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A Method for the Analysis of Tabun in Multisol Using Gas Chromatographic Flame Photometric Detection

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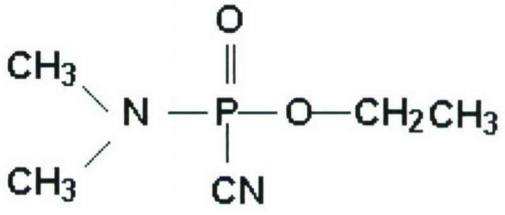
Preparation and analysis of tabun (GA) solutions are necessary for the continued development of countermeasures to this nerve agent. GA solutions must be stable and compatible for use in the test systems chosen for study; however, GA is very unstable in saline solutions. In the past we have found GA in saline at 2 mg/mL to be stable for a month or less at −70°C, whereas saline solutions of sarin (GB), soman (GD), and cyclosarin (GF) were stable for many months. Previous studies have shown that Multisol (48.5% H_2O, 40% propylene glycol, 10% ethanol, and 1.5% benzyl alcohol) provides stable solutions of GA. We confirmed the stability of GA in Multisol with phosphorus nuclear magnetic resonance (P—NMR) and developed a method for the analysis of GA in Multisol using gas chromatographic flame photometric detection (GCFPD) in the phosphorus mode. The GC method used acetonitrile (CH_3CN) for a dilution solvent because of its miscibility with GA in chloroform ($CHCl_3$) standards and GA in Multisol samples at 1% (v/v). Furthermore, the dilutions with CH_3CN made the phosphorus mode interference peak present in $CHCl_3$ analytically manageable, reduced the interferences of Multisol in the GC separation, and contributed to a safe and reliable analysis of GA at 20 μg/mL. We demonstrated the stability of GA in Multisol stored for more than a year at 70°C. This method contributes a suitable technique for the preparation and analysis of reliable solutions of GA in nerve agent medical research and demonstrates the extended stability of GA in Multisol.

Keywords Flame Photometric Detector, GA, Gas Chromatography, Multisol, Nuclear Magnetic Resonance, Tabun

INTRODUCTION

Tabun (GA) is a chemical warfare agent against which the United States is actively developing countermeasures. GA was developed by Schrader in 1936 as a pesticide and became one of the German (G) nerve agents.

GA continues to be of interest (Cabal et al. 2004; Kassa and Krejcova 2003; Krejcova and Kassa 2003; Kassa and Vachek 2002) as a chemical warfare agent due to its suspected use by Iraq, its potential use as a terrorist threat agent, and its historical reference as one of the original G nerve agents. Furthermore, GA-induced toxic effects are difficult to counteract due to the very low reactivating efficacy of pralidoxime chloride (2-PAMCl), the oxime currently used by the United States as a nerve agent antidote (Koplovitz et al. 1995; Koplovitz and Stewart 1994). Security (Army Regulation 2001a) and safety (Army Regulation 2001b) regulations of the U.S. Army constrain dilute nerve agent research to solutions of 2 mg/mL or less. The stability of these solutions is a concern for researchers. The structure and chemical name of GA are shown below:



Dimethylphosphoramidocyanidic acid, ethyl ester

This structure is highly susceptible to hydrolysis at the —CN, $-OC_2H_5$, and $-N(CH_3)_2$ bonds to phosphorus. In neutral or near-neutral aqueous solutions, GA hyrolyzes rapidly at the P—CN bond, giving ethyl hydrogen N,N-dimethylphosphoroamidate as the major product (Chemical and Instrumental Verification of Organophosphorus Warfare Agents 1977). GA is unstable in aqueous solutions (Larson 1953, 1958; Holmstedt 1951; Desire and Saint-Andre 1986; Epstein et al. 1973), even when prepared and immediately stored at –70°C. We developed a program to monitor the stability of dilute solutions of nerve agent. Our guideline for solution stability is

