ANALYTICAL

UNDERGRADUATE EXPERIMENT

Measuring Alcohol Content in Commercial Liquors via Benchtop qNMR

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INTRODUCTION

NMR spectroscopy is typically introduced to undergraduates in the organic chemistry with a focus on structure elucidation. Quantitative NMR (qNMR)[1,2] remains largely excluded despite being increasingly important in the pharmaceuticals and chemical industries to assess sample purity.[3]

Quantitative analytical methods introduced to undergraduates, i.e. GC and HPLC analysis, typically use the standard addition method where the peak intensity is correlated to a calibration curve generated from samples of known concentration.[4,5] This method has also been applied in qNMR spectroscopy,[6] and while valid, methods that employ an internal calibrant are more precise and result in lower levels of uncertainty.[7,8]

In this app note the alcohol content in commercial liquors will be measured with the NMReady-60 using two methods. First, a calibration curve was prepared, while in the second, an internal calibrant will be used. The results from the two methods will be compared and the advantages of using an internal calibrant will be highlighted.

PROCEDURE

Preparation of calibration curve

Ethanol solutions in D_2O were prepared (5, 10, 15, 20, 25, 30% v/v). Individually, 0.6 mL of each solution was transferred to an NMR tube & inserted in the NMReady-60. After equilibrating for 5 min the ^{1}H NMR spectrum was recorded in triplicate:

spectral width: 20 ppm	interscan delay: 32 sec
spectral center: 5 ppm	number of points: 4096
number of scans: 8	dummy scans: 0
receiver gain: 12dB	pulse angle: 90°

The ethanol triplet (δ = 0.84 ppm) in each spectrum was integrated and the absolute integral area recorded. The calibration curve was generated by plotting the known EtOH concentration vs. the absolute integral area (as an average of the triplicates recorded for each concentration).

Preparation of unknown samples for quantification via calibration curve

In an NMR tube, 0.3 mL of the commercial liquor was added to 0.3 mL of D_2O via micropipette. The sample was mixed thoroughly and then inserted into the NMR. After allowing the sample to equilibrate for 5 min, the ^{1}H NMR spectrum was recorded in triplicate with calibration curve parameters.

The alcohol content for each sample was quantified with the absolute integral of the ethanol triplet. The alcohol content was calculated by using this value in the calibration curve equation.

Preparation of unknown samples for quantification via internal calibrant

In an NMR tube, 0.3 mL of the commercial liquor was added by micropipette to 0.3 mL of the maleic acid solution (prepared by dissolving 1.6354 g (≥ 99% purity) in D_2O in a 5 mL volumetric flask). The sample was mixed thoroughly and placed into NMR spectrometer. After allowing the sample to equilibrate for 5 min, the ^{1}H NMR spectrum was recorded with the following parameters in triplicates:

spectral width: 20 ppm	interscan delay: 28 sec
spectral center: 5 ppm	number of points: 4096
number of scans: 8	dummy scans: 0
receiver gain: auto	pulse angle: 90°

The alcohol content was quantified by integrating the singlet from the maleic acid (δ = 6.09 ppm) and the triplet from the ethanol in the ^{1}H NMR spectrum and using the following equation:

$$m_{EtOH} = \frac{n_{MA} \times I_{EtOH} \times m_{MA} \times MW_{EtOH}}{n_{EtOH} \times I_{MA} \times MW_{MA}} \quad (1)$$

where m_{EtOH} = mass of ethanol
n_{MA} = number of protons in the maleic acid singlet (2)
I_{EtOH} = integral area of the ethanol triplet
m_{MA} = mass of maleic acid (0.0981 g)
MW_{EtOH} = molecular weight of ethanol (46.07 g/mol)
n_{EtOH} = number of protons in the ethanol triplet (3)
I_{MA} = integral area of the maleic acid singlet;
MW_{MA} = molecular weight of malec acid (116.07 g/mol)

By substituting in the known values, Eqn 1 can be simplified to the following:

$$m_{EtOH} = \frac{I_{EtOH} \times 9.041}{I_{MA} \times 348.21} \quad (2)$$

The mass of ethanol in each sample was obtained from Eqn 2, which can be converted to a volume using its density (ρ = 0.789 g/mL). From this volume percent of ethanol was determined.

