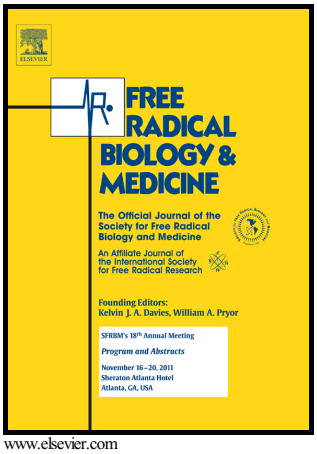

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Teng-Fei Yuan, Han-Qi Huang, Ling Gao, Shao-Ting Wang, Yan Li

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A Novel and Reliable Method for Tetrahydrobiopterin Quantification: Benzoyl Chloride Derivatization Coupled with Liquid Chromatography-Tandem Mass Spectrometry Analysis

Teng-Fei Yuan[1], Han-Qi Huang[1], Ling Gao, Shao-Ting Wang[*], Yan Li[*]

Department of Clinical Laboratory, Renmin Hospital of Wuhan University, Wuhan 430060, China

Tel.:+86-27-88041911; fax: +86-27-88041911. E-mail address: shaotingw@163.com

ABSTRACT

Tetrahydrobiopterin (BH_4) is a crucial cofactor for nitric oxide synthase, acylglycerol mono-oxygenase and aromatic amino acids hydroxylases. Its significant function for redox pathways in vivo attracted much attention for long. However, because of the oxidizable and substoichiometric nature, analysis of BH_4 has never been truly achieved with adequate sensitivity and applicability. In the present work, we pioneeringly stabilized BH_4 by derivatizing the active secondary amine on five-position with benzoyl chloride (BC). Benefiting from the favorable chemical stability and excellent mass spectrometric sensitivity of the product (BH_4-BC), ultra-sensitive and reliable quantification of endogenous BH_4 in plasma was achieved using liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. In such methodology, BH_4-BC-d_5 was introduced as stable isotopic internal standard. And the limit of quantification (LOQ) could reach 0.02 ng mL^{-1}. In the end, after investigation of plasma BH_4 in healthy volunteers (n=38), we found that the levels of

[1] These authors contributed equally to the present work.