Original article

Stability and sterility of extemporaneous 0.01% atropine eye drops

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Abstract:

Purpose: *When commercial atropine eye drops are not available, diluted atropine eye drops are used for myopia control. However, long-term stability and sterility of self-prepared 0.01% atropine, even using aseptic techniques, are a concern. The purposes of this study were to investigate the sterility and stability of extemporaneously prepared 0.01% atropine sulfate (atropine) eye drops during a 90-day period.* ***Methods:*** *A 0.01% atropine solution was extemporaneously prepared by diluting commercially available 1% atropine eye drops with two artificial tear solutions, namely hydroxypropyl methylcellulose and polyethylene glycol. The preparations were stored at 4°C and 25°C. Sterility and stability were determined every month on days 0, 30, 60, and 90 after the solutions were prepared. For stability analysis, the amount of atropine was quantified using high performance liquid chromatography and comparing to that at day 0 (percentage of initial content). Bacterial and fungal cultures were performed to evaluate sterility.* ***Results:*** *No bacterial or fungal growth was observed during the study period. The 0.01% content of atropine prepared from hydroxypropyl methylcellulose at 4°C remained constant throughout the study, while those kept at 25°C remained constant only on day 30. The atropine prepared from polyethylene glycol held at 4°C was inconclusive, contrary to those stored at 25°C significantly declined to approximately 20% of the initial amount.* ***Conclusions:*** *The extemporaneous preparations of 0.01% atropine eye drop showed no microorganism growth during the 90-day storage period. The extemporaneously prepared 0.01% atropine ophthalmic solution with hydroxypropyl methylcellulose kept at 4°C remained constant on day 90 of the study. However, preparations in different artificial tear solutions and storage temperatures can affect their stability.*

Keywords: ● *Stability* ● *Sterility* ● *0.01% atropine* ● *Eye drops* ● *Extemporaneous preparation*

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นิพนธ์ต้นฉบับ

ความคงตัวและความปราศจากเชื้อของยาหยอดตา atropine 0.01% สำหรับผู้ป่วยเฉพาะราย

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บทคัดย่อ

วัตถุประสงค์ *ในบริบทที่ไม่มียาผสม 0.01% atropine จำหน่าย การเตรียมยาเฉพาะรายถูกนำมาใช้เพื่อชะลอภาวะสายตาสั้น อย่างไรก็ดีข้อมูลด้านความคงตัวและความปราศจากเชื้อของยาเตรียมเฉพาะราย 0.01% atropine ยังเป็นที่ต้องคอยติดตามแม้จะถูกจัดเตรียมโดยวิธีปราศจากเชื้อปราศจากเชื้อก็ตาม วัตถุประสงค์ของงานวิจัยเพื่อศึกษาถึงความคงตัวและความปราศจากเชื้อของยาเตรียมเฉพาะราย 0.01% atropine ที่ระยะเวลา 90 วัน* ***วิธีการวิจัย*** *ยาเตรียมเฉพาะราย 0.01% atropine ผสมจากยา 1% atropine เข้ากับน้ำตาเทียมสองชนิดที่มีส่วนผสมของ hydroxypropyl methylcellulose และ polyethylene glycol ด้วยวิธีปราศจากเชื้อมาตรฐาน การเก็บยาเตรียมเฉพาะที่ผสมเสร็จแล้วไว้ที่อุณหภูมิ 4 และ 25 องศาเซลเซียส ทำการศึกษาถึงความคงตัวและปราศจากเชื้อ ทุก 30 วัน คือวันที่ 0, 30, 60, 90 หลังผสมยา ความคงตัวของยาศึกษาโดยวิธี high performance liquid chromatography โดยวัดผลเป็นเปอร์เซ็นต์ของปริมาณยาที่คงเหลือเทียบกับวันที่เริ่มผสมยา ส่วนความปราศจากเชื้อวัดจากผลการเพาะเชื้อทั้งชนิดแบคทีเรียและเชื้อรา* ***ผลการศึกษา*** *ตลอดระยะเวลาการศึกษาที่ 90 วัน ไม่พบทั้งเชื้อแบคทีเรียและเชื้อราปนเปื้อนในกลุ่มทดลอง ยาเตรียมเฉพาะราย 0.01% atropine ที่ผสมด้วย hydroxypropyl methylcellulose ที่ถูกเก็บที่อุณหภูมิ 4 องศาเซลเซียสมีความคงตัวของยาตลอดการศึกษา ในขณะที่ยาที่ถูกเก็บที่อุณหภูมิ 25 องศาเซลเซียสมีความคงตัวที่ระยะเวลา 30 วัน ส่วนยาเตรียมเฉพาะราย 0.01% atropine ที่ผสมด้วย polyethylene glycol ที่ถูกเก็บที่อุณหภูมิ 4 องศาเซลเซียสนั้นไม่สามารถสรุปผลได้ และพบว่าหากเก็บที่อุณหภูมิ 25 องศาเซลเซียส มีการลดลงของปริมาณยาลงอย่างมาก เหลือเพียง 21% เมื่อเทียบกับปริมาณยาเริ่มต้น* ***สรุป*** *ยาเตรียมเฉพาะราย 0.01% atropine ในการศึกษานี้ ที่ผสมด้วยยาน้ำตาเทียมมีความปราศจากเชื้อที่ระยะเวลา 90 วันหลังเตรียมยา ยาเตรียมเฉพาะราย 0.01% atropine ที่ผสมด้วย hydroxypropyl methylcellulose ที่ถูกเก็บที่อุณหภูมิ 4 องศาเซลเซียสมีความคงตัวของยาตลอดการศึกษา อย่างไรก็ดีการเตรียมยาเฉพาะราย 0.01% atropine ด้วยน้ำตาเทียมที่มีส่วนประกอบต่างชนิดกันรวมถึงอุณหภูมิที่เก็บรักษาที่ต่างกันมีผลต่อความคงตัวของยา*

