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Short communication

Capillary ion electrophoresis screening of nerve agent degradation products in environmental samples using conductivity detection

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Abstract

A method of detecting signature methylphosphonic acid (MPA) breakdown products of V and G nerve agents in environmental samples was developed using capillary ion electrophoresis with conductivity detection. The electrolyte (30 m*M* L-histidine, 30 m*M* 2-(N-morpholino)ethanesulfonic acid, 0.7 m*M* tetradecyltrimethylammonium hydroxide, and 0.03 weight% Triton X-100) allowed baseline separation of MPA, ethyl methylphosphonic acid (EMPA), isopropyl methylphosphonic acid (IMPA), and pinacolyl methylphosphonic acid (PMPA) in less than 10 min. Detector response was linear in the 6–60 μg/ml concentration range (correlation coefficient=0.99) with a detection limit around 6 μg/ml. The application of this method for screening MPA, EMPA, IMPA, and PMPA in surface water, groundwater, and soil extracts is demonstrated.

Keywords: Methylphosphonic acid; Ethyl methylphosphonic acid; Isopropyl methylphosphonic acid; Pinacolyl methylphosphonic acid

1. Introduction

In response to the US ratification of the Chemical Warfare Convention (CWC), this laboratory was tasked with developing a field screening method capable of detecting signature breakdown products of V and G nerve agents. The required capability of the methodology is to be able to detect methylphosphonic acid (MPA), ethyl methylphosphonic acid (EMPA), isopropyl methylphosphonic acid (IMPA), and pinacolyl methylphosphonic acid (PMPA) at the 10 μg/ml concentration level. The screening method shall not require quantitation but shall be used primarily to prioritize samples for more rigorous analysis by GC–MS. The chemical structures of these compounds are shown in Fig. 1.

Ion chromatography (IC) has been the method of choice for analyzing MPA, EMPA, IMPA, and PMPA. [1,2] However, capillary ion electrophoresis (CIE) could offer significant improvements in efficiency, sensitivity, ruggedness, and portability over IC for field screening applications.

The current on-site CIE method for MPA, EMPA, IMPA, and PMPA utilizes indirect ultraviolet (UV) detection. [3,4] The major drawback of this indirect

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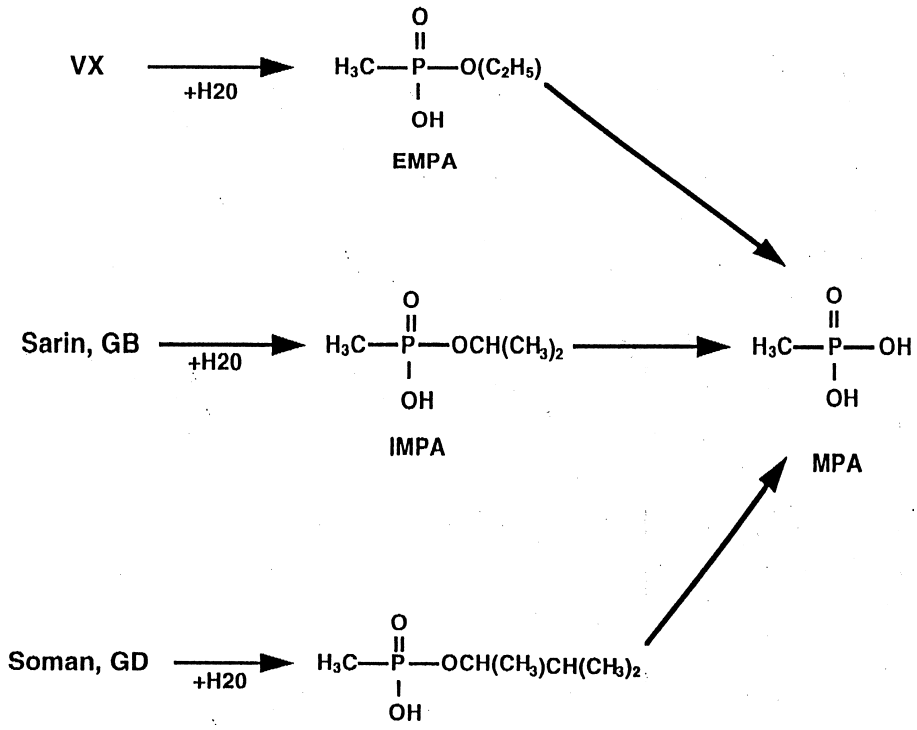

Fig. 1. Nerve agent degradation pathway for the three main alkyl methylphosphonic acids studied.

