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## Stability of extemporaneously prepared ophthalmic solutions for mydriasis

### Abstract

**Purpose** Results of an evaluation of the physical and chemical stability of extemporaneously prepared adult and pediatric ophthalmic solutions containing combinations of phenylephrine, tropicamide, and cyclopentolate are reported.

**Methods** A stability study was conducted to help determine the feasibility of innovative formulations to meet an unmet clinical need for combination mydriatic ophthalmic eyedrops. An adult mydriatic ophthalmic solution containing phenylephrine hydrochloride 2.5% and tropicamide 1.0% and a pediatric formulation containing phenylephrine hydrochloride 2.5%, tropicamide 0.5%, and cyclopentolate hydrochloride 0.5% were prepared using proper aseptic techniques. Triplicate samples of each formulation were stored for 60 days at refrigeration temperatures (2–8 °C) and analyzed on day 0 and days 7, 14, 28, and 60. At each time point, the stability samples were assessed by visual inspection, pH measurement, and stability-indicating high-performance liquid chromatography (HPLC) analysis.

**Results** Over the 60-day storage period, there was no significant change in the visual appearance or pH level of any of the adult or pediatric solution samples. The results of HPLC analysis indicated that all samples retained 97–102% of the initial drug concentrations for up to 60 days.

**Conclusion** Both adult and pediatric ophthalmic formulations containing combinations of phenylephrine, tropicamide, and cyclopentolate were stable physically and chemically for up to 60 days when stored at refrigeration temperatures (2–8 °C).

### Disciplines

Pharmacy and Pharmaceutical Sciences

### Comments

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## **TITLE**

### **Stability of Extemporaneously Prepared Ophthalmic Solutions for Mydriasis**

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## ABSTRACT

**Purpose.** Mydriatic ophthalmic solutions are commonly used in eye exams and pre-surgical treatment. Multiple drugs are often combined to achieve optimal clinical outcomes. The purpose of this study is to evaluate the physical and chemical stability of extemporaneously prepared adult and pediatric ophthalmic solutions containing combinations of phenylephrine, tropicamide, and cyclopentolate.

**Methods.** An adult formulation containing phenylephrine 2.5% and tropicamide 1.0%, and a pediatric formulation containing phenylephrine 2.5%, tropicamide 0.5%, and cyclopentolate 0.5% were prepared using proper aseptic techniques. Triplicate samples of each formulation were stored for 60 days at refrigeration (2-8 °C) and analyzed on day-0, 7, 14, 28 and 60. At each time point, the stability samples was assessed by visual inspection, pH measurement, and stability-indicating high-performance liquid chromatography (HPLC) analysis.

**Results.** Over the 60-day storage period, there was no significant change in visual appearance or pH level in any of the adult or pediatric solution samples. The HPLC analysis results also indicated that all samples retained between 97-102% of the initial drug concentrations for up to 60 days.

**Conclusion.** Both the adult and pediatric ophthalmic formulations, containing combinations of phenylephrine, tropicamide, and cyclopentolate, were stable physically and chemically for up to 60 days when stored at refrigeration (2-8 °C).

**Keywords.** Beyond use date, compounding, compounded sterile preparations, cycloplegia, mydriasis, stability

## Introduction

There are two pharmacologic classes of drugs to consider for use when mydriasis (pupil dilation) of the eye is needed: alpha-adrenergic agonists and anticholinergics.<sup>1</sup> Alpha-adrenergic agonists act by stimulating contraction of the dilator muscle, which enlarges the pupil. Anticholinergic agents cause dilation via relaxation of the iris sphincter muscle. Some anticholinergic agents also cause a cycloplegic effect, resulting in paralysis of the ciliary body in the eye. Most of these drugs are available commercially as single-ingredient products.<sup>1</sup> However, they are often used together as sequential drops, because no single drug has the ideal efficacy and safety profile for the wide range of patient parameters.<sup>2-4</sup>

Mydriatic ophthalmic solutions are routinely used for comprehensive eye exams and surgical procedures. Traditionally, the protocol has consisted of administering the required single-ingredient eye drops one at a time, separated by 5-10 minutes each. This is burdensome for patients and the healthcare team alike, and may lead to an increase in medication error. A preferred method consists of using a combination solution with all of the required ingredients that can be administered to the patient all at once. However, there is limited selection for commercially available combination mydriatic solution products.<sup>2</sup>

