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Screening of nerve agent markers with hollow fiber-chemosorption of phosphonic acids

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ABSTRACT

This report describes a method developed for extracting nerve gas markers such as phosphonic acids from urine and other aqueous samples. It involves single-step microextraction with chemosorption to hollow fibers that have been pre-soaked in a solution containing a derivatization reagent (3,5 triflouro methyl benzene diazomethane). The derivatives it forms with phosphonic acids can be sensitively detected by mass spectrometric detectors operating in negative chemical ionization (NCI) mode. Limits of quantification obtained in analyses of water and urine extracts by GC/MS in negative chemical ionization and selected ion monitoring mode were 0.1–10 and 0.5–10 ng/mL, respectively. Pentaflourophenyl diazomethane can also be used as a derivatization reagent, and the micro-extracts (which generate low background signals) can be sensitively analyzed by GC–MS/MS in NCI selected reaction monitoring (SRM) mode, using two specific transitions for both reagents. Thus, this sensitive approach can be flexibly modified to obtain confirmatory information, or address potential problems caused by interferences in some samples.

1. Introduction

The Chemical Weapons Convention (CWC) prohibits the use, preparation and stockpiling of chemical weapons (CWAs) and has been signed by most countries globally. Compliance with the convention is monitored by the Organization for the Prohibition of Chemical Weapons (OPCW). A key competence for this task is the ability to analyze CWAs, their degradation products and related compounds in environmental and biomedical samples. At the scene of an illegal use of chemicals methods for rapid provisional identification of the agent is required to provide the commander in chief with relevant information to manage the scene. These analysis can be performed in deployable laboratories supporting civilian hazmat teams, OPCW inspectors or military CBRN-units. Further on, verification analysis of the agents for legal and medical purposes will be done in the designated reach back laboratories. Fifteen (2015) countries have laboratories that are accredited for and designated by the OPCW for CWA analyses under the CWC. Nerve agents are highly toxic phosphor organic compounds that hydrolyze, forming phosphonic acids after environmental releases or in exposed humans. The formation of a specific O-alkyl methyl phosphonic acid is strictly linked to a specific nerve gas [1]. For example, soman and sarin respectively degrade to pinacolyl methylphosphonic acid and isopropyl methylphosphonic acid. Further slow 'aging' processes result in hydrolysis of the O-alkylchain, which eliminates the specificity of the phosphonic acids and hence identifiability of the original agent. The phosphonic acids are water-soluble and found in water, soil and on various surfaces after the use of nerve agents. Phosphonic acids, will be eliminated from the body in urine following exposure to non-lethal doses of nerve gas [2]. However, since nerve gases are highly toxic compounds and harmful to humans at low concentrations, concentrations of phosphonic acids in urine after exposure are also low [3].

Microextraction techniques are used in many applications, for example drug or pesticide tracing, using dispersive extractions [4] or various solid phase microextraction devices or fibers, including hollow fibers [5] [6] [7,8]. Porous hollow fibers have been used in chemical analyses for some time. Frequently a droplet of organic solvent is placed in them, then organic compounds are extracted into the solvent from aqueous samples. This approach can provide fast and convenient sample preparation and concentration of analytes [9]. The hollow fiber method can also be used in combination with derivatization [10,11]. For example, Ito et al. (2008) [7] have developed a method in which chlorophenols in urine samples are derivatized in solution, the derivatized compounds are extracted into a hollow fiber filled with toluene, and 2 μL of the toluene is

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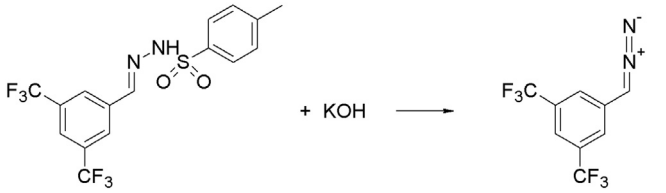

Fig. 1. The synthesis of the reagent 1-(diazomethyl)-3,5-bis(trifluoromethyl)benzene.

subsequently analyzed by GC/MS. Pardasani [12] have reported a similar approach for analyzing alkyl methylphosphonic acids (AMPAs) in aqueous samples, involving derivatization, extraction of the derivatives into a hollow fiber filled with trichloroethylene and analysis of 1 μL of the trichloroethylene by GC/MS. Another way to extract AMPAs from water or urine with hollow fibers, presented by Desoubries [13], involves use of a three-phase system with an acidic water/urine sample and a hollow fiber impregnated with octanol but filled with water at pH 14, followed by LC/MS analysis. Tak [14] have also used a three-phase system for extracting AMPAs from water into hollow fibers then analyzing the samples by LC/MS. In a refinement described by Lee [15], which reportedly affords significant improvements over SPME, CWA degradation products are derivatized "in situ" in a hollow fiber using the water-sensitive reagent *N*-*tert*-Butyldimethylsilyl-*N*-methyltrifluoroacetamide (protected in the solvent droplet), then analyzed by GC/MS.

Our key goal with this work is to develop analysis methods that can be used by deployable chemical analytical laboratories equipped with standard GC/MS for screening and if possible identification on site. The analysis will then be verified in a reach-back laboratory with sophisticated equipment e.g. GC–MS/MS, for verification. The work presented here is the development of a convenient, rapid and robust method for preparing samples (including urine and water samples) for analysis of phosphonic acids as the marker for illicit use of nerve gases. We explore the utility of a two-phase system with a hexane-reagent (3,5 triflouro methyl benzene diazomethane) phase in a hollow fiber performing chemosorption of phosphonic acids from a water sample. The sensitivity of the method was optimized with the GC/MS operated in negative ion chemical ionization (NCI) single ion recording (SIM) mode.

