Ultra-Trace Analysis of Organophosphorus Chemical Warfare Agent Degradation Products by HPLC-ICP-MS

Application

Homeland Security

Authors

Douglas D. Richardson, Baki B.M. Sadi,
and Joseph A. Caruso*
Department of Chemistry
University of Cincinnati
Cincinnati, Ohio 45221-0172
USA

Abstract

A coupled system of high performance liquid chromatography (HPLC) with inductively coupled plasma mass spectrometry (ICP-MS) as an element specific detector was used for analysis of three organophosphorus chemical warfare degradation products. Ethyl methylphosphonic acid (EMPA, the major hydrolysis product of VX), isopropyl methylphosphonic acid (IMPA, the major hydrolysis product of Sarin (GB)), and methylphosphonic acid (MPA, the final hydrolysis product of both) were separated by reversed phase ion-pairing high performance liquid chromatography (RP-IP-HPLC). The separated organophosphorus hydrolyzates were directly introduced into ICP-MS and detected at *m/z* 31. Detection limits for EMPA, IMPA, and MPA were found to be 263, 183, and 139 pg/mL, respectively, with separation in less than 15 minutes. The developed method was successfully applied to an environmental sample matrix.

Introduction

Recent increases in terrorist activity and the threat of chemical weapon attacks have led to the demand for a rapid and reliable method for the analysis of chemical warfare agents (CWA) and their degradation products. As a result of the Chemical Weapons Conventions (CWC), which banned the production, acquisition, retention, and direct or indirect transfer of chemical weapons, destruction of all chemical weapons held in reserve was mandated [1, 2]. These chemicals, which include nerve and vesicant agents, pose a deadly threat, not only to the human population, but also to vital aquatic and agricultural resources (Table 1.) [1, 3–6]. Based on these facts, the development of sensitive and selective analytical techniques for the analysis of CWA and their degradation products is of high importance to ensure homeland security.

Phosphorus containing nerve agents along with their degradation products present difficulties for ultra-trace analysis due to their high polarity, low volatility and lack of a good chromophore. Direct analysis of CWA degradation products provides an indirect technique for CWA detection (Figure 1). Previous studies have successfully utilized methods such as gas chromatography/mass spectrometry (GC-MS), ion mobility/mass spectrometry (IMMS), and liquid chromatography/mass spectrometry (LC-MS) for the analysis of organophosphorus containing degradation products with detection limits in the ng/mL range [4, 5, 7]. However, considering the lethal doses as reported in Table 1, lower detection limits in the pg/mL range are desirable for such nerve agents and their degradation products. To achieve this lower level detection requires a more sensitive and selective analytical detection technique, such as inductively coupled plasma mass spectrometry (ICP-MS).

*Published in: *Journal of Analytical Atomic Spectrometry*, vol. **21**, 396–403 (2006)

