DETERMINATION OF TIROFIBAN IN SERUM USING LIQUID CHROMATOGRAPHY WITH UV DETECTION

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The aim of this study is to develop a specific, sensitive, rapid, and simple HPLC method with UV detection to study pharmacokinetics of tirofiban in serum of rats alone or in the presence of heparin as simultaneous therapy. Thirty six Wistar rats were used randomly assigned in two groups: control group (n = 18), and group with venous thrombosis experimentally induced by ligature of the femoral vein (n = 18). Tirofiban hydrochloride was dissolved in sterile saline. The *i.v.* bolus doses (0.6 mg kg^{-1}; 0.8 mg kg^{-1}; 1 mg kg^{-1}) were injected using tail vein. Blood samples were taken in the presence of heparin during one hour: starting 15 minutes after injection in the intervals of 15 minutes. Tirofiban concentration was analysed immediately. Blood samples were centrifuged 5 min at 3500 rpm min^{-1}. The serum was treated carefully, then methanol was added for precipitation of proteins (in ratio serum: methanol = 1:3). The tubes were again centrifuged 5 min at 3500 rpm min^{-1} and supernatant was injected into the HPLC column. HPLC separation was carried out at ambient temperature, using reversed-phase LiChrospher® 100 RP-18 column (4.0 mm × 250 mm, 5 µm particle size). The chromatographic separation was performed by isocratic mode with of 0,1 M KH_2PO_4 (pH = 5.0, adjusted with 1.0 N sodium hydroxide) and acetonitrile in the ratio of 80:20 % *v/v* as mobile phase with a flow rate of 1.0 ml min^{-1}. UV detection was performed at 274 nm. The injection volume was 50 µl. Tirofiban concentration was also determined in spiked human and rat serum samples. System suitability was checked by evaluating different parameters (retention time, tailing factor, capacity factor, resolution, and selectivity). Tailing and capacity factors were obtained as 1.17 and 2.41 for tirofiban. Resolution factor for the system for tirofiban and heparin was 3.90. The retention times of tirofiban in methanol, human and rat serum samples were 9.1, 9.2, and 9.16 min, respectively (Figure 1.). The variation in retention time of tirofiban among five replicate injections of standard solution in methanol, human and rat serum samples was very little, giving relative standard deviations (RSD%) of 0.61%, 0.93%, and 0.82%, respectively. The response was linear over the range of 0.03 – 0.18 mg mL^{-1} in mobile phase and serum samples. The limit of detection (LOD) for tirofiban was 1.84, 13,8 and 14.6 µg mL^{-1} in methanol, spiked rat serum and spiked human serum, respectively (Table 1.). Plasma concentration-time profile obtained from the experimental group was significantly different from plasma concentration-time profile obtained from the control group. The proposed RP-HPLC method is simple, accurate, precise, and rapid for the determination of tirofiban in serum and for monitoring its concentration in serum. The described method can be readily applied, without any interference from endogenous substances, to study pharmacokinetics of tirofiban in serum given alone or in the presence of heparin as simultaneous therapy and for therapeutic monitoring of levels of tirofiban in serum samples.

Table 1. Characteristics of the linear regression analysis of tirofiban

	Methanol	Human Serum	Rat serum
Linearity range (mg mL^{-1})	0.03–0.18	0.03–0.18	0.03 -0.18
Slope	9200223	7942560	8795042
Intercept	2720.6	90750	23999
Determination coefficient (r^2)	0.9999	0.9943	0.9949
SE* of the intercept	5124.18	35082.2	36834.2
SE of the slope	43858.91	300276.9	315271.9
Detection limit (mg mL^{-1})	0.00184	0.0146	0.0138
Quantification limit (mg mL^{-1})	0.0056	0.0442	0.0419

*SE – Standard error

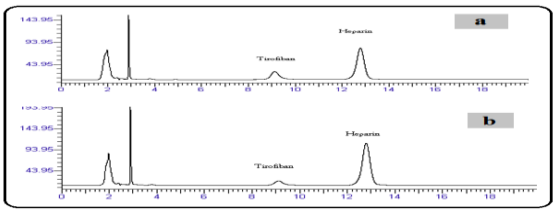

Figure 1. Chromatogram of standard solution of tirofiban and heparin in methanol (a) and chromatogram of serum spiked with tirofiban and heparin (b)

References

Oertel R, Köhler A, Koster A, Kirch W., Determination of Tirofiban in human serum by liquid chromatography-tandem mass spectrometry, J Chromatogr B Analyt Technol Biomed Life Sci. 2004 Jun 5;805(1):181-5.