Journal of Chromatography A, 1370 (2014) 80–92

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Simultaneous detection and identification of precursors, degradation and co-products of chemical warfare agents in drinking water by ultra-high performance liquid chromatography–quadrupole time-of-flight mass spectrometry

Vijay Tak[a], Ajay Purohit[a], Deepak Pardasani[a], D. Raghavender Goud[a], Rajeev Jain[b], D.K. Dubey[a,*]

[a] Vertox Laboratory, Defence Research and Development Establishment, Gwalior 474002, India
[b] School of Studies in Chemistry, Jiwaji University, Gwalior 474002, India

ARTICLE INFO

Article history:
Received 3 July 2014
Received in revised form 9 October 2014
Accepted 9 October 2014
Available online 22 October 2014

Keywords:
Chemical warfare agents
Chemical Weapons Convention
Quadrupole time-of-flight mass spectrometry
In-source collision induced dissociation
Tandem mass spectrometry
Ultra-high performance liquid chromatography

ABSTRACT

Environmental markers of chemical warfare agents (CWAs) comprise millions of chemical structures. The simultaneous detection and identification of these environmental markers poses difficulty due to their diverse chemical properties. In this work, by using ultra-high performance liquid chromatography–quadrupole time-of-flight mass spectrometry (UHPLC–QTOF), a generic analytical method for the detection and identification of wide range of environmental markers of CWAs (including precursors, degradation and co-products of nerve agents and sesqui-mustards) in drinking water, was developed. The chromatographic analysis of 55 environmental markers of CWAs including isomeric and isobaric compounds was accomplished within 20 min, using 1.8 μm particle size column. Subsequent identification of the compounds was achieved by the accurate mass measurement of either protonated molecule $[M+H]^+$ or ammonium adduct $[M+NH_4]^+$ and fragment ions. Isomeric and isobaric compounds were distinguished by chromatographic retention time, characteristic fragment ions generated by both in-source collision induced dissociation (CID) and CID in the collision cell by MS/MS experiments. The exact mass measurement errors for all ions were observed less than 3 ppm with internal calibration. The method limits of detection (LODs) and limits of quantification (LOQs) were determined in drinking water and found to be 1–50 ng mL^{-1} and 5–125 ng mL^{-1}, respectively. Applicability of the proposed method was proved by determining the environmental markers of CWAs in aqueous samples provided by Organization for the Prohibition of Chemical Weapons during 34th official proficiency test.

1. Introduction

After the recent use of Chemical Weapons (CW) in Syria, in August 2013 [1], despite the Chemical Weapons Convention (CWC) being in place, international community has apprehension that terrorist organization might be tempted to acquire and use CW against civilian people. The CWC, except for purposes that are not prohibited under the convention, prohibits the production, storage and usage of chemical warfare agents (CWAs). The treaty is administered by the Organization for the Prohibition of Chemical Weapons (OPCW) through its strict verification regime [2]. For the verification purpose, CWC enlisted millions of compounds having diverse chemical structures. However, very few specific compounds have been developed as CWAs, even though, verification program of CWC requires unambiguous identification of these enlisted compounds in the samples collected by the OPCW inspectors from the production, storage or suspected sites. Generally, OPCW inspectors perform on-site analysis; however, in case of any ambiguities samples may be sent to the designated laboratories certified by the OPCW for off-site analysis. The designated laboratories involved in off-site analysis aim to enhance their analytical capabilities to meet the challenges associated with verification program of the CWC. The major analytical challenge of verification program is the detection and identification of chemically diverse structures. This challenge becomes more difficult in the presence of high chemical background with low concentration level of analytes. Laboratories involved in off-site analysis use

* Corresponding author at: Vertox Lab, Defence R&D Establishment, Jhansi Road, Gwalior 474002, India. Tel.: +91 751 2233488; fax: +91 751 2341148.
E-mail address: dkdubey@rediffmail.com (D.K. Dubey).

