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Analysis of VX nerve agent hydrolysis products in wastewater effluents by ion chromatography with amperometric and conductivity detection

Haishan Piao [a], Randall B. Marx [a,*], Steven Schneider [b], David A. Irvine [a], John Staton [b]

[a] *SBR Technologies, 200 West Superior, Suite 200, Chicago, Il 60610, USA*
[b] *Perma-Fix of Dayton Inc., Laboratory Department, 300 S. West End Avenue, Dayton, OH 45427, USA*

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Abstract

An analytical method, based on the use of ion chromatography, was developed to monitor the levels of three regulated VX hydrolysis products in the effluent from a biological wastewater treatment process—ethylmethylphosphonic acid, methylphosphonic acid and 2-(diisopropyl)aminoethanethiol. Previous methods have not been applied to wastewater matrices or 2-(diisopropyl)aminoethanethiol. Despite the specificity and sensitivity constraints of this method, it was possible to measure the compounds in bioreactor effluents down to a level substantially below the US Army discharge limit of 0.1% (w/v). Analytical data was confirmed by liquid chromatography–mass spectrometry (LC–MS) at an independent laboratory.

Keywords: Warfare agents; Ethylmethylphosphonic acid; Methylphosphonic acid; Hydrolysis products; 2-(Diisopropyl)aminoethanethiol; Ion chromatography; Wastewater

1. Introduction

The United States Department of Defense was directed by Congress to destroy the US stockpile of chemical agent munitions and bulk agent by April 29, 2007, in accordance with the Chemical Weapons Convention (CWC) deadline. One destructive technology for VX nerve agent, *O*-ethyl *S*-[2-(diisopropylamino)ethyl] methylphosphonothiolate, is base-catalyzed hydrolysis. Base-catalyzed hydrolysis uses aqueous sodium hydroxide (NaOH) and heat to facilitate the hydrolysis of the VX molecule, destroying the agent and thereby neutralizing its toxicity.

Two phosphonic acids, ethylmethyl phosphonic acid (EMPA) and methyl phosphonic acid (MPA), as well as the organosulfur compound, 2-(diisopropyl)aminoethanethiol (DIAT), are products of VX base-catalyzed hydrolysis [1]. These compounds are regulated due to concerns that they could be used to reform VX.

Further processing of VX hydrolysate would be required to generate an effluent discharge that will meet the US Army limit of 0.1% (w/v) for all three of the previously-mentioned hydrolysis products. A study was conducted, by SBR Technologies and Perma-Fix of Dayton Inc., to design a treatment process for VX hydrolysate that would meet limits for hydrolysis products as well as all other discharge permit limits. The process includes chemical pre-treatment followed by biological wastewater treatment in an aerobic sequencing batch reactor (SBR). In order to verify that limits for VX hydrolysis products were met, it was necessary to have an analytical method available for the three regulated hydrolysis products present in VX hydrolysate.

It was important that the analytical method be designed to use financial resources and space efficiently, yet be sufficiently rigorous to meet quality assurance criteria. Methods based on capillary electrophoresis have shown detection limits for alkylphosphonates of less than 1 mg/L in a hydrolysate matrix [2], and about 6 mg/L in environmental matrices [3], however, this method would not be appropriate for uncharged analytes such as DIAT. While methods for all three hydrolysis

* Corresponding author. Tel.: +1 216 272 2469; fax: +1 212 988 9449.
E-mail address: randall.marx@sbrtechnologies.com (R.B. Marx).

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products were previously designed using liquid chromatography followed by mass spectrometry (LC–MS) [4], it was determined that LC–MS would be too expensive and difficult to operate for plant personnel. Experimental methods [5,6] were not considered sufficiently mature to be applied readily. As an alternative, liquid-based ion chromatography was proposed as a means to quantify the concentration of the compounds. Ion chromatographic methods have been developed for phosphonate hydrolysis products of nerve agents, with detection limits of less than 1 mg/L [7], however, these methods have not been applied to the thiol hydrolysis products, nor have the methods been applied to a wastewater matrix.

