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**QUANTITATIVE METHOD FOR THE DETECTION
OF TRIETHYL PHOSPHATE IN AQUEOUS SOLUTIONS**

by
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14. ABSTRACT A gas chromatography (GC) method has been developed to detect and quantify triethyl phosphate (TEP) at the parts per million level in aqueous solution. TEP was used as a chemical agent simulant to evaluate the penetration characteristics of clothing, gloves, boots and items of personal equipment. The analytical method, initially developed in 1981, combines gas chromatography (GC), gas chromatograph-mass spectrometry (GC-MS) and Fourier-Transform infrared (FTIR) techniques. Quality Assurance and Quality Control (QA/AC) procedures were built into the analytical method to ensure the integrity and reproducibility of the analytical results. The TEP was analyzed directly from aqueous samples without the need for sample extraction or concentration procedures.					
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Preface

The development of an analytical method for the quantitative analysis of aqueous solutions of triethyl phosphate was initiated in 1981 to assist in the evaluation of chemical agent penetration characteristics of clothing, gloves, boots and items of personal equipment provided to the individual soldier. The chemical agent penetration characteristics of materials were evaluated using the simulant triethyl phosphate (TEP). The TEP was analyzed directly from aqueous samples without the need for sample extraction or concentration procedures. Quality Assurance and Quality Control (QA/QC) procedures were built into the analytical method to ensure the integrity and reproducibility of the analytical method. The initial analytical method developed in 1981 combined gas chromatography with flame ionization detectors (GC-FID) and Fourier Transform Infrared Spectrometry (FTIR). This method was modified to its current form in 1999 to incorporate fused silica capillary columns and the high sensitivity/selectivity of mass spectrometry to eliminate potential analytical interferences.

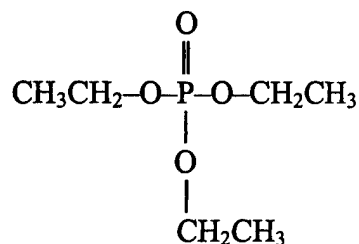
The research was conducted at the U. S. Army Soldier and Biological Chemical Command in Natick, MA during the period of October 1981 through March 2003 under program element number 622786 and project number AH98.

QUANTITATIVE METHOD FOR THE DETECTION OF TRIETHYL PHOSPHATE IN AQUEOUS SOLUTIONS

Introduction

The development and evaluation of materials designed for the protection of military personnel against chemical agents are essential to the Army's chemical protection programs. Materials such as clothing, gloves, boots and other items of individual protective equipment have been evaluated by subjecting them to penetration studies using the chemical agent simulant, triethyl phosphate (TEP). The resistance of a material to simulant penetration under various environmental conditions is indicative of its chemical protective properties. Consequently, the development of a sensitive, reproducible analytical method for the detection of TEP became necessary.

TEP is a colorless liquid whose empirical formula is $C_6H_{15}PO_4$ and whose structural formula is represented as follows:



M. W. = 182.16

TEP is readily soluble in water (Weast, R., 1972). Because of the phosphorus-oxygen structure, TEP is structurally similar to phosphorus-containing chemical agents and yet not considered a serious health hazard.

The method, initially developed in 1981, combined gas chromatography with flame ionization detection (FID) and Fourier Transform Infrared (FTIR) spectrometry. The gas chromatographic method provided a quantitative range of 0.5 parts per million to 1000 parts per million using packed, stainless steel gas chromatographic columns. Typically, FTIR methods were limited to confirmation for the presence of TEP; however, in instances where the concentration exceeded 500 parts per million, the samples can be analyzed directly by GC-FTIR as seen on preliminary runs. FID is more sensitive than FTIR. The analytical samples were virtually free of interferences to the extent that packed columns and FTIR provided the sensitivity and selectivity necessary to complete these studies. The absence of interferences also

allowed the samples to be analyzed without further intermediate preparation, purification or extraction procedures.

This method was modified in 1999 to accommodate fused silica capillary gas chromatographic columns and a mass spectrometer (MS) replacing the flame ionization detector and Fourier Transform Infrared Spectrometer. The current method provides superior sensitivity and selectivity when compared to the original infrared spectrometer technique. As with the flame ionization detector (FID), the lower detection limit of the mass spectrometer is 0.5 parts per million or 500 parts per billion. Whereas the FID method used retention times for the identification of the component peaks, MS uses molecular weight information extracted from each peak. The mass spectrometer was typically operated in the Selected Ion Monitoring (SIM) mode using the m/z 155 ion to quantify the triethyl phosphate concentration and the m/z 99, 127 and 109 fragments as confirmation ions for the presence of TEP.

The method was successfully applied to the analysis of TEP from samples obtained from penetration studies on military garments and fabric materials and also from sample penetration studies of butyl rubber and other polymeric materials used to fabricate protective gloves and boots.

Materials and Methods

Standards

TEP was obtained from the *Eastman Kodak*® Company and used without further purification. A series of standards (parts per million in water based on weight) were prepared and used for multilevel initial calibration.

Samples

The typical number of garment and fabric penetration samples evaluated varied from 6 to 33 for each individual analytical study. The number of butyl rubber penetration samples typically ranged from 14 to 30.

Quality Assurance/Quality Control (QA/QC)

Laboratory Control Samples (LCS) and Field Blanks containing water and no analyte were used to ensure that samples were free of cross-contamination. Any blank sample showing a positive concentration resulted in the qualification of an analytical sample if the analytical sample presented a concentration of less than 5 times the level observed in the blank. Data from samples whose concentrations were below 500 parts per billion were also reported, however their values were estimated.

Laboratory Duplicates were also used to measure the reproducibility of the analytical method. Duplicates that exhibited a deviation of greater than 25% were qualified and the analytical instrumentation was subjected to cleaning and maintenance.

An Initial Calibration was conducted prior to each analytical study. The initial calibration consisted of measuring TEP at five different concentration levels and determining the correlation coefficient for these standards. A Continuing Calibration consisting of one standard from the Initial Calibration was evaluated every 8 hours during the analytical study to ensure that instrumental variations would not affect the accuracy of the test results.

No Matrix Spike or Matrix Spike Duplicates was used in the QA/QC procedure because the aqueous matrix did not adversely interact with the TEP.

Gas Chromatography -Flame Ionization Detector

The method for TEP was first developed on a *Perkin-Elmer* Gas Chromatograph, Model Sigma 3B equipped with a flame ionization detector. A 12 foot long, 1/8 inch O. D. stainless steel column packed with 10% SP-2250 on 100/120 mesh *Supelcoport*® was used in the GC for component separation. Helium was used as the carrier gas and the flow rate was set at 40mLs per minute.

