

## ABSTRACT

High-performance liquid chromatography (HPLC) is a progressive analytical method used for qualitative and quantitative drug analysis.

This work describes various modifications of the chromatographic method for determining of BSIH (isonicotinic acid [2-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-benzylidene]-hydrazide) and SIH (salicylaldehyde isonicotinoyl hydrazone) in rabbit plasma. BSIH belongs to prochelators which convert to iron chelator SIH in the presence of hydrogen peroxide. Increased stability in plasma and lower toxicity even during repetitive administration is the advantage of BSIH.

Separation of BSIH, SIH and internal standard (o-108) was tested on various stationary phases using different chromatographic conditions. However, none of them provided better results than the initial one. The analysis was performed on chromatographic column Zorbax Bonus-RP (150 mm x 3,0 mm, particle size 3,5  $\mu\text{m}$ ,) with guard column using identical sorbent at a flow rate of 0,3 ml/min. Phosphate buffer (10 mM monosodium phosphate, pH6, adjusted with sodium hydroxide) : acetonitrile with methanol (60:40) (v/v) with the ratio of 60:40 (v/v) as the mobile phase. Detector response was registered at 297 nm. Linearity of the method was verified in the concentration range of 10  $\mu\text{M}$  to 100  $\mu\text{M}$  for both BSIH and SIH.

Stability of BSIH and SIH in rabbit plasma was tested during 10-hour incubation period at a temperature of 37 °C. Less than 10 % of the original concentration of SIH was determined after this period, whereas BSIH values fluctuated around 90 % of the original concentration. These results imply that BSIH is significantly more stable in plasma compared to SIH.