Abstract

Clinical trials clearly indicate a cause-and-effect relationship between metabolism disorders of sulfur compounds, such as homocysteine (Hcy), and progression of cancer, neurodegenerative and cardiovascular diseases. The presence of Hcy in the human organism in high levels can indicate a high risk of disease. Homocysteine thiolactone (HTL) is metabolically linked to Hcy and is formed by so-called error-editing reaction due to the capture of Hcy instead of methionine by methionyl-tRNA synthetase. HTL has a negative impact on human health, due to its high reactivity it forms isopeptide bonds with the amino groups of lysine in a process called *N*-homocysteinylation. In humans with a properly functioning excretory system, most of the toxic HTL is excreted with urine. Therefore, urine is the most commonly used matrix for HTL determination.

Taking into account above mentioned reasons, the main aim of the study was to develop new analytical methods for the determination of HTL in human urine using efficient and cheap tools. As a result of the research, three new methods were developed, that are based on a combination of two techniques, i.e. capillary electrophoresis (CE) and single drop microextraction (SDME). The CE is characterized by high resolution and separation efficiency, as well as very short analysis time and low sample and chemicals consumption. However, CE has one significant drawback, i.e. low concentration sensitivity. To improve CE sensitivity, SDME technique was utilized. SDME is a powerful tool characterized by several advantages that are crucial for CE determinations. It allows sample cleanup, significantly reduces the use of toxic organic solvents, the extraction process can also be easily automated and, most importantly, it has a high enrichment factor. During the experiments, the main focus was on three aspects: the selection of sample preparation conditions prior to the SDME procedure, the selection of optimal SDME conditions to obtain the highest yield, and the use of a method that allows drop stabilization, which has a direct impact on the repeatability of the extraction process. Owing to the automation of the SDME procedure in CE system, the number of steps in the procedure performed directly by the experimenter is reduced. It is also possible to combine in one procedure two techniques to improve CE concentration sensitivity, i.e. SDME in off-line mode and sample stacking by field amplified sample injection.

Elaborated procedures are characterized by a simple sample preparation by SDME extraction followed by CE analysis. Each of developed methodologies is characterized by satisfactory validation parameters, such as linearity, precision, accuracy and limit of quantification. These methods can be used in the future as analytical tools to help find evidence to support thesis of an association between elevated HTL levels and cardiovascular diseases. To the best of my

knowledge, the developed procedures for HTL determination in human urine are the first that are based on the usage of SDME and CE.