The oven temperature was maintained isothermally at 185°C throughout each run. The injector and detector temperatures were set at 250°C.

Peak heights and retention times for the standards and samples were measured with a Model 385 linear strip chart recorder and a *Hewlett-Packard* Model 3390A reporting integrator. The concentration of TEP in the samples was determined by comparing the response factors of the standard peaks in the multilevel initial calibration to the detector response of the sample peaks.

Due to the large number of samples generated, a *Hewlett-Packard* Automatic Sampler (Model 7672A) was used to automate the sample analysis procedure.

Figure 1 shows plots of standard concentration versus peak heights for three concentration ranges. Figure 2 shows chromatograms from the analysis of a standard and the analytical results for several fabric and butyl rubber glove penetration studies.

Gas Chromatography-Fourier Transform Infrared Spectrometry

A small amount (0.4 uL) of the 1000 parts per million standard TEP was injected into the *Hewlett-Packard* gas chromatograph interfaced with a *Nicolet* Fourier Transform Infrared Spectrometer Model 20SXB. The FTIR produced a reconstructed chromatogram for each peak that was eluted from the gas chromatograph. A search of the Environmental Protection Agency (EPA) Vapor Phase Infrared (IR) Spectral Library (Azarraga, Leo and Associates, 1984) was used to confirm the presence of TEP. See Figure 3 for an example of an FTIR reconstructed chromatogram of the TEP standard and the FTIR spectrum for that peak. Figure 4 contains the spectrum of the standard as well as the four close matches identified by the EPA Vapor Phase IR Spectral Library (Azarraga, Leo and Associates, 1984).

When 0.4 uL of a penetration test sample was injected into the GC-FTIR, two peaks were typically observed on the reconstructed chromatogram as shown by Figure 5. Figure 6 shows the

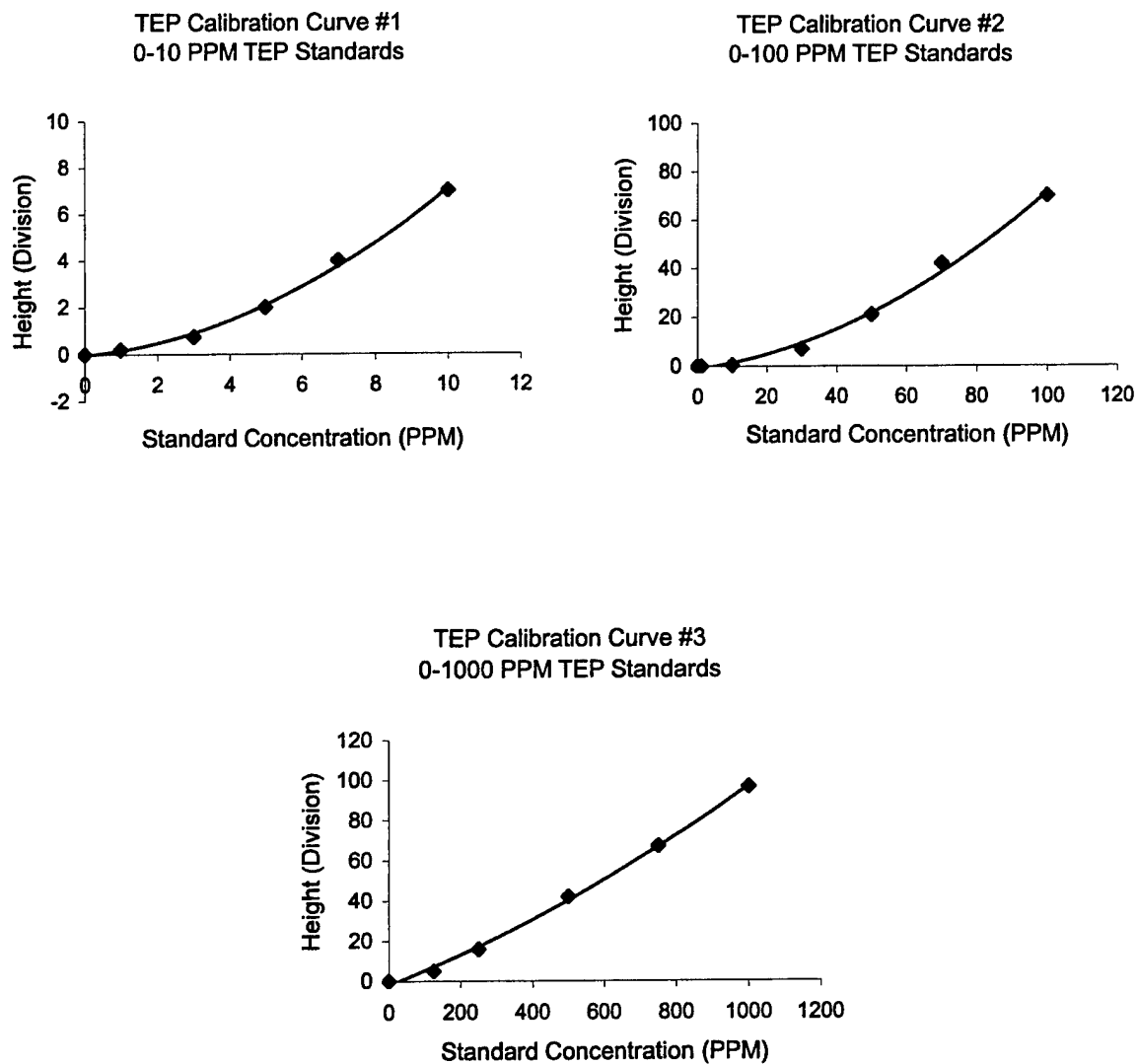
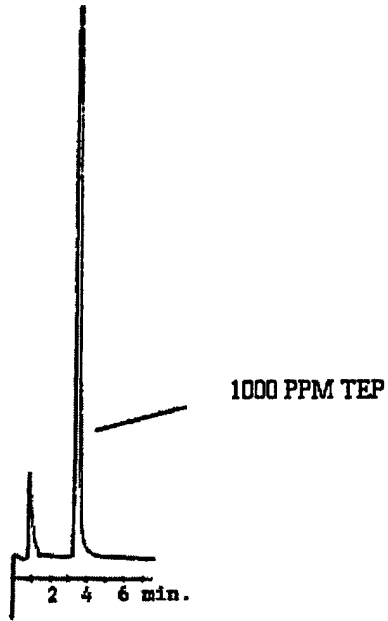
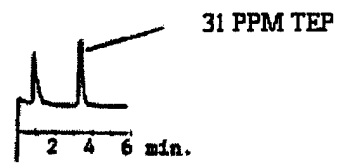


