Simultaneous Detection of 3-Nitrotyrosine and 3-Nitro-4-hydroxyphenylacetic Acid in Human Urine by Online SPE LC-MS/MS and Their Association with Oxidative and Methylated DNA Lesions

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ABSTRACT

Reactive nitrogen species (RNS) can modify proteins at tyrosine and tryptophan residues, and they are involved in the pathogenesis of various human diseases. In this study, we present the first liquid chromatography−tandem mass spectrometry (LC-MS/MS)-based method that enables the simultaneous measurement of urinary 3-nitrotyrosine (3-NTYR) and its metabolite 3-nitro-4-hydroxyphenylacetic acid (NHPA). After the addition of stable isotope-labeled internal standards, urine samples were purified and enriched using manual solid-phase extraction (SPE) and HPLC fractionation followed by online SPE LC-MS/MS analysis. The limits of quantification in urine were 3.1 and 2.5 pg/mL for 3-NTYR and NHPA, respectively. Inter- and intraday imprecision was <15%. The mean relative recoveries of 3-NTYR and NHPA in urine were 89−98% and 90−98%, respectively.

We further applied this method to 65 urinary samples from healthy subjects. Urinary samples were also analyzed for N-nitrosodimethylamine (NDMA) as well as oxidative and methylated DNA lesions, namely, 8-oxo-7,8-dihydroguanine (8-oxoGua), 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodGuo), N7-methylguanine (N7-MeG), and N3-methyladenine (N3-MeA), using reported LCMS/MS methods. Urinary 3-NTYR and NHPA levels were measured at concentrations of 63.2 ± 51.5 and 77.4 ± 60.8 pg/mL, respectively. Urinary 3-NTYR and NHPA levels were highly correlated with each other and with 8-oxoGua and 8-oxodGuo. Our findings demonstrated that a relationship exists between oxidative and nitrative stress. However, 3-NTYR and NHPA were correlated with N7-MeG and N3-MeA but not with NDMA, suggesting that NDMA may not be a representative biomarker of N-nitroso compounds that are induced by RNS.

Keywords: 3-nitrotyrosine, 3-nitro-4-hydroxyphenylacetic acid, LC-MS/MS, oxidative DNA lesion, methylated...

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