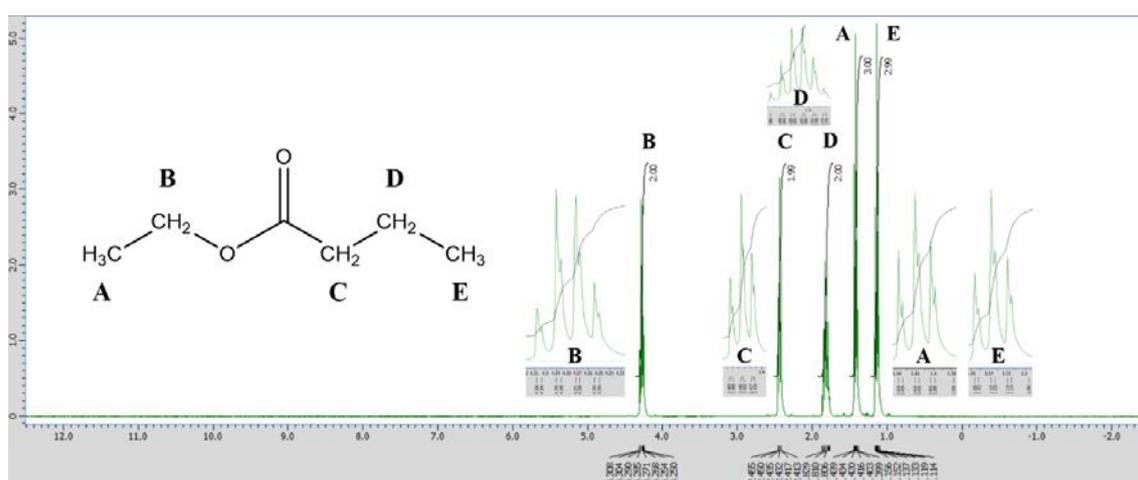


Figure 3. ^1H NMR spectrum of 100% ethyl butyrate, neat

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

Ethyl acetate (EtAc)				Ethyl propionate (EtPr)				Ethyl butyrate (EtBu)			
Proton	Shift (δ) ppm	Multiplicity	Integration	Proton	Shift (δ) ppm	Multiplicity	Integration	Proton	Shift (δ) ppm	Multiplicity	Integration
A	1.534	Triplet	2.98	A	1.446	Triplet	2.99	A	1.420	Triplet	3.00
B	4.372	Quartet	1.99	B	4.294	Quartet	2.00	B	4.285	Quartet	2.00
C	2.285	Singlet	3.00	C	2.489	Quartet	1.98	C	2.435	Triplet	1.99
				D	1.307	Triplet	3.00	D	1.810	Sextet	2.00
								E	1.137	Triplet	2.99

All ethyl esters possess methyl protons (A) on a carbon atom attached to another carbon atom containing methylene protons (B). The methylene protons are more downfield than the methyls due to their proximity to the ester oxygens, where they are less shielded from the applied magnetic field. The methyl and methylene protons have a normalized integration value of three and two, respectively, and are a triplet and quartet, respectively, as per the $n+1$ multiplicity rule of one-dimensional proton NMR. Increasing the parent chain decreases the chemical

shift of its methyl protons (C on EtAc, D on EtPr, and E on EtBu) due to their increasing distance from the electron-withdrawing ester oxygens.

Figure 4 - Figure 6 are the proton NMR spectra of 4:1 mixtures of EtAc: EtPr, EtAc:EtBu, and EtPr:EtBu, respectively. The percent composition was determined using the normalized integration areas at peaks specific to each component. Do note that each peak has shifted either downfield or upfield depending on the binary mixture. For the EtAc:EtPr and EtAc:EtBu mixtures, the acetate's

singlet methyl peak (C) was at 1.605 ppm and 2.531 ppm, respectively. For the EtAc:EtPr and EtPr:EtBu mixtures, the propionate was identified at 1.900 ppm, the quartet methylene peak (C), and 1.640 ppm, the triplet methyl peak (D), respectively. For the EtAc:EtBu and EtPr:EtBu mixtures, the butyrate's sextet methylene peak (D) was at approximately 2.165 ppm. Percent component composition

was calculated using the following equation, where X and Y are the components in the binary mixture in question, and N is the integration value:

