## K21 Assay of GHB Oxidation Activity Using a Succinic Semialdehyde-Hydrazine Adduct

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After attending this presentation, this presentation will provide attendees the opportunity to learn about the development of an assay for GHB oxidation, relying on the formation of a succinyl semialdehyde- hydrazine adduct, as determined by ultraviolet/visible absorbance (UV), high pressure liquid chromatography (HPLC) with UV and mass spectrometric detection (MS).

This presentation will impact the forensic and toxicological communities by introducing a new assay for the oxidation of GHB.

Forensic toxicologists and pathologists are regularly called on to evaluate the role and magnitude of effects of GHB in Drug-Facilitated Sexual Assaults (DFSA), accidental overdoses, and homicides. This laboratory is interested in the kinetics of GHB catabolism. The first step of GHB metabolism is oxidation to succinyl semialdehyde. Subsequently, oxidation of succinyl semialdehyde to succinate rapidly follows. To allow for an effective analysis of GHB metabolism based on product formation, or cofactor reduction, the assay methodology must effectively eliminate the oxidation of succinyl semialdehyde to succinate. The feasibility of utilization of hydrazine sulfate as an "aldehyde trap," allowing the termination of the reaction at succinyl semialdehyde via formation of a readily detectable, unique product, the succinyl semialdehyde-hydrazine adduct has been investigated. Previous studies suggest that hydrazine sulfate may be an effective means of trapping succinyl semialdehyde that would not be expected to interfere with the metabolism of GHB to succinyl semialdehyde.

The purpose of this study is to identify a succinic semialdehyde-hy- drazine adduct for use in an HPLC-MS assay for oxidation of GHB. An HPLC method has been previously developed for identification and quanti- tation of succinic semialdehyde in urine. The HPLC is operated at a flow rate of 1 mL/min with a mobile phase of 80 mg/L ammonium acetate buffer (pH 3.6) in 1:1 acetonitrile:water. Post column, the flow is split 1:4 producing a flow rate of 200 µL/min to the mass spectrometer. Electrospray ionization was used in the negative ion mode. The temperature of the electrospray was set at 400ºC (Struys EA et al, J Inherit Metab Dis, 28:913, 2005).

The succinyl semialdehyde-hydrazine adducts can take several forms. The following ions have been identified when excess succinyl semialdehyde is reacted with hydrazine sulfate: succinyl semialdehyde (m/z 102) and succinyl semialdehyde-hydrazine adducts (m/z 141, 183, 225, 257). The m/z 141 is consistent with an adduct comprising a heterocyclic ring using one succinyl semialdehyde molecule and hydrazine. Higher m/z values are expected to correlate with an adduct comprising two succinyl semialdehyde molecules with hydrazine. Increasing the amount of hydrazine used in the assay causes the heterocyclic product (m/z 141) to be favored; which offers a single product measurable by UV and MS detection.

Formation of adduct is reliant upon formation of aldehyde. Therefore, quantitation of the succinic semialdhyde-hydrazine adduct allows for determination of the rate of GHB oxidation to succinyl semialdehyde. By optimizing the HPLC-MS method for the detection of the oxidized product (in this case the succinyl semialdehyde-hydrazine adducts), the method is functional for determination of the kinetics of GHB oxidation.

**Gamma-Hydroxybutryrate, Assay, Succinyl Semialdehyde**

 ** Presenting Author*