



EduLab FOR INSTRUCTORS: FOURIER 80

Mixing it Up with NMR

Mixture Analysis of Cocktails

Authors & Affiliation:

Katrina Steiner, Kiera Ronda, Ronald Soong, Katelyn Downey, Peter Costa, William Wolff, Andre Simpson

Environmental NMR Center, University of Toronto, 1265 Military Trail, Toronto, ON, Canada, M1C 1A4

Experiment Hashtag #: #MixtUp #Educate2Resonate

Keywords:

Mixture Analysis, Patterning Matching, Spectral Fingerprint, Standard Addition, Food Science

Target group:

Undergraduate, General Chemistry, Analytical Chemistry, Food Chemistry

Objectives:

- Gain hands on experience on the Bruker Fourier 80 benchtop spectrometer while building upon the understanding of NMR in a practical setting.
- Apply NMR to real-world samples.
- Learn how to process and interpret 1D ^1H NMR data.
- Use spectral fingerprints to understand sample mixing.
- Learn how to confirm the presence of compounds via standard addition.

Background of the Experiment:

The fermentation of alcoholic beverages is an ancient practice. Evidence suggests that these beverages were made as far back as 7000 BCE.¹ Although alcohol production is rather old, cocktails did not originate until the 17th century. Cocktails are described as a blend of spirits and fruit juice mixers typically served on ice with garnishes. This EduLab investigates three popular cocktails at a molecular level using the Fourier 80. The first cocktail is a “Jäger Bomb” which consists of the German spirit, Jägermeister, and Sugar Free Red Bull. The second cocktail is fruitier with a tropical twist and consists of coconut flavored Malibu rum and pineapple juice. The third consists of Baileys Original Irish Cream and fresh espresso. These cocktails are specifically chosen as they have recognizable spectral profiles which are identifiable by 1D ¹H NMR. For example, Jägermeister is crafted using a blend of 56 herbs, blossoms, and roots which makes it high in aromatic content. In comparison, cocktails containing Baileys are more lipid rich due to its cream base. See Figure 1 to view what the largest lipid signals look like in 10% table cream.

Nuclear magnetic resonance (NMR) spectroscopy is a highly reproducible, non-invasive, and robust analytical technique that can rapidly analyze complex samples and mixtures. These key features of NMR are highly favorable for applications in food science. One dimensional NMR experiments, like the ones used in this EduLab, have been used to identify spectral fingerprints in various food samples, such as: virgin olive oil, dairy products, various meats, and cheeses.²⁻⁵ NMR spectral fingerprinting can even be used to identify the geographical origin and confirm the authenticity of foods (ex. honey) and beverages (ex. wine).^{6,7}

In this EduLab, we will see how 1D ¹H NMR spectral fingerprinting can be used to identify each cocktail representing a real-life complex sample mixture. Finally, we will investigate how the standard addition method can be used to experimentally confirm the position of the caffeine peak in the espresso sample.

Preparation and Prerequisite:

The cocktail experiments should take approximately four hours to perform (not including sample preparation time), an additional two hours for a report, and it is assumed that students have completed introductory concepts of 1D NMR and basics of spectral interpretation. The experiments are designed to be ideally completed in groups of 3-6 students. This investigation aims to demonstrate key NMR concepts, including interpretation of 1D spectra and standard addition experiments. This information is readily available in the version 001 Fourier EduLab Students Guide delivered on the USB stick with a Fourier 80. In addition, students should have a tutorial learning how to use processing software such as MestreNova.

It is strongly recommended that instructors set up the experimental templates prior to the laboratory as this exercise is meant to emphasize basic acquisition/processing and data analysis. Rather than focusing on advanced parameters, students should focus on calibrating and inputting basic parameters such as O1P, P1, DS and NS into prepared templates.

It is recommended that students complete the cocktail analysis lab before moving onto more difficult mixture analysis EduLabs such as the Red Bull mixture analysis (#NMRGivesYouWings).

To perform this experiment, a properly installed and adjusted Fourier 80 system with TopSpin Software is required. In addition, a sonicator, 1000 μ L micropipette, and 100 μ L micropipette should be available.