At the authors' healthcare facility, two combination mydriatic solutions are frequently prescribed for adult and pediatric patients, respectively. The adult formulation consists of phenylephrine 2.5% and tropicamide 1%, and the pediatric formulation consists of phenylephrine 2.5%, tropicamide 0.5%, and cyclopentolate 0.5%. Phenylephrine is an alpha-adrenergic agonist that causes dilation.<sup>1,5</sup> Cyclopentolate and tropicamide are both anticholinergics, which cause dilation and also produce a cycloplegic effect.<sup>1,6,7</sup> These drugs also differ in their potency, onset and duration of action, and tolerability. Both the adult and pediatric formulations are prepared

extemporaneously by the pharmacy department following the USP <797> requirements for sterile compounding.<sup>8</sup> Due to the high frequency of usage, it is desired to have a stock supply of both ophthalmic solutions available in the pharmacy inventory. However, in the absence of stability or sterility data, the default USP beyond-use date (BUD) of these two solutions stored at refrigeration is only three days.<sup>8,9</sup> The purpose of this study is to generate the physical and chemical stability data of the two mydriatic ophthalmic solutions in order to extend their BUD to facilitate the pharmacy inventory management and timely dispensing for patient use.

## **Methods**

### *Drug Source and Strength Labeling*

The stability samples of this study were prepared using phenylephrine hydrochloride powder (USP grade)<sup>a</sup>, tropicamide ophthalmic solution USP 1%<sup>b</sup>, and cyclopentolate hydrochloride ophthalmic solution USP 1%<sup>c</sup>. Both tropicamide and cyclopentolate solutions were manufactured sterile products containing commonly used excipients for ophthalmic dosage forms, such as boric acid, edetate disodium, and 0.01% benzalkonium chloride (preservative). The strengths of cyclopentolate and phenylephrine were expressed as the hydrochloride salt forms for all solutions used or prepared in this study. However, for simplicity, the hydrochloride salt designations are not restated in the remaining text.

### *Pediatric Formulation Preparation*

Using proper aseptic technique in a vertical laminar airflow hood, 30 mL each of tropicamide 1% and cyclopentolate 1% solutions was obtained from the source bottles and mixed in a suitable container. Approximately 50% of this tropicamide/cyclopentolate mixture was

added into a beaker pre-calibrated to 45-mL. An amount of 1.125 g phenylephrine powder was added to the beaker and stirred until fully dissolved. If necessary, the pH of the solution was adjusted to 4.2-5.8 using a small amount of 0.1 N HCl. Additional tropicamide/cyclopentolate mixture was added to the pre-calibrated beaker up to 45-mL for a final concentration of phenylephrine 2.5%, tropicamide 0.5%, and cyclopentolate 0.5%. The dilution due to the phenylephrine powder or pH adjustment was negligible as confirmed by the HPLC analysis of time zero samples (see Results and Discussion). The final solution was sterile-filtered and packaged into three sterile dropper bottles, approximately 15 mL each.

#### *Adult Formulation Preparation*

Using proper aseptic technique in a vertical laminar airflow hood, 45 mL of tropicamide 1% solution was obtained from source bottles and placed in a suitable container. Approximately half of the tropicamide solution was transferred to a beaker pre-calibrated to 45-mL. An amount of 1.125 g phenylephrine powder was added to the beaker and stirred until fully dissolved. Additional tropicamide solution was added to the pre-calibrated beaker up to 45 mL for a final concentration of phenylephrine 2.5% and tropicamide 1.0%. The pH adjustment, sterilization, and packaging were conducted in the same manner as the pediatric formulation preparation. The final solution was sterile-filtered and packaged into three sterile dropper bottles, approximately 15 mL each.

#### *Stability Study*

Three 15-mL bottles each of the compounded pediatric and adult ophthalmic solutions were used throughout the stability study. All samples were stored in the refrigerator (2-8° C) and

assayed on day-0, 7, 14, 28, and 60. At each time point, approximately 1.5 mL sample was dispensed from each bottle into a 2-mL clear centrifuge vial. Samples were visually inspected for clarity and particulates, tested for potential changes of pH, and analyzed by HPLC for drug concentration and potential degradation. For the HPLC analysis, 1 mL of each pediatric sample was diluted with purified water to the mark in a 25-mL volumetric flask. The nominal drug concentrations were 1.0, 0.2 and 0.2 mg/mL for phenylephrine, tropicamide, and cyclopentolate, respectively. The adult samples were diluted in the same manner but into 50-mL volumetric flasks. The nominal drug concentrations were 0.5 and 0.2 mg/mL for phenylephrine and tropicamide, respectively. Approximately 1 mL of each diluted sample was transferred to an HPLC vial for the analysis, and three injections were performed for each sample. Since the focus of this study was physical and chemical stability, sterility test was not performed.

