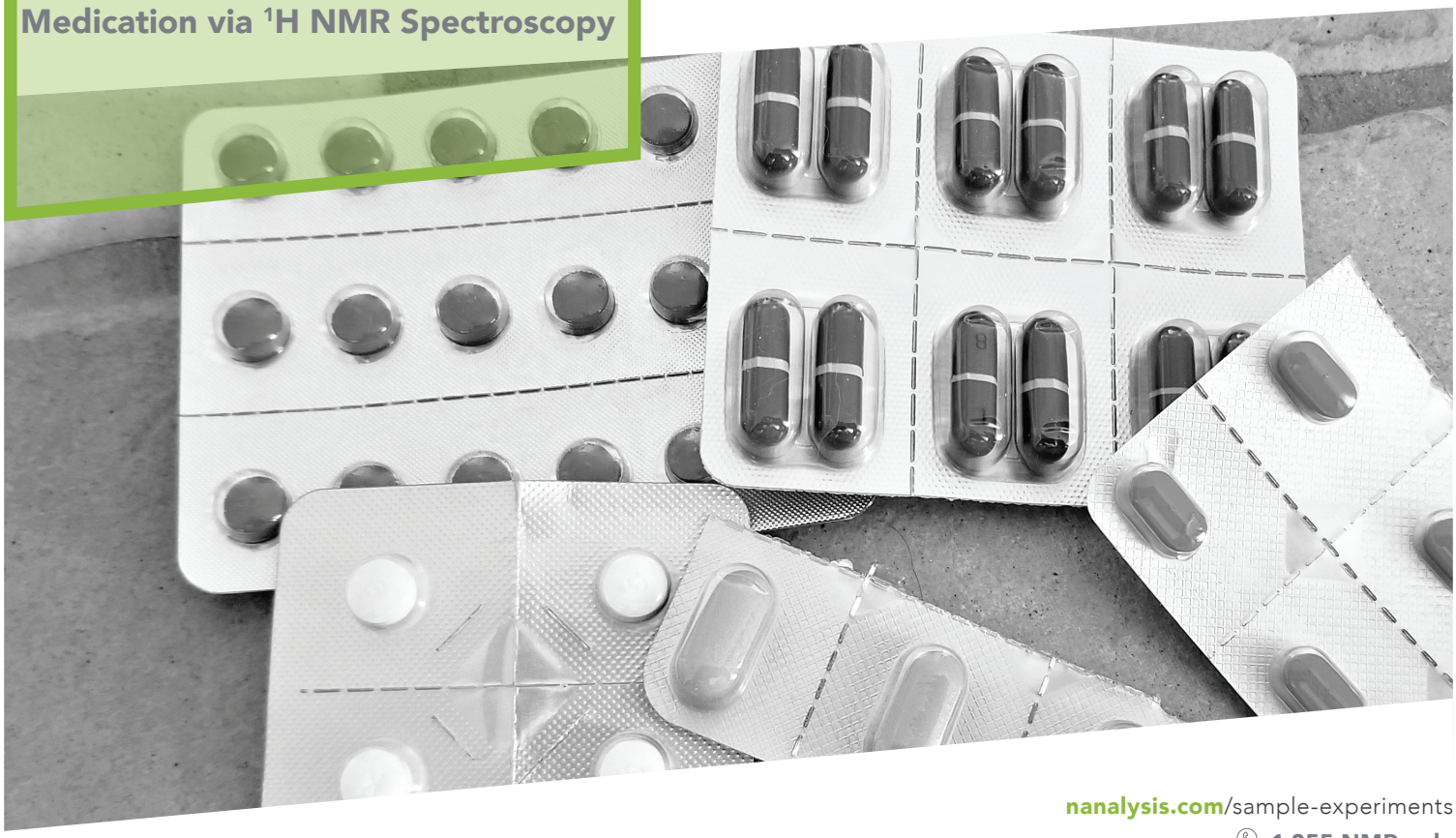
ORGANIC

UNDERGRADUATE EXPERIMENT

Quantification of Active Pharmaceutical Ingredients Present in Over-the-Counter Medication via ^{1}H NMR Spectroscopy



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INTRODUCTION

The use of over the counter (OTC) medications is quite popular for curing mild to moderate acute and chronic pain states. Common active pharmaceutical ingredients (APIs) such as acetaminophen, caffeine, and aspirin (**Figure 1**) are essential parts of many of these medications. Each of these components has a different effect on the body; for example, acetaminophen and aspirin have analgesic and antipyretic properties while caffeine works as a stimulant and an adjuvant.[1]

