Journal of Chromatography A, 1448 (2016) 32–41

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Analysis of chemical warfare agents in organic liquid samples with magnetic dispersive solid phase extraction and gas chromatography mass spectrometry for verification of the chemical weapons convention

Varoon Singh[a], Ajay Kumar Purohit[a], Sridhar Chinthakindi[a], Goud Raghavender D.[a], Vijay Tak[a], Deepak Pardasani[a], Anchal Roy Shrivastava[b], Devendra Kumar Dubey[a,*]

[a] *Vertox Laboratory, Defence Research and Development Establishment, Jhansi road, Gwalior 474002, India*
[b] *Electron Microscopy Division, Defence Research and Development Establishment, Jhansi road, Gwalior 474002, India*

ARTICLE INFO

Article history:
Received 10 February 2016
Received in revised form 5 April 2016
Accepted 19 April 2016
Available online 20 April 2016

Keywords:
Chemical weapons convention
Chemical warfare agents
Magnetic dispersive solid phase extraction
GC–MS
Sample preparation
Magnetic nanoparticles

ABSTRACT

A simple, sensitive and low temperature sample preparation method is developed for detection and identification of Chemical Warfare Agents (CWAs) and scheduled esters in organic liquid using magnetic dispersive solid phase extraction (MDSPE) followed by gas chromatography-mass spectrometry analysis. The method utilizes Iron oxide@Poly(methacrylic acid-co-ethylene glycol dimethacrylate) resin (Fe_2O_3@Poly(MAA-co-EGDMA)) as sorbent. Variants of these sorbents were prepared by precipitation polymerization of methacrylic acid-co-ethylene glycol dimethacrylate (MAA-co-EGDMA) onto Fe_2O_3 nanoparticles. Fe_2O_3@poly(MAA-co-EGDMA) with 20% MAA showed highest recovery of analytes. Extractions were performed with magnetic microspheres by MDSPE. Parameters affecting the extraction efficiency were studied and optimized. Under the optimized conditions, method showed linearity in the range of 0.1–3.0 $\mu g\,mL^{-1}$ ($r^2 = 0.9966$-0.9987). The repeatability and reproducibility (relative standard deviations (RSDs) %) were in the range of 4.5–7.6% and 3.4–6.2% respectively for organophosphorous esters in dodecane. Limits of detection (S/N = 3/1) and limit of quantification (S/N = 10/1) were found to be in the range of 0.05–0.1 $\mu g\,mL^{-1}$ and 0.1–0.12 $\mu g\,mL^{-1}$ respectively in SIM mode for selected analytes. The method was successfully validated and applied to the extraction and identification of targeted analytes from three different organic liquids i.e. *n*-hexane, dodecane and silicon oil. Recoveries ranged from 58.7 to 97.3% and 53.8 to 95.5% at 3 $\mu g\,mL^{-1}$ and 1 $\mu g\,mL^{-1}$ spiking concentrations. Detection of diethyl methylphosphonate (DEMP) and *O*-Ethyl S-2-diisopropylaminoethyl methylphosphonothiolate (VX) in samples provided by the Organization for Prohibition of Chemical Weapons Proficiency Test (OPCW-PT) proved the utility of the developed method for the off-site analysis of CWC relevant chemicals.

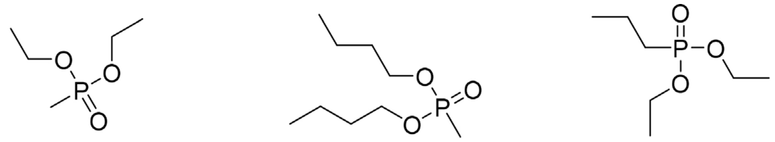
1. Introduction

Chemical warfare agents (CWAs) are powerful noxious chemicals which have been used as weapons of mass destruction [1–4]. These consist of nerve, blister, choking, blood and psychochemical agents. Among these, nerve agents classified as G-agents (sarin, soman, tabun and cyclosarin) and V-agents (VX) are the most lethal ones [4,5]. These chemicals have shown their horrifying capabilities in many incidents related to the military and terrorist activities [6]. An international treaty, known as Chemical Weapons Convention (CWC), came in the existence in the year 1997 which prohibits the production, storage and usage of CWAs other than the purposes not prohibited in the convention [7,8]. An organization known as the Organization for Prohibition of Chemical Weapons (OPCW) ensures implementation of CWC through its strict verification program [9,10]. To support its verification program OPCW maintains a global network of designated laboratories [11–13]. The laboratories achieve and/or sustain the status of designated laboratory through successful performance in international official proficiency tests (OPTs) organized by the OPCW [14]. Analytical work pertaining to the OPTs entails sample preparation followed by instrumental analysis by gas or liquid chromatography coupled to nitrogen-phosphorous detector (GC-NPD), flame photometric

