



EduLab FOR STUDENTS: FOURIER 80

# Mixing it Up with NMR

## Mixture Analysis of Cocktails

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**Experiment Hashtag #:** #MixItUp #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  $^1\text{H}$  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.<sup>1</sup> Although alcohol production is rather old, cocktails did not originate until the 17<sup>th</sup> 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 <sup>1</sup>H 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.

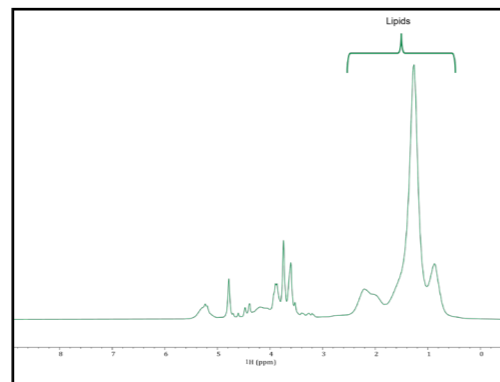
Nuclear magnetic resonance (NMR) spectroscopy is a highly reproducible, non-invasive, and robust analytical technique that can rapidly analyze complex samples and mixtures. These key features of NMR are highly favorable for applications in food science. One dimensional NMR experiments, like the ones 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.<sup>2-5</sup> NMR spectral fingerprinting can even be used to identify the geographical origin and confirm the authenticity of foods (ex. honey) and beverages (ex. wine).<sup>6,7</sup>

In this EduLab, we will see how 1D <sup>1</sup>H 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 minimal knowledge 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. Prior to carrying out these experiments, it is strongly recommended to be familiar with basic 1D NMR processing. This information is readily available in the version 001 Fourier EduLab Students Guide delivered on the USB stick with a Fourier 80. In addition, having a basic understanding on using processing software such as MestreNova is strongly recommended.

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



**Figure 1:** 1D <sup>1</sup>H 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, zgcprr

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>.<sup>8</sup>

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

## 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  $\mu$ L of the alcohol with 300  $\mu$ L of the mixer.
3. Prepare the Baileys and espresso cocktail → mix 50  $\mu$ L Baileys with 550  $\mu$ L 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.
2. In an Eppendorf tube, spike 50  $\mu$ L of the caffeine standard in 550  $\mu$ L 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.

## 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

### Qualitative Analysis

- Place the first NMR tube inside the spectrometer and acquire a 1D <sup>1</sup>H 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.
- Using the same sample, acquire a 1D <sup>1</sup>H NMR spectrum with water suppression. The pulse program zgpcpr 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.
- If using the zgpcpr, it is recommended the parameters D1=2, DS=8, and NS=256 are used. Make sure to also input the O1P and P1 determined in step 1.
- 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

- Place the caffeine spiked espresso sample in the spectrometer and acquire a 1D <sup>1</sup>H NMR spectrum (zg). the O1P and P1 for the sample.
- Using the same sample, acquire a 1D <sup>1</sup>H NMR spectrum with water suppression. The pulse program zgpcpr 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

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### Key Take Home Messages:

- NMR is a robust analytical tool that can be applied to real world samples without alteration.
- Each drink component has unique molecular fingerprints detectable by NMR which can be used for pattern matching in mixtures. This is one reason why NMR has major applications in food science.
- Standard addition is an effective method for confirming the identity of peaks such as caffeine in complex mixtures.

### Glossary

### Abbreviations

- D1:** Relaxation delay  
**DS:** Dummy scans  
**NS:** Number of scans  
**O1P:** O1 (or O1P for the value in ppm) is the carrier frequency used for the hard pulses  
**P1:** Length of the <sup>1</sup>H excitation pulse

### Results & Discussion:

To complete the exercise, answer the following questions:

- Discuss the fingerprint regions of each cocktail and identify at least one unique signal in each. Compare and contrast the cocktail spectra to the individual alcohol and mixer spectra. Label the spectra and if possible, suggest what the unique signals are. Hint: look at the ingredients for clues in assigning characteristic peaks. Only worry about assigning the unique peaks in each spectrum. Note: on one of the alcohols, there are unique peaks that are challenging to identify from the <sup>1</sup>H NMR alone. If you cannot assign them, feel free to label this as "unknown distinguishing aromatics".

Advanced (Interactive Mode): In 'multiple display' mode in TopSpin, you can overlay the individual alcohol and mixer spectra, and click the sum (Σ) button. Adjust the intensity of either the individual alcohol spectrum or mixer spectrum to see what it would look like in the summed result (i.e., mixture result). Can you see how this could be powerful for understanding the mixing in multicomponent systems?

- How can you confirm which peak in the aromatic region of the espresso is from caffeine? Demonstrate with a figure.

- What are some applications of low field NMR in the field of food science? Discuss the potential of NMR for understanding mixing of components. Can you think of industries or applications where such analysis or monitoring could be useful?

### Glossary

### Abbreviations

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