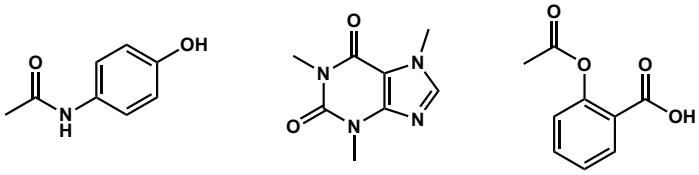

Figure 1. *Chemical structures of acetaminophen, caffeine, and aspirin.*

With such easy access to these common OTC medications, the quality of these pharmaceuticals is quite important. Quantitative analytical methods such as gas chromatography, high-performance liquid chromatography, UV-vis spectroscopy, and Fourier-transform infrared spectroscopy are commonly used techniques to ensure medicines adhere to pharmacopeia standards. Nuclear magnetic resonance (NMR) spectroscopy is an extremely powerful characterization technique that is often used to characterize chemical species, such as APIs. However, this technique has not been fully incorporated by most industries due to the drawbacks of traditional NMR spectroscopy (*i.e.*, large initial cost, specialized facilities, trained staff, cost of cryogens). With the emergence of benchtop NMR technology, this technique can be easily incorporated into workflows for more efficient and facile analysis, especially in the cases of underserved sectors within industry and academia.

NMR spectroscopy is typically introduced to undergraduate students in the organic chemistry curriculum with an emphasis on structural elucidation. Although this approach is great for qualitative analysis, students are not introduced to the quantitative aspect of NMR, termed quantitative NMR (qNMR), an equally important and powerful aspect of NMR spectroscopy, especially for industrial applications. In this sample experiment, adapted from an article published by Zivkovic *et al.*, common OTC medications are examined to quantify the amount of API present using qNMR.[2]

PROCEDURE

Each medicinal tablet was weighed and ground into a fine powder using a mortar and pestle. The fine powder was transferred into a vial for storage. ~20 mg of sample was accurately weighed out with ~10 mg of maleic acid and dissolved in 1 mL of DMSO-d_6. The mixture was heated and sonicated to ensure the formation of a homogenous mixture and an efficient extraction of APIs. The sample was then centrifuged, and an aliquot of the supernatant was analyzed via ^{1}H NMR spectroscopy using the Nanalysis 60 MHz instrument. The ^{1}H NMR spectra were subsequently obtained in triplicates using the following parameters:

Spectral Width: 40 ppm	Interscan Delay: 30 sec
Spectral Centre: 10 ppm	Number of Points: 16384
Number of Scans: 16	Dummy Scans: 0
Receiver Gain: Auto	Pulse Angle: 90°

Note: heating the sample solution is an important step for extraction. If a low extraction efficiency is observed, heating the mixture further should help.

The purity is then determined using the purity formula (**1**), and the purity formula is multiplied by the mass of the tablet to determine the mass of each API.

RESULTS & DISCUSSION

^{1}H NMR spectroscopy was used to qualify and quantify the APIs present in OTC medication. A stacked plot of maleic acid, caffeine, acetaminophen, and a mixture of OTC medication and maleic acid is presented in **Figure 2**. By comparing the spectra of the pure compounds to the spectrum of the OTC medication, the contents of the mixture can be easily qualified. **Figure 2** confirms that acetaminophen and caffeine are present in the medication, and by using the stacked plot, the representative signals of each component can be identified. Acetaminophen can be easily recognized through the aromatic signals between 7.41 and 6.58 ppm or through the methyl signal present at 1.98 ppm. Caffeine has characteristic signals between 3.88 and 3.22 ppm that make it simple to detect in this mixture. Although maleic acid is not present in the medicinal tablet, a small amount was added into the mixture and can be distinguished as a sharp singlet at 6.26 ppm.

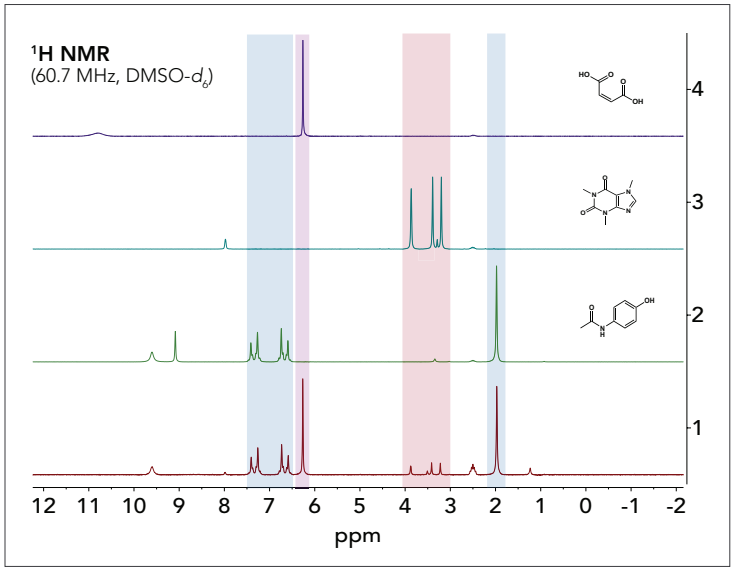

Figure 2. *Stacked plot of ^{1}H (60.7 MHz) NMR spectra representing OTC medication with maleic acid internal calibrant (1), acetaminophen (2), caffeine (3), and maleic acid (4).*

