EDULAB FOR INSTRUCTORS: FOURIER 80

NMR Gives You Wings

Analysis of Red Bull Energy Drink

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Experiment Hashtag #: #NMRGivesYouWings #Educate2Resonate

Keywords:

Compound identification, DOSY, HMQC, Selective excitation

Target group:

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

Objectives:

- Acquire, process and analyze basic 1D and 2D NMR spectra.
- Identify major components of Red Bull energy drink through fingerprint matching.
- Gain experience running specialized experiments (HMQC, DOSY, selective excitation).

Background of the Experiment:

Nuclear magnetic resonance (NMR) spectroscopy is a powerful analytical tool, one which has mainly been taught to undergraduates as a technique for identification and quantification of various chemical compounds. While historically, NMR has mainly been used in academia and industry for structure elucidation in organic chemistry, recent advancements have expanded its applications into many areas including metabolomics, pharmaceutical, environmental, and food quality research.[1-4]

In the food sector, NMR can be used to identify unknowns and quantify various metabolites in foods such as fish oils, wine, juice, coffee, meat, and beer.[4–11] In food research, the most commonly used NMR techniques involve one dimensional and two dimensional techniques, proton (^{1}H) and carbon (^{13}C) NMR, nitrogen (^{15}N) and phosphorus (^{31}P) NMR.[4,10,12]

This investigation aims to help students understand the growing applications of NMR, beyond that of its traditional uses in organic chemistry. The experiments are designed to introduce students to 1D and 2D NMR techniques through hands-on analysis of Red Bull energy drink. Red Bull is a popular beverage that traces its origins to a Thai energy drink (referred to as Krating Daeng) which was then modified and sold globally.[13-15]

Although other energy drinks can be substituted for this lab exercise, Red Bull is recommended to stay consistent with previously published high-field data which is used to help guide pattern matching and identification.

Preparation and Prerequisite:

The Red Bull experiments should take approximately four hours to perform, an additional two hours for a report, and it is assumed that students have completed introductory concepts of 1D and 2D 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 ^{1}H, HMQC, DOSY and selective excitation spectra. **It is strongly recommended that instructors setup 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 additionally recommended that students complete the Mixture Analysis of Cocktails prior to this lab as it introduces students to basic 1D mixture analysis before moving on to more advanced 2D and specialized experiments outlined in in the Red Bull lab.

Prior to completing the lab, students should be familiarized with processing of both 1D and 2D NMR spectra. This information is readily available in the version 001 Fourier EduLab Students Guide, which can be found on the USB stick delivered with the Fourier 80. In addition, students should have a tutorial learning how to use processing software such as MestreNova.

To perform this experiment, a properly installed and adjusted Fourier 80 system with TopSpin Software is required.

The lab requires the Fourier 80 to be equipped with gradients (important gradient coherence selection in HMQC needed to suppress the water, DOSY and selective excitation). In addition, a Sonicator (optional) and a 1000 µL micropipette should be available.

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.

Selective excitation:
Process of selecting a specific peak or set of peaks in the spectrum and exciting only those spins. This is done to minimize the interference from other peaks and is useful because it allows you to focus on the signal from a specific molecule.

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.

DOSY:
DOSY in NMR is the process of measuring the diffusion of a molecule in a sample. Diffusion Ordered Spectroscopy is done by measuring the intensity of the signal at different positions in the sample to identify the diffusion coefficient of a molecule.

1D ^{1}H WET

PULPROG	wet
TD	4096
SW (ppm)	14.8562
AQ (sec)	1.72032
RG	1
D1 (sec)	2
DS	8
NS	2048
O1 (ppm)	Determine from apex of water signal
P11 (µsec)	80000
SPNAM 7, SPNAM 8, SPNAM 9, SPNAM 10	Sinc1.1000
For power levels use these attenuations relative to calibrated power for an 80ms Sinc1.1000	use power levels sp7 +0.87 dB sp8 -1.04 dB sp9 +2.27 dB sp10 -5.05 dB
GPNAM 21, GPNAM 22, GPNAM 23, GPNAM 24	SINE.100
GPZ21 (%)	80.00
GPZ22 (%)	40.00
GPZ23 (%)	20.00
GPZ24 (%)	10.00
P16 (µsec)	2000.00

