

BASIC INVESTIGATIONS

Preparing for Chemical Terrorism: Stability of Injectable Atropine Sulfate

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Abstract

Objective: A massive nerve agent attack may rapidly deplete in-date supplies of atropine. The authors considered using atropine beyond its labeled shelf life. The objective was to determine the stability of premixed injectable atropine sulfate samples with different expiration dates.

Methods: This was an in-vitro study using gas chromatography and mass spectrometry (GC/MS). Four atropine solutions (labeled concentration of 400 $\mu\text{g}/\text{mL}$) ranging from in date to 12 years beyond expiration (exp) and an additional sample of atropine sulfate (labeled concentration of 2,000 $\mu\text{g}/\text{mL}$) obtained from a World War II era autoinjector were assayed for atropine stability. Standards of atropine sulfate and tropine were prepared and quantified by GC/MS. Study samples were prepared by adding a buffer solution to free the base, extracting with an isopropanol/methylene chloride mixture and followed by evaporating the organic layer to dryness. Pentafluoropropionic anhydride and pentafluoro-

propanol were then added as derivatization reagents. Study samples were heated, the derivatization reagents were evaporated, and the remaining compound was reconstituted in ethyl acetate for injection into the GC/MS. **Results:** All solutions were clear and colorless. Atropine concentrations were as follows: in date, 252 $\mu\text{g}/\text{mL}$; 2001 exp, 290 $\mu\text{g}/\text{mL}$; 1999 exp, 314 $\mu\text{g}/\text{mL}$; 1990 exp, 398 $\mu\text{g}/\text{mL}$; and WW II specimen, 1,475 $\mu\text{g}/\text{mL}$. Tropine was found in concentrations of <10 $\mu\text{g}/\text{mL}$ in all study samples. **Conclusions:** Significant amounts of atropine were found in all study samples. All samples remained clear and colorless, and no substantial amount of tropine was found in any study sample. Further testing is needed to determine clinical effect. **Key words:** atropine; organophosphate; drug stability. ACADEMIC EMERGENCY MEDICINE 2004; 11:329–334.

Current hospital stockpiles of antidotes for chemical terrorism are probably inadequate to deal with a mass casualty event.^{1,2} Organophosphorous compound toxicity commonly results in consumption of large amounts of atropine to control symptoms and prevent death.³ A mass exposure involving either commercial-grade insecticides or military-grade nerve agents may rapidly deplete in-date atropine supplies.²

Atropine is the tropine ester of tropic acid and is generally available as the sulfate salt. Nonenzymatic spontaneous hydrolysis of aqueous atropine yields tropine and tropic acid that are nontoxic but do not have the desired antimuscarinic activity.⁴ Expired atropine sulfate may still contain significant amounts of active drug. Since dosing is based on clinical effect, the potential benefit of using an expired drug in

a crisis situation should be examined. If an expired drug still contains a significant amount of atropine, then it may be useful in an emergency when in-date supplies are depleted. We hypothesized that premixed injectable atropine sulfate solution would retain a significant amount of its atropine concentration.

The primary objective of this study was to determine how much atropine remained in current and expired drugs. The secondary objective of this study was to identify the presence of tropine as a marker of atropine degradation in current and expired drugs.

METHODS

Study Design. This was an in-vitro study design that utilized gas chromatography and mass spectrometry (GC/MS).

Study Protocol.

Stock Standard Preparation. Primary standards of both atropine base and tropine (Sigma-Aldrich Corporation, St. Louis, MO; purity >99% by thin-layer or gas chromatography) were made by dissolving known measured amounts in methanol. Ten milliliters of methanol was used to dissolve 31.1 mg of atropine base and 20.3 mg of tropine to yield concentrations of 3.11 mg/mL (3.11 $\mu\text{g}/\mu\text{L}$) and 2.03

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mg/mL (2.03 $\mu\text{g}/\mu\text{L}$), respectively. Secondary standards were prepared by the combination of 1,608 μL of the atropine primary standard (5,000.88 μg) and 2,463 μL of the tropine primary standard (4,999.89 μg). Methanol was added to make a total volume of 10 mL. This yielded a final concentration of approximately 500 ng/ μL of atropine and tropine. Working standards were made by taking 20, 40, and 80 μL of the secondary solution, which corresponded to 10, 20, and 40 μg of compound.