There are other motivations to detect nerve agent hydrolysis products in wastewater. Non-incineration treatment processes for nerve agent generally involve the use of water. As many treatment systems use biological technologies, ultimately hydrolysis products are likely to enter a biological wastewater treatment process. It is necessary to monitor the effluents from these processes to meet regulatory limits.

The advantages of ion-chromatography are low-cost [8], maturity of the technology and simplicity of operation. While there are several types, ion chromatography generally involves separation of components in a liquid sample with an HPLC column and detection of the components with an electrical detector. If the analytes are ionic in solution, an ion-exchange column with organic modifiers [9] can be used to separate them and a conductivity detector is used for quantification. When combined with chemical eluent suppression, conductivity detection provides improved sensitivity and selectivity [8].

Some non-ionic compounds can also be detected by ion chromatography. For example, compounds containing less-than-fully oxidized sulfur can generate a signal as current from the detector is absorbed during oxidation. This technology is referred to as integrated pulsed amperometric detection. Amperometric detection has been used previously for detection of other thiol compounds [10]. Note that amperometric detection cannot be used for compounds that are recalcitrant to oxidation, such as alkyl phosphonates.

The disadvantages of ion chromatography for analyses of hydrolysis products in wastewater are low specificity and low sensitivity relative to LC–MS. Phosphonates will be detected by ion conductivity just as any other anions in the wastewater matrix such as nitrate, chloride, and sulfate. With capillary electrophoresis, the removal of such ions has been found to be critical to improving the sensitivity of the method in environmental samples [11]. In theory, unrelated anions may have retention times that are similar to one or both of the phosphonates, making analysis of phosphonates by ion chromatography unfeasible. Even if retention times make separation of the analytes and other ions possible, non-target ions may have signals that are so much stronger than the analytes that the analyte signal is not evident. Despite the apparent limitations, ion chromatography was selected as a potentially-appropriate method because the required detection limit for all hydrolysis products was quite large at 0.1% (1000 mg/L) and therefore only detection and quantification of a relatively strong analyte signal would be required.

2. Experimental

MPA standard was obtained as a powder (98%) from Sigma–Aldrich (St. Louis, MO). EMPA was obtained as a methanol solution (98%) from Sigma–Aldrich. DIAT hydrochloride (95%) was custom synthesized by Cerilliant Corporation (Round Rock, TX).

For analysis of phosphonates, EMPA and MPA, the following Dionex (Sunnyvale, CA) DX-600 IC system was used. The liquid chromatography system included an IonPac AS11 anion-selective ion-exchange analytical column, held at 30 °C with an LC25 chromatography oven. Samples were processed through an AS40 autosampler with a 25 μL injection volume. The mobile phase was distilled water, pumped with an IP25 isocratic pump and blended with concentrated potassium hydroxide using an EG40 eluent generator. The total flow rate was 1.0 mL/min. The CD25 conductivity detector was maintained with a current of 95 mA. The sample run time was 28 min. EMPA and MPA peaks have retention times of 3.7 and 9.5 min, respectively. The peaks are identified and quantified with software packaged as the PeakNet 6 Chromatography workstation.

For analysis of DIAT, a second Dionex DX-600 IC system was used. Only the computer components, hardware and software, were shared between the two systems. This second system used an Acclaim 120 column, held at 35 °C. Samples were injected manually with an injection volume set at 25 μL. The mobile phase was composed from three components with the listed volume percentages: 76% distilled water, 20% 500 mM sodium acetate (pH 3.75) and 4% acetonitrile (90% strength). Mobile phase was pumped with a GS50 Gradient pump, using the optional degas for 30 s every 2 min. The liquid flow rate was 1.0 mL/min. The run time was 10 min. For analyte detection, the ED50 electrochemical detector was used. The detector uses integrated pulsed amperometry, with gold electrode, and Ag/AgCl reference electrode components.

During oxidation, the DIAT absorbs current from the amperometric detector, thereby generating a signal. The cycle was optimized to effectively oxidize the DIAT to the corresponding disulfide. The waveform used for the ED50 integrated pulsed amperometric detector was run in one-second cycles with the program listed in Table 1.

