ABSTRACT

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Title of the Diploma Thesis: Method development for determination of 8-hydroxy-2-deoxy guanosine in urine for clinical research

This diploma thesis is based on the method development for determination of 8-hydroxy-2-deoxy guanosine, 8-hydroxyguanosine and creatinine using UHPLC. The aim was to develop optimal conditions for clinical research.

Experiments were carried out using UHPLC Nexera with mass spectrometer LCMS-8030, (Shimadzu, Japan). Two stationary phases were tested. The chromatographic separation was achieved using a Meteoric core C18 BIO 4.6 × 50 mm stationary phase with core-shell particles, particle size 2.7 µm (YMC, Germany) secured with a KrudKatcher Ultra 0.5 µm in-line filtr (Phenomenex, Germany). The used mobile phase consisted of water (pH 3 using acetic acid) and methanol (using formic acid 0.2 mM) in the ratio 90:10 (v/v). The temperature was maintained at 25 °C, a flow rate was set at 0.5 mL/min and 4 µl of sample was injected. After optimization of separation conditions, the method was applied to biological material (urine). The samples were prepared using solid-phase extraction. The method was validated.

The new method will be used in clinical research and practice for the needs of the physicians in University Hospital in Hradec Kralove as well as the others that have expressed interest (University Hospital Olomouc). The main advantage of this method is its ability to simultaneously monitor the effect of oxidative stress damage to DNA and RNA in patients with severe diseases. Advantageous is determination in urine as noninvasive sample collection method which is safe for the patients and which allow determination in time and analysis repeating. Important fact is the inclusion of creatinine for the correction of diuresis.

Keywords: 8-Hydroxy-2-deoxy guanosine, 8-Hydroxyguanosine, creatinine, UHPLC-MS/MS, urine, cancer, neurodegenerative disorders