

ABSTRACT

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Title of Thesis **Development and validation of HPLC methods for transport and metabolic studies**

The presented PhD thesis deals with the development and validation of chromatographic methods in bioanalysis of drugs and their metabolites. The work provides a theoretical introduction to the field of bioanalysis, using methods of liquid chromatography. Attention was paid to recent trends in the area, particularly with respect to the new types of stationary phases. In the experimental part of the thesis, reader is introduced to a development and validation of several bio-analytical methods using high performance liquid chromatography. The first method concerns with a determination of 5-fluorouracil and its metabolites by HPLC with gradient elution and UV/VIS detection on a column Luna PFP (2). Discussed are mainly specifics for the assay of this group of polar and poorly detectable compounds. The developed and validated method is being used for TDM of patients with rectal cancer. Another discussed method deals with a determination of methotrexate polyglutamates in erythrocytes using isocratic elution on a hybrid column Gemini C18 and fluorescence detection after post-column derivatization/photo-oxidation of the analyte. The developed and validated method is used for TDM of patients who suffer from certain autoimmune diseases. The last method discussed in the thesis is a method for an assay of a selective arginase inhibitor, N-(omega)-hydroxy-nor-L-arginine in rat plasma. For the separation was used Kinetex C18 column with core-shell technology. Gradient elution and fluorescence detection is used after on-line derivatization of substances with o-phthaldialdehyde. Biotransformation product of N-(omega)-hydroxy-nor-L-arginine was identified as nor L arginine by means of LC/MS/MS. The method was used for pilot pharmacokinetic experiment in rats, due to the absence of data about kinetics of this compound in the literature.