Figure 1. TEP Calibration Curves with Three Concentration Ranges from GC-FID Method

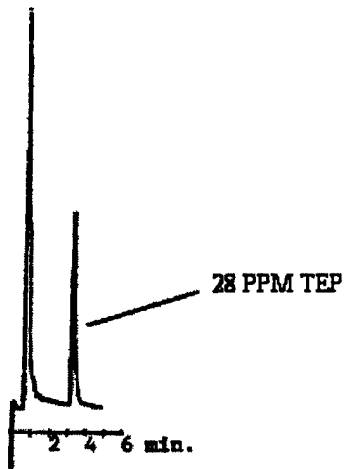
TEP Standard



Fabric Penetration Sample



TEP Standard



Rubber Penetration Sample

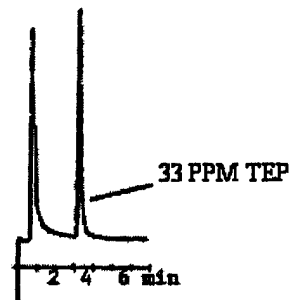
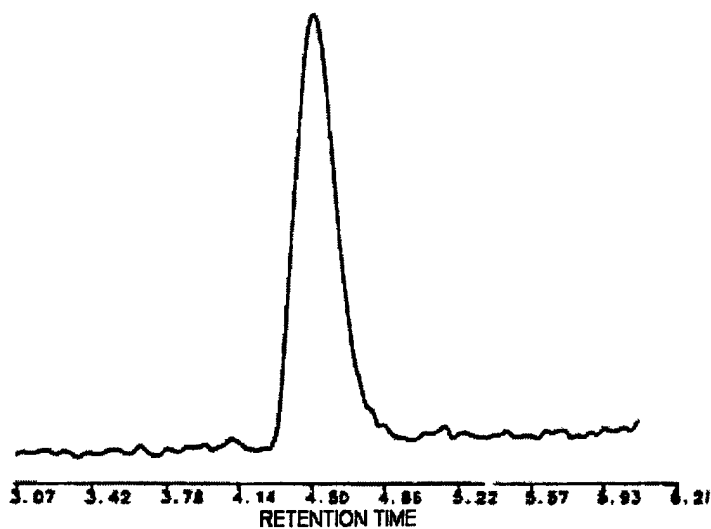


Figure 2. Gas Chromatograms of TEP Standards and Samples

FTIR Reconstructed TEP Standard Chromatogram



TEP Standard FTIR Spectrum

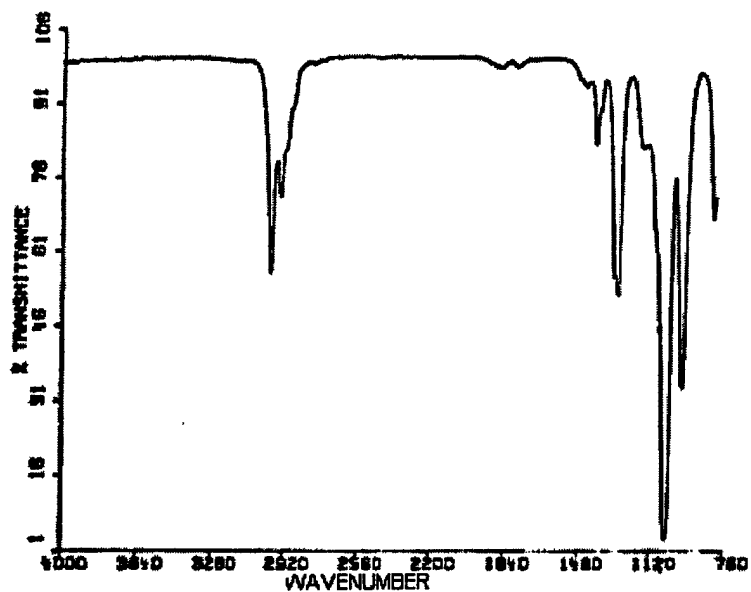


Figure 3. FTIR Reconstructed TEP Standard Chromatogram and TEP Standard FTIR Spectrum

