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Determination of Nerve Agent Degradation Products in Drinking Water

Waters Corporation

Abstract

This application note describes the use of a LC-MS method for the unambiguous identification of six AMPAs in drinking water. The analysis was performed by LC-ESI-MS and utilized an XBridge C₁₈ HPLC Column for

chromatographic separation.

Introduction

Nerve agents are among the most lethal of chemical warfare agents. In particular, the G-series nerve agents are of a concern due to their toxicity (LD50 human for GB = 25 mg/kg, GD = 5 mg/kg, GF = 5 mg/kg),[1] their environmental persistence (hydrolysis half-life of 1-3 days),[2] their accessibility and their recent use in Iraq[3] and Japan.[4] The potential use of these agents by terrorist organizations is of real concern and the ability to counter such attacks requires recognizing possible deployment scenarios, including the poisoning of drinking water supplies.

Once exposed to the environment, nerve agents degrade via hydrolysis to corresponding alkyl methyphosphonic acids (AMPAs) which do not exist in nature (Figure 1). Sarin (GB) to isopropyl methylphosphonic acid (IMPA), Soman (GD) to pinacolyl methylphosphonic acid (PMPA), VX to ethyl methylphosphonic acid (EMPA), Russian VX (RVX) to isobutyl methylphosphonic acid (i-BuMPA) and Cyclosarin (GF) to cyclohexyl methylphosphonic acid (CHMPA). These alkyl methylphosphonic acids are the hydrolyzed at a much slower rate to a common, stable product methylphosphonic acid (MPA). An analysis designed to detect nerve agents in water must be able to detect and distinguish the hydrolysis products at sufficiently low levels in order to prove useful.
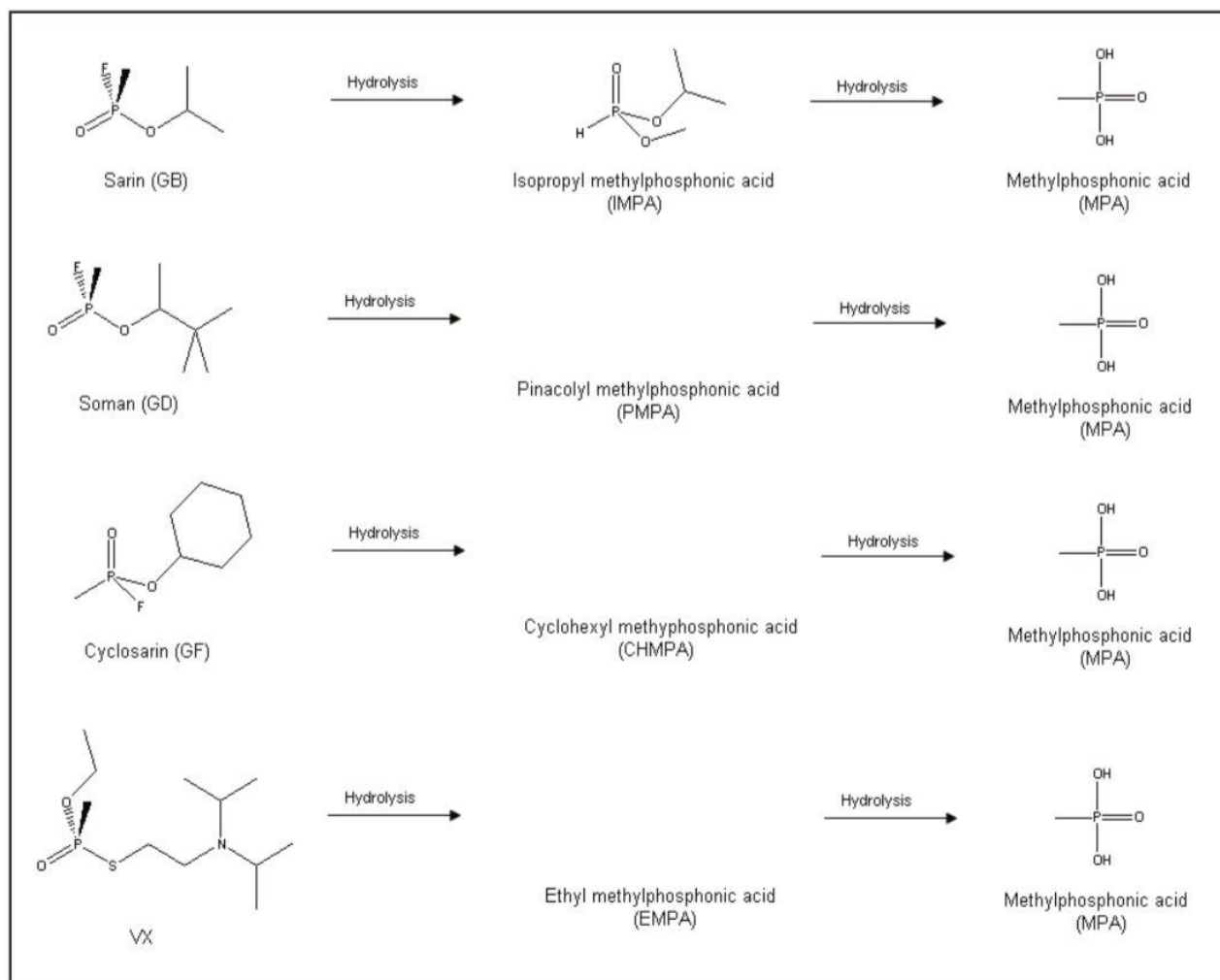
Figure 1. Degradation pathway for nerve agents GB, GD, GF, and VX.

