Abstract

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Title of Thesis: Development and validation of UHPLC-MS/MS method for the determination of maraviroc in placental perfusions

The goal of this work was to develop fast and selective method for the determination of maraviroc in human placental perfusions. The ultra-high performance liquid chromatography with tandem mass spectrometry (UHPLC-MS/MS) was used. BEH C18 column and gradient elution with the mobile phase A (water with 0.1% formic acid) and B (acetonitrile) at 0.35 mL/min flow-rate and 40 °C temperature were used for the separation. The mass spectrometry conditions were optimist and set up as follows: electrospray ionization in positive mode, capillary voltage 1.0 kV, cone voltage 35 V, extractor 3.0 V, RF lens 0.1 V, desolvatation gas flow 1000 L/h, temperature 450 °C, cone gas flow 100 l/h, ion source temperature 130 °C. Selected reaction monitoring (SRM) mode was used for quantitation. Liquid-liquid extraction (LLE) was chosen for sample preparation and was optimised. The best results were obtained when dichloromethane was used as the extraction agent (recovery >90%). The optimized method was fully validated in the range 1-1000 ng/mL at five concentration levels (1 ng/mL, 2.5 ng/mL, 50 ng/mL, 500 ng/mL and 1000 ng/mL) with the lower limit of quantification (LLOQ) 1 ng/mL and limit of detection (LOD) 0.3 ng/mL. Precision (RSD %) and accuracy (% bias) was determined for each concentration level: 1 ng/mL (RSD = 8.1%, bias = +17.5%), 2.5 ng/mL (RSD = 13.0%, bias = +13.5%), 50 ng/mL (RSD = 2.6%, bias = +3.3%), 500 ng/mL (RSD = 2.7%, bias = +0.4%), 1000 ng/mL (RSD = 1.7%, bias = -0.6%). Further parameters of validation were linearity (R^2 = 0.9983) and matrix effects (99.1% - 109.2%) at four concentration levels: 1 ng/mL, 50 ng/mL, 500 ng/mL and 1000 ng/mL. The stable isotopically labelled internal standard d_6-maraviroc, was used for quantification in all experiments. The method enabled sensitive and selective determination of maraviroc in placental perfusions.