ANALYSIS OF NERVE AGENT DEGRADATION PRODUCTS BY UPLC/MS/MS

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INTRODUCTION

The need for a rapid sensitive method for the detection and identification of the presence of Chemical Warfare Agents has been highlighted in recent years due to increased public concern about the use of chemical warfare. Although, more than 160 countries have signed the Chemical Weapons Convention (CWC) which prohibits the development, production, stockpiling and use of chemical weapons by the military [1] there is a serious threat of their use in a terrorist attack.

Nerve agents are organophosphate type compounds. The first class of nerve agents, the G-Series were synthesized in Germany during World War II. The G-series consists of GA (Tabun), GB (Sarin), GD (Soman) and GF (Cyclosarin) [2]. Sarin gained notoriety in 1995 due to its use in the Tokyo underground terrorist attack [3]. The second, more toxic series of nerve agents, the V-series consists of VX and R-VX (Russian VX).

All nerve agents are extremely toxic even in small amounts. 1 mg of VX on the skin can be fatal. Their mode of action in the body is to inhibit cholinesterase enzymes causing the accumulation of acetylcholine, resulting in prolonged stimulation and paralysis of muscles [4].

In the body and in the environment, nerve agents metabolize / degrade rapidly to form their corresponding Alkyl Methyl– Phosphonic Acid (AMPA). Figure 1 shows the nerve agents and their degradation products. A fast, easy analysis method for the AMPAs of the nerve agents, VX, GB, R-VX, GF and GD is presented here. This method is suitable for the analysis of biological and environmental samples, following appropriate sample preparation without the need for derivatization. Five nerve agent degradation products were analyzed using UPLC/MS/MS with the ACQUITY® UPLC TQD system in less than 1.8 minutes; the total cycle time was 4 minutes.

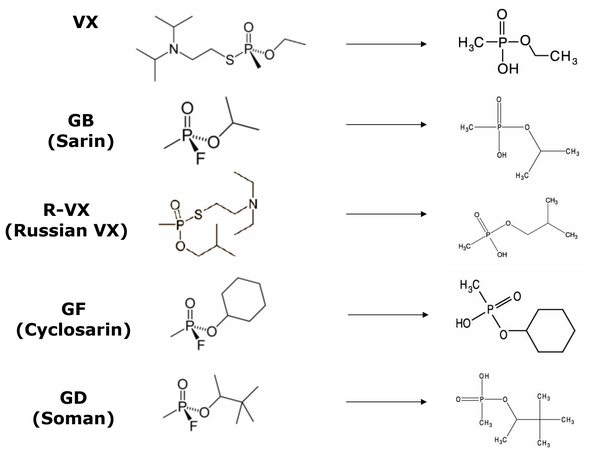

Figure 1: Nerve agents and corresponding degradation products (AMPAs)

Figure 2: Waters ACQUITY UPLC / TQD System

HYDROPHILIC INTERACTION CHROMATOGRAPHY (HILIC)

The AMPAs are low molecular weight, very polar compounds and difficult to retain on conventional reverse phase (RP) HPLC columns. Also, the retention of very polar analytes by RP chromatography often requires a non-volatile, highly aqueous mobile phase which is not ideal for electrospray MS. HILIC is a variation of normal phase chromatography with polar stationary phase columns and high organic content mobile phases. The advantage is the use of solvents miscible in water. HILIC is ideal for the direct analysis of plasma samples prepared by protein precipitation or environmental samples prepared by SPE. Both produce highly organic eluents which are compatible with the highly organic gradient starting conditions, thus eliminating the need for time consuming evaporation and reconstitution steps [5].

EXPERIMENTAL CONDITIONS

UPLC Conditions

Instrument: Waters ACQUITY UPLC
Column: Waters ACQUITY UPLC HILIC 2.1x100 mm, 1.7µm
Eluent A: 10 mM Ammonium Acetate in 90 / 10 Acetonitrile / Water
Eluent B: 10 mM Ammonium Acetate in 10 / 90 Acetonitrile / Water

Gradient:

Time (min)	Flow (mL/min)	A (%)	B (%)	Curve
0	0.7	97	3	6
2	0.7	97	3	6
3	0.7	60	40	6
3.1	0.7	97	3	1

Run Time: 4 minutes
Column Temperature: 35ºC
Injection volume: 10 µL

MS Conditions

Instrument: Waters ACQUITY TQD
Ionization Mode: ES-
Capillary Voltage: 3.6 kV
Source Temp: 150 ºC
Desolvation Temp: 400º C
Desolvation Gas: 800 L/hr

Metabolite	MRM Transition	CV (V)	CE (V)
EMPA	123.1 > 95	28	12
	123.1 > 79	28	20
IMPA	137.1 > 95	30	14
	137.1 > 79	30	21
iBuMPA	151.1 > 95	37	13
	151.1 > 77	37	20
CMPA	177.1 > 95	35	16
	177.1 > 79	35	31
PMPA	179.1 > 95	35	16
	179.1 > 79	35	32

RESULTS & DISCUSSION

Ultra Performance Liquid Chromatography (UPLC) enables faster and higher peak capacity separations than conventional HPLC. Using UPLC, baseline separation of 5 AMPAs was achieved in under 1.8 minutes compared with up to 20 minute literature reports of HPLC separations [6, 7]. Figure 3 shows the UPLC/MS/MS chromatogram of a 10 pg/µL standard.

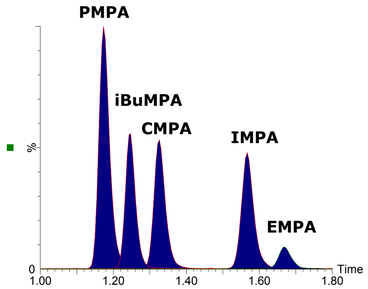

Figure 3: UPLC/MS/MS chromatogram of a standard containing five AMPAs

Two Multiple Reaction Monitoring (MRM) transitions were monitored for each of the AMPAs. The primary transitions were used for quantification and the secondary transitions for confirmation purposes. Waters TargetLynx™ Application Manager software was used to calculate the ion ratio between the primary and secondary transitions and for calibration and quantification.

Calibration curves for all 5 compounds were linear over the range 0.5 to 200 pg/µL with acceptable regression coefficients (r^2 > 0.996). Figure 4 shows a representative calibration curve for the compound IMPA.

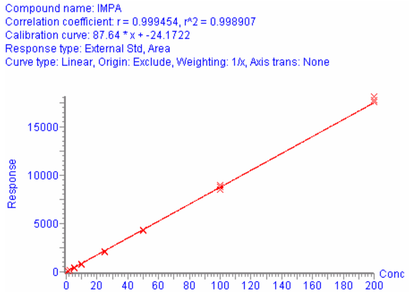

Figure 4: Calibration curve for IMPA

CONCLUSIONS

Quantification and confirmation of the presence of the nerve agents VX, GB, R-VX, GF and GD is achieved in one analysis. This was accomplished by applying a fast, sensitive UPLC/MS/MS method for the analysis of 5 nerve agent degradation products. Separation of the 5 compounds was achieved in less than 1.8 minutes. The method is based on hydrophilic interaction chromatography. This eliminates the time consuming evaporation and reconstitution steps following SPE or protein precipitation.

REFERENCES

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