

**TROPICAMIDE-PHENYLEPHRINE FIXED COMBINATION MICRODOSE OPHTHALMIC SOLUTION
EYENOVA CLINICAL STUDY PROTOCOL EYN-MYD-TP-41 VERSION B**

**PROTOCOL EYN-MYD-TP-41
VERSION B**

**A SINGLE-CENTER, ASSESSOR-MASKED, ACTIVE-CONTROLLED, PHASE 4 STUDY
EVALUATING SPEED OF PUPIL DILATION WITH THE MICRO-ARRAY PRINT (MAP)
DISPENSER WHEN COMPARING 2 DOSING REGIMENS OF TROPICAMIDE-
PHENYLEPHRINE FIXED COMBINATION OPHTHALMIC SOLUTION
(THE SPEED STUDY)**

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LIST OF ABBREVIATIONS

Abbreviation	Term
AC	Anterior chamber
AE	Adverse event
ADR	Adverse drug reaction
AT	Artificial tears
BCDVA	Best-corrected distance visual acuity
CF	Count fingers
CFR	Code of Federal Regulations
eCRF	Electronic case report form
FDA	United States Food and Drug Administration
GCP	Good Clinical Practice
HM	Hand motion
IB	Investigator brochure
ICF	Informed consent form
ICH	International Conference on Harmonization
IND	Investigational New Drug
IOL	Intraocular lens
IOP	Intraocular pressure
IRB	Institutional Review Board
ITT	Intent-to-treat
LP	Light perception
MAP	Eyenovia Micro-Array Print Dispenser
NLP	No light perception
OU	Both eyes (within this protocol, OU does not connote simultaneous treatment/evaluation)
PETG	Polyethylene terephthalate glycol
PP	Per-protocol
SAE	Serious adverse event
SD	Standard deviation
SLE	Slit lamp examination
TEAE	Treatment-emergent adverse event
TLR	Total letters read
UCDVA	Uncorrected distance visual acuity
US	United States
VA	Visual acuity
WMA	World Medical Association
µD	Microdose
µL	Microliter

NOTE: The first occurrence of some abbreviations is not spelled out in the document (e.g., units of measure).

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1. PERSONNEL AND FACILITIES

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2. STUDY SYNOPSIS

2.1. Study Objective

The primary objective of this study is to compare pupil dilation speed achieved with a single dose of MAP Dispenser-administered tropicamide-phenylephrine fixed combination to two doses of MAP Dispenser-administered tropicamide-phenylephrine fixed combination administered 5 minutes apart.

2.2. Study Drugs

- **Investigational Product, Dosage and Mode of Administration:** Commercially available tropicamide-phenylephrine fixed combination ophthalmic solution (T-P OFTENO SOLUCIÓN 50 mg/8 mg/ ml) delivered by the MAP Dispenser. In this study, drug will be administered at separate visits as a single mist to each eye or as 2 mists administered 5 minutes apart to each eye.

2.3. Study Population

At least 60 volunteer participants will be randomized to study drug administration in order to complete required follow-up treatments and evaluations of 56 or more subjects. Subjects will be enrolled at a single study site in Tijuana, Mexico.

2.4. Study Design

This trial is a single center, assessor-masked, active-controlled, cross-over noninferiority study evaluating tropicamide-phenylephrine fixed combination ophthalmic solution administered as a single mist to each eye and as two mists, administered 5 minutes apart in each eye.

Volunteer participants will be screened for study eligibility during a Screening Visit and enrolled after signing the study-specific informed consent form (ICF). Subjects meeting all inclusion/exclusion criteria will be scheduled for 2 treatment visits, which must occur at least 4 days, but no more than 7 days apart. At each of the two treatment visits, baseline measurements will be taken, then either 1 mist or 2 mists of the study drug will be administered to both eyes (OU). In the instances of 2 mists, the separate dispensing will occur approximately 5 minutes apart. Only 1 mist or 2 mists of the drug will be administered per treatment visit, and drug administration will occur in a randomized order using one of two sequences - AB and BA, where A is the dispensing of 1 mist of tropicamide-phenylephrine fixed combination and B is the dispensing of 2 mists of tropicamide-phenylephrine fixed combination. Afterwards, efficacy and safety assessments will be performed.

This study will be single-masked. There will be no differences in the presentation of the investigational drug administered and study personnel conducting ophthalmic assessments will be masked to study drug administration assignment. Personnel who administer study drug on a given day will not be allowed to perform post-study drug administration ophthalmic assessments on that day.

2.5. Inclusion Criteria

Subjects eligible for study participation must meet each criterion listed below. Ocular criteria must be met for both eyes.

1. Male or female 18 years old or above.
2. Female subjects must be either 1-year postmenopausal, surgically sterilized, or, if of childbearing potential, have a negative urine pregnancy test during the Screening Visit and agree to use an acceptable form of contraception throughout the study. *Acceptable methods include the use of at least one of the following: intrauterine (intrauterine device), hormonal (oral, injection, patch, implant, ring), barrier with spermicide (condom, diaphragm), or abstinence.*
3. Ability to provide signed written consent prior to participation in any study-related procedures.
4. Ability to return for all study treatment visits.
5. Photopic screening pupil diameter ≤ 3.5 mm in each eye.

2.6. Exclusion Criteria

Subjects with any of the following diseases, surgeries or conditions are ineligible for study participation. Subjects may not participate if either eye meets any of the ocular exclusion criteria.

1. Pregnancy or lactation.
2. Allergy to phenylephrine hydrochloride.
3. Allergy to tropicamide.
4. Allergy to benzalkonium chloride.
5. History of benign prostatic hyperplasia.
6. Use of benzodiazepines, monoamine oxidase inhibitors, tricyclic antidepressants, anticonvulsants, cholinergic drug at screening or anticipated during the study period.
7. Participation in any study of an investigational drug or device within 30 days prior to the Screening Visit, or at any time during the study period.
8. History of closed-angle glaucoma.
9. Anatomically narrow anterior chamber angles (Van Herrick grade ≤ 2 in either eye).
10. Ocular surgery or laser treatment of any kind prior to the Screening Visit.
11. History of chronic or acute uveitis.
12. History of traumatic iritis or hyphema.
13. History of traumatic mydriasis or angle recession.
14. History of heterochromia.
15. Irregularly-shaped pupil secondary to ocular trauma or congenital defect.
16. History of neurogenic pupil disorder (e.g., Horner's syndrome, third cranial nerve palsy, Adie's pupil, Argyll Robertson syndrome, etc.).
17. History of anterior chamber intraocular lens (IOL) or iris-fixated IOL.
18. History of iris surgery of any kind (e.g., iridotomy, iridectomy, coreoplasty).
19. History of iris atrophy.
20. History of iris – cornea apposition/touch.

21. Unwilling or unable to discontinue use of contact lenses at treatment visits.
22. Current active eye disease for which topical or systemic ophthalmic medication is necessary, except for dry eye disease managed using artificial tears (AT). *AT's must be discontinued on the day of each treatment visit.*
23. Presence of a severe/serious ocular condition, or any other unstable medical condition that, in the Investigator's opinion, may preclude study treatment and/or follow-up.

2.7. Primary Performance Endpoint

The primary performance endpoint is mean change in pupil diameter at 35 minutes versus per-visit baseline, as measured by digital pupillometry in highly photopic conditions. The highly photopic condition will be established using a fully-charged transilluminator (muscle light) at the brightest setting.

2.8. Exploratory Outcomes

- Proportion of eyes achieving pupil diameter of 6.0 mm or greater at 35 minutes
- Proportion of eyes achieving pupil diameter of 7.0 mm or greater at 35 minutes
- Mean change in pupil diameter at other timepoints (15, 55 minutes)
- Distribution of pupil diameters at 15, 35, 55 minutes
- Proportion of successful administrations on 1st attempt

These exploratory outcomes will also be measured using digital pupillometry in highly photopic conditions.

2.9. Safety Outcomes

- Slit-lamp examination findings
- Occurrence of AEs
- Intraocular pressure (IOP) measured at 60 minutes post-administration
- Visual acuity changes
- Proportion of administrations without Micro-Array Print (MAP) Dispenser touch to the eye

2.10. Schedule of Visits, Procedures and Evaluations

Study visits include a Screening Visit, which must occur between 1 and 14 days prior to treatment; followed by Treatment Day 1, then Treatment Day 2. Treatment visits must occur at least 4 days and not more than 7 days apart. The study visit schedule is presented in flow chart form in Figure 1.

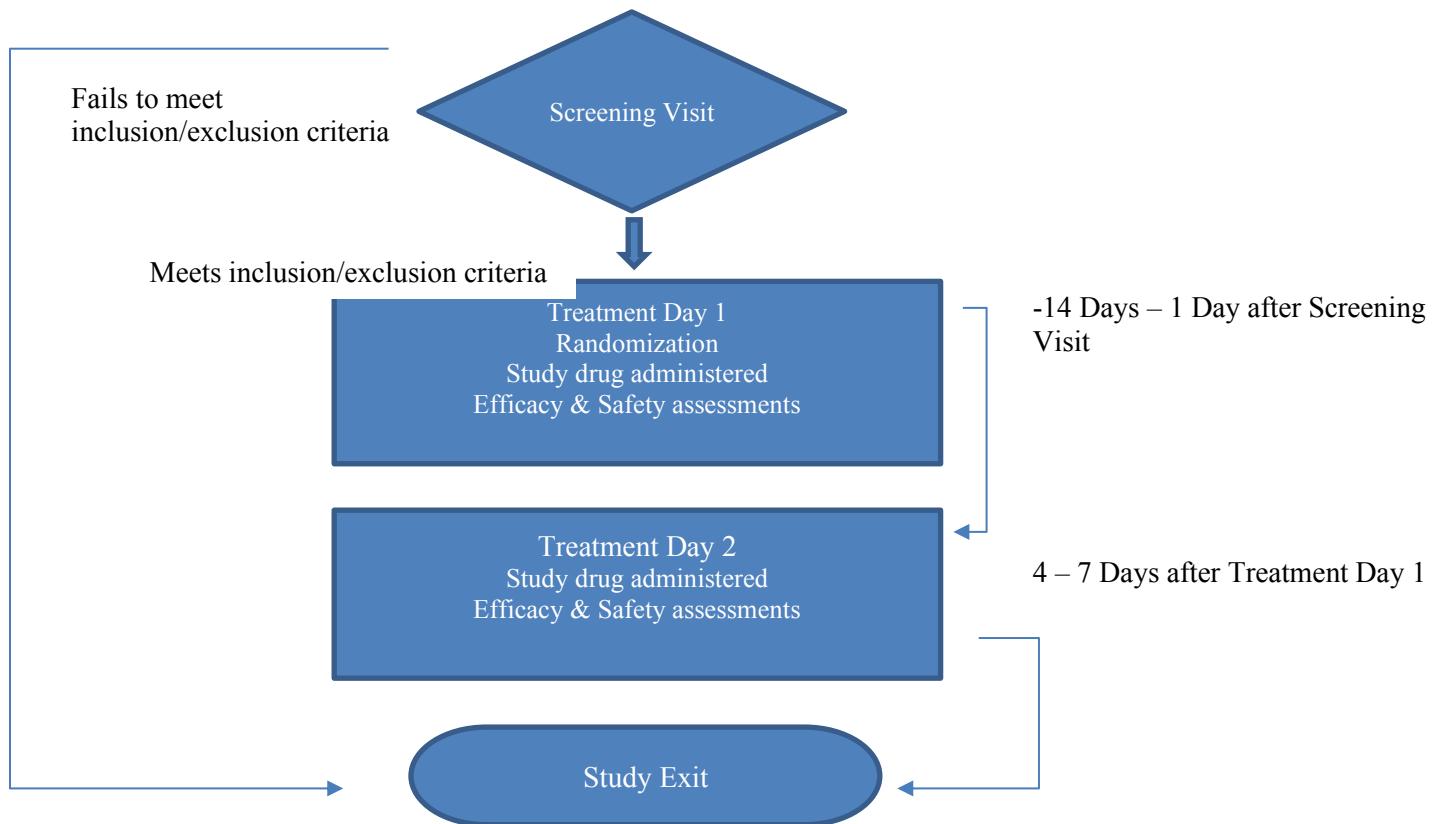


Figure 1: Study Visit Flow Chart

2.10.1. Study Medication Administration and Clinical Assessments

Study medication administration and clinical assessments will be performed at study visits as shown in **APPENDIX 1: SCHEDULE OF MEDICATION ADMINISTRATION AND EXAMINATIONS**.

