

Stability and Sterility of Extemporaneously Prepared 0.01% Atropine Ophthalmic Solution in Artificial Tears and Balanced Salt Solution

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ABSTRACT

Objective: The aim of this study was to investigate the physicochemical and microbiological stability of extemporaneously prepared 0.01% atropine ophthalmic solution in unopened eyedropper and in simulated use condition.

Materials and Methods: Two formulations of 0.01% atropine solutions, atropine in artificial tear and atropine in balanced salt solution (BSS), were prepared using 0.5 mL insulin syringes. In unopened conditions, 0.01% atropine solutions were stored for six months at refrigerated temperature (2-8°C) or room temperature (25±2°C). Visual inspection, atropine quantification, pH measurements, and sterility assay were analyzed at baseline, and every month for six months. In simulated use condition, 0.01% atropine solutions stored at refrigerated and room temperature were analyzed at 0, 15 and 30 days.

Results: In unopened conditions, both of 0.01% atropine formulations stored at refrigerated temperature showed satisfactory stability. Atropine remained within 90% to 110% of the initial concentration up to six months. Under room temperature, both formulations of atropine were less than 90% of their initial value after 4 months storage. In simulated use condition, atropine concentration was within 90% to 110% of initial value after 30 days at refrigerated and room temperature. All atropine solutions prepared in artificial tear and BSS were free from bacterial contamination throughout the study. No alteration of physical appearance (i.e., precipitation, discoloration) was observed, and pH values also remained nearly unchanged.

Conclusion: Both formulations of 0.01% atropine are physicochemically stable for up to 6 months when kept unopened in refrigerator, and for 1 month at refrigerated and room temperatures in simulated use condition.

Keywords: Myopia; atropine; stability; sterility; artificial tear; balanced salt solution (Siriraj Med J 2022; 74: 91-99)

INTRODUCTION

Myopia is an eye disorder and is the principal type of refractive error. Previous population-based studies have reported that the prevalence rates of myopia are highest in East Asian populations.^{1,2} Their findings showed that 80% of schoolchildren in Taiwan, Hong Kong, and China,

as well as up to 96% of schoolchildren in South Korea, suffered from myopia.³ It is estimated that myopia will affect nearly 5 billion people by 2050.^{2,4,5} Currently, there are many methods for controlling myopia progression, such as spectacles, contact lens, and pharmaceutical strategies. Most of the studies in this field use atropine

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eye drops to reduce the rate of myopia progression in children.⁶

Atropine is a nonselective muscarinic antagonist, it binds to and inhibit muscarinic acetylcholine receptors, producing a wide range of anticholinergic effects. The precise mechanisms underlying the efficacy of atropine in slowing myopia progression are remains unclear. Various hypotheses have been postulated, including the action via muscarinic receptor pathways in the retina, choroid and sclera. These resulting in the prevention of axial elongation, inhibition of scleral proliferation and matrix synthesis. Moreover, atropine may be exerting its effect via other receptors present in the eye.⁷⁻⁹

Most "Atropine for the Treatment of Myopia (ATOM)" studies focus on the efficacy and safety of 1%, 0.5%, 0.1%, and 0.01% atropine in myopic Asian children aged 6-12 years old. Their findings illustrated that 0.01% atropine is effective for retarding myopia with minimal side effects, compared with higher doses of atropine.¹⁰⁻¹³ Recently, the studies of Low-concentration Atropine for Myopia Progression (LAMP) have demonstrated the efficacy and safety of atropine concentrations of 0.05%, 0.025%, and 0.01% in China in children aged 4-12 years old with myopia. All these concentrations drastically reduced the rate of myopia progression without any vision-threatening side effects.¹⁴ Generally, 0.01% atropine is the most common strategy for managing childhood myopia and is widely used all over the world, including in Asian countries, such as Singapore, Taiwan, China, and Thailand.² The treatment period usually lasts for at least 2 years, and may take longer if myopia progression persists.¹⁵

Since 0.01% atropine ophthalmic solution is not commercially available in Thailand, eye drops are prepared by ophthalmologists or hospital pharmacists. The 1% commercial atropine is diluted with 0.9% sodium chloride solution, balanced salt solution, or various brands of artificial tears depending on the discretion of the ophthalmologist. Long-term treatment with atropine is required for myopia control, and hence a longer shelf-life is necessary to extend the follow-up intervals for patients. However, there is little data concerning the long-term stability of low-dose atropine eye drops. Only two studies have been published demonstrating that 0.01% atropine in 0.9% sodium chloride solution with or without preservatives is stable for six months in an unopened container, both at room temperature and refrigerated temperature.^{16,17} However, there are no studies on the stability of 0.01% atropine eye drops prepared in artificial tears (with preservatives) or balanced salt solution (without preservatives). The lack of long-term

stability and sterility data limits the conservation period of these preparations. Consequently, the aim of this study was to determine the long-term chemical, physical, and microbiological stability of extemporaneously prepared atropine in artificial tears containing preservatives and in balanced salt solution at refrigerated and room temperature. The chemical, physical, and microbiological stability of both formulations were also tested in simulated use conditions.

