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Stability of Dilute Tabun (GA) Solutions in Various Vehicles

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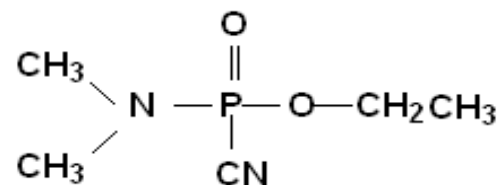
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14. ABSTRACT Tabun (GA) is unstable in aqueous media, which has created difficulties in supplying reliable preparations of GA in saline for toxicity studies. We previously demonstrated the stability of GA in Multisol (M), a four-component solvent (48.5% H ₂ O, 40% propylene glycol, 10% ethanol, and 1.5% benzyl alcohol) used in various chemical preparations. However, M's 10% ethanol content may alter toxicity findings. We used ³¹ P nuclear magnetic resonance (P-NMR) and gas chromatography mass spectrometry (GCMS) to investigate the stability of GA in two modifications of M. Mixture #1 contained 48.5% D ₂ O, 45% propylene glycol, 5% ethanol, and 1.5% benzyl alcohol; #2 contained 48.5% D ₂ O, 50% propylene glycol, and 1.5% benzyl alcohol. Using P-NMR we found GA to be stable for approximately 3 h at 25 °C in both mixtures. Using GCMS we found that GA was stable in both mixtures at -70 °C for more than 14 months. We performed separate dilutions of GA in mixture #1, mixture #2, and the original M with saline prepared with D ₂ O; we monitored GA's stability. Using P-NMR we demonstrated GA's stability at 25 °C for approximately 9 h in the dilutions of both mixtures and for at least 3 h in the original M dilution. This work demonstrated that the reduction or removal of ethanol from M can be accomplished without unduly influencing GA's stability for researchers and provides the length of time of GA's stability when diluting M vehicles with saline prepared with D ₂ O at 25 °C. These vehicle modifications may be helpful in future GA toxicity studies.					
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Abstract

Tabun (GA) is unstable in aqueous media. This instability has created difficulties in supplying reliable preparations of GA in saline for toxicity studies by our researchers. Multisol (M) is a four-component solvent (48.5% H₂O, 40% propylene glycol, 10% ethanol, and 1.5% benzyl alcohol) used to prepare various chemical preparations. We previously demonstrated the stability of GA in M. However, M's 10% ethanol content may alter toxicity findings when using it to investigate the toxicity of agents. We used ³¹P nuclear magnetic resonance (P-NMR) and gas chromatography mass spectrometry (GCMS) to investigate the stability of GA in two modifications of M. Mixture #1 contained 48.5% D₂O, 45% propylene glycol, 5% ethanol, and 1.5% benzyl alcohol, and #2 contained 48.5% D₂O, 50% propylene glycol, and 1.5% benzyl alcohol. Using P-NMR we found GA to be stable for approximately 3 h at 25 °C in mixtures #1 and #2. Using GCMS we found that GA was stable in mixtures #1 and #2 at -70 °C for more than 14 months. Furthermore, we performed separate dilutions of GA (1.9 mg/ml to 0.24 mg/ml) in mixture #1, in mixture #2, and in the original M with saline prepared with D₂O, and we monitored GA's stability. Using P-NMR we demonstrated GA's stability at 25 °C for approximately 9 h in the dilutions of mixture #1 and mixture #2, and for at least 3 h in the original M dilution. This work demonstrated that the reduction or removal of ethanol from M can be accomplished without unduly influencing GA's stability for researchers and provides the length of time of GA's stability when diluting M vehicles with saline prepared with D₂O at 25 °C. We concluded that these vehicle modifications may be helpful in future GA toxicity studies.

Introduction

Tabun's (GA) instability in aqueous media is a problem when preparing saline solutions of this analyte. GA's structure is highly susceptible to hydrolysis at the -CN, and -N(CH₃)₂ bonds to phosphorus.



In neutral or near neutral aqueous solutions, GA hydrolyzes rapidly at the P-CN bond, yielding ethyl hydrogen N,N-dimethylphosphoramidate as the major product.^{1,2} It has been our personal experience and the experience of others³ that the nerve agents sarin (GB), soman (GD), and cyclosarin (GF) in saline are stable for months when stored at -70 °C. However, GA in saline solutions decomposes after several weeks at this temperature.

