Abstract

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Title of diploma thesis: Monitoring of diclofenac metabolism in real time

This diploma thesis focuses on the optimization of experimental parameters of an analytical method for the sensitive and selective determination of diclofenac (DCF) using sequential injection analysis (SIA) in combination with spectrofluorimetric detection. The tested parameters influencing the determination of DCF using a complex with cyclodextrin (CD) include the selection of CD concentration, the DCF:CD ratio, the control program, the time interval between sample preparation and measurement, EX/EM bandwidth, calibration range, and storage conditions (container material and temperature regime). The optimized method is subsequently applied to real samples originating from a metabolic study and compared with data obtained from these samples using a reference off-line method of liquid chromatography with mass spectrometric detection (LC-MS). The results of this work demonstrate that the SIA method, in combination with spectrofluorimetry, generates results comparable to LC-MS in terms of analytical evaluation of DCF concentration decrease, thus approaching the sensitivity and accuracy offered by the LC-MS method. The thesis also focuses on the application of this method for monitoring DCF content during *in vitro* metabolism using primary human hepatocytes. This approach is based on the principle of SIA combined with spectrofluorimetric detection for fully automated determination of DCF in samples collected online from a 3D-printed module simulating *in vitro* metabolic conditions.