

## Targeted LC-MS quantification of non-enzymatically generated isoprostanes and neuroprostanes.

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Oxidative stress (OS) affects multiple levels of cellular organization including gene expression, mRNA and protein levels, and cellular metabolism. Lipids are crucial in maintaining cell and tissue structures, metabolism and signaling. Recent advances in MS-based lipidomics revealed an extremely high complexity of mammalian lipidomes with a large number of species still to be discovered. Furthermore, it appears that *in vivo* modifications of lipids are significant for organism homeostasis and play a key role in the pathophysiology of several human disorders. Thus, oxidation and nitration of lipids can induce diseases, such as atherosclerosis, obesity, inflammation and neurological diseases, and contribute to their development. Identification and quantification of all oxidized products is important not only in predictive diagnostics, but also for understanding the molecular mechanisms of oxidative stress in the context of related pathologies.

Since the discovery in 1990, isoprostanes (IsoPs) and neuroprostanes (NeuroPs) were intensively studied in the context of OS-mediated disorders. Due to their structural characteristics, high chemical stability, and presence in all biological fluids and tissues, IsoPs and NeuroPs are believed to be "golden biomarkers" of lipid peroxidation and OS. Immunoassays are widely accepted in clinical diagnostics, and perhaps represent the fastest screening test of F<sub>2</sub>-IsoP, but limited to one analyte per assay (e.g. 15-F<sub>2t</sub>-IsoP), thereby neglecting the possible presence of other IsoP and NeuroP series. Instead, targeted techniques such as liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) provides the required high sensitivity (pM range) and allow multiplexing and thus quantification of numerous IsoPs and NeuroPs in a single analysis. Although the biological significance of non-enzymatically formed IsoPs and NeuroPs was demonstrated, a lack of commercial standards limited the application of targeted LC-MS/MS. An innovative UPLC-ESI-QqLIT-MS/MS method was developed based on multiple reaction monitoring (MRM) for the quantification of 15 non-enzymatic isoprostane species using synthetic standards containing F<sub>2</sub>-IsoP (derived from AA), three F<sub>3</sub>-IsoP (from eicosapentaenoic, EPA), six F<sub>4</sub>-NeuroPs (from DHA) including three d4-IS, three dihomom-IsoPs, and one dihomom-isofuran (dihomom-IsoF) (from adrenic acid, AdA), and one F<sub>3</sub>-NeuroP (from n-6 docosapentaenoic, DPA). Using optimized LC-MS/MS it was possible to quantify two isoprostanes and three neuroprostanes from blood plasma and to demonstrate significant increase in 5-F<sub>2t</sub>-IsoP/5-*epi*-5-F<sub>2t</sub>-IsoP and 4(RS)-4-F<sub>4t</sub>-NeuroP levels in women with depression related disorders.