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Short communication

Solid supported in situ derivatization extraction of acidic degradation products of nerve agents from aqueous samples

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ABSTRACT

This study deals with the solid supported in situ derivatization extraction of acidic degradation products of nerve agents present in aqueous samples. Target analytes were alkyl alkylphosphonic acids and alkylphosphonic acids, which are important environmental signatures of nerve agents. The method involved *tert*-butyldimethylchlorosilane mediated in situ silylation of analytes on commercially available diatomaceous solid phase extraction cartridges. Various parameters such as derivatizing reagent, its concentration, reaction time, temperature and eluting solvent were optimized. Recoveries of the analytes were determined by GC–MS which ranged from 60% to 86%. The limits of detection (LOD) and limit of quantification (LOQ) with selected analytes were achieved down to 78 and 213 ng mL^{-1} respectively, in selected ion monitoring mode. The successful applicability of method was also demonstrated on samples of biological origin such as plasma and to the samples received in 34th official proficiency test conducted by the Organization for Prohibition the of Chemical Weapons.

1. Introduction

Alkyl alkylphosphonic acids (AAPA) and alkylphosphonic acids (APA) are persistent environmental degradation products of the most lethal type of Chemical Warfare Agents (CWA) known as Nerve Agents [1,2]. These phosphonic acids (PA) do not have any natural source of origin and therefore they serve as valuable markers for verification analysis of Chemical Weapons Convention (CWC) [2–4]. CWC which came into force in April 1997 prohibits the production, storage and use of CWA. The Organization for the Prohibition of Chemical Weapons (OPCW) situated in the Netherlands ensures the implementation of CWC through its strict verification regime [3,4].

Water may serve as an important matrix for investigation of the release of CWA in the environment. Abundant literature is available for the extraction and identification of CWA and related chemicals from aqueous samples. Most of the analytical methods rely on mass spectrometry coupled to a separation technique such as gas chromatography (GC–MS), liquid chromatography (LC–MS) and capillary electrophoresis (CE–MS) [5–10]. The later two techniques can directly handle aqueous samples but gas chromatography necessitates an efficient sample preparation protocol, essentially involving the removal of water and derivatization of non-volatile analytes (such as PA) [5–7].

Though a number of reactions are reported in literature for the conversion of PA into volatile derivatives, silylation is by far most common [11–13]. Because of its wide applicability, rich mass spectral database of silyl esters of PA is available commercially (NIST) as well as with the OPCW (The OPCW Central Analytical Database, OCAD) [14,15]. Also, a wide range of reagents are available commercially which can efficiently derivatize acids into corresponding silyl esters under anhydrous conditions [12,13,16]. Thus the GC–MS analysis of the PA present in aqueous matrix requires complete removal of water prior to silylation and instrumental analysis. Removal of water is achieved either by evaporation or by extraction of the analytes into suitable organic medium via silica solid phase (SAX-SPE) or mix mode anion exchange (MAX-SPE) [5,17]. No matter which procedure is adopted, it results into the addition of an extra step in the sample preparation, which in-turn increases the total analysis time as well as sources of errors.

In our previous studies, we have successfully demonstrated the derivatization of PA in water itself, leading to minimized sample preparation steps [18,19]. Two recent publications also deal with the same issue but the carcinogenicity of the reagents and requirement of large number of Tenax filled GC liners limit their use for practical purposes [20,21]. Hence the quest for a suitable

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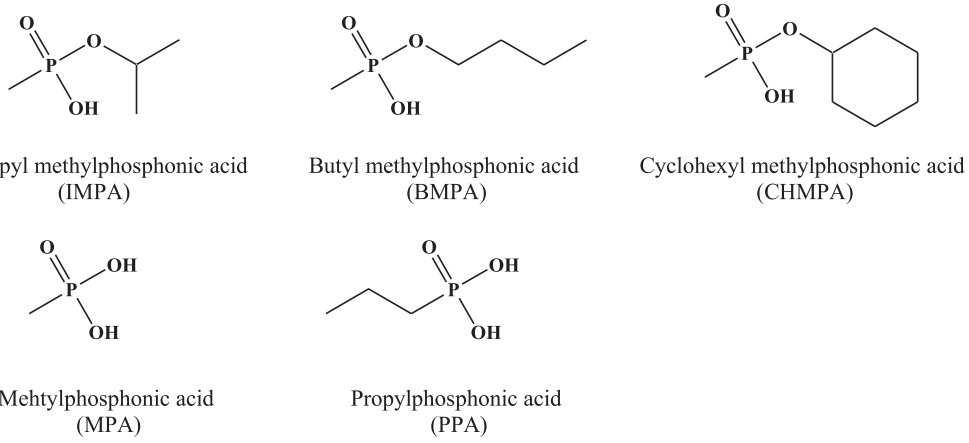

Fig. 1. Analytes selected for present study.

method for rapid screening of PA in water samples is still going on and herein we are reporting solid supported in situ derivatization extraction of selected PA from water (Fig. 1). The method involves absorption of aqueous sample on a commercially available diatomaceous sorbent (under various trade names such as Chem Elut, Cleanert, HyperSep SLE) followed by in situ derivatization and elution of the analytes. The method is very simple and straightforward, utilizes commercially available materials and chemicals and generates the typical silyl derivatives of PA for quick and easy identification.