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an analysis result that is within ±10% of the original gravimetric preparation concentration for that solution. It is our experience that saline solutions of sarin (GB), soman (GD), and cyclosarin (GF) remain stable for 6 months to a year at –70°C, while saline solutions of GA may be stable for 1 month at –70°C. Obviously, GA's instability is problematic when providing this agent in saline for the researcher. Therefore, an alternative solvent for GA was sought to replace saline in research investigating medical countermeasures to this toxic compound. A vehicle that we called Multisol has been used at this institute for years as a solvent for compounds that are insoluble in water. The composition of Multisol (48.5% H_2O, 40% propylene glycol, 10% ethanol, and 1.5% benzyl alcohol) is similar to the vehicle used in the commercial formulation of Valium for injection (Physicians' Desk Reference 2002). Multisol has been used to provide stable GA solutions (Joiner et al. 1989), and this four-component mixture was found to be an acceptable substitute for saline in subcutaneous injections of GA solutions.

Two instrumental methodologies, ^{31}P nuclear magnetic resonance (NMR) and gas chromatographic flame photometric detection (GCFPD), were used to demonstrate the stability of GA in Multisol. ^{31}P nuclear magnetic resonance (NMR) was used to compare the stability of GA in Multisol with the instability of GA in saline by monitoring the ^{31}P resonance of GA. The phosphorus mode GCFPD method was used to remove interferences from nonphosphorus components. The sensitivity of the phosphorus mode permitted the use of acetonitrile (CH_3CN) as a dilution solvent for GA in chloroform ($CHCl_3$) standards and GA in Multisol samples at 1% (v/v).

MATERIALS AND METHODS

Tabun (dimethylphosphoramidocyanidic acid, ethyl ester, GA) was obtained from the U.S. Army Edgewood Chemical Biological Center (ECBC), Aberdeen Proving Ground, MD; GA purity was 98.5% as determined by NMR spectroscopy. Deuterium oxide (D_2O) 99.9 atom % D and acetonitrile (CH_3CN), HPLC grade, were obtained from Aldrich Chemical Company, Milwaukee, WI. Absolute ethyl alcohol was obtained from Pharmco, Brookfield, CT. Propylene glycol was obtained from Phoenix Pharmaceutical Inc., St. Joseph, MO. Benzyl alcohol was obtained from Eastman Kodak, Rochester, NY. $CHCl_3$, pentene-stabilized HPLC grade, was obtained from Fisher Scientific Company, Pittsburgh, PA. Diisopropyl methanephosphonate (DIMP) was obtained from Lancaster Synthesis, Pelham, NH.

NMR measurements were made on GA in modified Multisol (48.5% D_2O, 40% propylene glycol, 10% ethanol, and 1.5% benzyl alcohol) and on GA in modified saline (D_2O containing 0.9 mg/mL NaCl). Solutions of GA were prepared gravimetrically at 2 mg/mL and stored at −70°C. Immediately upon thawing and mixing at room temperature, approximately 500 μL of the solution was transferred into a 5-mm o.d. NMR tube. All NMR data was collected on a Varian Unity Inova spectrometer equipped with 5 mm gradient Penta^R probe. Probe temperature was maintained at 25°C ± 0.1C and the VT air flow was maintained at 10 L per minute (LPM). Standard s2pul sequence with continuous proton decoupling [$^{31}P\{^1H\}$] was used and all the calibrations were done as per the installation procedure. Chemical shifts were reported in parts per million (ppm) using 85% phosphoric acid (H_3PO_4) as the external reference at −0.9 ppm.

Gas chromatographic analyses of GA in Multisol samples and GA in $CHCl_3$ standards were made after 1:100 dilution with CH_3CN containing 0.0056 mM DIMP as the internal standard. An HP 5890 series II Plus gas chromatograph/Flame photometric detector was used for detection of GA. The gas chromatograph was equipped with a 30 m, 0.25 mm i.d., 0.25 mm DB-5MS column (J&W Scientific, Folsom, USA). Initial temperature was 70°C for 2 min, and the temperature ramp rate was 30°C min^{-1} until the final temperature of 250°C was reached and held for 3 min. Samples were run in the split mode 20:1 with a helium carrier gas flow rate of 1 mL min^{-1} measured at 70°C.