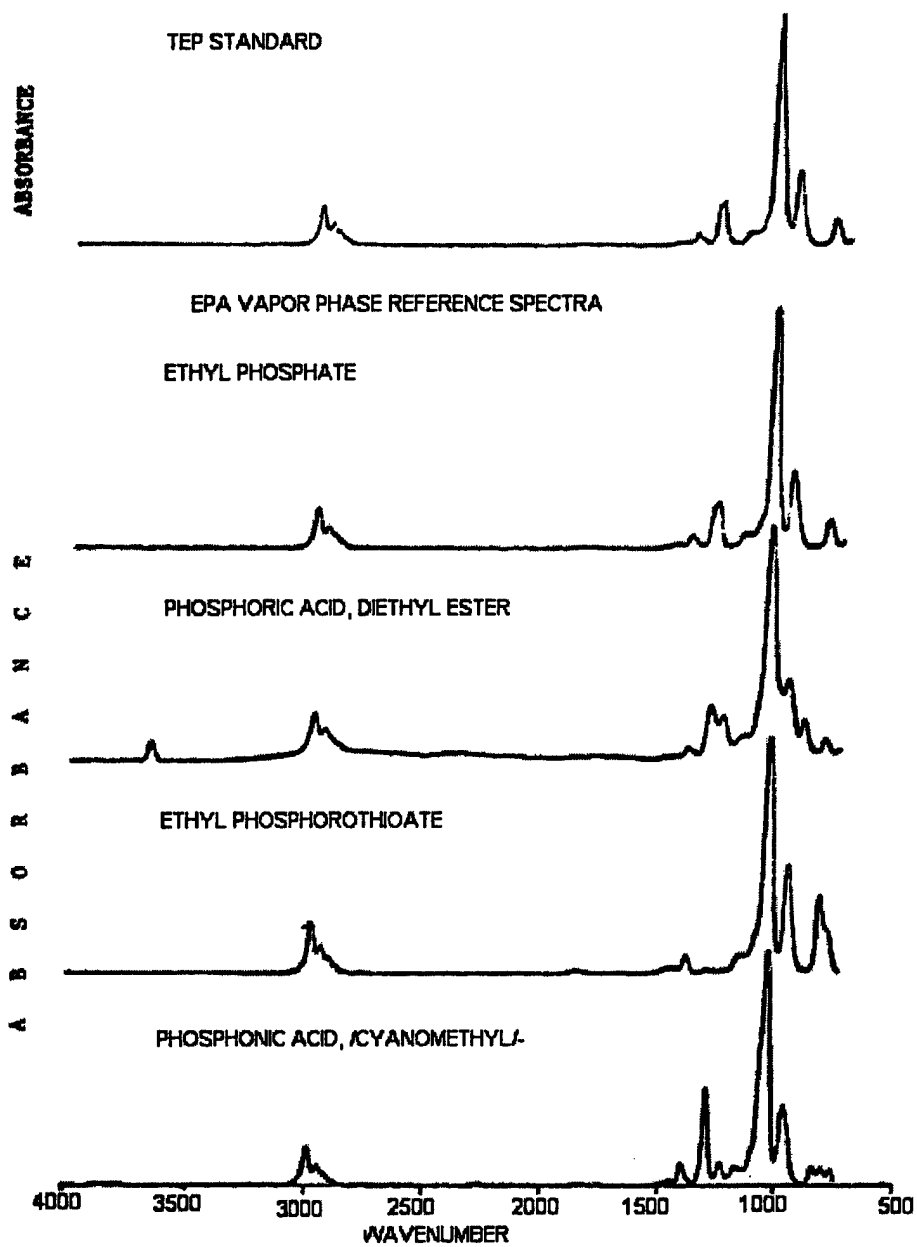


Figure 4. FTIR Spectra - TEP Standard and EPA Library Matches

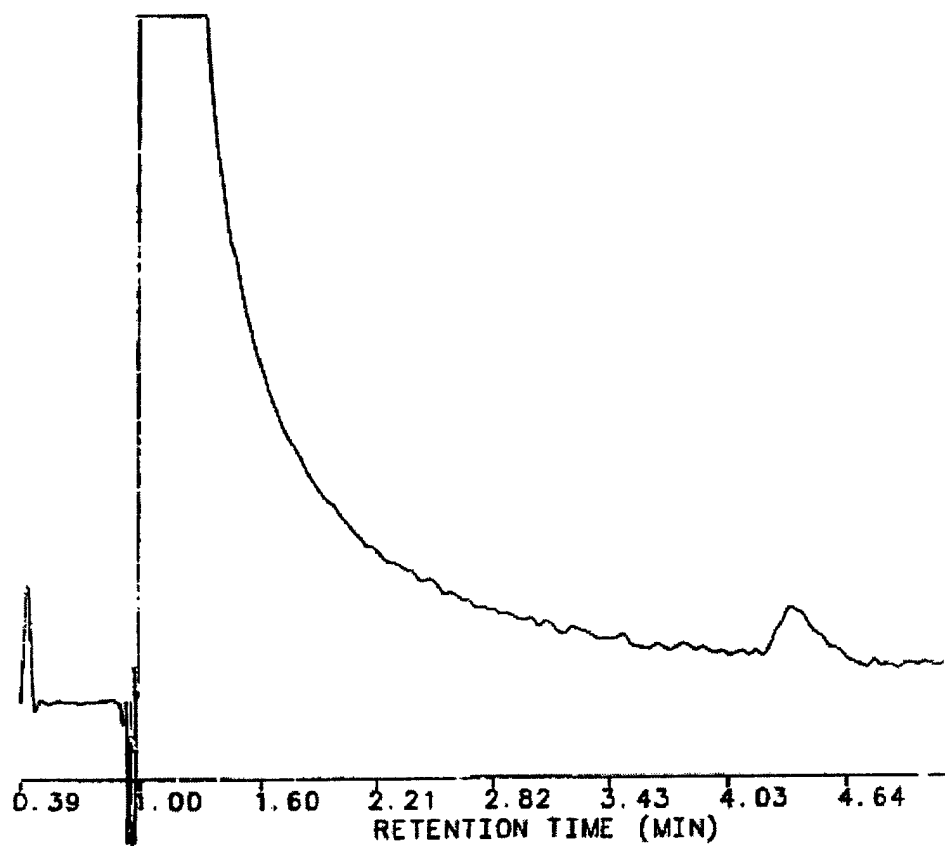


Figure 5. FTIR Reconstructed Sample #9 Chromatogram

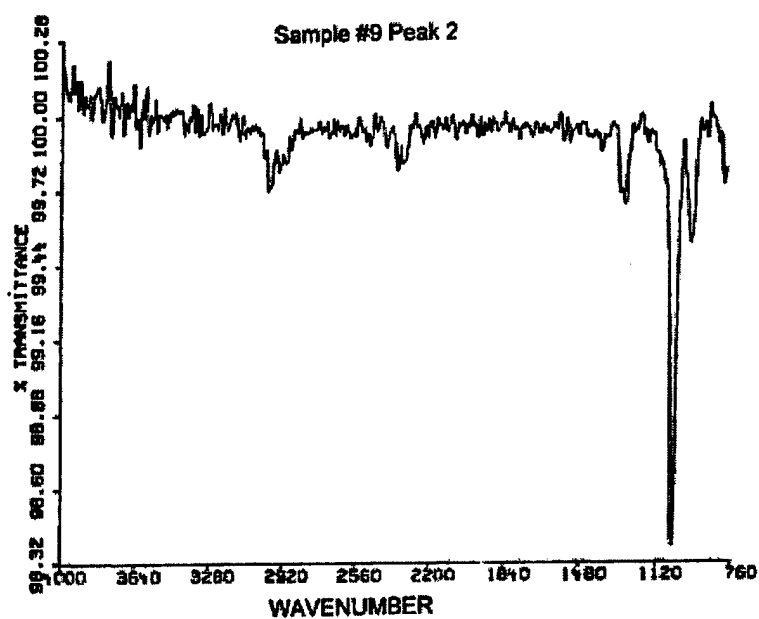
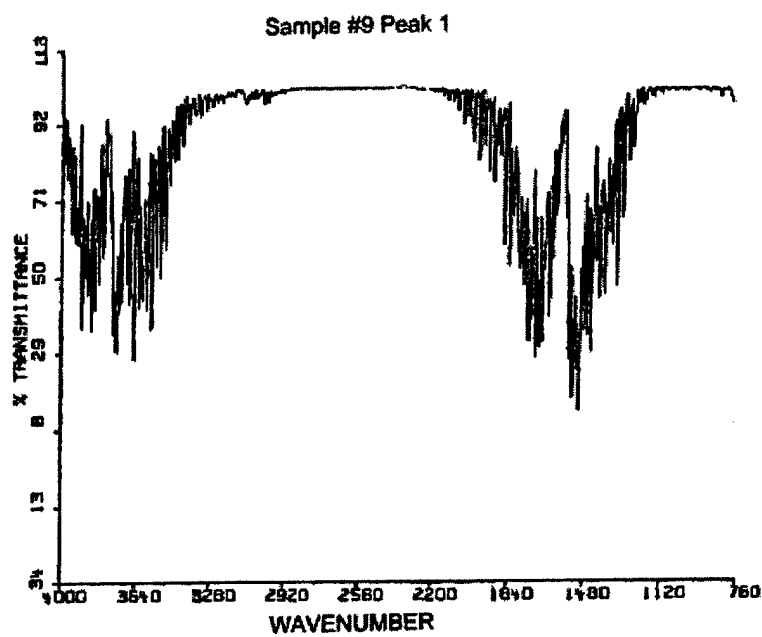