3. INTRODUCTION AND RATIONALE

3.1. Introduction

Eyenova, the Sponsor of this study, is a specialty pharmaceutical company pursuing development of a variety of ocular topical medications including mydriatics. Eyenova's drug formulations are administered using a proprietary system called the Micro-Array Print (MAP) Dispenser, which is a container closure system designed to accurately deliver precise low-volume doses of topical ocular pharmaceuticals.

Phenylephrine is an α_1 -adrenergic receptor sympathetic agonist that has been used for more than 70 years to dilate the pupil for diagnostic, therapeutic, and surgical procedures. Systemically, phenylephrine, when administered topically to the eye at higher doses, is known to cause serious cardiovascular reactions including ventricular arrhythmias, myocardial infarction, and elevated blood pressure in some patients^{1,2}. These risks are especially relevant when more mydriasis is required, and phenylephrine 10% is used, rather than the lower 2.5% concentration. Tropicamide is a synthetic derivative of tropic acid that became available for ocular use in 1959³. It is an anticholinergic mydriatic agent that acts by blocking the iris sphincter muscle and the ciliary muscle responses to acetylcholine. As an anticholinergic related to atropine and scopolamine, systemically absorbed tropicamide in higher doses has the potential to cause rare but potentially serious adverse events, particularly in the pediatric population.

The MAP Dispenser technology facilitates accurate topical ocular delivery of a controlled quantity of mydriatic agents in a precise manner to maximize therapeutic control and minimize systemic absorption and loss to washout.

3.2. Pupil Dilation

A variety of ophthalmic evaluations require pupil dilation to provide an unobstructed view of the lens, retina, and optic nerve. The degree of pupil dilation required depends on the procedure being performed. For routine diagnostic procedures, and in conditions where short term pupil dilation is desired, the goal is to achieve adequate mydriasis for visualization with minimal cycloplegic effect.

¹ Vaajanen A, Vapaatalo H. A single drop in the eye - Effects on the whole body? Open Ophthalmol J. 2017;11:305–314. doi: 10.2174/1874364101711010305

² Farkouh A, Frigo P, Czejka M. Systemic side effects of eye drops: a pharmacokinetic perspective. Clin Ophthalmol. 2016;10:2433–2441.

³ Merrill D L, Goldber, B, Zavell S. bis-Tropamide, A new parasympatholytic. Curr Ther Res. 1960;12:43–50.

3.3. Existing Mydriatic Options

Dilation of the pupil is currently produced with parasympathetic anticholinergics such as tropicamide, cyclopentolate, homatropine, and atropine; or with sympathetic agonists (sympathomimetics) such as phenylephrine⁴.

Parasympathetic antagonists block acetylcholine activation of muscarinic receptors, thereby paralyzing the iris sphincter muscle. The result is an enlarged pupil; however, at the same time, the ciliary muscle that regulates visual accommodation can be paralyzed, resulting in blurry vision that can persist for hours. Additionally, rare but serious systemic side effects have been observed after ocular administration of anticholinergics such as tropicamide, particularly in infants and children^{5,6}.

Sympathetic agonists produce dilation by stimulating the iris dilator muscle and constricting ophthalmic blood vessels. Although phenylephrine does not induce blurry vision, at lower concentrations; e.g., 2.5%, it generally does not dilate the pupil as well as parasympathetic agonists. Where full mydriasis is required, higher concentrations of phenylephrine; e.g., 10%, are required. Higher concentration phenylephrine, however, has been known to cause cardiovascular effects after systemic absorption, which include hypertension, tachycardia, and more rarely, arrhythmia and stroke, especially in patients with pre-existing heart conditions⁷.

To maximize dilation efficacy and minimize side effects, most ophthalmologists employ a combination of both classes of mydriatic agents. Tropicamide-Phenylephrine fixed combination ophthalmic solution is approved for eyedrop delivery and currently marketed (T-P OFTENO SOLUCIÓN 50 mg/8 mg/ ml, AUGEX – MEDICAMENTO – PR VADEMECUM) for dilation of the pupil.

3.4. Tropicamide-Phenylephrine Fixed Combination Ophthalmic Microdosing Solutions for Pupil Dilation

Eyenova's MAP Dispenser is designed to deliver precise microvolumes of topical ophthalmic medications using a piezo-generated microdroplet spray. When the dispenser is actuated, a precisely calibrated single microdose is delivered. It is anticipated that microdosing of ophthalmic drugs will enhance drug bioavailability and efficacy while minimizing systemic absorption and any associated side effects. Tropicamide-Phenylephrine fixed combination ophthalmic solution is approved for eyedrop delivery and currently marketed (T-P OFTENO SOLUCIÓN 50 mg/8 mg/ ml, AUGEX – MEDICAMENTO – PR VADEMECUM) for dilation of the pupil. The drug may be administered in approximately 8 µL microdoses using the MAP Dispenser.

⁴ Grob SR, Gonzalez LA, Daly MK. Management of mydriasis and pain in cataract and intraocular lens surgery: review of current medications and future directions. Clin Ophthalmol. 2014;8:1281–1289. doi: 10.2147/OPTH.S47569

⁵ Vaajanen A, Vapaatalo H. A single drop in the eye - Effects on the whole body? Open Ophthalmol J. 2017;11:305–314. doi: 10.2174/1874364101711010305

⁶ Farkouh A, Frigo P, Czejka M. Systemic side effects of eye drops: a pharmacokinetic perspective. Clin Ophthalmol. 2016;10:2433–2441.

⁷ Ibid.

Ocular microdosing with the MAP Dispenser has been evaluated in humans using phenylephrine 2.5%, phenylephrine 10%, and tropicamide 1.0% solutions.

3.5. Ophthalmic Microdosing

The rationale for Eyenovia's mydriatic development is the desire to optimize pupil dilation while reducing systemic side effects, improving local tolerability, and decreasing the amount of medication lost to drainage from increased tear turnover rate.

The average tear volume of an adult eye is 6–7 μ L^{8,9}. The estimated maximum volume of eyedrops that the cul de sac can contain, albeit momentarily, is approximately 30 μ L; this is reduced to 10 μ L with normal blinking¹⁰. The typical eyedropper (either unit dose or multidose), however, provides drops that range from 25 μ L to > 50 μ L. Even among bottles of the same size and shape, eyedrop sizes vary significantly depending on the angle of the bottle, the amount of ophthalmic solution remaining, and even among manufacturers¹¹.

Much of the eyedrop volume overflows into the nasolacrimal canal where the active drug product becomes available systemically. Ocular drugs that are absorbed by the nasolacrimal mucosa mimic intravenous injection delivery insofar as they are not susceptible to first-pass hepatic metabolism. Additionally, ocular medication in swallowed nasolacrimal secretions is theoretically available for absorption in the gastrointestinal tract. Taken together, only a small fraction of the applied medication is absorbed directly into the eye, while there remain multiple opportunities for systemic absorption¹². This can result in significant safety concerns, especially with drugs like phenylephrine and tropicamide that are known to produce dose-dependent systemic side effects^{13,14}. Additionally, excess drug in the eye may cause ocular surface toxicity and tolerability issues.

Instillation of eyedrops stimulates lacrimation and can increase the tear turnover rate more than 4-fold, which additionally dilutes the drug product administered¹⁵. If the eyedrop causes discomfort, the loss rate

⁸ Mishima S, Gasset A, Klyce SD Jr, Baum JL. Determination of tear volume and tear flow. *Invest. Ophthalmol.* 5(3), 264–276 (1966).

⁹ Scherz W, Doane MG, Dohlman CH. Tear volume in normal eyes and keratoconjunctivitis sicca. *Albrecht Von Graefes Arch. Klin. Exp. Ophthalmol.* 1974;192(2):141–150.

¹⁰ Shell JW. Pharmacokinetics of topically applied ophthalmic drugs. *Surv Ophthalmol.* 1982;26(4):207-18.

¹¹ Mammo ZN, Flanagan JG, James DF, Trope GE. Generic versus brand-name North American topical glaucoma drops. *Can J Ophthalmol.* 2012;47(1):55–61. doi: 10.1016/j.jco.2011.12.004.

¹² Urtti A, Salminen L. Minimizing systemic absorption of topically administered ophthalmic drugs. *Surv. Ophthalmol.* 37(6), 435–456 (1993).

¹³ Vaajanen A, Vapaatalo H. A single drop in the eye - Effects on the whole body? *Open Ophthalmol J.* 2017;11:305–314. doi: 10.2174/1874364101711010305

¹⁴ Farkouh A, Frigo P, Czejka M. Systemic side effects of eye drops: a pharmacokinetic perspective. *Clin Ophthalmol.* 2016;10:2433–2441.

¹⁵ Jordan A, Baum J. Basic tear flow. Does it exist? *Ophthalmology.* 1980 Sep;87(9):920–930.

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can be even higher¹⁶. Approximately 80% of a medication instilled as an eyeshot is lost to drainage during the first 15–30 seconds¹⁷.

Microdosed mydriatic medications have been demonstrated to provide similar or superior efficacy compared to traditional eyedropper systems. Microdosing can also reduce systemic absorption and side effects, improve patient comfort, and minimize drug dilution and spillage. Studies comparing microdosed phenylephrine and cyclopentolate to traditional eyedrops (26 – 30 μ L drop size) in premature babies¹⁸ and in full-term infants¹⁹ have shown equivalent pupil dilation with drop sizes ranging from 5 – 8 μ L^{20,21} while reducing systemic levels by more than 50%²².

Tropicamide 1.0% spray application caused similar pupil dilation as the same solution delivered as an eyeshot, but with less discomfort²³. The main reason microdosing has not become the accepted method for topical ocular dosing of ophthalmic medication is that no system has been developed that can consistently deliver accurate microdoses of these medications.

As shown in Figure 1, the Eyenova MAP Dispenser is designed to deliver a finely controlled microdroplet spray of tropicamide-phenylephrine fixed combination ophthalmic solution with precisely defined volume, velocity, and geometry.²⁴ These characteristics facilitate high-precision, piezo-generated medication delivery that gently coats the ocular surface.

¹⁶ Farris RL, Stuchell RN, Mandell ID. Basal and reflex human tear analysis. I Physical measurements: osmolarity, basal volumes, and reflex flow rate. *Am Acad Ophthal* 1981;88:852.