คำสำคัญ: ● *ความคงตัว* ● *ความปราศจากเชื้อ* ● *0.01% atropine* ● *ยาหยอดตา* ● *การเตรียมยาเฉพาะราย*

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Introduction

Myopia has become a global public health issue that leads to vision impairment[1]. According to the World Health Organization, the global abundance of myopia is rapidly increasing. Epidemiological estimation reveals that more than 28.3% (1,959 million) of people were myopic in 2010, and over 49.8% (4,758 million) people are predicted to have myopia in 2050, with Asia experiencing the greatest proportion of people with myopia[2,3]. Cochrane's review in 2011[4] indicated several methods to slow down the progression of myopia. Many studies[5-15] have reported that atropine slows down the progression of myopia in children, but it has side effects such as blurred eyes, sensitivity to sunlight, and irritation. A review of the published literature in 2016[16] showed that 0.01% atropine was the most effective concentration and had the least side effects[17-19], making this percentage of atropine popular for slowing down myopic progression in children, administered in the form of eye drops once a day. In Thailand, myopia is a major concern in children. Multimodal approaches, including the use of diluted atropine eye drops, are used to control myopia. However, there is no commercial drug for this type of eyedrop in the market, and there is no standard formula defined in any reference documents. A study conducted in Korea used a 1% atropine eye drop solution (Isopto 10 mg/mL; Alcon, Fort Worth, TX, USA) diluted with 0.9% normal saline, which showed sterility and stability for six months[20]. Our study focused on the following question: if commercial artificial tear eyedrops are used instead of normal saline, will the sterility and stability of the eye drops be maintained? Commercial artificial tear solutions may be better than normal saline as they have preservatives to ensure sterility. We postulate that the demulcent in artificial tear solutions would decrease the irritating, adverse effects of atropine. The data from Thailand's hospitals indicate that they self-mix 0.01% atropine using an aseptic technique, using 1% atropine with commonly available artificial tear solutions to achieve a 0.01% atropine solution. There are many brands and age-of-use of artificial tear solutions, which also have different preservation methods. As such, the hospitals have set the period limit for the usage of the prepared mixtures to 30 days; however, this is not supported by microbiological testing.

Therefore, in this study, the aseptic status of 0.01% atropine solutions self-prepared by the hospitals with artificial tear solutions was evaluated. Given the different methods of care and preservation temperatures, this study provides important data for hospitals in the preparation of eyedrop drugs by defining an optimal storage for 0.01% atropine eye drops to optimize the benefits for patients. If our study results demonstrate the stability and sterility of prepared atropine for a period longer than the current 30 days, scheduled follow-up of treatment for children with myopia may be practical and convenient. This can influence myopia control in the future.

Methods

Preparation of 0.01% atropine eye drops

There were two different types of artificial tear solutions used in this research (Table 1). Each bottle of either solution contains 10 mL of artificial tear solution.

Table 1 Artificial tears formula used in the study

Formula	Preservatives	Demulcent ingredients
A	Sodium perborate	Hydroxypropyl methylcellulose
B	Polyquaternium-1 (Polyquad)	Polyethylene glycol

The 0.01% atropine was mixed for individual patients at room temperature (25 °C) using the aseptic technique currently used in hospitals. This included the use of a clean and sterilized, long laboratory coat, cap, hat, mask, and gloves; a sterile syringe to extract and transfer 0.1 ml of the artificial tear solution from the bottle; and a new sterile syringe to extract 0.1 s of 1% atropine and inject it back into the artificial tear solution bottle. The solution was mixed, and the bottle was tightly closed. Preparation was conducted in a clean room under laminar flow. Each day at 14:00, the researcher opened the bottle and transferred 1 drop of the drug onto the prepared clean dish to simulate the activity of a patient using 0.01% atropine eyedrops daily to slow down myopic progression. The extemporaneous 0.01% atropine was done for 6 sets in either artificial tear formula. Five were experimental groups, and another one was the control group. The other 2 control groups were remained 1% atropine and artificial tear solution.