Figure 6. FTIR Spectra - Sample #9 Peaks 1 and 2

spectrum of the first peak whose FTIR spectral characteristics are consistent with the aqueous support media. Figure 6 also shows the spectrum of the second peak whose FTIR spectral characteristics are consistent with that of TEP. The identification of both peaks was confirmed by the spectral search of the EPA Vapor Phase IR Spectral Library. Figure 7 shows the spectra of the first peak and the four close matches from the spectral library, and Figure 8 shows those of the second peak and the four close matches from the same spectral library.

Variations in the retention times of the TEP peak on the GC chromatogram (Figure 2) and that on the FTIR reconstructed chromatogram (Figure 3) were the result of minor changes in the chromatographic conditions between the GC-FID technique and the GC-FTIR analytical method.

Gas Chromatography-Mass Spectrometry

A modified method was developed using a Model 6890N Gas Chromatograph, a Model 7683 Automatic Injector, and a Model 5893N Mass Spectrometer all manufactured by *Agilent Technologies, Inc.* A cross-linked fused silica capillary column 30 m long, 0.25 mm ID, and coated with a 0.25 μm film of 5%-phenyl-methylpolysiloxane was used in place of the previously described packed column for separating TEP from the other components in the sample. The flow rate of the helium carrier gas was 1.3 mLs per minute. The front inlet was split with a 1:1 ratio and its temperature was maintained at 130^o C while the MS detector's temperature was set at 280^o C.

Oven temperature was initially maintained at 100^o C for 8 minutes, then temperature programmed to a final temperature of 200^o C at a rate of 24^o per minute. The oven was held at the final temperature for 4 minutes. A chromatogram derived from the signal intensity of the m/z 155 fragment of TEP was developed and the presence of TEP was confirmed by a chromatographic overlap of the m/z 99, 127 and 109 fragment ions. At the beginning of each run, an initial calibration consisting of a multilevel standard was evaluated by GC-MS and the peak area for each standard was calculated. A standard curve was developed which correlated the response factor from each of the standards to the known concentration of the standards. Under these analytical conditions, the lower detection limit for this method was set at 0.5 part per million or 500 parts per billion.

Figure 9 shows several example plots of standard concentration versus response over several concentration ranges from the GC-MS method. Figure 10 shows an extracted ion chromatogram of a TEP standard; Figure 11 shows the mass spectrum of TEP (Stein, Levitsky, Fateev, Tchekhovski and Mallard, 2000). Figures 12 and 13 are an example of a TEP permeation study sample with the extracted ion chromatogram and mass spectrum, respectively (Stein, Levitsky, Fateev, Tchekhovski and Mallard, 2000).

