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In-line respeciation: an ion-exchange ion chromatographic method applied to the separation of degradation products of chemical warfare nerve agents in soil

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Abstract

The natural background of anions encountered when analyzing soil samples by ion chromatography (IC) present significant problems in the separation, detection and quantification of isopropyl methylphosphonic acid (IMPA) and methylphosphonic acid (MPA), the degradation products of sarin, a chemical warfare nerve agent. Using chemically-suppressed IC with conductivity detection, a commercially available ion-exchange column, and an isocratic binary eluent system, IMPA and MPA were determined in aqueous extracts of soil at sub-ppm (μg/g) concentrations without the need for gradient elution or organic solvent eluent modifiers. Common soil anions such as chloride, nitrate, sulfate and phosphate do not interfere with the analysis method due to the composition of the binary eluent allowing for greater mobilization of multivalent anions (e.g., MPA, carbonate, and sulfate) while monovalent anions (e.g., IMPA and nitrate) are relatively unaffected. Carbonate is selectively removed by in-line respeciation to bicarbonate.

Keywords: Warfare agents; Soil; Respeciation; Environmental analysis; Phosphonic acids; Sarin

1. Introduction

The production, destruction and verification of use of chemical warfare nerve agents in the post cold war world is a major national security issue for the USA as it considers the impact and ratification of the Chemical Warfare Convention. Contamination of the soil surrounding former production and future destruction facilities is also a potential environmental problem. Therefore, detection and monitoring of chemical nerve agent degradation products are important and immediate challenges to the research and development community. Isopropyl methylphosphonic acid (IMPA) and methylphosphonic acid (MPA) are two such degradation products. Fig. 1 shows the relationship of IMPA and MPA to the nerve agent GB (sarin). Under normal environmental conditions, GB is expected to degrade principally by hydrolysis in environmental samples to IMPA and then to MPA. Both IMPA and MPA are phosphonic acids (pK_a equals approximately 2.2), differing by the presence of the isopropyl ester and are expected to behave similarly in the soil with respect to their physiochemical properties.

Our laboratory was tasked to develop an analysis method for IMPA and MPA in soil at or below 1 μg/g. Both compounds are normally absent from

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uncontaminated soil. IC is an applicable method for the analysis of these compounds because they are readily ionized and therefore should be separable on ion-exchange resins. However the chemical similarities between IMPA and MPA have made them difficult to separate with the available ion-exchange resins. Some successful separations of the two compounds have been performed by taking advantage of the differing organic moieties of IMPA and MPA using mixed-mode columns, ion-exchange columns with reversed-phase characteristics [1]. Ion-pairing reversed-phase chromatography has also been used to separate the two compounds [2]. Non-suppressed IC has been applied by using UV absorbing eluents to effect an ion-exchange separation. The analytes are then detected spectrophotometrically in indirect (vacancy) mode [3]. The more work that is performed on the problem however the more complex the methods have become. In this study on the detection of IMPA and MPA in soil, the goal was to develop a simple analysis method requiring little sample preparation or ancillary equipment so that the analyses could be performed in the field in a mobile laboratory.

The method described here achieved this goal and does not require sample pre-treatment by solid-phase extraction, supercritical fluid extraction [4], or by pre-column or post-column derivatization [5]. The method does not require the use of organic solvents, mixed-mode analytical columns, gradient elution capabilities, anion trap columns, or ion-pairing techniques. This IC method demonstrates that by utilizing the selective power of the ion chromatograph in its simplest form, isocratic ion-exchange, the necessary separation of IMPA from MPA can be made by manipulation of column selectivity with a binary eluent. Conversion of carbonate, a known interfering anion indigenous to soil samples, to bicarbonate and thereby changing its retention time, is performed by in-line respeciation.

