COLLEGE OF SCIENCE
THE UNIVERSITY OF ALABAMA IN HUNTSVILLE

Honors Capstone Research (HCR) Summer Program 2020

Metabolomic Study of the LEW.1WR1 Rat: Serum Artificial Mixture Validation

Jennifer Nix, Dr. Vogler, Dr. Love-Rutledge,
Department of Chemistry

Overview/Introduction

Type-1 diabetes (T1D) is a complex genetic autoimmune condition where the body is unable to produce insulin units to meet metabolic needs. Without insulin, the body cannot lower its blood glucose which leads to a variety of problems, including loss of eyesight, loss of limbs, a compromised immune system, and more. Researchers looking to prevent T1D have been challenged with identifying all the genetic components of the disease for years. Our lab focuses on studying the metabolome of the LEW.1WRW1 rat (type-1 susceptible model) and looks to contribute to the growing research of elucidating biomarkers for T1D that will help save millions in the future. We planned to accomplish this by first validating an artificial mixture containing analytes of interest and validating such mixture by using nuclear magnetic resonance (NMR) for structure determination. After validation, we will apply the extraction method to blood serum for nontargeted metabolomic studies.

Methods

1. An artificial mixture mimicking observable metabolites in T1Ds was prepared by dissolving 100 mM of alanine, glutamic acid, glucose, aspartic acid, and 3 mM of bovine serum album (BSA) in 10 mM of pH 7 phosphate buffer.
2. 300 uL of chilled chloroform and 200 uL of chilled methanol were pipetted into mixture for extraction.
3. The upper layer of the mixture was placed into 10,000 MW cutoff micron ultracel YM-10 filters and centrifuged at 17,000 x g at 4°C for 10 minutes.
4. The filtrate was placed in a SpeedVac Vacuum concentrator for solvent removal.
5. The remaining pellet was re-diluted in 10 mM phosphate buffer and 150 uL of DSS in 10 % D_2O and transferred to a 5 mm NMR tube for analysis.
6. This artificial mixture was analyzed through the NMR method of nuclear Overhauser effect spectroscopy (NOESY) and performed on a Varian Unity Inova™ 500 MHz NMR spectrometer.

Results

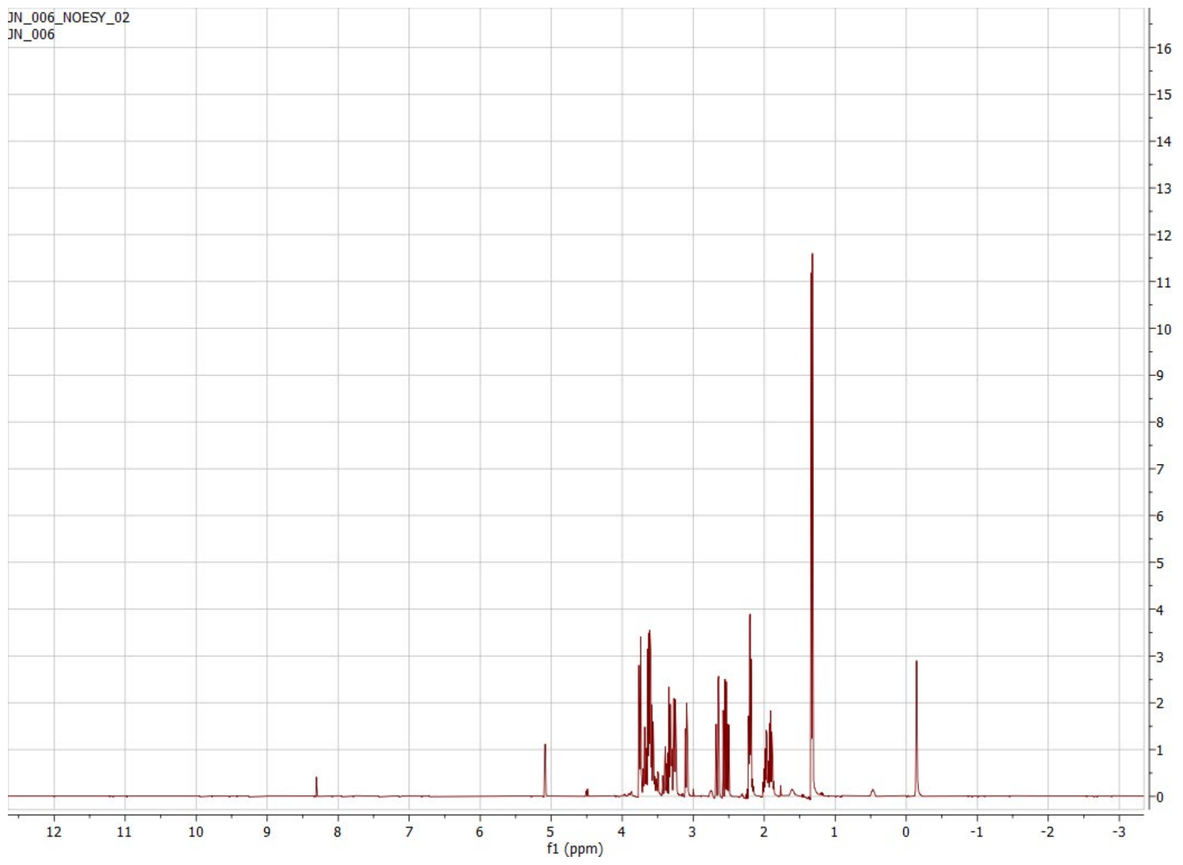

Figure 1. NOESY NMR spectrum of an artificial mixture containing 3 mM of BSA, 100 mM of alanine, glutamic acid, glucose, and aspartic acid dissolved in 10 mM ph 7 phosphate buffer.

Conclusion

NMR is a nondestructive method ideal for metabolomic studies aiming to determine concentrations of substances within a biological sample. Being able to identify signals belonging to metabolites help contribute to disease prevention research such as T1D. Developing proper extraction methods, validating them, and performing them on biological samples helps future researchers discover more unknowns of the inner workings of T1Ds.

Future Outlook

- The protocol still requires modifications for future replication.
- We plan to perform this extraction method on serum extracted from blood samples of our LEW.1WR1 rats.

References

1. Collins, Genoah. The LEW.1WR1 Rat Model is Glucose Intolerant. Chemistry, Master's Thesis, University of Alabama in Huntsville: Huntsville, AL, 2019.
2. Song, Z.; Wang, H.; Yin, X.; Deng, P.; Jiang, W. Application of NMR Metabolomics to Search for Human Disease Biomarkers in Blood. Clinical Chemistry and Laboratory Medicine. 2019. https://doi.org/10.1515/cclm-2018-0380.

Acknowledgements

I would like to thank Dr. Sharifa Love-Rutledge, Dr. Bernard Vogler, and James Wolfsberger for their mentorship, Dean Wilkerson of the Honors college for affording me this opportunity, and Dave Cook for his patience. Special thanks to Madushika Wilmarathne, Quiana Vidal, Vicky McConnell, and Hanna Underhill for their support over the COVID-19 summer.

ALABAMA SPACE GRANT CONSORTIUM — NASA — INDUSTRY — UNIVERSITY — COMMUNITY — 1989

HONORS COLLEGE
THE UNIVERSITY OF ALABAMA IN HUNTSVILLE

NASA