Abstract

Bisdioxopiperazines have been synthesized for the treatment of tumors with a significant antiproliferative effect. However, they have low bioavailability after oral administration. Consequently, sobuzoxane has been developed. It has been prepared as a prodrug of bisdioxopiperazine ICRF-154 to increase its bioavailability and ease the oral administration for the treatment of lymphomas and leukemias. It is assumed that sobuzoxane is metabolized to an active metabolite of ICRF-154, and then supposedly converted to open analog EDTA-diamide. During preclinical researches of bisdioxopiperazines it has been discovered that they reduce the adverse effects (cardiotoxicity) of anthracyclines. Dexrazoxane has the greatest cardioprotective potential, so it has been used for 20 years as cardioprotective drug. The exact mechanism of antiproliferative and cardioprotective effects has not been fully understood yet. Therefore, the aim of this work is to develop UHPLC-MS / MS method which enables the simultaneous analysis of sobuzoxane and it is anticipated metabolites, which would help in studying bioactivation of sobuzoxane in cardiac cells, in cell medium, and in plasma.

There was developed UHPLC-MS/MS method with gradient elution for the analysis of sobuzoxan, ICRF-154 and EDTAm in plasma and cell medium. For the separation of analytes was used chromatographic column Zorbax Sb-Aq (100 mm, 1,8 μm, Agilent) and as a mobile phase was used methanol and 1 mM ammonium formate. After the electrospray ionization the analytes were detected using triple quadrupole with the most intensive SRM transitions. The linearity of this method was verified with the use of working solutions with different concentration for every analyte. After that, the linearity was tested in plasma and cell medium in uniform concentrations 2,5 – 150 μM for sobuzoxane, ICRF-154 and EDTAm. The analyzes were difficult because of the different polarities of analytes and poor solubility of ICRF-154. This substance was only soluble in the formic acid, which decreased ionization of EDTAm. Then the stability of subozoxane in cell medium was tested, and there was a discovery that the concentration of subozoxane was falling very quickly. For the extraction of analytes from plasma was used precipitation of proteins with methanol. The cell medium was not adjusted, it was only diluted with 90% methanol.

The developed method should be further optimized for the use in the analysis of cardiac cells and fully validated. Subsequently, it shall be used to analyze samples from the pilot study bioactivation sobuzoxane.