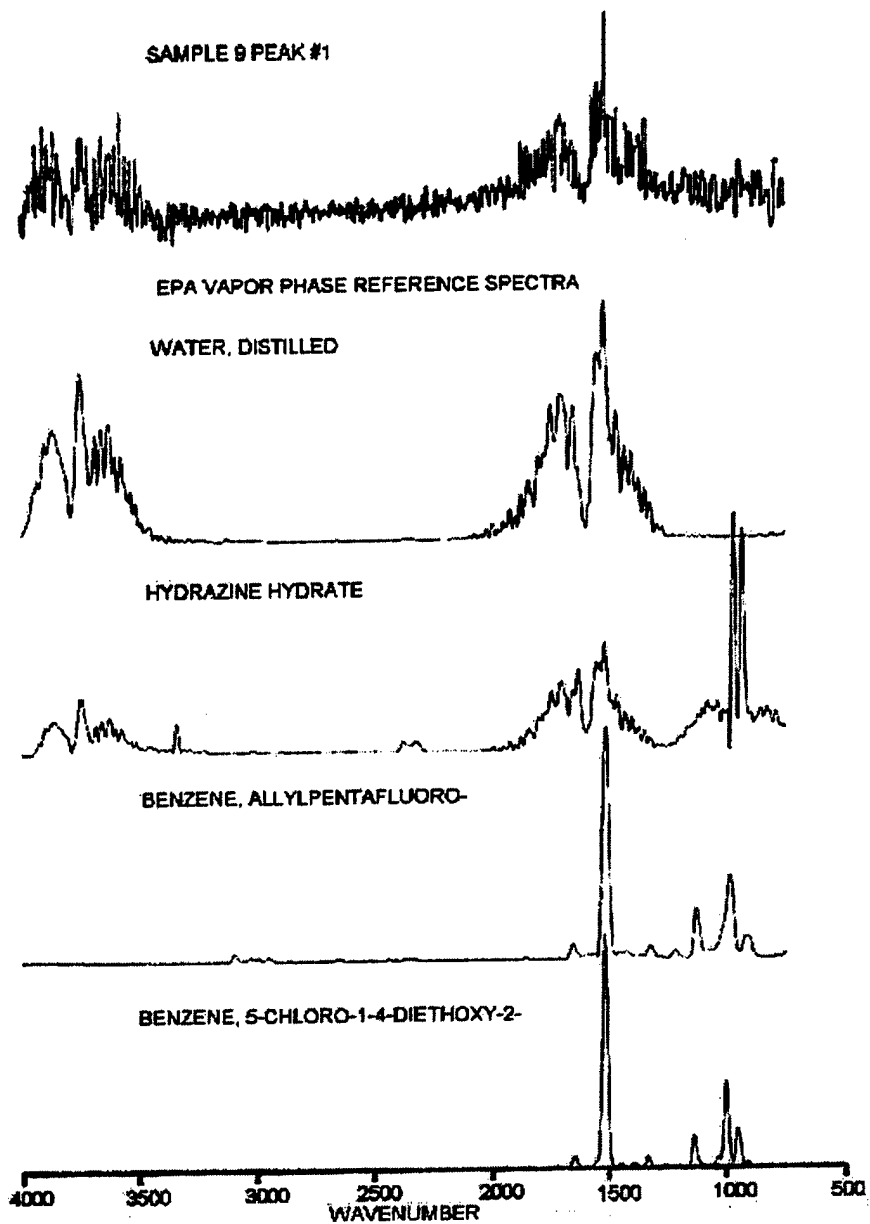


Figure 7. FTIR Spectra - Sample #9 Peak 1 and EPA Library Matches

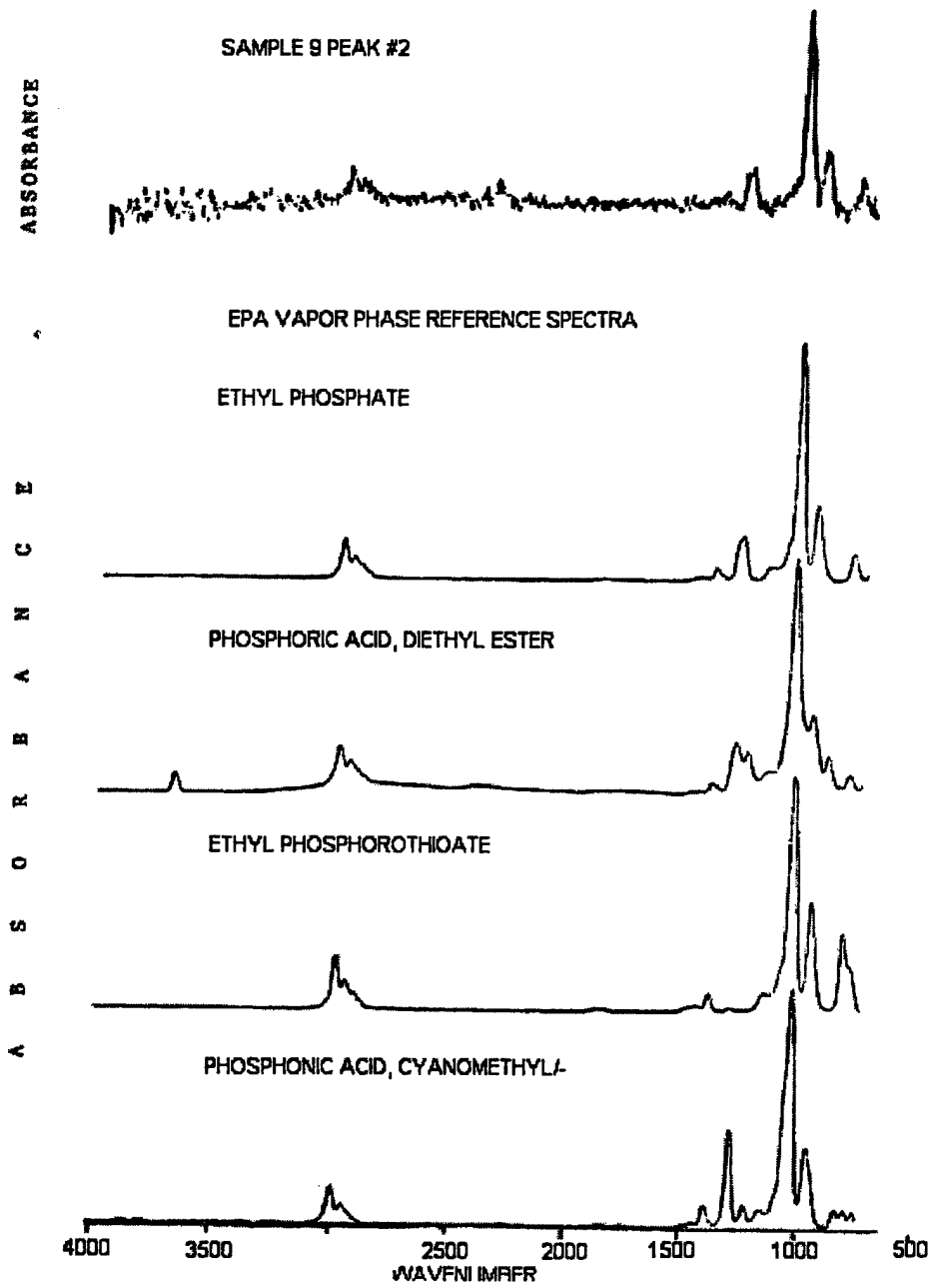


Figure 8. FTIR Spectra – Sample #9 Peak 2 and EPA Library Matches

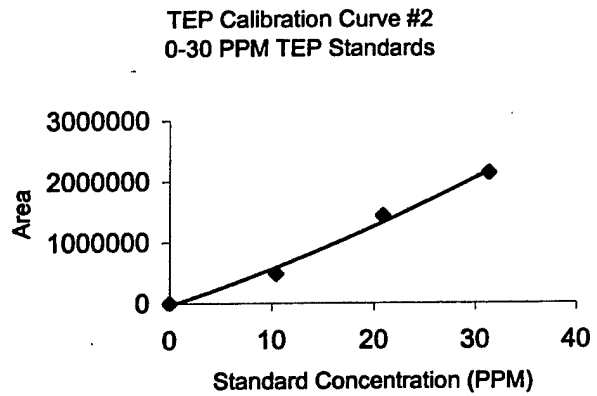
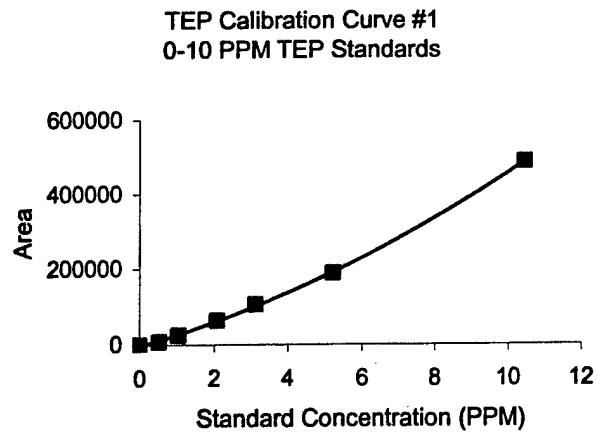


