Abstract

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Title of Thesis: Development of UHPLC-MS/MS method for analysis of selected drugs in a biological material

Dexrazoxane is a cardioprotective drug clinically used against anthracycline induced cardiotoxicity. ICRF-193 was synthetized as a novel, more effective analogue of dexrazoxan. It was reported that inhibition of topoisomerase II, which seems to be crucial for cardioprotective effect, is mediated by an *R,S* diastereomer (ICRF-193) while the racemic mixture of *R,R* and *S,S* enantiomers is almost ineffective. Nevertheless our *in vitro* experiments with racemic mixture showed some cardioprotective effect. The aim of this work was 1) to examine the possible role of contamination of standard of racemic mixture with the ICRF-193 and 2) to develop solid phase extraction (SPE) for ICRF-193 and its metabolite (ICRF-193$_{met}$) from plasma. The UHPLC coupled with a triple quadrupole mass spectrometer with electrospray ionization in positive mode. Bonus-RP column (100 × 3,0 mm, 1,8 μm) and formic acid (0,25%) with methanol as a mobile phase were used for separation of different forms of ICRF-193. Analysis of ICRF-193 and ICRF-193$_{met}$ was achieved on Luna Omega Polar column (100 × 2,1 mm; 1,6 μm) with a guard column. A mobile phase containing ammonium formate and acetonitrile were used. Four types of SPE columns (Discovery DSC-PH 100 mg/1 ml, Discovery DSC 18 100 mg/1 ml, Supel Select HLB 30 mg/1 ml, and Hypersep Verify AX 130 mg/1 ml), different types of washing solvents (H_2O, 5% MeOH, HCOOH) and elution solvents (ACN, MeOH, ACN + 10% HCOOH) were tested. Less than 0,035 % of ICRF-193 was detected in the racemic mixture. As ICRF-193 is a high potent agent, cardioprotective effect of racemic mixture can be partially caused by this contamination. The SPE procedure was optimized and the highest recovery of both, ICRF-193 and ICRF-193$_{met}$ from plasma was achieved on Hypersep Verify AX column. Nevertheless, the elution of target analytes required strongly acidify acetonitrile (10% HCOOH), which resulted into the signal supression. Therefore the sensitivity of the current method was increases only 10 times. When

focus only on the active compound ICRF-193, more effective SPE extraction (25 times lower LLOQ without pre-concentration step) employing HLB sorbent can be used.