The APIs present in OTC medications can be easily quantified using the formula below:

$$m_{API} = \frac{I_{API} * N_{IC} * M_{API} * m_{IC} * m_T}{I_{IC} * N_{API} * M_{IC} * m_s} \quad (1)$$

Where *I* represents the integral, *N* represents the number of protons associated with a signal, *m* represents the mass, *M* represents the molar mass, *API* represent the active pharmaceutical ingredient, *IC* represents the internal calibrant, *T* represents the tablet, and *s* represents the sample, where the sample is a portion of the tablet.

Figure 3 depicts the ^{1}H NMR spectrum of an OTC medication that was purchased at a local pharmacy. The tablet was dissolved in DMSO-d_6 and the internal calibrant, maleic acid, was added before data acquisition. Based on the label of the medication, each tablet should contain 500 mg of acetaminophen and 65 mg caffeine, resulting in relative compositions of 88.5% and 11.5%, respectively.

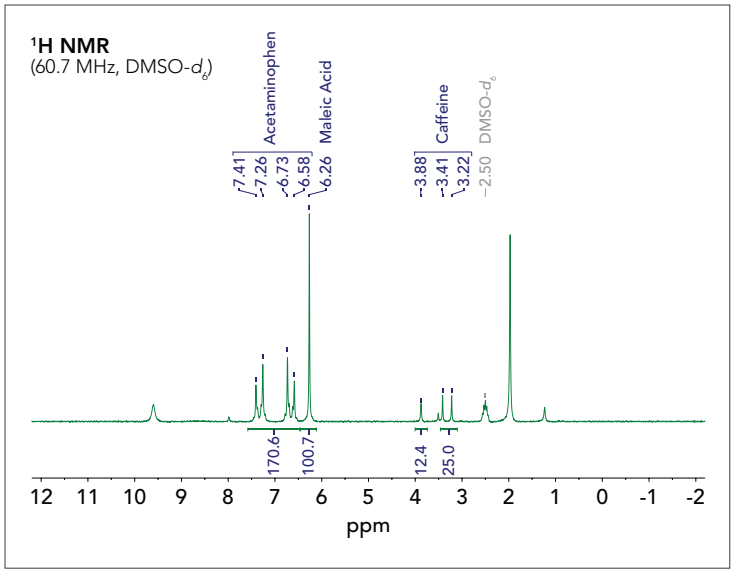

Figure 3. *^{1}H (60.7 MHz) NMR spectrum of an OTC medication and maleic acid dissolved in DMSO-d_6. Signals of interest for acetaminophen, maleic acid, and caffeine are annotated, peak picked, and integrated.*

Table 1. Comparison between masses obtained via ^{1}H NMR spectroscopy and manufacturer's label.

	Mass of Tablet (mg)	*Sample mass (mg)*	*Maleic Acid (mg)*	*Acetaminophen (mg)*	*Caffeine (mg)*
Tablet 1 NMR	*676.4*	*21.79*	*11.6*	*491.45 (0.25)*	*65.04 (1.72)*
Tablet 1 Label	-	-	-	*500*	*65*
Tablet 2 NMR	*706.45*	*21.05*	*13.23*	*493.35 (0.42)*	*61.99 (1.43)*
Tablet 2 Label	-	-	-	*500*	*65*

* Relative standard deviation (RSD) values shown in parentheses

Table 1 summarizes the results of these analyses for two different drug store brands, which were quantified for their acetaminophen and caffeine content, as well as the relative masses of the sample and the tablet. As shown, the relative amounts of acetaminophen and caffeine in tablet 1 is 491.45 mg and 65.04 mg, which correspond to 88.87% and 11.13% respectively. Tablet 2 shows similar results with the relative amounts of acetaminophen and caffeine obtained as 493.35 mg and 61.99 mg, which correspond to 88.43% and 11.57%, respectively. All values obtained compare well to the labeled amount, with some deviation from the label values possibly being attributed to extraction efficiency.

CONCLUSION

In this experiment, the relative compositions and quantities of APIs were determined using the Nanalysis 60 MHz instrument. The values obtained correlate well with the pharmaceutical labels. The experiment is simple to perform in undergraduate laboratories and highlights the important application of NMR spectroscopy as both a qualitative and quantitative analytical technique.

References

[1] Blough, E.R.; Wu, M. Front. *Pharmacol* **2011**, *2,* 72.
[2] Zivkovic, A.; Bandolik, J. J.; Skerhut, A. J.; Coesfeld, C.; Prascevic, M.; Zivkovic, L.; Stark, H. *J. Chem. Ed.* **2017**, *94,* 121-125.

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