UV method is carbonate interference with MPA detection.

Conductivity detection is a recent innovation in the field of CIE analysis. Strong ionic species can be detected and quantitated conductimetrically by the changes in the background conductivity of the carrier electrolyte. MPA, EMPA, IMPA, and PMPA are quite amenable to conductivity detection since they are strong acids and fully dissociate in an aqueous environment. The feasibility of applying this technique for detecting these compounds in spiked environmental water and soil extracts is demonstrated.

2. Materials[1]

2.1. Instrumentation

The CIE system employed in this study was the Crystal Model 300 Capillary Ion Analyzer-Crystal Model 1000 Conductivity Detector from Thermo CE (Boston, MA, USA). Computer control and data acquisition was carried out with a Waters Millennium 2010 Chromatography Manager (Milford, MA, USA). ConCap 1™ (ATI) fused-silica capillary columns (50 μm I.D.×60 cm) were used throughout the CIE separation.

2.2. Chemicals

Water used in this study was distilled and deionized (10–18 MΩ/cm) using a Millipore filtration unit (Bedford, MA, USA). L-Histidine, MES [2-(N-morpholino)ethanesulfonic acid], tetradecyltrimethylammonium bromide (TTAB), and Triton X-100 were obtained from Sigma (St. Louis, MO, USA). Tetradecyltrimethylammonium hydroxide (TTAOH) is prepared from the bromide salt using a styrene-based anion-exchange resin (OnGuard-A Sample Pretreatment Cartridges, Dionex, Sunnyvale, CA, USA). All reagent solutions were filtered and degassed prior to analysis.

[1]The use of trade names does not constitute an official US Department of the Army endorsement of these items.

The MPAs have been synthesized and analyzed for chemical structure and purity by teams within the Chemical, Biological Defense Command, Edgewood Research, Development and Engineering Center.

Characterized soils used in this study were prepared by Resource Technology (Laramie, WY, USA) and are available through the contracting office at US Army Dugway Proving Ground (UT, USA).

3. Methods

Instrumental methods conditions for this screening technique are as follows: electrolyte 30 m*M* L-histidine–30 m*M* MES–0.7 m*M* TTAOH (pH 6.5); 0.03 weight% Triton X-100; potential −25 V; and the capillary temperature 35°C. The capillary is regenerated before each analysis with electrolyte for 1.5 min.

The soils were extracted by weighing out 5 g samples of each matrix and adding 10 ml of deionized water. The soils were shaken for 1 min, decanted into a test tube, centrifuged at 1640 *g* for 20 min, and then filtered. The aqueous extract was spiked with a mixture of MPA, EMPA, IMPA and PMPA in acetone so that the final concentration of phosphonic acids in the water extract was 10 μg/ml.

The aqueous matrices were first filtered with 0.2 μm pore filter then spiked with the MPA mixture to obtain a final concentration of 10 μg/ml.

