EDULAB FOR STUDENTS

Fluorine Knows: A Simple Way to Measure Fluorinated Pharmaceuticals

An NMR Analysis of Pantoprazole

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Experiment Hashtag: #Educate2Resonate

Keywords:

API analysis, analytical chemistry, quantitative NMR, ^{19}F NMR, benchtop NMR

Target group:

Advanced Undergraduate or Graduate, General Chemistry, Analytical Chemistry, Medicinal Chemistry

Objectives:

NMR is a useful tool for structure determination as well as for quantitation. In this EduLab, students will refresh their knowledge of NMR structure elucidation using 1D ^{1}H and ^{19}F and 2D ^{1}H, ^{1}H-COSY experiments and explore quantitative NMR (qNMR) by being introduced to ^{19}F NMR on a Fourier 80 benchtop NMR spectrometer. Students will prepare samples, run NMR experiments and analyze their data to learn how to use NMR to determine concentration of a fluorinated compound in a complex sample.

Background of the Experiment:

Tablets are complex systems that contain not only the active pharmaceutical ingredient (API) but also a variety of excipients. These excipients play a crucial role in controlling the physical and chemical properties of the drug, as well as ensure the proper release and absorption of the API. A typical tablet consists of fillers (e.g. lactose, mannitol, starch), binders (e.g. starch, gelatine), glidants (e.g. talc, colloidal silica, starch), disintegrants (e. g. starch, polyvinyl-pyrrolidone, carboxymethyl-cellulose), lubricants (e. g. magnesium stearate, talc) and coating agents (e. g. Eudragit).[1] The careful selection and combination of these excipients are essential to produce tablets that are stable, effective, easy to dose, and convenient for the patient to use.

Fluorine has favourable properties including high electronegativity (3.98 on the Pauling electronegativity scale) and small atomic size with a van der Waals radius of 1.47 Å, resulting in a highly polarized C–F bond.[2] With such unique features, the use of fluorine in drug design has various benefits on the physicochemical properties. This leads to improved efficacy which contribute to the drug's pharmacokinetics, selectivity and as well as metabolic stability.[2,3] Therefore fluorinated pharmaceuticals have been used for a broad range of therapeutic purposes such as antifungal, antitumor, gastrointestinal and antipsychotic drugs and several of the most prescribed drugs have fluorine in their chemical structure.[2-5]

As such, it is important to establish a rapid, robust, selective and easy method for active ingredient concentration determination for fluorine-containing compounds. In this EduLab, the drug chosen is pantoprazole (Fig. 1), a first-generation proton pump inhibitor used mainly to lower stomach acid, and it is administered in case of gastro-oesophageal reflux disease (GERD/GORD) and for the gastric protection from NSAIDs and to prevent stomach ulcers.[6]

Here, a tablet formulation containing pantoprazole as the active pharmaceutical ingredient is analysed by NMR spectroscopy. Since pantoprazole contains fluorine atoms, while none the excipients in the formulation do, ^{19}F quantitative nuclear magnetic resonance (qNMR)[7,8] spectroscopy can be selectively employed for the precise and selective quantification of the active compound. ^{19}F NMR is advantageous as the natural abundance is 100% and as most solvents do not contain fluorine, solvent suppression is not necessary. Further on, the sample preparation is rapid and cost-effective compared to HPLC.

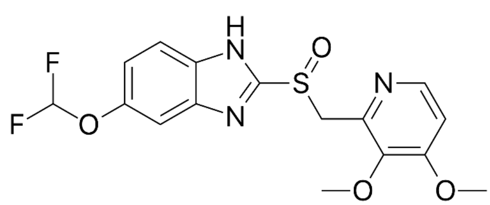


Figure 1: The chemical structure of pantoprazole

Experimental Setup:

Materials

- Commercially available pantoprazole (20 mg) tablets (note: most tablets contain the sodium salt of pantoprazole)
- ^{19}F external calibrant (e.g. trifluorotoluene, trifluoroethanol, pantoprazole precursor: 5-(difluoromethoxy)-2-mercapto-1*H*-benzimidazole)
- A suitable solvent (e.g. DMSO or methanol)
- 5 mm NMR tubes and caps
- Pipettes

Glossary

Active pharmaceutical ingredient (API): The biologically active component in a pharmaceutical drug, the substance responsible for the therapeutic effect of the medication.

Excipient: An inactive substance in a pharmaceutical drug that does not have therapeutic effects, used as a binder, to aid delivery, improve taste and/or appearance and so on.

qNMR: A method that uses the principles of NMR spectroscopy to quantify the amount of an analyte by comparing its signal to that of a known reference standard.