HMQC (NUC1=^{1}H, NUC2=^{13}C)

PULPROG	hmqcetgp
TD	512 (F2), 128 (F1)
SW (ppm)	12.9992 (F2), 199.987 (F1)
AQ (sec)	0.24576
RG	1
CNST2	145.00
D1 (sec)	1
DS	16
NS	128
O1 (ppm)	Determine from apex of water signal
O2 (ppm)	90
CPDPRG	garp4
P14 (µsec)	500.0
PCPD2 (µsec)	250.0
SPNAM 3	Crp60,0.5,20.1
GPNAM 1, GPNAM 2	SINE.100
GPZ1 (%)	80.00
GPZ2 (%)	40.00
P16 (µsec)	2000.00

Glossary

Abbreviations

NMR:
Nuclear Magnetic Resonance

DOSY:
Diffusion ordered spectroscopy

HMQC: Heteronuclear single quantum coherence spectroscopy

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

WET:
Water suppression enhanced through T1 effects

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 ^{1}H excitation pulse

DOSY

PULPROG	ledbpgppr2s
TD	4096 (F2), 16 (F1)
SW (ppm)	15.5990 (F2), 10.2288 (F1)
AQ (sec)	1.6384
RG	1
D1 (sec)	1
D20 (sec)	0.200
D21 (sec)	0.005
DS	2
NS	8
O1 (ppm)	Determine from apex of water signal
PLW9 (W)	1e-07 (~66 dB)
GPNAM 6, GPNAM 7, GPNAM 8	SMSQ10.100
GPZ6 (%)	100
GPZ7 (%)	-17.13
GPZ8 (%)	-13.17
P19 (µsec)	1000.00
P30 (µsec)	1000.00

Selective excitation

(will need to be run twice; by changing the O1P,
either the ethanol or propylene glycol can be selectively detected).

PULPROG	selgpse
TD	4096 (F2), 16 (F1)
SW (ppm)	15.5990 (F2), 10.2288 (F1)
AQ (sec)	1.6384
RG	1
D1 (sec)	1
DS	2
NS	8
O1 (ppm)	Determine from apex of water signal
P12 (µsec)	1e-07 (~66 dB)
SPNAM 2	SMSQ10.100
SPW2 (W)	SMSQ10.100
GPNAM 1	-17.13
GPZ1 (%)	-13.17
P16 (µsec)	1000.00

Experimental Setup:

- Red Bull energy drink
- 5 mm NMR tube and cap (1)
- 50 mL beaker and parafilm
- High-field Red Bull 1D and 2D data (found in Appendix I)
- Pulse programs: wet, hmqcetgp, ledbpgppr2s, selgpse

In Topspin, set up the following NMR experiments on the spectrometer (acquisition parameters are listed below): 1D WET (i.e. 1D ^{1}H NMR with WET water suppression), HMQC, DOSY,

Notes

and selective excitation (optional advanced experiment). Note that WET (water suppression enhanced through T_1 effects) was chosen as it is an effective water suppression technique that has become increasingly popular, but other water suppression techniques such as W5-WATERGATE could also be used, or zgcppr.

Template setup is done by copying over any previous parameter set, loading the chosen pulse program, and inputting the experimental values outlined below.

Sample Preparation:

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 in a 50 mL beaker 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. Transfer ~600 µL to a 5 mm NMR tube and cap it.

Experimental Procedure:

Insert sample in the spectrometer and determine the P1 and O1P (chemical shift of the water signal) of the sample. Record a 1D ^{1}H WET spectrum with 2048 scans, an HMQC with 128 scans, and a DOSY experiment with 8 scans. Lastly, run 2 selective excitation experiments with 32 scans; in the first, set the O1P value to that of the most downfield peak in the ethanol triplet, and in the second, set the O1P value to that of the most upfield peak of the propylene glycol doublet.