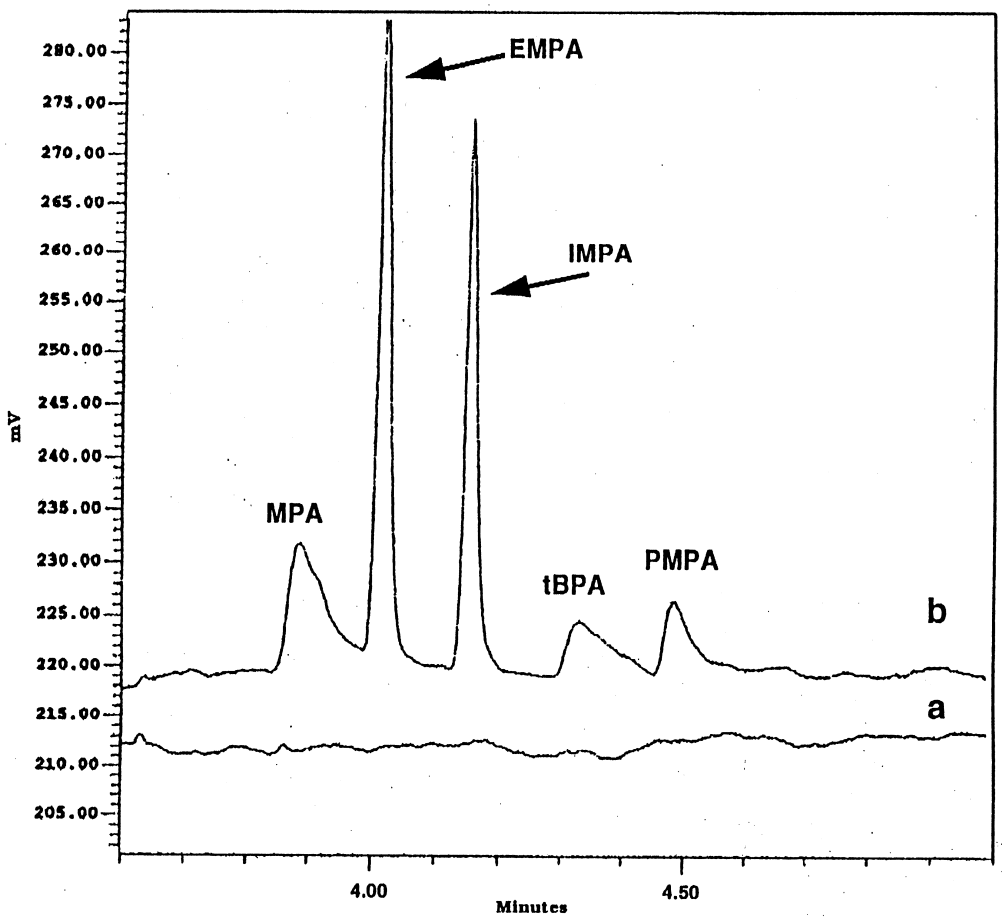

Fig. 2. Electropherograms of water blank (a) and water sample spiked at 10 μg/ml with each of the phosphonic acids (b).

4. Results and discussion

The hydrodynamic injection mode was used to load the capillary column with the sample. Samples were injected onto the capillary column at a pressure of 25 mbar for 12 s. Injection time was sufficient to give adequate analyte detector signal at the 10 μg/ml concentration level without creating undue matrix interference.

TTAOH was added to the running electrolyte as an osmotic flow modifier to ensure that the bulk flow of the electrolyte is directed toward the detector. Before preparing the running electrolyte, highly conductive TTAB had to be converted to TTAOH by replacing the bromide anion with hydroxide in order to avoid a spurious bromide peak in the electropherogram.

Triton X-100 was also added to the running electrolyte to improve analyte mobility by acting as a surfactant to decrease the surface tension on the coated layer of the flow modifier.

The phosphonic acid peaks are well separated from each other and nearly baseline resolved (see Fig. 2). The elution order of the analytes reveal that as the alkyl side chains of the methylphosphonate analytes increase both the charge to mass ratio and ion mobility decrease. For this reason, the migration time on the capillary column increases and detector sensitivity in the conductivity cell decreases. This relationship is mirrored in Hansch π-values [5] for the ethyl (1.0), isopropyl (1.2), and pinacolyl (2.6) alkyl side chains.

Calibration curves for each of these acids were generated over a concentration range of 6–60 μg/ml with four injections at each concentration level. A statistical program evaluated the best-fit line and provided an equation and a correlation coefficient to measure linearity. All calibration curves obtained a correlation coefficient of at least 0.99. In addition, the reproducibility of the instrumental system was tested by injecting ten repetitions of the 10 μg/ml standard. The peak area and retention time were measured and statistical data is presented in Table 1.

Minimal sample preparation of the matrices under field conditions is necessary and should not involve any offline desalting or ion-exchange. Therefore, the use of an internal standard is one means of negating the effect of retention time shift of the analyte peaks caused by high salt content of the sample.