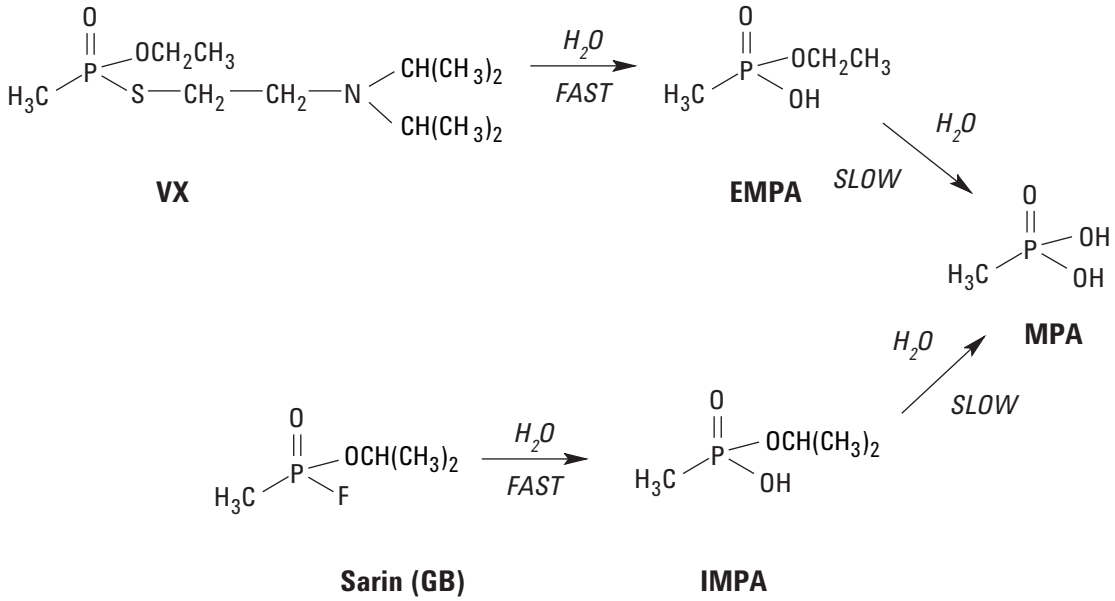
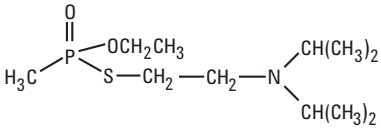
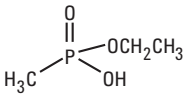
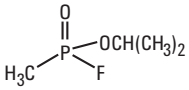
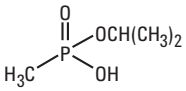
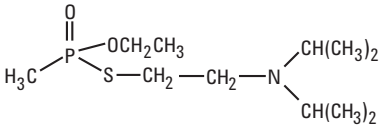
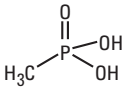
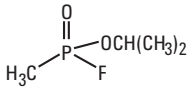


Figure 1. Degradation pathway of Sarin and VX.

Table 1. Chemical Warfare Agents and Degradation Products

Chemical warfare agent	Agent liquid LD_{50} (mg kg^{-1})[1]	Chemical warfare degradation products	Degradation product pKa	Degradation product Oral-human LD_{LO} (mg kg^{-1})
 VX	0.14	 EMPA	2.16	
 Sarin (GB)	24	 IMPA	2.24	143–428*
 VX	See above	 MPA	pKa_1 = 2.41 pKa_2 = 7.54	
 Sarin (GB)				

[1] Vapor form LD_{50} values range from ~0.09–2 mg-min/m^3 (Agent MSDS)

*Cerilliant MSDS

Elemental speciation analysis by ICP-MS allows for high sensitivity, low level detection, and elemental selectivity, making it the instrument of choice for ultra-trace elemental speciation studies [8–14]. Phosphorous (m/z = 31) analysis by ICP-MS until recently was limited due to its high first ionization potential (10.5 eV) and polyatomic interferences, including $^{14}N^{16}O^{1}H^{+}$ and $^{15}N^{16}O^{+}$ (m/z = 31) [15]. Sector-field MS detection with ICP sources do provide a potential resolution enhancement but at the expense of losing part of the beam. For elements with high ionization potentials the throughput is clearly diminished. Recent developments in collision/reaction cell (CRC) technology [16, 17] have allowed for the analysis of elements prone to isobaric and polyatomic interferences through removal by collisional dissociation (collision energy >> bond energy), chemical reaction, and/or energy discrimination [9].

In this study reversed phase ion-pairing chromatography was coupled with ICP-MS detection for the analysis of three organophosphorus degradation products of Sarin (GB) and VX. Helium CRC optimization experiments for the removal of polyatomic interferences through collisional processes and by application of an appropriate energy barrier are also described. Analytical figures of merit for each species studied, ethyl methylphosphonic acid (EMPA), isopropyl methylphosphonic acid (IMPA), and methylphosphonic acid (MPA), are presented. Finally, the HPLC-ICP-MS system was applied to spiked top soil samples for the determination of the three chemical warfare degradation products of nerve agents Sarin and VX.

