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Research Article

Determination of Alcohol Content in Alcoholic Beverages Using 45 MHz Benchtop NMR Spectrometer

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Alcohol or ethanol is considered the most widely used recreational drug worldwide, and its production, consumption, and sale are strictly regulated by laws. Alcohol content of alcoholic beverages (wine, beers, and spirits) is about 3–50% v/v. Analytical methods to determine the alcohol content must be reliable, precise, and accurate. In this study, the amount of ethanol in several alcoholic beverages was determined using a 45 MHz low-field benchtop NMR (nuclear magnetic resonance) spectrometer. Internal standard and standard addition analytical methods were utilized to quantify ethanol. For both methods, acetic acid or acetonitrile was used as internal standard to quantify alcohol content by using the peak area corresponding to the methyl peaks of ethanol, acetic acid, or acetonitrile. Results showed that internal standard method gave values of percent alcohol that are in close agreement with the indicated label as confirmed by running the samples in a 400 MHz high-field NMR spectrometer using acetic acid as internal standard. This study demonstrates the utility of a benchtop NMR spectrometer that can provide an alternative technique to analyze percent alcohol in alcoholic products.

1. Introduction

Ethanol, commonly called ethyl alcohol, is the principal alcoholic component in wine produced by fermentation of sugars by yeast. Wine-making is a rigidly controlled process and the final commercial product is subjected to stringent regulations regarding the alcohol content, acidity, sulfur dioxide quantity, sugar content, quality of the grapes, and amount of preservatives.

Several methods to quantify the alcoholic content in wine to ensure quality of highest standard are based on chemical (dichromate oxidation), physical (pycnometry, densitometry, refractometry, and hydrometry), chromatographic, and spectrometric analyses. The International Organization of Vine and Wine (OIV) has set up a compendium of official test methods to analyze wines and musts that vitivinicultural sectors need to follow to comply with international standards worldwide. Spectroscopic techniques such as infrared (IR) and NMR and mass spectrometry offer alternative methods other than traditional chemical testing and chromatographic methods such as high-performance liquid chromatography (HPLC) and gas chromatography (GC) [1–16]. Recently, Raman spectroscopy has been explored to quantify ethanol concentration in alcoholic beverages [17]. To maintain international oenological standards and to preserve high quality products, methods to determine alcohol content must be reliable to measure this very important parameter [9]. An additional advantage would be if the method implemented is officially recognized, but inexpensive and less prone to human errors.

Nuclear magnetic resonance (NMR) spectroscopy, a nondestructive noninvasive technique that provides a direct relationship between the measured spectra and chemical structure, is the primary standard and routine method for molecular structure characterization. The capability of NMR for quantitation (qNMR) to simultaneously detect a large number of compounds in a complex mixture without separation proves to be advantageous as a method to use for analysis of natural products [18, 19] including food, fruit juices, or alcoholic beverages [20–23]. In particular, ^{1}H NMR methodologies have been applied to determine chemical constituents (phenolics, sugars, and organic acids) and to measure the amount of alcohol for authenticating the quality of wine [24–27]. NMR analysis has been applied even in full

Table 1: Preparation of samples for standard addition method. Internal standards: acetic acid or acetonitrile.

% EtOH	EtOH (mL)	Sample (mL)	Internal standard (mL)	H_2O (mL)	Total volume (mL)
0	0	0.5	0.1	1.4	2.0
10	0.2	0.5	0.1	1.2	2.0
20	0.4	0.5	0.1	1.0	2.0
30	0.6	0.5	0.1	0.8	2.0
40	0.8	0.5	0.1	0.6	2.0
50	1	0.5	0.1	0.4	2.0

intact wine bottles to preserve the integrity of this valuable commodity [28, 29].

The objective of this study was to investigate the use of a low-field 45 MHz NMR spectrometer to determine the alcohol content of several alcoholic products. Benchtop NMR spectrometer has been used in teaching organic chemistry laboratory techniques [30, 31] and is gaining popularity due to ease and cost-effectiveness in its utility including in vivo imaging [32, 33] applications compared to high-field NMR instrumentation.