The migration time ratio and response factor, for each of the MPA analytes, was calculated using 1,1-dimethylethylphosphonic acid (t-BPA) as the internal standard. t-BPA was chosen as the internal standard due to similarities in chemical structure, detector response and pK_a-values to the methylphosphonic acid analytes. t-BPA would be especially useful in CWC field screening applications because it is chemically unrelated to any known chemical warfare agent. Response factors were determined by the average peak area response of ten repetitive

Table 2
Migration time ratios and response factors using t-BPA as the internal standard

Analytes	Migration time ratio[a]	Response factor (R_f)[b]
t-BPA	1.0000	1.0000
MPA	0.9044	2.6486
EMPA	0.9376	1.7374
IMPA	0.9667	0.8952
PMPA	1.0353	0.5672

[a] Migration time of analyte/migration time of internal standard.
[b] Response of analyte/response of internal standard.

Table 1
Statistical data for CIE using conductivity detection

Analyte	Correlation coefficient	Migration time[a] (min)	Standard deviation of migration time (min)	Peak area[a]	Standard deviation of peak area
MPA	0.997	3.716	0.007	407 783	15 648
EMPA	0.998	3.832	0.004	494 768	18 932
IMPA	0.999	3.938	0.008	335 506	14 634
PMPA	0.993	4.208	0.008	103 854	5 320

[a] Average of ten repetitions.

injections for each analyte at the 10 μg/ml concentration level (see Table 2).

Figs. 3–7 contain the electropherograms of different environmental matrices that have been spiked at the concentration level of 10 μg/ml with each of the MPA analytes. The MPA analytes in the electropherograms of the spiked samples of spring water (Fig. 3), Baltimore Harbor water (Fig. 4), and sandy loam extract (Fig. 5) are well separated from each other and nearly baseline resolved. The MPA analytes are also well resolved from the rapidly migrating common background anions (i.e. chloride, sulfate, nitrate, etc.). Interferences in the electropherograms of the loam and clay–loam spiked

