

Analysis of Organophosphorous Nerve Agent Metabolites by LC/MS/MS

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Introduction:

Nerve agents are the most well known and publicized chemical warfare agents. The chemical properties, lethality and history of these agents are well documented. Because of the potential use of nerve agents, methods for the detection and quantitation of these compounds are necessary. Of special interest is the development of methods to determine exposure levels to decrease the burden on the healthcare system. Our laboratory has developed and validated a method to measure the exposure to these agents by determination of the major metabolite the methyl phosphonic acids. This protocol uses solid phase extraction followed by electrospray negative ion LC/MS/MS analysis.

Experimental:

Four of the most common organophosphorous nerve agents were chosen for investigation; Sarin (GB), Soman (GD), Cyclosarin (GF), and VX. The corresponding metabolites are: methyl isopropyl phosphonic acid (GB acid), methyl pinococyl phosphonic acid (GD acid), methyl cyclohexyl phosphonic acid (GF acid), and methyl ethyl phosphonic acid (VX acid). Initial sample preparation consisted of adding a stable isotope analog of each of the metabolites to one mL of acidified urine and mixing. An anion exchange solid phase extraction (SPE) cartridge was conditioned using methanol and water. The sample was loaded, the column was rinsed with water and eluted with acidified methanol. The eluent was concentrated to ~300 μ L for LC/MS/MS analysis.

Instrumental:

The LC/MS/MS system was a Model 1100 capillary HPLC (Agilent, Wilmington DE) and a API 4000 mass spectrometer (MDS Sciex, Concord, ON). The column was an Agilent Zorbax SB-C18 150mm x 0.5 μ m id x 5 μ m. The product ion of all the metabolites and labeled internal standards was m/z 95.

Results:

The method was successfully validated by performing twenty calibration runs using five different analysts and measuring the results of a low QC (25 ng/mL) and a high QC (50 ng/mL). A sample chromatogram is shown in Figure 1 and sample calibration curves for these analytes are shown Figure 2. Results for the QC samples are shown in the table below:

20 Runs	VX Acid		GB Acid		GD Acid		GF Acid	
	QC Low	QC High						
Mean	25.9	50.4	25.9	50.1	26.1	49.7	26.3	50.0
SD	0.830	1.37	1.42	1.38	2.03	1.75	1.68	1.83
% RSD	3.1	2.7	5.5	2.8	7.8	3.5	6.4	3.6

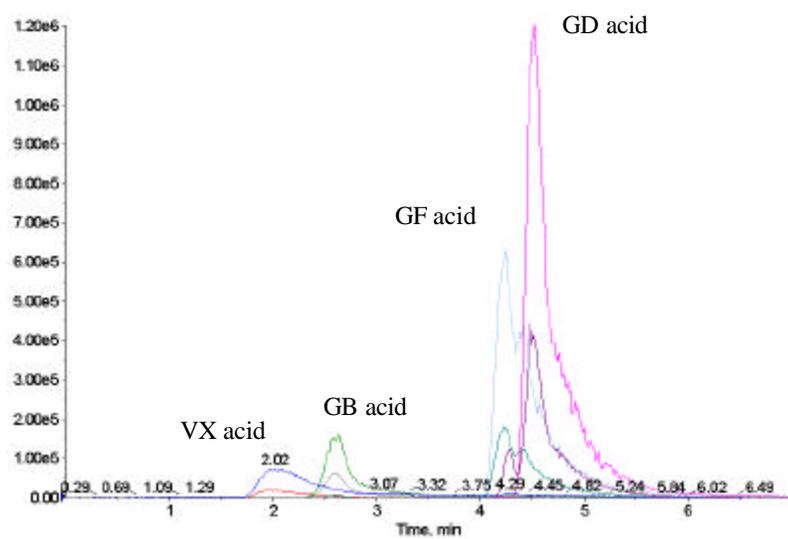


Figure 1 HPLC/MS/MS Chromatogram for the selected analytes

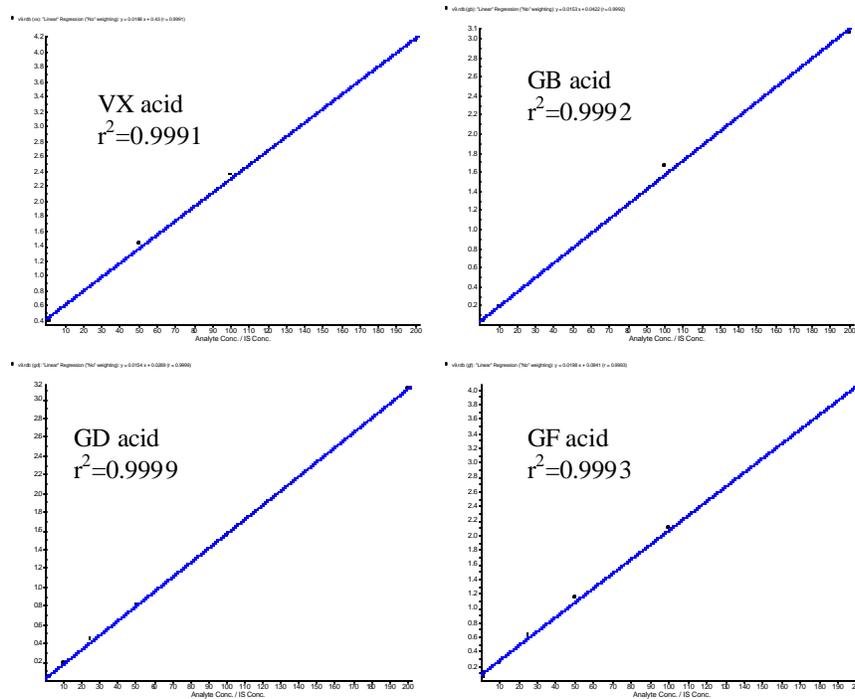


Figure 2 Typical Calibration Curves for the Nerve Agent Metabolites

Acknowledgements:

This work was funded by Public Health and Preparedness and Response to Bioterrorism Cooperative Agreement No. V90/CCU317014-03 CDC Focus Area D Chemical Agents