¹⁷ Lallemand F, Daull P, Benita S, Buggage R, Garrigue JS. Successfully improving ocular drug delivery using the cationic nanoemulsion, novasorb. *J Drug Deliv*. 2012;2012:604204.

¹⁸ Wheatcroft S, Sharma A, McAllister J. Reduction in mydriatic drop size in premature infants. *Br J Ophthalmol*. 1993;77(6):364-5.

¹⁹ Lynch MG, Brown RH, Goode SM, Schoenwald RD, Chien DS. Reduction of phenylephrine drop size in infants achieves equal dilation with decreased systemic absorption. *Arch Ophthalmol*. 1987;105(10):1364-5.

²⁰ Wheatcroft S, Sharma A, McAllister J. Reduction in mydriatic drop size in premature infants. *Br J Ophthalmol*. 1993;77(6):364-5.

²¹ Lynch MG, Brown RH, Goode SM, Schoenwald RD, Chien DS. Reduction of phenylephrine drop size in infants achieves equal dilation with decreased systemic absorption. *Arch Ophthalmol*. 1987;105(10):1364-5.

²² Ibid.

²³ Akman A, Aydin P. Comparison of mydriatic efficacy of spray application and drop instillation of tropicamide 1%. *Eye (Lond)*. 1999;13(5):653–655.

²⁴ Ianchulev T, Chayet A, Kahook M, Packer M, Pasquale L, Weinreb RN. Pharmacodynamic profile of mydriatic agents delivered by ocular piezo-ejection microdosing compared with conventional eyedropper. *Ther Deliv*. 2016;7(11):751-760.

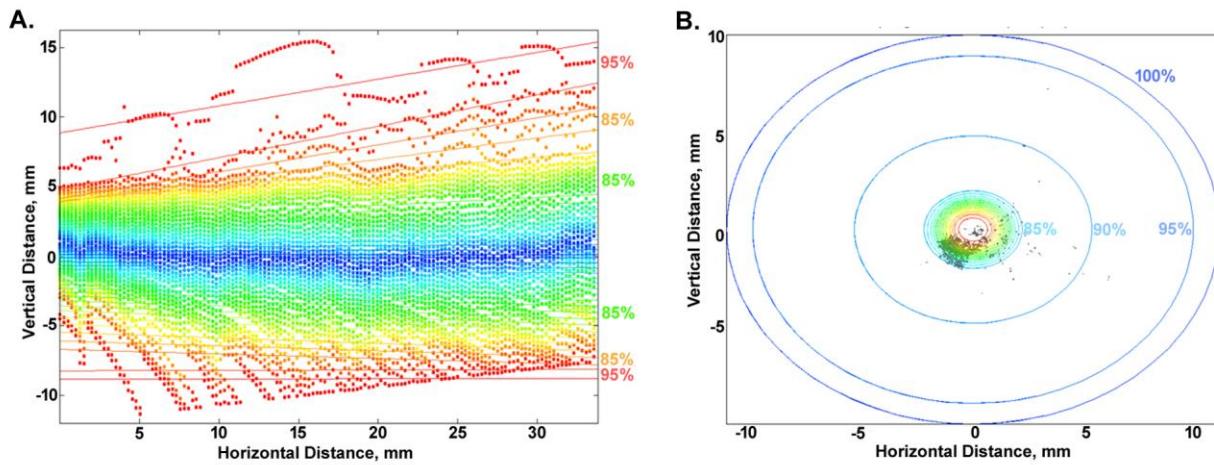


Figure 1: Microdroplet Distribution Pattern from the MAP Dispenser

Image analysis of high-speed photographs of the microdroplet plume quantitatively confirms precise medication microdroplet delivery. Shown are representative side (A) and cross-sectional (B) views of the droplet dispersal pattern. Color-coding indicates the percentage of the total medication dose present at different distances from the nozzle-to-target dead-center axis. The cross-sectional pattern on the right was captured 3 cm from the MAP Dispenser nozzle.

4. INVESTIGATIONAL PRODUCT DESCRIPTION

This study will evaluate pupil dilation speed subsequent to dosing with 1 mist and 2 mists of tropicamide-phenylephrine fixed combination ophthalmic solution (*T-P OFTENO SOLUCIÓN 50 mg/8 mg/ ml*) intended for mydriasis.

4.1 How Provided

The tropicamide-phenylephrine fixed combination ophthalmic solution to be studied in this protocol is contained sterile in the Micro-Array Print (MAP) dispenser, which is designed to deliver precise low-volume (~ 8 μ L) doses of topical ocular pharmaceutical ingredients. As shown in *Error! Reference source not found.*, the Micro-Array Print (MAP) Dispenser is composed of a “cartridge” and a “base”.

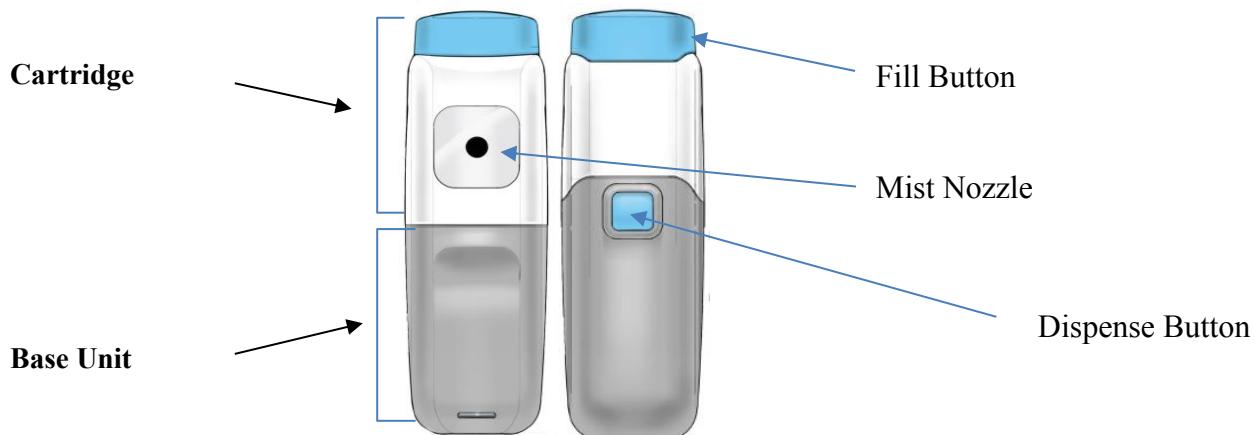


Figure 2: Micro-Array Print (MAP) Dispenser Front (L) and Back (R) View

The cartridge is the primary container closure system, which contains a vial filled with sterile study ophthalmic solution. At the time of initial Micro-Array Print (MAP) Dispenser actuation, the seal on the vial is punctured to allow the solution to flow to the Micro-Array Print (MAP) Dispenser delivery system. The cartridge also contains a piezo-delivery system designed to drive the solution through the Mist Opening. There is \leq 100 milliseconds time lapse between when the first solution droplet hits the corneal surface and completion of dose delivery, which is faster than the average involuntary blink response time (Kwon 2013).

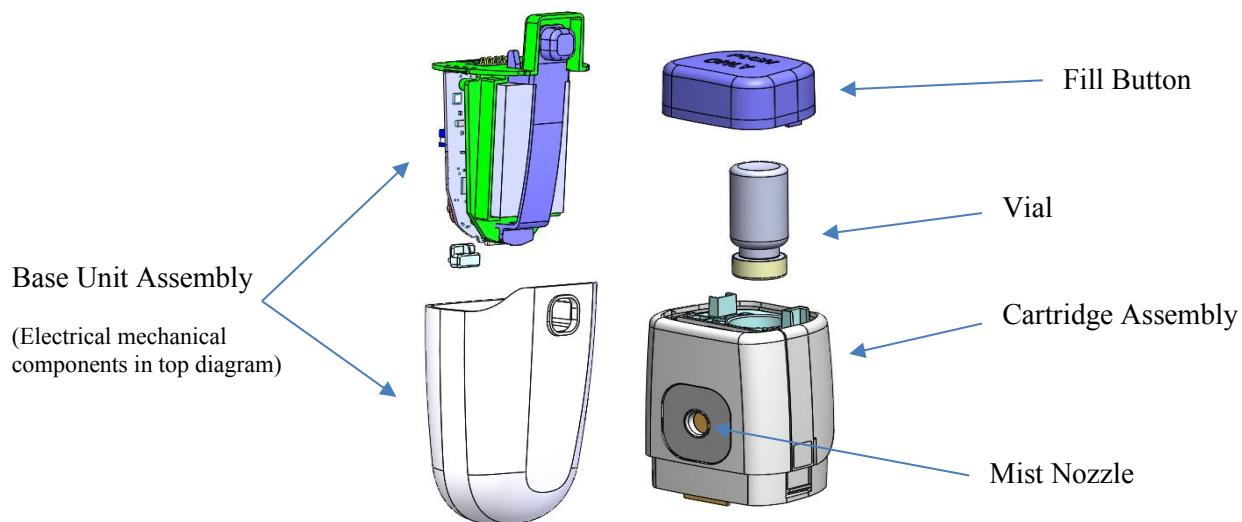


Figure 3: Diagram of the Micro-Array Print (MAP) Dispenser Base Unit (L) and Cartridge Assemblies (R)

Prior to initial Micro-Array Print (MAP) Dispenser use, tropicamide-phenylephrine fixed combination solution is aseptically transferred from the commercially available tropicamide-phenylephrine fixed combination ophthalmic solution bottle (T-P OFTENO SOLUCIÓN 50 mg/8 mg/ ml) in which it was received into the MAP Dispenser vial, the MAP Dispenser vial is inserted into the cartridge assembly, the cartridge and base are clipped together, and the seal on the vial containing the drug is punctured by depressing the MAP Dispenser Fill Button. Puncture of this seal creates a fluid path for the drug. Depression and release of the Fill Button approximately 5 times primes the system for initial drug delivery. At the time of dosing, the Fill Button is again pressed and released to activate the piezo element to create the aerosol containing the drug dose. When the Dispense Button is pressed, approximately 8 μ L of the drug is horizontally delivered through the Mist Nozzle to the cornea.

4.2 Route of Administration, Dosage, Regimen, Treatment Period(s)

The study medication will be administered by trained clinical study staff at two treatment visits in accordance with each subject's treatment assignment schedule. Either 1 mist in each eye or 2 mists administered approximately 5 minutes apart in each eye will be topically administered.

5. STUDY OBJECTIVE

The primary objective of this study is to compare pupil dilation speed achieved with a single dose of MAP Dispenser-administered tropicamide-phenylephrine fixed combination to two doses of MAP Dispenser-administered tropicamide-phenylephrine fixed combination administered 5 minutes apart.

6. STUDY DESIGN

This trial is a single center, assessor-masked, active-controlled, cross-over noninferiority study evaluating tropicamide-phenylephrine fixed combination ophthalmic solution administered as a single mist to each eye and as two mists, administered 5 minutes apart in each eye.

Volunteer participants will be screened for study eligibility during a Screening Visit and enrolled after signing the study-specific informed consent form (ICF). Subjects meeting all inclusion/exclusion criteria will be scheduled for 2 treatment visits, which must occur at least 4 days, but no more than 7 days apart. At each of the two treatment visits, baseline measurements will be taken, then either 1 mist or 2 mists of the study drug will be administered to both eyes (OU). In the instances of 2 mists, the separate dispensing will occur approximately 5 minutes apart. Only 1 mist or 2 mists of the drug will be administered per treatment visit, and drug administration will occur in a randomized order using one of two sequences - AB and BA, where A is the dispensing of 1 mist of tropicamide-phenylephrine fixed combination and B is the dispensing of 2 mists of tropicamide-phenylephrine fixed combination. Afterwards, efficacy and safety assessments will be performed.

