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Title of thesis: Development of method for the determination of entecavir in biological materials using UHPLC-MS/MS

Entecavir is a synthetic guanosine nucleoside that plays an important role in treatment of chronical hepatitis B virus infection. This work was focused on development of highly sensitive method to determine entecavir concentrations in rat urine by ultra-high performance liquid chromatography-tandem mass spectrometry. Entecavir $C_2{}^{13}N^{15}$, stable isotopically labeled internal standard, was used for quantitation. Hydrophilic interaction chromatography seems to be a suitable technique for retention and separation of polar compounds present in analyzed sample. One of the main goals was to confirm this fact by comparison with commonly used reverse phase chromatography mode (RP-UHPLC) on BEH C18 stationary phase. Conditions for RP-UHPLC were optimized as follows: mobile phase composed of acetonitrile/0.01 % formic acid (4:96). The HILIC conditions on BEH Amide stationary phase were optimized using isocratic elution with mobile phase composed of acetonitrile/5 mM ammonium acetate pH 4.0 (75:25). HILIC method provided much better results in terms of linearity and repeatability. Due to high polarity of entecavir Oasis HLB cartridge was chosen for solid phase extraction. Advantageously entecavir was eluted with 75 % acetonitrile in water, which was the same composition as HILIC mobile phase. Therefore, evaporation step could be omitted. Several strategies were used to overcome matrix effect, such as UHPLC separation, HILIC chromatography, SPE sample pre-treatment, stable isotopically labeled internal standard and dilution step. Therefore, influence of matrix effects, which were determined by post-column infusion and post-extraction addition method, was negligible in this method. The following validation parameters demonstrated the suitability of this method for the determination of entecavir in rat urine – accuracy (< 5% error), recovery (87 – 109 %), precision (< 3% RSD), selectivity and sensitivity (LOQ = 100 pg/ml).

Keywords: entecavir, hydrophilic interaction chromatography, UHPLC-MS/MS, solid phase extraction, matrix effects, validation