ANALYTICAL

UNDERGRADUATE EXPERIMENT

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

INTRODUCTION

The use of over-the-counter (OTC) medications is widespread for managing mild to moderate allergies. Active pharmaceutical ingredients (APIs) such as antihistamines, decongestants, and corticosteroids are crucial components of many allergy medications. Each of these ingredients has a unique effect on the body; for instance, antihistamines block histamine, which plays a role in causing allergy symptoms, decongestants alleviate nasal congestion, and corticosteroids reduce inflammation.[1] **Figure 1** displays some common antihistamines found in OTC allergy medications.

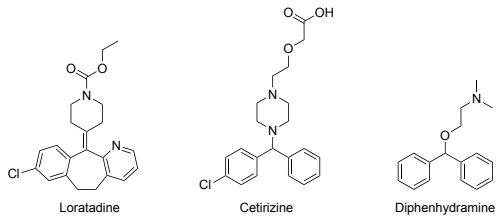

***Figure 1**. Chemical structures of common antihistamines found in OTC allergy medications.*

Ensuring the quality of these medications is crucial given their widespread availability. Various quantitative analytical methods are employed to confirm adherence to pharmacopeia standards and monographs, such as those published in the United States Pharmacopeia – The National Formulary (USP–NF). These include gas chromatography (GC), high-performance liquid chromatography (HPLC), UV-Vis spectroscopy, and Fourier-transform infrared spectroscopy (FTIR). Although nuclear magnetic resonance (NMR) spectroscopy is a highly effective technique for characterizing chemical species such as APIs, its use in industrial quality control (QC) has been limited by factors such as large initial costs, required specialized facilities, and the need for trained staff and cryogens to operate and maintain these systems. However, the advent of benchtop NMR technology has made the technique more accessible and affordable, enabling more efficient and streamlined analyses. This has been particularly beneficial for underserved sectors within industry and academia.

While NMR spectroscopy is commonly taught to undergraduate students in organic chemistry courses to emphasize structural

elucidation, the focus tends to be on qualitative analysis. While this is a powerful aspect of the technique, this approach often neglects the quantitative capabilities of NMR, known as quantitative NMR (qNMR). As such, students may miss out on valuable training that would be beneficial in industrial settings. In this sample experiment, common OTC allergy medications are examined to quantify the amount of API present using benchtop qNMR spectroscopy, and these results are compared to those provided on the product labels.

Procedure

An internal calibrant stock solution was prepared by accurately weighing and dissolving ~110 mg of maleic acid in 1 mL of D_2O using a volumetric flask.

Each medicinal tablet was weighed and ground into a fine powder using a mortar and pestle. The powder was transferred into a vial for storage. Then, ~50 mg of sample was accurately weighed in a microcentrifuge tube and dissolved in 1 mL of D_2O. The mixture was heated to approximately 50 °C for 10 minutes and sonicated to ensure an efficient extraction of APIs. The sample was then centrifuged, and an aliquot (0.6 mL) of the supernatant was transferred into a 5 mm NMR tube, along with 0.05 mL of internal calibrant stock solution. The sample was then analyzed via ^{1}H NMR spectroscopy using a Nanalysis 60 MHz instrument. The ^{1}H NMR spectra were subsequently collected in triplicates using the following acquisition parameters:

Spectral width: 40 ppm	Interscan delay: 30 sec
Spectral center: 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. Depending on the brand of antihistamine obtained, multiple sonication and centrifuge cycles may help increase extraction efficiency.

Results and Discussion

^{1}H NMR spectroscopy was used to quantify the cetirizine hydrochloride present in OTC allergy medication. **Figure 2** depicts the ^{1}H NMR spectrum of an extracted OTC allergy medication that was purchased at a local pharmacy. The samples were prepared as described in the **Procedure** above. Based on the medication's label, each tablet should contain 10 mg of cetirizine hydrochloride. The USP monograph for cetirizine hydrochloride tablets permits a variation of up to ±10% from the labeled value for the API, resulting in a permissible range of 9 to 11 mg of cetirizine hydrochloride per tablet.[2]

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

$$m_{API} = \frac{I_{API} * N_{IC} * M_{API} * m_{IC} * m_T}{I_{IC} * N_{API} * M_{IC} * m_s * 0.6}$$

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. The factor of 0.6 is included to consider the 0.6 mL aliquot of solution removed from the total sample solution for testing.

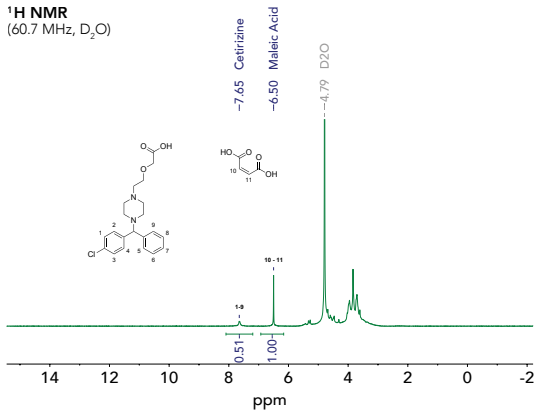

Figure 2. *^{1}H (60.7 MHz) NMR spectrum of an OTC medication and maleic acid dissolved in D_2O. Signals of interest for cetirizine and maleic acid are annotated, peak picked, and integrated.*

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

Brand	Mass of tablet (mg)	Sample mass (mg)	Maleic acid (mg/mL)	Cetirizine HCl (mg)	Label (mg)	% Difference*
Reactine®	176.2	61.8	110.6	9.70 (0.015)	10	3.0
Equate™	152.4	56.1	110.6	9.71 (0.009)	10	2.9

*Although the USP monograph for cetirizine HCl tablets allows for a tolerance range of up to ±10% from the labeled value, the percentage difference calculation was conducted using the labeled value.
**Residual standard deviation (RSD) values shown in parentheses.

The results of the cetirizine hydrochloride quantification analyses for two different drug store brands (Reactine® and Equate™) are presented in **Table 1**. The Reactine® tablet contained 9.70 mg of cetirizine hydrochloride, while the Equate™ tablet had a similar result with a cetirizine hydrochloride content of 9.71 mg. Notably, all values align well with the labeled amount, where the measured values of cetirizine hydrochloride content in both tablets fall within the acceptable range of ±10% from the labeled value (10 mg).

The published USP–NF monograph for cetirizine hydrochloride assays describes the use of HPLC as the method of choice.[2] For this procedure, a mobile phase consisting of acetonitrile, water, and 1M sulfuric acid is used, and a certified USP standard of cetirizine hydrochloride is required (~$360 CAD/250 mg). The use of expensive HPLC grade solvents (typically >99.9% purity) and reference standards makes this approach expensive and wasteful, especially when a non-destructive method such as benchtop NMR spectroscopy can be used.

Conclusion

In this experiment, the Nanalysis 60 MHz instrument was successfully utilized to the determine amount of cetirizine hydrochloride present in OTC allergy medication. The results obtained were in good agreement with the values provided on the labels. This experiment stands as evidence of the practical effectiveness of NMR spectroscopy as a quantitative analytical technique. Moreover, the straightforward procedure followed in this experiment makes it a valuable addition to undergraduate laboratory curricula.

References

[1] Allergy Medications: Know your options: https://www.mayoclinic.org/diseases-conditions/allergies/in-depth/allergy-medications/art-20047403 (accessed Feb 21, 2023)
[2] Cetirizine Hydrochloride Tablets (2021). The United States Pharmacopoeia – The National Formulary. Rockville, MD, USA.

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