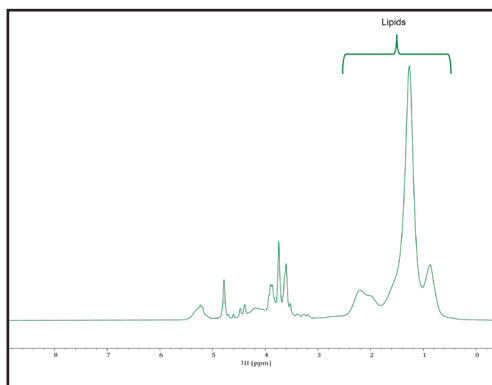


Figure 1: 1D ¹H NMR spectrum of 10% table cream. Note the strongest lipid signals arise in the 0.6-3.0ppm region.

Glossary

NMR:

Spectroscopic analytical technique based on radio frequency-induced transitions between energy levels that atomic nuclei adopt in an external magnetic field as a result of their own magnetic moment.

Cocktails: Blend of spirits and fruit juice mixers typically served on ice with garnishes.

NMR spectral

fingerprinting: The NMR spectrum of a sample can be seen as its spectral fingerprint showing the unique pattern of peaks characteristic for this sample. This can be helpful in targeted analysis, where an NMR spectrum of another sample does not need to be assigned, but screened against a dedicated target sample to see if their fingerprints match.

Abbreviations

NMR:

Nuclear Magnetic Resonance

1D:

one dimensional

Experimental Setup:

- Jägermeister
- Malibu Original
- Baileys Irish Cream
- Sugar Free Red Bull
- Pineapple Juice
- Espresso
- Pure caffeine
- 5mm NMR tubes with caps (10)
- Pulse programs: zg, zgcppr

In TopSpin, set up the following NMR experiments on the spectrometer (acquisition parameters are listed below): 1D zg (for O1P and P1 determination) and zgcppr (for sample analysis). Note that zgcppr was chosen as it is an effective water suppression, but other water suppression techniques such as WET (1D ^1H NMR with WET water suppression), or W5-WATERGATE could also be used. Template setup is done by copying over any previous parameter set, loading the chosen pulse program, and inputting the experimental values outlined below.

| PULPROG | zg | zgcppr |
|----------|-----------|-----------|
| TD | 8192 | 8192 |
| SW (ppm) | 15.599 | 15.599 |
| AQ (sec) | 3.2767999 | 3.2767999 |
| RG | 1 | 1 |
| D1 (sec) | 2.0 | 2.0 |
| DS | 4 | 8 |
| NS | 8 | 256 |

Table 1. Recommended parameters for 1D ^1H zg and zgcppr analysis.

When assigning peaks in the spectra, there are many NMR databases and literature sources that can be used to help with assignment if needed. This reference may help assign many key signals: <https://pubs.acs.org/doi/abs/10.1021/ed086p360>.⁸

For more background on standard addition in complex mixtures see this reference: <https://pubs.acs.org/doi/abs/10.1021/ed500364b>.⁹

Sample Preparation:

Preparing the cocktails:

1. Degas the Red Bull prior to use. This can be done by allowing an open can of Red Bull to stand in the fridge overnight then shaking the remaining carbonation out the day of use. In addition, sample can be sonicated for 2-3 hours if a sonicator is readily available.
2. Prepare the Malibu & pineapple juice, and Jägermeister & Sugar Free Red Bull cocktails → mix 300 μL of the alcohol with 300 μL of the mixer.
3. Prepare the Baileys and espresso cocktail → mix 50 μL Baileys with 550 μL Espresso.

Note: This cocktail is mixed in a different ratio due to the interactions the caffeine in the espresso has with the proteins and lipids in the Baileys. When the Baileys is in too high of concentration, the aromatic caffeine peak is no longer visible.

Preparing the caffeine sample and spiked sample:

1. Dissolve 30 mg of pure caffeine in 1 mL of water (0.154 mol/L). Note: Exceeding this concentration is not recommended as caffeine has low water solubility. To help with dissolution, sonicate the sample in an Eppendorf tube for 30 sec, then mix well.

Glossary

Abbreviations

O1P:

O1 (or O1P for the value in ppm) is the carrier frequency used for the hard pulses

WATERGATE:

WATER suppression by gradient tailored excitation

2. In an Eppendorf tube, spike 50 μL of the caffeine standard in 550 μL of the espresso and thoroughly mix. This sample is used for the standard addition experiment to identify which peak is caffeine.

Experimental Procedure:

1. Prepare the 10 NMR samples (3 alcohols, 3 mixers, 3 cocktails, 1 espresso + caffeine spike) following the instructions above and transfer 0.6 mL into clean 5 mm NMR tubes.
2. Seal the tubes with caps.