2. Experimental

Conditions

IC Conditions

Ion Chromatograph: Dionex DX 300

Detection: Conductivity

Eluent: 10 m*M* sodium tetraborate/3.75 m*M* sodium hydroxide

Flow-rate: 1.5 ml/min

Column: Sarasep AN300 100×7.5 mm (2 in tandem) and AN300 Guard column

Suppressor: Dionex Micromembrane

Regenerant: 50 m*M* sulfuric acid at 3 ml/min

Injection Volume: 250 μl

Soil sample preparation conditions

(1) Weigh 2 g of soil into a suitable extraction vial.

(2) Add 5 ml of extractant (5.1/5.4 m*M* sodium carbonate/sodium bicarbonate).

(3) Vortex briefly to wet soil.

(4) Mix on an inversion-type tumbler at 18 rpm for 30 min.

(5) Centrifuge out particulates at 3000 rpm for 15 min.

(6) Filter the extract with a 0.45-μm syringe filter.

(7) Analyze by IC.

(8) Dilute extract with deionized water as needed.

2.1. Analytical

The task of separating IMPA from MPA isocratically on an ion-exchange resin would require an

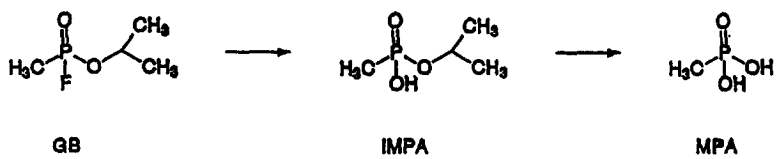

Fig. 1. Degradation of GB to IMPA and MPA.

anion-exchange column with unique performance characteristics. A column was chosen that had clearly demonstrated differing selectivities than the rest of the more commonly used anion-exchange columns. We chose the Sarasep AN300 (MetaChem Technologies, Torrance, CA, USA) analytical column (polystyrene–divinylbenzene 10-μm particles) because it was the first commercially available column to separate fluoride from the water dip. Although fluoride and the region of the chromatogram where it elutes was of little concern, the fact that the AN300 had this unique functionality made the column a good candidate. We chose sodium hydroxide (Fisher, Fair Lawn, NJ, USA) as the eluent because it elutes phosphate after sulfate on the AN300 column, a reversal of their normal elution order. This reordering of the normal elution profile for common anions was indicative of a selectivity that would prove to be beneficial to our goal. Other columns were also explored, but were not as applicable to this task.

Initial experiments were conducted using a 100× 7.5 millimeter column and 10 m*M* (millimolar) sodium hydroxide as the eluent at a flow-rate of 2.0 ml/min. To generate additional plate numbers the flow-rate was reduced to 1.5 ml/min and a second identical AN300 column was added in tandem. Both were preceded by an AN300 guard column. The ion chromatograph was a Dionex (Dionex, Sunnyvale, CA, USA) DX300 with a micromembrane suppressor and PED detector in conductivity mode. The suppressor regenerant was 50 m*M* sulfuric acid (J.T. Baker Ultrex, Phillipsburg, NJ, USA) applied at a flow-rate of 3 ml/min. The injection volume was 250 μl.

2.2. Sample preparation

The soil used in this study was a Tilsit soil (Table 1) from an active agricultural area of Ohio. The Tilsit soil was characterized (Agvise Labs, Northwood, ND, USA) as a silt loam soil with a pH of 7.3 and an organic content of 2.1%. The soil was used with no pretreatment other than passing it through a 2-mm sieve. Though we used only this soil in the study, we expect the anions present to be typical of most soils with variation seen primarily in concentration.

Soil extracts were prepared by fortifying 2.00 g of Tilsit soil with 2.5 μg each of IMPA and MPA dissolved in 25 μl of 80% acetone (Burdick and Jackson, Muskegon, MI, USA) and 20% deionized water solution. The samples were vortexed briefly to mix and incubated at room temperature for 1 h. The extractant was then added to the soil. The volume of the extractant was either 3 or 6 ml. The sample and extractant were vortexed briefly, and then mixed on an inversion-type tumbler at 18 rpm for 15, 30, or 60 min. At the end of the extraction the samples were centrifuged at 3000 rpm for 15 min to separate particulates from the extract. The extract was then decanted into a suitable vial for analysis by IC. Filtration of the extract was performed by the IC system autosampler.

