Analysis of Polar Nerve Agent Hydrolysis Products

by James Riches - Defence Science and Technology Laboratory (Dstl), Porton Down, Salisbury, Wiltshire, SP4 0JQ, UK.

The primary hydrolysis products of nerve agent chemicals such as sarin (isopropyl methylphosphonofluoridate) and VX (O-ethyl-S-2-diisopropylaminoethyl methylphosphonothiolate) that have been developed as chemical warfare agents (CWAs) are alkyl methylphosphonic acids (AMPAs). These chemicals, shown in Figure 1, hydrolyse further very slowly to methylphosphonic acid.

AMPAs are quite stable in the environment and are important indicators of the use of nerve agents (they have no common natural sources). They can be detected in soil, water, vegetation and other environmental samples including weapons system fragments [1] or in biological samples including blood, urine, hair or skin [2]. Related acids, shown in Figure 2, include the hydrolysis products of a large series of possible nerve agent chemicals controlled under the Chemical Weapons Convention (CWC), toxic hydrolysis products of the V class of nerve agents and the hydrolysis products of nerve agent precursor chemicals such as methylphosphonic difluoride (DF).

All of these acids present analytical challenges: they are not generally amenable to gas chromatography-mass spectrometry (GC-MS) without derivatisation due to their low volatility and high polarity, and even under direct analysis by liquid chromatography-mass spectrometry (LC-MS) non-standard techniques are required for optimum separation and sensitivity. For example the most polar of these acids, the alkylphosphonic acids (APAs), are retained poorly by standard C18 reverse phase columns, do not ionise efficiently and are strongly retained under hydrophilic interaction liquid chromatography (HILIC) conditions. Consequently, specialised approaches have been developed for their separation and detection in support of verification activities related to the CWC which is implemented by the Organisation for the Prohibition of Chemical Weapons (OPCW) [3]. Methods can be optimised for trace analysis through the use of sample preparation techniques and tandem or high resolution mass spectrometry. As a result the acids described can be detected at sub parts per billion (ppb) levels in a range of environmental and biological matrices in support of investigations of alleged use of nerve agent chemicals. This article describes some of the different analytical approaches used at Dstl for the identification and quantification of these polar acidic chemicals.

Direct analysis by liquid chromatography – mass spectrometry (LC-MS)

LC-MS can be used for the detection of AMPAs and APAs [4-5] and other degradation products of VX [6-7]. Screening methods [4-5] have been developed in positive ion mode; however, detection limits are modest (1 to 10µg·mL^{-1} in water in full-scan positive ion mode) particularly for the more polar hydrophilic APAs. Lower limits of detection are achievable using tandem mass spectrometry in negative ion mode. Highly sensitive and rapid methods using HILIC and tandem mass spectrometry (MS/MS) have been developed for AMPAs in urine [8] and water [9]. HILIC chromatography cannot be used for the APAs however, as they are too strongly retained. An example

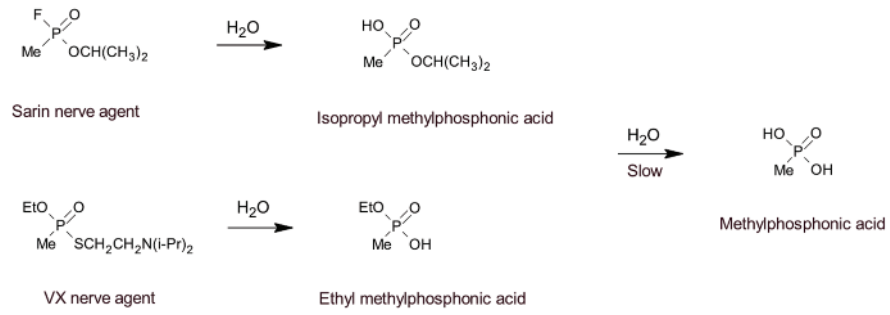

Figure 1. Chemical structures of selected nerve agent chemicals and their polar hydrolysis products.

