

## 2017 Research Week Proposal

Title – Comparison and Application of Fluorescent and Non-fluorescent Methods of Low Concentration Amino Acid Separation

Program of Study – Biomedical Sciences

Presentation Type – PowerPoint

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Category – Basic

### Abstract:

This research involves the examination of the properties and qualities of different techniques of separating mixtures of low-concentration amino acids using high-performance liquid chromatography (HPLC). Current methods on amino acid separation require the use of derivatizing agents to allow the amino acids to be detected with higher sensitivity than when they are in their underivatized state. Detection techniques include ultraviolet/visible (UV/Vis) spectroscopy and fluorescence detection. Two of these derivatizing agents give amino acids fluorescent properties and include ortho-phthaldialdehyde (OPA) and dansyl chloride [1,2]. In contrast to derivatization, a new method for HPLC separation of amino acids that is commercially available involves the use of a polar-endcapped HPLC column, which has unique properties compared to standard C18 columns which separate the samples slightly differently. This column has been claimed by the manufacturer to separate all 20 common amino acids underivatized and be detected using a set UV wavelength [3]. The goal of this research is to compare the efficiency of these three different HPLC methods of separating mixtures of amino acids and to develop the best method of detecting and separating them. This involves preparing standardized concentrations of common amino acids, such as glycine and alanine, in the millimolar range. These amino acids are then derivatized using the two different methods and processed through an HPLC run. The results are compared to determine what method would be the best to use at low-concentrations. Also, at Liberty University, we wish to develop this research into an educational component of the Organic Chemistry I laboratory curriculum, which would expose our students to practical analytical chemistry techniques as well as the Urey-Miller experiment, which was used by evolutionary scientists to prove that life was formed from the natural prebiotic environment.

### References:

- [1] Tapuhi, Y., Schmidt, D. E., Lindner, W., Karger, B. L. (1980). Dansylation of amino acids for high-performance liquid chromatography analysis. *Analytical Biochemistry* 115:123-129.
- [2] Zhao, M., & Bada, J. L. (1995). Determination of  $\alpha$ -dialkylamino acids and their enantiomers in geological samples by high-performance liquid chromatography after derivatization with a chiral adduct of o-phthaldialdehyde. *Journal of Chromatography A*, 690, 55-63 and references cited within.

[3] Butchart, K., Woodruff, M. (2012). A simple method for resolution of 22 amino acids in LC. <http://www.fortis-technologies.com/resources/Fortis+Poster+HPLC+2012+Amino+Acids+v2.pdf>; accessed Feb-28-2017.