This study will be single-masked. There will be no differences in the presentation of the investigational drug administered and study personnel conducting ophthalmic assessments will be masked to study drug administration assignment. Personnel who administer study drug on a given day will not be allowed to perform post-study drug administration ophthalmic assessments on that day.

7. STUDY OUTCOMES

7.1 Effectiveness Outcomes

The primary performance endpoint is mean change in pupil diameter at 35 minutes versus visit-specific baseline, as measured by digital pupillometry in highly photopic conditions. The highly photopic condition will be established using a fully-charged transilluminator (muscle light) at the brightest setting. The difference in mean change in pupil diameter will be compared between the single dose of MAP Dispenser-administered tropicamide-phenylephrine fixed combination and two doses of MAP Dispenser-administered tropicamide-phenylephrine fixed combination administered 5 minutes apart. The primary objective is to demonstrate non-inferiority of the single dose to the two doses, with a non-inferiority margin of 0.5 mm.

The study's sample size will provide 90% power to demonstrate non-inferiority of the single dose regimen assuming a true difference of 0.0 mm, a standard deviation of 0.85 mm, and a non-inferiority margin of 0.5 mm.

Additional exploratory outcomes (also to be measured in highly photopic conditions) include:

- Proportion of eyes achieving pupil diameter of 6.0 mm or greater at 35 minutes
- Proportion of eyes achieving pupil diameter of 7.0 mm or greater at 35 minutes
- Mean change in pupil diameter at other timepoints (15, 55 minutes)
- Distribution of pupil diameters at 15, 35, 55 minutes
- Proportion of successful administrations on 1st attempt

These exploratory outcomes will also be measured using digital pupillometry in highly photopic conditions.

7.2. Safety Outcomes

A safety analysis will be conducted to compare one dose vs. two doses of fixed combination of phenylephrine 2.5%-tropicamide-phenylephrine fixed combination μ D for the following outcomes:

- Slit-lamp examination (SLE) findings
- Anterior segment exam by transilluminator findings
- Occurrence of AEs
- Intraocular pressure (IOP) measured at 60 minutes post-administration
- Visual acuity changes
- Proportion of administrations without Micro-Array Print (MAP) Dispenser touch to the eye

8. STUDY POPULATION

Volunteer participants will be considered enrolled after signing the study-specific ICF(s) and screened for study eligibility during a Screening Visit. Subjects meeting all inclusion/exclusion criteria will be scheduled for 2 treatment visits. It is assumed that approximately 5% of subjects (n = 4) will fail to complete the study visits after randomization to treatment; therefore, to have 56 subjects evaluable for the primary efficacy analysis, a minimum of 60 subjects will be randomized to study drug administration at Treatment Visit 1 (Day 1). To account for possible screen failures, the study will enroll up to 70 subjects.

8.1. Inclusion Criteria

Subjects eligible for study participation must meet each criterion listed below. Ocular criteria must be met for both eyes.

1. Male or female 18 years old or above.
2. Female subjects must be either 1-year postmenopausal, surgically sterilized, or, if of childbearing potential, have a negative urine pregnancy test during the Screening Visit and agree to use an acceptable form of contraception throughout the study. *Acceptable methods include the use of at least one of the following: intrauterine (intrauterine device), hormonal (oral, injection, patch, implant, ring), barrier with spermicide (condom, diaphragm), or abstinence.*
3. Ability to provide signed written consent prior to participation in any study-related procedures.
4. Ability to return for all study treatment visits.
5. Photopic screening pupil diameter \leq 3.5 mm in each eye.

8.2. Exclusion Criteria

Subjects with any of the following diseases, surgeries or conditions are ineligible for study participation. Subjects may not participate if either eye meets any of the ocular exclusion criteria.

1. Pregnancy or lactation.
2. Allergy to phenylephrine hydrochloride.
3. Allergy to tropicamide.
4. Allergy to benzalkonium chloride.
5. History of benign prostatic hyperplasia.
6. Use of benzodiazepines, monoamine oxidase inhibitors, tricyclic antidepressants, anticonvulsants at screening or anticipated during the study period.
7. Participation in any study of an investigational drug or device within 30 days prior to the Screening Visit, or at any time during the study period.
8. History of closed-angle glaucoma.
9. Anatomically narrow anterior chamber angles (Van Herrick grade \leq 2 in either eye).
10. Ocular surgery or laser treatment of any kind prior to the Screening Visit.
11. History of chronic or acute uveitis.
12. History of traumatic iritis or hyphema.

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13. History of traumatic mydriasis or angle recession.
14. History of heterochromia.
15. Irregularly-shaped pupil secondary to ocular trauma or congenital defect.
16. History of neurogenic pupil disorder (e.g., Horner's syndrome, third cranial nerve palsy, Adie's pupil, Argyl Robertson syndrome, etc.).
17. History of anterior chamber intraocular lens (IOL) or iris-fixated IOL.
18. History of iris surgery of any kind (e.g., iridotomy, iridectomy, coreoplasty).
19. History of iris atrophy.
20. History of iris – cornea apposition/touch.
21. Unwilling or unable to discontinue use of contact lenses at treatment visits.
22. Current active eye disease for which topical or systemic ophthalmic medication is necessary, except for dry eye disease managed using artificial tears (AT). *AT's must be discontinued on the day of each treatment visit.*
23. Presence of a severe/serious ocular condition, or any other unstable medical condition that, in the Investigator's opinion, may preclude study treatment and/or follow-up.

9. STUDY METHODS

9.1. Subject Informed Consent

The EC-approved study ICF must be used for administration of informed consent.

The investigator or designee will explain the study purpose, procedures and responsibilities to the potential participant and provide sufficient opportunity to ask questions, while allowing adequate time for consideration of the information provided. Written consent for study participation must be present prior to initiation of any study-specific procedure; subjects will be considered enrolled in the study upon their signature on the consent form(s). It is the responsibility of the Investigator to complete the informed consent process, maintain a copy of the signed consent forms in the subject's medical records, and provide each subject with a copy of their fully-executed consent documents.

9.2. Subject Selection and Screening Procedures

Study participants will be recruited from the Investigator's patient population, referrals, or other outreach methods. Written recruitment materials directed to potential study participants must be approved by the EC.

Where possible, candidate participants may be pre-screened via review of their medical charts to evaluate potential eligibility based on study inclusion/exclusion criteria. During the informed consent discussion, the potential participant's willingness and ability to meet the follow-up requirements will be evaluated. Those who elect to sign the study consent form(s) will be considered enrolled in the study and given a study identification code. At/after the time of enrollment, the subject will be evaluated at the Screening Visit for study eligibility based on inclusion/exclusion requirements established in **Sections 8.1 and 8.2**. Subjects who fail to qualify for the study will be considered "screen failures" and exited from the study. Subjects who successfully complete the Screening Visit will be scheduled for their next visit at which the first study medication will be administered.

9.3. Randomization to Study Drug Administration Sequence

Each eligible subject will be randomly assigned to receive one of 2 sequences, AB and BA, where A is the dispensing of 1 mist of tropicamide-phenylephrine fixed combination and B is the dispensing of 2 mists of tropicamide-phenylephrine fixed combination. After Treatment Visit 2, each study subject should have received both 1 mist of tropicamide-phenylephrine fixed combination and the 2 mists of tropicamide-phenylephrine fixed combination. The study drug administration schedule is equally distributed across the 2 randomization sequences. Subject drug administration assignments will be prepared using a computer-generated randomization scheme by an independent statistician who is not involved in the day-to-day conduct of the study. The drug assignment sequence (Study Drug AB or BA) will be provided in paper format contained in a sealed envelope for each subject randomized to treatment.

This study will be single-masked. There will be no differences in the presentation of the investigational drug administered and study personnel conducting ophthalmic assessments will be masked to study drug administration assignment. Personnel who administer study drug on a given day will not be allowed to

perform post-study drug administration ophthalmic assessments on that day.

9.3.1. Procedure for Unmasking Study Drug Assignment

Unmasking of study drug assignments can only occur in the event of a medical emergency or occurrence of an AE that, in the opinion of the investigator, warrants such action. In the absence of medical need, the study drug assignment will not be available to the investigator, site staff, or Sponsor (or designee) representative involved in day-to-day study management until study completion and database lock.

In the event an investigator considers unmasking of a subject's study drug assignment to be necessary, he/she must first contact the study Medical Monitor (or designee) to discuss the case. Only after consultation with the study Medical Monitor will the decision be made regarding unmasking. Study drug assignments will be revealed only for the subjects approved for unmasking, thus leaving masking on remaining subjects intact.

9.4. Study Drug Packaging, Labeling and Storage

The investigational product will be delivered in microdose form using the Eyenova MAP Dispenser. The product must be aseptically transferred from the commercially available ophthalmic solution bottle (T-P OFTENO SOLUCIÓN 50 mg/8 mg/ ml for dilation of the pupil) in which it is received to the sterile vial provided with the MAP Dispenser cartridge. After aseptic transfer, the filled vial is re-inserted into the MAP Dispenser cartridge.

To maintain masking of the study drug assignments for study personnel performing clinical evaluations, study inventory storage and management will be performed by a sterile drug transfer technician not allowed to administer drug and not allowed to perform ophthalmic assessments. The Sponsor, or designee, will provide study drug dispensers to the site.

Instructions regarding study medication preparation are included in **APPENDIX 2: STUDY MEDICATION PREPARATION PROCEDURE**. Each designated sterile drug transfer technician will be trained and certified by the Sponsor, or designee, prior to study medication preparation.

Prior to use, study medication must be stored in a secure area. To maintain product stability, keep the bottle tightly closed in no more than 30 °C.

New MAP Dispensers must be prepared by the sterile drug transfer technician for use by site personnel on each treatment day. To maintain masking of site personnel, the MAP Dispensers containing study drug will be labeled with the pharmacy preparation date and "use by" date, then forwarded to site personnel for use. The date on which the MAP Dispenser is used will be added by site personnel. At the end of the treatment day, each MAP Dispenser provided will be returned to storage. **Please note that each MAP Dispenser prepared must be used on the day of preparation; the MAP Dispenser may not be re-used on subsequent days.**

9.5. Study Drug Administration

Site personnel designated to administer study medication will be trained by the Sponsor on proper use of the MAP Dispenser prior to study initiation. A single MAP Dispenser should be used for study drug administration to all subjects as indicated by their study drug assignment on that treatment day. At each treatment visit, after collection of baseline BCDVA, IOP and SLE data, study medication will be administered by designated site personnel in accordance with the subject's assigned sequence. Subjects will be administered either 1 mist of tropicamide-phenylephrine fixed combination OU or 2 mists of tropicamide-phenylephrine fixed combination OU. With the dispensing of 2 mists of tropicamide-phenylephrine fixed combination, approximately 5 minutes should elapse between the first and the second dose administration. Study drug administration procedures are further detailed in **APPENDIX 3: STUDY MEDICATION ADMINISTRATION PROCEDURE**.

9.6. Study Visits and Clinical Assessments

Study participants will be evaluated at a Screening Visit. Subjects who qualify for further study participation will be scheduled for visits on Treatment Day 1 and Treatment Day 2 at which the assigned study drug is administered and examinations are performed. Data collected from each visit will be documented in the source records and recorded on study electronic Case Report Forms (eCRF).