MATERIALS AND METHODS

Reagents and materials

Atropine sulfate monohydrate, the reference standard of atropine, and scopolamine hydrobromide, the internal standard for atropine, were obtained from The United States Pharmacopeial Convention, Inc., USA. 1% Atropine sulfate solution was obtained from Alcon-Couvreur, Belgium. Balanced salt solution (BSS) was obtained from Alcon Research LLC, USA. Hydroxypropyl methylcellulose (HPMC), an artificial tears solution with sodium perborate as a preservative, was obtained from Silom Medical Co., Ltd., Thailand. LC/MS grade acetonitrile and formic acid were obtained from Scharlau, Barcelona, Spain. HPLC-grade methanol was obtained from Fisher Scientific UK, the United Kingdom. Type I water was produced using a Milli-Q water purification system from Millipore Corporation, USA.

0.01% Atropine eye drops preparation

The preparation processes were undertaken by scientists in a clean room of class 1.0×10⁵ (air cleanliness level of a maximum of 2.93×10⁴ particles (≥0.5 μm) per cubic meter). Two formulations of 0.01% atropine ophthalmic solutions were prepared aseptically using a 0.5 mL insulin syringe:

- Atropine in preserved artificial tears (HPMC), prepared by dissolving 0.1 mL of 1% atropine sulfate solution into 10 mL artificial tears.
- Atropine in balanced salt solution, prepared by dissolving 0.15 mL of 1% atropine sulfate solution into 15 mL balanced salt solution (BSS).

Clear low-density polyethylene commercial eyedroppers of HPMC and BSS were used as the containers in this study.

Study design

The stabilities of the 0.01% atropine ophthalmic solutions were studied at refrigerated temperature (2-8 °C) or room temperature (25±2 °C). The durations of the study for the unopened eyedroppers and in simulated use conditions were 6 months and 1 months, respectively.

In short, there were 4 subgroups in each study (for the unopened eyedroppers and simulated use conditions) as shown below:

- (i) 0.01% atropine in HPMC at refrigerated temperature,
- (ii) 0.01% atropine in HPMC at room temperature,
- (iii) 0.01% atropine in BSS at refrigerated temperature,
- (iv) 0.01% atropine in BSS at room temperature.

The eyedroppers stored at room temperature were kept on the shelf, protected from light in their commercial packages at 50%±10% residual humidity (RH).

Physicochemical and microbiological stability of the 0.01% atropine ophthalmic solutions in simulated use conditions

At day 0, 60 eyedroppers with two formulations of 0.01% atropine solutions were prepared, with 15 eyedroppers for each subgroup (i–iv). For illustration purposes, subgroup (i) with a total number of 15 eyedroppers was used as an investigation process example. Each of the 15 eyedroppers was emitted daily (1 drop of the 0.01% atropine solutions), that is, a drop was squeezed out of the eyedropper and collected for analysis instead of being dropped into the eye. Out of the 15 eyedroppers, 10 eyedroppers were obtained for visual inspection and sterility assay. Next, 5 eyedroppers were tested at day 0, 15, and discarded. Another 5 eyedroppers were tested at day 0 and 30. It is important to note the reason why the eyedroppers were discarded after the sterility assay on day 15. Namely, subgroups (i) and (ii) both had an approximate volume of 10 mL, while the sterility assay required at least 4 mL. Hence, after the daily emission and two sterility assays, there would be an insufficient amount of solution remaining for another sterility assay and so these were discarded. The remaining 5 eyedroppers from the 15 totals were used for the atropine quantification and pH measurements at days 0, 15, and 30.