Except for calibration standards, all analytical samples were from some point of the treatment process in the pilot-scale system. The three matrices—untreated, chemically-treated, and chemically- and biologically-treated VX hydrolysate—are similar to those expected for the full-scale system, which includes chemical treatment to oxidize a majority of the hydrolysis products followed by biological treatment in a sequencing batch reactor to remove more than 90% of the influent biochemical oxygen demand (BOD).

Table 1
Waveform for amperometric detector

Time (s)	Potential (V)	Integration (begin/end)
0.00	0.24	
0.05	0.24	Begin
0.09	1.34	
0.61	0.24	
0.65	1.34	
0.69	0.24	End
0.70	−1.50	
0.71	−1.50	
0.89	−0.21	
1.00	−0.21	

In the laboratory, samples from two types of 5.8 L biological treatment reactors were used for analytical tests: controls were fed wastewater from the full-scale facility which contained no hydrolysis products and the test reactors were fed 80% (v/v) full-scale wastewater blended with VX hydrolysate that had been chemically treated (test reactor) at 20% (v/v). Control bioeffluent contains effluent from a parallel biological treatment system that receives no hydrolysate but does receive all other wastewaters. The latter samples were the chemically- and biologically-treated VX hydrolysate samples. The 80:20 volume ratio matches the design for a proposed full-scale facility. Duplicates were maintained for each type of reactor. Both reactors were fed 4–5 g total organic carbon (TOC) daily. While chemical treatment was responsible for more than 85% degradation of hydrolysis products, no degradation of these compounds could be attributed to the bioreactors.

3. Results and discussion

Substantial dilution of the bioreactor effluent matrix was required for analysis of hydrolysis products. In tests with 0.01% (w/v, or 100 ppm) spikes of hydrolysis products, and a 1/10 (v/v) dilution of the wastewater samples with distilled water, EMPA was found to bleed into the void volume and show signs of tailing while thiolamine peaks were found to be blunt-shaped. It was suspected that high concentrations of non-analyte anions, such as sulfate and chloride, were saturating the ion-exchange column at dilutions of 1/10 or less. At a 1/100 dilution, the previously noted problems were no

