Endogenous GHB levels in ante-mortem whole blood and urine – the effect of different additives and storage conditions

Sørensen L.K[1], Faldborg K.B[1], Andersen C.U[1]. and Hasselstrøm J.B.[1*]

[*]lead presenter, jbha@forensic.au.dk
[1]Section for Forensic toxicology and drug analysis, Department of Forensic Medicine, Aarhus University, Denmark

Detection γ-hydroxybutyric acid (GHB) intake is crucial in drug facilitated crimes, but difficult due to latency between intake and sampling, endogenous levels, and production after sampling. This study aimed to develop a liquid-chromatography-tandem-mass-spectrometry method for selective determination of endogenous GHB in human ante-mortem whole blood and urine. Furthermore, we investigated the stability of GHB after sampling and endogenous levels in samples taken under controlled conditions.

Whole blood proteins were precipitated using methanol and acetonitrile, and the extract was cleaned-up by anion exchange. Separation of the analytes from structural isomers was obtained using a reversed-phase column with anion properties. The selective clean-up and the use of 13C-labelled analogues as internal standards made it possible to use pure solvent calibrants. The validated lower limits of quantification were 0.005 µg/mL in blood and 0.010 µg/mL in urine. The relative intra-laboratory reproducibility standard deviation and numerical bias were less than 15% at these concentrations. The mean true extraction recovery was greater than 90% for both matrices.

At room temperature and 4°C the GHB concentration rapidly increased several fold in fluoride citrate (FC) preserved blood. However, in fluoride oxalate (FX) preserved blood the mean concentration only increased slightly to 0.070 µg/mL after 28 days at 4°C. GHB was stable at -20°C. Endogenous GHB levels ranged from 0.0069-0.050 and 0.016-0.41 µg/mL in 95 whole blood and 120 urine samples, respectively, from 15 participants receiving placebo in a clinical GHB study. All GHB concentrations were below 0.066 and 1.3 µg/mL in 105 FX-preserved whole blood and urine samples, respectively, submitted to routine forensic antemortem toxicological analysis.

Based on these results, we argue that the current cut-off levels for discrimination between endogenous and exogenous GHB could be lowered considerably in especially whole blood samples, if FX-preserved blood is sampled and stored at -20°C until analysis.