State the objective of the project

The implementation of internal standards in quantitative bioanalysis is an accepted and commonly used procedure. In most cases internal standards used in quantitative LC-MS analysis are stable isotopically labeled analogues of the analyte, i.e. compounds in which several atoms in the analyte are replaced by their stable isotopes. Usually, ^{2}H, ^{13}C, ^{15}N and ^{18}O isotopes are used. However the deuterated isotopologues are not considered as good internal standards due to the possibility of deuterium affecting their co-elution during LC-MS. Additionally, preparation of deuterated standards of drugs and their metabolites involves complicated and time consuming multi-step *de novo* synthesis, using expensive isotopically labeled substrates. Therefore, there is a strong need of low-cost methods for isotope-labeled standards preparation for quantitative analysis by LC-MS.

The main goal of this project is to adopt and optimize the method of deuterium labeling of peptides containing sarcosine (*N*-methylated glycine) residue, via hydrogen/deuterium exchange (HDX) of α-carbon hydrogen atoms in D_2O under basic conditions, developed previously by Chemistry and Stereochemistry of Peptides and Proteins Research Group from Faculty of Chemistry UWr, for the qualitative and quantitative LC-MS analysis of sarcosine-containing drugs including racetams, tadalafil and their metabolites in blood and urine samples as well as extending the group of compounds to be tested with sulfinylacetamide derivatives such as armodafinil. We also plan to determine the acidity of the exchanged hydrogen atoms using the computational "proton affinity" method. Presented project will be performed in collaboration with scientists form Jan Mikulicz-Radecki University Teaching Hospital, Wrocław.

Methodology

The proposed project involves the preparation of deuterated standards of sarcosine and sulfinylacetamide moiety containing drugs and their metabolites by mentioned hydrogen/deuterium exchange method, determination of the kinetics of isotope exchange, isotope effect and their application for qualitative and quantitative LC-MS analysis. The isotopic labeling will be optimized for the reaction time and H/D exchange efficiency. The influence of used amine, pH, solvent compositions and microwave irradiation on HDX process will be analyzed. The kinetics of the HDX reaction will be determined by mass spectrometry and nuclear magnetic resonance spectroscopy. Additionally we plan to develop a direct hydrogen-deuterium isotope exchange method of sarcosine- and sulfinylacetamide-containing compounds present in urine and blood samples for their qualitative and quantitative analysis. Such process requires HDX reaction conditions optimization (pH, applied base, reaction time, temperature). Each sample after optimization will be analyzed by LC-MS and LC-MS/MS method. Such analysis may give us information about deuteration rate, possible side products and their chemical nature. To achieve this goal the blood (or urine) sample will be divided into two equal volume portions. One of them will be treated by D_2O/base/organic solvent mixture to perform the sample deuteration while the second part will be dissolved in H_2O/base/organic solvent mixture. After incubation, samples will be lyophilized, dissolved, base will be removed, mixed and analyzed by LC-MS. The HDX conditions for deuterated standards of armodafinil, racetams and tadalafil preparation will be investigated. The developed and optimized HDX reaction conditions will be used for global investigation of sarcosine- and sulfinylacetamide-containing compounds in blood and urine samples. The project will also include the use of the "proton affinity" computational method to determine the acidity of exchangeable hydrogen atoms.

Expected impact of the research project

It may be expected that the results obtained during this project may provide new insights into the qualitative and quantitative analysis of drugs including sarcosine and sulfinylacetamide moieties, like racetams, tadalafil and armodafinil create a novel tool for researchers, clinicians and forensic scientists working on improvement of diagnostic accuracy, evaluation of treatment efficacy, early diagnosis of disease and forensic investigation of some not approved sarcosine- and sulfinylacetamide-containing drugs for any medical or dietary administration. **We believe that the proposed method of deuterated standards of sarcosine- and sulfinylacetamide-containing drugs preparation may significantly reduce the costs of their analysis by LC-MS making it more common. While determining the acidity of individual hydrogen atoms, it will be possible to later predict the applicability of the described isotope exchange method to a wider group of compounds with the potential using as internal standards.** We hope that the proposed solution may also enable the development of a new branch of metabolomics that could be called sarcosinomics. The proposed strategy may allow for the analysis of still unknown endogenous sarcosine-containing compounds.