LARGE VOLUME SAMPLE STACKING CAPILLARY ELECTROPHORESIS FOR ANALYSIS OF 3-NITROTYROSINE IN CEREBROSPINAL FLUID

Ebru TÜRKÖZ ACAR, A. Nur ONAR

Department of Chemistry, Faculty of Science and Art, Ondokuz Mayis University, Samsun, Turkey
(eturkoz@omu.edu.tr)

Key Words: 3-nitrotyrosine, capillary electrophoresis, stacking, oxidative stress, cerebrospinal fluid, peroxynitrite

Nitric oxide (NO or NO\(^+\)) is a ubiquitous gaseous free radical which is important in regulating numerous biological processes. NO can react with superoxide anion to yield toxic oxidizing agents called reactive nitrogen species (RNS), such as peroxynitrite (ONOO\(^-\)). Peroxynitrite (ONOO\(^-\)) can react with tyrosine residues in proteins to form 3-nitrotyrosine (3-NT). Elevated levels of 3-NT have been reported in many diseases including acute lung injury, some kinds of cancer neurodegeneration, atherosclerosis, multiple sclerosis, Alzheimer’s and Parkinson’s disease. Since peroxynitrite has a very short half-life at physiological pH, the stable 3-NT induced by oxidation of tyrosine residues in protein was considered an important biomarker of RNS production in various tissues [1]. Therefore it is necessary to develop an accurate, sensitive and simple analysis method for determination of 3-NT in biological fluids. For this purpose, large volume sample stacking- capillary electrophoretic (LVSS-CE) method has been developed in this study. Cetyltrimethylammonium bromide (CTAB) was used as an electroosmotic flow modifier. The content of running buffer used was as phosphate buffer (0.1 M pH 7.0) with 0.5 mM CTAB. Optimum conditions were selected as temperature of 30°C, -20 kV separation potential. For sample introduction hydrodynamic injection for 500 s period was applied. LOD and LOQ values were calculated as 6.56x10^{-10} and 2.19x10^{-9} M respectively. Recovery of for this method was found as 106.23± 11.93%. The proposed method was successfully applied to plasma and cerebrospinal fluid (CSF) without any extraction procedure.

References

1. M. Du, W. Wu, N. Ercal, Y. Ma, Simultaneous determination of 3-nitrotyrosine, \(\alpha\)-, \(m\)-, and \(p\)-tyrosine in urine samples by liquid chromatography-ultraviolet absorbance detection with pre-column cloud point extraction, 803, 321-329, 2004