ABSTRACT

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Title: Analysis of drugs and potential drugs in biological material using liquid chromatography

Bioanalytical methods play important role during development of new drugs. This is because determining the concentration of the drug of interest in biological matrices can help to reveal its mechanism of action, determine the values of basic pharmacokinetic parameters or study the fate of a potential drug during *in vitro* experiments. Considering the complexity of biological samples and low concentrations of studied compounds, the sample preparation prior to analysis is really important. Miniaturized techniques derived from standard extraction methods have significant presence in modern bioanalysis, mainly because they decrease both required sample volume and consumption of laboratory material to a minimum, and they enable automatization.

One of the main areas of drug discovery is currently the treatment of cancer. Unfortunately, development of highly efficacious drugs and prolongation of patient survival have resulted in the risk of developing late complications associated with anticancer treatment including serious side effect such as damage of the heart after anthracycline administration, leading in some cases to heart failure.

Experimental work of this thesis consists of 2 sections focused on bioanalytical evaluation of 1) selected anticancer agents and 2) potential cardioprotectants.

In the first part solid-phase microextraction is utilized as an alternative method for determination of plasma protein binding of thiosemicarbazone DpC. The suitability of the optimized protocol was verified by evaluating repeatability, linearity, and recovery of extraction from PBS buffer or plasma. The results of *in vitro* assay showed that DpC is highly bound to plasma proteins, which corresponds to the values previously published for other thiosemicarbazone Dp44mT.

Next part is focused on development of rapid UHPLC-MS method for determination of anthracycline daunorubicin (DAU) and its metabolite in various biological matrices, enabling

in vitro study of the fate of DAU during incubation with NO-donor – molsidomine, more precisely its active form SIN-1. It was observed that SIN-1 at higher concentrations accelerates the degradation of DAU, which is most likely to be the cause of unexpectedly low cytotoxicity of DAU in incubations with both cardiac and tumour cells.

The aim of the third part of this work was to develop first UHPLC-MS method suitable for simultaneous determination of prodrug sobuzoxan, its active form (ICRF-154) and metabolite (EDTA-diamide) in biological samples. Based on the subsequent analysis of samples from the *in vitro* evaluation of sobuzoxan activation and metabolism, several important conclusions were drawn. 1) It gradually degrades in the cell culture medium; 2) readily penetrates into cardiac cells, where it is actively metabolized to ICRF-154 and EDTA-diamide and 3) it is rapidly decomposed in plasma.

Furthermore, several simple chromatographic methods with UV detection have been developed to compare the stability of a number of newly synthesized aroylhydrazone analogues. Surprisingly, it has been shown that substitution of a salicylaldehyde group with 2,6-dihydroxybenzaldehyde does not significantly increase stability of hydrazone bond. The most stable analogue (compound 5) was subsequently tested to determine its ability of chelating and protection of cardiac cells against the oxidative stress induced by various pro-oxidants.