Materials and Methods

Reagents

The three chemical warfare degradation products (ethyl methylphosphonic acid (EMPA), isopropyl methylphosphonic acid (IMPA), and methylphosphonic acid (MPA)) used were obtained from Cerilliant (Austin, TX) as 1 mg/mL certified reference materials (CRMs). CRMs are used as standard analytical solutions for analysis of Schedule 1, 2, or 3 toxic chemicals, their precursors, and/or degradation products as mandated by the CWC for verification [1, 5]. Stock solutions of 10 mg/mL for each degradation product were prepared through dilution in HPLC buffer. Further dilution of these stock solutions in HPLC buffer as well as preparation of standard mixtures over the range 20–400 ng/mL were performed as needed. Instrument tuning was accomplished through the use of a 30 ng/mL adenosine 5'-triphosphate (Sigma, St. Louis, MO) corresponding to a phosphorus concentration of 5 ng/mL.

A 50 mmol/L ammonium acetate (Fisher Scientific, Fairlawn, NJ) solution with 5 mmol/L myristyltrimethylammonium bromide (Aldrich, Milwaukee, WI) ion pairing agent and 2% methanol (TEDIA, Fairfield, OH) at pH 4.85 was used as the chromatographic buffer. The buffer was prepared fresh from stock solution before starting the experiments. Adjustment of the pH was accomplished through addition of glacial acetic acid (Fisher Scientific, Fairlawn, NJ).

Environmental top soil was collected from outside of the laboratory at the University of Cincinnati. Preparation of the soil samples consisted of placing 1.0 g solid material in 5.0 mL DDI water and stirring for 15 minutes. The resulting solution was filtered through 0.20 µm Nalgene nylon/cellulose acetate syringe filters (Nalge Nune International Corporation, Rochester, NY). Environmental soil samples were processed as blanks and 100 ng/mL spiked mixtures (prior to filtration) of ethyl methylphosphonic acid (EMPA), isopropyl methylphosphonic acid (IMPA), and methylphosphonic acid (MPA).

Instrumentation

HPLC Conditions

An Agilent 1100 (Agilent Technologies, Palo Alto, California) high performance liquid chromatograph (HPLC) equipped with a binary pump, autosampler, vacuum degasser, thermostated column compartment, and diode array detector was used for the separation of the three chemical warfare degradation products. A C_8 column (Alltima C_8,100 Å, 3.2 × 150 mm, 5 µm, Alltech Associates Inc, Deerfield, IL) with a guard column (Alltima C_8, 7.5 × 3.0 mm, 5 µm, Alltech Associates Inc, Deerfield, IL) was used for all separation experiments. A detailed description of the HPLC separation conditions is provided in Table 2.

Table 2. HPLC-ICP-MS Instrumental Parameters

ICP-MS parameters	
Forward power	1500 W (with shielded torch)
Plasma gas flow rate	15.6 L/min
Auxiliary gas flow rate	1.0 L/min
Carrier gas flow rate	1.20 L/min
Nebulizer	Glass expansion micro-concentric
Spray chamber	≈2 °C (Scott double channel)
Sampling depth	6 mm
Sampling and skimmer cones	Nickel
Dwell time	0.1 s
Isotopes monitored (*m/z*)	^{31}P and $^{47}PO^{+}$
Octopole reaction system	He (Flow optimized prior to experiment)
HPLC parameters	
Instrument	Agilent 1100 HPLC
Flow rate	0.5 mL/min
Injection volume	100 µL
	50 mM Ammonium acetate; 2% Methanol
Buffer	5 mM Myristyltrimethylammonium bromide
pH	4.85
Column	Alltima C8 (3.2 × 150 mm) 5 µm

ICP-MS

An Agilent 7500ce (Agilent Technologies, Tokyo, Japan) ICP-MS equipped with shielded torch and collision/reaction cell technology was used for the element specific detection of ^{31}P and $^{47}PO^{+}$ throughout this experiment. The collision/ reaction cell consisted of an octopole ion guide operated in rf only mode and also served for the removal of polyatomic interferences. Electronic coupling of the ICP-MS with the HPLC was accomplished through the use of a remote cable which allowed for simultaneous starting prior to each chromatographic run. A detailed description of ICP-MS operating conditions is provided in Table 2.

