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Determination of gamma-hydroxybutyric acid (GHB) and its precursors in blood and urine samples: salting- out approach

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AIMS: The recreational use of gamma-hydroxybutyric acid (GHB) and its precursors gamma-butyrolactone (GBL) and 1,4-butanediol (BD) at dance clubs and rave parties has become increasingly popular in the Western world, including Finland. Thus a validated method for GHB determination is important in every forensic laboratory. To study the possible interconversion of GHB and GBL in the samples during storage, the method should be able to identify both GHB and GBL. In addition, we also wanted to include the other GHB precursor, BD in our analysis. Thus the aim of this study was to develop a procedure for simultaneous, quantitative GC-MS analysis of GHB, GBL and BD in blood and urine samples.

METHODS: In early stages of method development two aspects become clear: first, in order to analyse GHB, the salting-out approach should be utilized, and secondly, incorporation GBL in same method proved difficult. Thus, we developed a separate application for GBL. GHB and BD were extracted from blood or urine samples as follows: first, 200-µl aliquots of the samples diluted with 400 µl of purified water and mixed with 5 ml of t-butylmethylether (plus internal standard benzyl alcohol) were added to clean test tubes preloaded with approximately 0.5 g of NaCl. Then, during mixing, a 100-µl aliquot of 0.5 M HCl was added. After centrifugation the solvent phase was transferred to a clean test tube preloaded with approximately 10 mg of Na2SO4. The test tubes were capped and left at room temperature for 30 minutes. After centrifugation, the solvent was poured to a new test tube and evaporated in vacuum. When analyzing GHB or BD the residue was dissolved in 100 µl of acetonitrile, 30 µl of N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) was added and mixed, and finally injected into an Agilent GC/MS (EI) apparatus equipped with a DB-5MS capillary column (length 30 m, ID 0.32 mm, film 1,0 µm). When analyzing GBL, the sample treatment procedure was similar, except that salting-out step was omitted. GBL was analyzed underivatized with an Agilent GC-FID apparatus equipped with an Innowax capillary column (length 30 m, ID 0.53 mm, film 1.0 µ m). As a part of the validation the performance of the GHB-analysis was also tested by analysing blood and urine samples from rats treated with GBL.

RESULTS: The limit of quantitation (LOQ) for GHB was 3 mg/l in blood and urine, and 1 mg/l for GBL in urine (GBL was not analysed in blood). For BD, the limits were 3 and 25 mg/l in blood and urine, respectively. In a working range of 3-75 mg/l both intra-assay and intermediate accuracy and precision for GHB and BD in blood and urine were found acceptable (bias and RSD < 15%, at the LOQ bias and RSD < 20%), as were those for GBL in working range of 1-75 mg/l. As expected, GHB was found in blood and urine samples of rats treated with GBL.

CONCLUSIONS: As revealed by the validation data this procedure is suitable for quantitative determination of GHB and its precursors in blood and/or urine samples. Concerning analysis of the precursor drugs, it must be noted, that their conversion to GHB is very fast, thus it is unlikely that these compounds would be encountered in samples.

KEYWORDS: *GHB, GBL, 1,4-butanediol*

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