## ABSTRACT

My doctoral dissertation consists of five papers published in prestigious, specialist scientific journals of international and national scope, devoted to the preparation of "tools" that can be used to monitor the presence of important from a medical point of view of lipoyllysine (LLys) and lipoic acid (LA) in biological samples.

The subject of my dissertation research was the elaboration of new, previously undescribed procedures and analytical methods for the simultaneous determination of (i) total LA and weakly protein bound LA by hydrogen bonds (ProtS-LA) in plasma, (ii) total LA and LLys content in urine, and (iii) total LA and LLys content in animal tissue homogenates. The elaboration of the analytical procedures consisted of the steps of sample preparation, chromatographic separation and determination. Each of the above-mentioned analytical procedures was subjected to a validation process, the purpose of which was to determine the extent of their usefulness and to assess their credibility. In research the high-performance liquid chromatography technique with a diode array detector (HPLC-DAD) was used. All optimized and validated analytical procedures have been successfully applied to the therapeutic monitoring of the amount of LLys and/or LA in urine and plasma of apparently healthy volunteers after oral LA supplementation as well as monitoring the concentration of LLys and LA in the meat samples of various origins. Tissues such as liver, heart, kidney and stomach from cows, calves, pigs, chickens and turkeys were used for the research. The results of presented doctoral dissertation showed that the developed methods allow for the simultaneous determination of LLys and/or LA at low concentration levels, which can be useful in pharmacokinetic, biochemical and biomedical studies. Moreover, the elaborated methods are relatively cheap, have a very good repeatability of results, a simple sample preparation procedure and are eco-friendly (low consumption of the sample and chemical reagents). It should also be emphasized that the application of the HPLC technique with the use of the UV-DAD detector makes these methods easily available for most analytical or clinical laboratories.

Additionally, in this research work the antioxidant properties of LA, LLys, dihydrolipoic acid (DHLA) and dihydrolipoyllysine (DHLLys) using the model 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) were examined. The ability to neutralize free radicals was determined by the spectrophotometric method. It was found that LA and LLys are not characterized by the capability to quench the DPPH radical, while the reduced form of LLys i.e. DHLLys possesses antiradical activity. However, a higher DPPH radical quenching

capacity was observed for DHLA than for DHLLys due to the presence of two free thiol (–SH) groups in their molecule.

The subject of the doctoral dissertation was also two reviews concerning homogenization of biological samples and chemical derivatization, which are used on a large scale in almost every analytical laboratory to facilitate the sample preparation step. The knowledge obtained during the writing of these papers helped in planning the experiments necessary to develop the analytical methods being the subject of the doctoral thesis.