The timing and frequency of assessments/procedures to be performed at each visit is outlined in **APPENDIX 1: SCHEDULE OF MEDICATION ADMINISTRATION AND EXAMINATIONS**. Methodology for study examinations is presented in **APPENDIX 4: EXAMINATION PROCEDURES**.

9.6.1. Screening Visit (-14 Days to Day 0)

The Screening Visit must occur no more than 14 days prior and no less than 1 day prior to Treatment Visit 1. At the Screening Visit, the following activities will be performed:

- Documentation of study informed consent/assent using EC-approved consent form(s)
- Collection of demographic data (gender, age, ocular comorbidities)
- Collection of medical/ocular history and medication use data
- Administration of urine pregnancy test, if applicable
- BCDVA – OU
- Pupil diameter measurement – OU
- Slit lamp biomicroscopy including Van Herrick angle assessment – OU
- Dilated Fundus Exam – OU
- IOP measurement - OU
- Determination of study eligibility

9.6.2. Treatment Visit 1 (Day 1)

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Subjects will return to the site at least 1 day and no more than 14 days after the Screening Visit. The subject will be queried regarding the occurrence of any adverse events since the time of the Screening Visit. The following baseline measurements will be taken prior to study drug administration:

- Pupil diameter measurement – OU
- BCDVA – OU
- Slit lamp biomicroscopy – OU
- IOP measurement - OU

The first study drug dosing sequence will be either 1 mist or 2 mists of the study drug administered to both eyes (OU). With the 2 mists sequence, the 2 dispensations will occur approximately 5 minutes apart. With the 2 mists sequence, the completion of the first administration of medication will be considered Time 0 (T0). The following assessments will be done at the timepoints specified below:

Timepoint	Clinical Assessment (to be performed in the specified order)
T0 + 15 minutes (+/- 2 minutes)*	<ul style="list-style-type: none">• Pupil diameter measurement – OU• AE assessment
T0 + 35 minutes (+/- 5 minutes)**	<ul style="list-style-type: none">• Pupil diameter measurement – OU• AE assessment
T0 + 55 minutes (+/-10 minutes)***	<ul style="list-style-type: none">• Pupil diameter measurement – OU• Anterior segment exam by transilluminator – OU• AE assessment

* The pupil diameter is the only measurement that must be within the window (\pm 2 min)

** The pupil diameter is the only measurement that must be within the window (\pm 5 min)

*** The pupil diameter is the only measurement that must be within the window (\pm 10 min)

9.6.3. Treatment Visit 2 (Day 5 to Day 8)

Subjects will return to the site. Subjects will be queried regarding the occurrence of any adverse events since the previous visit and the following baseline measurements will be taken prior to study drug administration:

- Pupil diameter measurement – OU
- BCDVA – OU
- Slit lamp biomicroscopy – OU
- IOP measurement - OU

The second study drug dosing sequence will be either 1 mist or 2 mists of the study drug administered to both eyes (OU). With the 2 mists sequence, the 2 dispensations will occur approximately 5 minutes apart. With the 2 mists sequence, completion of the first administration of medications will be considered Time 0 (T0). The following assessments will be done at the timepoints specified below:

Timepoint	Clinical Assessment (to be performed in the specified order)
T0 + 15 minutes (+/- 2 minutes)*	<ul style="list-style-type: none">• Pupil diameter measurement – OU• AE assessment
T0 + 35 minutes (+/- 5 minutes)**	<ul style="list-style-type: none">• Pupil diameter measurement – OU• AE assessment
T0 + 55 minutes (+/-10 minutes)***	<ul style="list-style-type: none">• Pupil diameter measurement – OU• Anterior segment exam by transilluminator – OU• AE assessment

* The pupil diameter is the only measurement that must be within the window (\pm 2 min)

** The pupil diameter is the only measurement that must be within the window (\pm 5 min)

*** The pupil diameter is the only measurement that must be within the window (\pm 10 min)

If applicable, a urine pregnancy test will be administered at this visit.

9.4.5. Unscheduled Visits

Unscheduled visits are those which are not required by the study protocol, but which occur due to an ocular intervention or a subject complaint regarding their eye. No specific testing is required at unscheduled visits; rather, the investigator and/or qualified study staff will perform the procedures necessary to treat/evaluate the subject at these visits. Clinical data from these visits will be recorded on the relevant Unscheduled Visit eCRF.

9.4.6. Missed Visits

Subjects who miss a scheduled visit should be contacted by site personnel to encourage the subject to return to clinic as soon as possible after the missed visit. In the event a subject does not return for multiple consecutive examinations, site personnel must make a minimum of 3 documented attempts to contact the subject. If the subject does not reply to any of these attempts, site personnel should send a letter by certified mail (with request for notification of delivery receipt) to the subject. If a subject is non-responsive to each of these follow-up attempts, he/she will be terminated from the study and considered lost to follow-up.

9.7. Study Exit

9.7.1. Subject Termination

Subjects may be terminated from the study due to:

- Failure to meet protocol eligibility criteria
- Investigator decision that termination is medically indicated
- Voluntary withdrawal from the study
- Loss to follow-up
- Other administrative reasons (e.g., study terminated by Sponsor, technical problems, non-compliance with study medication administration or other study requirements, etc.)

Subjects who terminate due to the occurrence of an AE will be followed until resolution or stabilization of the event.

Subjects who exit the study due to failure to meet eligibility criteria may be replaced. Subjects who exit the study prematurely for other reasons will not be replaced. The Sponsor (or designee) should be promptly notified of a subject's early termination, and information regarding the termination (e.g., date and reason) should be documented in the source records and on the Study Exit eCRF.

9.7.2. Subject Completion

Subjects will be considered to have completed the study if they did not terminate study participation prior to completion of primary endpoint assessment at Treatment Visit 2.

10. ADVERSE EVENTS

Throughout the course of the study, all efforts must be made by the investigator to remain alert to possible AEs or untoward findings. All AEs must be assessed for severity and relationship to the study drug, and the investigator must take all appropriate and necessary therapeutic measures required for AE resolution. Adverse events must be evaluated until resolution or, if the AE is assessed as chronic, until stable.

10.1. Adverse Event Definitions

Most of the AE definitions provided are published in or **ICH E6 – Good Clinical Practice: Consolidated Guidance.**

10.1.1. Adverse Event (AE)

Any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug-related.

10.1.2. Adverse Drug Reaction (ADR)

Any noxious and unintended response to a medicinal product administered at any dose. A reasonable possibility must exist that the adverse reaction is related to the medicinal product administered.

10.1.3. Unexpected Adverse Event or Unexpected Suspected Adverse Reaction

An AE or suspected adverse reaction is considered "unexpected" if it is not listed in the Investigator Brochure (IB) or is not listed at the specificity or severity that has been observed; or, if an IB is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended. "Unexpected," as used in this definition, also refers to AEs or suspected adverse reactions that are mentioned in the IB as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug but are not specifically mentioned as occurring with the particular drug under investigation.

10.1.4. Suspected Adverse Reaction

Any AE for which there is a reasonable possibility that the drug caused the event. "Reasonable possibility" means there is evidence to suggest a causal relationship between the study drug and the AE. Suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any AE caused by a drug.

10.1.5. Life-Threatening Adverse Event or Life-Threatening Suspected Adverse Reaction

An AE or suspected adverse reaction is considered "life-threatening" if, in the view of either the Investigator or Sponsor, its occurrence places the subject at immediate risk of death. Life-threatening AEs do not include AEs or suspected adverse reactions that, had they occurred in a more severe form, might have caused death.

10.1.6. Serious Adverse Event (SAE) or Serious Suspected Adverse Reaction

An AE or suspected adverse reaction that, in the view of either the Investigator or Sponsor, results in any of the following outcomes:

- Death
- Is life-threatening²⁵
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Persistent or significant disability/incapacity, meaning there is a substantial disruption of the subject's ability to conduct normal life functions
- A congenital anomaly or birth defect

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.²⁶

10.1.7. Serious Ocular Adverse Events

Serious ocular AEs include, but are not limited to:

- A decrease in BCDVA of ≥ 30 total letters read (TLR) or an increase of ≥ 0.6 logMar from the most recent previous measurement of BCDVA
- A decrease in BCDVA to light perception (LP) or worse that lasts > 1 hour
- An ocular event that, in the opinion of the investigator, requires medical or surgical intervention to prevent permanent loss of sight
- Severe intraocular inflammation; e.g., 4+ anterior chamber cell/flare or 4+ vitritis
- Corneal decompensation
- Retinal tear or detachment
- Central or branch retinal vein occlusion

10.1.8. Treatment-Emergent Adverse Event (TEAE)

An AE not present prior to initiation of therapy, or an already present event that worsens either in intensity or frequency following initiation of therapy.

10.2. Adverse Event Assessment and Documentation

²⁵ A life-threatening SAE is any AE or adverse reaction that places the subject, in the view of the Investigator, at immediate risk of death from the reaction as it occurred (*i.e.*, it does not include a reaction that, had it occurred in a more severe form, might have caused death).

²⁶ CFR 312.23 – IND Safety Reporting,

<https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=312.32>

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All AEs that occur from the time the subject receives the first dose of study drug²⁷ until study participation has been completed (e.g., TEAEs) must be documented. Any medical condition present prior to the first study drug dose that remains unchanged or improves will not be recorded as an AE; however, a worsening of the condition after dosing with the study drug, will be considered an AE.

Adverse events may be determined by evaluating the following in relation to the study subject:

- Observed or volunteered problems
- Complaints
- Physical signs and symptoms
- The occurrence of a medical condition during the study, which was absent at baseline
- The worsening of a baseline medical condition during the study

Treatment-emergent AEs, regardless of causal relationship, must be assessed by the investigator and recorded in the source documentation and the appropriate eCRF. Each AE must be described as ocular or non-ocular along with the following information: date of onset, date of resolution, severity, frequency of the event (single episode, intermittent, continuous), action taken (none, medical and/or surgical), relationship to study drug, and seriousness criteria. Any medication necessary for the treatment of an AE must be recorded on the Concomitant Medication eCRF and any surgical intervention necessary for AE treatment must be recorded on the Concomitant Procedure eCRF. If more than one distinct AE occurs, each event should be recorded separately.

Adverse events will be documented beginning at the time of onset, and documentation must continue until recovery is noted. Events that are ongoing at the time of study exit must be followed until resolution or stabilization.

10.2.1. Adverse Event Severity

Adverse event severity will be assessed by the investigator using the following definitions:

Mild	Subject is aware of sign or symptom, but it is easily tolerated
Moderate	Subject experiences discomfort enough to cause interference with usual activity
Severe	AE is incapacitating to subject, causing inability to work or do usual activity

10.2.2. Adverse Event Relationship to Study Drug

The relationship of the AE to study drug will be assessed by the investigator using the following definitions:

²⁷ Events that occur between the Screening Visit and initial administration of study drug should be recorded as Medical History, and not as an AE.