Table 1
Tilsit soil characteristics

Characteristic	
Textural class (USDA)	Silt loam
Percent sand	12.1
Percent silt	66.4
Percent clay	21.5
Bulk density (disturbed) g/cm^3	1.19
pH	7.3
Organic matter (%)	2.1
Cation-exchange capacity (mequiv./100 g)	10.0
Base saturation	
Calcium (%)	59
Magnesium (%)	34
Sodium(%)	3.9
Potassium (%)	2.8
Available nutrients	
Calcium (μg/g)	1180
Magnesium (μg/g)	408
Sodium (μg/g)	90
Potassium (μg/g)	110

3. Results and discussion

3.1. Analytical

Results obtained by the initial chromatographic experiments using 10 m*M* sodium hydroxide eluent indicated that the common soil anions (fluoride, chloride, nitrate, carbonate, phosphate, and sulfate) eluted in a typical profile, phosphate being the exception. Fig. 2a shows the common soil anions plus IMPA and MPA separated on the AN300

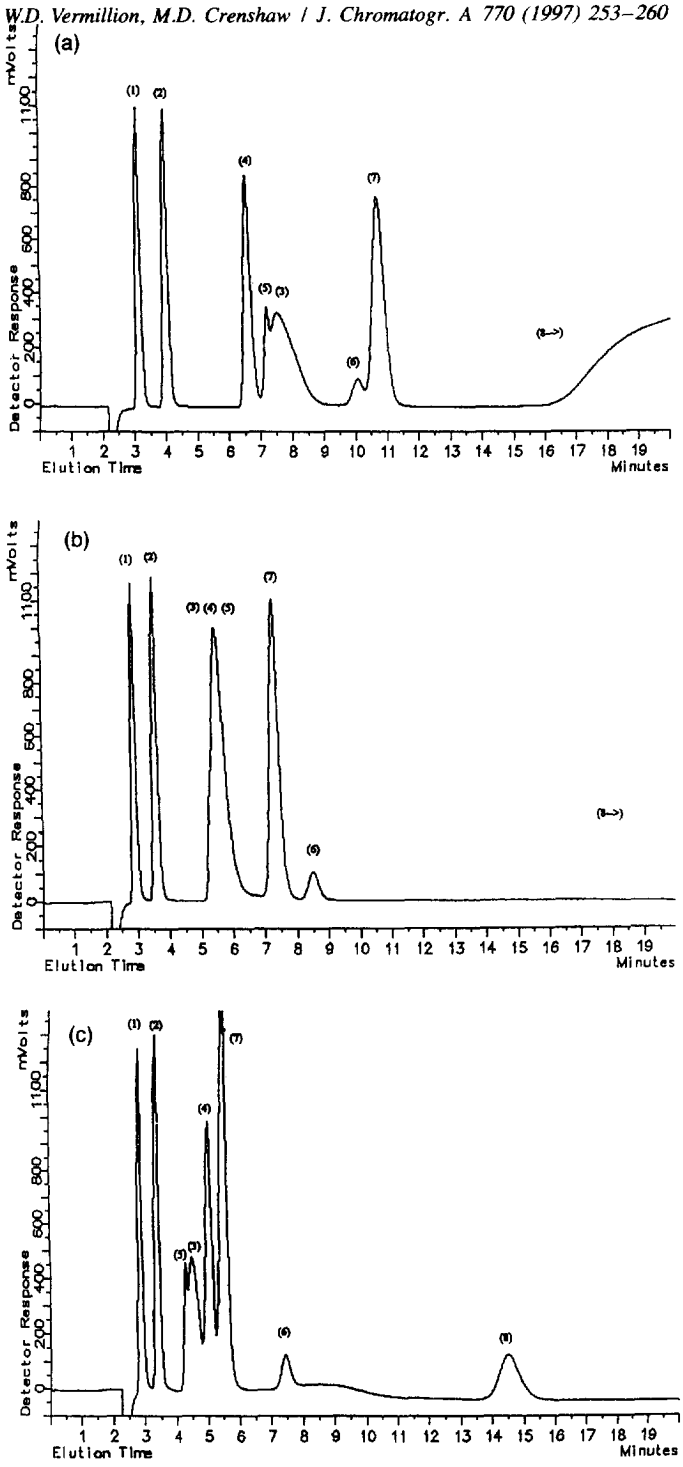

Fig. 2. (a) Common soil anions and IMPA/MPA separated on an AN300 with 10 m*M* NaOH. (1) Fluoride, (2) chloride, (3) carbonate, (4) nitrate, (5) MPA, (6) IMPA, (7) sulfate, (8) phosphate. (b) Common soil anions and IMPA/MPA separated on an AN300 with 15 m*M* NaOH. (1) Fluoride, (2) chloride, (3) carbonate, (4) nitrate, (5) MPA, (6) IMPA, (7) sulfate, (8) phosphate. (c) Common soil anions and IMPA/MPA separated on an AN300 with 20 m*M* NaOH. (1) Fluoride, (2) chloride, (3) carbonate, (4) nitrate, (5) MPA, (6) IMPA, (7) sulfate, (8) phosphate.

column with 10 m*M* sodium hydroxide as the eluent. Note the position of the monovalent anions, fluoride, chloride, nitrate, and IMPA, and the divalent anions, carbonate, MPA, and sulfate. As the eluent strength is doubled to 20 m*M* (Fig. 2c) the di-anions have eluted approximately 50% faster while the mono-anions have eluted only about 20% faster. Fig. 2a–c, show this elution profile progressively as divalent sulfate, which elutes at 11 min in 10 m*M* eluent, elutes sooner