2. Materials and Method

2.1. Materials. Commercially available alcoholic beverages were obtained from local commercial vendors and analyzed for alcohol content. Fourteen different alcoholic samples used were Banana liqueur (KY, USA), Black Magic rum (KY, USA), Fleischmann's vodka (LA, CA, USA), Smirnoff vodka (Norwalk, CT, USA), Stolichnaya vodka (Latvia), Three Olives vodka (Rochester, NY), Dr. McGillicuddy's root beer liquor (WI, USA), Merlot red wine (Modesto, CA), Cruzan Key Lime rum (IL, USA), Pinot Noir (Modesto, CA, USA), Canadian ice wine (Niagara on the Lake, ON, Canada), Tour de Soleil Rosé (Côtes de Gascogne, France), Beringer White Zinfandel (Napa, CA, USA), and Moscato white wine (Modesto, CA, USA). Absolute ethanol (200 proof) used as calibration standard was obtained from Mallinckrodt Chemicals (St. Louis, MO, USA). Acetic acid and acetonitrile were obtained from Sigma Aldrich (St. Louis, MO, USA). Deuterated water (D_2O, 99.9% D) was purchased from Cambridge Isotope Laboratories (Tewksbury, MA, USA).

2.2. Sample Preparation. NMR samples for internal standard method were prepared by measuring 900 μL of alcoholic samples and 100 μL of glacial acetic acid into a small capped 3 mL vial for a total volume of 1 mL. Five different mixtures were prepared. The solutions were mixed well and immediately used for analysis in a 45 MHz NMR spectrometer. For comparison, NMR samples were prepared for 400 MHz NMR spectrometer by placing 100 μL of the acetic acid-alcohol sample mixture into a 5 mm NMR tube combined with 600 μL of D_2O. Additionally, acetonitrile was also used as an internal standard instead of acetic acid. NMR samples for the calibration curve were prepared by mixing 10–90% of absolute ethanol and a fixed quantity of acetic acid (10%) as internal standard for a total volume of 1 mL in milliQ water. Samples for standard addition method were prepared with absolute ethanol concentration ranging from 0 to 50% volume/volume being added into the mixture. Preparation of six samples for the standard addition method is shown in Table 1.

2.3. ^1H NMR Spectroscopy. About 200–300 μL of the alcohol-acetic acid mixture was injected into the inlet port of the 45 MHz NMR instrument. Sample loop holds about 20 μL of liquid. A sample of approximately ten times the sample loop volume is injected to ensure that sample to be analyzed is inside the loop, and droplets of liquid drained out of the outlet port. ^{1}H NMR spectra were obtained on a picoSpin 45 MHz NMR spectrometer equipped with a fixed magnet. Shimming on water was utilized prior to spectral acquisition. One-dimensional one-pulse ^{1}H NMR spectra generated as exponentially decaying sine wave or free induction decay (FID) were acquired using the one-pulse sequence available in the picoSpin pulse script library. FIDs were collected at 298 K by employing a relaxation delay of 8 s, a pulse width of 90° (43 μs), and 16 scans. ^{1}H NMR spectra were also obtained on a 400 MHz Bruker Avance NMR spectrometer equipped with an autosampler. One-dimensional one-pulse ^{1}H NMR spectra were acquired using the one-pulse sequence in the Bruker pulse sequence library. FIDs were collected at 298 K by employing a relaxation delay of 1 s, a pulse width of 90° (14.9 μs), and 16 scans. Both FIDs were apodized with an exponential multiplication prior to Fourier transformation. Chemical shifts (δ) are given in ppm relative to acetic acid (or acetonitrile) set at 2.04 ppm. Processing was performed using ACD/Labs NMR Processor Version 12.01 and TopSpin 2.1.6 processing data software for the 45 and 400 MHz NMR instruments, respectively.