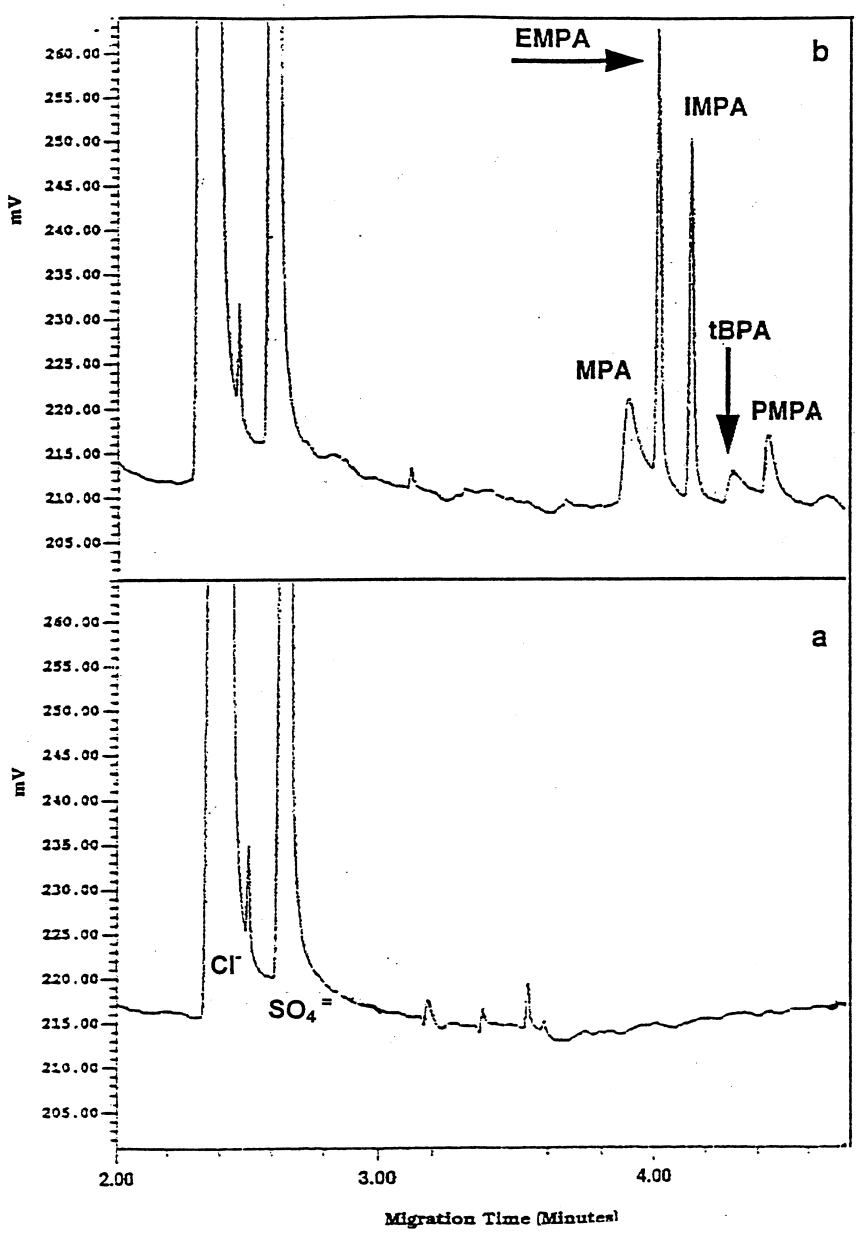

Fig. 3. Electropherograms of spring water blank (a) and spring water spiked at the 10 μg/ml concentration level (b).