RESULTS

P-31 NMR demonstrated the stability of GA in modified Multisol for 14 h compared with the instability of GA in modified saline for 5 h. GCFPD was used to quantify GA in Multisol samples against stable GA in chloroform standards.

Figure 1 shows the stability of GA in modified Multisol for 14 h at 25°C using ^{31}P NMR to monitor the GA and potential decomposition product resonances in a sample at 2 mg/mL. The (−10) ppm peak is the GA resonance that remains the major component of the 28 spectra (Chemical and Instrumental Verification of Organophosphorus Warfare Agents 1977). In addition, a small resonance at (−0.9) ppm can be seen early but remains a minor component. This result was a clear indication that GA in Multisol could be used for several hours at 25°C before the onset of decomposition.

Figure 2 demonstrates the instability of GA in saline, modified with D_2O, over a 5-h period at 25°C using 31-P NMR to monitor the GA and decomposition product resonances. The (−10) ppm peak is the GA resonance that decreases through the 11 spectra. When Figures 1 and 2 are compared, the instability of GA is readily apparent.

Figure 3 shows the chromatogram obtained for the analysis of a 20 ug/mL solution (1 uL injection slit 20/1) obtained from the dilution of GA in $CHCl_3$ with CH_3CN containing DIMP. Peak #1 is a chloroform impurity, peak #2 is DIMP, and peak #3 is GA.

Figure 4 shows the chromatogram obtained for the analysis of a 20 ug/mL solution (1 uL injection slit 20/1) obtained from the dilution of GA in Multisol with CH_3CN containing DIMP. Peak #1 is DIMP and peak #2 is GA.

Table 1 contains the GCFPD analysis results for a GA in Multisol (GA-M) sample gravimetrically prepared at 1.89 mg/mL. The first analysis result, 1.82 mg/mL, was determined 1 day after preparation and storage at −70°C from three analyses of a single

