ABSTRACT

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Title of diploma thesis: Study of chiral aspects of drug metabolism using instrumental analytic methods

For the evaluation of stereospecificity of rat and human cytosolic carbonyl reductases and activity of microsomal oxidases involved in nabumetone biotransformation, a new chiral high-performance liquid chromatographic method was developed. The prepared LLE-HPLC-PDA method enabled extraction, separation and ultraviolet detection of prochiral nabumetone and its five phase I metabolites including enantiomers of two chiral biotransformation products of carbonyl reduction. Methyl ester of naproxen served as an internal standard. Diethyl ether was used for liquid-liquid extraction (LLE) of biomatrices. Chiralcel OD-R 250 mm×4.6 mm column with a mobile phase methanol–1M $NaClO_4/HClO_4$ aqueous solution pH=3 (75:25, v/v) were employed in isocratic sufficient separation of nine analytes. The whole analysis lasted 60 minutes at the flow rate of 0.5 ml/min. The column effluent was monitored using a photodiode-array detector (scan or single wavelength at λ=265 nm). The results of in vitro nabumetone biotransformation in cytosole and microsomal fraction of liver cells of two species (rat vs. human) were compared and enantiomeric excess of chiral nabumetone metabolites was evaluated. Stereospecificity in the rat cytosolic carbonyl reductases to nabumetone was observed: enantiomeric ratio of (+)-reduced nabumetone vs. (-)-reduced nabumetone was found to be 10 % vs. 90 %). The proposed chiral HPLC-PDA method complements previous chromatographic methods developed for the study of nabumetone phase I and phase II biotransformation and was successfully employed in drug-metabolism-tracking.