Storage

The preserved control drug and the 0.01% atropine eye drops obtained by mixing were stored at two temperatures: in a refrigerator at 4°C and at room temperature at 25°C (Table 2). The refrigerator was validated weekly to ensure that temperatures never fell below freezing. This study provided the data logger for continuous temperature monitoring to ensure that investigations were performed at the desired temperature.

Sterility evaluation

The evaluation of the sterility of one set of culture media consisted of bacterial (blood agar, MacConkey agar, and thioglycollate broth) and fungal (Sabouraud agar) culture tests. The culture media were incubated at 30-35°C for bacteria and 20-25°C for fungus. The

Table 2 Four experimental groups used to determine the stability and sterility of extemporaneously prepared 0.01% atropine eye drops

Experimental group	Subset of experimental group	Number of sets
Group 1 : Formulation A (hydroxypropyl methylcellulose) stored at 4 °C	- Hydroxypropyl methylcellulose + atropine (0.01% atropine)	5 sets
	- Hydroxypropyl methylcellulose + atropine (0.01% atropine) (control)	1 set
	- Hydroxypropyl methylcellulose (control)	1 set
	- 1% atropine (control)	1 set
Group 2 : Formulation A (hydroxypropyl methylcellulose) stored at 25 °C	- Hydroxypropyl methylcellulose + atropine (0.01% atropine)	5 sets
	- Hydroxypropyl methylcellulose + atropine (0.01% atropine) (control)	1 set
	- Hydroxypropyl methylcellulose (control)	1 set
	- 1% atropine (control)	1 set
Group 3 : Formulation B (polyethylene glycol) stored at 4 °C	- Polyethylene glycol + atropine (0.01% atropine)	5 sets
	- Polyethylene glycol + atropine (0.01% atropine) (control)	1 set
	- Polyethylene glycol (control)	1 set
	- 1% atropine (control)	1 set
Group 4 : Formulation B (polyethylene glycol) stored at 25 °C	- Polyethylene glycol + atropine (0.01% atropine)	5 sets
	- Polyethylene glycol + atropine (0.01% atropine) (control)	1 set
	- Polyethylene glycol (control)	1 set
	- 1% atropine (control)	1 set

incubation days were days 0, 30, 60, and 90 (after mixing the drug) when the researcher transferred the single drop samples onto the culture media. Petri dishes were then incubated, and results were determined on days 0, 30, 60, and 90.

Stability evaluation

All groups of low-concentration atropine sulfate solutions were evaluated using *high performance liquid chromatography* (HPLC) on days 0, 30, 60, and 90. Three samples from each group were analyzed and compared with the initial amount (day 0), which was considered 100%.

This method was validated according to the International Conference on Harmonisation (ICH) guidelines[21]. 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 95% confidence interval limit of the measures)[22,23].

The need for consent was waived by Institutional Review Board Royal Thai Army Medical Department. The study protocol was reviewed and approved by the Institutional Review Board of the Royal Thai Army Medical Department (approval number: R183b/62_Exp).

Statistical evaluation

Statistical analysis of data was performed using Stata/BE 17. The svy suite of commands in Stata was applied. Repeated measures analysis of variance (ANOVA) was used to detect significant differences in means over time.

Results

Sterility data

All samples showed no evidence of microbial growth during the experimental period at both storage temperatures.

Stability data

The stability analysis results of the remaining atropine content in self-prepared 0.01% atropine solutions are shown in Table 3. The content of self-prepared 0.01% atropine from hydroxypropyl methylcellulose at 4 °C was constant over the period of study. The content of self-prepared 0.01% atropine from hydroxypropyl methylcellulose at 25°C remained constant at only day 30 of the study. The content of self-prepared 0.01% atropine from polyethylene glycol maintained at 4°C was inconclusive. While atropine prepared in polyethylene glycol kept at 25°C showed significantly faster degradation than other groups, resulting in ~80% loss of the initial amount by day 90. The repeated measures ANOVA was used to compare the mean of remaining atropine content

Table 3 Stability analysis results of remaining atropine content (%) in self-prepared 0.01% atropine solutions (mg/mL)

Day	Formulation A at 4°C (%)	Formulation A at 25°C (%)	Formulation B at 4°C (%)	Formulation B at 25°C (%)
0	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
30	97.16±1.71	93.89±8.99	84.39±3.18	69.88±8.80
60	97.86±6.70	72.18±8.58	88.24±28.49	27.87±1.41
90	99.24±3.64	92.4±14.65	96.56±6.92	21.01±2.89