---

* Corresponding author.
E-mail addresses: dkdubey@rediffmail.com, vertoxdrde@gmail.com (D.K. Dubey).

http://dx.doi.org/10.1016/j.chroma.2016.04.058



***O,O′*-Diethyl methylphosphonate (DEMP)**

***O,O′*-Dibutyl methylphosphonate (DBMP)**

***O,O′*-Diethyl propylphosphonate (DEPP)**

***O,O′*-Diethyl *N,N*-diethylphosphoramidate (DEDEP)**

***O*-Cyclohexyl methylphosphonofluoridate (Cyclosarin, GF)**

3,3-dimethylbutan-2-yl methylphosphonofluoridate (Soman, GD)

***O*-Ethyl *N, N*-dimethylphosphoramidocyanidate (Tabun, GA)**

Fig. 1. Analytes selected for the study.

detector (GC-FPD), mass spectrometer (GC–MS and LC–MS), Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance spectroscopy (NMR) [14,15]. However, GC–MS is most widely used technique because of its high sensitivity and unequivocal identification of CWC relevant chemicals (CRCs) [16]. However, the key for successful detection and identification of CRCs in spiked matrix during OPTs lies in sample preparation [17,18].

Organic liquid samples such as *n*-hexane, dichloromethane, ethyl acetate and dodecane are commonly submitted matrices in OPTs for detection and identification of CRCs. Extraction and enrichment of analytes (e.g. hydrophobic CWAs) from organic liquid samples pose difficulty due to similar chemical nature of analytes and matrix. Methods, presented in the recommended operating procedures (ROPs) for organic samples, include concentration, dilution, solvent exchange, liquid–liquid extraction (LLE), and solid phase extraction (SPE) [17–22]. Dilution and concentration have their own limitations. Dilution increases the chances of false negative identification due to probability of decreased concentration of analytes below the detection limits of instruments, whereas the concentration increases the background chemicals which can mask the peaks of interest. SPE may adsorb the analytes strongly resulting to low recoveries and may even trap high hydrocarbon background. This increases the possibilities of analytes loss. Further, the method is not suitable for organic samples which are non-volatile and difficult to concentrate such as dodecane and silicon oil. Thus, solvent exchange method is still most effective method for sample preparation of organic liquid samples [21]. This method was reported by our group and since then no significant development has taken place in this field.

Magnetic dispersive solid phase extraction (MDSPE) has gained attention in the immediate past, due to its virtues like high surface area, magnetic retrievability, operational simplicity, high extraction efficiency, ability to extract analytes from large volume samples and selective functionalization of materials [23]. The key feature of the MDSPE is tailored functionalization of magnetic sorbents to aid the extraction chemistry of analytes present in samples [23–25].

A vast literature is available for extraction of non-polar compounds like pharmaceuticals [26,27], pesticides [28], phenols [29], polar contaminants [30], sweeteners [31], illicit drugs [32], personal care products [33], CWAs and their degradation products [34–37] from aqueous matrix. But till date no report is available on MDSPE of analytes from organic liquids. The scarcity of sample preparation methods for CWAs from organic liquid, and virtues of MDSPE prompted us to develop an efficient method. Herein, we are reporting the MDSPE of CRCs from organic liquid assisted by Fe_2O_3@Poly(MAA-co-EGDMA) functionalized nanoparticles.

To the best of our knowledge this is the first report on sample preparation by using magnetic polymer based resin for extraction of CWAs and CRCs from organic liquid.

2. Experimental section

2.1. Chemicals

$Fe_2O_3 < 50$ nm, 2,2-azobis(2-methyl-propionitrile) (AIBN), methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA), alumina column, dodecane, tri-*n*-butyl phosphate (TBP) (chromatographic standard) were purchased from Sigma-Aldrich (New Delhi, India). AIBN was recrystallized twice from ethanol and dried in a vacuum oven for 24 h. MAA and EGDMA were passed through alumina column and then vacuum distilled before use. MAA and EGDMA were distilled at 50 °C at 1.5 mm Hg and 40 °C at 1.0 mm Hg respectively. HPLC grade solvents acetonitrile, *n*-hexane, chloroform and silicon oil were purchased from E. Merck (Mumbai, India) and used as received. CWAs simulants such as *O,O′*-diethyl methylphosphonate (DEMP), *O,O′*-diethyl propylphosphonate (DEPP), *O,O′*-dibutylmethylphosphonate (DBMP), *O,O′*-diethyl *N,N*-diethylphosphoramidate (DEDEP) and real agents 3,3-dimethylbutan-2-yl methylphophonofluoridate (soman), *O*-cyclohexylmethylphosphonofluoridate (cyclosarin), *O*-ethyl-*N,N*-diethylphosphoramidocyanidate (tabun) were synthesized in our laboratory in microgram quantities and purified