Qualitative Analysis

1. Place the first NMR tube inside the spectrometer and acquire a 1D ^1H NMR spectrum (zg). The water solvent peak in this spectrum will be used to establish the frequency of the proton channel (O1P) for the subsequent experiment. It is important that the O1P is identified as precisely as possible as it plays an important role for the water suppression. The O1P should be set to the apex of the water peak. P1 should additionally be determined.
2. Using the same sample, acquire a 1D ^1H NMR spectrum with water suppression. The pulse program zgcppr is recommended; however, others can be alternatively used. The chosen experiment should suppress the water solvent signal and produce a relatively flat baseline around the water.
3. If using the zgcppr, it is recommended the parameters D1=2, DS=8, and NS=256 are used. These parameters ensure that lower intensity peaks are visible. This is especially important for the aromatics. Use a pre-saturation field of $\sim 10\text{-}12$ Hz. Edprosol can be used to calculate the pre-saturation field if required. More information on how to use edprosol can be found in the TopSpin EDPROSOL Manual. Alternatively, a value of 66 dB can be simply entered into parameter "PLdB9".
4. Repeat these steps for the other nine samples. For the spectra to be comparative, all parameters should be kept the same except for the O1P which is sample specific.

Caffeine Standard Addition

1. Place the caffeine spiked espresso sample in the spectrometer and acquire a 1D ^1H NMR spectrum (zg). Determine the O1P for the sample.
2. Using the same sample, acquire a 1D ^1H NMR spectrum with water suppression. The pulse program zgcppr is again recommended. It is crucial that this experiment is collected with identical parameters of the pure espresso spectrum.

Data Processing:

Process all data following standard protocol, which includes properly phasing each spectrum and correcting the baselines. An exponential apodization corresponding to a line broadening of 0.1 is recommended.

Calibrate the ppm axis of the alcohol spectra and the cocktail spectra using the center of the ethanol triplet. The center should be at 1.188 ppm. Using the calibrated cocktail spectra, calibrate the individual mixers.

Notes

Glossary

Water Suppression:

A technique used in NMR to minimize the usually strong signal deriving from water. This is important because the water signal can interfere with the signal from other molecules you are interested in investigating, resulting in information loss.

Abbreviations

TD:

Number of FID points

SW:

Spectral width

AQ:

Acquisition time

RG:

Receiver gain

D1:

Relaxation delay

DS:

Dummy scans

NS:

Number of scans

P1:

Length of the ^1H excitation pulse

Notes

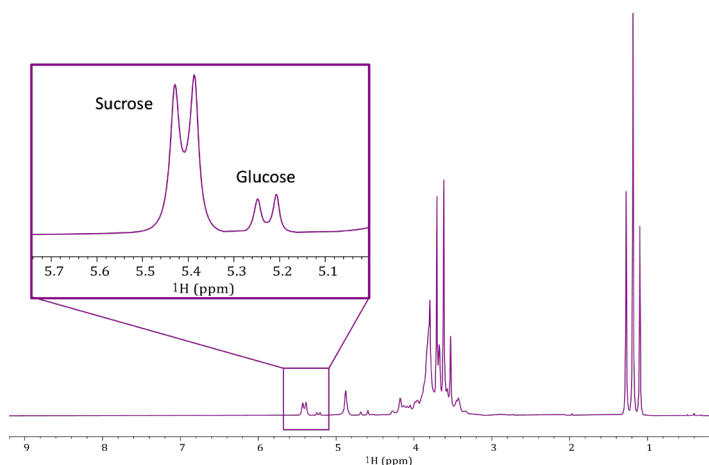


Figure 3. Cocktail: Malibu and pineapple juice. Together this cocktail contains distinguishing sugars from both the Malibu and the pineapple juice as well as the ethanol triplet.

This cocktail will be the hardest to distinguish for students. However, they should be able to recognize it due to its comparative simplicity.