http://dx.doi.org/10.1016/j.chroma.2014.10.030
0021-9673/

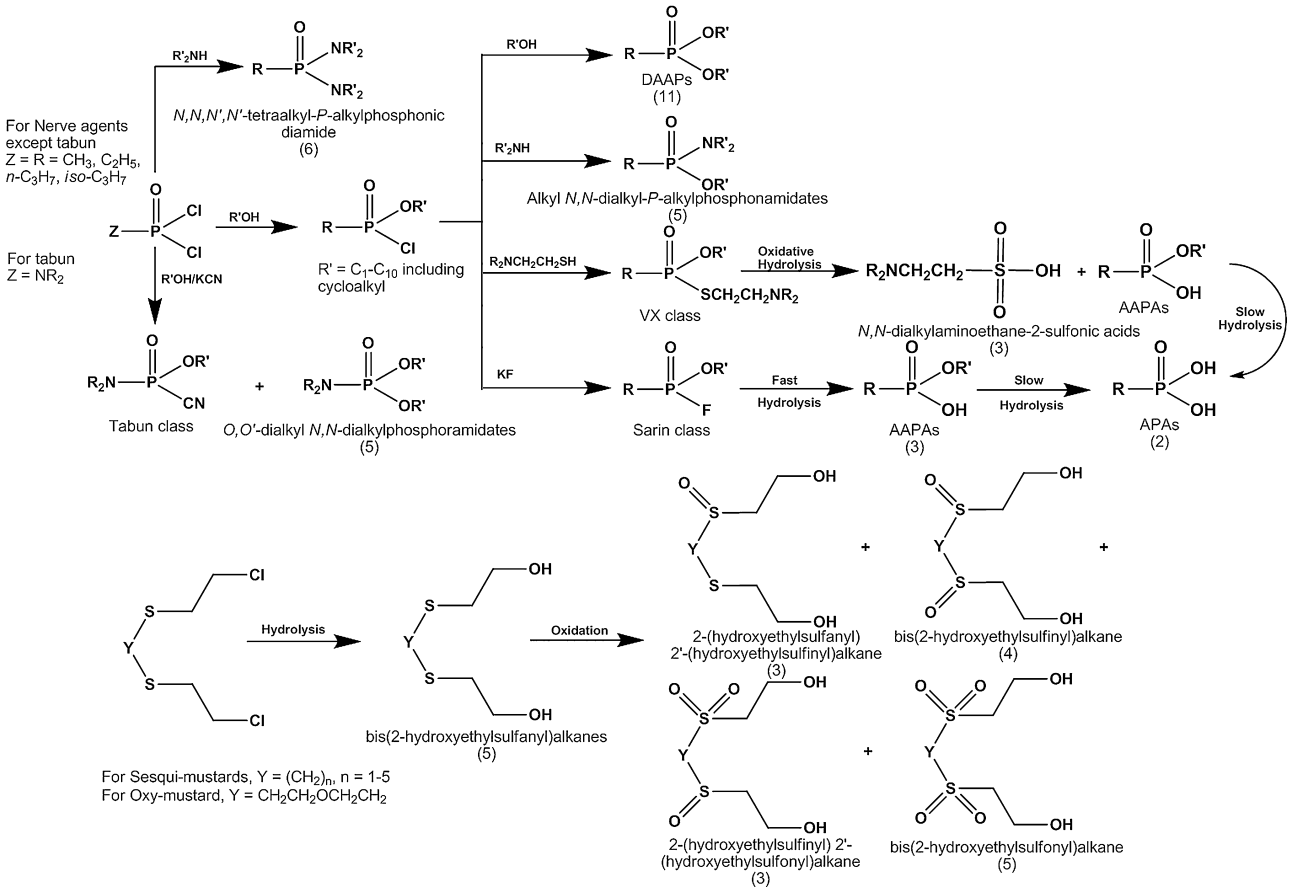

Fig. 1. Environmental markers of nerve agents and sesqui-mustards. Number in parenthesis indicates the number of analytes included in study from particular class. For details, see Section 2.

different instrumental techniques such as gas chromatography–mass spectrometry (GC–MS), liquid chromatography–mass spectrometry (LC–MS), Fourier transform infrared spectroscopy and nuclear magnetic resonance spectroscopy to screen different class of compounds. Therefore, a prerequisite is to have a generic method which has the ability to identify the large number of compounds simultaneously.