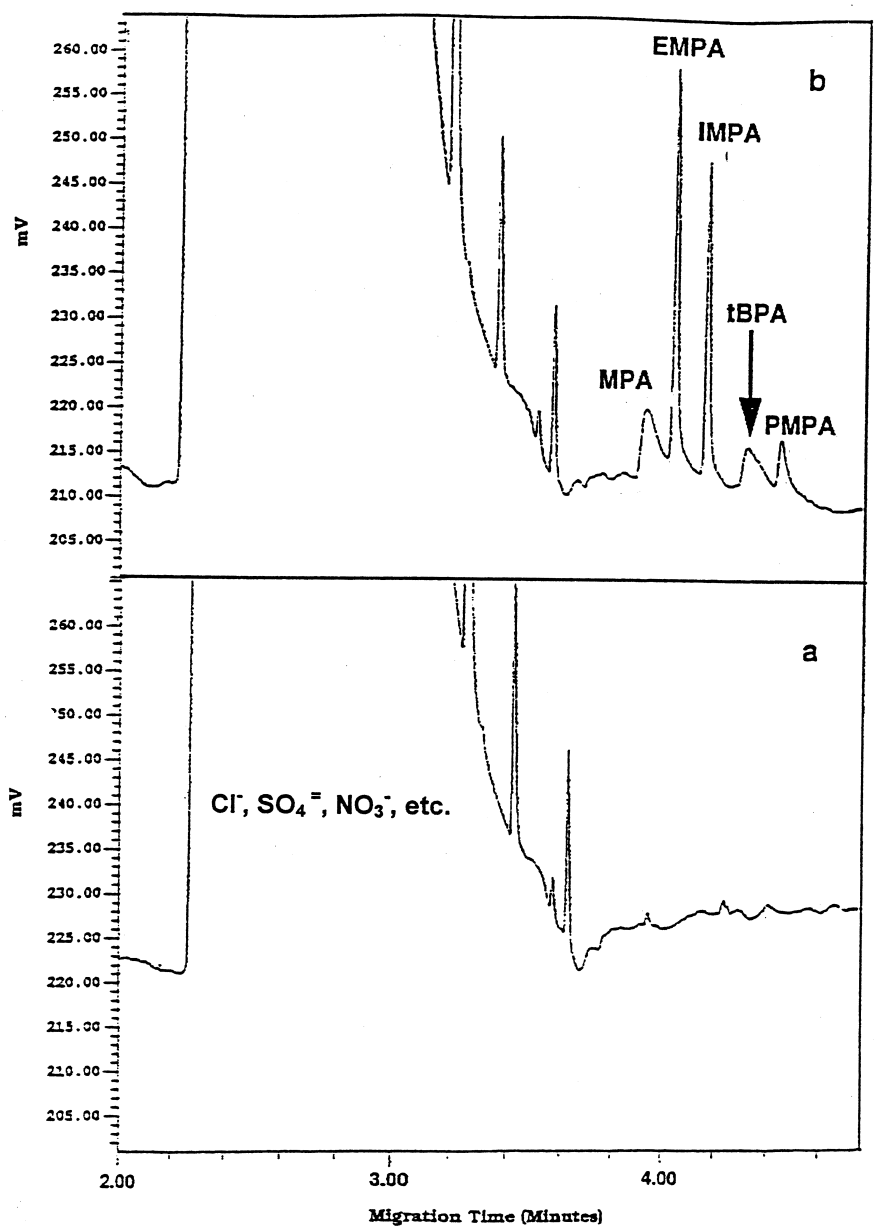

Fig. 4. Electropherograms of Baltimore Harbor water blank (a) and Baltimore Harbor water spiked at the 10 μg/ml concentration level (b).

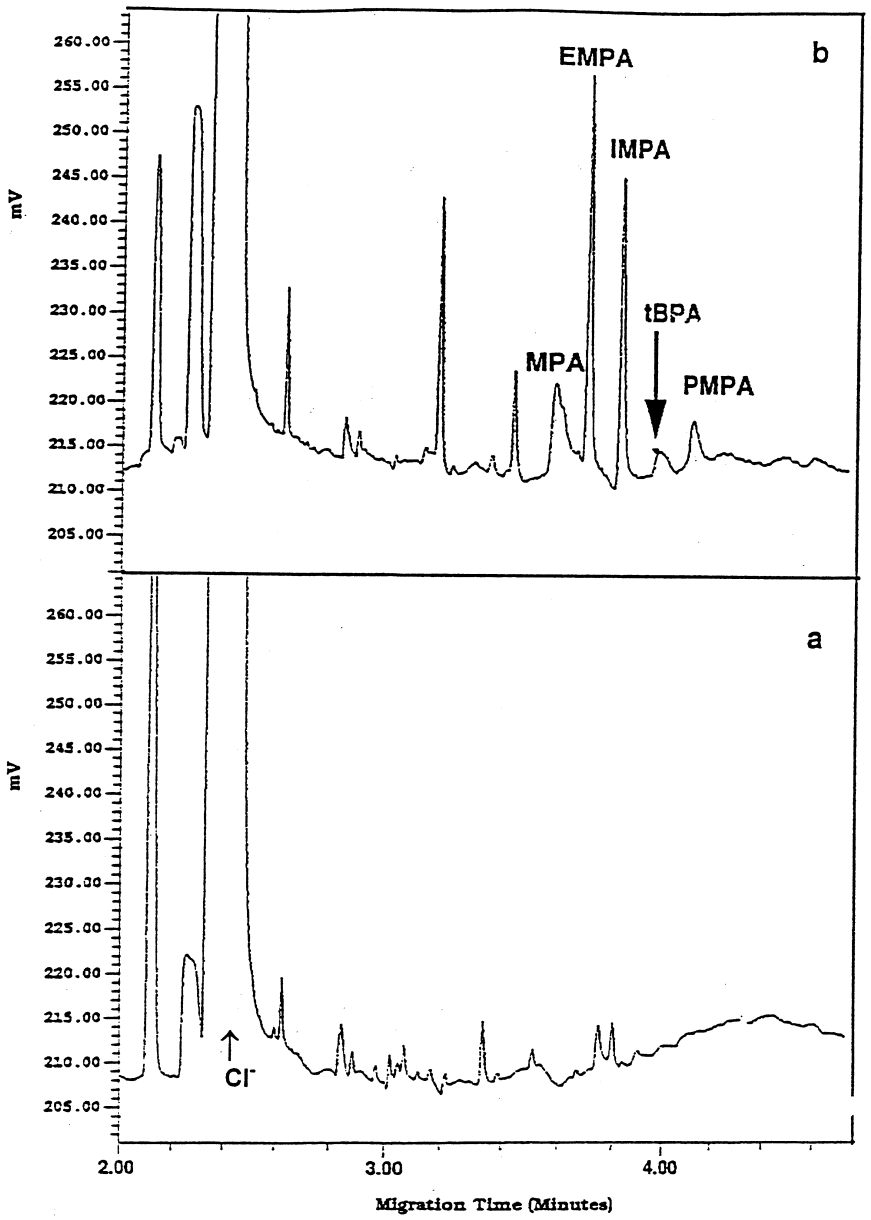

Fig. 5. Electropherograms of a sandy loam extract blank (a) and a sandy loam extract spiked at the 10 μg/ml concentration level (b).