Multisol (M) (48.5% H₂O, 40% propylene glycol, 10% ethanol, and 1.5% benzyl alcohol) has been used to solubilize compounds that were found to be relatively insoluble in saline.⁴⁻⁸ The composition of M is similar to the vehicle used with injectable Valium™.⁴ Other workers found that M provided stable GA solutions.^{3,9,10} However, the 10% ethanol component in M concerns researchers in that it may alter toxicity findings for an analyte in a solution of M. We therefore evaluated changes in M's composition, first reducing and then removing ethanol. We chose ³¹P nuclear magnetic resonance (P-NMR) to investigate the stability of GA at 25 °C in two modifications of M, referred to as mixture #1 (m#1) and mixture #2 (m#2). The ethanol content was reduced to 5% in m#1 and removed completely in m#2; H₂O was replaced with D₂O in both mixtures. In each mixture we increased propylene glycol to compensate for ethanol changes. We then investigated the stability of GA in m#1 and m#2 after storage at -70 °C for 14 months using gas chromatography mass spectrometry (GCMS).

Another GA stability question arises when researchers further dilute the solutions of GA in M with saline for toxicity studies since they then need to know how long GA is stable in the diluted solutions. P-NMR was again used to investigate the stability of GA in M, m#1, and m#2 after each of these three solutions was diluted with saline prepared with D₂O.

Materials and Methods

Materials. Tabun (Dimethylphosphoramidocyanidic acid, ethyl ester, GA) was obtained from the US Army Edgewood Chemical Biological Center (ECBC), Aberdeen Proving Ground, MD; GA purity was 98.5% as determined by NMR spectroscopy. Deuterium oxide 99.9 atom % D and acetonitrile (CH₃CN), HPLC grade were obtained from Aldrich Chemical Company, Milwaukee, WI. Absolute 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. Chloroform (CHCl_3), high purity solvent, was obtained from Burdick and Jackson, Muskegon, MI. Diisopropyl methanephosphonate (DIMP) was obtained from Lancaster Synthesis, Pelham, NH.

P-NMR measurements were made of GA in m#1, GA in m#2, and separate dilutions of GA in M, m#1, and m#2 made with D_2O saline. Solutions of GA in M, m#1, and m#2 were prepared gravimetrically at 1.9 mg/ml with D_2O substituted for H_2O and stored at $-70\text{ }^\circ\text{C}$. Immediately upon thawing and mixing at room temperature, 700 μL of the solution was transferred into a 5 mm o.d. NMR tube. P-NMR measurements were made of 0.24 mg/ml GA solutions prepared by mixing 88 μL of the gravimetrically prepared solutions of GA with 612 μL of D_2O saline in an NMR tube. All NMR data was collected on Varian Unity Inova spectrometer equipped with a 5 mm gradient Penta^R probe. Probe temperature was maintained at $25\text{ }^\circ\text{C} \pm 0.1\text{ }^\circ\text{C}$, and the VT air flow was maintained at 10 LPM. Standard s2pul sequence with continuous proton decoupling [$^{31}\text{P}\{^1\text{H}\}$] was used, and all the calibrations were done as per the installation procedure. Chemical shifts are reported in ppm (parts per million) using 85% phosphoric acid (H_3PO_4) as external reference at -0.9 ppm.

Gas chromatographic analyses of GA in M, GA in m#1, GA in m#2, and CHCl_3 were made after 1:100 dilution with CH_3CN containing 0.014 mM DIMP as the internal standard. An Agilent 6890 gas chromatograph interfaced with a 5973 mass selective detector was used with electron energy set to 70 electron volts. GA was analyzed in the selected ion monitoring (SIM) mode using the three principal ions of GA (m/z 70, 133, 162) and the three principal ions of DIMP (m/z 97, 123, 79) using dwell times of 30 microseconds for each ion. The gas chromatograph was equipped with a 30 m, 0.25 mm i.d., 0.25 mm HP-5MS column. The injection port was at $250\text{ }^\circ\text{C}$, and the initial oven temperature was $70\text{ }^\circ\text{C}$ for 2 min, and the temperature ramp rate was $30\text{ }^\circ\text{C min}^{-1}$ until the final temperature of $250\text{ }^\circ\text{C}$ was reached and held for 3 min. Samples were run in the splitless mode with a helium carrier gas flow rate of 1 ml min^{-1} measured at $70\text{ }^\circ\text{C}$.