Experimental Procedure:

Sample Preparation

1. Prepare three pantoprazole solutions by dissolving one tablet in 2 mL solvent. The tablets contain an enteric coating to protect pantoprazole from the acidic environment, therefore, to aid dissolution, it is advisable to cut the tablet in half or crush it and to use an ultrasonic bath. Approximately 15 minutes of stirring time are required. Since some excipients cannot be dissolved in methanol or DMSO, the suspension has to be centrifuged or filtered.

2. Transfer 600 µL of the tablet solution into an NMR tube. You will have 3 samples with unknown concentration.

3. Prepare a series of five external calibration solutions with a fluorine content in the range of 20–100 mM. You can use any of the above-mentioned calibrants, just make sure the range of fluorine concentration is the same.

4. Record ^{19}F {^{1}H} spectra of the tablet and calibration solutions using the parameters below. Record three replicates of each tablet sample. Pay attention to the T_1 values of the pantoprazole and the calibrant as they might differ.

5. Process and analyze the data using the procedure described below.

NMR experiment set-up

The quantitative pantoprazole determination, the ^{19}F {^{1}H} spectra should be recorded using the *zgiglh* pulse sequence on the Fourier 80 benchtop NMR spectrometer. The suggested TopSpin experiment parameters are given in the table below:

Parameter	Abbreviation, unit	Value
Spectral width	SW, ppm	50
Transmitter offset	O1P, ppm	–75
Number of transients	NS	16
Number of dummy scans	DS	4
Number of points in the time domain	TD	32k
Inter-scan delay	D1, s	see pre-lab Qs
Exponential line broadening	LB, Hz	1
Size of real spectrum	SI	64k

Abbreviations

qNMR: quantitative Nuclear Magnetic Resonance

ERETIC: Electronic REference To access In vivo Concentrations

NSAID: Non-Steroidal Anti-Inflammatory Drug

GERD: Gastroesophagial Reflux Disease

Data Processing:

1. Process all the spectra using standard protocols, including phase and baseline correction.

2. Determine the integral of the fluorine peak on the spectra of the dilution series.

3. Represent the F concentration against integrals; and determine the linear equation of the obtained calibration curve.

4. Use the obtained equation from the calibration curve to determine the fluorine concentration of the unknown pantoprazole sample, and from this calculate the amount of pantoprazole in the tablet. Pay attention to the number of F atoms in pantoprazole.

Results & Discussion:

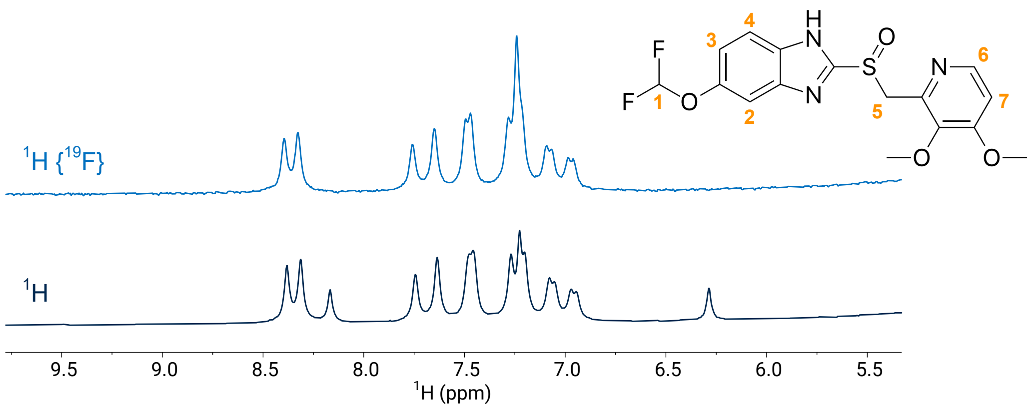
After processing the data, answer the following questions:

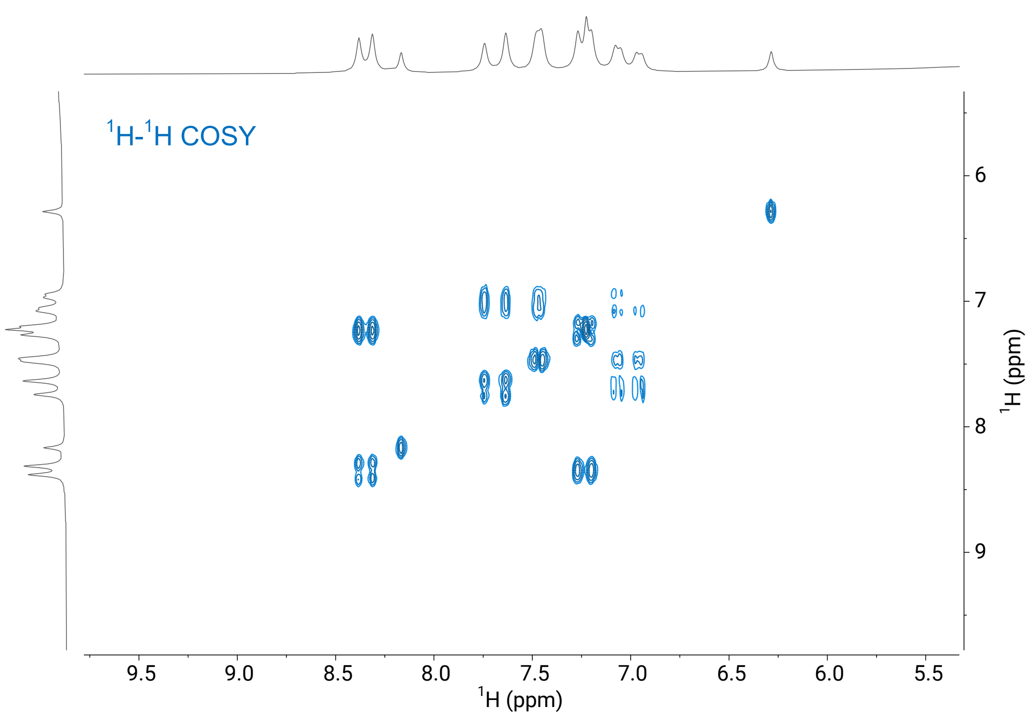
1. Discuss the results obtained using the calibration curve. Does the calculated mass of pantoprazole match the mass stated on the packaging? Discuss the accuracy of this method and state possible sources of error.

2. Does the calculated mass vary significantly between samples or is it explained by the instrumental variability between the replicates of the same sample? Use a statistical test – analysis of variance – to justify your answer.

Notes

3. Assign the 80 MHz ^{1}H spectrum of pantoprazole using the spectral data provided below. Explain your reasoning. The signal for proton 5 is not shown.





Notes

Optional Questions for Students:

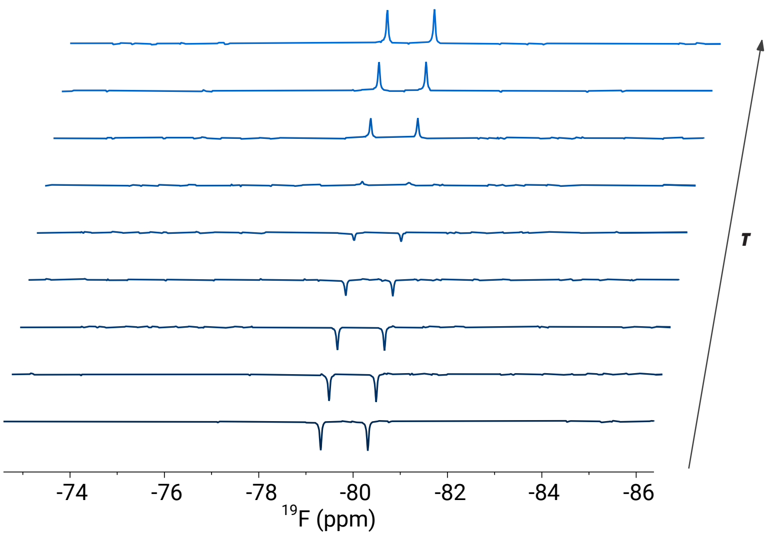
Pre-Laboratory Questions:

1. Give at least 3 advantageous properties of ^{19}F as an NMR active nucleus.

2. What is T_1 relaxation and why is it important for quantitative NMR measurements?

3. Use the ^{19}F inversion recovery experimental data below to calculate the ^{19}F T_1 time of pantoprazole at 80 MHz. Propose what relaxation delay value should be used in the experiment.

Notes



τ / s	Intensity
0.05	−4.2404
0.10	−4.0042
0.25	−3.4159
0.50	−2.3837
0.80	−1.3155
1.50	0.5065
3.00	2.9612
5.00	4.2761
10.0	5.0490

Notes

Key Take Home Messages:

- Learn how to perform quantitative NMR
- Learn how to use ^{19}F NMR to analyse fluorine-containing compounds
- See an example of benchtop NMR applications in pharma

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