3. Results and Discussion

An NMR spectrum is a plot of the radio frequency applied against absorption, and an NMR signal is referred to as a resonance expressed as chemical shift in ppm, which is independent of the spectrometer frequency [34]. Important features of an NMR spectrum that provide information pertaining to the structure of the molecule include chemical shift, multiplicity, peak integration, and coupling constant. Chemical shift depends on the environment of the nucleus (such as hydrogen or H) being observed. Hydrogens in the vicinity of electronegative atoms (O, N, and halogens) experiencing reduced electron density will be deshielded and will resonate downfield or at higher ppm. In addition, protons in aromatic compounds (benzene) and alkenes (double bonds)

are downfield shifted as a consequence of induced magnetic field due to circulating electrons or anisotropic effects. Multiplicity or spin-spin splitting refers to the interaction of the nucleus with adjacent nuclei, or a hydrogen with neighboring hydrogens, and can be evaluated empirically by the $(n + 1)$ rule in which the resonance peak is split into $(n + 1)$ components, where n refers to the number of equivalent hydrogens (n) on the adjacent atom(s). The observed multiplicity which is dependent on the number of adjacent hydrogens will give rise to splitting patterns designated as singlet (no adjacent hydrogen), doublet (1 adjacent H), triplet (2 adjacent H's), quartet (3 adjacent H's), and so forth. Integration refers to the area under the peak(s) or multiplets which is a measure of the number of hydrogens in that particular resonance or chemical shift. The coupling constant (or J value) is a measure of the interaction or coupling of adjacent nuclei so that hydrogens separated by three bonds will be coupled to each other and will have the same coupling constant or J value. For ethanol (CH_3CH_2OH), there are three unique hydrogens which will appear as three separate NMR signals: CH_3 (methyl) at 1.18 ppm, CH_2 (methylene) at 3.64 ppm, and the OH (hydroxyl) at 4.80, which overlaps with water peak. Splitting pattern for ethanol will be triplet for CH_3, quartet for CH_2, and singlet for OH, with peak integration ratio of 3 : 2 : 1 corresponding to CH_3, CH_2, and OH, respectively. Hydroxyl proton will appear as a broad singlet in both 45 and 400 MHz spectrometers due to presence of water and residual hydrogens in deuterated solvents. For absolute or ultra-pure anhydrous ethanol, splitting of the OH peak is observed in the 45 MHz NMR spectrometer used for this study [35] since the liquid sample is run neat and not dissolved in deuterated solvent which can cause broadening of the hydroxyl group in the sample due to proton exchange. J value for the coupling of CH_3 and CH_2 is ~6 Hz.

Figure 1 shows the NMR spectra of alcoholic samples acquired with 45 MHz benchtop and 400 MHz NMR spectrometers. The triplet assigned to the methyl (CH_3) group at 1.18 ppm was the peak used for quantification from the formula shown in (1), wherein the amount of ethanol, which is the main component, is calculated directly from the NMR spectrum. This is based on standard analytical procedures for determining analyte concentrations [13–33, 36]. The singlet for the methyl peak at 2.04 ppm corresponding to acetic acid or acetonitrile was used for calibration and as reference set to 3 for peak integration to determine the alcohol content of the samples. The methylene (CH_2) peak tends to have an overlapping peak with the alcoholic product probably due to sugar molecules and other dissolved solutes and hence is not utilized for analysis. Determination of alcohol content in alcoholic beverages is shown as follows:

$$\%\ \text{Alcohol}_{\text{sample}} = \frac{(\text{Peak Area})_{\text{sample}}}{(\text{Peak Area})_{\text{standard}}} \times \frac{N_{\text{standard}}}{N_{\text{sample}}} \times \frac{V_{\text{standard}}}{V_{\text{sample}}} \times 100, \quad (1)$$

where N is the number of hydrogens; V is the volume.

Determination of the percent alcohol in the sample was a straightforward calculation based on the average of 5 different sample preparations from the same alcoholic product. Acetic acid is utilized as the internal standard for quantification by peak integration [16, 28] since it is readily available, cheap, stable, nonvolatile, and soluble in aqueous ethanolic solutions. The reference signal for the methyl group at 2.04 ppm is a singlet and widely separated from the ethanol peaks. Acetonitrile was also used as an internal standard utilizing the methyl peak also at 2.04 ppm. In addition, one sample for each alcoholic product was run in a 400 MHz NMR spectrometer (see Figure 1) and the results obtained for the benchtop NMR are comparable.

Obviously, higher resolution is observed for 400 MHz than 45 MHz as shown in Figure 1. Sample preparations for low- and high-field strength instrumentations are different. Less sample preparation is involved for the benchtop instrument, while for the high-field NMR instrument the alcohol-acetic acid mixture is dissolved in deuterated water (D_2O) for locking purposes. Alcoholic products that are thick and milky such as Tequila Rose and Chiata Rum cannot be determined for the 45 benchtop NMR due to difficulty in injecting the samples.