Results

P-NMR was used to investigate the stability of GA in two modifications of M (m#1 and m#2) at $25\text{ }^\circ\text{C}$. Figure 1 shows the stability of GA in m#1 over a 13-h period in a sample of GA prepared at 1.9 mg/ml and using D_2O in place of H_2O . The -10 ppm peak is the GA resonance,² and it remained the major component throughout the run. Small resonances that do not increase in size are impurities. Small resonances appeared and began to grow in size after 3.5 h. We attributed these resonances to P containing decomposition products of GA. Figure 2 follows the stability of GA in m#2 over a 13-h period in a sample of GA at 1.89 mg/ml and using D_2O in place of H_2O . Again the GA resonance at -10 ppm peak remained the major component after 13 h. Decomposition appeared to be under way at the 5-h point.

Figure 1

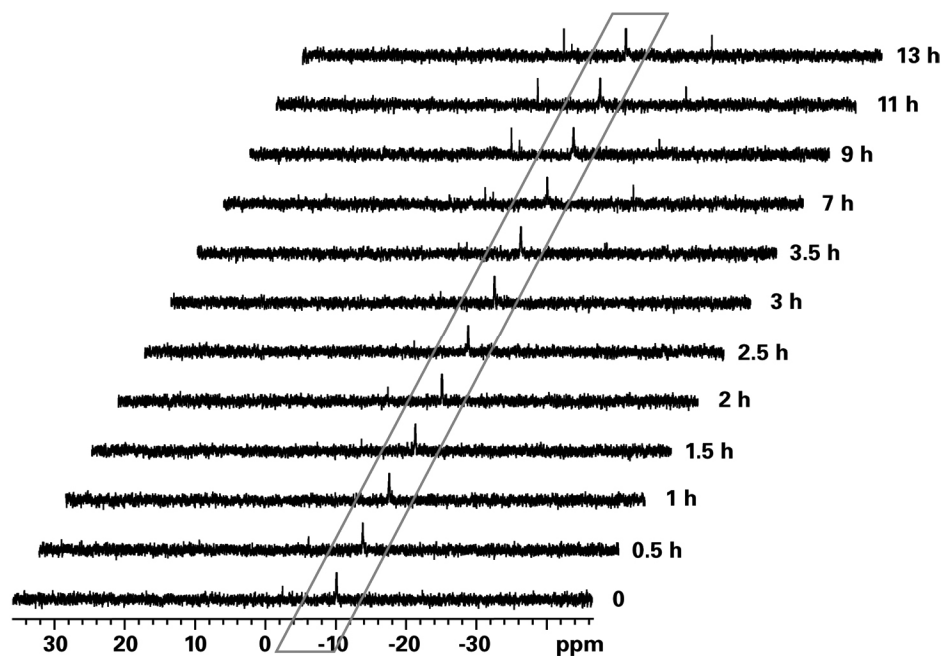


Figure 1 followed the stability of GA in m#1 over a 13-h period at 25 °C using P-NMR to monitor the GA and decomposition product resonances in a sample of GA at 1.9 mg/ml. The GA resonance at -10 ppm² remained the major component for 13 h. Small resonances that proceed to grow in size occurred at 3.5 h. We attributed these resonances to P containing decomposition products of GA.