Data Processing:

All spectra should be processed using standard protocols, including baseline correction and phasing, and calibrated using the sucrose doublet at 5.4181 ppm. More information can be found here: https://pubs.acs.org/doi/10.1021/ed086p360

In the HMQC spectrum, the 1D ^{1}H WET spectrum shall be used as the external projection. Completely removing the water streak from the HMQC can be challenging; options include qfil or qpol (both topspin processing options), t1away (a topspin au) or scaling T_1 noise in commercial packages such as MestraNova. Identification of Red Bull components can be done by comparison with provided high field data. All corresponding 500 MHz data is provided.

More processing information can additionally be found in the version 001 Fourier EduLab student guide.

Results & Discussion:

For all compound identification in the 80 MHz Red Bull spectra, corresponding Red Bull data analyzed at high-field (500 MHz) is provided in Appendix I for reference.

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To complete the exercise, let students answer the following questions:

1. **Identify and label sucrose, β-glucose, α-glucose, citric acid, niacin, and caffeine in the 1D ^{1}H WET spectrum. Discuss one advantage and one disadvantage of using 1D NMR for compound identification.**

   Data analysis and discussion primarily focus on identification of components in Red Bull energy drink, and more advanced experiment types are used to identify compounds not visible in standard 1D ^{1}H NMR. Various NMR prediction software programs are commercially available and could be used, but for simplicity, corresponding Red Bull data analyzed at high-field (500 MHz) is provided for comparison purposes, and is discussed in the education paper by Simpson et al.[1].

   In the aliphatic region of the 1D ^{1}H WET spectrum, the sucrose anomeric proton (doublet) can be identified at ~5.420 ppm. As the most concentrated component (listed first in the ingredients after water), this should be concentrated and resolved enough to clearly assign and was used here for calibration against the Bruker Bio-reference spectral database. The anomeric protons of alpha and beta glucose can also be identified at ~5.240 ppm and ~4.650 ppm respectively. Compared to the high-field data, each doublet's pair of peaks appear more spread out, and this is because the bandwidth of a spectrum on the 80 MHz NMR is less than that of a higher field instrument, whereas the J-coupling value remains the same (i.e. chemical shift scales with field strength but J-coupling does not). In high-field spectra, citric acid appears as a pair of doublets that roof towards each other, each representing a CH_2 group. In the low-field data, the citric acid is well-resolved and easily identifiable, but collapses into 2 singlets.

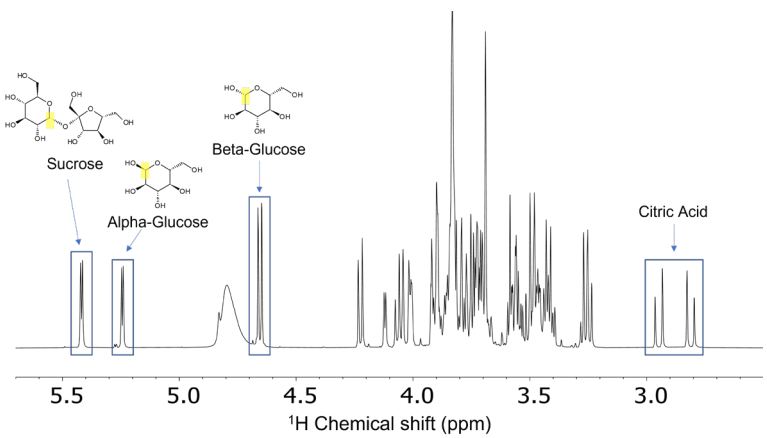

Figure 1. The 2.0-6.0 ppm range of the 80 MHz 1D ^{1}H WET spectrum of Red Bull.

In the aromatic region, 3 of the 4 niacin peaks can be clearly observed (see figure 2) and caffeine can be identified as the high intensity singlet at ~7.910 ppm. Since it is relatively well-resolved, an optional additional aspect to this lab could be to perform a standard addition to quantify the caffeine content and compare it to the listed concentration on the Red Bull can (80 mg/250 mL).

One advantage of using 1D NMR is that experiment time is generally much shorter than in 2D NMR. A disadvantage, particularly for compound identification, is that 1D NMR spectra have less dispersion, meaning that many peaks may be hidden under spectral overlap.