### *HPLC Analysis*

HPLC analysis for both the pediatric and adult ophthalmic samples was performed using a Shimadzu model LC-2010A instrument equipped with a programmable auto-injector and dual-wavelength UV detector<sup>d</sup>. A Phenomenex C18 column<sup>e</sup> was used and maintained at 40° C during the analysis. The mobile phase consisted of a linear gradient method from 98:2 to 60:40 water:acetonitrile v/v in 15 minutes with a constant level of 0.1% v/v trifluoroacetic acid as the buffer. The injection volume was 10 µL, and the UV detection wavelengths were set at 210 nm and 254 nm. The 254 nm wavelength was used to analyze phenylephrine and tropicamide, and the 210 nm wavelength for cyclopentolate. Under these conditions, all three drugs were well separated with satisfactory UV responses for quantitation. As shown in Figure 1, the retention

times of phenylephrine, tropicamide, and cyclopentolate were approximately 5, 11, and 14 minutes, respectively.

At each stability study time point, five standard solutions for each formulation were analyzed for calibration purpose. The standards were prepared from pure drug powders<sup>f,g,h</sup> and encompassed the 80% - 120% range of the nominal drug concentrations in the diluted stability samples for HPLC analysis. Calibration curves were constructed via linear regression of the peak area versus the concentration for each drug. Each curve was found to be linear over the concentration range of the standards with  $r^2 \geq 0.995$ . All standard solutions were injected three times on each analysis day to assess the method precision. The intraday and interday coefficients of variation were found to be within 2.0%.

#### *Forced Degradation Study*

A short-term forced degradation study was performed to evaluate whether the HPLC method was stability indicating, thus capable of separating the potential degradation products from the parent drugs. Aqueous solutions of phenylephrine, tropicamide, and cyclopentolate at concentrations of 1.0, 0.2, and 0.2 mg/mL respectively were prepared from pure drug powders.<sup>f,g,h</sup> Samples of all three drug solutions were treated with 1N hydrochloric acid<sup>i</sup> (final pH ~2), 1N sodium hydroxide<sup>j</sup> (final pH ~12), and 30% hydrogen peroxide<sup>k</sup> (final concentration 3%). The samples treated with acid and base were incubated at 60° C. Those treated with hydrogen peroxide were incubated at 60° C or subjected to sunlight. All samples were assayed at time zero and subsequent time points ranging from 24 hours to 3 days until a minimum of 20% degradation had been reached in at least one sample for each drug. Significant degradation was observed in all three drug samples treated with hydrogen peroxide and in cyclopentolate sample

at pH 12. All degradation products were separated from the three parent drugs by the gradient HPLC method, and no major interfering peaks were noted. Therefore, the HPLC method was considered as stability indicating and suitable for the stability study.

## **Results and Discussion**

There is an unmet need for combination mydriatic ophthalmic solutions to accommodate the variety of medical procedures and patient parameters. The three active ingredients used in this study are all generic drugs available as manufactured ophthalmic solutions or pure powders (USP grade) which are convenient source materials for compounding combination solutions. Since ophthalmic solutions must be sterile, it is important to consider the quality of the source materials and follow the pertinent requirements in USP general chapter <797>.<sup>8</sup> To minimize potential microbial contamination, it is preferred to use manufactured ophthalmic solutions (sterile) as the source materials. However, the use of non-sterile pure powders may be necessary to achieve desired drug concentrations. In that case, the final solution requires sterilization and is considered high-risk for inadvertent microbial contamination according to USP <797>.<sup>8</sup> In this study, the stability samples were prepared from phenylephrine powder and manufactured ophthalmic solutions of tropicamide and cyclopentolate. The final solutions were sterilized by cold sterile filtration.

The freshly prepared pediatric and adult ophthalmic solutions appeared clear, colorless, and free of particulates. The pH and the initial drug concentration values are reported in Table 1 and Table 2, respectively. Throughout the 60-day storage at refrigeration, no significant changes of visual appearance were observed in any of the stability samples. The pH of all samples remained within the pre-defined acceptable range of 4.2 to 5.8 (Table 1). The stability-indicating

HPLC analysis also confirmed that all pediatric samples retained 99-102% of the initial drug concentrations and all adult samples retained 97-100% (Table 2). No significant degradation peaks were observed in the chromatograms for either the pediatric or the adult formulations. Overall, both pediatric and adult ophthalmic solutions exhibited satisfactory physical and chemical stability at refrigeration for up to 60 days.

There are two major limitations to the study. First, sterility testing was not performed for any of the stability samples, because the study aims were limited to chemical and physical stability. However, it is essential to follow USP <797> requirements and perform the necessary testing for the actual preparations for patient use. Secondly, this study utilized two manufactured ophthalmic solutions which contained additional excipients, such as boric acid, edetate disodium, and benzalkonium chloride. It was not studied whether the stability results would remain the same without these excipients. Therefore, it is advised that the stability results from this study may be applicable to only formulations prepared using similar source materials.

