Abstract

This bachelor thesis is focused on development of a specific UHPLC-MS/MS method for the determination of rivaroxaban in rat serum.

At first, the setting of the mass spectrometer was optimized. Suitable MRM transitions were found for rivaroxaban and its isotopically labeled internal standard. For rivaroxaban was found the MRM transition 436.1 → 145.05 with optimal energy levels Q1 = -12.0 V; CE = -30.0 V; Q2 = -27.0 V. For rivaroxaban D4 was found the MRM transition 440.1 → 145.0 with optimal energy levels Q1 = -22.0 V; CE = -31.0 V; Q2 = -25.0 V. The ion source setting parameters were as follows: nebulizing gas flow 3 l/min; heating gas flow 10 l/min; interface temperature 300 °C; desolvation temperature 526 °C; DL temperature 250 °C; heat block temperature 400 °C; drying gas flow 10 l/min. The optimal chromatographic method was as follows: A Poroshell 120 SB AQ, 100 × 2.1 mm, 2.6 μm (Agilent) chromatographic column; the mobile phase consisted of acetonitrile with the addition of 0.1% formic acid (A) and distilled water with the addition of 0.1% formic acid (B); flow rate of the mobile phase 0.5 ml/min; gradient elution (time: 0-1-2-3.5-4-6.5 min; A: 20-20-80-80-20-20 % *v/v*); autosampler temperature 15 °C; column temperature 40 °C; time of analysis 6.5 minutes; injection volume 2 μl.

The optimized UHPLC-MS/MS method was linear (weighted linear regression $1/x^2$) in the concentration range of 0.15-383.33 ng/ml with a coefficient of determination of 1.000 means excellent linearity. The accuracy (relative standard deviation) for concentrations of 12.27 ng/ml and 76.67 ng/ml is in the range of 2 to 5 %. The trueness (relative error) for these concentrations is also in the range of 2 to 5 % and thus meet the criteria for bio-analytical application. For the concentration of 0.77 ng/ml was 58.7 % accuracy and 33.6 % trueness, which probably indicates a mistake in the preparation of the solution.

Key words: rivaroxaban, rat serum, UHPLC-MS/MS