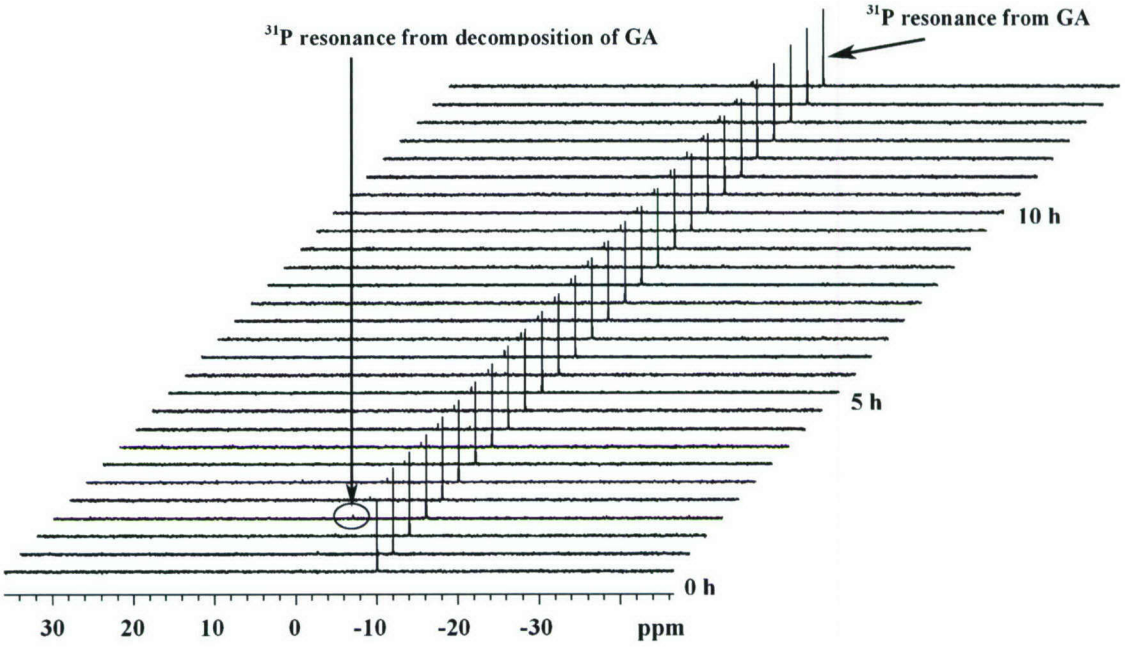


FIG. 1. Stability of GA in Multisol demonstrated with 31-P NMR.

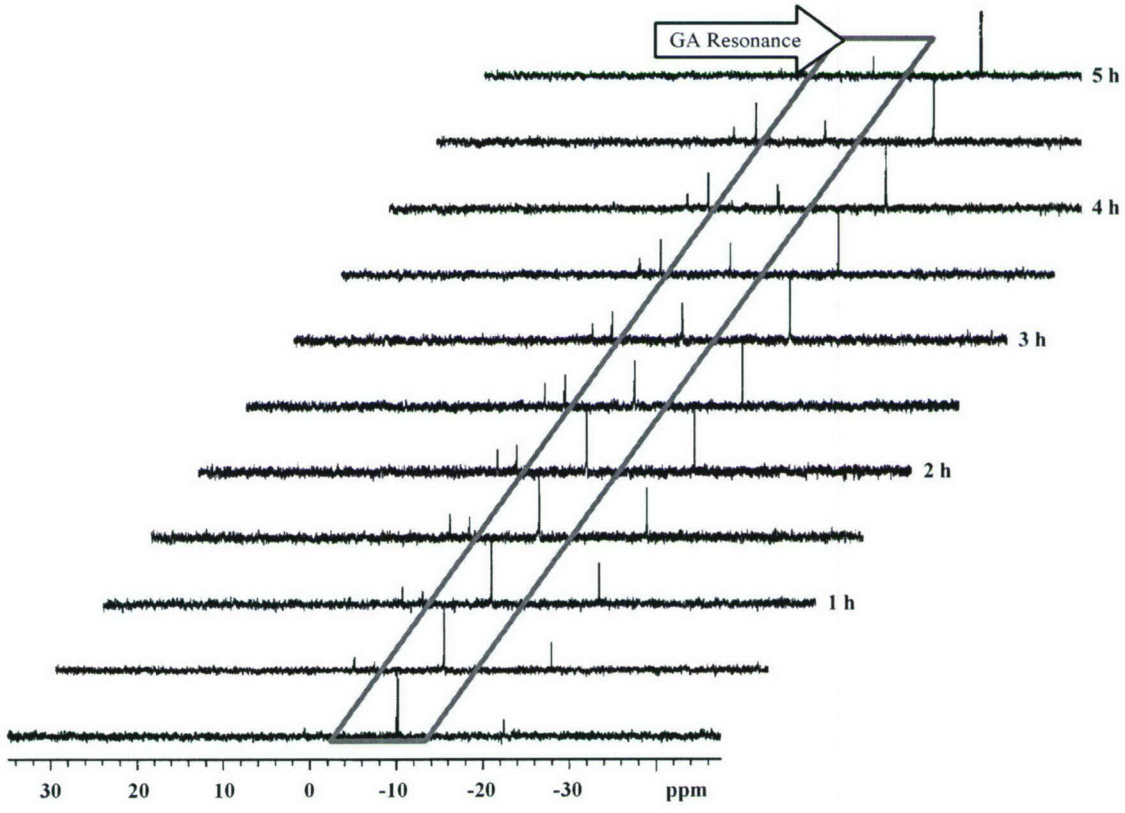
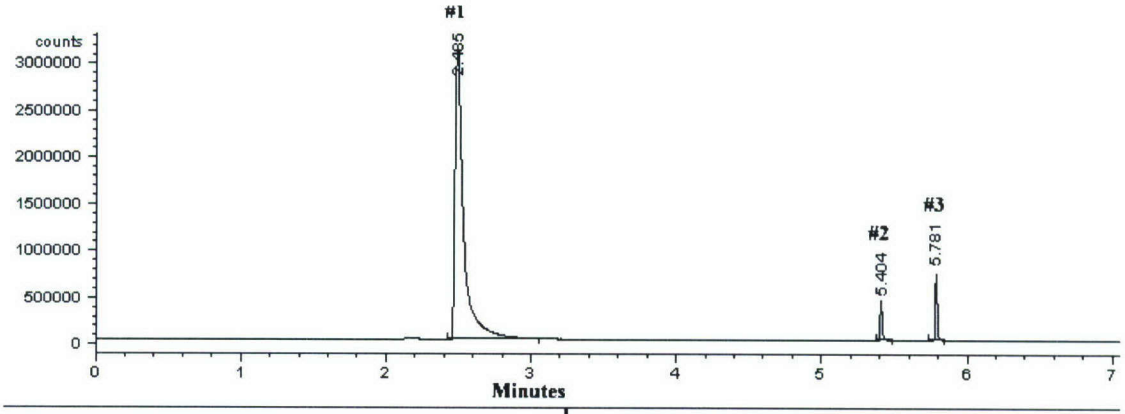