Not Related	Evidence exists that the AE has a cause other than the study drug (e.g. pre-existing condition or underlying disease, intercurrent illness, or concomitant medication) and does not meet any other criteria listed.
Possibly Related	A temporal relationship exists between event onset and administration of study drug. Although the AE may appear unlikely to be related to the study drug, it cannot be ruled out with certainty; and/or the event cannot be readily explained by the patient's clinical state or concomitant therapies.
Probably Related	A temporal relationship exists between the event onset and administration of study drug; it appears with some degree of certainty to be related based on known therapeutic and pharmacologic actions of the study drug. It cannot be readily explained by the patient's clinical state or concomitant therapies.
Definitely Related	Strong evidence exists that the study drug caused the AE. There is a temporal relationship between the event onset and administration of the study drug. There is strong therapeutic and pharmacologic evidence that the event was caused by the study drug. The patient's clinical state and concomitant therapies have been ruled out as a cause.

10.3. Adverse Event Reporting

Any treatment-emergent AE occurring in the study must be reported on the AE eCRF. Expedited reporting of an AE is required if the event is considered “serious”, regardless of relationship to the study drug or whether the event is expected or unexpected.

10.3.1. Expedited Adverse Event Reporting

Any SAE that occurs during the study, regardless of relationship to the study drug, or whether the event is expected or unexpected, ***must be reported to the Sponsor, or the Sponsor's designee, within 24 hours*** of the investigator's becoming aware of the event.

Reports should be made by completing the SAE eCRF. Information provided on the SAE eCRF should be supplemented with hospitalization records, death certificate, clinic notes from specialists evaluating the subject's condition, etc. as applicable for the event. The urgency for reporting SAEs is 3-fold:

- to facilitate discussion [and implementation, if necessary] by the Sponsor and the Investigator of appropriate follow-up measures;
- To facilitate Investigator reporting of unanticipated problems involving risk to human subjects to the IRB/EC; and
- To enable the Sponsor to fulfill the reporting requirements to the appropriate regulatory authority.

11. STATISTICAL METHODS

The primary objective of this study is to compare pupil dilation at 35 minutes achieved with a single dose of MAP Dispenser-administered tropicamide-phenylephrine fixed combination to the pupil dilation achieved with two doses of MAP Dispenser-administered tropicamide-phenylephrine fixed combination administered 5 minutes apart.

11.1. Effectiveness Endpoints

Primary Effectiveness Endpoint:

The primary effectiveness endpoint is mean change in pupil diameter at 35 minutes versus visit-specific baseline, as measured by digital pupillometry in highly photopic conditions. The highly photopic condition will be established using a fully-charged transilluminator (muscle light) at the brightest setting.

Exploratory Effectiveness Outcomes:

- Proportion of eyes achieving pupil diameter of 6.0 mm or greater at 35 minutes
- Proportion of eyes achieving pupil diameter of 7.0 mm or greater at 35 minutes
- Mean change in pupil diameter at other timepoints (15, 55 minutes)
- Distribution of pupil diameters at 15, 35 and 55 minutes
- Proportion of successful administrations on 1st attempt

These additional efficacy analyses will also be measured using digital pupillometry in highly photopic conditions.

11.2. Sample Size

Sixty (60) subjects who have provided informed consent for study participation will be enrolled and randomized with the goal of having 56 subjects evaluable for the primary efficacy endpoint.

Sample size can be calculated using methods for cluster randomized trials²⁸. The sample size calculated assuming independence is multiplied by the design effect ($DE = 1 + (m - 1)\rho$, where m is the cluster size and ρ the intraclass correlation) to account for correlation between eyes. In this situation the correlation between eyes in an individual is quite high, approximately 0.8 from previous studies, thus with two eyes per person the DE is 1.8.

Assuming a standard deviation of 0.85 mm for change-from-baseline pupil diameter (from previous studies) and a true difference of 0.0 mm, a sample of 62 independent eyes would provide 90% power to show non-inferiority (NI) of one dose to two, using a NI margin of 0.5 mm and a one-sided t-test at alpha = 0.025.

²⁸ Campbell, M.J., and S.J. Walters, 2014. How to Design, Analyse and Report Cluster Randomized Trials in Medicine and Health Related Research. Wiley, NY.

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Adjusting for correlation between eyes results in a required sample of $62*1.8=112$ “clustered” eyes, or 56 enrolled subjects.

Allowing for approximately 5% for loss to follow-up results in an enrolled sample size of 60 subjects.

11.3. Safety Outcomes

A safety analysis will be conducted to compare a single dose of MAP Dispenser-administered tropicamide-phenylephrine fixed combination to two doses of MAP Dispenser-administered tropicamide-phenylephrine fixed combination administered 5 minutes apart for the following outcomes:

- Slit-lamp examination findings
- Anterior segment exam by transilluminator findings
- Occurrence of AEs
- IOP measured at 60 minutes post-administration
- Visual acuity changes
- Proportion of administrations without Micro-Array Print (MAP) Dispenser touch to the eye

11.4. Analysis Populations

Subjects who are missing efficacy assessments at any of the time points or are otherwise unevaluable for efficacy considerations will not be replaced.

11.4.1. Intent to Treat Population

The intent to treat (ITT) population will consist of all randomized subjects who received a dose of study drug. Subjects will be analyzed according to the planned sequence of treatments.

11.4.2. Per Protocol Population

The per protocol (PP) population will consist of all ITT subjects who completed all planned assessments without major protocol violations. Subjects to be excluded from the PP population will be selected prior to unmasking drug assignment. Subjects will be analyzed according to the sequence of study treatments received.

11.4.3. Modified Per Protocol Population

The modified per protocol (MPP) population is defined similarly to the PP population, except that classification will be made for each subject at the individual treatment visit level, rather than at the subject level. Unlike the PP population, subjects who only complete 1 of the 2 treatment visits may be included.

The MPP will consist of all ITT subjects who completed all planned assessments without major protocol violations for the given treatment visit. Subjects to be excluded from the MPP population will be selected prior to unmasking treatment

11.4.4. Safety Population

The safety population will consist of all ITT subjects. Subjects will be analyzed according to the sequence of study drugs received

11.5. General Statistical Considerations

Primary efficacy analyses will be conducted on the PP population. The primary performance endpoint and exploratory outcomes will be summarized with descriptive statistics, and point estimates with 95% confidence intervals will be calculated for each outcome.

Safety analyses will be performed using the safety population. The number of subjects reporting at least 1 AE of a given type will be summarized. Each AE will be summarized by incidence and percentage and by eye. Slit lamp (including hyperemia assessment) findings at scheduled follow-up visits will be tabulated such that the number and percentage of subjects in each category will be summarized.

All primary analyses will be performed in both eyes. The pupil diameter measurement at each corresponding time point after administration will be compared between study treatments.

Descriptive statistics will be used to summarize continuous outcomes (number of subjects [N], mean, standard deviation or standard error of the mean, median, maximum, and minimum) and categorical variables, i.e. race, sex, iris color (frequency and percentage) at each assessment time point.

The AB/BA crossover design used in this study allows for within-eye estimates of both treatment effects (A and B) and carryover (AB, BA). Thus, there is no need to include random effects for eye in the analysis model. However, to account for the clustering of eyes within individuals the analysis will include a specification of repeated measurements per person (e.g. using a REPEATED statement in SAS or an appropriate correlation structure in the R nlme package).

This study has a “period-dependent” covariate (baseline pupil measurement) as discussed in Jones and Kenward²⁹ (section 5.4.4). We are assuming that the time between visits is sufficiently long that the baseline pupil measurement at each visit is unaffected by carryover.

Unless explicitly stated otherwise, all statistical tests and confidence intervals will be two-sided, alpha = 0.05.

11.5.1. Analysis of Primary Effectiveness

All time points for pupil diameter will be analyzed separately; however, only the 35-minute time point will support primary effectiveness.

²⁹ Jones, B, and M.G. Kenward, 2015. Design and Analysis of Cross-Over Trials, 3rd ed. CRC Press, London.

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Success is defined as non-inferiority of the single-dose MAP Dispenser-administered tropicamide-phenylephrine fixed combination compared to the two-dose MAP Dispenser-administered tropicamide-phenylephrine fixed combination administered 5 minutes apart.

Comparisons of mean pupil diameter between treatments will be based on an ACOVA model for longitudinal data. The model will contain an effect due to subject x eye, period (1 or 2), direct effect of drug dosing, AB/BA carryover, and baseline pupil diameter. The model will include a compound symmetric correlation structure between eyes. Hypothesis tests will be based on confidence intervals for least squares mean (expected marginal mean, see Dean et. al., 2017³⁰) contrasts between treatments.

Let M_1 denote the expected marginal mean from the single-dose MAP Dispenser treatment change-from-baseline pupil diameter and M_2 denote the expected marginal mean from the two-dose MAP Dispenser treatment, both calculated at the observed mean baseline pupil diameter.

The null and alternative hypotheses are defined as follows:

$$H_0: M_1 - M_2 \leq -0.5 \text{ mm}$$
$$H_A: M_1 - M_2 > -0.5 \text{ mm}$$

The non-inferiority null hypothesis will be tested by constructing a 95% confidence interval for the contrast for the difference between M_1 and M_2 , based on the fitted model. If the lower bound of the 95% confidence interval is above -0.5 mm then the null hypothesis will be rejected and the single-dose MAP Dispenser treatment will be declared non-inferior to the two-dose MAP Dispenser treatment.

11.5.2. Additional Efficacy Analyses

The proportion of eyes achieving pupil size of 6.0 mm or greater and 7.0 mm or greater at 35 minutes post-dose will be summarized by eye and by treatment group with descriptive statistics.

For each of these proportions, a comparison of the single-dose MAP Dispenser treatment to the two-dose MAP Dispenser treatment will be conducted using logistic regression, with generalized estimating equation (GEE) methods used to account for correlation between eyes.

The logistic regression model will use the same fixed effects as the primary analysis. Comparisons between treatment groups will be based on a contrast between estimated marginal means for the log odds ratios. Confidence intervals will be produced for odds ratios. In the event that the logistic regression fails to converge, the model may be simplified to obtain convergence. In the event of complete separation (no responses in one or more groups), the Firth method (Firth 1993, Heinze, 2006³¹), will be used to produce

³⁰ Dean, A., D. Voss, D. Draguljić, 2017. Design and Analysis of Experiments, Springer.

³¹ Firth D. Bias reduction of maximum likelihood estimates. Biometrika 1993; 80:27-38.

estimates, with bootstrap confidence intervals. If possible (if the fitting is numerically stable) then a similar model will be fit to the proportions and estimated marginal means for the difference in proportions will also be produced.

In addition, a cumulative proportion of responders analysis (CPRA) will be created to graphically display the proportions of subjects with a pupil size at 35 minutes post-dose over the entire range of possible pupil size cut-offs (Farrar 2006), with separate lines for each treatment and eye combination.

The same model used for the primary endpoint will be fitted to the mean change in pupil diameter at other timepoints (15, 55 minutes). For each of these time points, estimated marginal means and associated 95% confidence intervals will be produced, as in the primary analysis.

The distribution of pupil diameters at 15, 35, 55 minutes will be summarized for each dosing group with descriptive statistics, including quantiles (at $q = 0.05, 0.10, 0.25, 0.50, 0.75, 0.90, 0.95$). Plots will be created to show change in the distribution through time. Pupil diameters through time will also be graphically compared to results from the earlier MIST-1 and MIST-2 studies.

The proportion of successful administrations on 1st attempt will be summarized overall and for each dosing group, with associated (Wilson score) 95% confidence intervals.

11.5.3. Sensitivity Analyses

The primary efficacy analysis described above will be rerun using the MPP population as a sensitivity analysis. No further imputation of missing data will be attempted.

11.5.4. Interim Analyses

No interim analyses are planned for this study.

11.5.5. Analysis of Baseline Data

No separate analyses of baseline data are planned for this study.

