K62 Determination of Gamma-Hydroxybutyric Acid (GHB) in Hair Using Alternative Derivatization Techniques

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After attending this presentation, attendees will better understand a new method of derivatization for improved separation of GHB and urea in hair samples, following methanolic incubation, by Gas Chromatography/Mass Spectrometry (GC/MS). Attendees will also be provided with an overview of the effects of external factors such as diet, drug use, etc., on the levels of endogenous GHB in human hair.

This presentation will impact the forensic science community by providing information about an alternative derivatization technique which allows for better separation of silylated GHB and urea in hair than what is currently achieved by using N,O-Bis(trimethylsilyl) Trifluoroacetamide (BSTFA) as a derivatizing agent.

GHB is an important forensic drug due to its potential for abuse and its implication in drug-facilitated crimes; however, it is also an endogenous compound and is therefore found in low concentrations in human hair. GHB is only detectable in blood and urine samples for up to 12 hours. Therefore, in cases of suspected GHB use, hair can be used as an alternative matrix. When analyzing hair samples for GHB, the most commonly used derivatization reagent is BSTFA with 1% Trimethylchlorosilane (TMCS). One issue that is commonly encountered using BSTFA is the incomplete separation of GHB and urea by GC/MS.

Derivatization with BSTFA is a suitable method when analyzing hair samples by Two-Dimensional Gas Chromatography/Time-of-Flight/Mass Spectrometry (GCxGC/TOF/MS) or by Gas Chromatography/Tandem Mass Spectrometry (GC/MS/MS), which are both able to sufficiently separate GHB and urea, but most forensic laboratories do not have access to this instrumentation. Therefore, an alternative method of derivatization is needed which will allow for better separation by GC/MS. The following derivatization reagents were analyzed for their ability to separate GHB and urea: BSTFA with 1% TMCS, N-methyl-N(tert-butyldimethylsilyl trifluoroacetamide) (MTBSTFA) with 1% tert-butyldimethylchlorosilane (TBDMCS), boron trifluoride ($BF_{3)}$ in methanol, pentafluorobenzyl bromide (PFBBr), and pentafluoropropionic acid anhydride (PFPA). MTBSTFA was found to yield enhanced separation compared to BSTFA. The alkylation and acylation reagents, BF_3, PFBBr, and PFPA, were not found to sufficiently derivatize GHB or urea for GC/MS analysis.

The determined method of derivatizing with MTBSTFA was used to analyze hair samples collected from volunteers in a university community (students, faculty, and staff). Each participant answered questions about their diet, hair treatments, personal life, and drug and alcohol use. For each sample, the provided answers were compared to the detected GHB concentrations in order to determine any effect of the external factors on endogenous GHB concentrations. Concentrations were between 0.2ng/mg and 1.0ng/mg. Some outliers were detected, indicating possible links between diet, lifestyle, hair treatment, and GHB concentration.

GHB, Derivatization, GC/MS