FIG. 2. Instability of GA in saline demonstrated with 31-P NMR.



File Information	
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File Path	C:\HPCHEM\2\DATA\TEST\
Date	23-Jan-04, 09:55:22
Sample	GADIMPC,CH3CN

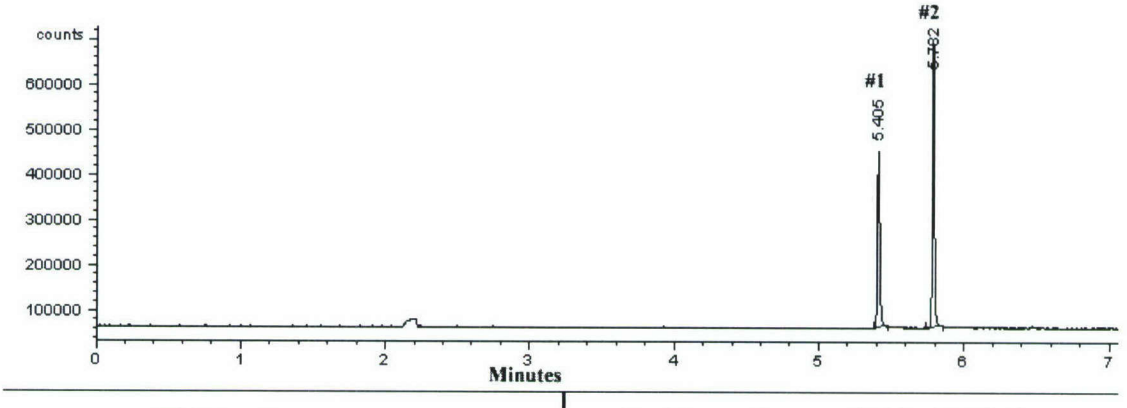
#	Time	Area	Height	Width	Symmetry
1	2.485	12951076.0	3122523.8	0.060	0.33
2	5.404	413578.6	418480.1	0.015	0.70
3	5.781	686582.8	726711.0	0.015	0.88

FIG. 3. Chromatogram of GA in $CHCl_3$ after dilution with CH_3CN containing DIMP as the internal standard. Peak #1 is $CHCL_3$ impurity. Peak #2 is DIMP. Peak #3 is GA.

dilution of a GA-M sample. The second analysis result, 1.76 mg/mL, was obtained following 5 months of storage at −70°C using three analyses of three separate dilutions of a GA-M sample. The third analysis result, 1.75 mg/mL, was obtained 14 months after preparation and storage at −70°C using three analyses of three separate dilutions of a GA-M sample.

