K52 A Segmental Analysis of Endogenous Gamma-Hydroxybutyric (GHB) Acid in Human Hair

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After attending this presentation, attendees will be informed about a validated Liquid-Liquid Extraction (LLE) and Liquid Chromatograph/Tandem Mass Spectrometric (LC/MS/MS) method for the detection of GHB in human head hair. The validated method uses multi-point calibration curves and quality control samples. The research presented also investigates segmental analysis of hair samples and inter- and intra-variation among individuals.

This presentation will impact the forensic science community by providing information regarding endogenous GHB concentrations in hair. The research presented will address baseline levels of GHB within and between individuals to determine if a background threshold can be established for an unexposed population. Segmental analysis of hair samples will provide information regarding the variation of endogenous concentrations within an individual over a period of time that corresponds to hair growth rates.

GHB has been used in drug-facilitated crimes and is also a popular recreational drug of abuse. Ingestion of this drug may induce euphoria, amnesia, dizziness, and unconsciousness, depending on dosage. Because GHB is a natural chemical found in humans, it can be difficult to separate naturally occurring levels from levels following ingestion. GHB poses an additional challenge to the forensic community in that it is rapidly excreted by the body. Hair analysis is a good alternative to blood and urine due to the longer detection window available to establish the involvement of drugs in reported crimes. The scientifically accepted mean growth rate of human head hair is 1cm per month, which can be used to estimate the time period of ingestion. This research utilizes segmental hair analysis to determine baseline GHB concentrations among non-GHB users and to evaluate variability along the length of the hair. Knowing how much variation is present within an individual will help determine if an individual can serve as their own control in cases of ingestion.

Before collecting hair samples, a full quantitative validation was performed for the extraction procedure. The parameters assessed were: accuracy, precision, calibration model, carryover, interferences, Limit Of Detection (LOD), Limit Of Quantitation (LOQ), and processed sample stability. Due to the endogenous nature of GHB in hair, ionization suppression/enhancement experiments were not completed. Instead, the study relied on the deuterated GHB to compensate for any suppression or enhancement that may occur. Accuracy and precision were found to be within ±20% at low (1.2ng/mg), medium (4.0ng/mg), and high (9.6ng/mg) concentrations. A linear model was obtained from 0.4ng/mg to 12ng/mg and no carryover was observed in unspiked synthetic hair samples following injections of 12ng/mg or 24ng/mg GHB. No interfering signals (not including background GHB) were observed in hair extracts. The LOD and LOQ of the method were experimentally determined to be 0.4ng/mg. Lastly, extracts were observed to be stable after eight days while being stored at ≤14°C.

To evaluate the baseline GHB concentrations, hair collected from non-GHB users was segmented into 1cm increments based on proximity to the scalp. The segments were washed with organic solvents, cryogenically ground, and digested with sodium hydroxide. After digestion, the samples were neutralized with sulfuric acid and extracted via LLE with ethyl acetate. The extracts were then evaporated to dryness, reconstituted in mobile phase, and filtered for analysis by LC/MS/MS to determine the levels of GHB present. Initial results for 37 non-GHB users reveal an average baseline GHB concentration of 0.90ng/mg, with a minimum of 0.43ng/mg, maximum of 3.49ng/mg, and median of 0.84ng/mg. The average variation within individuals was 13%. Work continues on the processing of additional hair samples from other non-drug users.

GHB, Hair Analysis, LLE