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Thermally assisted methylation and subsequent silylation of scheduled acids of chemical weapon convention for on-site analysis and its comparison with the other methods of methylation☆

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ABSTRACT

On-site verification of the chemical weapon convention (CWC) requires provision for the detection and identification of alkyl phosphonic acids as well as some organic acids that are amenable to GC–MS only after derivatisation. Various derivatisation methods have been used for the identification of these acids and for many cases the methyl derivatives are less prone to artifacts possibly leading to false positive identification. Methylation with diazomethane is widely used but, especially for on-site analysis it has limitation due to the potential explosive and health hazards. Other methylation procedures like trimethylsilyldiazomethane (TMSD), thermally assisted methylation (TAM) by trimethylphenylammonium hydroxide (TMPAH) and trimethylsulfonium hydroxide (TMSH) are evaluated. Data for methylation for the alkyl alkylphosphonic acids, alkylphosphonic acids and benzilic acid are reported. In addition, TAM followed by the silylation in the same sample without any additional sample preparation is also reported. Several parameters such as solvent, temperature, amount of reagents, time, etc. were studied. The two commercially available reagents namely, TMPAH and TMSH for TAM and subsequent silylation were evaluated. The LOD with TMPAH was below 0.5 ng per injection since all of the acids were detected by GC–MS with the S/N of >3 in full scan mode by AMDIS and their inter day relative standard deviation was from 4.7% to 10.8%.

1. Introduction

The chemical weapon convention (CWC) prohibits the development, production, stockpiling and use of chemical weapons [1]. The Organisation for the Prohibition of Chemical Weapons (OPCW), The Hague, The Netherlands ensures implementation of CWC by a verification program [2]. For verification, "sampling and analysis" is one of the important activities and it is accomplished by using on-site analysis—primarily with gas chromatography–mass spectrometry (GC–MS) analysis [3].

Particularly in investigations of alleged use of chemical weapons samples of soil and water contaminated during deliberate or inadvertent spread of chemical warfare agents (CWA) [4,5] are important. The nerve agent CWA typically undergo hydrolysis in the environment resulting in alkyl alkylphosphonic acids (AAPAs) and alkylphosphonic acids (APAs) [6,7]. The detection and identification of these acids indicate the probable prior presence of the parent compound (for example; nerve agents) in a given sample and is an important aspect of verification analysis of CWC [5,8].

Although many different instrumental techniques have been used successfully to detect these phosphonic acids [9–13], GC–MS is the most preferred one because of its adequate sensitivity and selectivity. In addition, only a very limited range of equipment is approved for on-site analysis by the OPCW inspectors. The phosphonic acids are amenable to GC–MS only after derivatisation and have been reviewed in the recent articles [14–18]. The most important and common derivatisation methods are silylation, methylation and pentafluorobenzylation [19–21].

The analyses of these acids are cumbersome, since either they are present in the aqueous matrices or water is required to extract them from any other matrix and the water must be subsequently removed prior to derivatisation [14]. The use of strong anion exchange (SAX) for on-site sample preparation procedure had reduced the time for the evaporation of water [22] but further improvements are needed. In the on-site sample preparation protocol of OPCW, trimethylsilyl is the only derivative currently used for alkyl phosphonic acids. Trimethylsilylation is a quite effective derivatisation but identification of alkyl alkylphosphonic acids due to spectral similarity of trimethylsilyl derivatives of O-alkyl alkylphosphonic acids when O-alkyl is higher than

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C4 and especially for O-cyclic alkyls is ambiguous. The corresponding methyl derivatives give additional information about the type of O-alkyl groups and thus it offers an alternate method of identification.

Methylations with diazomethane is widely used and although, the reaction is fast and the yield of the products is high, alternative methylation methods are preferred due to the potential hazards, difficulty in the preparation of diazomethane and especially transportation for on-site analysis. Trimethylsilyldiazomethane (TMSD) is a methylation reagent that is commercially available in standardised solution, it does not require cumbersome preparation steps and is neither mutagenic nor explosive. TMSD has been suggested as an efficient alternative to diazomethane [23–25]. Similarly thermally assisted or pyrolytic alkylation reactions are also well documented in the literature for various kinds of organic chemicals and have been reviewed in some recent reviews [26–30]. The pyrolytic alkylation reactions are generally conducted with tetra-alkylammonium salts. The analytes which are acidic in nature are mixed with the tetra-alkylammonium salts/hydroxides and injected into the GC injection port operated at 250–300 °C, where the analytes are derivatised.

Methylation of the scheduled acids gives additional information for the unambiguous identification of these acids. To include the methylation in the on-site analysis protocol, all of these methylation procedures were evaluated. Here the comparison of these methylation procedures in context with the alkyl alkylphosphonic acids, alkylphosphonic acids and benzilic acid are reported. There is a limited time for the on-site analysis so a procedure that can give information on both methyl and trimethylsilyl derivatives without additional sample preparation time is valuable. Prior to this study trimethylsilyldiazomethane is referred as synthetic procedure for methylation of various phosphonic acid [25], in this study the evaluation of TMSD as an analytical derivatisation method has been reported. In this study, thermally assisted methylation followed by the silylation in the same sample without any additional sample preparation is also reported. The two commercially available reagents namely, trimethylphenylammonium hydroxide (TMPAH) and trimethylsulfonium hydroxide (TMSH) for thermally assisted methylation (TAM) and subsequent silylation were also evaluated. Finally, comparison of all the above stated methylation processes for the scheduled acids, application of TAM and subsequent silylation on OPCW organised proficiency test is reported.