(A) Formulations prepared from hydroxypropyl methylcellulose kept in 4°C. (B) Formulations prepared from hydroxypropyl methylcellulose kept in 25°C. (C) Formulations prepared from polyethylene glycol kept in 4°C. (D) Formulations prepared from polyethylene glycol kept in 25°C.

on day 0 and day 90 of the study. The result shows a significantly statistical difference between groups of the formulation prepared with hydroxypropyl methylcellulose at 4°C and polyethylene glycol at 25°C, formulation prepared with hydroxypropyl methylcellulose at 25°C and polyethylene glycol at 25°C, formulation prepared with polyethylene glycol at 4°C and polyethylene glycol at 25°C, throughout the study (Table 4). An analysis of the difference in the remaining atropine content between day 0 and a latter-day within a group revealed that the content of atropine prepared in polyethylene glycol at 25°C had significantly decreased by day 60 of the study (Table 5).

Discussion

During the 90-day period, no microbial contamination was detected, despite the patient-administered, once-daily dose simulating the potential in-use shelf life of self-prepared eye drops for daily use in pediatric patients. Most studies prepare 0.01% atropine solutions by diluting 1% atropine sulfate solutions in 0.9% normal saline[20,24]. This is a preliminary study involving extemporaneously prepared solutions from artificial tear solutions containing ocular lubricants and preservatives to improve antimicrobial activity. Our findings demonstrate that atropine sulfate diluted in hydroxypropyl methylcellulose confers a more stable drug content after 90 days because atropine sulfate has the same vehicle as hydroxypropyl methylcellulose.

The strength of the study is the application in regular medical practice. The long shelf-life of 0.01% atropine with preserved sterility and stability is valuable in hospitals where the commercial drug is unavailable. One limitation of the present study is the use of pipette extraction of the drug to provide an accurate measurement. Further study is required to optimize long-term stability and sterility of eye-drop solutions. Moreover, a study of cost and effective analysis should be done to encourage extemporaneous 0.01% Atropine prepared by the pharmaceutical department in a general hospital.

Conclusion

This study demonstrated that extemporaneously prepared 0.01% atropine ophthalmic solution with hydroxypropyl methylcellulose kept at 4°C remained constant on day 90 of the study. However, preparations in different artificial tear solutions and storage temperatures can affect their stability. All prepared solutions preserved sterility on day 90 of the study.

Table 4 Mean difference in remaining atropine content between groups at day 90 of the study

(X) group	(Y) group	Mean Difference (X-Y)	Standard error	*p*-value
A (4 °C)	A (25 °C)	8.9485	4.76062	0.102
A (4 °C)	B (4 °C)	6.2647	4.76062	0.230
A (4 °C)	B (25 °C)	43.8761*	5.32253	< 0.001*
A (25 °C)	B (4 °C)	- 2.6838	4.76062	0.591
A (25 °C)	B (25 °C)	34.9276*	5.32253	< 0.001*
B (4 °C)	B (25 °C)	37.6114*	5.32253	< 0.001*

* The mean difference is significant at $p < 0.05$.

(A) Formulations prepared with hydroxypropyl methylcellulose. (B) Formulations prepared in polyethylene glycol.

(X) Mean remaining atropine content of the prepared formulation in the initial group. (Y) Mean remaining atropine content of the prepared formulation in the latter group.

Table 5 Mean difference in remaining atropine content between the initial study and later within the same group

X	Y	Mean Difference (X-Y)	Standard error	*p*-value
A (4 °C)				
Day 0	Day 30	2.837	0.989	0.103
Day 0	Day 60	2.143	3.870	0.635
Day 0	Day 90	0.757	2.103	0.753
A (25 °C)				
Day 0	Day 30	6.111	5.191	0.360
Day 0	Day 60	27.821	4.956	0.030*
Day 0	Day 90	7.600	8.456	0.464
B (4 °C)				
Day 0	Day 30	15.605	1.834	0.014*
Day 0	Day 60	11.756	16.450	0.549
Day 0	Day 90	3.435	3.996	0.481
B (25 °C)				
Day 0	Day 30	30.120	6.224	0.130
Day 0	Day 60	72.131	0.996	0.009*
Day 0	Day 90	78.991	2.041	0.016*

* The mean difference is significant at $p < 0.05$.

(A) Formulation prepared in hydroxypropyl methylcellulose. (B) Formulation prepared in polyethylene glycol. (X) Remaining atropine content of the initially prepared formulation. (Y) Remaining atropine content of the same prepared formulation on a later day.

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Conflict of Interest

The authors have no conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article, and the authors will bear all liabilities about claims relating to the content of this article. Sunita and Yuttaphong conceived and designed the study. Nattapon and Saowaluck conducted the experiment. Sunita collected and analyzed the data. Sunita and Worapot wrote the manuscript. All authors read and approved the manuscript for publication.

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