$$\% \text{ Composition} = \frac{N_X}{N_X + N_Y} * 100.$$

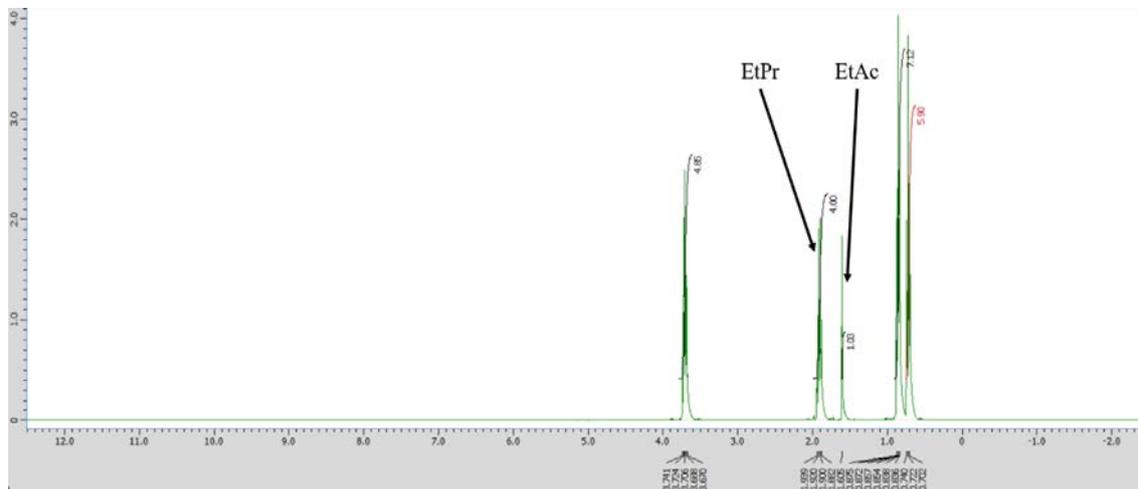


Figure 4. ^1H NMR spectrum of a 1:4 mixture of EtAc:EtPr, neat

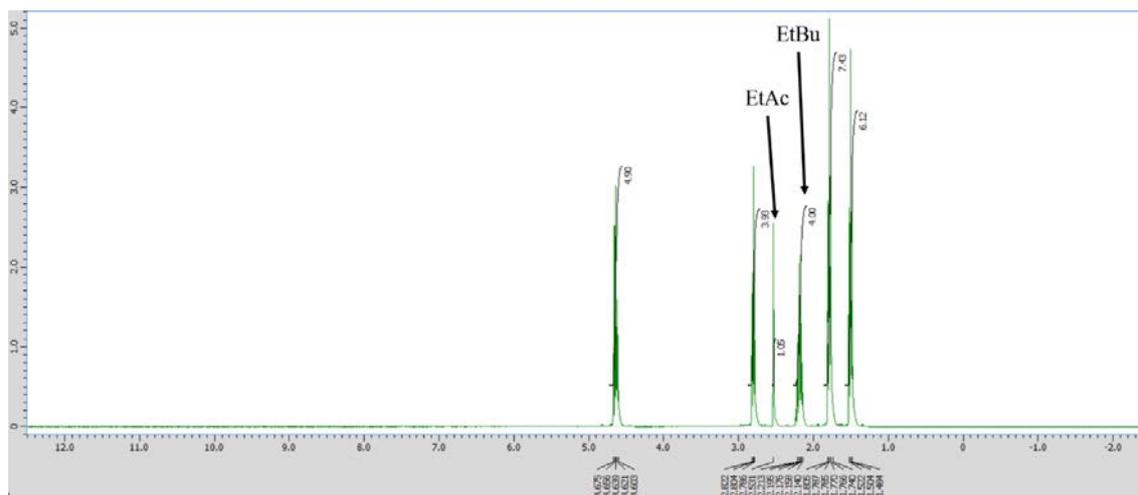


Figure 5. ^1H NMR spectrum of a 1:4 mixture of EtAc:EtBu, neat

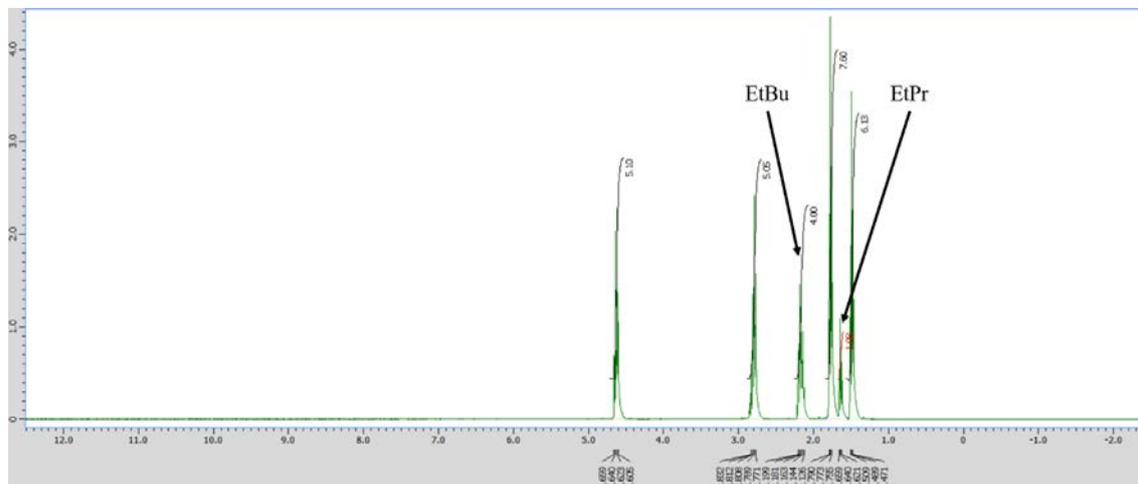


Figure 6. ^1H NMR spectrum of a 1:4 mixture of EtPr:EtBu, neat

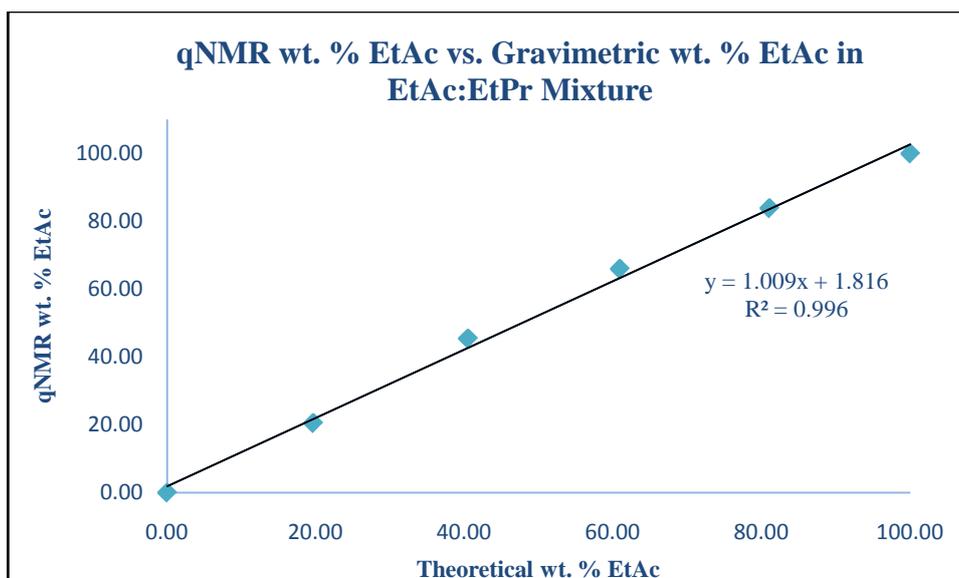


Figure 7. qNMR wt. % EtAc vs. Gravimetric wt. % EtAc in EtAc:EtPr mixture

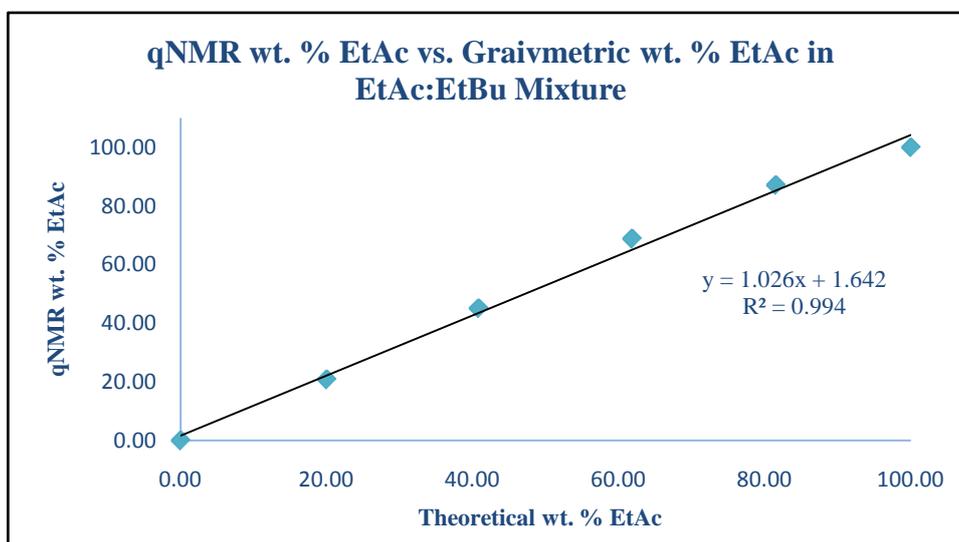


Figure 8. qNMR wt. % EtAc vs. Gravimetric wt. % EtAc in EtAc:EtBu mixture