Each working standard was evaporated over a water bath to remove the methanol (Lab-Line Instruments, Melrose Park, IL; Model # 18005) at 45°C for 5–10 minutes using nitrogen. Pentafluoropropionic anhydride (100 μL) and pentafluoropropanol (50 μL) were added to the 20- μL standard as derivitization reagents. Derivatization reagent amounts were doubled and quadrupled for the 40- and 80- μL standards, respectively. Each sample was vortexed for 1 minute and then heated to 90–92°C for 20 minutes (Pierce, Rockford, IL; Series 344 heating block). The derivatization reagents were evaporated over a water bath as previously described.

Each standard was dissolved in 50 μL ethyl acetate to produce final concentrations of 200, 400, and 800 ng/ μL . One microliter of each concentration was injected into a GC/MS (Mass Selective Detector Model 5973, Hewlett-Packard; Series 6890 Gas Chromatograph System, Hewlett-Packard, Palo Alto, CA) for analysis. Sample injection parameters for the GC/MS system were as follows: injection temperature, 250°C; column, capillary HP5-MS; ramp, 10°C/min; and flow gas, helium, and flow rate, 0.8 mL/min.

Sample Preparation. Four vials of premixed atropine sulfate injection solution with a labeled concentration of 400 $\mu\text{g}/\text{mL}$ and different dates of manufacture (mnf) and expiration (exp) were tested. An additional sample from a World War II era autoinjector (Figure 1)

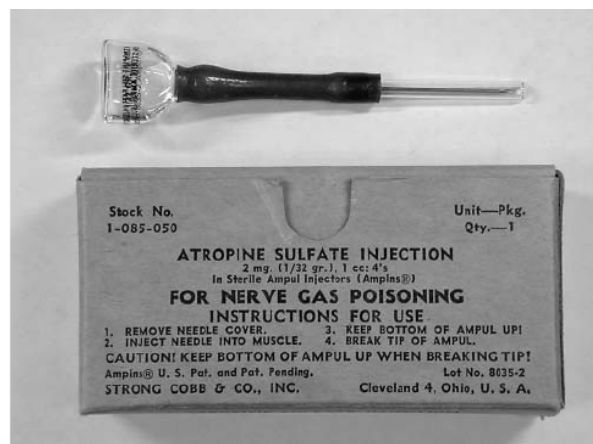


Figure 1. A World War II era autoinjector found in a civil defense nerve gas kit.

containing injectable atropine sulfate solution with a labeled concentration of 2,000 $\mu\text{g}/\text{mL}$ (expiration date not listed) was also tested. All samples were stored according to the manufacturer's recommendations since time of original purchase in accordance with good pharmacy practices to the best of the study investigators' knowledge. The study samples included Elkins-Sinn (400 $\mu\text{g}/\text{mL}$), Lot No. 087047, exp 2/90, mnf 8/87; Elkins-Sinn (Cherry Hill, NJ; 400 $\mu\text{g}/\text{mL}$), Lot No. 046153, exp 4/99, mnf 4/96; Elkins-Sinn (400 $\mu\text{g}/\text{mL}$), Lot No. 058111, exp 5/01, mnf 5/98; American Pharmaceutical Partners, Inc. (Champaign, IL; 400 $\mu\text{g}/\text{mL}$), Lot No. 312122, exp 1/04, mnf 1/01; and Strong, Cobb & Co., Inc. (Cleveland, OH; 2,000 $\mu\text{g}/\text{mL}$) Lot No. 8035-2. Concentrated ammonia solution was added to a saturated solution of ammonium chloride until a pH of 9.5 was reached. Two milliliters of this solution was added to 250 μL of the 400 $\mu\text{g}/\text{mL}$ specimens and 50 μL of the 2,000- $\mu\text{g}/\text{mL}$ specimen (100,000 ng of drug) in order to free the base. The organic components (i.e., atropine and any breakdown products) were extracted using 4 mL of a 1:9 isopropanol/methylene chloride mixture. Each sample was vortexed for 1 minute and then centrifuged for 5–10 minutes to separate out the organic layer. Four milliliters of water was added to each sample, and it was vortexed, centrifuged, and the water aspirated off. The organic layer was evaporated on a water bath as previously described. Each study sample was derivatized with 500 μL of pentafluoropropionic anhydride and 250 μL of pentafluoropropanol and vortexed for 1 minute. Study samples were then heated at 90–92°C for 20 minutes and the organic layer evaporated on a water bath (as previously described for the standard sample preparation). The remaining compound was dissolved in 100 μL ethyl acetate and 1 μL injected into the GC/MS for analysis. Samples were also pH tested.

