ABSTRACT

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Title of Thesis Analytical evaluation of salicylaldehyde isonicotinoyl hydrazone derivatives

Následuje překlad abstraktu práce do anglického jazyka

High performance liquid chromatography (HPLC) is one of the most modern and widely used analytical separation methods. Its advantage is that it enables qualitative and quantitative determination of substances even in a complex biological matrix.

Biocompatible iron chelators are used in therapy primarily for the treatment of iron overload. However, due to their antioxidant and antiproliferative effects, they could also find application in the treatment of other diseases (Parkinson's and Alzheimer's disease, cancer).

The biocompatible iron chelator salicylaldehyde isonicotinoyl hydrazone (SIH) from the group of aroylhadrazones was very promising, with low toxicity and significant antioxidant and cytoprotective effect. However, its use is complicated by the short half-life in plasma. Therefore, chelators were derived from its structure that are expected to have higher stability. The tested substances were: chloroacetophenone isonicotinoyl hydrazone (CAF-INH) and hydroxymethyl acetophenone isonicotinoyl hydrazone (HMAF-INH).

The aim of this work was to develop optimal chromatographic conditions for HPLC analysis of these chelators and a sample treatment method in order to determine the analyte in a biological material.

The analysis was performed on a Merck 250x4 mm I. D. column packed with LiChrospher® 100, RP-18e (5 µm) with a Purospher® 100, RP-18e (5 µm) precolumn.

For the analysis of the CAF-INH chelator in plasma, a mixture of phosphate buffer, methanol and acetonitrile, in a volume ratio of 40:30:30 (v / v / v), was selected as the mobile phase. The UV detector was set to wavelengths of 251 and 285 nm. For the HMAF-INH chelator, a mixture of phosphate buffer, methanol and acetonitrile in a volume ratio of 50:25:25 (v / v / v) was chosen as the mobile phase. The UV detector was set at 275 nm and 328 nm. The flow rate in both cases was 1 ml / min. The retention time was 3.4 min for (Z) resp. 7.0 min for (E) CAF-INH isomer and 7.2 min for HMAF-INH.

Precipitation deproteination with acetonitrile (0.4 ml to 0.2 ml plasma) was chosen for sample preparation, an extraction yield of 80% (CAF-INH), 93% (HMAF-INH) was achieved.

The linearity of the method for the determination of both analytes was verified in the concentration range of 10-100 µmol / l. The precision and accuracy of the method were verified for the determination of both substances in plasma and both validation parameters met the acceptance criteria for both analytes [12].

A 10-hour plasma stability study was performed. At its end, 40% CAF-INH and 47% HMAF-INH remained. Both chelators thus proved to be significantly more stable than the parent compound SIH. The increased stability may be due to an increase in the electron density on the carbonyl carbon and steric hindrance of the hydrazone bond.