CWAs are generally unstable, especially in the presence of water. Hydrolysis of CWAs leads to a multitude of stable degradation products. These degradation products are relatively non-toxic and have negligible industrial importance. However, there are exceptions which have industrial importance like thiodiglycol in textile industries and dimethyl methylphosphonate as a flame retardant. But their presence in the environmental samples is a good indication of possible use or production of CWAs.

Munitions grade mustard formulations typically contains additional sulphur vesicants including bis(2-chlorethylthio)ethane (sesqui-mustards), bis[(2-chloroethylthio)ethyl]ether (oxy-mustard) and longer chain sulphur vesicants [3]. Under ambient conditions, degradation of sulphur mustard and its analogs occur through hydrolysis and oxidation that give rise to thiodiglycol (TDG), bis(2-hydroxyethylsulfanyl)alkanes, 2,2′-bis(2-hydroxyethylsulfanyl)diethyl ethers and their multiple oxidation products, Fig. 1.

In water, nerve agents hydrolyze to non-toxic alkyl alkylphosphonic acids (AAPAs) and subsequently slowly to alkylphosphonic acids (APAs). AAPAs and APAs are persistent in environment. Thus, they are significant for verification of the CWC, as they would not be routinely detected in environmental samples. Furthermore, co-products generated during the synthesis of nerve agents, are also important markers of nerve agents as depicted in Fig. 1. For example, *O*,*O*′-dialkyl alkylphosphonates (DAAPs) and *O*,*O*′-dialkyl *N*,*N*-dialkylphosphoramidates are inevitable co-products of nerve agents. *N*,*N*-Dialkylaminoethane-2-sulfonic acids are non-scheduled markers of *O*-alkyl *S*-2-dialkylaminoethyl alkylphosphonothiolates (VX type). Moreover, *N*,*N*,*N*′,*N*′-tetraalkyl-*P*-alkylphosphonic diamides and alkyl *N*,*N*-dialkyl-*P*-alkylphosphonamidates are also important markers of nerve agents which are included in Schedule 2.B.4 of CWC [2]. Consequently, a reliable and sensitive technique is required for the simultaneous detection of these different classes of markers of CWAs in aqueous samples.

Chemical diversity of environmental markers of CWAs ranges from highly polar (AAPAs, APAs, *N*,*N*-dialkylaminoethane-2-sulfonic acids, bis(2-hydroxyethylsulfanyl)alkanes and their oxidized products) to non-polar (DAAPs, *O*,*O*′-dialkyl *N*,*N*-dialkylphosphoramidates, alkyl *N*,*N*-dialkyl-*P*-alkylphosphonamidates and *N*,*N*,*N*′,*N*′-tetraalkyl-*P*-alkylphosphonic diamides), volatile (phosgene) to non-volatile (salts of VX), acids (pKa = 2, AAPAs and APAs) to bases (pKa = 9.2, triethanolamine), and alcohols (pinacolyl alcohol) to amides (*N*,*N*,*N*′,*N*′-tetraalkyl-*P*-alkylphosphonic diamides and alkyl *N*,*N*-dialkyl-*P*-alkylphosphonamidates).

Currently, most reliable analytical technique for the detection and identification of CWAs and their markers is based on GC–MS [4]. GC–MS provides required sensitivity for chromatographic separation and identification of CWAs and their markers. Since aqueous samples or extracts, which are common environmental matrix, cannot be directly analyzed by GC–MS, it requires compatible organic phase transfer and in case of polar analytes, it also requires time