

<http://www.sciencedirect.com/science/article/pii/S1096719213002114>

Quantitation of gamma-hydroxybutyric acid in dried blood spots in newborn screening

Christophe P. Stove, Ann-Sofie M.E. Ingels, Willy E. Lambert
Laboratory of Toxicology, Ghent University, Belgium
E-mail address: Christophe.Stove@UGent.be (C.P. Stove).

When reporting on new methods for the detection of analytes in biological matrices, it is important to make adequate referral to the existing state-of-the-art ones. We feel that the article by Forni et al. [1] has failed in doing so. This paper cites neither existing publications on the use of dried blood spots (DBS) for gamma-hydroxybutyric acid (GHB) quantitation [2,3], nor recent relevant similar LC-MS/MS procedures for GHB detection in biofluids [4,5]. Moreover, based upon the reported limited validation data, we consider it premature to state that this article provides data for true quantitation of GHB in DBS (as stated in the title). Given the absence of referral to a lower limit of quantitation (LLOQ), one can assume that the LLOQ corresponds to the lowest point of the calibration curve, i.e. 8 nM. Importantly, intra- and interday imprecision and bias were not reported at this level. In this context, it is not clear how quantitative data were obtained for over 1000 data points that lie below this presumed LLOQ, which, per definition, is a limit below which no quantitation should be performed. Hence, rather than providing a mean GHB concentration, reporting a median value would be recommendable in this case. In addition, the calibration line has been extended through the origin, which is contraindicated in quantitative bioanalysis; and, for the value of 4851 nM, it is not clear whether this has been obtained by extrapolating the calibration curve or by diluting the sample (no dilution integrity experiment is discussed). Last but not least (as can readily be deduced from the preparation of the calibrators), all reported concentrations (nM levels) are incorrect: nM should be replaced by μM throughout the article (as well as throughout this letter). In conclusion, while we agree that the authors have provided evidence suggesting that their method may be used for semi-quantitative screening purposes (to screen for outliers, using a cut-off value), it cannot be concluded from the presented data that the method is capable of truly quantitatively determining endogenous GHB concentrations.

References

- [1] S. Forni, P.L. Pearl, K.M. Gibson, Y. Yu, L. Sweetman, Quantitation of gammahydroxybutyric acid in dried blood spots: feasibility assessment for newborn screening of succinic semialdehyde dehydrogenase (SSADH) deficiency, *Mol. Genet. Metab.* 109 (2013) 255–259.
- [2] A.M.E. Ingels, W.E. Lambert, C.P. Stove, Determination of gamma-hydroxybutyric acid in dried blood spots using a simple GC–MS method with direct “on spot” derivatization, *Anal. Bioanal. Chem.* 398 (2010) 2173–2182.
- [3] A.S. Ingels, P. De Paepe, K. Anseeuw, D.K. Van Sassenbroeck, H. Neels, W.E. Lambert, C.P. Stove, Dried blood spot punches for confirmation of suspected gammahydroxybutyric acid intoxications: validation of an optimized GC–MS procedure, *Bioanalysis* 3 (2011) 2271–2281.
- [4] L.K. Sørensen, J.B. Hasselstrøm, A hydrophilic interaction liquid chromatography electrospray tandem mass spectrometry method for the simultaneous determination of γ -hydroxybutyrate and its precursors in forensic whole blood, *Forensic Sci. Int.* 222 (2012) 352–359.
- [5] S.R. Dahl, K.M. Olsen, D.H. Strand, Determination of γ -hydroxybutyrate (GHB), β -hydroxybutyrate (BHB), pregabalin, 1,4-butane-diol (1,4BD) and γ -butyrolactone (GBL) in whole blood and urine samples by UPLC–MSMS, *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* 885–886 (2012) 37–42.