than monovalent IMPA in 15 m*M* eluent. The same behavior can be observed with the divalent MPA/carbonate co-eluters eluting sooner than monovalent nitrate as the eluent is strengthened to 15 m*M* and then 20 m*M*. This manipulation in relative retention times of the monovalent and divalent anions allows the desired separation of IMPA from MPA.

After IMPA and MPA were successfully separated, the next task was to separate both from the anionic interferences which would be encountered in an environmental soil sample. Using sodium hydroxide as an eluent creates the problem of having carbonate as a potentially interfering anion in the chromatogram. As shown in Fig. 2a Fig. 2b Fig. 2c, carbonate and MPA co-elute. Because each is divalent, strengthening or weakening the sodium hydroxide eluent will not separate them on the AN300 column. Therefore to separate MPA from carbonate the concentration of sodium hydroxide was reduced to an optimal 3.75 m*M* (pH 11) which positioned the co-eluting MPA/carbonate critical pair and IMPA between nitrate and sulfate. Then to remove carbonate from its co-eluting position with MPA, 10 m*M* sodium tetraborate (Aldrich, Milwaukee, WI, USA) was added which lowered the eluent pH to 9.5 and converted carbonate to bicarbonate (pK_a carbonic acid equals 10.3). As shown in Fig. 3, bicarbonate elutes much earlier in the chromatogram and is therefore not an interfering peak. This in-line re-speciation of carbonate to bicarbonate is the key discovery of the method development activity.

Fig. 3 shows the elution order of the common interfering anions, in deionized water, plus IMPA and MPA, after optimization with tetraborate. The trade-off to this optimization is that sulfate, in this system, elutes at 45 min and makes overall analysis times approximately 50 min. Also, with the tetraborate addition, phosphate returns to its typical