Results and Discussion

Due to the nature of the compounds of interest, ion-pairing chromatography was investigated as the chromatographic separation technique. The acid dissociation constants for the chemical warfare degradation products are provided in Table 1. Based upon these values a buffer system (acetic acid/ ammonium acetate; pKa 4.8) at pH 4.85 was used in the separation experiments. It was believed that the hydrophobicity and difference in effective charges of the different species would allow for separation by the proposed chromatography. Myristyltrimethylammonium bromide along with an ammonium acetate/acetic acid buffer (pH 4.85) and 2% methanol for the mobile phase allowed separation of methylphosphonic acid, ethyl methylphosphonic acid, and isopropyl methylphosphonic acid with the selected column in less than fifteen minutes (Figure 2A).

ICP-MS Detection

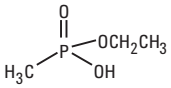
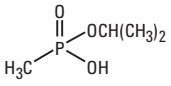
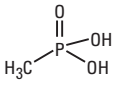
Element specific detection by ICP-MS is a popular analytical technique based on the high sensitivity and selectivity offered by this instrument. In this experiment, instrument sensitivity and selectivity was vital because of the need for element specific detection of phosphorus (m/z = 31) and the complex nature of the environmental matrix analyzed. Recently phosphorus analysis by ICP-MS has grown in popularity due to the ability to remove nitrogen-based polyatomic interferences on ^{31}P, and the ability to ionize phosphorous sufficiently, in spite of its high first ionization potential. Other researchers depended upon the formation of PO^{+} (m/z = 47) [19, 20], or the use of high-resolution mass spectrometers to differentiate between the polyatomic interferences and the phosphorus signal at m/z = 31 [12, 21–23]. Monitoring PO^{+} in these experiments was performed to ensure no loss of ^{31}P signal by oxide formation.

This study involved the use of helium collision cell for the removal of $^{14}N^{16}O^{1}H^{+}$ and $^{15}N^{16}O^{+}$ interferences through a collision/energy discrimination process. Any fragmentation of the polyatomic interferences would need to overcome the nitrogen-oxygen bond energy by using helium [9, 21]. After overcoming the polyatomic interferences with collisional dissociation, selective ion transmission by adjusting the pole bias plays a vital role in analyte response. Helium was chosen as the collision gas for all experiments due to its light/non-reactive nature to allow for reduction of the background signal at *m/z* 31. Optimization of the helium gas flow rate was accomplished through the use of a mass flow control valve and constant introduction of 30 ng/mL adenosine 5'-triphosphate (corresponding to 5 ng/mL phosphorus) in buffer. Phosphorus response versus helium flow rate was plotted and the flow rate corresponding to optimal signal and the lowest background (buffer signal *m/z* = 31) was selected (Table 2.). The gas flow used for all experiments ranged from 3.5–4.0 mL/min helium for all experiments based upon the optimization results.

Analytical Figures of Merit

Calibration curves were prepared through the use of standard mixtures ranging from 20–400 ng/mL. All regression coefficients (r^2) values were acceptable with the lowest value being 0.993. Detection limits (3σ) based on three times the standard deviation of seven replicates of the blank peak areas (IUPAC) for the analysis of MPA, EMPA, and IMPA were found to be 139, 263, and 183 pg/mL, respectively. The detection limits are an improvement of at least one order of magnitude compared with those reported in other analytical techniques for these warfare agent hydrolyzates (Table 3), although the detection limits reported here were calculated based on a concentration that would give a signal three times that of the noise. The precision for repeated injections of a 20 ng/mL standard mixture was lower than 1% for retention times and lower than 6% for peak areas. Column recovery was calculated to evaluate the extraction efficiency for the sample preparation and separation techniques. These values ranged from 69%–86%. The analytical figures of merit are summarized in Table 4.