Table 2 summarizes the results obtained for the determination of ethanol content of several commercially available alcoholic beverages using both 45 and 400 MHz instruments. The methodologies used to determine the alcohol content involve the use of an internal standard and standard addition method. The use of an internal standard in which acetic acid or acetonitrile was added directly to the sample increases reliability and accuracy of the calculations for the determinations used for both instruments. Standard addition method as shown in Figure 2 includes adding known quantities of ethanol to correct for matrix effects. Both methods (internal standard or standard addition method) require the use of an internal standard to calibrate for peak integration. The internal standard addition method involves less sample preparation and would be the preferred method of choice if substantial amounts of samples were to be determined. Linearity curve for standard concentrations ranging from 10 to 90% ethanol in water with acetic acid as reference is shown in Figure 3 for the 45 MHz ^{1}H NMR spectrometer. External standard method using a calibration curve such as shown in Figure 3 with the use of acetic acid for peak integration produced erroneous calculations of the alcohol content and was not utilized for this study. Final determination of the alcohol content was primarily based on the peak integration of methyl (CH_3) peaks referenced to acetic acid or acetonitrile using (1) since this NMR spectral region was observed to be significantly less affected by overlap caused by other components in the sample compared to the methylene (CH_2) region, where other peaks were observed (see Figure 1). A reference or standard added internally will be needed *always* to quantify the amount of alcohol. Acetic acid and acetonitrile were chosen as the internal standards due to cost, molecular structure similarity to ethanol, and ease of preparation since these are liquid standards compared to 3-(trimethylsilyl)-1-propane sulfonic acid sodium salt (DSS) or $(CH_3)_3Si$