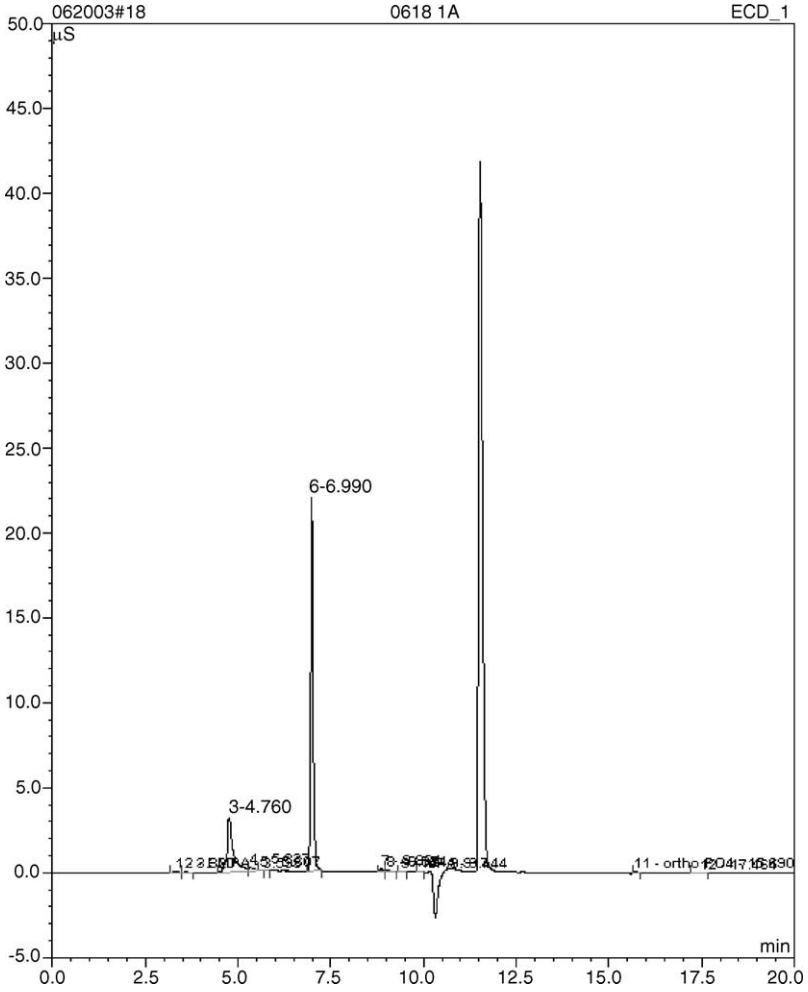

Fig. 1. Chromatogram of biologically-treated control wastewater (1/100 dilution) with conductivity detection.

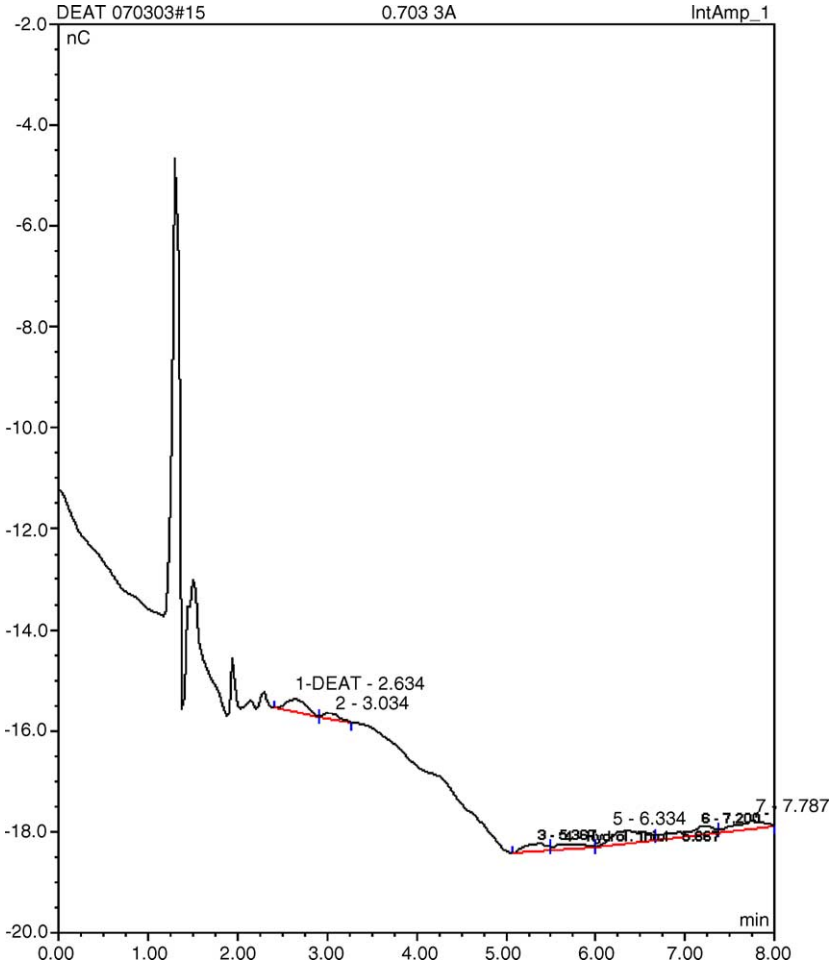

Fig. 2. Chromatogram of biologically-treated control wastewater (1/100 dilution) with amperometric detection.

longer present. While possible, greater dilutions would have decreased the sensitivity of the method.

Using a dilution of 1/100, peaks could be detected for hydrolysate compounds in biologically-treated wastewater where none were present previously in control samples, the latter of which are shown in Figs. 1 and 2. As shown in Fig. 3, phosphonate and phosphate peaks were sharp and symmetrical. DIAT peaks were slightly skewed and elongated (Fig. 4).