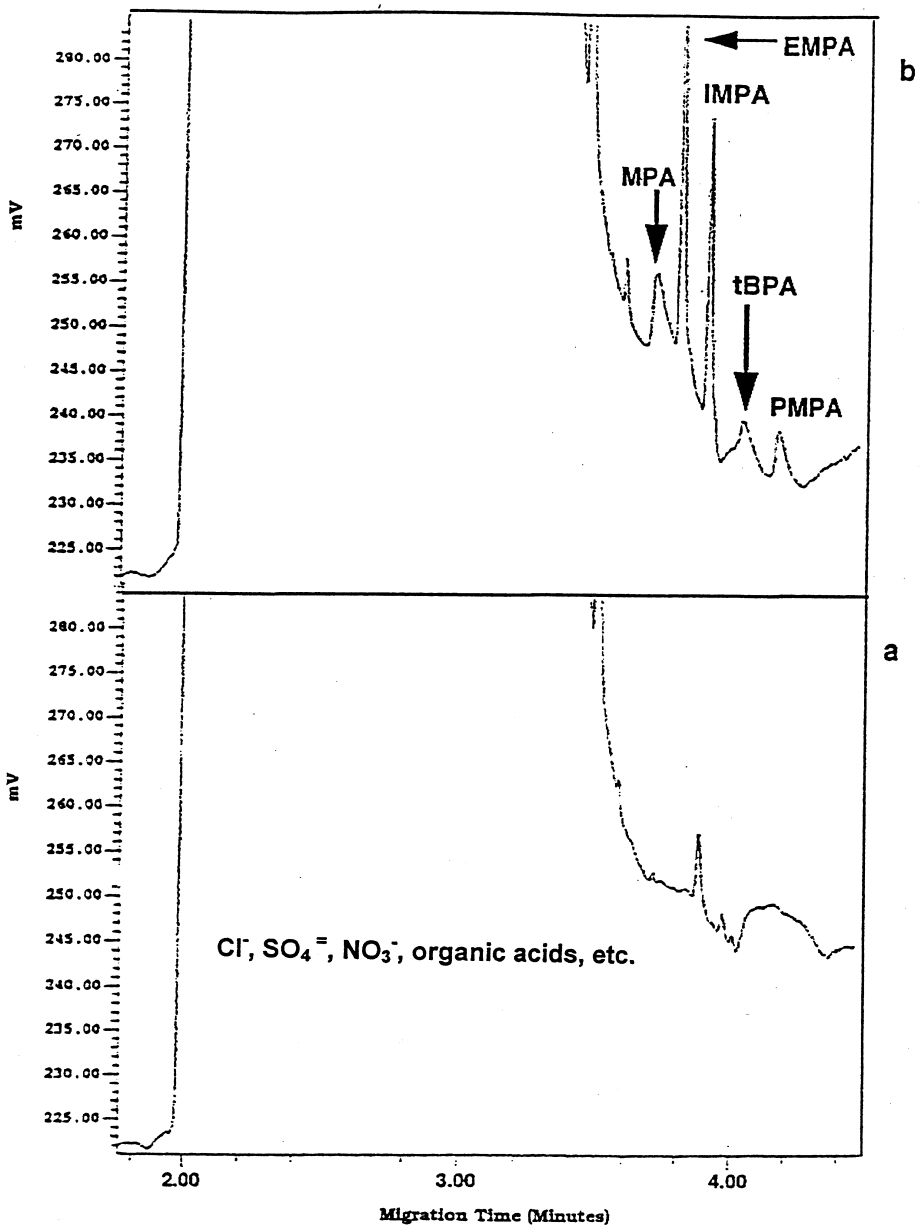

Fig. 6. Electropherograms of a sandy clay–loam extract blank (a) and a sandy clay–loam extract spiked at the 10 μg/ml concentration level (b).