Atropine Concentration Determination. Peaks from the standard samples found on gas chromatography were identified using MS. The areas under the curve were measured by computer algorithm. The results were then plotted as a function of increasing standard sample concentration. Linear regression using the least squares method was used to determine atropine concentration of the study samples. The R^2 value was calculated for the linear regression analysis. These results were then corrected for atropine concentration per milliliter. The 95% confidence intervals (CIs) for the measured concentrations of the atropine study samples were then calculated based on the 95% CI of the slope of the regression line.

Tropine Concentration Determination. Tropine peaks from the atropine study samples found on GC were identified using MS. The amounts were so small that manual determination was necessary. A known

amount of tropine was injected into the gas chromatography column and the area under the curve measured. A ratio of the individual tropine peaks from the study samples to the known value was made and the tropine concentration determined.

RESULTS

Standard Samples. GC/MS analysis of all three standard specimens yielded peaks appearing at the same retention time for the pentafluoropropionic ester (PFPe) of tropine and atropine. In the cases of the 200 and 400 ng/ μ L (Figure 2) concentrations, a small peak representing excess derivatization reagent was also identified.

Study Samples. GC/MS analysis of the five study samples yielded major peaks for the PFPe of atropine, minor peaks for underivatized atropine, and in all cases insignificant peaks for tropine. Minor peaks corresponding to excess derivitization reagent were found in all samples except for the 1990 sample. Minor to insignificant peaks on GC/MS corresponding to ethanol 2-butoxyphosphate and bis (2-ethyl-hexyl) phthalate (by MS) were found in all samples except the World War II era specimen. GC results for the 1999 (Figure 3) and World War II (Figure 4) samples are shown as representative examples.

The R^2 value of the standard curve was calculated to be 0.8591. The measured atropine concentration of

each of the study samples is listed, along with its measured pH and calculated 95% CI, in Table 1. Significant atropine concentrations (both the PFPe of atropine and underivatized atropine) were found in all specimens. All atropine study samples had <10 μ g/mL of tropine.

DISCUSSION

The catabolic degradation of aqueous atropine is well described. Instability is due to either hydrolysis, which yields tropine and tropic acid, or dehydration, which yields apoatropine.⁵⁻⁷ Apoatropine may undergo further hydrolysis to atropic acid and tropanol (tropine), and dimerization of these compounds may produce belladonnine and isotropic acid, respectively.⁷ However, dimerization of these compounds occurs only under either extreme conditions such as high temperatures or following long periods of time and not under normal storage conditions.^{6,7} Thus, the main pathway of instability in aqueous atropine solution is the hydrolytic reaction yielding tropine and tropic acid.⁸

The stability of aqueous atropine sulfate is dependent on several variables. It is well established that stability is enhanced in acid solution.⁹ Ideal storage pH for aqueous atropine sulfate solution ranges approximately between 3 and 4.^{5,9,10} Atropine in solution has a significantly shorter half-life in neutral (pH 7–8) and basic (pH 8–10; >11.5) conditions.^{8,10} High experimental temperatures significantly decrease half-life as

