Stability of 0.1% and 0.01% Atropine Eye Drops in Tears Naturale Forte Stored in Low Density Polyethylene Dropper Bottles for 102 Days at 4°C and 25°C

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INTRODUCTION

Low dose atropine ophthalmic drops (0.01-0.05%) can be used to slow the progression of myopia in children. However, the only commercially available formulation is atropine 1% ophthalmic drops. To prepare a more dilute formulation, we selected Tears Naturale Forte as the diluent due to being commercially sterile and has an effective non-benzalkonium preservative. We sought to conduct a stability study since there is no published stability data for atropine ophthalmic drops at concentrations less than 1%.

OBJECTIVES

The objective of the study was to evaluate the stability of 0.1% and 0.01% atropine eye drops in Tears Naturale Forte stored in low density polyethylene dropper bottles for 102 days at 4°C and 25°C.

METHODS

Liquid Chromatographic Method

The liquid chromatographic system consisted of a mixture of 20% methanol and 80% 0.05 M phosphoric acid which was pumped through 150 mm x 4.6 mm reverse-phase C18, 5µm column (Supelcosil ABZ+; Supelco, Toronto, ON) at 1.0 mL/min. The effluent was monitored with UV detection (Waters 998 photodiode array detector, Toronto ON) at 215 nm.

Assay Validation

The method was evaluated to ensure reproducibility, accuracy and assay specificity. The system was shown to be capable of separating atropine from its degradation products and excipients present in the Tears Naturale Forte formulation. Accuracy and reproducibility of standard curves was tested over 5 days. Inter- and intra-day errors of reproducibility were assessed by the coefficients of variation (CV) and the standard deviation of regression.

Stability Study

On study day 0, 3 mL atropine 1% eye solution (Alcon, Lot: 21G16AA, exp: June 2023) was drawn up and diluted in 27 mL Tears Naturale Forte (Alcon, lot: 10YMN, exp: 2023-Oc) to a final concentration of 0.1% and then filtered with a 5 µm sterile filter into six 30 mL low density polyethylene (LDPE) dropper bottles each.

To prepare he 0.01% atropine eye drops, 0.3 mL atropine 1% eye solution (Alcon, lot: 21G16AA, exp: June 2023) was drawn up and diluted with 29.7 mL of Tears Natruale Forte (Alcon, lot: 10YMN, exp: 2023-Oc). The solution was then filtered through a 5 µm filter into six 30 mL LDPE dropper bottles each.

Three LDPE dropper bottles of each concentration were stored at 4°C and 25°C. The concentration of atropine was measured on study days 0, 2, 4, 8, 15, 21, 28, 49, 64, 79, 102 using a validated stability indicating liquid chromatographic method with UV detection. Physical inspection was conducted on each study day.

Data Reduction and Statistical Analysis

The concentration of a solution on a particular day was considered "acceptable" or "within acceptable limits" if it was greater than 90% of the initial concentration (as determined on day 0) and the amount found on that day, with 95% confidence, was also greater than 90% of the initial concentration.

Analysis of variance was used to test differences in degradation rate between the different storage temperatures and initial concentrations. The 5% level was used as the *a priori* cut-off for significance.

