

Physical Compatibility and Chemical Stability of a Concentrated Solution of Atropine Sulfate (2 mg/mL) for Use as an Antidote in Nerve Agent Casualties

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INTRODUCTION

In light of tragic events that have occurred, such as the sarin gas attack in the Tokyo subway and the attack on the World Trade Center on September 11, 2001, it has become apparent that we cannot avoid the threat of terrorism and that there is a great need for us to be prepared.¹ Nerve agents, which include sarin, soman, tabun, and other organophosphates, are named most often as threats for chemical terrorism.² One way to be prepared for such threats is to stockpile appropriate quantities of the antidotes. For nerve gas attacks, these consist of atropine (initial dose of 2 mg to 6 mg intramuscular/intravenous), pralidoxime, and diazepam.³ In Canada, atropine is commercially available in several strengths up to 1 mg/mL but is not normally stocked. The most appropriate of these to stockpile is the 0.6 mg/mL ampules, which are commonly used in the majority of hospitals. This would allow for rotation of the stock, which would minimize the cost of stockpiling. A cost-effective alternative to this is preparation of concentrated atropine 2 mg/mL solution from atropine powder.^{4,5} This form is most convenient for administration to exposed individuals. The cost of the powder is minimal and storage space is not an issue.

Atropine sulfate is reported to be stable for 3 months at room temperature when prepackaged in Tubex syringes.⁶ When

ABSTRACT

Atropine is one of the preferred antidotes for nerve gas exposure. Effective therapy requires large doses. Stockpiling of atropine in forms currently available commercially can be costly and can require a large storage area. Other problems in using the commercial product are the time required to break the large number of ampules needed for treatment, and the large volume of medication that must be administered. A preparation of concentrated atropine for injection from atropine powder is the ideal solution, but the stability of this preparation is unknown. A formulation for concentrated atropine sulfate solution (2 mg/mL) was developed using pharmaceutical-grade powder. The solution was prepared in isotonic saline solution and packaged in polypropylene syringes, which were sealed with syringe caps. Assessment of physical stability under several storage conditions was based on clarity, color, and pH monitoring. Chemical stability of the preparation was determined by using a stability-indicating high-performance liquid chromatographic method. All solutions remained clear and colorless, and the pH changed only slightly over the study period. The potency of the atropine sulfate solution remained above the *United States Pharmacopeia* limit of 93% for the duration of the study. The solutions were physically compatible and chemically stable when stored for 28 days at 35°C and exposed to light, 364 days at 23°C and exposed to light, or 364 days at 5°C and protected from light. Concentrated solutions of atropine sulfate can be compounded quickly and easily by using commercially available pharmaceutical-grade powder as the active ingredient. The extended shelf-life allows the preparation to be readily available in the field as required.

atropine solution was packaged in polypropylene syringes, spectrophotometric and potentiometric analyses found the solution to be stable for 4 weeks when stored at room temperature and protected from light.⁷

The commercially available atropine sulfate product (1 mg/mL) has a pH of approximately 3 to 6.5.⁶ A pH of 3.5, which is reported to have minimum degradation, was chosen for the concentrated preparation.⁸

The objectives of this study were twofold: (1) to develop a formulation for a concentrated atropine sulfate solution (2 mg/mL) that could be prepared easily from pharmaceutical-grade powder, and (2) to determine the physical compatibility

and chemical stability of the solution when packaged in polypropylene syringes and stored for 28 days at 35°C and exposed to light, 364 days at 23°C and exposed to light, or 364 days at 5°C and protected from light. The goal was development of a cost-effective, easily prepared atropine sulfate formulation that can be readily available in the field when needed.

MATERIALS AND METHODS

All chemicals and reagents were of *United States Pharmacopeia*, high-performance liquid chromatographic (HPLC), or American Chemical Society laboratory grade. No chemicals were purified any further before use.