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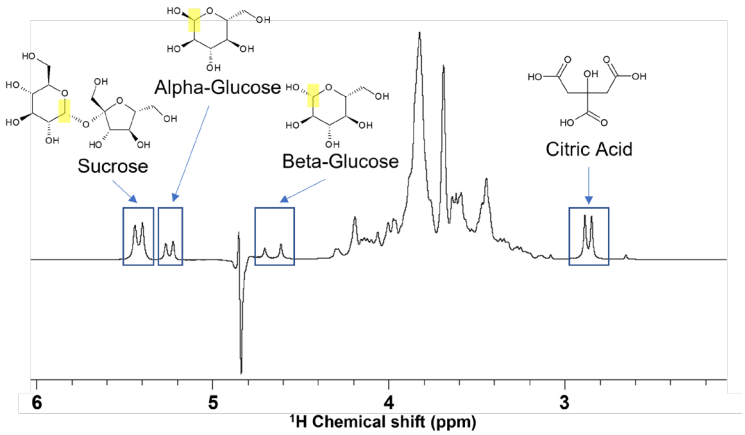

Figure 2. The 2.0-6.0 ppm range of the 80 MHz 1D ^{1}H WET spectrum of Red Bull.

2. Taurine is not visible in the 1D ^{1}H WET spectrum as it overlaps with the sugars, but can be observed in the HMQC spectrum. Using a figure, identify and label the taurine peaks, and explain the advantage of using HMQC NMR for compound identification.

Although a handful of Red Bull components could be identified in the 1D ^{1}H WET spectrum alone, many peaks are hidden by spectral overlap. Therefore, 2D NMR experiments are advantageous in compound identification as they increase spectral dispersion and enable identification of compounds that are not visible in 1D ^{1}H NMR. Here, a 2D ^{1}H-^{13}C NMR experiment that depicts C-H bonds is used (heteronuclear multiple quantum coherence, HMQC). In the HMQC, the taurine peaks can now be observed (in addition to previously assigned peaks), which were hidden under spectra overlap in the 1D spectrum.

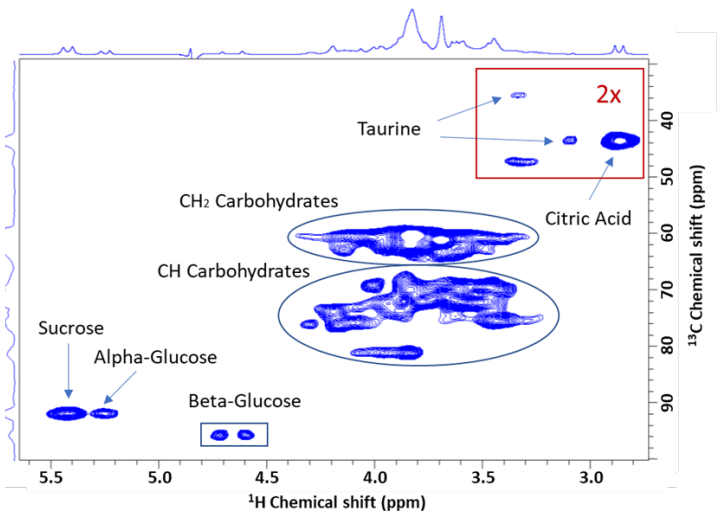

Figure 3. The 80 MHz HMQC spectrum of Red Bull.

3. What information does the DOSY experiment provide? Using a labelled figure, discuss how it can be used to differentiate between sucrose and the glucose anomers.

The Diffusion Ordered SpectroscopY experiment (DOSY) is a 2D experiment that can be used to increase dispersion and differentiate between molecules of different sizes. The DOSY spectrum displays the 1D ^{1}H spectrum on one axis and the diffusion rate on the other, where large, slow-diffusing molecules remain near the top of the spectrum and

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small, fast-diffusing molecules move towards the bottom of the spectrum. Since alpha and beta glucose have the same hydrodynamic radius, they diffuse at roughly the same rate, and DOSY can therefore be a highly effective approach for confirming the positions of the glucose anomers. Sucrose is roughly twice as large as glucose, and therefore diffuses at a slower rate, thereby effectively differentiating it from the glucose peaks in the DOSY spectrum. Figure 4 depicts the carbohydrate region of the Red Bull DOSY.