2. Experimental

2.1. Chemicals and reagents

The following phosphonic acids were used: methyl (MPA), ethyl methyl (EMPA), ethyl Cd_3 (EMPA- d_3), isopropyl methyl (IMPA), isopropyl Cd_3 (IMPA- d_3), butyl methyl (BMPA), pinacolyl methyl (PinMPA), pincolyl Cd_3 (PinMPA- d_3) and cyclohexyl methyl (CHMPA). PinMPA and EMPA were acquired from Sigma Aldrich (St. Louis, USA), all other phosphonic acids were synthesized in-house. 1000 ppm (weight/volume) stock solutions of the AMPAs were prepared in acetonitrile. The d_3 AMPAs were used as internal standards. A 10 μL portion of a 100 ppb solution of C^{13} hexachlorobenzene (Cambridge Isotope Laboratories, Andover, MA, USA) in hexane, prepared by diluting a 5 ppm stock solution in hexane, was added as a recovery standard (RS) before all GC/MS analyses.

1-(diazomethyl)-3,5-bis(trifluoromethyl)benzene (35F) was synthesized in-house, Fig. 1. (*E*)-*N*′-(3,5-bis(trifluoromethyl)benzylidene)-4-methylbenzenesulfonohydrazide (0.049 g, 0.12 mmol) were dissolved in a metanolic solution of potassium hydroxide (1.2 mL, 0.2 M) and heated to 80 °C in a microwave reactor for 2 min. After being cooled to room temperature, the methanolic solution was diluted with a small volume of pentane and washed with NaHCO3 (10% aq) and NaCl (30% aq), and dried over Na2SO4. Finally, the volume was adjusted with heptane to give a 0.25 M solution of 35F. As a complement, pentaflourophenyl diazomethane (PFB, a 0.25 M solution in hexane) was also tested as a derivatization reagent. All solutions were stored in a refrigerator except the 1 M stock solution of 35 F, which was stored at −20 °C. The solvents used were: dichloromethane and acetonitrile from Fisher (Loughborough, UK); heptane, benzene, toluene and chloroform from Merck (Damstadt, Germany); and dichloroethane, tetrahydrofuran and trimethyl benzene from Sigma Aldrich (St. Louis, USA). All solvents were of at least p.a. grade. In addition, NaCl and HCl were purchased from VWR Chemicals (Fontenay-sous-Bois, France), respectively, and used to prepare 1 M stock solutions that were stored at room temperature until use.

2.2. Sample preparation

The hollow fibers used were Accurel PP Q3/2 polypropylene fibers, supplied by Membrana GmbH (Wuppertal, Germany), that were cut into 3 ± 0.2 cm pieces, cleaned by immersion in acetone and sonication for 15 min, dried at room temperature, then stored in glass vials until use. The test matrixes were tap water from municipal supplies in Umeå (Sweden), water from the river Umeälven in Umeå and urine samples from five volunteers. Pooled urine samples from two individuals were used during optimization study. Samples were stored at +8 °C. EMPA, IMPA, BMPA, PinMPA and CHMPA GC/MS calibration curves were obtained using samples containing 4, 8, 16, 80 and 800 ng/mL of the respective analytes, 10 μL of the derivatization reagent (0.25 M) and 10 μL of the RS (100 ng/mL) in final volumes of 100 μL.

2.2.1. Hollow fiber characterization with SEM

The procedure for preparation of the specimens for SEM imaging is as follows: polypropylene fibers with and without exposure to reagent were deliberately fractured using cutting tools, mounted onto aluminum stub using carbon adhesive tape and silver paint, and sputter-coated with 5 nm Au/Pd (Quorum Q150T ES). The morphology of the samples was examined by field emission scanning electron microscope (FESEM; Carl Zeiss Merlin) using in-chamber secondary electron detector at accelerating voltage of 4 kV and probe current of 120 pA.

2.2.2. Optimization of experimental conditions

Any solvent used in the AMPA chemosorption process had to be capable of solubilizing the derivatization reagent, immiscible in water and not too volatile. Nine solvents were tested in attempts to identify the optimal choice: heptane, dichloromethane, toluene, tetrahydrofuran, benzene, dichloroethane, chloroform, acetonitrile and trimethyl benzene. We also tested a refinement applied by Desoubries [13] involving use of one solvent inside a hollow fiber and another on the outside, using heptane inside and acetonitrile outside. The samples used in the solvent study were 1 mL tap water samples spiked with 1–5 μg/mL (ppm) of EMPA, IMPA, BMPA and PinMPA (Fig. 2), saturated with salt and adjusted to pH 1–2 by adding HCl. The fibers were 1 cm long, filled with 5 μL of solvent using a syringe, and placed in solvent for 2 s to coat their outer surfaces and fill their pores with solvent. They were then placed in water samples for 90 min at room temperature. After this sampling period, the fibers were transferred to vials filled with 150 μL of heptane and sonicated for 5 min to extract the derivatized AMPAs from them into the solvent. Finally the fibers were removed and the extracts were analyzed by GC/MS in full-scan electrospray ionization (EI) mode.

In order to maximize recoveries of derivatized AMPAs, we first explored effects of pH and salinity in a screening design on extrac-