ABSTRACT

At present, the High Performance Liquid Chromatography (HPLC) is still the dominant separation method, it is widely used in all areas of drug analysis. HPLC provides qualitative and quantitative evaluation of the separated components.

This thesis describes the development of the method for HPLC analysis of daunorubicin (DAU) and its metabolite daunorubicinol (DAU-OL) in a rabbit plasma. The analyzed compounds were first separated from interfering substances in plasma due the deproteinization. For a precipitation was chosen methanol (600 μl) with the highest extraction efficiency.

The separation of DAU and its metabolite was reached by Zorbax SB-Aq column (3,5 μm, 150 x 4,6 mm; Agilent Technologies, USA), the mobile phase consisted of a mixture of water with phosphate butter and phosphoric acid (pH 2,4) and acetonitril (74:26, v/v). The analysis ran at isocratic mode during 15 minutes. The mobile phase was delivered at a rate of 1,1 ml/min, an aliquot of 40 μl was injected into the chromatographic system. The fluorescence detector was operated at an excitation wavelength of 480 nm and an emission wavelength of 560 nm. Doxorubicin was used as an internal standard.

The methodology was validated with respect to linearity (4 – 83 ng/ml for DAU, 25 – 500 ng/ml for DAU-OL), precision and accuracy. The stability of this method was set in the laboratory conditions during 0,5 hours and after freezing the sample during 24 hours. It showed that for long-term storage is essential to keep a limited stability.

The limiting factor in the use of anthracycline chemotherapy drug DAU is cardiotoxic effects. In clinical practice is available only drug dexrazoxane, which is able to protect the myocardium. The results of this study can be applied to developing new cardioprotective agents during the effectiveness testing.