After completing the 1-month study under simulated use conditions, further investigations were planned, with the aim to extend the experimental period of both formulations at refrigerated temperature to 2 months. There were 2 subgroups of eyedroppers here: (i) 6 eyedroppers of 0.01% atropine in HPMC at refrigerated temperature, and (ii) 6 eyedroppers of 0.01% atropine in BSS at refrigerated temperature. These two subgroups were investigated in the exact same manner as in the 1-month study. Out of the 6 eyedroppers in each subgroup, 4 eyedroppers were obtained for visual inspection and sterility assay at day 0, one at another time point (days 15, 30, 45, or 60), and one discarded ($n = 1$ for each time point/subgroup). The remaining 2 from the 6 eyedroppers were used for atropine quantification and pH measurements at days 0, 15, 30, 45, and 60 ($n = 2$ for each time point/subgroup).

Physicochemical and microbiological stability of 0.01% atropine ophthalmic solutions in the unopened eyedroppers

In total, 120 eyedroppers of 0.01% atropine solutions were prepared, comprising 30 eyedroppers for each subgroup: atropine in HPMC at refrigerated temperature, atropine in HPMC at room temperature, atropine in BSS at refrigerated temperature, and atropine in BSS at room temperature. In each subgroup, 5 unopened eyedroppers were used for the analysis at days 30, 60, 90, 120, 150, and 180 ($n = 5$ for each time point/subgroup). Each eyedropper was subjected to the following analyses: visual inspection, atropine quantification, pH measurement, and sterility assay. The baseline values for atropine quantification, pH measurement, and the sterility assay were obtained from the studies of the 0.01% atropine ophthalmic solutions under the simulated use conditions.

Analyses

Quantification of atropine

The liquid chromatography with tandem mass spectrometry (LC-MS/MS) method was applied for quantitative analysis of the extemporaneously prepared atropine solution. LC-MS/MS analysis was performed using an Acquity Ultra Performance LCTM (Waters, Co., Ltd. USA) coupled to a Quattro Premier XE Mass Spectrometer (Micromass Technologies, UK) equipped with an electrospray interface. For data acquisition and processing, a MassLynx 4.1 SCN627 system (Micromass Technologies, UK) was used.

Scopolamine hydrobromide was used as an internal standard (IS). The chromatographic separation of atropine and the internal standard was performed using a Kinetex C18 column (50×2.10 mm, 1.7 μ m; Phenomenex Ltd., USA). The mobile phase was an 85:15 (v/v) mixture of 0.1% (v/v) formic acid and acetonitrile in an isocratic elution mode over a 2 min total run time. The flow rate was 0.3 mL/min and the column temperature were set at 30±5 °C. The injection volume was 1 μ L. MS analyses were carried out using the multiple reaction monitoring (MRM) mode with positive electrospray ionization (ESI+). The mass transition ion-pair was selected as m/z 290.1 to 124.1 for atropine and m/z 304.1 to 138.1 for the IS.

Validation of this method was performed according to the International Conference on Harmonisation (ICH) guidelines.¹² Linearity was determined by preparing one calibration curve daily using six concentrations of atropine (50, 100, 150, 200, 300, and 400 ng/mL), obtained from atropine standard solution diluted in diluent solutions (methanol and Milli-Q water at a ratio of 1:1, v/v). The influence of different weighting factors ($1/x$ and $1/x^2$) on the sum of the percentage relative error

was evaluated and the results were compared with an unweighted calibration curve. Accuracy was tested by spiking the atropine reference standard with the atropine test sample (at a concentration of 200 ng/mL) to obtain three concentration levels, namely 80%, 100% and 120%, of the test sample concentration. The accuracy was evaluated on the basis of the calculated recovery values, and the results should be found within the range of 95%-105%. The precision of the methods was determined in terms of the intra-day precision (repeatability) and intermediate precision (within-laboratory reproducibility). The intra-day precision was assessed by injecting six replicates of three different concentrations of atropine standard solutions (100, 200, and 300 ng/mL) on the same day. The intermediate precision was determined by injecting the same solutions for three consecutive days. The intra-day and intermediate precisions are expressed as the relative standard deviation (RSD, %). A value of less than 5% was acceptable for both RSDs.

For sample preparation, 0.01% atropine solution from each eyedropper was diluted with the diluent solution to obtain a theoretical concentration of 200 ng/mL. A 100 μ L aliquot of diluted atropine was transferred into a 1.5 mL micro tube and mixed with 20 μ L internal standard solution at a concentration of 1,000 ng/mL. The micro tubes were thoroughly mixed by vortex mixing for 10 seconds. Then, 1 μ L of the mixed solution was collected and transferred into an autosampler vial and submitted to LC-MS/MS analysis.