Liquid chromatography-mass spectrometry (LC-MS) would seem an attractive for this analysis owing to its sensitivity and because it allows direct analysis of aqueous samples with little or no sample preparation. In recent years, numerous publications have illustrated the analysis of chemical warfare agent degradation products using LC-MS. Black and Read developed a screening procedure for the hydrolysis products of nerve agents using liquid chromatography electrospray ionization mass spectrometry (LC-ESI-MS).[5] D'Agostino, *et al* documented the use of a packed capillary LC-ESI-MS to detect and identify nerve agents.[6] They also developed a method based on aqueous extraction and liquid chromatography-time-of-flight mass spectrometry with electrospray ionization (LC-ESI-ToF-MS) to determine trace levels of nerve agents in soil.[7]

This report will describe the use of a LC-MS method for the unambiguous identification of six AMPAs in drinking water. The analysis was performed by LC-ESI-MS and utilized an XBridge C_{18} HPLC Column for chromatographic separation.

Experimental

Materials

MPA, EMPA, IMPA, i-BuMPA, CHMPA and PMPA were provided by the Research Institute of Chemical Defense (Beijing, China). They were dissolved in methanol (10 mg/ml) and stored at 4 °C as stock solution. The working solution was prepared by diluting to the appropriate concentration with distilled water prior to use. Methanol and acetonitrile were of HPLC grade, and all other chemicals used were of analytical grade.

HPLC Conditions

Column:	XBridge C_{18}, 2.1 x 150 mm, 3.5 µm
Part number:	186003023
Mobile phase:	10 mM ammonium formate in water (solvent A) and 10mM ammonium formate in methanol (solvent B)
Elution gradient:	99% A (0-2 min), 99-30% A (2-17 min), 30% A (17-25 min)
Flow rate:	0.2 mL/min
Injections:	5 µL
Instrument:	Waters Alliance 2690

MS Conditions

Instrument:	Micromass LCT time-of-flight Mass Spectrometer with standard Z-spray electrospray interface

ESI conditions:	Negative-ion electrospray
Capillary voltage:	– 2 kV
Sample cone voltage:	10 V
Extraction cone voltage:	5 V
Desolvation temp.:	300 °C
Source temp.:	120 °C
Desolvation gas flow rate:	540 L/h

Samples

The environmental water sample was tap water (reflecting a possible contamination of the public drinking water supply). The spiked sample for this experiment was prepared by adding known amounts of nerve agents or AMPAs to blank water. The blank sample was also analyzed to ensure absence of AMPAs.

Five milliliters of water sample was spiked with solutions of GB, GD, GF, VX and RVX in methanol to the appropriate concentration and then stored at room temperature for 5 days prior to analysis (ensuring that hydrolysis occurred). The water sample was filtered through a 0.2 μm membrane filter and then analyzed by LC-MS.