Using spikes of the control bioreactor matrix and dilutions of 1/100, it was possible to determine the detection limit, precision, accuracy, and calibration statistics. For the method detection limit (MDL), 7 replicate spikes of 0.01% were diluted 1/100. As shown in Table 2, the MDLs for all analytes are more than an order of magnitude lower than the regulatory discharge limit of 0.1%. It should also be noted that the spike level ranged from 3 to 10 times the MDL. The same seven-replicate study was used to determine the percent recovery as measured against a calibration curve for standards in distilled water. Here, the percent recovery for all three hydrolysis products fell within the normally accepted range of 80–120%, signifying that the matrix had an acceptably low effect on the response of the analytical instrument. The calibration curves for EMPA, MPA and DIAT are shown in Figs. 5–7.

A low range concentration of 0.01% was chosen for several reasons. This value is three times greater, or more, than the method detection limits for all analytes. Furthermore this value is an order of magnitude less than the effluent discharge

Table 2
Analytical statistics for hydrolysis products in bioreactor effluent matrix

Compound	Method detect. limit (%)	Percent recovery	Low concentration (%)	High concentration (%)	Number of data points	R^2	Response factor[a]
EMPA	0.001	103	0.01	0.3	7	0.9997	0.09
MPA	0.002	87	0.01	0.3	7	0.9999	0.12
DIAT	0.003	112	0.01	0.05	4	0.9780	5.6

[a] Units for EMPA and MPA are uS min/ppm. Units for DIAT are uC min/ppm.

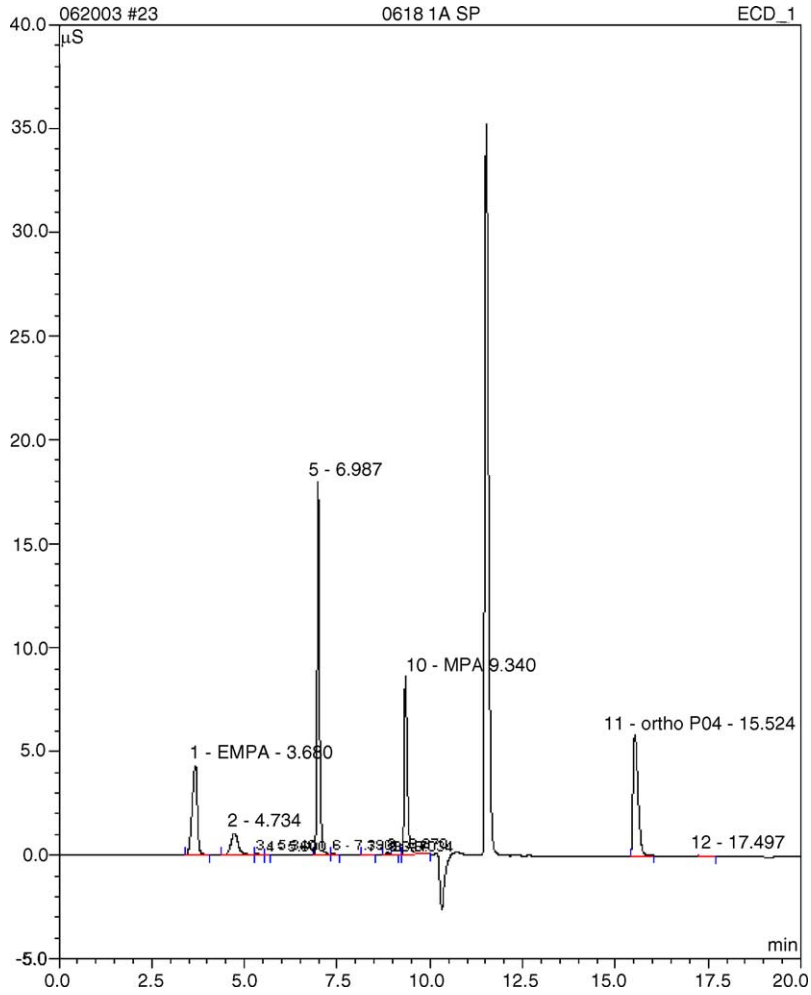

Fig. 3. Chromatogram of spiked, biologically-treated, control wastewater (1/100 dilution) with conductivity detection. The sample contained 1000 ppm spikes of MPA, EMPA and orthophosphate prior to dilution.

limit of 0.1%. The highest acceptable concentration was limited to 0.05% in the DIAT. Above this concentration, the response became non-linear (see Fig. 7); with spikes up to 0.3%, DIAT curves had an R squared value that was diminished to 0.96. In general, the concentration range and linearity of the response were diminished with DIAT as compared to the phosphonates.