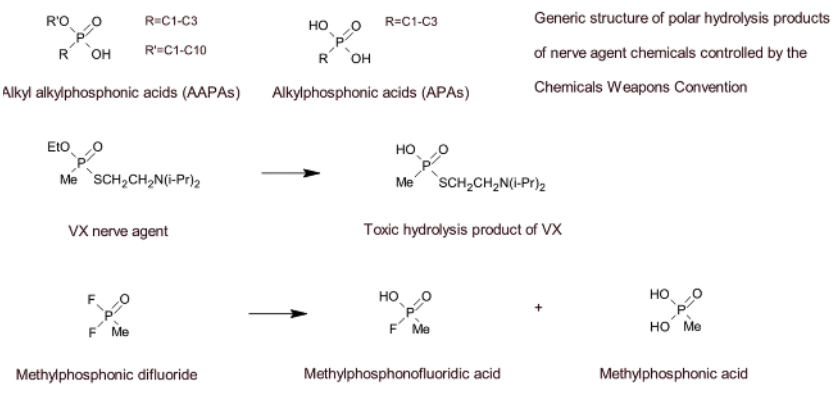

Figure 2. Chemical structures of polar hydrolysis products of nerve agent chemicals and precursor chemicals.

chromatogram using a HILIC-MS/MS method [8] for high sensitivity analysis of the urinary metabolite of the nerve agent sarin is shown in Figure 3. The method uses a Supelco Ascentis Express HILIC (silica) column (50 mm by 2.1 mm with 2.7µm particle size) from Sigma-Aldrich (Dorset, UK). The mobile phase composition is 14% 0.02 M aqueous ammonium formate and 86% acetonitrile.

More specialised methods using ion chromatography based column technology can be used when it is important to analyse alkyl alkylphosphonic acids (AAPAs) and APAs simultaneously [10] such as in environmental samples that have weathered for a long time (hydrolysis of AAPAs to APAs is very slow). As well as commonly-used sample preparation techniques such as solid phase extraction other specialised approaches to enhance chromatography and sensitivity have included derivatisation [11] and the use of ion-pairing [12] and post-column [13] reagents.

At Dstl a Primesep SB mixed mode reversed phase-anion exchange column from SIELC Technologies Inc (IL, US) has been applied to separate the APA methylphosphonic acid from methylphosphonofluoridic acid. Peak shape and sensitivity depend upon the buffer concentration and mobile phase composition. The approach also shows promise for optimising the sensitivity of direct analysis of the APAs and the development of sensitive methods for simultaneous analysis of APAs and AAPAs. An example separation of methylphosphonic acid and methylphosphonofluoridic acid using this mixed mode chromatography is shown in Figure 4. The mobile phase consisted of 90% 40mM ammonium formate (adjusted to pH 4.0 with formic acid) and 10% acetonitrile. The column dimensions were 150 mm by 2.1 mm (5 µm particle size).

