K20 Stability of Exogenous GHB in Antemortem Blood and Urine Under Various Temperature and Storage Conditions

Albert A. Elian, MS, Massachusetts State Police Crime Laboratory, 59 Horse Pond Road, Sudbury, MA 01776*

After attending this presentation, attendees will learn how storage conditions will effect GHB concentration in blood and urine.

This presentation will impact the forensic community and/or humanity by demonstrating the effect of long storage on GHB levels in blood and urine.

The stability of exogenous GHB in three blood and three urine samples under a variety of storage conditions; room temperature, 4°C and
-20°C, was evaluated over a period of six months. GHB concentration increased the most at room temperature, with almost no change at the lower temperature.

Gamma-hydroxybutyric acid (GHB) is an endogenous substance found in the body. This central nervous system depressant, which was first synthesized in the 1960s, has been used for induction of anesthesia, treatment of narcolepsy, and for alcohol and opiates withdrawal. Recently, GHB has been used illicitly by bodybuilders to increase the release of growth hormone, ravers attendees for its euphoric, sedation and muscle relaxation after ecstasy use, and victims of drug-facilitated sexual assault (DFSA) [8-10].

Due to the increased demands on forensic toxicologists to analyze GHB in cases such as DFSA and operating motor vehicles under the influence, there are often variable time intervals between collection of the specimen and analysis. A literature review has revealed no stability study on antemortem blood or urine exogenous GHB levels. However, one study reported the effect of storage on endogenous GHB antemortem urine levels, and another study investigated the effect of storage conditions on GHB-free and spiked urine antemortem concentration.

Quantitation of GHB was achieved by liquid-liquid extraction, followed by concentration of the extracts and derivatization with BSTFA. Analysis was performed on an Agilent 6890 gas chromatograph interfaced with an Agilent 5975 mass selective detector. A 12m x 0.25mm (internal diameter), 0.25mm (film thickness), HP-1MS column (100% polydimethylsiloxane) was used with helium as the carrier gas at a flow rate of 2.0 mL/min. An Agilent 7683 automatic sampler was used for injection into the gas chromatograph. The splitless injection mode was used with the valve closed for 0.25 min, and 2ml samples being injected. The operating conditions for the analyses were injection port, 280°C; the detector, 300°C; initial oven temperature, 60°C for 2 min increased at 30°C/min to 300°C, holding for 1 min. The mass spectrometer was operated in the SIM mode. The ions selected for monitoring were chosen from full scan mass spectral analyses of the analytes that gave minimum interference. The following ions were monitored: GHB: m/z 233,234,235 and GHB-d_6: m/z 239,240,241.

Three actual blood (20, 50, and 75 mg/L) were submitted to the laboratory in test tubes containing sodium fluoride. Three actual urine samples (33, 108, and 220 mg/L) were submitted in plastic jars with no preservative added. The samples were chosen, from casework, to cover a wide range of concentrations. The specimens were analyzed at the time of arrival in the laboratory and then divided into three sets as described above.

For the blood stored at -20°C there was an increase in GHB concentration of 1-12%, at 4°C 3.4-16%, and 20°C 9.6-28% (Fig. 1-3). For the urine stored at -20°C there was an increase in GHB concentration of 1-15%, at 4°C 1-27%, and at 20°C 3.6-44% (Fig. 4-6), with the highest increase in GHB concentration in the lower concentrations (Fig. 1 and 4). This could be attributed to the fact that a small increase in the GHB level would be enough to significantly change to the measured level.

Storage, Gamma-Hydroxybutyric Acid (GHB), Exogenous

 ** Presenting Author*