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Single vial sample preparation of markers of nerve agents by dispersive solid-phase extraction using magnetic strong anion exchange resins

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ABSTRACT

A sample preparation method involving extraction, enrichment and derivatization of acidic degradation products of nerve agents was developed using magnetic strong anion exchange resins (MSAX). The method was performed in a single vial involving magnetic dispersive solid phase extraction (MDSPE). Analytes were derivatized with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) in the presence of resins. MSAX were custom synthesized using Fe_3O_4 nanoparticles as core, 4-vinylpyridine-co-divinylbenzene as polymer shell and quaternary pyridinium function as anion-exchanger. Hydroxide ions were the counter-anions of MSAX to effectively capture the acidic alkyl alkylphosphonic acids (AAPAs) and alkylphosphonic acids (APAs). Quantitative measurements of analytes were performed in the selected ion monitoring mode of GC–MS. Full scan mode of analysis was followed for identifications. Under the optimized conditions analytes were recovered in the range of 39.7–98.8% ($n = 3$, relative standard deviations (RSD) from 0.3 to 6.5%). Limits of detection (LODs) were in the range of 0.1–1.1 ng mL^{-1}; and the linear dynamic range was 5–1000 ng mL^{-1} with r^2 of 0.9977–0.9769. Applicability of the method was tested with rain-, tap-, muddy-water and Organization for Prohibition of Chemical Weapons (OPCW) Proficiency Test samples.

1. Introduction

Extraction and derivatization of hydrolyzed products of nerve agents are essential for the forensic and verification analysis of chemical weapons convention (CWC) [1–3]. To accomplish this task, Organization for Prohibition of Chemical Weapons (OPCW) maintains a global network of designated laboratories. Laboratories are designated through international official proficiency tests (PTs). Sample preparation in combination with analytical techniques such as gas chromatography–mass spectrometry (GC–MS) is used for identification of the CWC related compounds. Thus, efficient sample preparation is the key to meet analytical challenges of degradation products of chemical warfare agents (CWAs).

Nerve agents are highly toxic organophosphorous compounds [4]. They hydrolyze successively to alkyl alkylphosphonic acids (AAPAs) and alkylphosphonic acids (APAs) in water [5]. Hence, AAPAs and APAs are considered chemical markers of nerve agents. Identifications of AAPAs and APAs are achieved by sample preparation followed by analysis using GC–MS and/or liquid-chromatography–mass-spectrometry (LC–MS) [6]. Before GC–MS analysis, phosphonic acids are derivatized to impart volatility [6,7]. Various sample preparation methods are reported to extract AAPAs and APAs from water. These include disk-based solid phase extraction [7] solid-phase micro-extraction (SPME) [8,9], zirconia based solid phase extraction [10,11], molecular imprinted polymer isolation [12], on-matrix derivatization-extraction [13], sorbent trapped thermal desorption [14] and evaporation followed by cation removal [15]. For derivatization of phosphonic acids, trimethylsilylation is the frequently used method amongst the methylation, *tert*-butyldimethylsilylation and pentafluorobenzylation [16].

Recent advances in sample preparation have led to the development of magnetic dispersive solid phase extraction (MDSPE) [17–22]. MDSPE overcomes several limitations associated with the conventional solid phase extraction (SPE), such as handling of large volume samples and samples containing particulates [17,18]. MDSPE, owing to high surface area of the sorbent and magnetic

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collectivity, offer high extraction efficiency with operational simplicity. Reports on the use of magnetic nano-spheres for extraction and enrichment of CWAs and their degradation products are scarce [22,23]. Magnetic carbon nano-tubes have been used to extract CWAs from water [23], and sulfonic acid functionalized magnetic spheres have been reported to isolate basic degradation products of CWAs [22]. In comparison to conventional extraction materials, magnetic nano-particles offer several advantages such as operational simplicity, efficiency and applicability to turbid samples [17–23]. To the best of our knowledge, no reports are available on the use of magnetic nano-spheres for extraction and enrichment of AAPAs and APAs from aqueous samples. Prompted by the extraction virtues of MDSPE, herein, we report extraction and derivatization of AAPAs and APAs with chemically modified magnetic nano-spheres. To synthesize the magnetic nano-spheres with anion exchange capability, Fe_3O_4 nanoparticles were encapsulated in polyvinylpyridine through suspension polymerization [22,24]. To these polymer coated nano spheres, anion-exchange property was imparted by the quaternization of surface pyridine functions. Since these nano-spheres possessed the magnetic and anion-exchange properties, they were termed as magnetic strong anion exchange resins (MSAX). Using these MSAX, phosphonic acids were extracted from water through anion-exchange process.

