

The subject of the research conducted as a part of the dissertation was focused on elaboration of a new, reliable and robust methods based on gas chromatography-mass spectrometry technique (GC-MS), enabling determination of homocysteine thiolactone (HTL) in biological specimens in the presence of low molecular mass thiols, methionine (Met), homocysteine (Hcy), and cysteine (Cys). As a result the work provided convincing evidence that GC-MS technique can be successfully used in analysis of human urine and saliva for mentioned above non-volatile sulfur-containing compounds.

Taking into account the requirements of GC-MS, one of the main goals of my studies was to elaborate reliable and useful sample preparation procedures. On this field special attention I have paid on the possibilities of using extraction techniques, deproteinization, analytes preconcentration by drying under vacuum and appropriate chemical modification/derivatization of the compounds studied.

During the study three original methods I have developed. Moreover, I have proven that each of them is able to deliver valuable data concerning concentration of HTL in human urine and saliva. In this way, I was able to show for the first time that HTL is present in human saliva. It should be noted that saliva exhibit multiple distinct advantages over blood (plasma/serum) and urine as a diagnostic specimen. Measured values for salivary HTL are the first ever reported. Moreover, I have also examined a correlation between salivary concentration of HTL and its urinary levels. One of elaborated methodologies has been designed to simultaneous determination in a single run of HTL in the presence of a number of salivary biologically relevant compounds, such as Met, total Cys and Hcy. To the best of my knowledge, this is the first and the only available GC-MS based assay dedicated to salivary methionine related sulfur-containing compounds determination.

Elaborated assays are primarily characterized by a robust sample preparation procedure followed by GC-MS analysis. Finally, each of developed methodologies is characterized by satisfactory validation parameters, expressed as linearity, precision, accuracy and limit of quantification (LOQ). I have also shown that elaborated assays can act as an exquisite tools for determination of HTL in saliva as well as urine samples donated by apparently healthy volunteers. Thus, despite some limitations, they can be used in the future as analytical tools facilitating finding of evidences supporting thesis dealing with association of aminothiols with cardiovascular diseases.