K37 A Novel LC/MS/MS Method for the Detection and Quantitation of GHB

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After attending this presentation, attendees will be introduced to a new LC/MS/MS method that can be used for the detection of gamma-hydroxybutyric acid (GHB) using Hydrophilic Interaction Liquid Chromatography (HILIC).

This presentation will impact the forensic science community by supplying an effective method of GHB analysis that is time efficient, involves very little sample preparation, and does not require sample derivatization.

GHB is a common Schedule I central nervous system depressant that is often implicated in the commission of many drug-facilitated sexual assaults because it leaves the victim confused, unable to resist, incapacitated, and promotes memory loss. GHB is also clinically used for the treatment of cataplexy. HILIC is a newer technology, and although there has not been extensive research done on this particular chromatography, it has been shown to be useful for the analysis and separation of polar compounds. Due to this property, it has been speculated that it would work very well for GHB, but has yet to be applied to biological samples, such as urine. HILIC works by applying a water-miscible mobile phase across a strongly hydrophilic stationary phase. This current research focused on the development of a new LC/MS/MS method that can be used in conjunction with HILIC to identify and quantify GHB in urine samples. This method included a Zwitterion Chromatography (ZIC^{O}) HILIC column (3.5mm, 100 x 2.1mm) from SeQuant. The mobile phase consisted of 10mM ammonium formate in water, pH 6.38, for Solvent A, and 100% acetonitrile for Solvent B. The following gradient was employed: 90% organic to 40% organic in 20 minutes (~2.5% per minute), then back up to 90% organic at 10% per minute, maintaining a flow rate of 0.2 mL/min for the duration of the run. The gradient allowed sufficient time for the compound to come off the column, and also for the column to re-equilibrate, which is important due to the sensitivity of the column. Additionally, the gradient provided a wash step to clean the column of any impurities that had the potential to be carried over into subsequent samples. LC/MS/MS was performed using electrospray ionization with negative ion mode monitoring. Ions were examined in MRM mode, and the 103 to 85 and 103 to 101 transitions were used. GHB-d6 was used as the internal standard, and the 109 to 90 transition was used. A linear range of GHB standards were observed from 0.01ug/mL to 10 g/mL. GHB was also detected in synthetic urine in concentrations as low as 0.04mg/mL. It was found that injecting straight synthetic urine did result in some ion suppression; however, when the samples were diluted by 75% with acetonitrile, there was not as much ion suppression. Potential interferences, such as gamma-butyrolactone (GBL) and 1,4-butanediol, will also be tested to ensure that drugs with similar structures to GHB will not provide any interference with the results on this particular column. Blind studies will also be performed.

GHB is often difficult to detect due to its increased rate of metabolism and excretion in the body; this new method will provide a simple, quick method for the detection of the drug in urine without extensive extraction or derivatization procedures using ZICÒ-HILIC and LC/MS/MS.

LC/MS/MS, ZIC-HILIC, GHB

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