EQUIPMENT

The HPLC system consisted of an isocratic pump (Model LC-10AT; Shimadzu Corporation, Kyoto, Japan), a photodiode array detector (Model SPD-M6A; Shimadzu Corporation) set at 230 nm, and an auto-injector (Model SIL-10A_{XL}; Shimadzu Corporation), which injected 50- μ L samples. The column used was a 5 micron, 4.6 $\times$ 150 mm C18 column (Luna; Phenomenex, Torrance, California). The flow was set at 1.0 mL/minute. Class-VP software (Version 4.2; Shimadzu Corporation) was used for data collection and analysis.

Color and clarity were monitored against a black/white background. A calibrated pH meter was used to measure the pH (Model Accumet 25; Fisher Scientific Ltd., Nepean, Canada) at every sampling interval.

Chromatographic Conditions

The assay was conducted according to a modified *United States Pharmacopeia* HPLC method.⁹ The modification was a reduction in the organic phase from 5% to 3%. Parahydroxybenzoic acid 0.1 mg/mL (Lot 12819C0P0; Aldrich Chemical Company, Inc., Milwaukee, Wisconsin) was used as an internal standard.

Assay Validation

The assay method was proven to be stability indicating by monitoring forcibly degraded samples for interfering peaks. Samples subjected to degradation by addition of an acidic (pH $\approx$ 1.2), alkaline (pH $\approx$ 12.8), or oxidizing (0.1 mL of 30% hydrogen peroxide per 10 mL) substance were monitored for 698 hours. The HPLC method was further validated by preparing a standard curve over the range of 0.05 mg/mL to 0.40 mg/mL and determining a coefficient of variance for intraday and interday samples. Sensitivity and accuracy, based on sample recovery, also were determined. Multi-wavelength (220 nm and 230 nm) and ultraviolet (UV) spectral analysis (200 nm to 350 nm) were used to test peak purity.

Sample Preparation

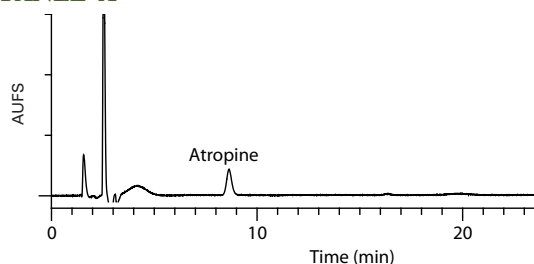
A stock solution of atropine sulfate was prepared by dissolving pharmaceutical-grade powder (Lot 80241; PCCA, London, Canada) in a sufficient amount of sodium chloride 0.9% solution (Lot W3E23A1; Baxter Corporation, Toronto, Canada) to make a 2-mg/mL solution. The pH of the solution was adjusted to approximately 3.5 by adding 0.1 N sulfuric acid. This solution was filtered through a 0.2-micron mixed cellulose esters membrane into an appropriate sterile evaluated bottle. Polypropylene syringes, 5 mL (Becton Dickinson and Company, Franklin Lakes, New Jersey), then were filled aseptically with 3 mL of solution. Sealed syringes were stored for 28 days at 35°C and exposed to light, 364 days at 23°C and exposed to light, or 364 days at 5°C and protected from light. Samples were collected on days 0, 7, 14, and 28 (35°C), or days 0, 28, 56, 84, 168, and 364 (23°C and 5°C), and stored at -70°C for analysis at a later date.

RESULTS AND DISCUSSION

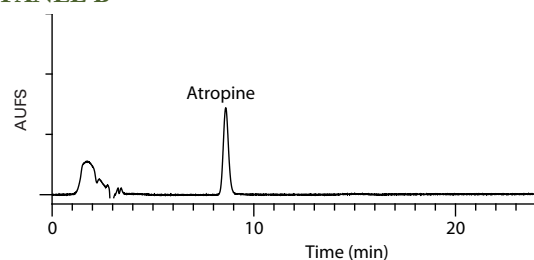
Figure 1 shows how degradation samples created under acidic, alkaline, and oxidative conditions were successfully monitored without interference with the parent peak. Exposure of the atropine to acidic, alkaline, and oxidative conditions validated the stability-

FIGURE 1. Sample chromatograms of atropine sulfate and degradation products.

