Toxichem Krimtech 2013;80(Special Issue):224

LC-MS/MS of GHB in head hair and beard

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Abstract

Aim: The purpose of the presented work was the development of a method to quantify GHB in head hair and beard as well as the evaluation of co-extracted substances on quantification. Endogenous GHB concentrations in hair were determined with LC-MS/MS and compared to concentrations measured in segmented scalp hair and beard after a single GBL administration.

Methods: Endogenous GHB concentrations were determined by multiple standard addition and compared to calibration with spiked preextracted hair matrix using LC-MS/MS with on-line SPE for sample analysis. Matrix effects were investigated by post-column infusion.

Results: The endogenous GHB concentrations measured in 27 hair samples ranged up to 1.28 ng/mg. Elevated concentrations of 1.64 ng/mg and 3.53 ng/mg were measured in two cases where repeated GHB or GBL consumption was suspected, while a single intake of a "therapeutic" dose (1.5 mL GBL) by two volunteers was detected neither in segmented scalp hair (7 mm segments) nor in beard after shaving every second day. Significant inter-individual variations of the ion suppression were noted with a matrix effect of 40 – 77 % (n = 6 different hair samples). However, the matrix effect was found to have no negative impact on quantification, since the analyte and the internal standard are subject to the same extent of ion suppression.

Conclusion: A single administration of a "therapeutic" dose of GBL caused no increase of the GHB concentration measured in hair segments. In view of the observed inter-individual variations of the ion suppression, great importance must be attached to the matrix effect when validating methods for hair analysis.

1. Introduction

γ-hydroxybutyric acid (GHB) is a mammalian neurotransmitter related to the well-investigated γ-aminobutyric acid (GABA). After its synthesis as GABA-analogue in the early 1960s [1], GHB was tested for a variety of medical applications. Today it is prescribed for symptomatic treatment of narcolepsy with cataplexy.

Moreover, GHB has been abused as club drug in Europe since the 1990s. The sought-after effects of GHB are similar to alcohol and include disinhibition and feelings of euphoria. At higher doses GHB can induce coma and cause memory loss [2]. GHB is commonly synthesised illicitly by hydrolysis of γ-butyrolactone (GBL), which is used as chemical solvent and yet legally available in some European countries. In the human metabolism GBL is rapidly converted to the acid, thus GBL can be consumed directly.

Regular recreational abuse of GHB appears to be restricted to small sub-population [3]. Its administration for drug facilitated sexual assault is much more disconcerting and presents a challenge for forensic investigation. The short half-life of GHB (20-30 min) often impedes detection of drug administration in body fluids, as blood or urine samples have to be collected within a few hours after the incident. Due to the prolonged detection window, hair may serve as alternative matrix for forensic analysis. The endogenous GHB content of hair appears to be

a few ng per mg hair and is discernible from GHB incorporated after regular drug abuse [4-6]. The detectability of a single intake of the substance, however, remains highly controversial.

2. Material and Methods

2.1. Materials

GHB and GHB-d6 were obtained from Lipomed (Arlesheim, Switzerland). Formic acid (49–51 % and 98 %) and GBL were from Fluka (Sigma-Aldrich Chemie GmbH, Buchs, Switzerland). HPLC-grade acetonitrile was from Biosolve (Chemie Brunschwig, Basel, Switzerland). Water was purified in-house with a Milli-Q water system (Millipore (Billerica, USA).

2.2. Study design

Hair samples of 27 GHB-free individuals were collected to determine the range of endogenous GHB. Two hair samples from cases of suspected GHB or GBL abuse were available as positive control samples.

In order to investigate the detectability of a single abuse of the substance, two male volunteers each consumed 1.5 mL GBL dissolved in a glass of Coke. Assuming complete conversion to the acid, this dose corresponds to 27 mg/kg bodyweight and 28 mg/kg bodyweight GHB, respectively, for test person 1 and 2. The probands shaved with an electric razor every other day for three weeks after the intake and collected the beard samples on aluminium foil. Beard samples were obtained from test person 1 on days 1, 2, 5, 6, 8, 10, 13, 14, 18, and 20 after intake, and from test person 2 right before intake (day 0) and on days 2, 4, 6, 10, 12, 14, 16, 19, and 24 after intake. Head hair samples were collected from the back of the head before the intake of GBL. Further hair samples were taken from test person 1 on days 9, 25, and 82 after intake and from test person 2 on days 8, 17, 25, and 37 after intake. All head hair samples were wrapped in aluminium foil for storage. Segmental analysis of all head hair samples was performed. The proximal part of the sample was analysed in four segments of 7 mm length each, the distal part was analysed in a fifth segment of 3-5 cm length.

