K63 The Simultaneous Determination of Gamma-Hydroxybutyric Acid (GHB) and GHB-Glucuronide (GHB-Gluc) in Urine Using Hydrophilic Interaction Liquid Chromatography-Tandem Mass Spectrometry (HILIC-MS/MS)

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After attending this presentation, attendees will better understand a new method using HILIC-MS/MS to determine GHB and GHB-Gluc in urine. A new separation method is necessary to detect GHB and its glucuronide metabolite in urine to better understand the role of the glucuronide in the metabolism of GHB and the potential usefulness of the metabolite in the analysis of GHB in urine samples.

This presentation impacts the forensic science community by introducing a novel separation procedure using HILIC-MS/MS to simultaneously determine GHB and its glucuronide metabolite in urine.

GHB is an endogenous compound in mammalian tissue. It is classified as a Schedule I controlled substance that is highly addictive with low medicinal properties and has been abused in health clubs, raves, and in Drug-Facilitated Sexual Assault (DFSA) cases.[1] GHB is rapidly eliminated from the body after its absorption, making it difficult to detect.[2] GHB-Gluc is a recently discovered metabolite of GHB whose role in the metabolism of GHB still requires investigation and is not well understood.[3] There is currently no method to detect GHB and its metabolite, GHB-Gluc, simultaneously in biological fluids. Difficulty in the analysis of GHB and its glucuronide metabolite can arise due to the polarity of the compounds. Because they are small and polar molecules, HILIC can be utilized to achieve optimum separation.[4]

A Macherey-Nagel NUCLEODUR® HILIC column (100mm x 2mm, 3µm) connected to an MS/MS with an Electrospray Ionization (ESI) source operated in the negative ion mode was used for all analyses. Mass spectrometric analysis was performed in the Multiple Reactions Monitoring (MRM) mode using appropriate collision energy for each selected precursor ion. MRM transitions monitored for GHB included m/z of 103 to 85, 103 to 101, and 103 to 59 for quantitation. The MRM transitions monitored for quantitation of GHB-Gluc were m/z of 279 to 103, 279 to 113, and 279 to 59. Chromatography was performed at 50°C using a binary flow method with mobile phases of 0.1% (v/v) formic acid in water (pH=7) as the strong phase and 0.1% (volume/volume (v/v)) formic acid in acetonitrile for the weak phase. The weak phase was held at 90% for two minutes, then decreased to 60% for five minutes, and held for three minutes. The weak phase was increased back to 90% for five minutes to allow the column to re-equilibrate for the next sample. The total acquisition time was 18 minutes. GHB and GHB-Gluc eluted at approximately two and nine minutes, respectively. The spiked urine samples were diluted 1:4 with deionized water, filtered, then 5µL was injected into the HILIC column. The method displayed good linearity in the concentration range of 1µg/mL to 100µg/mL for GHB and GHB-Gluc with a R^2=0.99. The Limit of Detection (LOD) and Limit of Quantitation (LOQ) for GHB and GHB-Gluc were approximately 13µg/mL and 40µg/mL. This method was calculated to be repeatable through Analysis of Variance (ANOVA) testing.

The method was validated in urine samples with regard to linearity, sensitivity, selectivity, precision, accuracy, and recovery. This method could be used in forensic toxicology laboratories for victims of DFSA, Driving Under the Influence (DUI) suspects, and postmortem investigations.

Reference(s):

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3. Petersen I.N., Tortzen C., Kristensen J.L., Pedersen D.S., Breindahl T. Identification of new metabolite of GHB: gamma-hydroxybutyric acid glucuronide. *J Anal Toxicol.* 2013 Jun;37(5):291-7.
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GHB, Glucuronide, HILIC