K13 Determination of GHB in Blood and Urine Using GC/MS/MS Without Derivatization

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After attending this presentation, attendees will learn about a new method for determining concentrations of Gamma-Hydroxybutyric Acid (GHB) and Gamma-Butyrolactone (GBL) in toxicology specimens by Gas Chromatography Tandem Mass Spectrometry (GC/MS/MS), without the need for derivatization. Compared to ethanol, GHB is a more powerful central nervous system depressant that can be used and abused for multiple purposes with deadly consequences. Forensic toxicology laboratories that analyze specimens from Driving While Intoxicated (DWI) suspects, victims of Drug-Facilitated Sexual Assault (DFSA), and postmortem investigations need a durable method for GHB/GBL analysis to invoke when necessary.

This presentation impacts the forensic science community by relaying a novel procedure that utilizes the sensitivity and selectivity of GC/MS/MS, while promoting a more routine workflow by negating the need for hazardous derivatization agents. GHB is a controlled substance used for medicinal therapy of cataplexy, insomnia, and fibromyalgia. Because of its intoxicating effects, GHB can be abused in bodybuilding, lowering inhibitions or rendering a victim defenseless in DFSA, and as a recreational club drug. GHB is a relatively small polar molecule that can be difficult to detect and is rapidly eliminated from the body after ingestion. Low levels of endogenous GHB are common in all mammals, complicating detection and toxicological interpretation.

Derivatization is a common method for GHB analysis; however, derivatizing agents increase costs, are harmful to gas chromatography columns, and are hazardous to the analytical chemists who use them. Acid-catalyzed conversion of GHB to GBL is a much cleaner and less expensive way to analyze GHB. In the presence of concentrated sulfuric acid, GHB converts from its linear polar form to GBL, a less polar-cyclic lactone which is less damaging to the GC column since it does not need to be derivatized. By avoiding derivatization, both time and money are saved by cutting out extra steps with derivatizing agents. In this method, extracts of GHB from the sample are split into cyclized and non-cyclized preparations for analysis. Any GBL already present in the sample will be revealed by its inherent volatility, while the remaining non-cyclic GHB is converted to GBL using sulfuric acid and therefore detected as GBL in the acquisition method.

Specifically, a liquid-liquid extraction employing methylene chloride was used to extract blood and urine samples fortified with varying concentrations of GHB. This novel method produced an average R^2 value of 0.99 in urine (n=3) and 0.99 in blood (n=3). The range of the calibration curve was linear from 10mg/L to 400mg/L of GHB in blood and urine. The acquisition method involved a splitless injection of 1µL of extract at 250°C into a DB35 ultra inert GC column. The oven program was set to an initial temperature of 50°C for 2 minutes, then increased at a rate of 50°C/min to 280°C with a hold of 6.5 minutes. The flow rate was set to 1.5mL/min with a total run time of 13.1 minutes. A modest 10V collision energy potential was sufficient to produce optimal fragmentation ions. The transition product ions for GBL were identified at 86m/z to 42m/z for quantitation and 86m/z to 39m/z for qualifier ions. Due to its similar size and properties, delta-valerolactone was used as an internal standard for this analysis. By using this non-deuterated internal standard, the R^2 value of the calibration curve only needed to be above .98 to be acceptable. The transition product ions of delta-valerolactone were determined to be 100m/z to 41.1m/z for quantitation and 100m/z to 56.1m/z for qualifier ions.

The method was validated in blood and urine for routine screening of DFSA casework, as well as evaluating DWI suspects and postmortem cases. Other published studies have recommended a 10mg/L cutoff to accommodate a low level of GHB while recognizing a level above endogenous concentration. Meanwhile, the upper limit of quantitation extends well into the range of blood concentrations reported to cause fatal toxicity. This procedure will reduce costs and improve time management, while providing a sensitive and selective method for GHB analysis.

Gamma-Hydroxybutryic Acid, Gas Chromatography Tandem Mass, Gamma-Butyrolactone

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