ABSTRACT

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Title of Thesis: Application of LC-MS/MS methods to *in vitro* transport experiments

Gilteritinib, an antineoplastic drug, is used for targeted anticancer therapy. It belongs to the group of receptor tyrosine kinase inhibitors. Its structure is based on a pyrazinecarboxamide skeleton. So far, it has been chromatographically determined in biological material only from plasma. In this diploma thesis, gilteritinib was analyzed from Opti-MEM cell medium to be used *in vitro* transport experiments. Subsequently the LC-MS/MS method was optimized and validated. During progression and optimization of the LS-MS/MS method, the Thermo Finnigan LCQ Advantage Max Ion Trap was observed that with an increasing number of injections had been shown increasing signal suppression for gilteritinib and its internal standard (maraviroc), which reached up to 75 % after only 30 sprays. Contamination of the inlet to the ion transfer capillary, to which the ion source (electrospray) is placed diagonally, was also visible. Thereafter it was tested whether another construction of the ion source (orthogonal position of electrospray) will not solve the problem. After the transfer of the method to the Shimadzu LCMS 8030 (triple quadrupole), no significant suppression of the analyte signal or contamination was observed even after 100 injections. Final chromatographic separation was performed on a Hypersil Gold C18 column (100 × 4,6 mm, 3 μm) at 40°C in isocratic mode, where the aqueous part of the mobile phase consisted of 0,1% formic acid in water and organic phase composed of methanol (1:1, v/v) at flow rate 0,350 mL/min. The following transitions were used for the analysis: analysis was performed in SRM mode with the following ionic transitions: GIL $[M+H]^+$ m/z = 553 (CE -27, -34) → m/z 436, 353 a IS $[M+H]^+$ m/z = 514 (CE -20) → m/z 389.

In the case of validation of the method, in which requirements were imposed on selectivity, linearity, accuracy and precision, matrix effect and stability, values obtained meets the requirements of the European Medicines Agency (EMA). As such, the method is ready for application to real samples.