In the chemical stability assessment, the baseline concentration (day 0) was defined as 100% and the subsequent concentrations of each time point were calculated as percentages of the initial concentration. Acceptance criteria for the stability were defined as 90%-110% of the baseline concentration (including the limit of a 95% confidence interval of the measures).^{13,14}

Visual inspection and pH measurements

During the study period, the physical appearance of the solutions was examined when the samples were taken from each eyedropper for the sterility assay. An approximately 4 mL sample was dispensed from each eyedropper into a 5 mL sterilized tube. Before sending the sample for the sterility assay, the atropine solutions were visually inspected under white light. The transparency, color, and presence of visible particles or haziness were noted.

For pH measurement, a 0.5 mL aliquot of 0.01% atropine from each sample was transferred into a 2.0 mL micro tube. Hand-held pH testing was performed on a SevenCompact S220 pH/ion meter with an InLab

Micro Pro-ISM electrode (Mettler Toledo, Switzerland), which was calibrated at 25 °C in pH 4.01, 7.00, and 9.21 buffer solutions (Mettler Toledo, Switzerland). The pH change was considered acceptable if it did not vary by more than one pH unit from the initial value.¹⁴

Sterility assay

The sterility assay was carried out by the Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University, in line with the United States Pharmacopeia (USP) for pharmaceutical microbiology testing.¹⁵ First, 4 mL of 0.01% atropine solution from each eyedropper was aseptically taken and sent to the Department of Microbiology in a 5 mL sterilized tube for the sterility assay, using a direct inoculation method. Each sample was transferred directly to a fluid thioglycolate medium and soybean casein digest medium, and then incubated at 30-35 °C and 20-25 °C, respectively, for 14 days. The culture medium was then carefully examined for microbial growth.

RESULTS

Quantification of atropine

The retention times were 1.02 min for atropine and 0.70 min for scopolamine. The method was shown to be selective, as no interferences were observed at the retention times corresponding to 0.01% atropine in the artificial tears or in the BSS (Figs 1A-E). The calibration curve was linear for the concentrations ranging from 50-400 ng/mL and the determination coefficient R^2 was greater than 0.999 (Fig 1F). The weighting factor of $1/x$ was selected, since it was the one that reproduced the least sum of percentage relative errors (%RE). This method showed acceptable accuracy as the percentage recovery ranged from 99.42%-102.18% in the three different concentrations of atropine standard solutions. The precision was satisfactory, with the RSD of the intra-day and intermediate precision ranging from 1.05%-2.99% and 1.66%-2.94%, respectively.

Chemical stability

In the simulated use study, both formulations demonstrated chemical stability (concentration range between 90%-110% of the initial concentration) for up to 30 days at room temperature and 60 days at refrigerated temperature. The concentrations of the 0.01% atropine solutions stored at room temperature were between 97.60%-99.44% of the initial concentrations in HPMC and 102.26%-106.93% in BSS, and the 95% confidence interval was a maximum of +4.34%. For the 0.01% atropine solutions stored in refrigerator, the concentrations were

