Comparison of different derivatization techniques for LC-MS/MS analysis of urinaryoxidative stress markers

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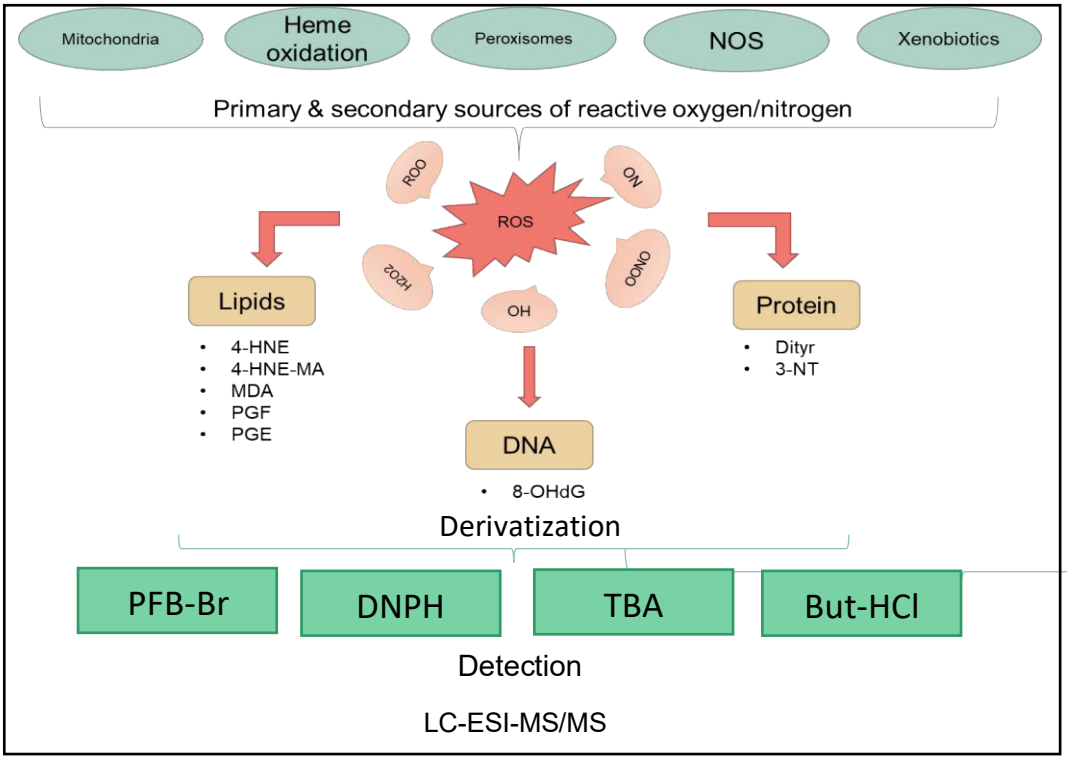

Figure 1. Quanitification of urinary oxidative stress markers with LC-MS/MS

Novel studies suggest that free radicals and oxidative stress contribute to various chronic diseases such as diabetes, cancer, Alzheimer's disease, etc. Reactive oxygen species (ROS) and free radicals are very reactive molecules, which can easily react with molecules nearby and cause damage known as oxidative stress. The products formed during oxidation are referred to as secondary ROS products. Lipid peroxidation products are the most studied analytes in oxidative stress studies, but ROS can also lead to DNA oxidation, as well as protein oxidation or nitration. The quantification of ROS within the body remains a difficult task, due to the reactivity of ROS. ROS levels are indirectly measured via colorimetric and immuno assays such as UV absorbance and enzyme linked immuno-sorbent assays (ELISA), but these assays lack specificity and sensitivity for simultaneous quantification of different types of reactive molecules. Here, we aim to develop a method that can simultaneously quantify different urinary analytes that can characterize the oxidative stress profile of an individual. Since the chemical and physical properties of the secondary products hurdle one's ability to quantify them simultaneously with liquid chromatography tandem mass spectrometry (LC-MS/MS), derivatization is required. We, therefore, compared different derivatization techniques to optimally quantify these metabolites, including thiobarbituric acid (TBA), acetyl butanol- hydrochloride, Pentafluoro benzyl-bromide (PFB-Br), and Dinitrophenylhydrazine (DNPH). These derivatization reagents react with specific functional groups that are present in our analytes of interest. Thus far, our study revealed that even though the derivatised metabolites showed better response and retention, the simultaneous detection remains a challenging task. Overall, we conclude that parallel derivatization may have to be done to detect all molecules of interest.

Keywords: Oxidative stress, ROS, derivatization, LC-MS/MS.