Development of new methods for the treatment and analysis of blood biomarkers of chemical warfare agents by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS

Blister agents are highly alkylating toxic compounds used as chemical warfare agents. They can react with blood proteins, including albumin, forming stable adducts. These adducts, which have a lifespan of 20 to 25 days, can be used as biomarkers to retrospectively demonstrate exposure to such agents. The aim of this PhD was the development of a sample preparation and LC-MS analysis method. Monitoring biomarkers of five different blister agents in pure media was achieved using both reversed-phase (RP) and hydrophilic interaction chromatography (HILIC) modes. The sensitivity of these methods towards two families of albumin-adduct biomarkers of blister agents allowed for the selection of the enzyme to be used for the digestion step, namely proteinase K. An online solid-phase extraction (SPE) procedure was developed using commercial supports to treat the digests and facilitate the monitoring of the adducted peptides in real matrices. Protein precipitation, commonly used in published procedures, and protein reduction with DTT, which had never been applied to blister agent analysis, were investigated. The impact of these steps on the LC-MS signal of the SM-adducted tripeptide in plasma incubated with SM was evaluated. These steps did not result in increased sensitivity and were therefore omitted to reduce the overall analysis time. Online digestion on an immobilized enzyme reactor (IMER), replacing the enzyme-intensive in solution digestion step, was considered in order to automate the entire analytical workflow. The proteinase K IMERs developed showed lower activity compared to the free enzyme but still enabled the detection of SM adduction at the monitored site through the associated biomarker ion a fully automated procedure.