K8 Double-Suicide by Gamma-Butyrolactone (GBL) Ingestion: An Analysis by Headspace/Solid-Phase Microextraction Coupled to Gas Chromatograph/Mass Spectrometer (HS/SPME/GC/MS) and Tandem Columns

Brian Joseph Waters, MS, Fukuoka University, Department of Forensic Medicine, Fukuoka 814-0180, JAPAN; Kenji Hara, PhD, Fukuoka University, Fukuoka 814-0180, JAPAN; Natsuki Ikematsu, BPharm, Fukuoka University, Fukuoka 814-0180, JAPAN; Mio Takayama, PhD, Fukuoka University, Fukuoka 814-0180, JAPAN; Aya Matsusue, PhD, Fukuoka University, Fukuoka 814-0180, JAPAN; Masayuki Kashiwagi, PhD, Fukuoka University, Fukuoka 814-0180, JAPAN; Shin-ich Kubo, PhD, Fukuoka University, Fukuoka 814-0180, JAPAN*

Learning Overview: After attending this presentation, attendees will better understand the toxicological findings that indicate death due to ingestion of GBL, a metabolic precursor to Gamma-Hydroxybutyrate (GHB).

Impact on the Forensic Science Community: This presentation will impact the forensic science community by demonstrating an efficient and robust method for the determination of GBL and GHB from biological fluids.

In May of 2018, a man (Case 1) in his early forties went missing from the hospital room where he was staying. He left a handwritten will stating he would die in the backyard of a former male coworker (Case 2) in his late sixties. The note included the name and address of the coworker. Both men were found dead lying side by side on a blue tarp in the backyard of the address written on the will. Near their feet were many empty packages of sleep medication, wine and whisky bottles, unlabeled brown bottles containing an unidentified liquid, and other trash.

Blood and gastric samples from both men were obtained for toxicological analysis. Prior to analysis, ethanol quantitation and screening by Liquid Chromatography/Mass Spectrometry (LC/MS) had been performed by another laboratory. Routine GC/MS screening revealed the presence of GBL, a colorless liquid with a characteristic odor that is often used as a precursor to other chemicals. GBL undergoes rapid enzyme-mediated hydrolysis or oxidation to GHB *in vivo*. The presence of GHB was confirmed by GC/MS analysis after Trimethylsilyl (TMS) derivatization.

For quantitation, duplicate aliquots of 200µL of blood or diluted gastric contents were added to 10-mL headspace vials with an equal volume of distilled water. Sodium chloride was added along with an internal standard, GBL-d_6; 20µL of 5N hydrochloric acid was added to one sample and 20µL of water was added to the other. The vials were capped and pre-heated for 5min at 70°C. An HS/SPME fiber was used to extract the GBL from the samples. The SPME fiber was inserted and exposed to the headspace and continued to be heated at 70°C for 10min. After adsorption, the fiber was inserted into the GC inlet and desorbed for 2min at 250°C. In the vial containing acid, any GHB in the sample was converted to GBL. In the vial omitting the acid, the GHB was not converted. The difference between the GBL quantitation in the acid sample and the non-acid sample represented the amount of GHB in the specimen.

Separation was performed on a Shimadzu® TQ8030 GC/MS with tandem columns consisting of a Rtx®-200 (5m x 0.15mm x 0.25µm df) pre-column connected serially to an Rtx®-200 (8m x 0.18mm x 0.4µm df) separation column. Injection was in the split mode at 1:15. Helium was the carrier gas with an initial pressure of 259.7kPa. The interface and ion source temps were 260°C and 230°C, respectively. The initial column temp was 60°C for 0.8min, ramped to 250°C at 70°C/min, and held for 3min. The MS was operated in the scan-SIM mode (scan: *m/z* 40-550; SIM: *m/z* 42, 86 for GBL, *m/z* 48, 92 for GBL-d_6) with electron impact ionization at 70eV.

Case 1 had absolute GBL concentrations in the blood and gastric contents of 75µg/mL and 695µg/mL, respectively. Absolute GHB concentrations (as total GHB-absolute GBL) in the blood and gastric contents of Case 1 were 404µg/mL and 103,955µg/mL, respectively. Absolute GBL concentrations in the blood and gastric contents in Case 2 were 64µg/mL and 782µg/mL, respectively. Case 2 had absolute GHB concentrations in the blood and gastric contents of 367µg/mL and 43,436µg/mL, respectively.

The GBL and GHB levels were consistent with other fatalities attributed to GHB/GBL intoxication in the literature. Both men had several different compounds in their system, including sleep medication and alcohol, in various amounts.

Gamma-Butyrolactone, Gamma-Hydroxybutyrate, Postmortem