K41 Gamma-Hydroxybutyrate (GHB) in Saliva: A GC/MS Method Applicable to Toxicological and Physical Evidence

Giorgia De Paoli, PhD, West Virginia University, Oglebay Hall, 1600 University Avenue, Morgantown, WV 26506; and Suzanne C. Bell, PhD, West Virginia University, Department of Chemistry, PO Box 6045, Morgantown, WV 26506-6045*

The goal of this presentation is to introduce attendees to the potential use of saliva as an alternative biological matrix and as a tool in GHB screening analysis and to establish GC/MS as a sensitive analytical technique for the detection of GHB in saliva.

This presentation will impact the forensic science community by describing a proposed method for the rapid, selective and accurate toxicological screening of saliva analysis for forensic purposes. The use of a surrogate standard provides a quantitative measure of extraction and prepa- ration efficiency that is matrix specific. The method described here could be applied to swabs, neat saliva, and possibly physical evidence such as saliva on drink glasses. Current research is focused on the latter application.

GHB and related compounds have been known for years because of their illicit use in drug facilitated sexual assault (DFSA) and to a lesser extent, as party drugs. This problem is exacerbated by GHB's rapid clearance rate and short half life of ~30 min. For this reason, it would be useful to develop a rapid screening analysis from a biological matrix that predictably tracks plasma drug concentrations. Oral fluids, which can be collected non- invasively, are an attractive option. Unfortunately, saliva drug concentra- tions are generally significantly lower than those in urine, which creates challenges for method development.

A sensitive and specific gas chromatography-mass spectrometer (GC- MS) method has been developed using selective ion monitoring (SIM) for the identification and quantification of gamma-hydroxybutyric acid (GHB) in saliva. In this approach, 1.0 µl of synthetic saliva was spiked with 1.0 µl of GHB-d6 as the internal standard. As an added quality assurance method,
1.0 µl of 1,7-heptanediol is added to all samples as a surrogate spike. The purpose of the surrogate is to track the efficiency of extraction and preparation procedures.

After a silyl-derivatization the sample was injected at a split ratio of 10:1. The following ions were monitoring: GHB 233, 234; GHB-d6: 239,
240, 241; 1,7-heptanediol: 55, 73, 97. No interferent peaks were observed. The LOQ was determined to be 0.5 ppm with a linear dynamic range of 0.5 ppm to 50 ppm. Quality-control samples (5 ppm, 20 ppm, 30 ppm) were prepared for evaluation of analytical precision. Variation was found to be from 1.07 to 9.44% in both intra-day and day-to -day experiments respectively. Surrogate recovery from saliva samples fell in the range of
94.6 to 100% with an average of 98.37% and a corresponding % RSD of 1.2%. Data obtained from validation were compared with results from sample prepared drying saliva before the derivatization process. Blank samples from lab staff were analyzed to estimate endogenous GHB in saliva. Values in the range of 2-3 ppm were typical. These results will be presented.

The success of this method suggests a novel extension to physical evidence. Victims of sexual assault may leave biological evidence such as saliva on surfaces like the exterior of glasses, tissues, and cigarettes. If the saliva was deposited after the illicit drugging occurred, the deposited saliva may provide valuable investigative information and evidence of a drug- facilitated assault. With drugs such as GHB that have rapid clearance rates, the capability to detect elevated GHB in deposited saliva samples could be significant.

In the present work, we utilized the GC/MS method for a screening test for GHB in saliva on objects such as cigarettes, bottles, cups, plastic glasses. Blank saliva was spiked with GHB at different concentrations. 5 µl of those samples were then spread out on the surfaces. At time intervals, saliva samples were extracted from the objects with a swab saturated with methanol. After a centrifugation, the supernatant was dried and reconstituted in 500uL of methanol, of which 1 uL was injected into the instrument. This methodology precludes quantitation, but does afford reliable qualitative results at low concentrations.

Gamma-Hydroxybutyric acid (GHB), GC-MS, Saliva

 ** Presenting Author*