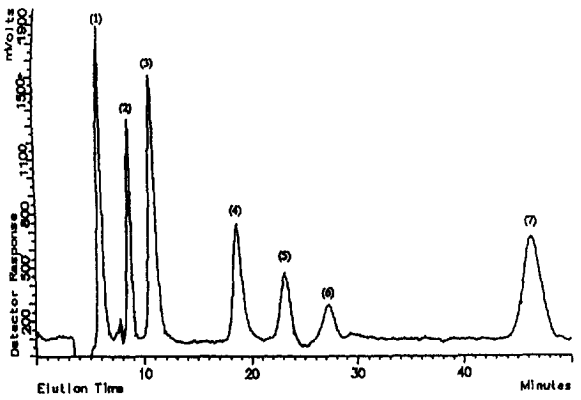

Fig. 3. Mixture (100 ng/ml) of common anions and IMPA/MPA in deionized water separated on the AN300 column using 3.75 m*M* sodium hydroxide/10 m*M* sodium tetraborate eluent. (1) Fluoride, (2) chloride, (3) carbonate (bicarbonate), (4) nitrate, (5) MPA, (6) IMPA, (7) sulfate.

position eluting just before sulfate. Phosphate is not detected when present at 200 ng/ml but is detected at 35 min when present at a concentration of 20 μg/ml. Gradient elution would not shorten analysis times significantly because it would require 30 min for the peaks of interest to elute and approximately 20 min to strip and re-equilibrate the column.

The AN300 column and the sodium hydroxide/sodium tetraborate eluent allowed the band spacing and elution order necessary to avoid the numerous interfering anions indigenous to soil extracts. Late eluting peaks which are separated isocratically on polymer packings tend to broaden considerably; however, with sufficient separation from the interferences and if the longer analysis times are tolerable, gradient elution is not necessary nor applicable. Under these conditions, 50–60 min analysis times are recommended to ensure that sulfate is eluted from the column prior to the analysis of the next sample.

Maintaining freshly prepared eluent is important for retention time precision. Continuous helium sparging of the eluent minimizes absorption of ambient carbon dioxide. Two liters of fresh eluent under continuous helium sparge used over a two day period produced no significant change in retention time.

Instrument detection limits (IDLs) for pure compounds diluted in deionized water responding with a

Table 2
Extraction of IMPA and MPA from 2 g of soil with water

	Extraction method					
	1	2	3	4	5	6
Initial amount of IMPA (μg)	1.25	2.5	2.5	2.5	2.5	2.5
Initial amount of MPA (μg)	1.25	2.5	2.5	2.5	2.5	2.5
Volume of each extraction (ml)	3	3	6	3	6	6
Duration of each Extraction (min)	15	30	30	30	30	60
Number of extractions	3	3	3	3	1	1
Total volume of extract (ml)	9	9	18	9	6	6
Number of replicates	2	3	3	3	3	3
Average recovery of IMPA (%)	72.8	83.6	54.9	97.8	74.7	81.2
S.D.	12.7	7.7	8.06	10.7	2.0	2.3
R.S.D. (%)	17.4	9.2	14.7	10.9	2.6	2.9
Average recovery of MPA (%)	26.6	32.8	40.2	31.3	20.9	25.9
S.D.	0.14	3.9	2.3	2.4	3.8	1.7
R.S.D. (%)	0.53	12.0	5.8	7.6	18.1	6.7

3:1 *S/N* ratio were 10 ng/ml for MPA and 20 ng/ml for IMPA.

3.2. Sample preparation

Sample preparation, extraction of the soil, was explored by varying extraction time, pH, extractant volume, and number of extractions. Tables 2–5 show the results of these tests. The results shown in Table 2 indicate that there is no clear advantage to one method over the other when extracting with water when varying the extraction time, extractant volume or number of extractions and that a 30 min extraction time and 3 ml of extractant (methods 2 and 5 in Table 2) works as well or better than the other combinations.

Table 3
Extraction efficiencies for IMPA and MPA from soil with water

	IMPA recovery (%)				MPA recovery (%)			
	1st extract	2nd extract	3rd extract	Total IMPA recovered	1st extract	2nd extract	3rd extract	Total MPA recovery
Sample 1	94.5	20.3	0	114.8	9.3	10.6	8.8	28.7
Sample 2	97.8	24.4	0	122.2	10.1	10.2	9.0	29.3
Sample 3	95.1	21.6	0	116.7	10.1	9.9	7.9	27.9
Average	95.8	22.1	0	117.9	9.8	10.2	8.6	28.6
S.D.	1.76	2.10	na	3.84	0.462	0.351	0.586	0.702
R.S.D. (%)	1.8	9.5	na	3.3	4.7	3.4	6.8	2.5

