

Abstract

The thesis deals with the chromatographic determination of O-alkyl methyl phosphonic acids in human plasma and the treatment of biological material before the actual analysis. The theoretical part deals with the problems of individual groups of warfare agents, their mechanism of action and metabolism in the human body. The experimental part was devoted to the treatment of a human plasma sample in order to develop a procedure that would allow the subsequent determination of the concentration of free O-alkyl methyl phosphonic acids as metabolites of chemical warfare agents and thus help to distinguish them from those released from binding to human blood proteins. The second objective was to refute possible interference between the derivatization reagent and free O-alkyl methyl phosphonic acids.

The methodology and chromatographic analysis were applied to human plasma samples spiked with pinacolyl methyl phosphonic acid (PMPA) and ethyl methyl phosphonic acid (EMPA). These samples were first treated by centrifugal filtration, the procedure for which was under development. Centrifugation was carried out in Vivaspin 6 centrifuge tubes. The most efficient procedure was one in which the tubes were first rinsed with 400 μ l of water and centrifuged for 10 min at 8 000 *g*. Subsequently, the tubes were spotted with sample, centrifuged (20 min, 8 000 *g*) and washed again with 400 μ l of water (centrifugation 10 min, 8 000 *g*). After this step, precipitation and solid-phase extraction were carried out. LC-MS analysis followed. This developed procedure proved successful and led to the separation of free O-alkyl methyl phosphonic acids from those bound to plasma proteins.

In the second part of the thesis, a plasma sample with the addition of PMPA and with the addition of soman was analyzed. These samples were subjected to fluoride recovery and solid-phase extraction. Simultaneously, both samples were derivatized with 2[(dimethylamino)methyl]phenol (2-DMAMP). The second objective was also fulfilled; the given interference between the acid and 2-DMAMP was not confirmed, therefore there should be no false positive determination of soman in the sample even if it contained the given O-alkyl methyl phosphonic acid.

Key words: O-alkyl methyl phosphonic acids, chemical warfare agent, nerve agents, acetylcholinesterase, butyrylcholinesterase