ABSTRACT

Charles University in Prague, Faculty of Pharmacy in Hradec Králové

Department of	Department of Pharmaceutical Chemistry and Drug Control
Candidate	Mgr. Ján Stariat
Supervisor	Prof. RNDr. Jiří Klimeš, CSc.
Title of Doctoral Thesis	Analytical evaluation of drugs and drug candidates from the group of iron chelating agents

High performance liquid chromatography (HPLC) ranks among the most important analytical techniques in the field of pharmaceutical analysis thanks to its robustness and the ability to separate and qualitatively and quantitatively analyse the compounds presented in various samples. The ongoing progress in the development of novel stationary phases and detection techniques allows rapid analysis and to get comprehensive characterization of the analytes presented in the complex samples even at very low concentrations.

Cancer still remains a leading cause of death, thus the need for novel, efficient treatment strategies is of crucial importance. The mechanism based on iron chelation (Fe) inside the tumour cells represents one of the most promising strategy which could enhance the prognosis of patients suffering from cancer resistant to standard chemotherapy. Thiosemicarbazone iron chelators are currently under intensive development as novel anticancer drugs. Their strong antiproliferative activity has been already described both *in vitro* and *in vivo*. However, the further progress of their development strongly requires proper analytical techniques for their analysis in biological materials.

The mechanism of action of the only clinically used cardioprotectant efficient against antracycline toxicity - dexrazoxane is also supposed to be mediated by iron chelation. However, this assumption has never been unambiguously confirmed. This could be partially caused by the lack of bioanalytical methods capable to simultaneously evaluate the drug and its active metabolites in the target organ – in a myocardium.

The first part of this doctoral thesis was focused on analytical evaluation of the thiosemicarbazone iron chelators. At the beginning, novel HPLC-UV methods were developed and validated for the determination of *in vitro* stability of 2-hydroxy-1-naphthylaldehyde-4-methyl-3-thiosemicarbazone – (N4mT) and di(2-pyridyl)ketone-4,4-dimethyl-3-thiosemicarbazone (Dp44mT) in plasma. Their stability was subsequently compared with stability of the parent compound of the aroylhydrazone chelators - pyridoxal isonicotinoyl hydrazone (PIH). The results of these experiments pointed on apparently higher stability of thiosemicarbazones in comparison to their aroylhydrazone forerunners.

Novel HPLC-MS/MS method capable to quantify *E/Z* izomers of 2-benzoylpyridine-4-ethyl-3-thiosemikarbazone (Bp4eT) was developed and validated. The method was successfully applied to solubility study of Bp4eT in various aqueous media as well as pharmaceutical cosolvents. Furthermore, it was utilized to propose the composition of a pharmaceutical formulation suitable for *i.v.* application of this drug.

The phase I metabolism of Bp4eT was studied *in vitro* and *in vivo*. Two metabolites were detected *in vitro* and their chemical structures were proposed based on HPLC-MS/MS experiments. These suggestions were subsequently confirmed by the analysis of their standards characterized by NMR and IR spectrometry. Both oxidative metabolites were also

detected *in vivo* together with the newly found hydroxylated metabolic product.

The last work of the first part was focused on identification of *in vitro* phase I/II metabolites of newly developed thiosemicarbazone analog - di(2-pyridyl)ketone-4-methyl-4-cyclohexyl-3-thiosemicarbazone (DpC) using UPLC-QTOF. Initially, the chemical structures of the metabolites were proposed based on the high resolution analysis with accurate mass measurement. These were subsequently confirmed by the fragmentation study. Ten phase I metabolites were detected, among which the products of the thiocarbonyl group oxidation, *N*-demethylation, hydroxylation, cyclohexyl ring opening coupled with water addition as well as the various combinations of these reactions were identified. In the case of phase II conjugates, only two glucuronides were detected, while the sulphates and glutathione conjugates were not found.

The second part of the thesis was intended to investigate the feasibility of the HILIC mode for the simultaneous analysis of dexrazoxane and its polar metabolites (B, C, ADR-925). The particular attention was also pay on description of the retention mechanism under MS compatible chromatographic conditions. The separation of all compounds in one analytical run was achieved only on the zwitterion-based stationary phase. This systematic study of the retention behavior confirmed the participation of mixed mode retention mechanism, among which the partitioning, adsorption and ion-exchange could be involved.