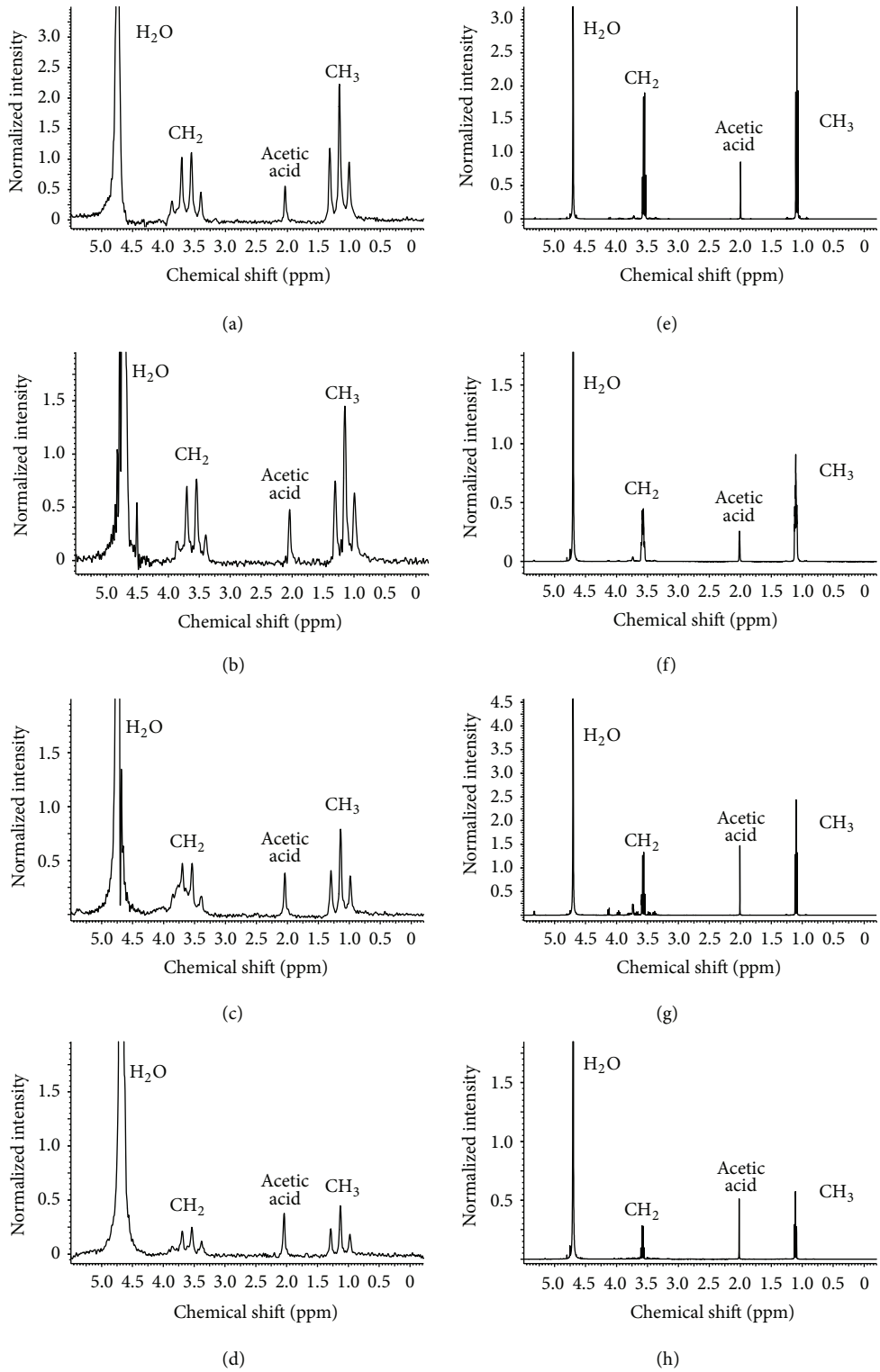

Figure 1: ^{1}H NMR spectra of alcoholic products acquired with a 45 (a–d) and 400 (e–h) MHz NMR spectrometer. (a, e) Black Magic rum; (b, f) Smirnoff vodka; (c, g) Dr. McGillicuddy's root beer liqueur; (d, h) Tour de Soleil Rosé. Triplet at 1.16 and quartet at 3.56 ppm correspond to hydrogens in methyl (CH_3) and methylene (CH_2) groups, respectively, in ethanol, while the singlet at 2.04 ppm corresponds to the methyl peak of acetic acid. Acetic acid CH_3 peak was used for peak integration.