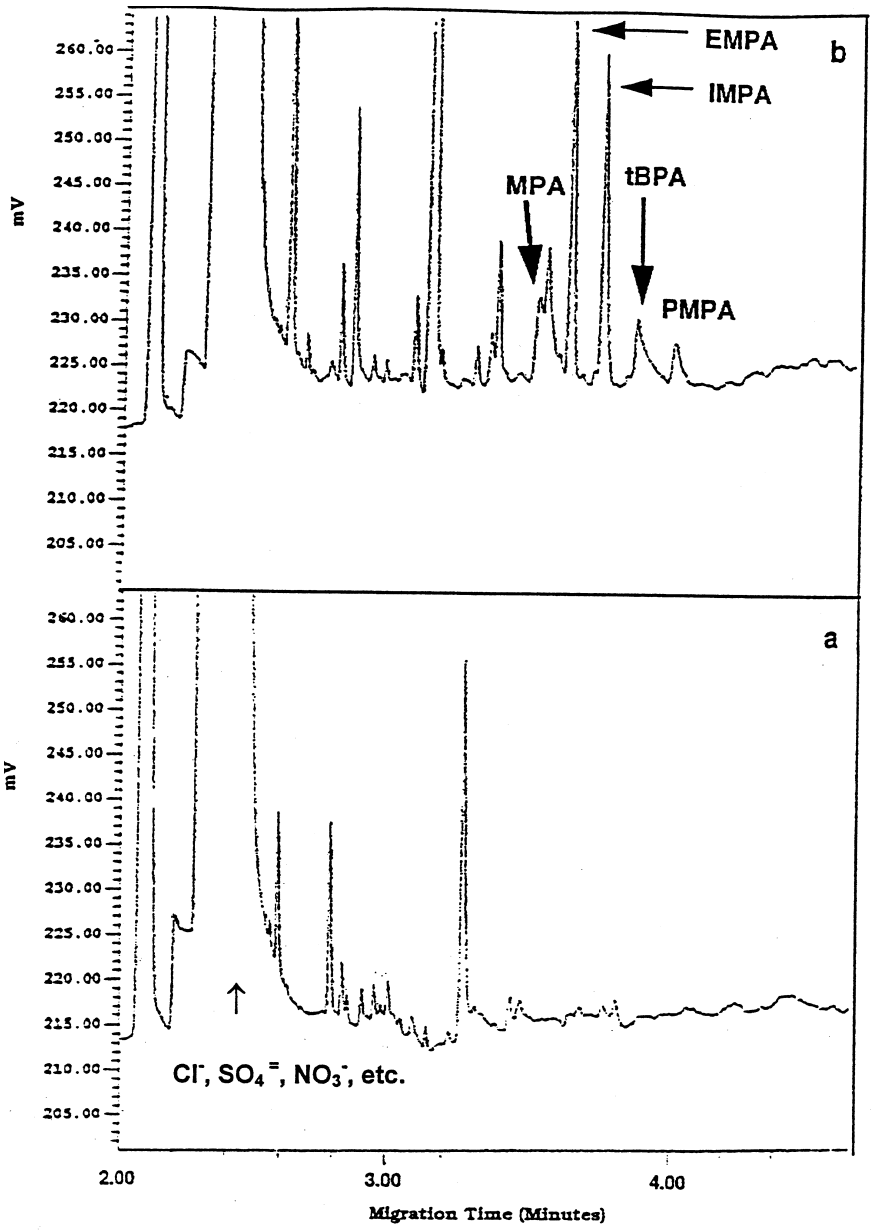

Fig. 7. Electropherograms of a loam extract blank (a) and a loam extract spiked at the 10 μg/ml concentration level (b).

extract (Figs. 6 and 7) appear to be due, in part, to high concentrations of organic acids in the soil matrix.

5. Conclusion

CIE with conductivity detection serves as a useful screening method for the separation of G and V type chemical warfare related compounds. The method is rapid and sensitive (CWC requirement of 10 μg/ml) and requires little or no sample preparation, uses little sample volume and generates minimal waste.

Acknowledgements

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