ABSTRACT

Reactive oxygen and nitrogen species have a physiological role in the organism, but their extensive production can damage cells and result in many diseases. By interaction of biomolecules with reactive oxygen and nitrogen species are biomolecules damaged. Damaged lipid, protein and DNA molecules then serve as biomarkers of oxidative stress and allow its evaluation. Oxidative stress can be caused by external factors such as drugs and then it may occur as adverse and toxic effects. It is important to monitor a drug's ability to induce oxidative stress to monitor drug safety. A high-performance liquid chromatography method coupled with tandem mass spectrometry has been developed to determine malondialdehyde from the cell pellet as a biomarker of lipoperoxidation. The greatest emphasis was given to the optimization of sample preparation by deproteination, derivatization and solid-phase extraction. The method was validated with acceptable specificity, accuracy, precision, recovery and matrix effect. The method complies with the requirements of the European Medicines Agency for the validation of bioanalytical methods. The method can determine intracellular malondialdehyde in the concentration range 0,1–2 $\mu mol \cdot dm^{-3}$. This method was successfully applied to the analysis of biological samples from *in vitro* experiment, where the levels of malondialdehyde in HepG2 cells exposed to acetylcholinesterase reactivators K048, K074, K075 and K203 were evaluated. The aim of the experiment was to find relationship between the structures of reactivators and their ability to induce oxidative stress. We also tried to modify the high-performance liquid chromatography method coupled with tandem mass spectrometry for the determination DNA damage so that it was possible to determine the protein damage marker 3-nitrotyrosine simultaneously. We focused mainly on the sample preparation by solid-phase extraction which was originally developed only for 2-deoxyguanosine and 8-oxo-2-deoxyguanosine.