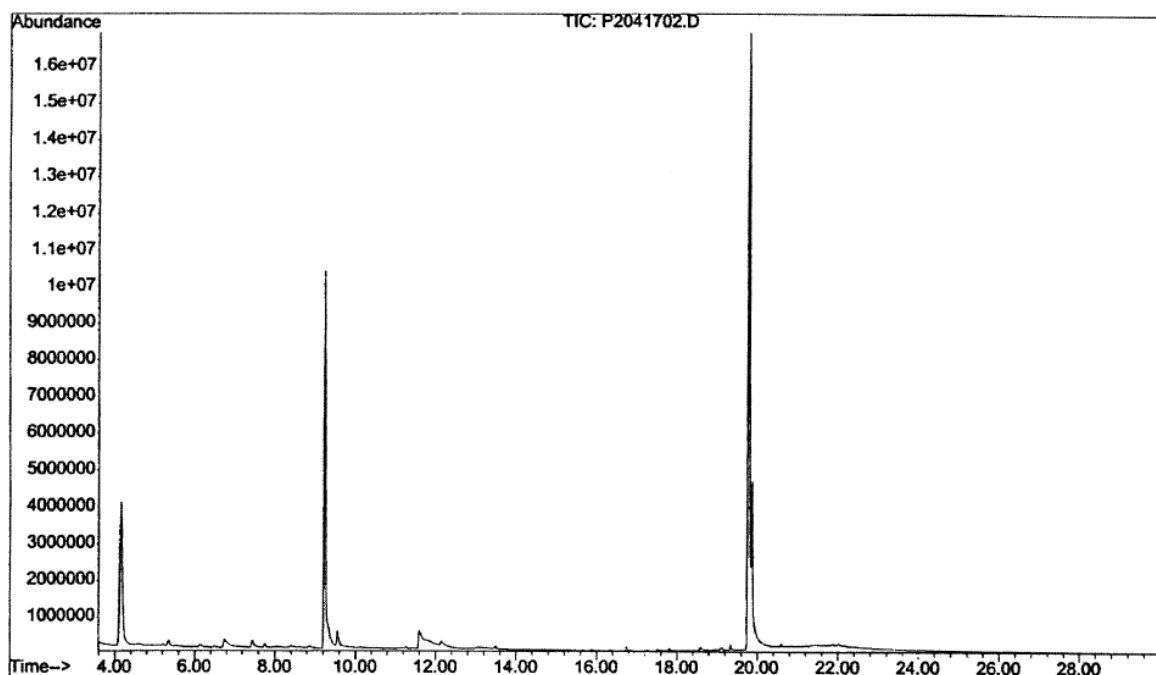


Figure 2. The gas chromatography results for the 400 ng/ μ L standard sample. Significant peaks are easily identifiable for (moving left to right): excess derivatization reagent, the pentafluoropropionic ester (PFPe) of tropine, and the PFPe of atropine.