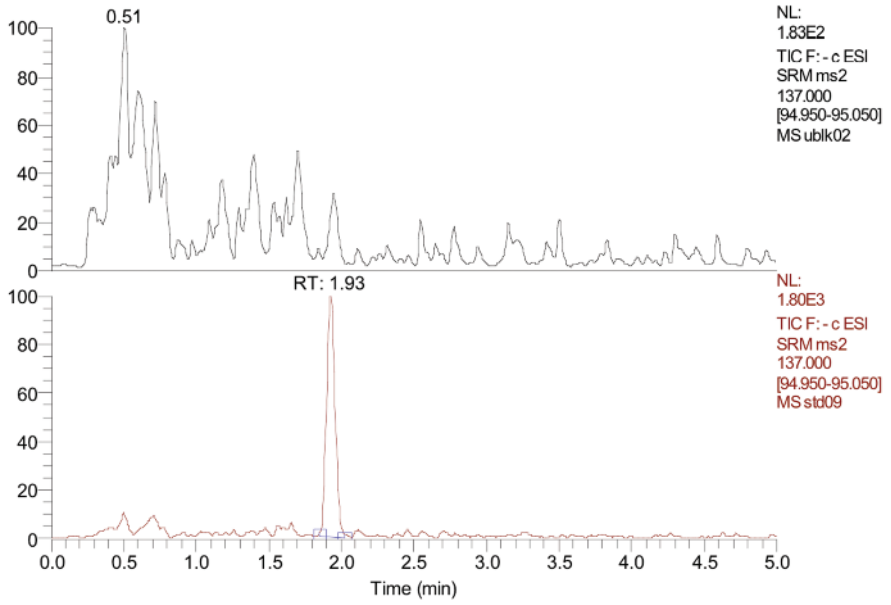

Figure 3. Chromatogram from HILIC-MS/MS analysis showing control urine (top trace) and urine spiked with isopropyl methylphosphonic acid at 0.5 ng·mL^{-1} (bottom trace).

Chromatographic conditions
Column: Supelco Ascentis Express HILIC (silica) 50 mm x 2.1 mm (2.7 µm particle size)
Eluent composition: 0.02 M ammonium formate in 14% water / 86% acetonitrile (isocratic)
Flow rate: 500 µL·min^{-1} (0 - 2.9 min); 1000 µL·min^{-1} (3.0 - 4.0 min)
Detection: Triple quadrupole mass spectrometer in selected reaction monitoring mode (negative ion)

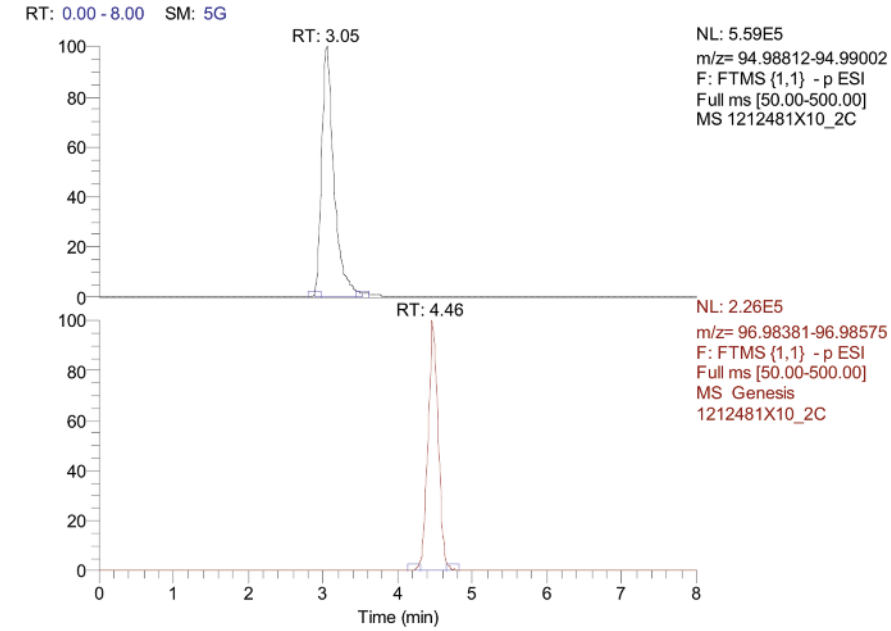

Figure 4. Separation of nerve agent precursor hydrolysis products methylphosphonic acid (top trace) and methylphosphonofluoridic acid (bottom trace) using a mixed mode LC column.

Chromatographic conditions
Column: SIELC Technologies Inc. Primesep SB mixed mode reversed phase-anion exchange 150 mm x 2.1 mm (5.0 µm particle size)
Eluent composition: 0.04 M ammonium formate in 90% water (adjusted to pH 4) / 10% acetonitrile (isocratic)
Flow rate: 400 µL·min^{-1}
Detection: Orbitrap™ mass spectrometer (negative ion)

Analysis by gas chromatography – mass spectrometry (GC-MS) following derivatisation

