ABSTRACT

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Title of Thesis: HPLC-MS/MS analysis of selected drug candidates in biological materials

Substances with codenames JM-298 and JM-299 are positional isomers that are currently under intensive research as potential drugs used in the treatment of metabolic and liver diseases. The aim of this work was to develop and validate the UHPLC-MS/MS method for the determination of these analytes from plasma and to determine their pharmacokinetic profile in this material after administration to mice.

Several precipitating agents (acetonitrile, acidified acetonitrile, methanol and acidified methanol) were tested in plasma sample preparation (25µl), where the highest recoveries and the lowest matrix effects were achieved when 250 µl of methanol was used. The analysis was carried out using the UHPLC-MS/MS instrument with electrospray ionisation in positive mode. Separation was carried out using an Acquity UPLC BEH C18 (2.1×50 mm, 1.7 µm) protected with a pre-column with the same sorbent. The column oven was heated to 30 °C and the sample injection volume was 1 µl, the flow rate of the mobile phase was 0.3 ml/min. As part of the optimisation, various mobile phases were tested, for example mixtures of acetonitrile or methanol with different concentrations of formic acid in water during isocratic and gradient elution. The highest response with optimal peak symmetry was achieved by gradient elution with a mobile phase consisting of 0.1% formic acid in water and acetonitrile. The developed method was validated for both substances in the concentration range of 0.25 to 25.00 µg/ml in plasma and subsequently used for the determination of the pharmacokinetic profile in mice plasma after the administration of JM-298 or JM-299.