Abstract

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Title: Use of HPLC-HRMS for the study of tacrine group compounds potentially useful in the therapy of Alzhemier‘s disease.

High-performance liquid chromatography coupled with high-resolution mass spectrometry (HPLC-HRMS) is an analytical technique very often used in the new drugs development. This technique is widely used, from the confirmation of the proposed structure and identification of present impurities, through the assessment of absorption, distribution, metabolism and excretion to the monitoring of poisoning and intoxications.

In the theoretical part, the presented dissertation thesis is focused on the basic characteristics of Alzheimer's disease, its currently available treatment and new treatment strategies. In addition, attention is paid to areas closely related to the experimental part, namely: the study of pharmacokinetics, analytical tools for determining pharmacokinetic parameters, liquid chromatography, mass spectrometry and the specifics of the bioanalytical methods.

The experimental work is in the commented form of four published works dealing with the development and preclinical evaluation of substances from the tacrine group, which are promising candidates for the treatment of Alzheimer's disease. The work is primarily focused on studying of absorbtion and biotransformation of tacrine molecules, 7-methoxytacrine and its derivatives, 6-chlorotacrine and 7-phenoxytacrine. Since tacrine is a molecule that has been withdrawn from clinical use due to the established hepatotoxic effects of its 7-hydroxytacrine metabolite, it is necessary from the point of view of the safety of pharmacotherapy to monitor hepatotoxicity in newly developed tacrine derivatives as well.

One of the main objectives of the work was to develop and optimize the HPLC-MS method, which will enable comprehensive monitoring of the biotransformation of

tacrine, 7-methoxytacrine, 7-phenoxytacrine and 6-chlorotacrine molecules. Thanks to the application of a newly developed highly selective HPLC-HRMS method, it was determined that 7-phenoxytacrine forms only a trace amount of 7-hydroxytacrine after incubation with human liver microsomes and that 6-chlorotacrine does not form this metabolic precursor of toxic action at all. Furthermore, the work pays attention to the analytical confirmation of the identity and the determination of impurities of the newly synthesized substances, the study of their ability to pass through the blood-brain barrier, and a more advanced study of the biotransformation and toxicity of selected tacrine derivatives. The results of this dissertation thesis provide the data that contribute to the rational development of new tacrine derivatives with reduced or no hepatotoxicity.