Samples of the test reactor effluent were used to confirm the quality of the analytical method in effluents from bioreactors that had been fed treated VX hydrolysate. In one confirmation test, duplicate sets of samples were tested by ion chromatography at Perma-Fix and subsequently by liquid chromatography–mass spectrometry at Southwest Research Institute. The set of three samples consisted of bioreactor effluent samples from three different days of operation for the test reactor, which was fed treated hydrolysate and wastewater daily. Analysts at Southwest Research Institute were not informed of the results from analysis at Perma-Fix. The results of both tests are shown in Table 3. Clearly the results of IC analysis match those of LC–MS analysis with an average percentage difference between the two types of analysis of less than 10%.

Analyte spikes of the test reactor effluent matrix were a second measure of the quality of the analytical method. In this analysis, test reactor effluent matrix samples were spiked with 0.02–0.10% of the analytes and concentrations

Table 3
Test bioreactor effluents analysed by Perma-Fix (IC) and SwRI (LC–MS)

Sample no.	EMPA (%, w/v)		MPA (%, w/v)		DIAT (%, w/v)	
	SwRI	Perma-Fix	SwRI	Perma-Fix	SwRI	Perma-Fix
1	0.03	0.04	0.08	0.07	<0.01	<0.01
5	0.03	0.04	0.09	0.08	<0.01	<0.01
10	0.03	0.03	0.08	0.08	<0.01	<0.01

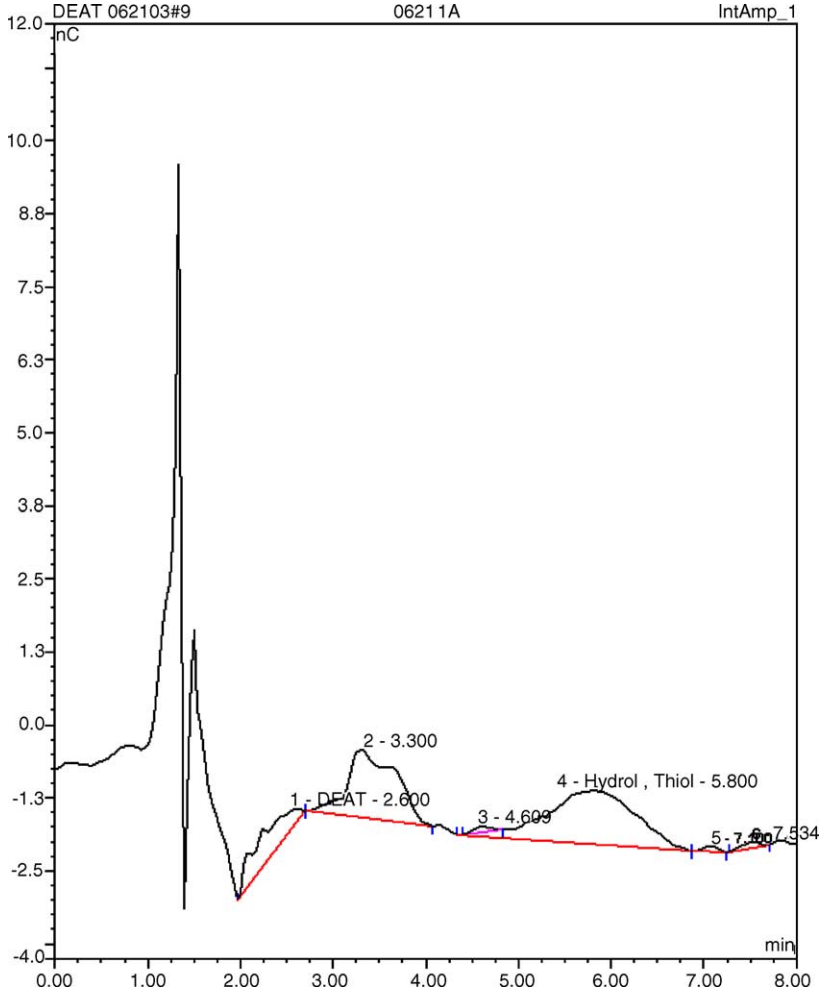

Fig. 4. Chromatogram of spiked, biologically-treated, control wastewater (1/100 dilution) with amperometric detection. The sample contained 200 ppm spikes of DIAT prior to dilution. Note: in this figure, DIAT is referred to as "Hydrol. Thiol.".