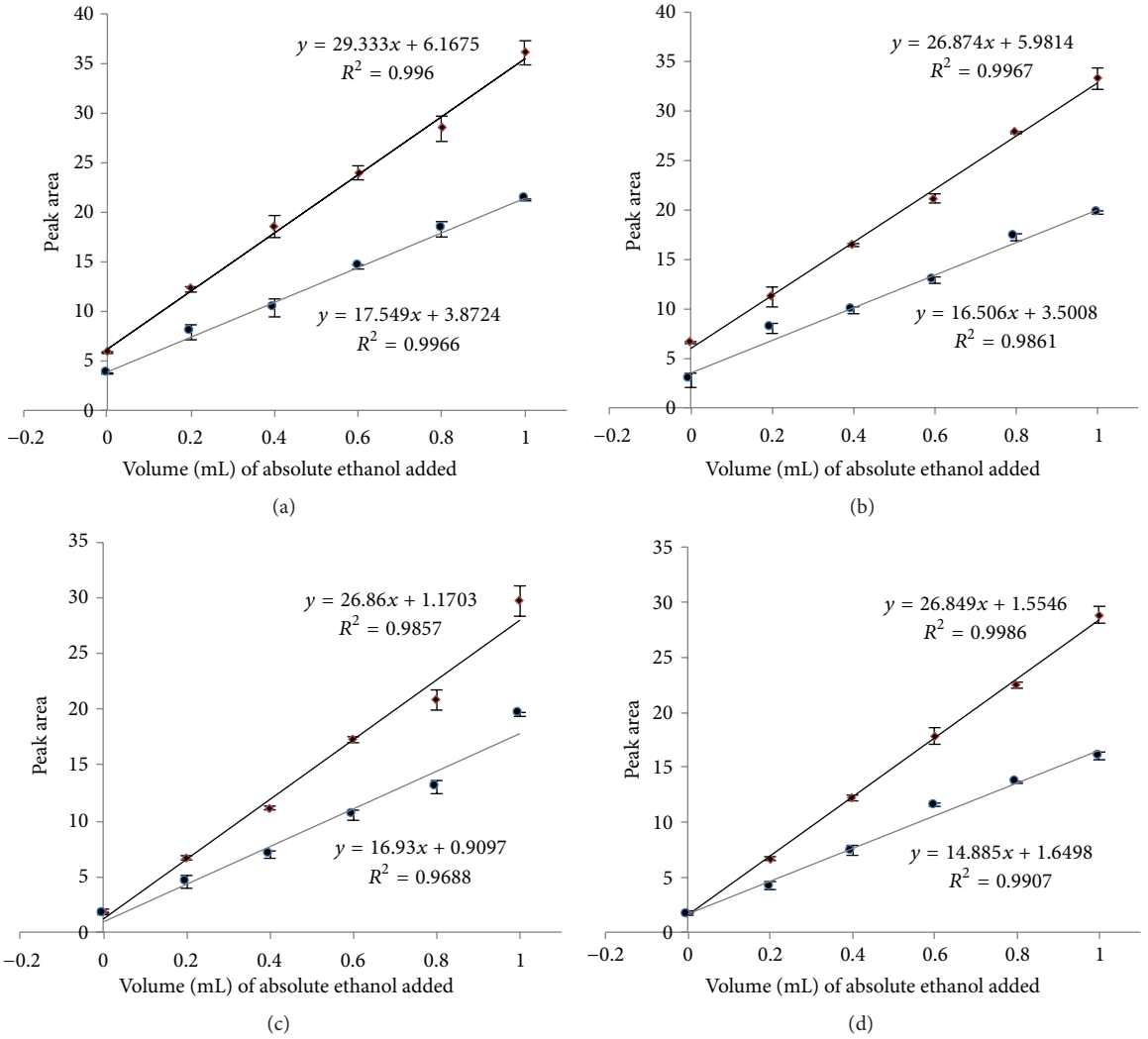

FIGURE 2: Standard addition method using a 45 MHz ^{1}H NMR spectrometer. Black (top) and gray (bottom) lines correspond to methyl (CH_3) and methylene (CH_2) peak areas, respectively. (a, b) Smirnoff vodka using HOAc and ACN standards, respectively. (c, d) Pinot Noir white wine using HOAc and ACN standards, respectively.

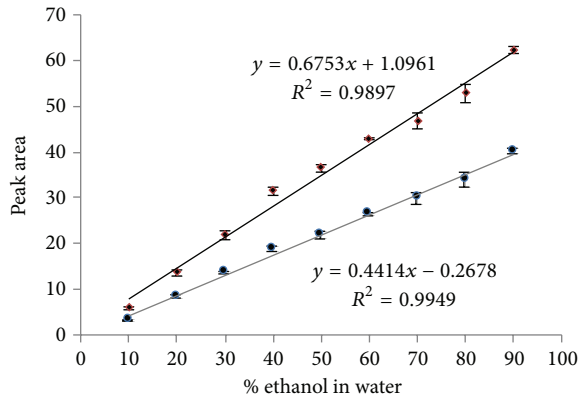

FIGURE 3: Linearity curve for 45 MHz ^{1}H NMR spectrometer. Black (top) and gray (bottom) lines correspond to methyl (CH_3) and methylene (CH_2) peak areas, respectively.

$(CD_2)_2CO_2Na$, sodium 3-trimethylsilyl-propionate-2,2,3,3-d_4 (TMSP), both of which are solids. No significant amount of acetic acid was detected in the alcoholic beverages that were used in this study as shown in Figures 4(a) and 4(c) in which alcoholic beverages were run as neat samples in the 45 MHz spectrometer and confirmed when the same samples (dissolved in D_2O) were run in the 400 MHz instrument (see Figures 4(b) and 4(d)). If acetic acid were present in significant amount in the alcoholic beverage, then the amount of acetic acid originally present would be calculated by running two samples: one with acetic acid standard of known amount and the other without acetic acid standard. The peak area for the sample with added acetic acid standard would be greater than without it. Consequently, the amount of ethanol present can be corrected accordingly.

To obtain accurate peak integration for quantitative NMR experiment, a relaxation delay of approximately $5 \times T_1$ value

Table 2: Percent alcohol content of alcoholic beverages using 45 and 400 MHz NMR spectrometers. Standard errors were calculated for 5 determinations performed for the 45 MHz instrument. HOAc = glacial acetic acid; ACN = acetonitrile; IS = internal standard; SA = standard addition method.