RESULTS

Table 1. Percent remaining of Initial Atropine Concentration on Each Study Day

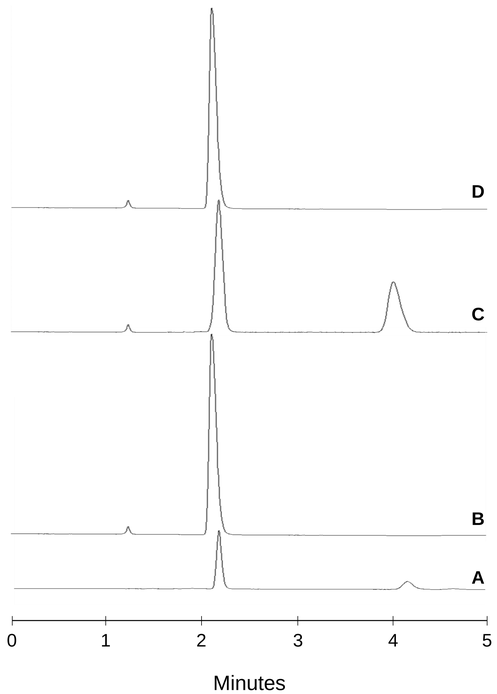
Storage Temperature	4°C	25°C	4°C	25°C
Initial Nominal Concentration	0.01% (0.1 mg/mL)	0.01% (0.1 mg/mL)	0.1% (1 mg/mL)	0.1% (1 mg/mL)
Actual Concentration (mg/mL ± CV)	0.1 ± 0.20	0.1 ± 0.78	1.0 ± 0.44	1.0 ± 0.44
Study Day 0	100	100	100	100
Study Day 2	99.15±0.23	97.32±0.61	99.88±1.09	97.84±0.38
Study Day 4	99.20±0.46	97.04±1.13	100.61±0.24	97.73±0.43
Study Day 8	100.81±0.21	94.58±0.28	100.43±0.19	95.23±0.16
Study Day 15	99.11±0.51	88.46±0.59	100.59±0.49	91.18±0.28
Study Day 21	98.75±0.21	85.08±0.74	100.98±0.46	87.84±0.45
Study Day 28	99.44±0.78	80.83±0.84	101.09±0.25	84.66±0.36
Study Day 49	100.37±0.31	67.85±0.80	100.42±0.36	75.29±0.43
Study Day 64	99.94±0.29	61.53±0.62	100.76±0.42	68.98±0.66
Study Day 79	102.53±0.39	55.20±1.23	101.64±0.19	64.96±0.09
Study Day 102	101.18±0.67	56.47±1.05	99.47±0.24	63.68±0.81
Rate of Concentration Change (%/day)	0.022	-0.480	0.001	-0.389
Intercept	99.303	96.634	100.501	97.449
Std Error in Slope	0.007909	0.039281	0.005791	0.026792
Confidence Interval for slope	0.01789	0.08886	0.01310	0.06061
Fastest Slope 95% Confidence	0.00396	-0.56902	-0.01215	-0.4492
Upper Limit 95% Confidence	0.0397	-0.3913	0.0140	-0.3280
Shortest T-90 (95% CI)	-2524.82	17.57	823.03	22.26

1. CV: coefficient of variation

Figure 1. Representative Chromatograms

Chromatogram A represents the atropine standard at time 0. Chromatogram B represents the same sample after degradation with heat (85°C) for 80 hours. 29.4% of the initial concentration remained and the degradation product eluted at 4.2 minutes.

Chromatogram D represents a solution of atropine 0.1% in Tears Naturale Forte on study day 0 with storage at 25°C. Chromatogram C represents the same solution after storage at 25°C for 102 days. There was 56.5% of the initial concentration remaining.



Concentration Results

The percent remaining of atropine on each study day is reported in Table 1. Solutions stored in the refrigerator (4°C) retained more than 98% of their initial concentration for the entire study duration. Solutions stored at room temperature (25°C) degraded rapidly. The calculated time to achieve 90% of the initial concentration with 95% confidence was 17 days while study day 8 was the last observed date with >90% of the initial concentration. All solutions remained clear and colourless for the entire study duration.

Analysis of variance revealed significant differences in percent remaining due to study day ($p=0.02$) and temperature ($p<0.01$) but not initial concentration ($p=0.44$).

Assay Validation

Assay validation demonstrated that atropine was separated from its degradation products and Tears Naturale Forte did not contain any ingredients that interfered with its measurement (Figure 1). Atropine was measured specifically, accurately (deviations from known averaged 0.82%) and reproducible (within day replicate error averaged 0.32% and between day replicate error averaged 0.65%). Another measure of replicate error, the standard deviation of the regression, averaged 2.19. Therefore, the assay was deemed to be stability indicating.

CONCLUSIONS

Atropine 0.1 and 0.01% ophthalmic drops in Tears Naturale Forte is chemically stable for at least 102 days when stored in the fridge, but only 8 days at room temperature.

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