Determination of Biomarkers of Organophosphorus Agent Intoxication by Ion Chromatography and Tandem Mass Spectrometry

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Introduction

As a result of a growing international political instability, the use of chemical weapons (CW) became a frequent and powerful tool of various terrorist groups. Nerve agents are highly toxic chemicals, some of them have been applied in terrorist and military activities. Nerve agents are often divided into two classes: G-series and V-series. The first one is O-alkyl methylphosphonofluoridates, such as (RS)-O-isopropyl methylphosphonofluoridate (sarin or GB), pinacolyl methylphosphonofluoridate (soman or GD) and cyclohexyl methylphosphonofluoridate (cyclosarin or GF). V-series type includes substituted aminoethyl-O-alkyl methylphosphonothioates, such as [2-(diisopropylamino)ethyl]-O-ethyl methylphosphonothioate (VX) and [2-(diisopropylamino)ethyl]-O-isobutyl methylphosphonothioate (VR). These substances have relatively short lifetime in human body and are hydrolyzed to their corresponding alkyl methylphosphonic acids (AMPAs), which are the specific markers of a certain nerve agent. The final and most stable hydrolysis product of all AMPAs is methylphosphonic acid (MPA). It is less specific than AMPAs, but can persist in samples for a long period of time without any degradation. This is particularly crucial in cases where access to the intended place of application of nerve agents is difficult or impossible for a long period of time. Ethylphosphonic acid (EPA), propylphosphonic acid (PPA) and isopropylphosphonic acid (iPPA) are similar in properties to MPA and can be formed during degradation of second-generation nerve agents.

Experimental part

IC–MS/MS data were obtained using a liquid chromatograph (Dionex Ultimate 3000) connected to an AB Sciex Qtrap 3200 mass spectrometer (AB Sciex, Canada) and a Shimadzu LCMS-8050 system (Shimadzu, Japan). Chromatographic separation was carried out on the anion-exchanger packed into a stainless steel chromatographic column (100 mm × 4 mm i.d.) which was made in our laboratory. The column was maintained at 35 °C. Mobile phase A consisted of a mixture of acetonitrile and water 23:77 (v/v) containing 27 mM of ammonium acetate. Mobile phase B consisted of a mixture of acetonitrile and water 70:30 (v/v) containing 27 mM of ammonium acetate. The following elution gradient was applied: 0-4.5 min (100% A), 4.5-9.5 min (100% A – 100% B), 9.5-13.5 min (100% B), 13.5-14.5 min (100% B – 100% A), 14.5-18.5 min (100% A). The injection volume was 10 µl for each sample.

The ESI-CID-MS/MS conditions on the AB Sciex Qtrap 3200 were as follows: negative ESI mode; ion spray voltage, –4500 V; ion source heater temperature, 350 °C; ion source gas (air) for nebulizing, 30 psi; ion source gas (air) for drying solvent, 40 psi; curtain gas (N_2), 15 psi; collision gas (N_2), high.

The Parameters of MS instrument on the Shimadzu LCMS-8050 were as follows: negative ESI mode; capillary voltage, –3000 V, nebulizing gas flow, 3 L/min, heating gas and drying gas flow, 10 L/min both, interface temperature, 300 °C, heat block temperature, 400 °C, temperature of desolvation line, 250 °C, CID gas pressure 270 kPa.

Strong anion-exchange cartridges (Chromabond SB), cartridges based on copolymer of styrene and divinylbenzene (Chromabond HR-P) and self-made hypercrosslinked polystyrene cartridges (HCLPS) were used for the extraction of APAs and AMPAs from urine.

Results

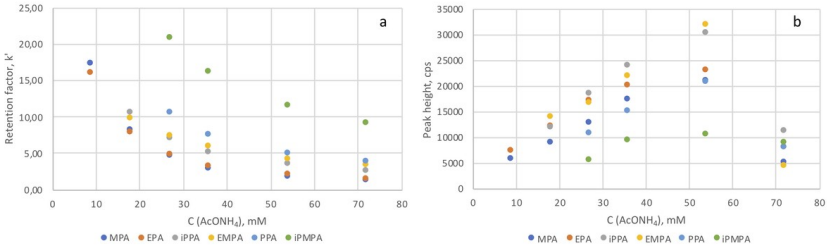

Figure 1. Dependence of retention factors (a) and peak heights (b) of phosphonic acids on the concentration of ammonium acetate in the mobile phase. Flow rate 0.8 mL min^{-1}.