Table 3. Chemical Warfare Degradation Product Detection Limits

Chemical Warfare Degradation Products	Analytical Method	Detection Limits ng mL^{-1}
 EMPA	Ion mobility mass spectrometry[A]	560–1700[5]
	LC-ESI-TOF[B]	80–1000[3]
 IMPA	Electrophoresis microchip with contactless conductivity detector[C]	48–86[24]
 MPA	RP-IP-HPLC-ICP-MS[D]	0.139–0.263*

[A] Based on concentration producing a signal three times that of the noise.

[B] Estimated in SIM mode at concentrations down to 50 ng/mL for signal-to-noise ratio of 3:1.

[C] Estimated from signal-to-noise characteristics (S/N = 3) of the response for 150 ng/mL mixture.

[D] Based on IUPAC.

*This work

Table 4. Analytical Figures of Merit Based on 20 ng/mL Mixture

Chemical warfare degradation product	Detection limit pg/mL	Column recovery	RSD (%) peak area	RSD (%) retention time
MPA	139	86.2	2.75	0.38
EMPA	263	69.2	5.39	0.55
IMPA	183	73.0	5.96	0.65

Complex Samples

Investigation of complex sample matrix effects on the method led to the collection of top soil samples. Samples were treated with the sample preparation procedure described in the experimental section. Figure 2B provides a chromatogram of spiked top soil samples. The blank chromatogram (not shown) did not show the presence of any unknown peaks and the spiked top soil sample demonstrates the same separation profile compared to the standard sample chromatogram (Figure 2A).

Conclusion

In this work the coupling of ion-pairing reversed phase HPLC with ICP-MS equipped with collision/reaction cell allowed for trace analysis of three organophosphorus chemical warfare degradation products: MPA, EMPA, and IMPA. Ion-pairing chromatography offered a good separation based on interactions of the analyte between the stationary and mobile phases as well as slight charge differences between the species of interest. This method provides a highly sensitive and selective technique with baseline separation of the three species within 15 minutes and detection limits of less than 263 pg/mL. Application of the developed method to environmental soil demonstrated the RP-IP-HPLC-ICP-MS technique as high potential for complex sample speciation analysis. Investigation of alternative liquid and gas chromatographic separation techniques coupled with atomic mass spectrometric detection are currently underway.

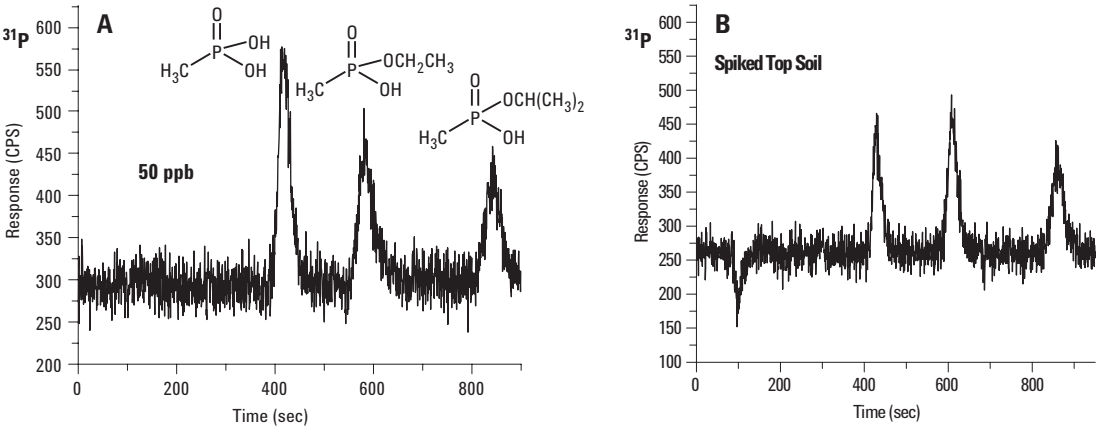


Figure 2 Separation of MPA, EMPA, and IMPA in a standard mixture (A) and spiked topsoil (B).