Figure 2

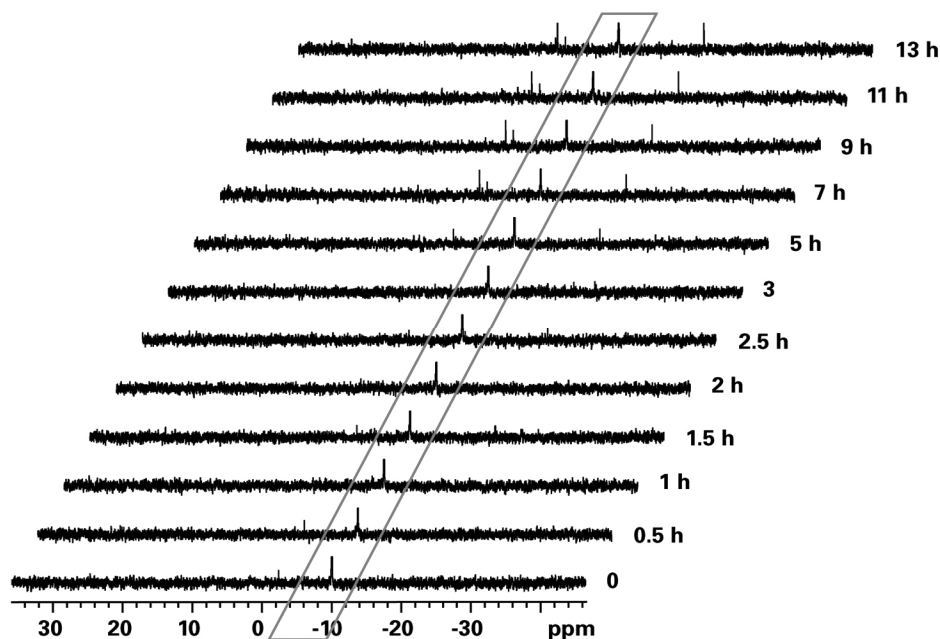


Figure 2 followed the stability of GA in m#2 over a 13-h period at 25 °C using P-NMR to monitor the GA and decomposition product resonances in a sample of GA at 1.9 mg/ml. The GA resonance at -10 ppm² remained the major component for 13 h. Small resonances that proceeded to grow in size occurred at 5 h. We attributed these resonances to P containing decomposition products.

The stability of diluted solutions of GA in M, m#1, and m#2 with D₂O saline is shown in Figures 3, 4, and 5. We did not detect evidence of GA decomposition for 9 h after diluting m#1 with D₂O saline (Figure 3). Similarly, we did not detect evidence of GA decomposition until the 9-h time point after diluting m#2 with D₂O saline (Figure 4). Finally we did not detect GA decomposition in the comparatively short time of 3 h after diluting M with D₂O saline (Figure 5).

Previously, we studied the stability of 1.9 mg/ml solutions of GA in M after storage at -70 °C. In this study, preparations of GA in M, m#1, and m#2 using D₂O to replace H₂O were stored for more than 14 months at -70 °C. We chose GCMS to analyze these solutions using GA in CHCl₃ as a reference standard. Figures 6A and 6B are typical chromatograms of standard and sample m#2 used for the results in Table 1.

