ABSTRACT

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Title of Diploma Thesis: **Optimization and validation of HPLC method for the determination of sodium diclofenac and its degradation product in tablets**

This diploma thesis deals with the optimisation and the validation of the HPLC method determination of sodium diclofenac (DF) and its degradation product 1-(2,6-dichlorphenyl)-indolin-2-on (DPI) in tablets. DF is a non-steroidal anti-inflammatory drug with analgesic, antipyretic and anti-inflammatory effects. The degradation product DPI has been produced in formulations after a long-term storage, especially after exposure to light or heat. The method development was based on a method created by the Pharmaceutical Faculty of Charles University for the determination of DF, its degradation product and preservatives in topical emulgel and the method was optimised for determination of DF and DPI in tablets. The monolithic column Chromolith® Performance RP-18e (100×3 mm, Merck) and flurbiprofen as an internal standard were chosen. The mobile phase was prepared of methanol and aqueous solution of phosphoric acid (pH 2,5) in the ratio of 65:35 and the flow rate of 1 ml/min. An UV detector at 254 nm was used. The suitability of chromatographic system was tested and the method was validated. The following parameters were verified: accuracy (relative standard derivation $RSD_{\%}$ 0,96% for DPI, 0,46% for DF), linearity (linear dependence for DPI was demonstrated in range of 0,05-0,5 mg/100 ml, regression line y = 0,4202x + 0,0019, R = 0,99978, for DF on 10-35 mg/100 ml, y = 0,2788x - 0,09, R = 0,99951), precision (only for DF, recovery in range of 100,51-102,78%, $RSD_{\%}$ 0,90%), the limit for detection (0,0028 $mg.l^{-1}$) and limit of quantitation (0,0095 $mg.l^{-1}$) was determined for the degradation product. The robustness and selectivity were also tested. According to the results of validation, the method provides precise and accurate results and it is suitable for the determination of sodium diclofenac and its degradation product in Veral tablets. The use of monolithic column reduced the time of analysis by 75% (the original method < 17 min, now < 4 min).