Figure 9. TEP Calibration Curves with Two Concentration Ranges from GC-MS Method

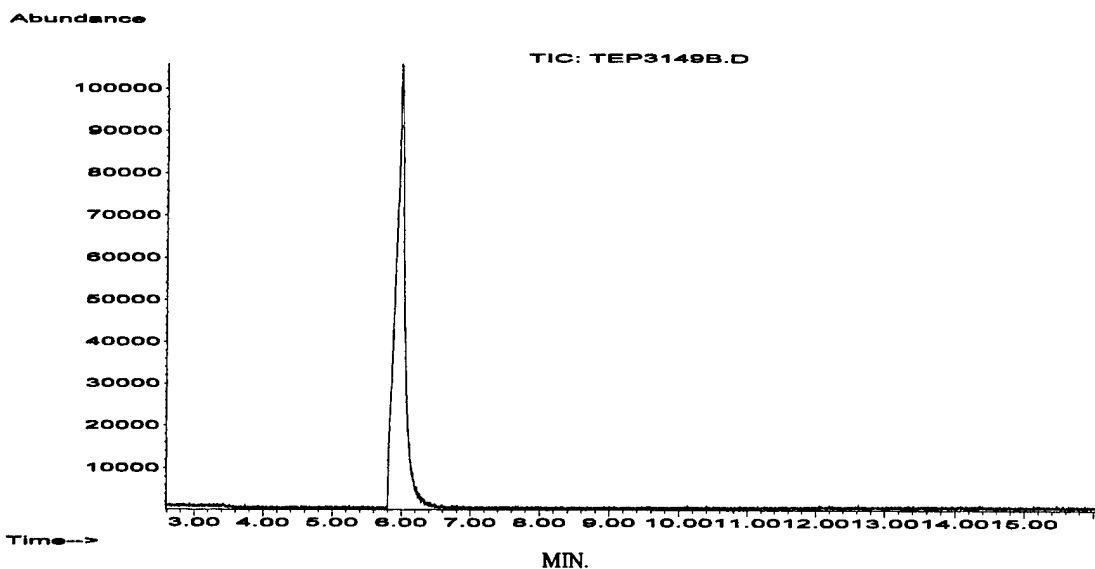


Figure 10. Extracted Ion Chromatogram of TEP Standard

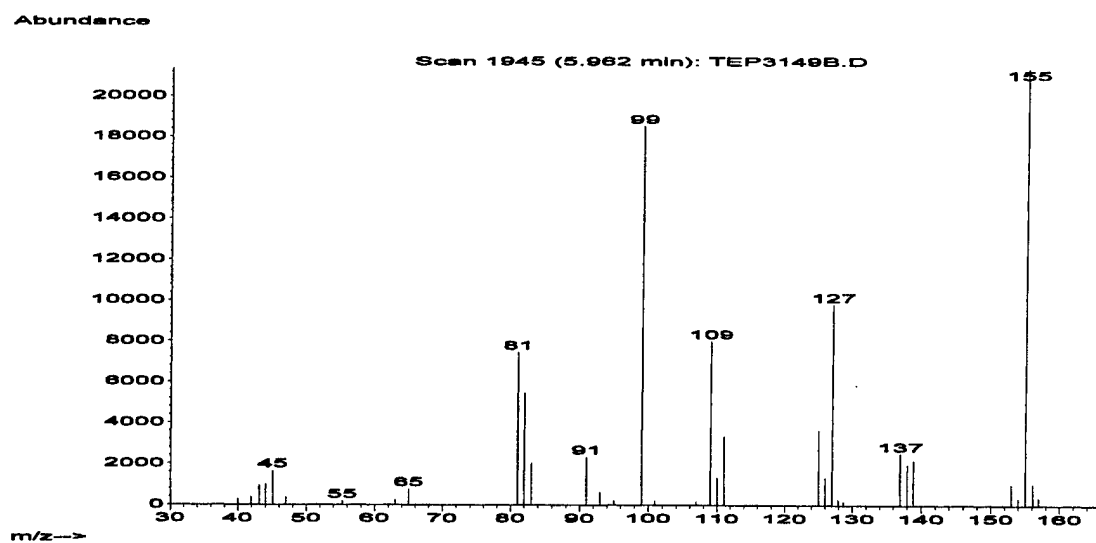


Figure 11. Mass Spectrum of TEP Standard Peak

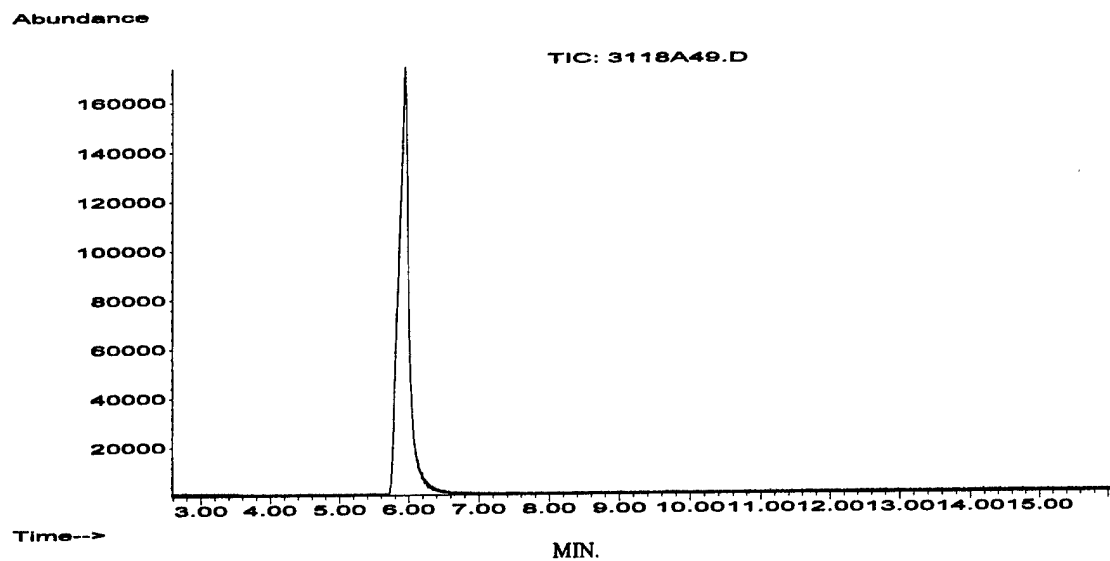


Figure 12. Extracted Ion Chromatogram of TEP Sample

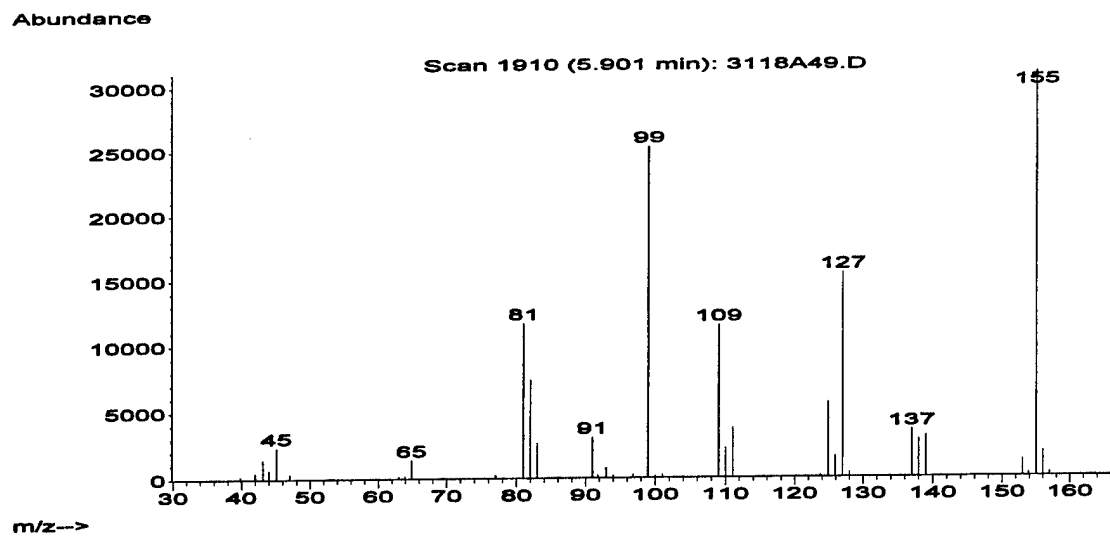


Figure 13. Mass Spectrum of TEP Sample Peak

Results

Table 1 shows the concentrations of TEP from the fabric penetration samples. Table 2 shows those from butyl rubber glove samples. Tables 3 and 4 show concentrations of TEP from the garment penetration samples of a recently analyzed sample lot.

Table 1. *TEP Concentrations in Samples from Fabric Penetration Studies*

Sample Number	TEP (ppm)	Sample Number	TEP (ppm)
791	30	825	4
792	5	826	0
793	37	828	0
794	82	829	0
807	0	830	0
811	1	831	0
815	0	832A	0
816	18	832B	8
817A	2	833	13
817B	4	835	4
818	0	836	1
819	0	838	0
820	0	839	0
821	0	840	0
822	0	841	0
823	2	842	0
824	2		

Table 2. *TEP Concentrations in Samples from Butyl Rubber Penetration Studies*

Sample Number	TEP (ppm)	Sample Number	TEP (ppm)
4A	0	4N	124
4B	0	4O	249
4C	0	4P	455
4D	0	4Q	755
4E	0	4R	>1000
4F	0	4S	>1000
4G	0	4T	>1000
4H	0	4U	>1000
4I	0	4V	>1000
4J	0	4W	>1000
4K	1	4X	>1000
4L	2	4Y	>1000
4M	33	4Z	>1000

Table 3. *TEP Concentrations of Samples from Garment Penetration Studies*

Sample Number	TEP (ppm)	Sample Number	TEP (ppm)
6-1	11	8-1	0
6-2	0	8-2	13
6-3	1E	8-3	2E
6-4	2	8-4	3
6-5	0	8-5	44
6-6	68	8-6	17
6-7	0	8-7	0
6-8	0	8-8	5
6-9	174	8-9	0
6-10	53	8-10	0
6-11	0	8-11	0
6-12	0	8-12	0
6-13	0	8-13	0
6-14	0	8-14	0
6-15	0	8-15	0
6-16	1E	8-16	0

NOTE: E = Result is estimated when it is lower than or equal to 5X the concentration detected in the blank.

Table 4. *TEP Concentrations of Samples from Garment Penetration Studies*

Sample Number	TEP (ppm)	Sample Number	TEP (ppm)
7105-9	0*	4116-9	0
5114-9	30	1114-9	70
3118-9	500	7108-9	10
7101-3	0	4124-9	0
4126-9	390	4102-9	50
1101-3	3	5126-3	0
7101-13	0*	3117-3	>1000
2103-9	80	7101-09	0*
5113-3	30	1118-9	0