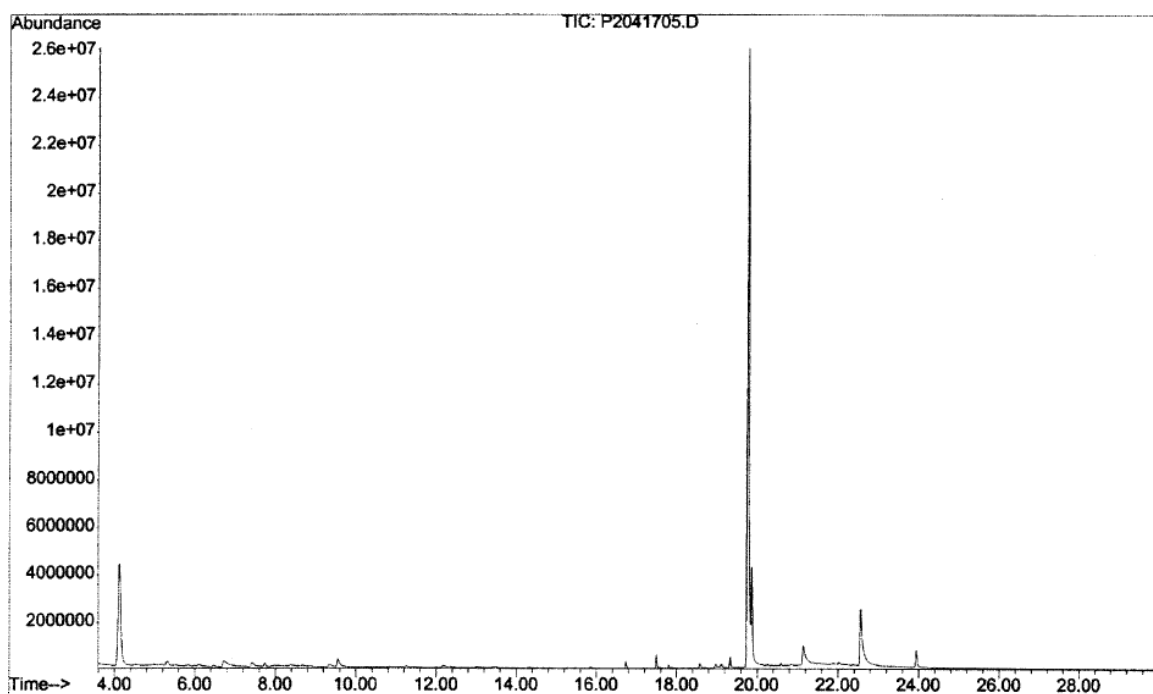


Figure 3. The gas chromatography results for the sample that expired in 1999. Moving left to right, peaks are as follows: excess derivatization reagent, the pentafluoropropionic ester (PFPe) of atropine, underivatized atropine, ethanol 2-butoxyphosphate, and bis (2-ethylhexyl) phthalate.

well.^{8,10} One study calculated the half-life of aqueous atropine to be greater than 1,700 years at a pH of 3.0 and at 20°C.⁵ Another study predicted the half-life to be 1,800 years at a pH of 4.0 and a constant temperature of 20°C.¹⁰ A temperature increase of 10°C reduced that predicted half-life to 473 years.¹⁰ Conversely, experimental and calculated half-lives of atropine at pH values >11.5 ranged between 18 and 137 minutes at temperatures of 25°, 32.5°, and 40°C.⁸ Experimental and predicted half-life values for aqueous atropine solutions ranging from pH 8 to 10.5 at 40°C varied from 3.4 to 116 hours.⁸

A similar study evaluated the stability of a multicomponent organophosphate antidote formulation (atropine sulfate, tremedoxime bromide, and benactyzine) in plastic and glass containers over a range of storage temperatures and time periods.¹¹ Samples were prepared at a pH of 2.8 and stored under the following conditions: 80°C for 3 weeks; 65°C for 7 weeks; 52°C for 3 months; and in a water-jacketed incubator at 40°C for 4 months or in a refrigerator at 5°C for 4 months.¹¹ No significant loss of atropine sulfate was found for any condition, although breakdown products were not specifically tested for.¹¹ Atropine sulfate was stable over a six-month period in another multicomponent organophosphate antidote formulation with no degradation products detected.¹² In this study, aqueous atropine samples were maintained at a pH of 4 and temperatures of 5°C, 25°C, and 40°C.

The small amounts of tropine found in the atropine study samples are suggestive of some degradation. However, it is also possible that some tropine was included during the manufacturing.

When the existence of significant amounts of atropine remaining in our study samples is coupled with the absence of substantial amounts of tropine and what is already known about aqueous atropine stability, our findings suggest that expired atropine sulfate solution could still be efficacious. Indeed, the expiration date is usually two to three years from the date of manufacture and is based on retaining >90% of drug potency in its original packaging.¹³ This date does not indicate loss of potency after expiration but rather that potency is known until the expiration date from real-time or extrapolated data.¹³ Retained potency varies among drugs and is dependent on storage conditions.¹³ Many drugs retain 90% of their potency for at least five years past the labeled expiration date if stored under reasonable conditions.¹³ Since atropine dosing in the treatment of a cholinergic crisis is based exclusively on clinical response, properly stored drug may still be useful even past its expiration date.

LIMITATIONS

A major limitation of this study is the possible existence of other breakdown products; however, the