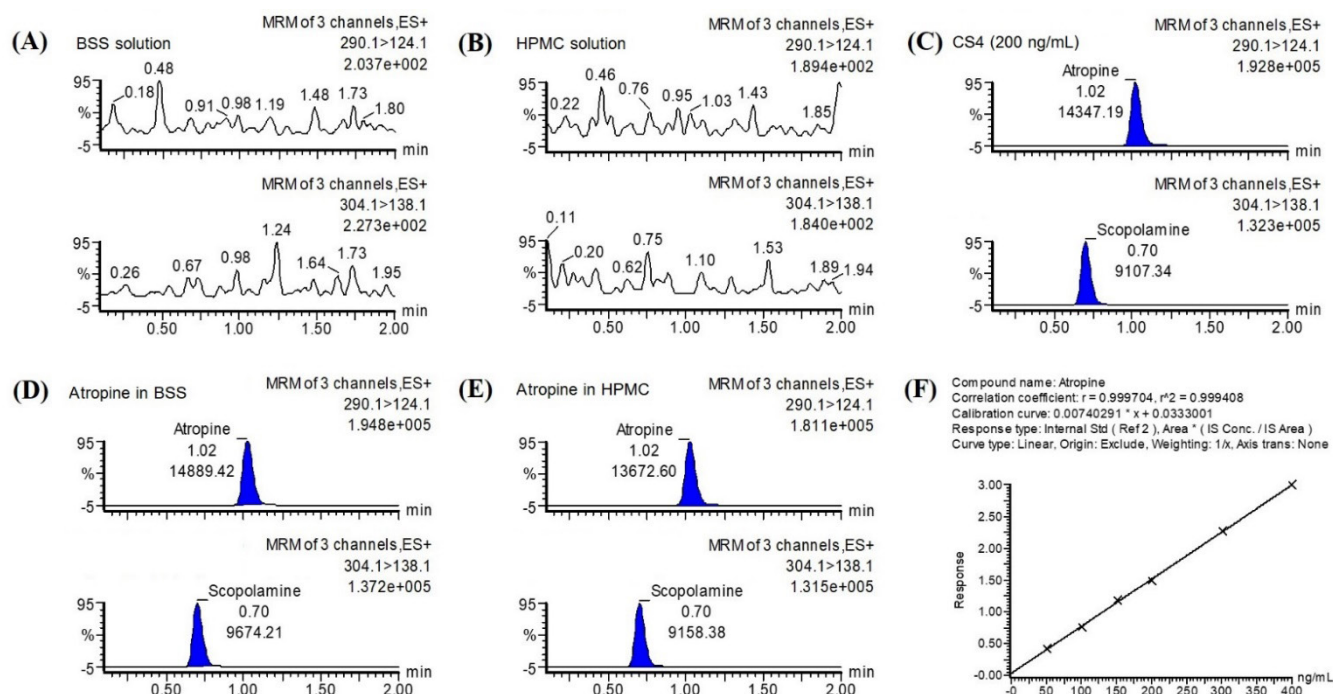


Fig 1. Chromatograms of: (A) BSS solution, (B) HPMC solution, (C) atropine reference standard spiked at 200 ng/mL, (D) atropine test sample in BSS at 200 ng/mL, and (E) atropine test sample in HPMC at 200 ng/mL. (F) Calibration standard of atropine.

between 96.57%-105.80% of the initial concentration in HPMC and 97.40%-104.50% in BSS, and the 95% confidence interval was a maximum of $\pm 3.70\%$. The chemical stability results for the simulated use conditions are presented in Table 1.

In the unopened study, the 0.01% atropine in HPMC and BSS stored at refrigerated temperature remained stable up until 180 days of storage. The concentrations were between 93.61%-102.99% of the initial concentrations in

HPMC and 92.66%-105.11% in BSS, with the maximal and the 95% confidence interval at a maximum of $+4.61\%$. At room temperature, the 0.01% atropine solutions were still within an acceptable range for 60 days in HPMC and for 90 days in BSS. The chemical stability results for the unopened study are presented in Table 2. The chemical stability trend for all the conditions are presented in Fig 2.

TABLE 1. Percentage of atropine concentration remaining (mean \pm 95% CI) of 0.01% atropine for each formulation and conservation condition in the simulated use study.

Storage conditions	Solutions	Percentage of atropine concentration remaining (mean \pm 95% CI)				
		Day 0	Day 15	Day 30	Day 45	Day 60
At room temperature (25 \pm 2 °C)	HPMC	100	97.60 \pm 4.10	99.44 \pm 4.34	ND	ND
	(n=5)	(n=5)	(n=5)	(n=5)		
	BSS	100	106.93 \pm 1.15	102.26 \pm 2.72	ND	ND
	(n=5)	(n=5)	(n=5)	(n=5)		
In refrigerator (2–8 °C)	HPMC	100	101.44 \pm 2.20	104.86 \pm 2.47	96.57 \pm 1.53	105.80 \pm 3.53
	(n=7)*	(n=7)	(n=7)	(n=7)	(n=2)	(n=2)
	BSS	100	104.50 \pm 2.29	103.73 \pm 2.31	102.75 \pm 3.70	97.40 \pm 1.87
	(n=7)*	(n=7)	(n=7)	(n=7)	(n=2)	(n=2)