12. QUALITY ASSURANCE AND QUALITY CONTROL

The Sponsor or designee will perform quality control and quality assurance checks for this study. Before initiation of study enrollment, the following documents will be reviewed with the investigator and site study staff:

Heinze, G. A comparative investigation of methods for logistic regression with separated or nearly separated data., Statistics in Medicine 2006; 25:4216-4226

- Study protocol
- Study medication preparation procedures
- Study medication administration procedures
- Electronic Case Report Forms (eCRF) and procedures for completion
- Informed consent process
- Adverse event (AE) reporting procedures

12.1. Study Monitoring

During the study, the Sponsor or designee may perform periodic on-site or remote site visits. During these visits, information recorded on the study eCRFs will be verified against source documents to confirm data capture completeness, accuracy and logical consistency. Study documents will be reviewed to confirm protocol compliance and adherence to EC and Sponsor-specified reporting requirements, and product accountability will be checked.

12.2. Study Product Accountability

Accurate records of each MAP Dispenser base unit and cartridge shipped must be maintained by the Sponsor, or designee, and study investigator. Study drug will be purchased in Mexico on-site. Study drug purchased and used will be documented and the dispensers received and used will be documented.

The study investigator is responsible for documenting each MAP Dispenser used, the date of use, the identifiers for subjects who received study drug with the MAP Dispenser, and the number of mists administered to each subject eye.

MAP Dispenser product accountability will be performed by reconciling all dispensers provided to the site with dispensers used for study drug administration, subjects for whom the dispenser was used, and dispensers returned to the Sponsor or designee. Additionally, for tropicamide-phenylephrine fixed combination product, each commercial bottle purchased will be reconciled with vials prepared, the associated dispensers used in study drug administration, subjects for whom the dispenser was used, and the dispenser returned to the Sponsor or designee.

13. ETHICAL AND REGULATORY CONSIDERATIONS

This protocol was designed and will be conducted, recorded, and reported in compliance with the principles of Good Clinical Practice (GCP)³². Sponsor and Investigator responsibilities associated with adherence to GCP are specified in **APPENDIX 5: SPONSOR AND INVESTIGATOR**

OBLIGATIONS. The study will be performed in accordance with the recommendations guiding physicians in biomedical research involving human subjects adopted by the 18th World Medical Assembly, Helsinki, Finland, 1964 and later amendments (see **APPENDIX 6: WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI**).

13.1. Institutional Review

Before enrollment of study subjects, this protocol and the study-specific consent form must be reviewed and approved by an Ethics Committee (EC) operating in accordance with local regulatory requirements. Any changes to the study protocol or consent form must be approved by the EC prior to implementation. Materials for study patient recruitment and study-specific written materials provided to the subject must also be approved by the EC prior to use.

Ongoing study progress reports will be submitted to the EC at least annually, and more frequently if specified by the EC. Reports of safety events and any protocol deviations that affect the safety and welfare of a study subject will be submitted to the EC in accordance with EC requirements.

13.2. Informed Consent

The study consent form has been developed in compliance with ICH E(6). Study consent form must be submitted to and approved by the EC prior to implementation. Any EC-requested modifications to the consent form must remain in compliance with ICH E(6).

As discussed in **Section 9.1**, each participant must be provided with a copy of the EC-approved consent form for their review, and the participants written approval(s) must be provided prior to initiation of study procedures.

13.3. Subject Confidentiality

The Investigator will maintain the confidentiality of the identity of subjects enrolled in the study and the information contained in the study records. The records will be made available as required for review by a reviewing EC; however, to the extent possible, the subject's identity will not be disclosed.

³² Good Clinical Practice: Integrated Addendum to ICH E6(R1) Guidance for Industry, March 2018.

14. DATA HANDLING AND RECORD KEEPING

Procedures for the handling and analysis of data will be conducted using good computing practices meeting ICH guidelines for the handling and analysis of data for clinical trials.

14.1. Data Quality Control and Reporting

After data have been entered into the study database, a system of computerized data validation checks will be applied and written queries pertaining to data omissions and discrepancies will be forwarded to study sites for resolution. Study staff will update the database as appropriate to resolve queries generated. All changes to the study database will be documented.

14.2. Data Archiving

Archived versions of the database will be saved by the Sponsor or designee consistent with ICH GCP Guidelines, complying with whichever of the requirements is longer. The Sponsor will notify the investigator when documents should be returned.

14.3. Records Retention

The Investigator's site will retain all records related to the study in compliance with ICH GCP Guidelines.

14.4. Protocol Amendments

Modifications to the approved protocol are only possible using approved protocol amendments and with the agreement of all responsible persons. The procedure for approval of a protocol amendment is identical to that for approval of the protocol. The EC must be informed of all protocol amendments and should be asked for its opinion as to whether a full re-evaluation of the ethical aspects of the study is necessary. This should be fully documented.

The investigator must not implement any deviation from or change to the protocol, without discussion with, and agreement by the Sponsor and prior review and documented approval/favorable opinion of the amendment from the relevant EC, except where it is necessary to eliminate an immediate hazard to study patients, or where the change(s) involves only logistical or administrative aspects of the study (e.g., change in monitor(s), change of telephone number(s)).

Protocol amendments will be submitted to the appropriate authority(ies) as required by the applicable regulatory requirement(s).

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APPENDIX 1: SCHEDULE OF MEDICATION ADMINISTRATION AND EXAMINATIONS

Assessment/Procedure	Screening Visit (Day -14 to Day -1)	Treatment Visits 1 and 2 ¹				
		Baseline ⁰	Time 0 ²	Time 1 T0+15 min (\pm 2 min) ⁸	Time 2 T0+35 min (\pm 5 min) ⁹	Time 3 T0+55 min (\pm 10 min) ¹⁰
Informed consent	X					
Demographics	X					
Medical history	X					
Ocular history	X					
Prior/concomitant medication use	X					
Urine pregnancy test ³	X ³					
Manifest refraction (OU)	X					
BCDVA (OU) ⁴	X	X				
Study drug administration sequence determination (OU)		X (at Treatment Visit 1 only)				
Study drug administration (OU)			X			
Slit lamp biomicroscopy (OU)	X	X				
Anterior segment exam by transilluminator (OU)						X
Van Herrick Angle Assessment (OU) ⁵	X					
IOP (OU) ⁶	X	X				
Pupil diameter assessment (OU) ⁷	X	X		X	X	X
Study eligibility determination	X					
Dilated fundus exam (OU)	X					
AE assessment		X		X	X	X

⁰ Baseline refers to evaluations made at each Treatment Day prior to study medication administration.

¹ Treatment Visit 1 and 2 must be separated by at least 4 days; but may be up to 7 days apart to allow for scheduling flexibility.

² Time 0 starts at the point that either the 1 mist dose or first of two mists of study medication doses is administered in second eye of the study subject

³ A urine pregnancy test will be conducted in females of childbearing potential at the Screening Visit and at the last visit (Treatment Visit 2). The test may be administered at any time during the Screening and Treatment Day 2 Visits. If the subject terminates study participation prior to Treatment Visit 2, this test must be administered promptly at the time of termination.

⁴ BCDVA to be measured using Snellen methods. BCDVA to be assessed using Screening Visit manifest refraction at each Treatment Visit.

⁵ Performed as part of slit lamp examination.

⁶ IOP to be measured without anesthesia using the Oculus Orvis tonometer.

⁷ Performed using Neuroptics pupillometer – VIP 300.

⁸ The pupil diameter is the only measurement that must be within the window (\pm 2 min)

⁹ The pupil diameter is the only measurement that must be within the window (\pm 5 min)

¹⁰ The pupil diameter is the only measurement that must be within the window (\pm 10 min)

APPENDIX 2: STUDY MEDICATION PREPARATION PROCEDURE

To maintain masking of the drug assignments for study staff performing clinical evaluations, study medication preparation and inventory management will be performed by a sterile transfer technician. The Sponsor, or designee, will provide the following to the site:

MAP Components
<ul style="list-style-type: none">• 6 x MAP Dispenser base units• 12 x MAP Dispenser cartridges containing empty 2 mL vial (sterile) + loose adhesive label to be applied to cartridge• Accessories for sterile transfer• 8 x Micro USB charging cable/connector plug

Prior to initial MAP Dispenser use, the study drug must be aseptically transferred from the commercial product bottle in which it is received into the MAP Dispenser vial. The vial is then inserted into the MAP Dispenser cartridge assembly, the cartridge and base are clipped together, and the seal on the vial containing the drug is punctured by depressing the MAP Dispenser Fill Button. Puncture of this seal creates a fluid path for the drug. Depression and release of the Fill Button approximately 5 times primes the system for drug delivery. At the time of dosing, the Fill Button is again pressed and released to activate the piezo element to create the aerosol containing the dose. When the Dispenser Button is pressed, approximately 8 μ L of the ophthalmic solution is delivered horizontally through the Spray Nozzle to the cornea.

The site will be trained and certified by the Sponsor, or designee, regarding MAP Dispenser cartridge assembly processes prior to their performance.

MAP Dispenser cartridges and base units will be provided by Eyenova to site personnel for use. A cartridge and base unit will be assembled to be used that day and the “date used” for the MAP Dispenser will be added to the product preparation/use label. At the end of the treatment day, each MAP Dispenser provided will be returned to storage. **Please note that each MAP Dispenser must be used on the day of preparation; the MAP Dispenser may not be re-used on subsequent days.**

APPENDIX 3: STUDY MEDICATION ADMINISTRATION PROCEDURE

This study will be single-masked. There will be no differences in the presentation of the investigational drug administered and study personnel conducting ophthalmic assessments will be masked to study drug administration assignment. Personnel who administer study drug on a given day will not be allowed to perform post-study drug administration ophthalmic assessments on that day. Site personnel designated to administer study medication will be trained by the Sponsor on proper use of the MAP Dispenser prior to study medication administration. In order to reduce the potential for operational bias, study personnel who administer study medication on a given day will not be allowed to perform post-study drug administration ophthalmic assessments on that day. Study drug must be administered in location unattended by examining staff so that examiners are not aware of which drug dose the subject received.

For each treatment day, one MAP Dispenser base units and 2 cartridges containing the study drug to be administered should be provided for use. Each MAP Dispenser will appear the same. The cartridge and base unit will be assembled, then the study drug administration day will be added to the MAP Dispenser label and the system will be primed for use by depressing and releasing the Fill Button, then depressing and releasing the Dispense Button. This sequence must be performed up to 5 times to complete the priming process.