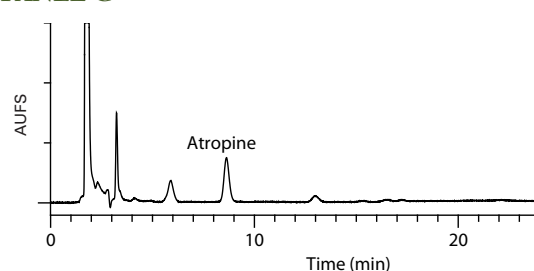
PANEL A



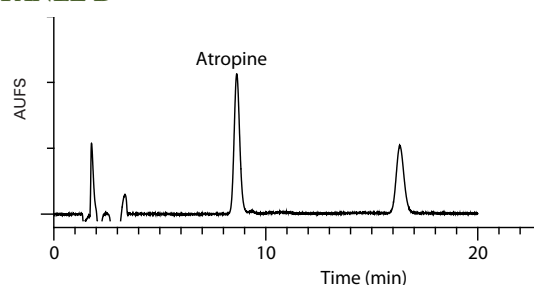
PANEL B



PANEL C



PANEL D



Panel A represents acid-degraded atropine after 698 hours. Panel B represents alkali-degraded atropine after 698 hours. Panel C represents an oxidized sample after 384 hours. Panel D represents atropine after 364 days storage at 23°C and exposed to light.

AUFS = absorbance units full scale

TABLE 1. Stability of Atropine Sulfate 2 mg/mL in Isotonic Saline Solution in Polypropylene Syringes.

Initial Concentration (mg/mL) ^a	Day	Percentage of Initial Concentration Remaining ^a		
		5°C	23°C	35°C
1.9 ± 0.01	7	ND	ND	101.0 ± 1.1
	14	ND	ND	100.1 ± 0.7
	28	99.9 ± 0.8	ND	102.1 ± 0.4
	56	100.0 ± 0.7	ND	ND
	84	100.8 ± 0.8	ND	ND
	168	100.4 ± 0.6	ND	ND
	364	99.4 ± 0.5	ND	ND
1.9 ± 0.03	7	ND	ND	ND
	14	ND	ND	ND
	28	ND	102.1 ± 0.2	ND
	56	ND	102.4 ± 0.5	ND
	84	ND	102.4 ± 0.7	ND
	168	ND	102.2 ± 1.0	ND
	364	ND	103.0 ± 0.5	ND

^aMean ± standard deviation (*n* = 6)
ND = not determined

indicating nature of the assay method. The atropine was sensitive to degradation under acidic, alkaline, and oxidative conditions.

The standard curve was linear over the range of 0.05 mg/mL to 0.40 mg/mL ($R^2 = 0.9985$). The coefficients of variance of intraday and interday testing were 0.7% and 1.8%, respectively. The sensitivity of the method was calculated to be 203.4 ng. Sample recovery was determined to be 99.7% ± 0.52 from five separate samples. Multi-wavelength and UV spectral analysis confirmed all atropine peaks to be pure.

None of the samples underwent change in color or clarity over the course of the study. There were slight increases in pH, of 1.08, 0.77, and 0.41 at 35°C, 23°C, and 5°C, respectively.

The atropine solution showed no signs of chemical degradation at any of the storage temperatures over the course of the study. The percentages of initial concentration remaining were 102.1% after 28 days storage at 35°C and exposed to light, 103.0% after 364 days storage at 23°C and exposed to light, and 99.4% after 364 days storage at 5°C and protected from light, as shown in Table 1.

CONCLUSION

A concentrated atropine sulfate solution 2 mg/mL can be prepared easily and economically from pharmaceutical-grade powder in isotonic saline solution and packaged in sealed polypropylene syringes. Our results show that this concentrated atropine sulfate solution was stable for 28 days when stored at 35°C and exposed to light, for 364 days when stored at 23°C and exposed to light, or for 364 days when stored at 5°C and protected from light. This information allows prefilled syringes, with an acceptable shelf life, to be prepared in a quick and economical manner for immediate use.

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