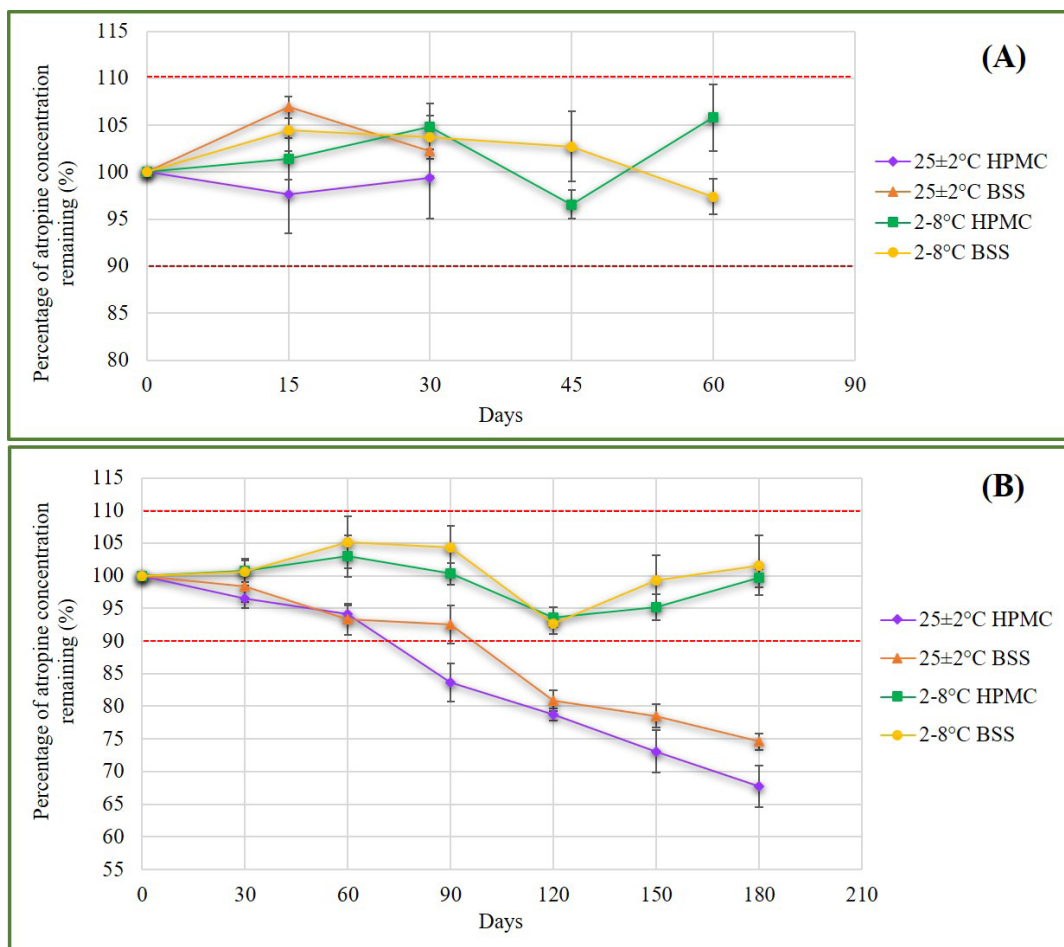
* n = 5 in the 1-month study and n = 2 in the 2-month study.

ND = not determined.

TABLE 2. Percentage of atropine concentration remaining (mean \pm 95% CI) of 0.01% atropine for each formulation and conservation condition in the unopened eyedroppers.

Storage conditions	Solutions	Percentage of atropine concentration remaining (mean \pm 95% CI)						
		Day 0	Day 30	Day 60	Day 90	Day 120	Day 150	Day 180
At room temperature (25 \pm 2 °C)	HPMC	100	96.51 \pm 1.46	94.07 \pm 1.36	83.67 \pm 2.94	78.71 \pm 0.92	73.09 \pm 3.21	67.74 \pm 3.15
	(n=30)	(n=5)*	(n=5)	(n=5)	(n=5)	(n=5)	(n=5)	(n=5)
	BSS	100	98.32 \pm 2.36	93.32 \pm 2.38	92.54 \pm 2.92	80.90 \pm 1.61	78.53 \pm 1.84	74.57 \pm 1.25
	(n=30)	(n=5)*	(n=5)	(n=5)	(n=5)	(n=5)	(n=5)	(n=5)
In refrigerator (2–8 °C)	HPMC	100	100.71 \pm 1.69	102.99 \pm 3.19	100.30 \pm 1.62	93.61 \pm 1.53	95.17 \pm 1.95	99.62 \pm 1.38
	(n=30)	(n=5)*	(n=5)	(n=5)	(n=5)	(n=5)	(n=5)	(n=5)
	BSS	100	100.57 \pm 2.04	105.11 \pm 3.93	104.27 \pm 3.32	92.66 \pm 1.63	99.34 \pm 3.78	101.58 \pm 4.61
	(n=30)	(n=5)*	(n=5)	(n=5)	(n=5)	(n=5)	(n=5)	(n=5)

* Baseline values of the atropine concentration were obtained from the studies of 0.01% atropine ophthalmic solutions in 1-month simulated use conditions.