Figure 3

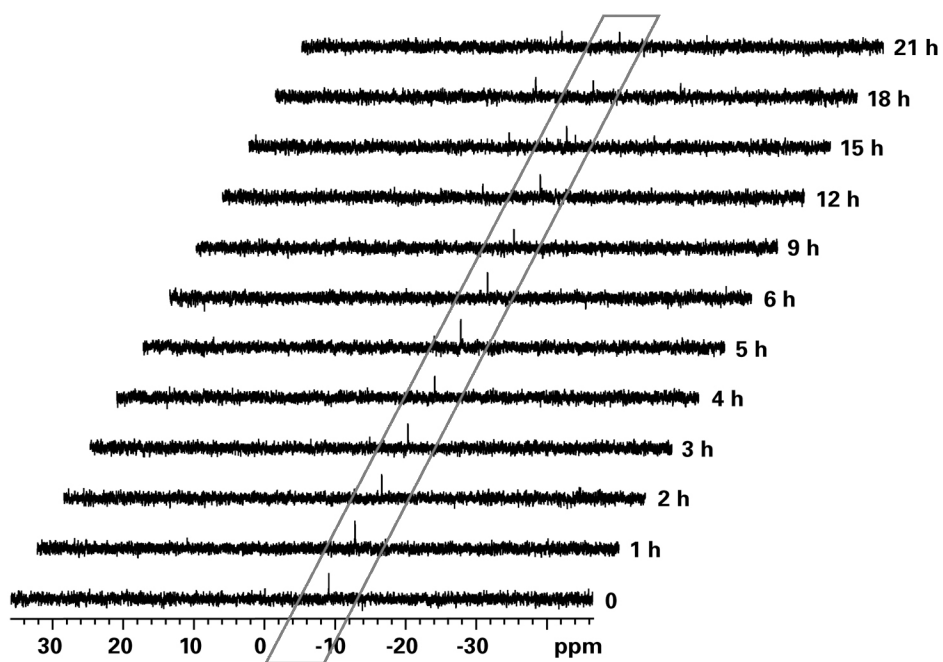


Figure 3 shows the stability of GA in a diluted sample for 21 h at 25 °C using P-NMR to monitor the GA and decomposition product resonances. The 0.24 mg/ml sample was prepared by mixing in GA in m#1 with D_2O saline. We did not detect evidence of GA decomposition for 9 h.

Figure 4

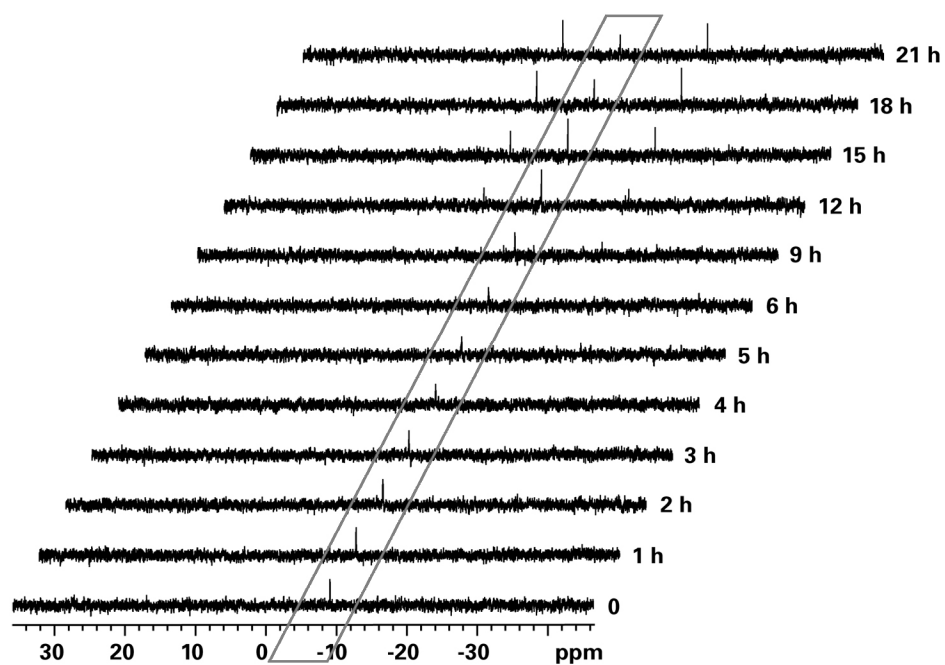


Figure 4 shows the stability of GA in a diluted sample for 21 h at 25 °C using P-NMR to monitor the GA and decomposition product resonances. The 0.24 mg/ml sample was prepared by mixing in GA in m#2 with D₂O saline. We saw evidence of GA decomposition at 9 h.