2. Experimental

2.1. Chemicals and materials

For this study, O-ethyl methylphosphonic acid (EMPA), methylphosphonic acid (MPA), ethylphosphonic acid (EPA), O-pinacolyl ethylphosphonic acid (PinEPA) and benzilic acid (BA) were used as a model compounds. Standards of these acids were purchased as neat commercial chemicals from Aldrich (Seelze, Germany) with purity higher than 95%.

The analytical grade solvents hexane, methanol (MeOH), 1-propanol (1-PrOH), 2-propanol (isopropanol; 2-PrOH) and acetonitrile were from J.T. Baker, (Deventer, The Netherlands), ACS grade ethanol (EtOH) from Riedel De Haen (Germany), dichloromethane from Fluka (Buchs, Switzerland), ACS grade benzene and tetrahydrofuran (THF) 99% from Aldrich (Steinheim, Germany), Ultra residue analysed grade toluene, ethyl acetate and chloroform from J.T. Baker (Phillipsburg, USA), concentrated 37% hydrochloric acid (HCl) from Aldrich (Seelze, Germany). The derivatising agent *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and Diazald were purchased from SUPELCO (Bellefonte, PA, USA) and 2.0 M solution of trimethylsilyldiazomethane in hexane from Aldrich (Steinheim, Germany). Thermally assisted methylating agents 0.5 M solution of trimethylphenylammonium hydroxide in methanol and 0.25 M trimethylsulfonium hydroxide were purchased from Fluka (Switzerland). The internal standard tri-*n*-butyl phosphate (TBP) was purchased from Aldrich (Seelze, Germany). Millipore water (18 MΩ cm) was used as deionized water. The standard water for spiking was prepared by spiking magnesium sulfate heptahydrate and calcium chloride dihydrate procured from Aldrich (Seelze, Germany), sodium carbonate procured from J.T. Baker (Deventer, The Netherlands), sodium sulfate procured from Fluka (Buchs, Switzerland) at concentration of 250 μg/mL.

The Accubond II SAX cartridges (silica, 200 mg, 3 mL) were obtained from Agilent Technologies (Milwaukee, WI, USA).

2.2. Standard solutions

Stock standard solutions (5 mg/mL) for each of the acids as described earlier were prepared from the neat commercial chemicals without further purification by separately weighing 20 mg of the chemical into a 4 mL vial and diluting with 4 mL of acetonitrile. Stock solutions of the TBP (5 mg/mL and 1 mg/mL) were prepared by weighing 20.0 mg and 4.0 mg, respectively, into a 4 mL vial and diluting with 4 mL of acetonitrile. A working solution was prepared from the stock solutions of MPA, EPA, PinEPA, EMPA and BA with a concentration of 100 μg/mL (100 ppm) of each of the acids.

2.3. Instrumentation

The samples were analysed by GC–MS in electron ionization mode and dual flame photometric detector (dual-FPD) in phosphorus {P} and sulfur {S} channel, using an Agilent 6890N gas chromatograph equipped with a 5975 inert XL mass selective detector (Agilent Technologies, Milwaukee, WI, USA). A Restek Rxi-5ms capillary column, 30 m × 0.25 mm × 0.25 μm, was used. The column oven temperature was programmed from 40 °C (hold for 2 min) to 280 °C at 10 °C/min, and hold at 280 °C for 6 min. Helium (99.999%) at a constant flow rate of 0.9 mL/min was used as a carrier gas. The samples were analysed in the splitless mode at an injection temperature of 250 °C. Injected volume was 1 μL using a Combi PAL autosampler (CTC Analytics, Zwingen, Switzerland) equipped with a 10 μL Hamilton syringe. GC interface temperature was set at 280 °C. Mass spectra were obtained with electron energy of 70 eV, and mass spectral data were acquired over a mass range of 40–450 amu. EI source temperature and the quadrupole temperature were set at 230 °C and 150 °C, respectively.

2.4. Analytical procedures

During this study each and every experiment was repeated three times and the data reported here are the averages of these data.

2.4.1. Strong anion exchange extraction procedure

The analytes were extracted from aqueous samples using strong anion exchange SAX [22]. The SAX cartridge was conditioned by passing 1 mL of methanol followed by 1 mL of Milli Q water, 2 mL of aqueous sample was loaded on the cartridge and washed with 4 mL of water and 4 mL of methanol. The acids were extracted from the cartridge by eluting with 2 mL of 0.1 N hydrochloric acid in methanol. This eluent was evaporated to dryness and analysed after derivatisation.

2.4.2. Methylation by diazomethane

Diazomethane was freshly prepared by slowly adding potassium hydroxide into *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide ethereal solution. Diazomethane was condensed with diethyl ether and stored at −20 °C.