Derivatisation reactions for the analysis of APAs and AAPAs by GC-MS include methylation using diazomethane [14] and trimethylphenylammonium hydroxide (TMPAH) or trimethylsulfonium hydroxide (TMSH) [15], silylation [16-17] or acylation [18-19]. The advantages and disadvantages of strategies for derivatising nerve agent degradation products have been reviewed [20]. Methylation and silylation are useful for analysis and screening methods at parts per million (ppm) levels. Methods with the lowest limits of detection involve derivatisation with molecules containing electron-capturing groups, for example pentafluorobenzyl bromide (PFBBr), followed by analysis using negative ion tandem mass spectrometry [18-19]. The main advantages of these methods are high sensitivity and good chromatographic resolution. However, they are relatively slow in comparison with LC-based methods for which run times can be less than five minutes. The extremely high sensitivity of these methods can also lead to carry-over problems making them difficult to use from a practical point of view. Nevertheless, developments are likely to continue for high sensitivity methods where lengthy sample preparation and analysis times are acceptable. An example of the detection of isopropyl methylphosphonic

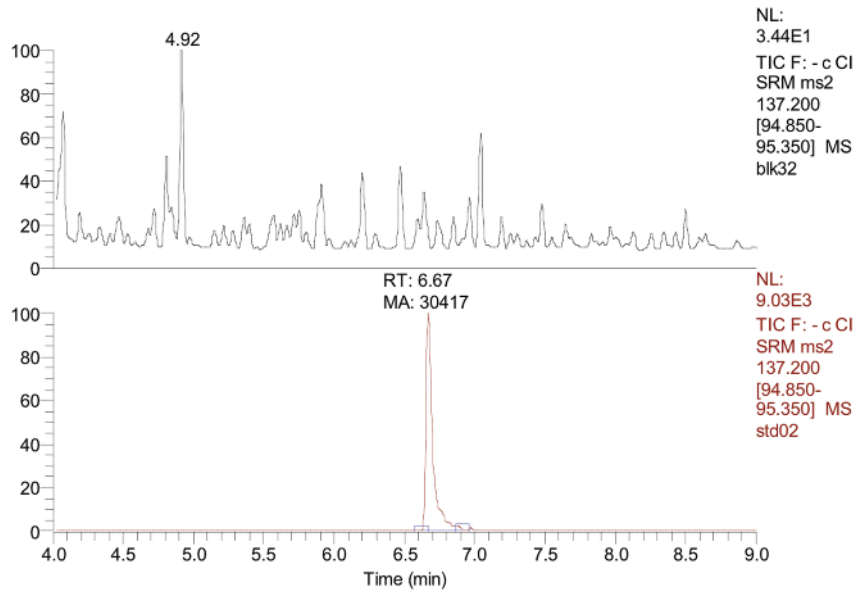

Figure 5. Chromatogram from GC-MS/MS analysis following derivatisation with PFBBr showing control urine (top trace) and a urine sample spiked with isopropyl methylphosphonic acid at 1.0 ng·mL^{-1} (bottom trace).

Chromatographic conditions
Column: Restek Rxi-XLB, arylene / methyl modified polysiloxane 30 m x 0.25 mm (0.25 µm film thickness) with 1 m x 0.25 mm intermediate polarity deactivated guard column
Temperature programme: 50 °C (0 - 1 min), 30 °C·min^{-1} to 180 °C, 10 °C·min^{-1} to 280 °C (2 min hold)
Injector temperature: 250 °C (splitless mode)
Flow rate: 1.1 mL·min^{-1}
Detection: Triple quadrupole mass spectrometer in selected reaction monitoring mode (negative ion chemical ionisation)

acid spiked into a urine sample at 1 ng·mL^{-1} by GC-MS/MS based on its PFBBr derivative is shown in Figure 5. The method uses a Restek Rxi-XLB, arylene/methyl modified polysiloxane column (30m by 0.25 mm with a 0.25µm film thickness) from Thames Restek Ltd, (Berkshire, UK).

The future development of reagents that are more stable in water and easier to use than PFBBr [21-22] is a promising development as is the host of new sample preparation techniques such as hollow fibre membrane liquid phase micro-extraction (HF-LPME) and dispersive liquid micro-extraction, which combine extraction and derivatisation in one step.