Table 4
Extraction efficiencies for IMPA and MPA from soil with weak bicarbonate/carbonate

	IMPA recovery (%)				MPA recovery (%)			
	1st extract	2nd extract	3rd extract	Total IMPA recovered	1st extract	2nd extract	3rd extract	Total MPA recovery
Sample 1	98.5	18.9	11.3	128.7	16.3	14.2	11.7	42.2
Sample 2	101	20.1	14.9	136.0	17.0	12.6	12.8	42.4
Sample 3	95.4	20.4	14.6	130.4	15.8	11.4	13.0	40.2
Average	98.3	19.8	13.6	131.7	16.4	12.7	12.5	41.6
S.D.	2.81	0.794	2.0	3.82	0.603	1.40	0.700	1.22
R.S.D. (%)	2.1	4.0	14.7	2.9	3.7	11.1	5.6	2.9

Table 5
Extraction efficiencies for IMPA and MPA from soil with strong bicarbonate/carbonate

	IMPA recovery (%)				MPA recovery (%)			
	1st extract	2nd extract	3rd extract	Total IMPA recovered	1st extract	2nd extract	3rd extract	Total MPA recovery
Sample 1	86.0	20.0	0	106.0	25.0	24.5	10.2	59.7
Sample 2	90.1	22.2	0	112.3	26.4	22.7	10.2	59.3
Sample 3	85.2	na	0	85.2	26.4	24.2	11.0	61.6
Average	87.1	21.1	0	109.2	25.9	23.8	10.5	60.2
S.D.	2.63	1.56	na	4.45	0.808	0.964	0.462	1.23
R.S.D. (%)	3.0	7.6	na	4.1	3.1	4.0	4.4	2.0

Table 3 shows that when the individual extracts were not combined, an average of 95.8% of the IMPA is extracted in the first extract and therefore subsequent extractions diluted the sample. An average of 9.5% of MPA is recovered in each of the three succeeding extracts so additional extractions do not increase the MPA concentration in the extract but only increase the total quantity of MPA recovered.

We also explored the use of alkaline solutions as extractants. These solutions were a weak bicarbonate/carbonate (J.T. Baker) solution (1.7/1.8 m*M*) and a strong bicarbonate/carbonate solution (5.1/5.4 m*M*). Table 4 lists the recovery of IMPA and MPA by extraction with the weakly alkaline solution. An average of 98.3% of the IMPA is extracted by the first extraction. The overall average recovery (13.9%) of MPA in each of the three succeeding extracts is nearly constant but the first extract contains slightly more MPA than the others. The total amount of MPA recovered is greater than that obtained with water alone. The results listed in Table 5 show the recoveries of IMPA and MPA using the strong bicarbonate/carbonate solution. Again most (87.1%) of the IMPA is extracted in the first extraction and an even greater amount (25.9%) of MPA is recovered by the first extraction. Total MPA recovery is also increased (60.2%) from the combined extractions. When the samples were acclimated for 24 h instead of 1 h prior to strong bicarbonate/carbonate extraction, an average of 85% of the IMPA was recovered and 58% of the MPA was recovered in three extracts (data not listed). These recoveries are similar to those obtained after a 1-h acclimation period so the alkaline extraction may overcome adsorption of these compounds to the soil.

Once the strong bicarbonate/carbonate was established as the best extractant for both IMPA and MPA, the data was further reviewed to determine if the process could be further simplified. The results showed that 26% of the MPA was recovered in the first extraction, 24% in the second extraction, and 10% was found in the third. For IMPA, 87% was recovered in the first extraction, 21 percent was found in the second, and none was found in the third. Because most of the recovery is obtained in the first two extractions (6 ml) for both IMPA and MPA, the extractant volume was adjusted to one 5-ml extraction as described in the sample preparation method. This adjustment not only simplified the method and saved processing time, but it concentrated the extracted analytes and maximized the method sensitivity.

