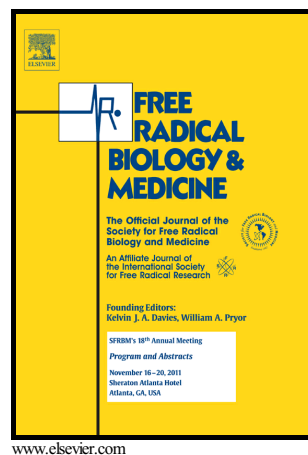


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A Novel and Reliable Method for Tetrahydrobiopterin Quantification: Benzoyl Chloride Derivatization Coupled with Liquid Chromatography-Tandem Mass Spectrometry Analysis

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ABSTRACT

Tetrahydrobiopterin (BH₄) 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 BH₄ has never been truly achieved with adequate sensitivity and applicability. In the present work, we pioneeringly stabilized BH₄ 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 (BH₄-BC), ultra-sensitive and reliable quantification of endogenous BH₄ in plasma was achieved using liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. In such methodology, BH₄-BC-d₅ 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 BH₄ in healthy volunteers (n=38), we found that the levels of

¹ These authors contributed equally to the present work.

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