2.3. Sample Preparation

Hair samples were washed with water, acetone and dichloromethane. After drying the samples were manually cut to short pieces of 1-3 mm length. 1 mL water and 10 µL GHB-d6, 4 µg/mL were added to 20 mg cut hair, resulting in 2.0 ng GHB-d6 per mg hair. The sample was agitated for 4 min, centrifuged for 1 min and then placed into an ultrasonic bath for 2 h. The sample was shaken for 4 min and centrifuged for 10 min. The aqueous extract was evaporated in 2 mL glass vials and reconstituted in 100 µL 99:1 water:acetonitrile + 0.1 % formic acid. 20 µL were injected.

Calibration samples were extracted with 1 mL water for 2 h before spiking to remove endogenous GHB. Afterwards, 1 mL fresh water was added and the samples were spiked with GHB and GHB-d6 to obtain the final concentrations of 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, and 10.0 ng GHB per mg hair, respectively, and 2.0 ng/mg GHB-d6.

2.4. Instrumentation

A Dionex 3000 HPLC system was used for chromatographic separation. Sample analysis was conducted by reversed-phase chromatography with water (0.1 % formic acid) and acetonitrile (0.1 % formic acid) on a Thermo Scientific Hypercarb column. The procedure included on-

line SPE for sample purification. For detection an AB Sciex 5500 QTrap mass spectrometer operated in negative MRM mode was used. Two MRM-transitions were recorded each for GHB and the internal standard.

3. Results and Discussion

3.1. Matrix Effect

Vast signal suppression shortly before the GHB retention time and significant inter-individual variation of the matrix effect were observed in post-column infusion experiments. These observations were confirmed by quantitative determination of the matrix effect on the internal standard. The measured matrix effect was generally around 60-77 %, but could be as low as 40 % for some hair samples. Fortunately, the suppression of the analyte and the internal standard product ions is equivalent and does not affect quantification. This could be shown by standard addition experiments: The endogenous concentration determined from a standard addition experiment was in good agreement with results calculated from a calibration curve.

3.2. Endogenous GHB

Two thirds of all hair samples obtained from 27 GHB free individuals contained less than 0.2 ng GHB per mg hair. The determined endogenous range, however, extended up to 1.28 ng/mg. The measured concentrations are smaller than the physiologic levels found in literature. This discrepancy is most likely due to different extraction procedures. Differentiation between the endogenous GHB and elevated GHB levels due to regular drug consumption was confirmed by hair analysis in two cases of suspected GBL abuse. The determined concentrations in the respective hair samples were 1.64 ng/mg and 3.53 ng/mg, respectively.

3.3. Single Intake of GBL

No increase of the measured GHB concentration was observed after a single ingestion of 1.5 mL GBL, neither in head hair nor in beard samples. Segmental analysis of head hair samples indicated very constant GHB concentrations along the hair shaft for all samples. However, the four head hair samples collected from test person 1 before intake and on days 9, 25, and 82 after intake revealed significant variation in the GHB content. The day-to-day variation was much less pronounced in the head hair samples of test person 2.

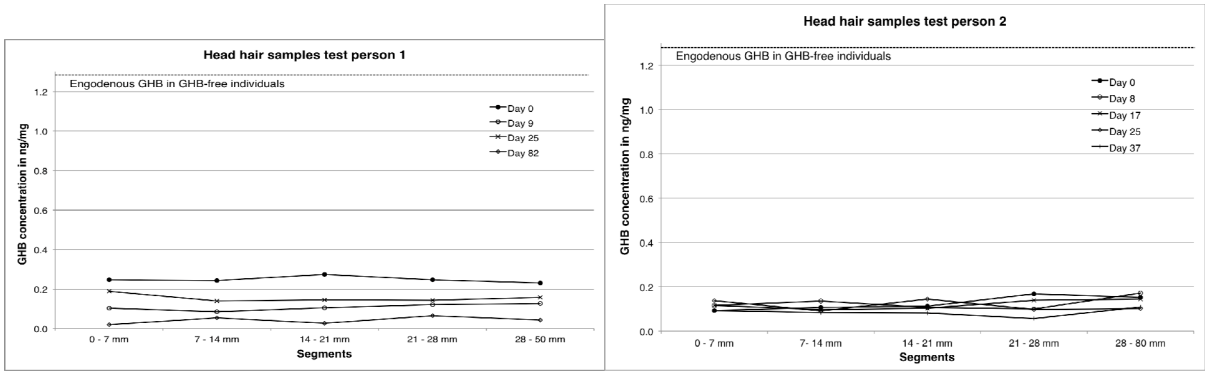

Fig. 1. Segmental analysis of head hair samples of test person 1 (left) and test person 2 (right).