Alcoholic beverages	% alcohol on label	% alcohol 400 MHz HOAc IS	% alcohol 45 MHz HOAc IS	% alcohol 45 MHz ACN IS	% alcohol 45 MHz HOAc SA	% alcohol 45 MHz ACN SA
Banana liqueur	49.5	46.80	49.03 ± 0.24	53.69 ± 0.19	54.56 ± 0.46	56.46 ± 0.58
Black Magic rum	47	46.07	48.81 ± 0.19	49.71 ± 0.34	41.40 ± 0.48	47.41 ± 0.35
Fleischmann's vodka	40	37.07	39.15 ± 1.13	40.04 ± 2.07	44.56 ± 0.28	44.62 ± 1.42
Smirnoff vodka	40	37.83	43.39 ± 0.15	42.21 ± 0.25	42.05 ± 0.78	44.50 ± 0.49
Stolichnaya vodka	37.5	37.33	37.84 ± 0.90	38.99 ± 0.04	44.81 ± 0.86	44.61 ± 0.51
Three Olives vodka	30	28.05	33.54 ± 0.26	32.39 ± 0.62	30.04 ± 0.59	34.05 ± 0.42
Dr. McGillicuddy's root beer liqueur	21	22.15	20.51 ± 1.10	21.47 ± 0.60	22.26 ± 0.35	22.25 ± 0.56
Cruzan Key Lime rum	21	20.50	26.33 ± 0.21	20.34 ± 0.45	22.94 ± 0.25	23.96 ± 0.31
Merlot red wine	13	11.30	13.36 ± 0.09	13.41 ± 0.15	15.89 ± 0.32	15.32 ± 0.31
Pinot Noir	11.5	10.13	11.47 ± 0.07	11.25 ± 0.11	10.22 ± 0.37	11.58 ± 0.39
Canadian ice wine	11	10.23	11.87 ± 0.10	11.38 ± 0.04	9.10 ± 0.53	12.46 ± 0.30
Tour de Soleil Rosé	11	11.50	9.94 ± 0.28	11.51 ± 0.43	13.51 ± 0.20	14.49 ± 0.76
Beringer White Zinfandel	10	10.56	9.26 ± 0.32	13.10 ± 0.24	10.92 ± 0.21	13.13 ± 0.22
Moscato white wine	9	7.69	9.09 ± 0.28	9.69 ± 0.06	10.62 ± 1.50	10.40 ± 0.90

