Abstract

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Title: Ultilization of LC-MS in bioanalysis of anthracyclines and potential cardioprotective compounds

Coupling of liquid chromatography to mass spectrometry (LC-MS) represents a near-ideal analytical tool for drug bioanalysis. This instrumentation allows identification and quantification with high selectivity and sensitivity, and provides information on the structure of tested compounds. An integral part of bioanalytical methods is the sample treatment prior to analysis, which should include isolation of the analytes, remove ballast from the matrix and ideally preconcentrate the sample. The sample treatment should be effective, quick, simple and repeatable. Current trends also emphasis process automation, high throughput and low consumption of organic solvent, which is in accordance with green chemistry.

Dexrazoxane (DEX) is the only approved, clinically used drug, preventing chronic cumulative cardiotoxicity caused by anthracycline (ANT) therapy – commonly used anticancer drugs. The development of novel and more effective cardioprotective drugs is limited by uncertainties regarding (1) information about the pathophysiological mechanism of chronic ANT cardiotoxicity (2) the mechanism of action of DEX and (3) the structure-cardioprotective activity relationship of bisdioxopiperazines.

The theoretical part of this dissertation focused on utilization of LC-MS instrumentation in drug bioanalysis, on conventional sample treatment methods, on current trends in microextraction and their application in bioanalysis. Furthermore, validation guidelines for bioanalysis are discussed and, finally, the analyzed substances are presented. The experimental part is conceived as a commentary on four published articles. This part is divided into two thematic units: (1) bioanalytical evaluation of new

potential cardioprotectives – dexrazoxane analogues and (2) development and optimization of microextraction techniques for isolation of anthracyclines from rabbit plasma.

In the first part, bioanalysis of new DEX analogues and prodrugs, and their cardioprotective potential against ANT-induced toxicity was examined. The LC-MS methods for analysis of new DEX analogues and their potential prodrugs have been developed and validated in relevant biological matrices. The methods were utilized for *in vitro* stability, bioactivation and metabolism studies, and for determination of phramacockinetics *in vivo*. We revealed a very close structure-cardioprotective activity relationship in bisdioxopiperazine group. Furthermore, selected DEX analogs were characterized in terms of stability, metabolism and bioactivation. And finally, we discovered a promising drug candidate for further comprehensive *in vivo* examinations on chronic ANT-cardiotoxicity model.

The second part focused on determination of ANT in rabbit plasma using liquid phase microextraction methods (LPME). A 96-well LPME and electromembrane extraction (EME) were both tested. The optimized EME was subsequently compared with conventional extraction techniques, namely with protein precipitation and liquid-liquid extraction. The EME outperformed the conventional methods in all tested parameters, which included recovery, matrix effects and purification of the sample evaluated by the phospholipid content. In this work an alternative microextraction method for isolation of anthracyclines from plasma was developed. The EME proved to be fast, efficient, reliable and a green technique.