Method detection limits (MDLs) were determined by fortifying 2 g of Tilsit soil with 0.2, 0.4, and 0.8 μg of IMPA and MPA, extracting with strong bicarbonate/carbonate extractant, and then analyzing the extracts by IC against a calibration curve ranging from 20 ng/ml to 500 ng/ml. Fig. 4 demonstrates the method detection limits as 400 ng of IMPA and MPA per gram of soil. The chromatogram shows the typical soil extraction profile chromatographed at the detection limit for this method. The nitrate peak elutes at about 17 min and returns to baseline (R_s = 1.6) before MPA elutes. IMPA is resolved from MPA. The negative response seen just after the elution of IMPA was determined to be associated with the extractant and does not interfere with IMPA identification or determinations above the method detection limit. Dilution of the sample after extraction with deionized water eliminates this negative response.

Although it is clear that a reduction in the total concentration of anions loaded onto the column would benefit this method, pre-treatment of the soil

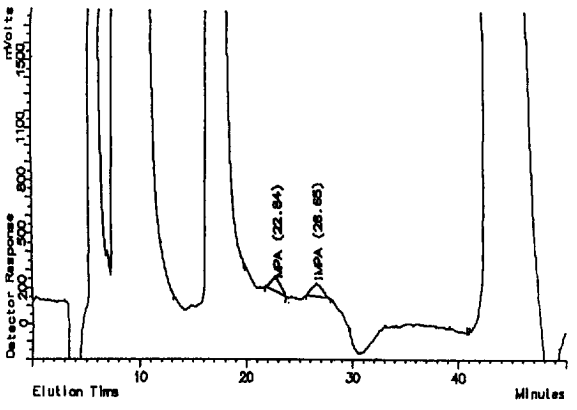

Fig. 4. MDL (400 ng/g) for IMPA and MPA in Tilsit soil extract separated on the AN300 column using 3.75 m*M* sodium hydroxide/10 m*M* sodium tetraborate eluent.

extracts with solid-phase extraction cartridges or the use of supercritical fluid extraction was not within the goals of this project.

4. Conclusions

The total soil extraction efficiency was studied by comparing deionized water with bicarbonate/carbonate as the extractant. The bicarbonate/carbonate extractant was more effective than deionized water for the recovery of MPA. The average recovery for MPA extracted with three 3-ml aliquots of water was 29%, while the same bicarbonate/carbonate extraction averaged 60%. Recovery of IMPA was nearly 100% with either water or the bicarbonate/carbonate solution.

The described IC analysis method was developed by optimizing selectivity with the appropriate stationary and mobile phases and then further refining that selectivity by developing in-line respeciation. The method is a simple isocratic IC method requiring minimal sample preparation for determining two degradation products of chemical warfare nerve agents in soil. The method provides baseline separation of IMPA and MPA from each other and from common anions found in the soil such as: fluoride, chloride, carbonate, nitrate, phosphate, and sulfate. The analytes are separated by ion-exchange mechanisms alone using conventional chemical-suppression IC. The sample preparation process is quick and simple and requires no special laboratory equipment. Method detection limits are 400 ng/g of soil for both IMPA and MPA in a 2-g soil sample.

Acknowledgments

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References

[1] A.F. Kingery, H.E. Allen, Anal. Chem. 66 (1994) 155–159.
[2] P.C. Bossle, D.J. Reutter, E.W. Sarver, J. Chromatogr. 407 (1987) 399–404.
[3] G.A. Pianetti, L.M. Moreira de Campos, P. Chaminade, A. Baillet, D. Baylocq-Ferrier, G. Mahuzier, Anal. Chim. Acta 284 (1993) 291–299.
[4] W.H. Griest, R.S. Ramsey, C.H. Ho, W.M. Caldwell, J. Chromatogr. 600 (1992) 273–277.
[5] P.C. Bossle, J.J. Martin, E.W. Sarver, H.Z. Sommer, J. Chromatogr. 267 (1983) 209–212.