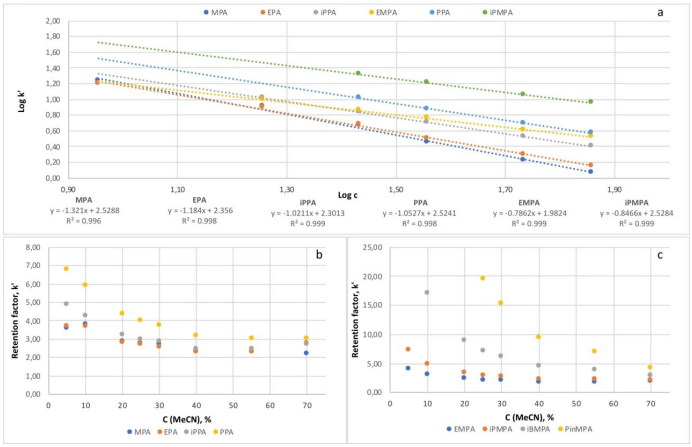

Figure 2. Plots of log k′ versus log c of ammonium acetate concentration in the mobile phase (a). Plots of retention factors of APAs (b) and AMPAs (c) versus acetonitrile volume fraction in the mobile phase (ammonium acetate concentration was constant and equaled to 27 mM).

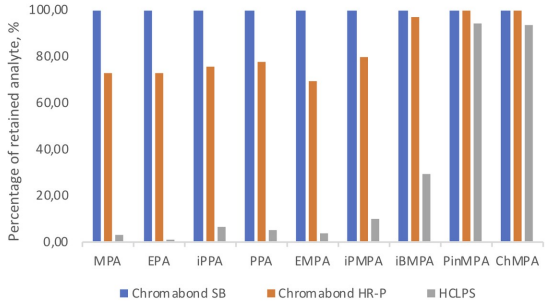

Figure 3. Percentage of retained analyte, obtained by comparison of peak areas of corresponding acids in the eluates of the cartridges and in the standard solution, which was not passed through the cartridge.

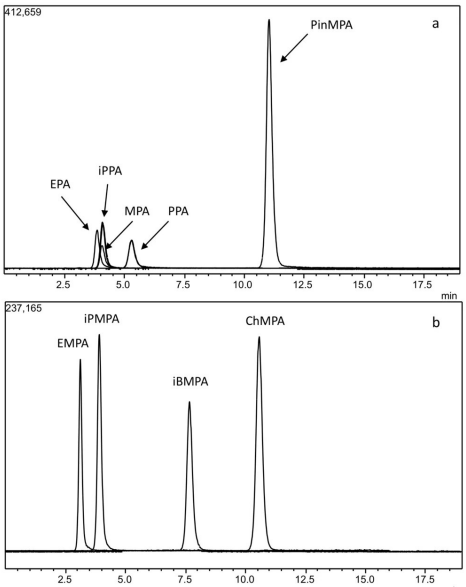

Figure 4. MRM chromatograms of the mixture of phosphonic acids in water (t_0=1.0 min) summarized by two individual transitions (Table 1) for each analyte. The peaks of MPA, EPA, PPA, iPPA, PinMPA (a) and EMPA, iPMPA, iBMPA, ChMPA (b) are presented as two separate chromatograms for a better illustration of the separation.

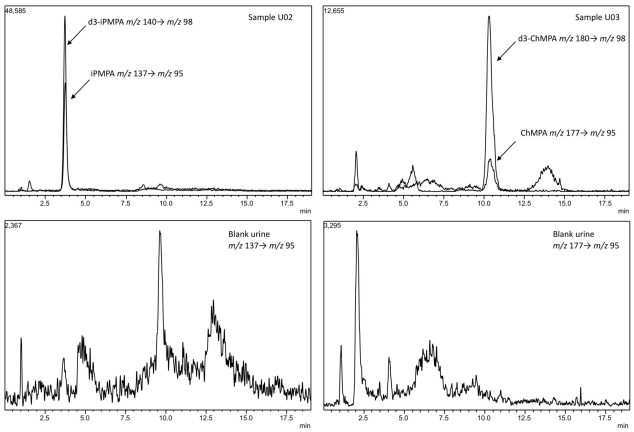

Figure 5. MRM chromatograms of blank urine samples (bottom) and urine samples U02 and U03 (top), that contain 28±3 ng mL^{-1} of iPMPA and 6±1 ng mL^{-1} of ChMPA.

Conclusion

In this work, a simple and multianalyte IC-MS/MS approach was developed and validated for determination of a wide range of alkylphosphonic acids and alkyl methylphosphonic acids in urine. The study of analyte retention on the in house made PS/DVB-based anion-exchange column showed that APAs are mainly retained due to ionic interaction, while AMPAs – due to hydrophobic interactions. This enabled simultaneous determination of both highly polar compounds and compounds with hydrophobic fragments. The developed sample preparation procedure using anion-exchange cartridges for solid-phase extraction provides high recovery values of the analytes and it can be successfully combined with the further anion-exchange separation. An application of deuterated internal standards and tandem mass spectrometry led to high accuracy and sensitivity of APAs and AMPAs determination in urine. The proposed procedure was validated using two different MS instruments and successfully tested on urine samples from the Biomedical Proficiency Test. Simultaneous determination of APAs and AMPAs allows to reveal the fact of using chemical weapons, establish its type and sometimes origin. The developed IC-MS/MS approach can be considered as one of the most effective ways to determine a wide range of phosphonic acids without the use of derivatization. This method has a potential for application in OPCW designated laboratories. It can be used to analyze biological or environmental samples taken by an inspection team in the area of the alleged use of chemical weapons in order to prove this fact and define the agent that was used.

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