**Fig 2.** (A) Percentage of atropine concentration remaining (mean \pm 95% CI) for each formulation and conservation condition in the simulated use study. (B) Percentage of atropine concentration remaining (mean \pm 95% CI) for each formulation and conservation condition in the unopened eyedroppers.

Visual inspection and pH measurements

All the samples that were sent for the sterility assay (from 168 eyedroppers) remained clear and colorless, with no precipitation or visible particles observed during the study period in all the study conditions. The pH of all the samples showed insignificant changes throughout the study. For both formulations, when stored at refrigerated temperature and room temperature, the pH did not vary by more than 0.20 and 0.23 pH units from the initial value for the simulated use conditions and unopened

conditions, respectively (Table 3 and 4).

Sterility assay

The results indicated that the sterility was preserved in all the samples, i.e., for every subgroup in the simulated use and unopened conditions. No microbiological growth was observed when incubated for 14 days at 30–35 °C in fluid thioglycolate medium and at 20–25 °C in soybean casein digest medium.

TABLE 3. The pH value of 0.01% atropine sulfate for each formulation and conservation condition in the simulated use study.

Storage conditions	Solutions	pH value (mean ± SD)				
		Day 0	Day 15	Day 30	Day 45	Day 60
At room temperature (25±2 °C)	HPMC	6.93±0.02	6.90±0.04	6.92±0.01	ND	ND
	(n=5)	(n=5)	(n=5)	(n=5)		
	BSS	7.02±0.04	6.82±0.08	6.88±0.04	ND	ND
	(n=5)	(n=5)	(n=5)	(n=5)		
In refrigerator (2–8 °C)	HPMC	6.95±0.05	6.86±0.05	6.93±0.02	6.89±0.02	6.90±0.03
	(n=7)*	(n=7)	(n=7)	(n=7)	(n=2)	(n=2)
	BSS	6.98±0.08	6.92±0.06	6.90±0.06	6.81±0.05	6.78±0.07
	(n=7)*	(n=7)	(n=7)	(n=7)	(n=2)	(n=2)

* n = 5 in the 1-month study and n = 2 in the 2-month study.

ND = not determined.

TABLE 4. The pH value of 0.01% atropine sulfate for each formulation and conservation condition in the unopened eyedroppers.

Storage conditions	Solutions	pH value (Mean ± SD)						
		Day 0	Day 30	Day 60	Day 90	Day 120	Day 150	Day 180
At room temperature (25±2 °C)	HPMC	6.93±0.02	6.91±0.01	6.92±0.01	6.91±0.01	6.83±0.00	6.85±0.01	6.90±0.02
	(n=30)	(n=5)*	(n=5)	(n=5)	(n=5)	(n=5)	(n=5)	(n=5)
	BSS	7.02±0.04	6.86±0.05	6.89±0.02	6.81±0.07	6.79±0.10	6.81±0.06	6.83±0.05
	(n=30)	(n=5)*	(n=5)	(n=5)	(n=5)	(n=5)	(n=5)	(n=5)
In refrigerator (2–8 °C)	HPMC	6.97±0.03	6.90±0.01	6.88±0.03	6.92±0.01	6.87±0.01	6.91±0.03	6.92±0.03
	(n=30)	(n=5)*	(n=5)	(n=5)	(n=5)	(n=5)	(n=5)	(n=5)
	BSS	7.02±0.04	6.88±0.04	6.85±0.06	6.85±0.04	6.85±0.02	6.86±0.05	6.88±0.05
	(n=30)	(n=5)*	(n=5)	(n=5)	(n=5)	(n=5)	(n=5)	(n=5)

* Baseline values of the pH measurement were obtained from the studies of 0.01% atropine ophthalmic solutions in the 1-month simulated use conditions.

DISCUSSION

To assess the accuracy of the extemporaneous prepared ophthalmic solutions in clinical practice, this study investigated the accuracy of using 0.5 mL and 1 mL insulin syringes compared to an auto pipette for the extemporaneous preparation. The 0.01% atropine solutions were prepared using an auto pipette, and 0.5 mL and 1 mL insulin syringes. Here, 0.1 mL of 1% atropine sulfate was mixed with 9.9 mL of HPMC and 0.15 mL of 1% atropine sulfate was mixed with 14.85 mL of BSS ($n = 5$ for each apparatus in each formulation). The preparation using the 1 mL insulin syringe was the same as for the preparation using the 0.5 mL insulin syringe. The mean concentration of atropine in HPMC compared to the expected concentration ranged from 98.24%-104.37%, 100.25%-102.91%, and 154.29%-157.05% for the auto pipette, and the 0.5 mL and 1 mL insulin syringes, respectively. The mean concentration of atropine in BSS ranged from 98.00%-103.03%, 100.01%-104.79%, and 140.63%-145.89% for the auto pipette, and the 0.5 mL and 1 mL insulin syringes, respectively. In this study, a 0.5 mL insulin syringe was then used in the preparation process, since it was more accurate than the 1 mL insulin syringe.