of hydrolysis products in the spiked samples were compared with the unspiked samples to determine the recovery. As in all other analysis, it was necessary to dilute the matrix samples and spike matrix samples 1/100 (v/v) with distilled water. As shown in Table 4, the average spike recovery was lowest for MPA, which was the only compound whose spike recovery was outside the range of 80–120%. For all of the individual 10 samples as well as the averages, the recovery of MPA was lower than that of EMPA, which was in turn

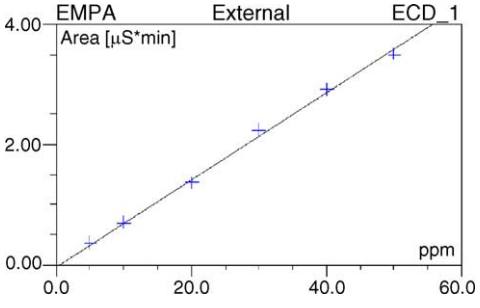

Fig. 5. Calibration curve for EMPA.

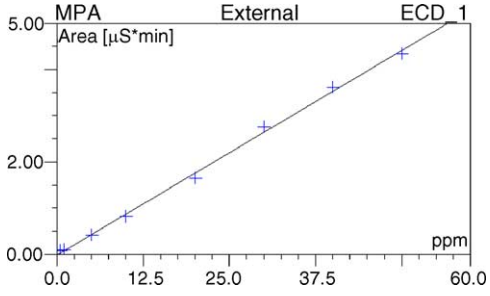

Fig. 6. Calibration curve for MPA.

Table 4
Spike recoveries of test reactor effluents, 10-sample results

	Spike concentration	Average spike recovery (%)	Average percentage standard spike duplicates
EMPA (%, w/v)	0.10	87	2
MPA (%, w/v)	0.10	73	2
DIAT (%, w/v)	0.02	116	9

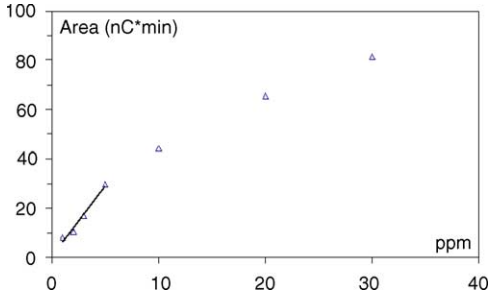

Fig. 7. Calibration curve for DIAT. Notes: only the linear portion (<5 ppm) of the curve was used. Calibration was performed in wastewater matrix; similar responses were obtained in deionized water.

lower than that of DIAT. This pattern was also apparent in spikes of the control matrix, as seen in Table 2. The average percentage standard deviation of spike replicates was less than 10% for all analytes.

4. Conclusion

Taken as a whole, data on the analysis of VX-related hydrolysis products show that it is possible to use Ion Chromatography to meet the analytical needs of a wastewater treatment process for VX hydrolysate. Analysis in wastewater effluent was possible without sample cleanup. Due to the simplicity and maturity of the technology, it was brought online in a short time, only several months. Ion chromatography method also has important limitations, notably the required dilution of the matrix of at least 100-fold, high detection limits of 100–300 mg/L, and a moderately low recovery of MPA in the test reactor effluent matrix.

The appropriateness of an IC analytical method for phosphonates in wastewaters suggests other applications. Phosphonates are present in other compounds, such as the hydrolysis products of pesticides [12], which may be present in the wastewater distribution system. To screen for the hydrolysis products of organophosphorus compounds rapidly, ion chromatography may serve as a useful method.

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