B196 Determination of the Stoichiometry in the Modified Ferric Hydroxamate Test for Gamma-Hydroxybutyric Acid (GHB)

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After attending this presentation, attendees will understand how the method of continuous variation and mole ratio method, utilizing Ultraviolet/Visible (UV/Vis) spectrophotometry, can be used for the determination of the stoichiometry of the purple-colored metal-ligand complex formed in the modified hydroxamate test for GHB.

This presentation will impact the forensic science community by providing an understanding of the mechanism and final structure of the complex that forms in the modified ferric hydroxamate test for GHB.

A rapid color test was developed to detect GHB in human urine in 2002. The test involves converting GHB to Gamma-Butyrolactone (GBL) with acid followed by a ferric hydroxamate reaction with a monohydroxamic acid intermediate. A purple-colored complex with a 3:1 stoichiometry of drug ligand to iron metal was proposed and formed at pH 3; however, results of multiple, previous monohydroxamic acid studies contradicted that finding. Two of the previous studies found a 1:1 stoichiometry between a monohydroxamic acid ligand and iron metal ion and that the complex forms the most intense color at approximately 510nm below a pH of 2. The goal of this study was to further investigate the modified ferric hydroxamate test for GHB.

The methods of continuous variation and mole ratio were used to determine the stoichiometry of the purple complex formed in the modified ferric hydroxamate test for GHB. GBL was used as the starting material for research purposes. The monohydroxamate acid intermediate was formed after the addition of 0.5M hydroxylamine hydrochloride in 95% ethanol and 6M sodium hydroxide. The purple complex formed after the addition of iron (III) chloride and an adjustment of pH using 6M hydrochloric acid. UV/Vis spectrophotometry was used for the analysis and absorbance values were collected at 500nm (l_{MAX} of the complex). Studies of pH were performed by adding 0.2 mL and 0.1mL increments of hydrochloric and sulfuric acids to observe at what pH the complex is formed. Concentration studies were also performed to determine figures of merit including Limit Of Detection (LOD), Limit Of Quantitation (LOQ), Linear Dynamic Range (LDR), and molar absorptivity of the complex (e). These studies were also repeated using lactones of different ring sizes, Beta-Butyrolactone (BBL) and Delta-Valerolactone (DVL). The effect of ionic strength by the addition of sodium chloride was also studied. Lastly, the modified ferric hydroxamate test was analyzed using GHB as the starting material.

It was determined that the purple complex formed in the modified ferric hydroxamate test had a 1:1 stoichiometry of drug ligand to iron metal. This stoichiometry was consistent with GBL, BBL, and DVL. The results of the pH study indicated that the complexes do not form until the pH was below 2. This result was achieved using two different acids with two different concentrations. Results from a concentration study for GBL indicated a LOD of 7.7×10^{-4}M, LOQ of 1.4×10^{-3} M, LDR of 1.4×10^{-3}-5.0×10^{-2}M, and e of 352.2 Liters/mol×cm. The ionic strength studies indicated that the decrease in pH using an acid, not just the addition of chloride ions, was crucial to the formation of the purple complex. Preliminary results from the application of GHB to the modified ferric hydroxamate test indicated that it might not be necessary to add acid for the last step if enough acid was used to convert a sample containing GHB to GBL.

It is important for forensic chemists to understand the chemistry behind this color test because it reinforces validity of their results. Furthering the research behind the modified ferric hydroxamate reaction for GHB may allow for its future use in crime laboratories.

Stoichiometry, GHB, GBL