The GHB concentration was found to be higher in beard samples than in head hair samples. While all concentrations measured in head hair samples were within the endogenous range,

the beard samples contained up to 3.11 ng/mg for test person 1 and up to 3.48 ng/mg for test person 2. The high GHB content of beard samples cannot be attributed to the single intake of GBL, as the GHB concentration was not increased in beard samples collected shortly after intake. The elevated GHB concentrations probably originate from contamination by external sources like sweat. These contaminations may also explain the tremendous day-to-day variation of the GHB in beard samples observed for both subjects.

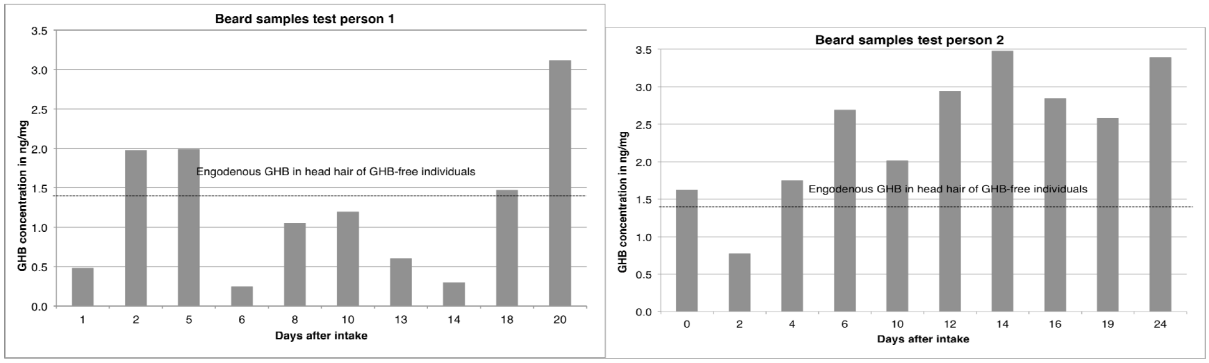

Fig. 2. GHB concentrations in beard samples of test person 1 (left) and test person 2 (right).

4. Conclusions

Despite the inter-individual variation of the matrix effect, quantitative sample analysis was possible. The highest endogenous GHB concentration in a GHB-free individual was 1.28 ng/mg, which is clearly lower than the concentrations measured in positive control samples.
The single consumption of 1.5 mL GBL was detectable neither in head hair nor in beard samples. The concentrations determined in beard samples were higher than the concentrations in head hair. However, this is not attributable to the GBL consumption but much rather due to contamination of the beard samples by sweat. The variation of the GHB content determined for hair samples collected from one individual on different days is noteworthy, both for head hair and beard samples. The variation was more pronounced for beard samples, which can be seen as further indication for contamination by sweat.

5. References

[1] Laborit H. Sodium 4-Hydroxybutyrate. Int J Neuropharmacol 1964;3:433-451.

[2] Chin R L, Sporer K A, Cullison B, Dyer J E ,Wu T D. Clinical course of gamma-hydroxybutyrate overdose. Ann Emerg Med 1998;31:716-722.

[3] European Monitoring Centre for Durgs and Drug Abuse. GHB and its precursor GBL: an emerging trend case study. 2008.

[4] Goulle J P, Cheze M ,Pepin G. Determination of endogenous levels of GHB in human hair. Are there possibilities for the identification of GHB administration through hair analysis in cases of drug-facilitated sexual assault? J Anal Toxicol 2003;27:574-580.

[5] Kintz P, Cirimele V, Jamey C ,Ludes B. Testing for GHB in hair by GC/MS/MS after a single exposure. Application to document sexual assault. J Forensic Sci 2003;48:195-200.

[6] Martz W. Intraindividual variation of endogenous GHB in hair. 17th Annual Meeting of the Society of Hair Testing. 2012;Toronto.