Conclusions

Highly sensitive analytical techniques can be developed for the analysis of the polar acid degradation products of nerve agents and their precursor chemicals. Direct analysis by LC-MS can offer sub-ppb sensitivity with short (<5 min) run times. This is currently the preferred method at Dstl for trace analysis of these chemicals. Future developments look likely to include automation of sample extraction from difficult matrices such as soil and urine, use of high resolution mass spectrometry for increased confidence in identification and the development of combined sample extraction and derivatisation techniques.

The sensitivity of modern analytical techniques and the use of accredited procedures to control chain of custody during sampling and analysis provide a sound framework for investigations in support of allegations of illegal use of chemical weapons. These procedures could be used to provide credible and usable forensic evidence, months, if not years, after an incident has taken place.

Acknowledgements

The author is grateful to Robert Read, Sarah Stubbs, Neil Roughley, Andy Webb, Richard McColm and Peter Burbage at Dstl who provided the data reported in this article.

References

1. N B Munro, S T Talmage, G D Griffin, L C Waters, A P Watson, J F King, V Hauschild. Environmental Health Perspectives, 107 (1999), 933-974.

2. R M Black, D Noort. Methods for the retrospective detection of exposure to toxic scheduled chemicals. Part A: Analysis of free metabolites in Chemical Weapons Convention Chemicals Analysis (M Mesilaakso (Ed.), Wiley, Chichester, UK, 2005.

3. http://www.opcw.org/

4. R M Black, R W Read. J. Chromatogr. 759 (1997) 79-92.

5. R M Black, R W Read. J. Chromatogr. 794 (1998) 233-244.

6. R M Black, R W Read. Liquid chromatography/mass spectrometry in analysis of chemicals related to the Chemical Weapons Convention, in: Chemical Weapons Convention Related Analysis (M Mesilaakso, Ed.), Wiley, Chichester, UK, 2005.

7. R Subramaniam, C. Åstot, L Juhlina, C Nilsson, A Östin. J. Chromatogr. A 1229 (2012) 86-94.

8. D B Mawhinney, E I Hamelin, R Fraser, S S Silva, A J Pavlopoulos, R J Kobelski. J. Chromatogr. B 852 (2007) 235-243.

9. B T Roen, S R Sellevag, E Lundanes. Anal. Chim. Acta 761 (2013) 109-116.

10. H Piao, R B Marx, S Schneider, D A Irvine, J Staton. J. Chromatogr. A 1098 (2005) 65-71.

11. V Tak, D Pardasani, A Purohit, D K Dubey. Rapid Commun. Mass Spectrom. 25 (2011) 3411-3416.

12. U Saradhi, S Prabhakar, T J Reddy, M Murty. J. Chromatogr. A 1157 (2007) 391-398.

13. D B Mawhinney, R D Stanelle, E I Hamelin, R J Kobelski. J. Amer. Soc. Mass Spectrom. 18 (2007) 1821-1826.

14. R S Macomber. Synth. Commun. 7 (1977) 405-407.

15. K Amphaisri, M Palit , G Mallard. J. Chromatogr. A 1218 (2011) 972-980.

16. S V Vasilevskii, A F Kireev, I V Rybal'chenko, V N Suvorkin. Anal. Chem. 57 (2002) 491-497.

17. M Kanamore-Kataoka, Y Seto. J. Health Sci. 54 (2008) 513-523.

18. S A Frederiksson, L G Hammarstrom, L Henriksson, H A Lakso. J. Mass Spectrom. 30 (2005) 1133-1143.

19. J Riches, I Morton, R W Read, R M Black. J. Chromatogr. B 816 (2005) 251-258.

20. R M Black, R Muir. J. Chromatogr. A 1000 (2003) 253-281.

21. R Subramaniam, C Åstot, L Juhlin, C Nilsson, A Östin. Anal. Chem. 82 (2010) 7452-7459.

22. R Subramaniam, A Östin, C Nilsson, C Åstot. J. Chromatogr. B 928 (2013) 98-105.