

8. Abstract

High-performance liquid chromatography (HPLC) is one of the most frequently used separation technique for quantitative and qualitative evaluation of drugs. The present paper deals with optimization of the chromatographic conditions and its application to evaluating the stability of two novel iron chelators 2,6DHAF-INH and AHC-INH in rabbit plasma. The derivatives were synthesized so as to increase stability of their hydrazone bond compared to their mother compound of salicylaldehyde isonicotinoyl hydrazone (SIH).

The best differentiation of the two evaluated derivatives, products of their decomposition and internal standard (SIH) was achieved by means of a chromatographic column with a reverse stationary phase LiChroCART[®] HPLC – cartridge LiChrospher[®] 100 RP – 18e (15 μ m) with a precolumn. The mobile phase for analysis of 2,6DHAF-INH was selected to be a mix of phosphate buffer (0.01 M aqueous solution NaH₂PO₄ · 2 H₂O s 2 mM EDTA; pH 6.0) and methanol in 47:53(v/v) ratio. Detection at 300 nm wave length, flow 1.0 ml/min, column temperature 25 °C were used. The mobile phase for analysis of AHC-INH was selected to be a mix of phosphate buffer (0.01 M aqueous solution NaH₂PO₄ · 2 H₂O s 2mM EDTA; pH 6.0) and methanol in 50:50(v/v) ratio. Detection at 325 nm wave length, flow 1.0 ml/min, column temperature 25 °C were used.

The stability of the two chelators pursued during 10 hours in rabbit plasma s at the temperature of 37 °C showed different results. A drop in concentration down to 63 % of the original amount was observed in AHC-INH in three hours and further down to 51 % in ten hours, while 2,6 DHAF-INH showed a more rapid degradation decreasing to as low as 18.7 % of the original quantity in three hours and falling down to 0 % in 10 hours. The results demonstrate that the AHC-INH chelator displays stability markedly superior to the maternal substance of SIH which decayed to 7.64 % of the original quantity in three hours. The other evaluated derivate 2,6DHAF-INH showed a similar rate of decay as SIH did.