Figure 5

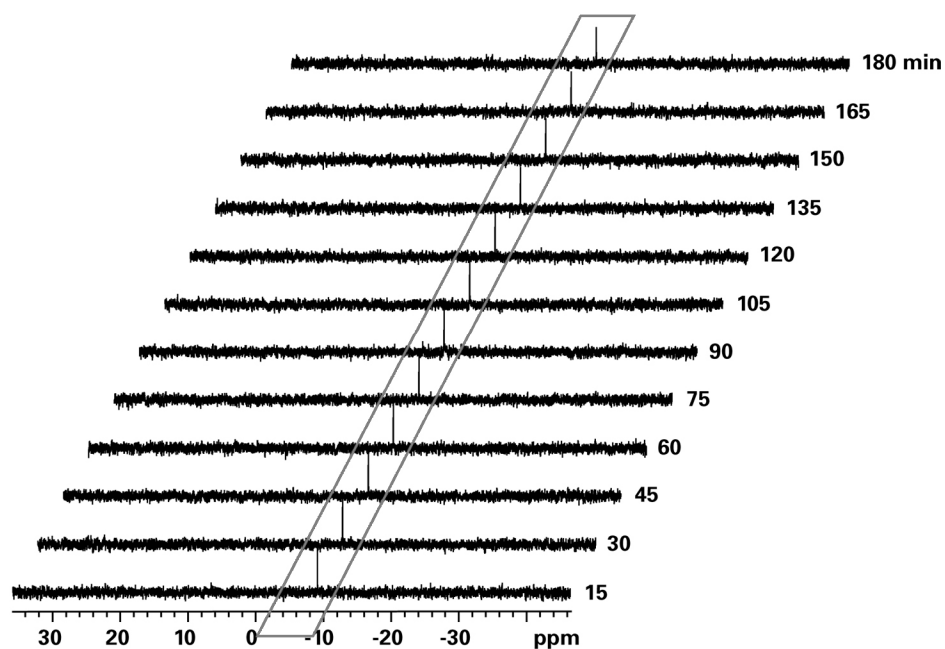


Figure 5 shows the stability of GA in a diluted sample for 3 h at 25 °C using P-NMR to monitor the GA and decomposition product resonances. The 0.24 mg/ml sample was prepared by mixing in GA in M with D₂O saline. GA appeared stable during this 3-h period.

Figure 6A

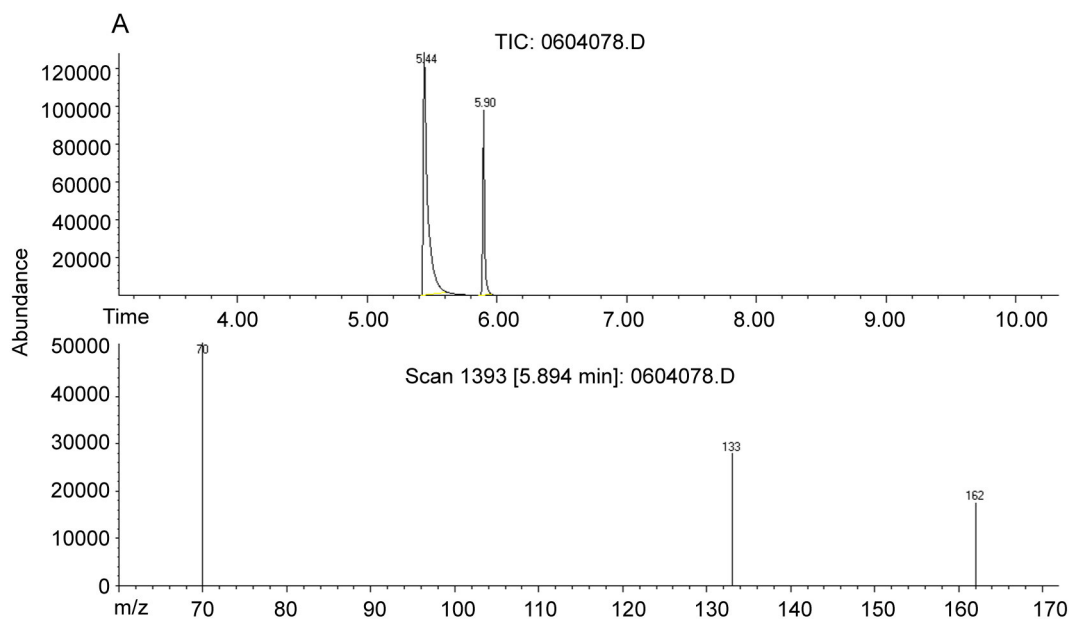
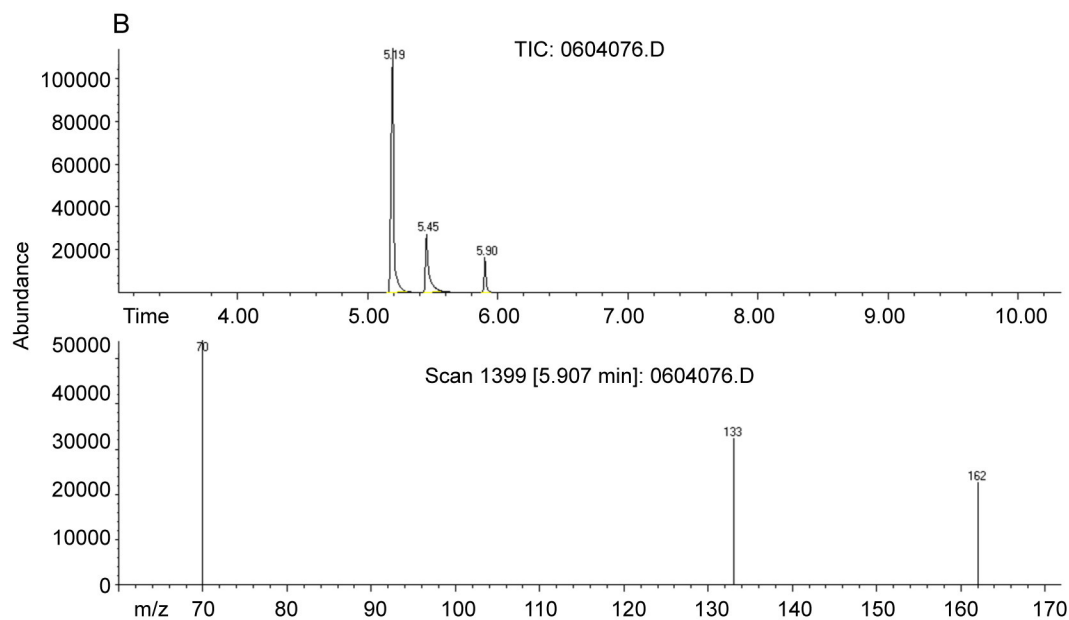