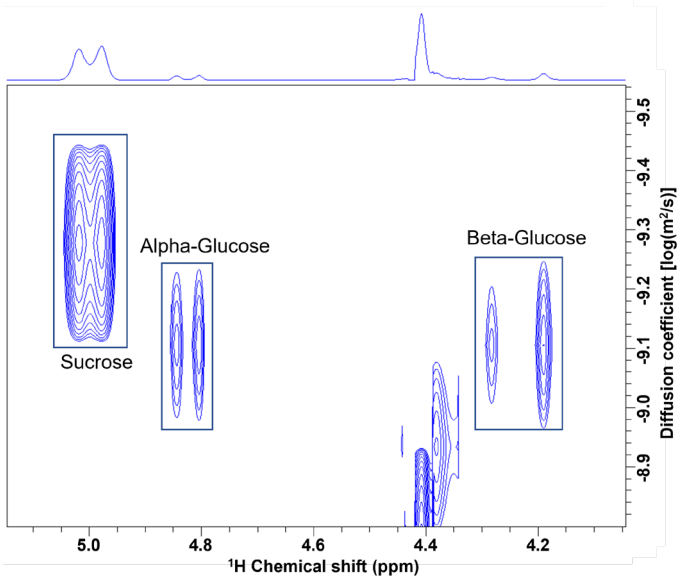

Figure 4. The 80 MHz DOSY spectrum of Red Bull.

4. Using the 1D ^{1}H WET spectrum and the selective excitation results, explain how ethanol and propylene glycol can be differentiated from each other. Demonstrate with a labelled figure. (optional)

An additional experiment that can be run with more advanced NMR teaching labs is the selective excitation experiment; this 1D ^{1}H experiment can be used to selectively detect ethanol or propylene glycol (1,2-propanediol), which overlap around 1.185 ppm in the standard 1D ^{1}H NMR spectrum. Propylene glycol is an ingredient in Red Bull that acts as a flavouring and a preservative, whereas ethanol is not listed as an ingredient as it is only present in very low concentrations as a flavoring. The green spectrum in Figure 5 depicts part of the aliphatic portion of the standard 1D ^{1}H spectrum, wherein ethanol and propylene glycol overlap. In the blue spectrum, only the ethanol is recovered by selective excitation at position A, a peak in the ethanol triplet. In the black spectrum, only the propylene glycol is recovered by selective excitation at position B, a peak in the propylene glycol doublet. Selective experiments such as the one presented here may be particularly useful in low-field NMR analysis, wherein spectral overlap is more challenging than in high-field NMR.

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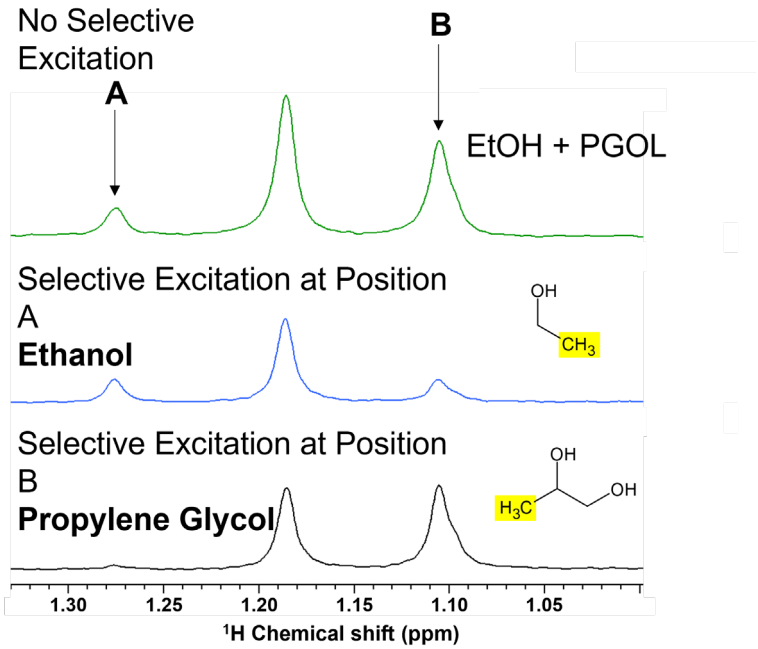

Figure 5. The 80 MHz 1D 1H (green), selective excitation of ethanol (blue) and selective excitation of propylene glycol (black) spectra of Red Bull.