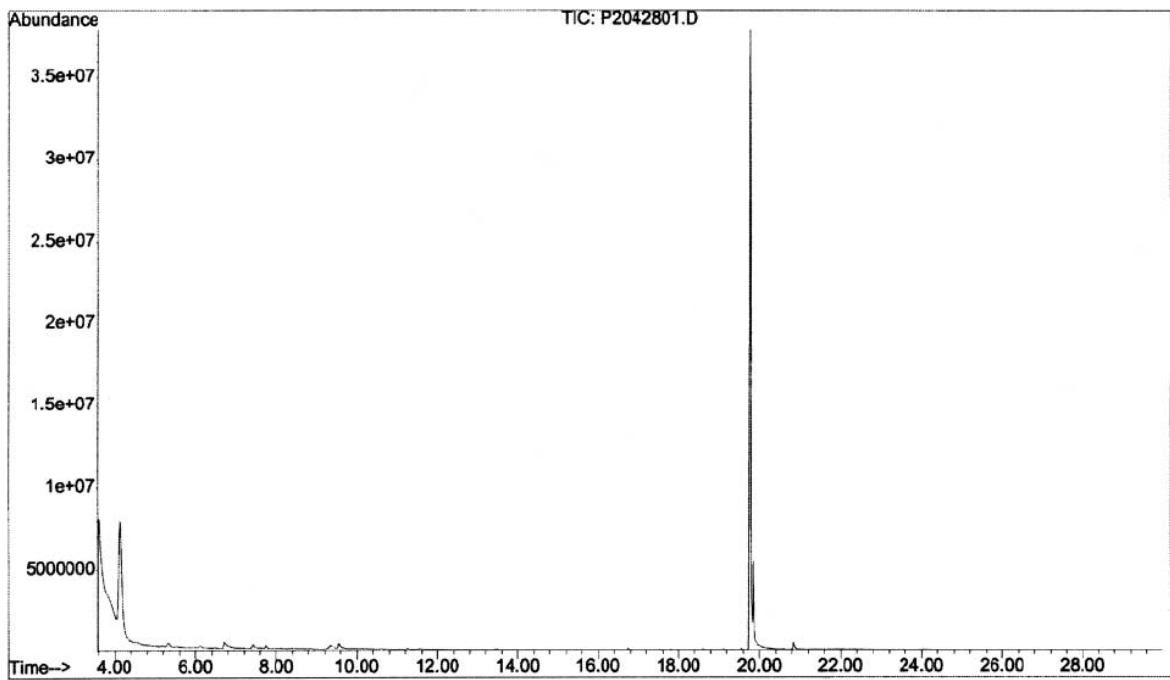


Figure 4. The gas chromatography results for the undated World War II specimen. Moving from left to right, peaks are as follows: excess derivatization reagent, the pentafluoropropionic ester (PFPe) of atropine, and underivatized atropine.

presence of significant amounts of atropine coupled with the absence of any other significant peak on GC argues against this possibility. More likely, the extraction process resulted in some lost drug. Since the study samples underwent the extraction process individually, this might have resulted in variation between the amounts of drug lost between them. Although there is no reason to believe the ammonia buffer solution would have interfered with tropine identification, that possibility also exists. The discovery of minor amounts of ethanol 2-butoxyphosphate and bis (2-ethylhexyl) phthalate in the 1990 sample may reflect imperfect technique but probably reflect buffering agents added by the manufacturer. We did not specifically test for apoatropine; however, as it does have inherent anticholinergic properties, it might be useful as an antidote.¹⁴ Therefore, presence of this breakdown

product is probably not of concern. We also did not assess sample potency (i.e., clinical effect) in this study.

One final limitation is that we tested only one vial from each lot. Although all vials in each lot were manufactured and stored in a similar fashion, it is possible, although unlikely, that we selected an aberrant vial for study. Because of our need to maintain this supply in the event of a disaster and our desire to continue this study in the future, multiple samples were not tested.

CONCLUSIONS

Significant amounts of atropine were found in all samples tested. Tropine, as a marker of degradation, was not found in a substantial amount in any tested product. Stability of aqueous atropine sulfate is

TABLE 1. Measured Atropine Concentration, 95% Confidence Intervals (CIs), and Measured pH

Sample	Manufacturer	Labeled Concentration (μg/mL)	Measured Concentration (μg/mL)	Calculated 95% CI = (μg/mL)	pH Value
In date	American Pharmaceutical Partners, Inc.	400	252	(235, 268)	3.88
Exp 2001	Elkins-Sinn, Inc.	400	290	(272, 308)	3.93
Exp 1999	Elkins-Sinn, Inc.	400	314	(295, 333)	3.82
Exp 1990	Elkins-Sinn, Inc.	400	398	(375, 420)	4.02
WWII	Strong, Cobb & Co, Inc.	2,000	1,475	(1,385, 1,565)	4.85

enhanced if maintained at low pH values and temperatures. Further testing is needed to determine clinical effect. Since dosing of atropine is based on clinical parameters and there is no known toxicity from expired agents that maintain clarity, sterility, and solubility, we would consider using expired atropine during a mass casualty event.

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