Results & Discussion

As noted in the procedure, the ^{1}H NMR spectra were acquired with very long interscan delays; this is crucial to obtain consistent and accurate results. The length of the interscan delay is directly related to the T_1 relaxation times of the NMR sample and is typically set to the longest T_1 relaxation time multiplied by 5 times to ensure that every nucleus in the sample has fully relaxed before the next pulse is applied. To ensure that this criterion is met, the T_1 relaxation times were measured for the maleic acid singlet (~2 sec) and the triplet of the ethanol (~4-7 sec) to find a suitable interscan delay.

The calibration curve generated by plotting the ethanol concentration versus the absolute integral area is displayed in figure 1. The data points were fitted very well (R^2 = 0.9992) to a linear equation that will be used to quantify the alcohol content in the commercial liquor samples.

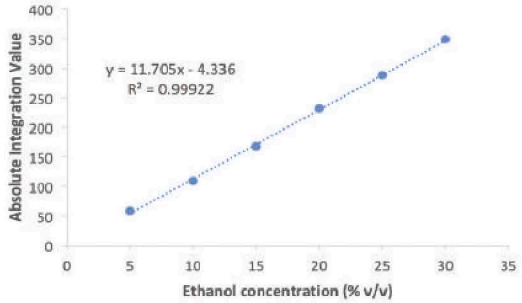

Fig 1. Calibration curve prepared to determine alcohol content.

Table 1. Alcohol content measured via calibration curve

	Alcohol Content (% v/v)		
Commercial Liquor	**Manufacturer**	**Experimental[a]**	**% Error**
Chambord	16.5	15.94	3.39
Frangelico	20.0	19.71	1.45
Disaronno	28.0	27.57	1.54
Crown Royal Apple	35.0	34.88	0.34
Grand Marnier	40.0	38.96	2.60
Bombay Sapphire	40.0	38.74	3.15

[a]The experimental values are an average of 3 separate acquisitions.

With the calibration curve in hand, the alcohol content of the commercial liquors was determined and the results are presented in Table 1.

It is clearly seen that the results obtained match well with the alcohol content reported by the manufacturer for a variety of samples with percent errors ranging from 0.34 to 3.39.

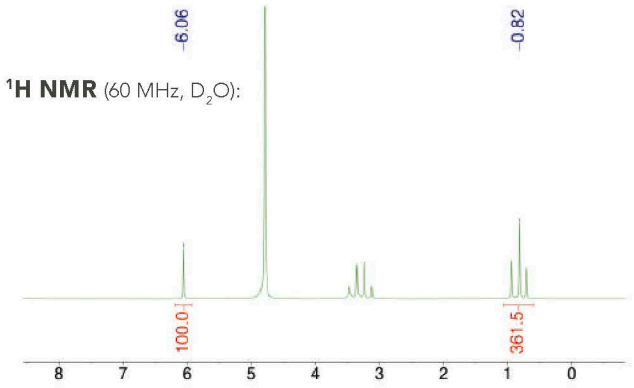

Fig 2. Example ^{1}H NMR spectrum acquired to quantify alcohol content with an internal calibrant.

A representative ^{1}H NMR spectrum (fig 2) highlights commercial liquor sample with internal calibrant, maleic acid. The maleic acid singlet appears at δ = 6.06 ppm while the triplet from the ethanol in the commercial liquor is present at δ = 0.82 ppm. The alcohol content determined with the internal calibrant method is shown in table 2. There is excellent correlation between the alcohol content determined with the NMReady-60 and what is stated by the manufacturer. Besides negating the need to prepare a separate calibration curve, the internal calibrant also gave more accurate results compared to those obtained with the calibration curve, with lower percent errors ranging from 0.13 to 1.90.

Table 2. Alcohol content measured via internal standard

	Alcohol Content (% v/v)		
Commercial Liquor	**Manufacturer**	**Experimental[a]**	**% Error**
Chambord	16.5	16.81	1.88
Frangelico	20.0	20.38	1.90
Disaronno	28.0	27.94	0.21
Crown Royal Apple	35.0	35.7	2.00
Grand Marnier	40.0	39.67	0.83
Bombay Sapphire	40.0	40.05	0.13

[a]The experimental values are an average of 3 separate acquisitions.

Conclusions

In this experiment the alcohol content of a variety of commercial liquors was accurately determined with the NMReady-60. Two methods were employed and complementary results were obtained. However, by using an internal calibrant, more accurate results were determined, and preparation of a calibration curve was avoided. The experiment is simple to perform and provides an excellent introduction to the principles and application of qNMR in undergraduate labs.

References

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Data Accessibility

The data can be processed directly on the NMReady-60 and printed and/or exported directly to a USB or networked file where it can be worked up using third party NMR processing software.

For more examples:
www.nanalysis.com/sample-experiments