Figure 6B



Figures 6A and 6B are GCMS chromatograms of GA in CHCl_3 and GA in m#2 respectively. Figure 6A is one of the reference chromatograms used for the calculation of GA's concentration in M, m#1, and m#2. The peak at 5.44 min is DIMP and at 5.90 min is GA. The large first peak in Figure 6B is benzyl alcohol.

Table 1

GA solution and preparation date (ddmmyy)	Gravimetric preparation Concentration mg/ml	GCMS Analysis 060407 Concentration mg/ml {%Coefficient of Variation, [CV], n = 3}
GA in M 081205	1.87	1.94 (5.1%)
GA in m#1 180106	1.90	1.86 (3.6%)
GA in m#2 180106	1.89	1.97 (1.8%)
GA in CHCl ₃ 090905	1.89	reference standard

Table 1 contains the GCMS analysis results of GA in three solvent vehicles after more than 14 months of storage at -70 °C, demonstrating GA's stability in M, m#1, or m#2.

Discussion

The results of this study demonstrated that GA (1.9 mg/ml) was stable for approximately 3 h at 25 °C in m#1 and in m#2. We previously¹⁰ demonstrated GA's stability for 14 h in M and less than 1 h in D₂O saline. Furthermore, we can reduce or remove ethanol's influence in GA toxicity studies by using m#1 and m#2. Although GA's stability at 25 °C was reduced to 3 h in these two mixtures from 14 h in M, the researcher still has ample time for its use in toxicity studies at 25 °C.

The GCMS analysis indicates that GA has stability in m#1 and m#2 for more than 14 months when stored at -70 °C. This is similar to the stability of GA in M at -70 °C previously reported,¹⁰ and that is a large improvement compared to GA's stability of weeks in saline solution. It should be noted that M, m#1 and m#2 were prepared with D₂O instead of H₂O and that may have improved the stability of GA.

Researchers who dilute GA-Multisol, GA-mixture #1, and GA-mixture #2 in saline have 3 h (Figure 3) or longer (Figures 4 & 5) before decomposition occurs. GA's stability in these dilutions is remarkable, and we suspect that propylene glycol is contributing to this stabilization in the presence of D₂O saline. A toxicity study has confirmed that GA-Multisol can be diluted from 1.9 mg/ml to 0.24 mg/ml with D₂O saline at room temperatures without loss of anticipated potency.¹¹

In conclusion the vehicle modifications we have described may be helpful to researchers who are concerned with the potential toxicity of ethanol and the stability of dilute solutions of GA.

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