DISCUSSION

GA is unstable in aqueous solutions. Our analysis results routinely show the concentration of GA in saline to be less than 90% of the gravimetric concentration within a month of preparation while kept at −70°C. Because the toxicity of GA is a frequently used reference in research to develop medical countermeasures to chemical warfare nerve agents, we sought a suitable solvent substitute for saline in the preparation and analysis of stable GA solutions. We chose Multisol as a substitute for saline because it was used previously to prepare anticholinergic (Capacio and Shih 1991; McDonough et al. 2000), anticonvulsant (Physicians' Desk Reference 2002; McDonough et al. 1999); McDonough et al. 2000, and GA solutions (Joiner et al. 1989). that were stable and suitable for research. We confirmed the stability results (Joiner et al. 1989) and extended the shelf life of GA in Multisol to 14 months.



File Information	
GC-File	SIG20065.D
File Path	C:\HPCHEM\2\DATA\TEST\
Date	23-Jan-04, 10:45:32
Sample	GADIMPM,CH3CN
Sample Info	Spilt flow 20:1, M=Multisol(H20), C=CHCL3, Jan 23

#	Time	Area	Height	Width	Symmetry
1	5.405	403844.8	401652.5	0.016	0.69
2	5.782	628419.9	655925.7	0.015	0.85

FIG. 4. Chromatogram of GA in Multisol after dilution with CH_3CN containing DIMP as the internal standard. Peak #1 is DIMP. Peak #2 is GA.

TABLE 1

GCFPD analysis results at selected times after preparation demonstrate GA in Multisol within ±10% of the gravimetric concentration at 14 months

Time after preparation	Concentration mg/mL (deviation from gravimetric concentration)
1 day	1.82 (−4%)
5 months	1.76 (−7%)
14 months	1.75 (−7%)

As part of our investigation we used 31-P NMR as a methodology separate from gas chromatography to further investigate and confirm GA's extended stability in Multisol, Figure 1, versus GA's instability in saline, Figure 2. In Figure 1 the Multisol stabilizes the GA resonance at (−10) ppm against rapid hydrolysis as shown by the slow rise over 14 h of the resonance at (−0.9) ppm, which we attribute to the first hydrolysis product, ethyl hydrogen N,N-dimethylphosphoroamidate (Chemical and Instrumental Verification of Organophosphorus Warfare Agents 1977). In Figure 2 we see the rapid onset of multiple hydrolysis product resonances as the GA resonance (−10) ppm decreases over 5 h.

We developed a relatively simple GCFPD method in the phosphorus mode for a routine and sensitive GC method of analysis for GA in Multisol. The phosphorus mode removed potential interferences from components in Multisol that do not contain phosphorus. This GC method by virtue of dilution in CH_3CN reduced and made manageable a peak present in $CHCl_3$ and detected in the phosphorus mode, Figure 3. The dilution with CH_3CN also removed the problem of sample immiscibility that arose with other solvents and reduced the GC column interferences due to the components of Multisol. Using this method we demonstrated that GA in Multisol solutions is stable for 14 months when stored at −70°C. The extended shelf life at −70°C of GA in Multisol versus GA in saline provides more reliable solutions of this nerve agent for our research.

In this work, we contributed a suitable technique for the preparation and analysis of reliable solutions of GA.

We offer Multisol for consideration as a possible solvent system for researchers working with analytes that are unstable or insoluble in completely aqueous based solvents. Currently we are investigating reduced concentrations of ethanol in the Multisol mixture and their effect on the stability of GA preparations.

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