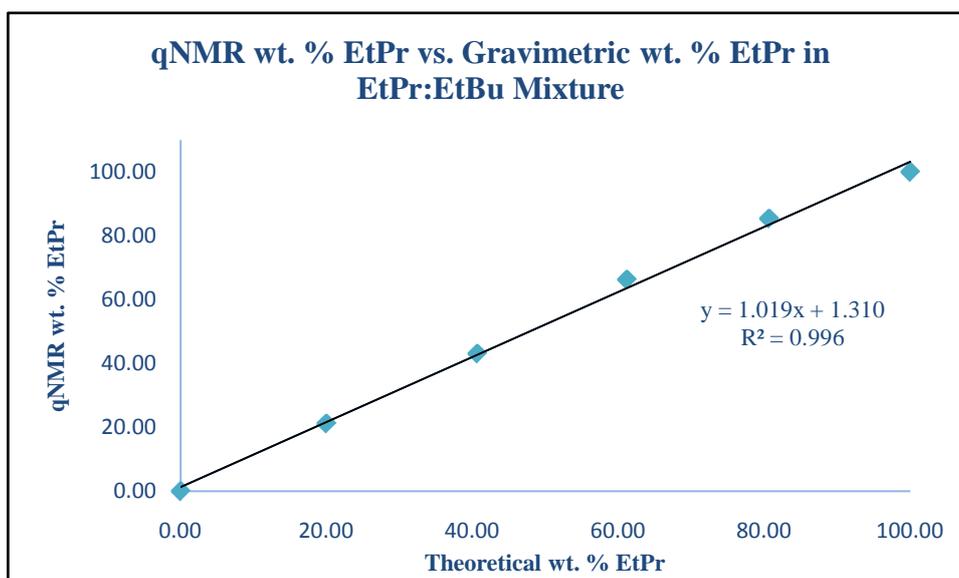


Figure 9. qNMR wt. % EtPr vs. Gravimetric wt. % EtPr in EtPr:EtBu mixture

Figure 7 - Figure 9 shows the calibration curves of the qNMR calculated wt. % versus theoretical wt. % of a chosen component in a binary mixture. All calibration curves displayed excellent agreement between the wt. % calculated from qNMR and the gravimetrically (theoretical) wt. % values. The correlation coefficient (R^2) in all cases was greater than 0.99.

4. Conclusions

The results reaffirm the quantitative uses of proton NMR. The calibration curves can be used to accurately determine the wt. % composition of a component in a binary mixture using different ethyl esters. There is a strong, linear correlation between the wt. % calculated from qNMR and the theoretical values determined gravimetrically. This experiment can be adapted for different reagents, has substantiated previous experiments [5,6], demonstrated the quantitative usage of proton NMR, and serves as an excellent tool for the undergraduate chemistry laboratory.

Acknowledgments

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