5. **Optional Question: Discuss the water suppression method used and compare it to at least 3 other water suppression techniques from the literature.**

   This is an optional short essay which encourages students to conduct a literature search and learn about different water suppression techniques.

Notes

Key Take Home Messages:

- Recent developments in NMR have expanded its applications beyond historical uses as an analytical tool for structural elucidation in organic chemistry.
- Typically, a combination of 1D and 2D NMR experiments are required to provide information to elucidate and identify components in food samples.
- The DOSY experiment enables differentiation between different sizes of molecules at similar chemical shifts.
- Selective excitation experiments can be used to selectively detect compounds that overlap in 1D spectra.

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Appendix I – High-field (500 MHz) spectra of Red Bull:

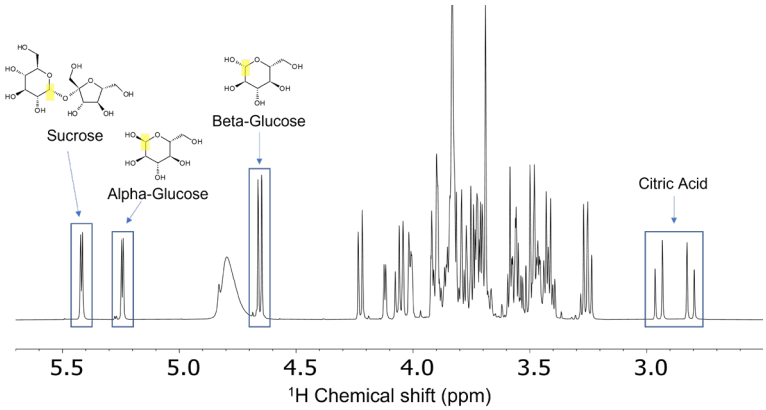

Figure A1. The 2.0-6.0 ppm range of the 500 MHz 1D ^{1}H NMR spectrum of Red Bull (for comparison purposes).

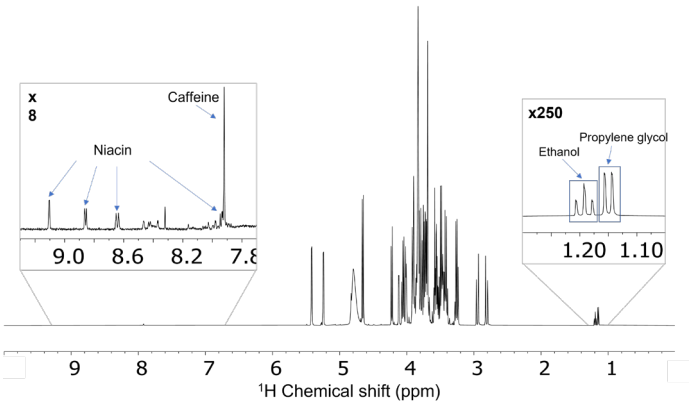

Figure A2. The 500 MHz 1D ^{1}H NMR spectrum of Red Bull, with insets depicting the aromatic region, as well as the ethanol and propylene glycol peaks.

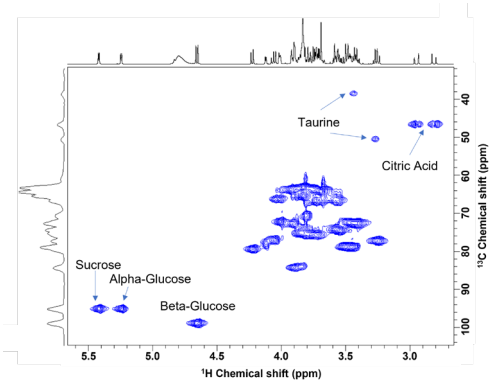

Figure A3. The 500 MHz HMQC spectrum of Red Bull.

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