References

1. United States Bureau of Arms Control and Disarmament Agency, “Chemical Weapons Convention” (CWC), Washington D. C., April 29 1997.
2. J. F. Weimaster, W. T. Beaudry, P. C. Bossle, M. W. Ellzy, L.G Janes, D. W. Johnson, J. M. Lochner, S. G. Pleva, J. H. Reeder, et al. *J. Chem. Technol.Biotechnol.* 1995, **64**, 115–128.
3. Q. Liu, X. Hu, J. Xie, *Anal. Chim. Acta* 2004, **512**, 93-101.
4. J. R. Smith, M. L. Shih, M. L., *J. Appl. Toxicol.* 2001, **21**, S27–S34.
5. W. E. Steiner, B. H. Clowers, L. M. Matz, W. F. Siems, H. H. Hill, *Jr. Anal. Chem.* 2002, **74**, 4343–4352.
6. R. M. Wester, H. Tanojo, H. I. Maibach, R. C. Wester, *Toxicol. Appl.Pharmicol.* 2000, **168**, 149–152.
7. R. M. Black, R. W. Read, *J. of Chromatogr., A* 1998, **794**, 233–244.
8. J. A. Caruso, B. Klaue, B. Michalke, D. M. Rocke, *Ecotox. Environ. Safety* 2003, **56**, 32–44.
9. B. B. M. Sadi, A. P. Vonderheide, and J. A. Caruso, *J. Chromatogr., A* 2004, *In Press.*
10. J. A. Caruso, K. L. S. a. K. L. A. “Elemental speciation new approaches for trace element analysis”: By J. A. Caruso, K. L. Sutton, and K. L. Ackley – eds, 2002.
11. A. Montaser, “Inductively Coupled Plasma Mass Spectrometry”; Wiley-Vch: New York, 1998.
12. J. S. Becker, S. F. Boulyga, C. Pickhardt, J. Becker, S. Buddrus, M. Przybylski, *Anal. Bioanal. Chem.* 2003, **375**, 561–566.
13. S. Wilbur, E. McCurdy, Agilent Technologies, app. note 2001, 5988-4286EN.
14. P. Leonhard, R. Pepelnik, A. Prange, N. Yamada, T. Yamada, *J. Anal. At. Spectrom.* 2002, 17, 189-196.
15. C. D. Stalikas, C. N. Konidari, *J. Anal. At. Spectrom.* 2001, **907**, 1–19.
16. S. D. Tanner, V. I. Baranov, D. R. Bandura, *Spectrochim. Acta, Part B* 2002, **57**, 1361–1452.
17. D. R. Bandura, V. I. Baranov, S. D. Tanner, *Anal. Bioanal. Chem.* 2001, **370**, 454–470.
18. P. C. Bossle, D. J. Reutter, E. W. Sarver,. *J. Chromatogr.* 1987, **407**, 399–404.
19. A. H. Kudzin, D. K. Gralak, J. Drabowicz, J. Luczak, *J. Chromatogr. A* 2002, **947**, 129–141.
20. Z. H. Kudzin, D. K. Gralak, G. Andrijewski, J. Drabowicz, J. Luczak, *J. Chromatogr. A* 2003, **998**, 183–199.
21. C. Siethoff, I. Feldmann, N. Jakubowski, M. Linscheid, *J. Mass Spectrom.* 1999, **34**, 421–426.
22. M. Wind, I. Feldmann, N. Jakubowski, W. D. Lehmann, *Electrophoresis* 2003, **24**, 1276–1280.
23. S. Kozono, S. Takahashi, H. Haraguchi, *Anal. Bioanal.Chem.* 2002, **372**, 542–548.
24. J. Wang, M. Pumera, G. E. Collins, A. Mulchandani, *Anal. Chem.* 2002, **74**, 6121–6125.

For More Information

For more information on our products and services, visit our Web site at www.agilent.com/chem.

Printed in the USA
July 21, 2006
5989-5346EN

Agilent Technologies