## **Conclusion**

Two mydriatic ophthalmic solutions were prepared extemporaneously for pediatric and adult patients respectively. The pediatric solution contained phenylephrine 2.5%, tropicamide 0.5%, and cyclopentolate 0.5%. The adult solution contained phenylephrine 2.5% and tropicamide 1.0%. Both solutions were physically and chemically stable for up to 60 days at refrigeration (2-8° C). These results can be used to aid in the selection of an appropriate beyond-use date. Additionally, proper sterile compounding practice and microbiological testing as per USP <797> must be strictly followed when preparing these ophthalmic solutions for actual patient use.

**Footnotes**

- a. Phenylephrine hydrochloride powder, Letco, Decatur, AL, LOT# 1406030115, EXP 12/2016.
- b. Tropicamide Ophthalmic Solution USP, 1%, Bausch & Lomb, Tampa, FL, LOT# 24033, EXP 02/2017; NDC 24208-585-64.
- c. Cyclopentolate Hydrochloride Ophthalmic Solution USP, 1%, Bausch & Lomb, Tampa, FL, LOT# 238872, EXP 01/2017; NDC 24208-735-06.
- d. HPLC system, Model 2010A, Shimadzu Scientific Instruments, Marlborough, MA.
- e. HPLC column, C18 Kinetex, 5  $\mu$ m, 100A, 150 $\times$ 4.6 mm, Phenomenex Inc., Torrance, CA.
- f. Tropicamide powder, PCCA, Houston, TX, LOT# C168671, EXP 07/2019.
- g. Cyclopentolate hydrochloride powder, PCCA, Houston, TX, LOT# C163864, EXP 07/2018.
- h. Phenylephrine hydrochloride powder, PCCA, Houston, TX, LOT# C164766, EXP 11/2018.
- i. Hydrochloric acid, 1N standard solution, Thermo Fisher Scientific, LOT# B00N7235.
- j. Sodium hydroxide, 1N standard solution, Thermo Fisher scientific, LOT# B00N0974.
- k. Hydrogen peroxide, 30%, Thermo Fisher Scientific, LOT# B0526846.

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**Key Points**

1. There is an unmet clinical need for combination mydriatic ophthalmic eye drops. Two formulations, adult and pediatric, were prepared by extemporaneous compounding and evaluated for physical and chemical stability.
2. The adult formulation contained phenylephrine 2.5% and tropicamide 1.0%. The pediatric formulation contained phenylephrine 2.5%, tropicamide 0.5%, and cyclopentolate 0.5%.
3. Both adult and pediatric formulations were found to be stable physically and chemically for up to 60 days when stored at refrigeration (2 – 8 °C).

Table 1. pH Results of Compounded Pediatric and Adult Ophthalmic Solutions Stored at Refrigeration<sup>a</sup>

Formulation	Mean ± S.D. pH				
	Day 0	Day 7	Day 14	Day 28	Day 60
Pediatric Ophthalmic Solution	5.08 ± 0.01	5.04 ± 0.03	5.01 ± 0.01	4.96 ± 0.01	4.88 ± 0.02
Adult Ophthalmic Solution	4.75 ± 0.06	5.02 ± 0.01	5.03 ± 0.01	4.95 ± 0.01	4.93 ± 0.03

<sup>a</sup> Triplicate samples (n = 3).

Table 2. HPLC Results of Compounded Pediatric and Adult Ophthalmic Solutions Stored at Refrigeration<sup>a</sup>

Formulation	Drug	Mean ± S.D. Initial Drug Concentration (mg/mL) <sup>b</sup>	Mean ± S.D. Initial Drug Concentration Remaining (%) <sup>b</sup>			
			Day 7	Day 14	Day 28	Day 60
Pediatric Ophthalmic Solution	Phenylephrine	0.957 ± 0.018	99.6 ± 0.9	99.7 ± 2.0	99.7 ± 2.0	102.1 ± 1.1
	Tropicamide	0.190 ± 0.001	99.2 ± 1.0	99.8 ± 1.8	99.9 ± 1.2	101.3 ± 1.0
	Cyclopentolate	0.197 ± 0.004	98.9 ± 1.2	99.5 ± 1.6	100.6 ± 1.7	101.1 ± 1.4
Adult Ophthalmic Solution	Phenylephrine	0.494 ± 0.005	97.0 ± 1.1	98.0 ± 1.5	95.9 ± 0.8	99.9 ± 0.6
	Tropicamide	0.211 ± 0.001	97.2 ± 1.1	97.7 ± 1.2	99.8 ± 0.8	99.7 ± 0.9

<sup>a</sup> HPLC = high performance liquid chromatography.

<sup>b</sup> Triplicate samples (n = 3).

**Figure 1.** Representative Chromatogram of Pediatric Formulation with UV detection at (A) 210 nm and (B) 254 nm. The retention times are approximately 5, 11, and 14 minutes for phenylephrine, tropicamide, and cyclopentolate, respectively.