4116-3	530	2113-3	90
3105-3	360	2118-3	70
1126-9	10	3105-9	450
1118-9	10	4117-9	0
7113-13	0*	5114-3	0
1101-9	0		
2118-9	70		

* - The blotter paper did not get wet.

Conclusions

The GC analytical method for TEP was found to provide the sensitivity, selectivity and reliability to accurately monitor and evaluate the penetration characteristics of the chemical agent simulant TEP through military fabrics and butyl rubber protective items. The method is simple and direct requiring no sample preparation. Analysis time per sample was sufficiently fast to allow the analytical method to be automated. The initial studies involving the FTIR spectrometer interfaced with the gas chromatograph qualitatively confirmed the presence of TEP. In addition, the FTIR provided a means to observe the presence of potential interferences possibly generated by TEP degradation or matrix interactions.

Studies indicated that the water used as solvent for TEP reacted with the packing materials inside the chromatographic column when the column was operated at constant temperature of 185°C. Therefore, to ensure the integrity of the column and to verify the validity of the results, several standards were run before and after analysis of each group of samples in support of QA/QC procedures.

When the analytical method was converted from packed columns to fused silica capillary columns with bonded phases the problem with water degradation of the chromatographic packing materials was eliminated but the column integrity is still evaluated as part of the QA/QC process. The mass spectrometer provides increased sensitivity and a larger analytical range than was observed with the GC-FTIR method. The presence of TEP in the GC-MS runs are easily confirmed by retention time as well as characteristic fragment ions in the mass spectrum.

This document reports research undertaken at the U.S. Army Soldier and Biological Chemical Command, Soldier Systems Center, Natick, MA, and has been assigned No. NATICK/TR-041002 in a series of reports approved for publication.

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