2. Experimental

2.1. Solid supported in situ derivatization extraction procedure

A typical procedure is described as follows: Spiked water (1.0 mL) was loaded on Chem Elut (1 mL, un-buffered) solid supported liquid extraction (SLE) cartridge and was allowed to percolate the bed (sorbent bed became wet only three fourth of its total length). After 15 min, 0.5 mL of *tert*-butyldimethylchlorosilane (TBDMCS) solution (40% w/v in dichloromethane, DCM) was loaded and cartridge was placed in a dry bath set at 40 °C. After 30 min, the analytes were eluted with 2× 2 mL of eluting solvent (typically DCM). The eluate was dried over anhydrous sodium sulphate and concentrated to 500 μL under a gentle nitrogen stream. Appropriate aliquot of chromatographic internal standard tripropylphosphate (TPP) was added to achieve the final concentration of 10 μg mL^{-1}. One microliter of this extract was injected in the GC–MS for analysis.

3. Results and discussion

Solid supported liquid extraction (SLE) offers various advantages over conventional liquid-liquid extraction methods such as, support for automation for high throughput, and better recoveries and precision by removing the issues with emulsions that are often formed when performing liquid-liquid extraction [22–24]. It involves the immobilization of aqueous matrix over solid diatomaceous earth sorbent. The analytes enriched in a thin aqueous film over solid sorbent surface are subsequently eluted with water immiscible organic solvent. The high surface area at the interface between the organic and aqueous phases determines the overall efficiency of extraction process. The efficacy of the technique has been successfully demonstrated for the extraction of variety of compounds (including CWA) from aqueous samples of environmental and biological origin [22–24].

For present study however, the SLE process was slightly modified to achieve in situ derivatization extraction of selected PA. Parameters optimized for this purpose are discussed in the following sub-sections.

3.1. Selection of derivatizing reagent

A wide choice of reagents was available for performing the derivatization of PA adsorbed on sorbent surface. However, silylation reactions were preferred over others because of the availability of mass spectral data in commercial as well as OPCW databases [14,15]. A number of silylating reagents (ESI, S1.1) were first screened for their efficacy against the selected analytes adsorbed on damp diatomaceous sorbent surface. Only *N*-*tert*-butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA) and TBDMCS could produce the acceptable results in the GC–MS analyses. The failure of trimethylsilyl (TMS) reagents could be attributed to the hydrolytic instability of the formed esters and/or reagents under moist conditions. *tert*-Butyldimethylsilyl (TBDMS) derivatives on the other hand, show better tolerance to the moisture and thus could produce the derivatives, easily detectable in subsequent GC–MS analyses.

Between TBDMCS and MTBSTFA, the former is less reactive and therefore rarely used for derivatization purposes. MTBSTFA on the other hand is more reactive and has strong silyl donor reactivity [11,13]. In present case however, fast reactivity of the reagent may lead to rapid hydrolysis of the reagent itself. Hence TBDMCS is expected to produce better results as compared to MTBSTFA and actually it happened so. Lower recoveries (21–34%, data not shown) were observed where the derivatization of analytes adsorbed on SLE cartridges was carried out with 0.5 mL of MTBSTFA (in place of TBDMCS solution, Section 2.1). Less reactive TBDMCS ensured its availability to the analytes for sufficiently long period, producing excellent results. Since TBDMCS is solid at room temperature, a suitable organic solvent was required for its dissolution and subsequent application to the analytes enriched over sorbent surface. Toluene, acetonitrile, THF, pyridine, DCM and MTBSTFA were screened for this purpose and amongst these, later two produced best results. In subsequent studies however, DCM was preferred because of less artefacts and cleaner total ion chromatograms (GC-TIC) [25].

Finally, the concentration of TBDMCS was varied from 5 to 40% (w/v) in DCM and effect was observed on the recoveries of the analytes. Higher amount of reagent ensured its availability to the analytes for the reaction and therefore recoveries of all the analytes exhibited positive response towards TBDMCS concentration, reaching to >80% for selected AAPA. Recoveries of APA lagged behind,