Results and Discussion

It is known that ion intensity obtained in ESI-MS frequently depends largely on the concentration of the buffering salt in the mobile phase. In an optimization study, the highest intensity for every AMPA was obtained at the concentration of ammonium formate below 10 mM. A concentration of 10 mM ammonium formate in the mobile phase was chosen on consideration of peak shape in the chromatogram. Reduction of pH from approximately 6.4 to 4.0, by addition of formic acid, resulted in poorer peak shape for all analytes.

Additionally, since AMPAs are closely related compounds, to optimize the separation of methylphosphonic

acids, gradient elution had to be performed in one LC run. Injections of 5 µl or less gave the best resolution. Larger volume injections gave improved detection limits but at the expense of resolution between MPA, EMP and IMPA. An ion chromatogram showing the separation and detection of the AMPAs using selected ion monitoring is shown in Figure 2.
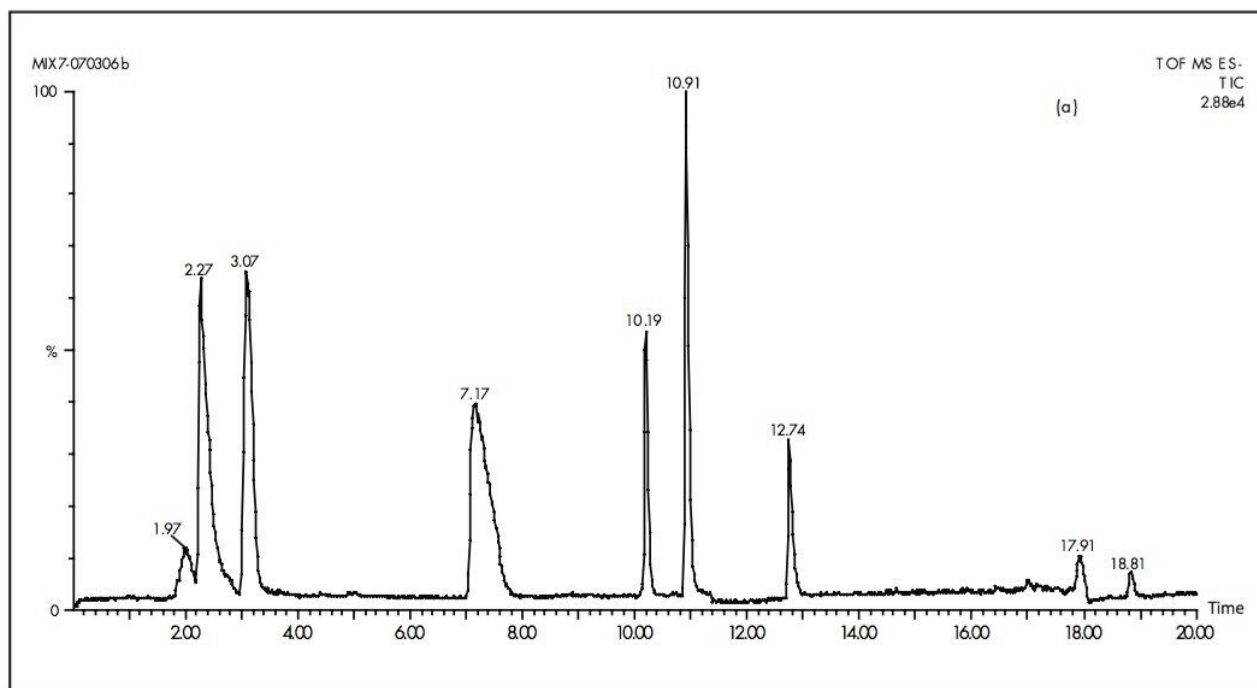

Figure 2. LC-ESI-MS total ion current chromatograms of seven chemical warfare agents degradation products: (a) XBridge C₁₈ 3.5 µm

Compounds: (1) methylphosphonic acid; (2) ethyl methylphosphonic acid; (3) isopropyl methylphosphonic acid; (4) isobutyl methylphosphonic acid; (5) cyclohexyl methylphosphonic acid; (6) pinacolyl methylphosphonic acid; (7) benzilic acid

Chromatogram courtesy of Dr. Jian-wei Xie, Beijing Institute of Pharmacology and Toxicology.

Conclusion

A rapid, accurate and simple procedure for screening nerve agent degradation product AMPAs was developed by LC-MS, utilizing XBridge C₁₈ chromatographic separation. Effective separation was achieved for six closely related AMPAs in an environmental water sample in less than 13 minutes.

References

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