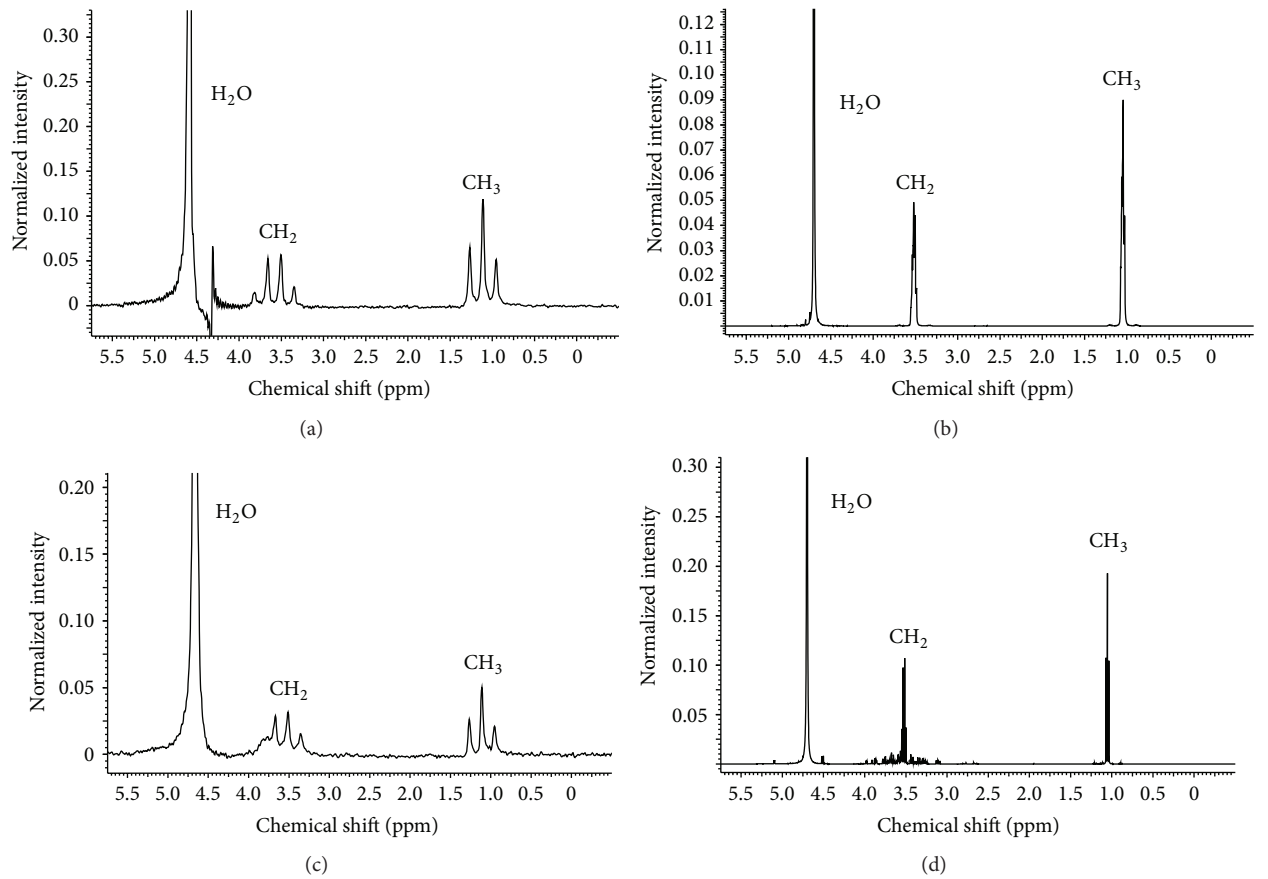

Figure 4: ^{1}H NMR spectra of alcoholic products acquired with a 45 MHz instrument (left) as neat samples and with a 400 (right) MHz NMR spectrometer of the sample dissolved in D_2O. (a, b) Smirnoff vodka; (c, d) Dr. McGillicuddy's root beer liqueur. OH peak in ethanol overlaps with the water peak.

is typically utilized [19]. T_1 refers to a time constant or relaxation time for the nucleus to fully relax between pulses and return to equilibrium. Each nucleus in a molecule will have different T_1 values due to different magnetic environments. A relaxation delay of 8 seconds was used for the 45 MHz spectrometer. However, a 1-second relaxation delay was used to reduce run time for 400 MHz FT instrument since an insignificant difference of about 0.1–0.3% was observed when the value was changed from 1-second to 8–10-second delay.

Based on the results of this study, internal standard method performed in the low-field 45 MHz benchtop NMR spectrometer produced percent alcohol levels that are more comparable to the label than the standard addition method and therefore was the method utilized to compare with the high-field 400 MHz NMR spectrometer. Results obtained for 45 MHz and 400 MHz spectrometer differ slightly, most likely due to variation in the sample preparation. Samples for the 400 MHz were 100 μL aliquots derived from the samples prepared for the 45 MHz and the aliquot diluted with deuterated water. Discrepancy in exact volume measurements could have introduced this difference.

The most important advantage for using the 45 MHz benchtop NMR spectrometer over the high-field instrument is the cost. Low-field fixed magnet NMR compared to superconducting high-field spectrometer requires less maintenance because cryogens are not needed avoiding additional task for personnel to manage the instrument. NMR tubes and deuterated solvents required for field/frequency stabilization for high-field instrumentation [37] are not necessary reducing additional expenses incurred to run the samples.

4. Conclusion

Benchtop NMR technology provides a cost-effective and low maintenance equipment compared to the high-field NMR instrumentation and can be a promising technique in the future for the wine industry to analyze the alcoholic content to authenticate quality of wine or other alcoholic beverages. Compared to other techniques whereby correction methods need to be applied during the analysis due to presence of sugars and other dissolved components in the alcoholic products that may lead to unacceptable errors, NMR technique is a straightforward method for quantification purposes.

Competing Interests

The author declares that they have no competing interests.

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