In the stability assessment of the ophthalmic solutions, the physicochemical and microbiological stability should be evaluated. Previous recent studies have also focused on the long-term stability of ophthalmic atropine solutions. Saito et al.¹⁶ demonstrated that the physical, chemical, and microbiological stability of 0.01%, 0.10%, 0.25%, and 0.5% atropine in 0.9% sodium chloride solution were maintained for at least 6 months when stored unopened in polyethylene bottles at 25 °C or 5 °C. Berton et al.¹⁷ showed that 0.01% atropine in 0.9% sodium chloride solutions with and without antimicrobial preservative were physicochemically stable for 6 months when stored unopened in low-density polyethylene bottles at 25 °C. The aim of our study was to investigate the long-term stability of 0.01% atropine in HPMC and BSS when stored unopened at room temperature (25 °C) or at refrigerated temperature (5 °C), and the stability of the 0.01% atropine solutions in a simulated use condition for up to 2 months.

In the simulated use study, 0.01% atropine in HPMC and BSS demonstrated physicochemical and microbiological stability for up to 30 days at room and refrigerated temperature. For the 2-month extension study at refrigerated temperature, 0.01% atropine in HPMC and BSS also maintained its physicochemical and microbiological stability throughout the study period.

In the unopened conditions, 0.01% atropine in HPMC

and BSS stored at refrigerated and room temperature showed both physical and microbiological stability over 6 months. The pH values remained nearly constant, and no visual changes or microbial contamination were observed over the study period. Regarding the chemical stability, the mean atropine concentrations in HPMC and BSS remained well within 90%-110% of the initial concentration for 6 months at refrigerated temperature. However at room temperature, the mean atropine concentrations in HPMC and BSS were considered to be at an acceptable level of stability for only 2 and 3 months, respectively. These results supported the effect of temperature on the chemical stability. The differences in chemical stability at room temperature between our study and previous studies¹⁶⁻¹⁸ are particularly related to the formulation. In previous studies, atropine was mostly prepared in 0.9% sodium chloride solution with a pH value of 5.3-6.2, compared to the pH value ranging from 6.8-7.0 for atropine in HPMC and BSS. The stability of atropine sulfate solution is enhanced in acidic conditions, as it has a lower degree of hydrolysis. Atropine sulfate solution is most stable at a pH between 3-6, and the ideal storage pH ranges between 3-4.¹⁹⁻²⁰ However, ophthalmic solution should better fall within the ocular comfort range (pH 6.6-7.8) to avoid eye discomfort and irritation.²¹

From the results from the unopened study, the conservation period of 0.01% atropine in HPMC and BSS could be ensured for 6 months when stored at 5 °C and for 2 months when stored at 25 °C.

Hence, the follow-up intervals for patients receiving these formulations could be extended to up to 6 months when a refrigerator is available.

There are some limitations of this study to note. First, the number of samples in the 2-month extension simulated use study was limited. Second, the room temperature in this study was 25 ± 2 °C, which is actually lower than the average indoor temperature in most parts of Thailand. Since the storage temperature significantly affects the chemical stability, the conservation period for 0.01% atropine in HPMC and BSS outside the refrigerator might be, consequently, shorter than in our study.

CONCLUSION

This study demonstrated that 0.01% atropine solution both in HPMC and BSS retained good physicochemical and microbiological stability for 6 months both when left unopened and when stored at 5 ± 3 °C; whereas, the atropine concentration in unopened eyedroppers stored at 25 ± 2 °C generally declined over time. This study also confirmed the physicochemical and microbiological stability of both formulations at 5 ± 3 °C or 25 ± 2 °C for 30

days after opening. In conclusion, the extemporaneously prepared 0.01% atropine ophthalmic solution both in HPMC and BSS could be kept for up to 6 months in the refrigerator at a temperature of 2-8 °C until the bottle is opened.

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Potential conflicts of interest

The authors have no conflicts of interest with the manufacturers or suppliers of any of the products or materials in this study. It is to be noted though that the authors were supported by a Chalermprakiat grant from the Faculty of Medicine Siriraj Hospital, Mahidol University.

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