2. Experimental

2.1. Materials

Iron (III) oxide nanoparticles <50 nm (Fe_3O_4), linoleic acid, 4-vinylpyridine (4-VP), divinylbenzene (DVB), azo(bis)isobutyronitrile (AIBN), polyvinyl alcohol (PVA), propyl chloride, polypropylene SPE tubes (3 mL), PTFE frits (20 μm) and tributylphosphate (TBP) were purchased from M/s Sigma–Aldrich Chemical Co. Pvt. Ltd., (Bangalore, India). To remove inhibitors, 4-VP and DVB were passed through alumina column (Aldrich, Cat. No. 306312, Milwaukee, WI, USA), vacuum distilled and stored at −5 °C. AIBN was recrystallized twice from methanol and stored in a vacuum oven for about 24 h. Polyethylene glycol (PEG-200), sodium chloride (NaCl), sodium hydrogen carbonate ($NaHCO_3$), sodium sulphate (Na_2SO_4), sodium nitrate ($NaNO_3$), calcium chloride ($CaCl_2$), sodium hydroxide (NaOH), hydrochloric acid (HCl) and all HPLC grade solvents were purchased from E. Merck (Mumbai, India) and used as received. Rain water, tap water (supplied from local municipal corporation) and muddy water (pooled rain water from a nearby pond of the laboratory) were used for the validation study. Nerve agent degradation products methylphosphonic acid (MPA), ethylphosphonic acid (EPA), isopropylphosphonic acid (IPA), propylphosphonic acid (PPA) and *O*-cyclohexyl methylphosphonic acid (CHMPA) selected for the study (Fig. 1) were synthesized in house by the reported procedures [25,26].

2.2. Standard spiking solutions

Single analyte stock standard solution was prepared in tetrahydrofuran at a concentration of 1 mg mL^{-1}. The stock standard solution of internal standard (tributylphosphate) was prepared in acetonitrile at a concentration of 1 mg mL^{-1}. Intermediate working standards containing 500 μg mL^{-1} of each MPA, EPA, IPA, PPA and CHMPA were prepared in tetrahydrofuran. For optimization of extraction parameters, water was spiked with an appropriate amount of intermediate working standard to get the final concentration of 1 μg mL^{-1} of each analyte.

2.3. Synthesis and functionalization of magnetic strong anion-exchange resins (MSAX)

MSAX resins were synthesized by the suspension polymerization [22] with modification as described below.

2.3.1. Grafting of linoleic acid on Fe_3O_4 nanoparticles

Fe_3O_4 nanoparticles (0.5 g) and linoleic acid (0.5 mL) were mixed in a 10 mL beaker for 10 min. After mixing, they were washed with 2 × 10 mL ethanol to remove excess of linoleic acid. Linoleic acid was grafted on the surface of Fe_3O_4 nanoparticles by chemisorption. Grafting took place due to innate binding of two oxygen atoms of carbonyl group with iron atoms located on the surface of Fe_3O_4 particles. Unbound linoleic acid was washed away with ethanol.

2.3.2. Synthesis of magnetic poly(4-vinylpyridine-co-divinylbenzene) (PVPDVB) resin

In a three necked (100 mL) round bottom flask PVA (0.25 g) was dissolved in 50 mL Milli-Q water and purged with nitrogen gas for 10 min. Fe_3O_4 nanoparticles (0.5 g) grafted with linoleic acid were added to this solution. 4-VP (2.5 mL), DVB (0.5 mL) and AIBN (0.03 g) were mixed in a beaker and added to the suspension. The mixture was stirred at 800 rpm at 60 °C for 1 h on a magnetic stirrer under N_2 atmosphere. After 1 h, the stirring rate and temperature were raised to 1100 rpm and 90 °C, respectively. The mixture was allowed to react for 5 h. PVPDVB coated magnetic resins were collected with

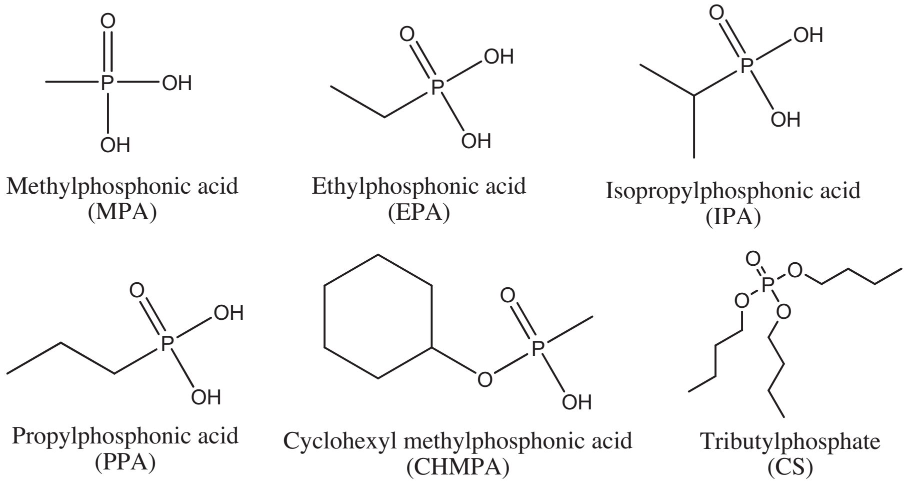

Fig. 1. Analytes selected for the study.

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