色谱 » 2010, Vol. 28 » Issue (12) :1123-1127 DOI: 10.3724/SP.J.1123.2010.01123

超高效液相色谱-串联质谱法测定氧化损伤标志物8-羟基脱氧鸟苷

宋玉玲, 汪海林*

中国科学院生态环境研究中心, 环境化学与生态毒理学国家重点实验室, 北京 100085

Analysis of 8-oxo-7,8-dihydro-2′-deoxyguanosine using ultra high performance liquid chromatography-tandem mass spectrometry

SONG Yuling, WANG Hailin*

State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

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摘要 发展了一种超高效液相色谱-串联质谱法(UPLC-MS/MS)检测脱氧核糖核酸(DNA)分子中8-羟基脱氧鸟苷(8-OHdG)的方法。DNA分子在酶解过程中,脱氧鸟苷(dG)易被氧化形成8-OHdG,从而使得8-OHdG的检测结果不准确。通过加入甲磺酸去铁铵作为保护剂,有效地避免了酶解过程造成的dG氧化。酶解液通过超滤膜(截留相对分子质量为3000的分子)处理,有效去除大量蛋白分子后,直接进行UPLC-MS/MS测定。采用外标法定量,在17.6～1400 fmol范围内,8-OHdG的峰面积与其物质的量具有良好的线性关系,相关系数为0.9998。利用本方法测定了小牛胸腺DNA中8-OHdG的含量(用比值8-OHdG/106dG表示)为12.9±2.35,与前人报道的检测结果一致。本方法也可以应用于评价各种氧化因素引起的DNA氧化损伤。

关键词： 超高效液相色谱-串联质谱 8-羟基脱氧鸟苷 脱氧鸟苷 脱氧核糖核酸 小牛胸腺

Abstract： An ultra high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method for the analysis of biomarker 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-OHdG) in deoxyribonucleic acid (DNA) was developed. The artificial oxidation of 2′-deoxyguanosine (dG) at numerous stages in the sample preparation bring a challenge to the accurate measurement of 8-OHdG in DNA. To avoid the artificial oxidation during the enzymatic digestion, desferrioxamine mesylate as a protectant was added into the mixtures. By utilizing YM-3 Centricon membrane (3000 of relative molecular mass cut off), excess proteins in enzymatic solutions were effectively removed, allowing direct UPLC-MS/MS analysis. The UPLC-MS/MS analysis showed a linear relationship between the peak areas and the amounts of 8-OHdG in the range of 17.6~1400 fmol, and the correlation coefficient was 0.9998. By using the developed method, the content of 8-OHdG in calf thymus DNA (CT DNA) was estimated about 12.9±2.35 (calculated as 8-OHdG/106 dG), which was consistent with the previous work. This method can also be applicable for the detection of 8-OHdG in DNA under various oxidative stresses.

Keywords： ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) 8-oxo-7,8-dihydro-2`-deoxyguanosine (8-oxodGuo) 2`-deoxyguanosine (dGuo) calf thymus DNA (CT DNA)

Received 2010-09-02; published 2010-12-27

Fund:

国家“973计划”支持项目(No. 2007CB407305).

Corresponding Authors: 汪海林,博士,研究员,博士生导师,研究方向主要包括高灵敏生物分离分析、生物分子相互作用研究和DNA损伤的检测及修复机制研究. Tel: (010)62849600, E-mail: hlwang@rcees.ac.cn. Email: hlwang@rcees.ac.cn

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引用本文:

宋玉玲, 汪海林*.超高效液相色谱-串联质谱法测定氧化损伤标志物8-羟基脱氧鸟苷[J] 色谱, 2010,V28(12): 1123-1127

SONG Yuling, WANG Hailin*.Analysis of 8-oxo-7,8-dihydro-2′-deoxyguanosine using ultra high performance liquid chromatography-tandem mass spectrometr [J] Chinese Journal of Chromatography, 2010,V28(12): 1123-1127

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