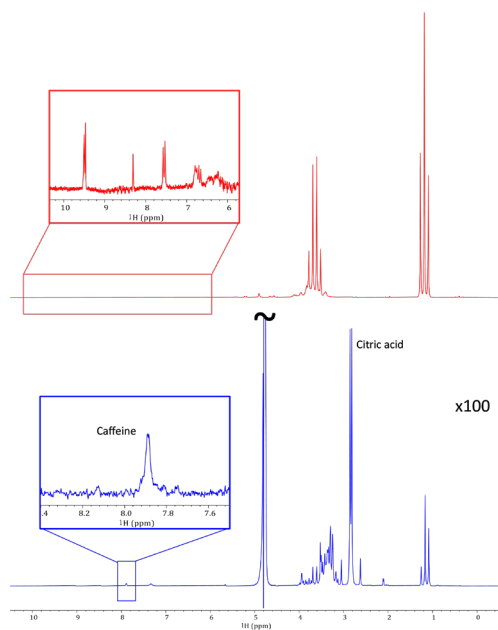


Figure 4. Mixers: Jägermeister (red), Sugar Free Red Bull (blue). Students should be able to recognize Jägermeister based on its unique aromatic region and ethanol triplet. Sugar Free Red Bull can be identified using the caffeine and citric acid signals.

Although Red Bull and espresso both contain caffeine, espresso lacks the distinct citric acid signal. Note that the students do not need to know the exact identity of the aromatic peaks. These peaks come from the variety of 56 herbs, blossoms, and roots in the Jägermeister. That is why it is important for the students to know the ingredients in each sample. That way they can make the connection that the Jägermeister will contain aromatics.

Note: The lipid region of the Baileys overlaps with the CH₃ ethanol multiplet. The lipid spectrum in Figure 1 may help visualize the lipids in Baileys more easily. Also, if students are having a hard time assigning caffeine, tell them to come back to this cocktail after the standard addition experiment.

References:

1. McGovern, P.E.; Zhang, J.; Tang, J.; Zhang, Z.; Hall, G.R.; Moreau, R.A.; Nunez, A.; Butrym, E.D.; Richards, M.P.; Wang, C.; Cheng, G.; Zhao, Z.; Wang, C. Fermented beverages of pre- and proto- historic China. *PNAS* **2004**, 101(51), 17593-17598.
2. Alonso-Salces, R.; Gallo, B.; Collado, M.I.; Sasia-Arriba, A.; Viacava, G.E.; Garcia-Gonzalez, D.L.; Toschi, T.G.; Servili, M.; Berrueta, L.A. ¹H-NMR fingerprinting and supervised pattern recognition to evaluate the stability of virgin olive oil during storage. *Food Control* **2021**, 123, 107831.
3. Karoui, R.; De Baerdemaeker, J. A review of the analytical methods coupled with chemometric tools for the determination of the quality and identity of dairy products. *Food Chem.* **2007**, 102(3), 621-640.
4. Decker, C.; Krapf, R.; Kuballa, T.; Bunzel, M. Differentiation of meat species of raw and processed meat based on polar metabolites using ¹H NMR spectroscopy combined with multivariate data analysis.
5. Kandasamy, S.; Yoo, J.; Yun, J.; Kang, H.B.; Seol, K.; Ham, J. ¹H HRMAS-NMR based metabolic fingerprints for discrimination of cheeses based on sensory qualities. *Saudi J. Bio. Sci.* **2020**, 27(6), 1446-1461.
6. Boffo, E.F.; Tavares, L.A.; Tobias, A.C.T.; Ferreira, M.M.C.; Ferreira, A.G. Identification of components of Brazilian honey by ¹H NMR and classification of its botanical origin by chemometric methods. *LWT* **2012**, 49(1), 55-63.
7. Godelmann, R.; Fang, F.; Humpfer, E.; Schutz, B.; Bansbach, M.; Schafer, H.; Spraul, M. Targeted and nontargeted wine analysis by ¹H NMR spectroscopy combined with multivariate statistical analysis. Differentiation of important parameters: grape variety, geographical origin, year of vintage. *J. Agric. Food Chem.* **2013**, 61(23), 5610-5619.
8. Lefebvre, B.; Corrin, T.; Simpson, A.J.; Shirzadi, A., Burrow, T.E.; Dicks, A.P. Use of NMR and NMR Prediction Software To Identify Components in Red Bull Energy Drinks. *J. Chem. Ed.* **2009**, 86(3), 360-362.
9. Simpson, A.J.; Mitchell, P.J.; Masoom, H.; Mobarhan, Y.L.; Adamo, A.; Dicks, A.P. An Oil Spill in a Tube: An Accessible Approach for Teaching Environmental NMR Spectroscopy. *J. Chem. Ed.* **2015**, 92(4), 693-697.