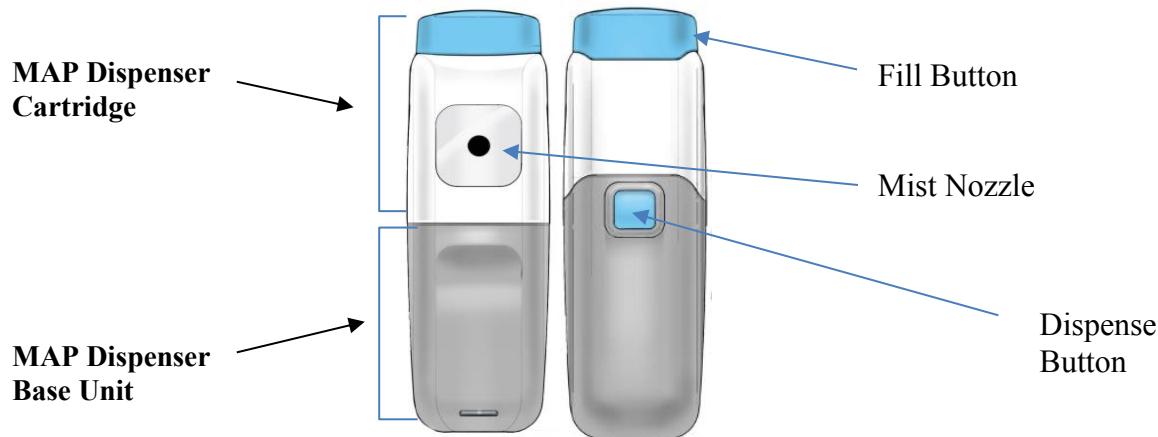


Figure 1: Eyenova MAP Dispenser Front (L) and Back (R) View

At the time of study medication administration, the following steps should be followed sequentially:

1. Fill MAP Dispenser dose by depressing the Fill BUTTON once.
2. Hold MAP Dispenser such that the thumb and 3rd finger are positioned on each side of the cartridge and index finger is positioned on Dispense BUTTON at the back of the MAP Dispenser. *Note: Holding MAP Dispenser with fingers around the front of the dispenser may affect dispenser alignment with the eye, resulting in MIST administration outside of the targeted corneal area.*

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3. Begin to position the MAP Dispenser over the patient's eye such that the MIST NOZZLE is facing the cornea and the DISPENSE BUTTON is facing the medication administrator. While positioning, it is suggested to use the little finger of the hand holding the MAP Dispenser to open the lower lid and the thumb of the opposite hand to hold the upper lid open.
4. Lower the MAP Dispenser toward the eye such that it is almost touching the patient's brow and cheek. The MAP Dispenser may be rotated slightly to avoid interference that might affect stability during spray administration; however, it is important that the MIST NOZZLE be aligned directly toward the patient's cornea.
5. Ask patient to first look up, then look at the blue light around the MIST NOZZLE
6. When blue light is centered on the patient's iris, depress, then quickly release DISPENSE BUTTON to administer medication. Visually confirm medication delivery to the cornea.
7. If medication delivery missed the cornea, withdraw MAP Dispenser from eye, then reload by depressing the FILL BUTTON once and repeat Steps 1-6.
8. Repeat steps 1-7 for the contralateral eye.

APPENDIX 4: EXAMINATION PROCEDURES

All study personnel conducting ophthalmic assessments will be masked to the study drug administered.

1. Visual Acuity

The evaluation of VA should be performed before the subject's pupils have been dilated. Distance VA measurements should be obtained at a testing distance of 4 meters by trained personnel using Snellen lighted charts. Chart background luminance should be between 80 cd/m² and 160 cd/m², inclusive (85 cd/m² is recommended).

Subjects' BCDVA will be determined using subjective manifest refraction.

The subject should be seated so that the distance from the subject's eyes to the Snellen chart is 4 meters (157.5 inches). The middle line of letters to approximately one-third of the distance from the top line of letters should be at eye level for the subject. The subject should be asked to start reading letters at the top line of the chart. Document the number of letters correctly read the line. Move to the next line down if 4 or more letters are read correctly and document the number of letters correctly read on this line. Continue moving down the lines (documenting the number of letters read for each line) until the subject reads 2 or more letters incorrectly, then stop. *Note: If the total number of letters read (TLR) correctly is less than 20 letters, position the subject 1 meter from the chart and add +0.75 D sphere, then resume testing in the same manner.* If the subject is unable to read letters correctly at both 4.0 meters and 1.0 meters, then the subject should be tested to determine if vision is count fingers (CF), hand motion (HM), light perception (LP), or no light perception (NLP). All visual acuities will be expressed in standard notation for Snellen.

2. Pupil Diameter Measurement

Pupil diameter should be measured prior to additional study-specified examinations using the Neuroptics VIP 300 pupillometer; the same pupillometer should be used for each evaluation on any study visit day for a given subject. The subject should be asked to focus straight ahead at a target placed at 3 meters to avoid accommodation. A fully-charged transilluminator, or muscle light, will be shone into one eye while the pupillometer is used to measure the pupil diameter in the fellow eye. After a brief rest period, the process will be repeated to measure pupil diameter in the other eye.

Pupillometry – Suggestions for Obtaining Consistent Readings

As pupil diameter is the primary endpoint in this study, it is very important to have consistent pupillometry measurements at baseline (prior to medication administration) and at each measurement timepoint subsequent to medical administration. Two factors that can affect pupil dilation during the measurement process include the brightness of the muscle light and the distance of the light from the eye. The following steps should be taken to minimize variability in these factors.

MUSCLE LIGHT

1. Make sure the muscle light is stored in the charger between subject exams in order to maintain full charge.
2. Make sure the muscle light is adjusted to the brightest setting during the pupil measurement.

PUPILLOMETER (VIP-300)

1. Bring the VIP-300 Pupillometer in position on the eye to be measured.
2. When the “circle” appears in the screen bring the fully charged muscle light into the visual field of the contralateral eye.
3. The bend in the muscle light should be aligned with the handle of the pupillometer and shining directly in the pupil of the contralateral eye. (See Figure 1)
4. Release the measurement button on the pupillometer to obtain the measurement.



Figure 1: Proper alignment and position of the muscle light

4. Intraocular Pressure Measurement

After completion of slit lamp biomicroscopy, IOP measurements with the Oculus Corvis Tonometer. Recent tonometer calibration must be documented.

Two consecutive IOP measurements of each eye must be obtained. If the 2 measurements differ by more than 2 mmHg, a third measurement must be obtained. IOP will be analyzed as the mean of these 2 measurements, or as the median of the 3 measurements.

5. Slit Lamp Biomicroscopy or Anterior segment exam by transilluminator

The eyelids, conjunctiva, sclera, cornea, anterior chamber, lens, iris and anterior vitreous of the eye will be examined with the aid of a table-mounted binocular microscope, called a slit lamp or a transilluminator. The patient will be seated during this examination and fluorescein dye should be instilled into the ocular cul-de-sac to facilitate the examination. Grading will be assigned as follows:

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LID

Erythema

None (0)	Normal, without any redness, or less than mild
Mild (+1)	A low grade flushed reddish color
Moderate (+2)	Diffused redness encompassing entire lid margin
Severe (+3)	Deep diffused reddish color of lid margins and superior or inferior eyelid

Edema

None (0)	Normal, no swelling of the lid tissue, or less than mild
Mild (+1)	Slight diffuse swelling above normal
Moderate (+2)	General swelling
Severe (+3)	Extensive swelling of the eyelid, with/without eversion of upper or lower lids

CONJUNCTIVA

Hyperemia

None (0)	Normal. Appears white with a small number of conjunctival blood vessels easily observed
Mild (+1)	Prominent, pinkish-red color of both the bulbar and palpebral conjunctiva
Moderate (+2)	Bright, scarlet red color of the bulbar and palpebral conjunctiva
Severe (+3)	“Beefy red” with petechiae. Dark red bulbar and palpebral conjunctival with evidence of subconjunctival hemorrhage

Edema

None (0)	Normal, no swelling of the conjunctiva or less than mild
Mild (+1)	Slight diffuse or regional swelling of the conjunctiva
Moderate (+2)	General swelling of the conjunctiva
Severe (+3)	Extensive swelling of the conjunctiva

SCLERA

Normal
Abnormal

CORNEA

Edema

None (0)	Transparent and clear, or less than mild
Mild (+1)	Dull glassy appearance
Moderate (+2)	Dull glassy appearance of epithelium with large number of vacuoles
Severe (+3)	Stromal edema, localized or diffuse, with stromal striae

Staining

None (0)	No fluorescein staining of epithelium, or less than mild
Mild (+1)	Slight punctate fluorescein staining
Moderate (+2)	Regionally dense coalescent fluorescein staining
Severe (+3)	Marked fluorescein staining with immediate stromal leakage as a result of epithelial loss

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ANTERIOR CHAMBER

Cells

None (0)	No cells seen, or less than mild
Mild (+1)	+ cells
Moderate (+2)	++ cells
Severe (+3)	+++ cells
Hypopyon (+4)	++++ cells, hypopyon formation (indicate hypopyon size)

Flare

None (0)	No Tyndall effect, or less than mild
Mild (+1)	Tyndall beam in the AC has mild intensity
Moderate (+2)	Tyndall beam in the AC is of strong intensity
Severe (+3)	Tyndall beam is very intensive. Aqueous has a white, milky appearance

IRIS

Normal
Abnormal

LENS

Lens Status

Phakic*

*Indicate Lens Opacity Grade:

None (0)	None present, or less than mild
Mild (+1)	Subtle
Moderate (+2)	Moderate
Severe (+3)	Dense

Pseudophakic
Aphakic

ANTERIOR VITREOUS

Normal
Abnormal

6. Dilated Fundus Examination

Using ophthalmoscopy, the fundus will be examined and evaluated by the Investigator to be either normal or abnormal at the Screening Visit.

APPENDIX 5: SPONSOR AND INVESTIGATOR OBLIGATIONS

SPONSOR OBLIGATIONS

The Sponsor is committed to:

1. Complying with all applicable health authority regulations governing the conduct of clinical research studies.
2. Protecting the rights, health, safety and welfare of study subjects.
3. Informing clinical investigators of any new information about the study which may affect the health, safety or welfare of the subjects, or may influence their decision to continue participation in the study.
4. Providing clinical investigators with the study protocol, and CRFs on which to document the study evaluation variables for each subject entered into the study.
5. Providing the statistical analysis and study report writing resources necessary to complete reporting of study results.
6. Ensuring equity of consideration among all investigators in multicenter studies in all matters of publications, meeting presentations, etc.
7. Certifying that IRB/EC approval of the protocol and Investigator Agreement will be completed prior to treatment at an investigational site.

The Sponsor shall have the right to terminate the study at any time by written notice to the investigator. Without limiting this right, the Sponsor will normally only terminate the study under the following circumstances:

- If severe and/or SAEs associated with the study medication in human and/or animal studies indicate discontinuation of the study
- If the Sponsor wishes to discontinue the study for commercial reasons
- If the Sponsor has reasons to believe that the study cannot be satisfactorily completed due to insufficient patient enrollment, or an insufficient number of participating study sites identified within a reasonable time frame.

INVESTIGATOR OBLIGATIONS

Each Investigator must be a licensed physician who has completed a residency or preceptorship in ophthalmology. The Investigators have the following responsibilities:

1. Subject Selection

The Investigator is responsible for assuring that all subjects entering the study conform to the patient selection criteria.

2. Informed Consent

The Investigator is responsible for fully reviewing the nature of the study, the possible risks, and alternative treatments with prospective patients prior to their enrollment in the study. The Investigator is responsible for obtaining written informed consent in compliance with ICH E(6) for each patient, prior to enrollment in the trial. A copy of the signed Informed Consent Form will be maintained in the patient's medical record.

3. Ethics Committee Approval

The Investigator must obtain approval for participation in this protocol from the EC for the institution at which the procedure will be performed prior to entering any patients in the study. The ICF to be used will also be submitted to the EC for approval prior to initiation of the study.

Assurance that EC approval of the study protocol and ICF has been obtained will be provided to the Sponsor, or Sponsor designee, prior to initiation of the study.

4. Subject Evaluations and Data Reporting

The Investigator is responsible for complying with the requirements of the study protocol and any amendment or clarification as published by the Sponsor, or Sponsor designee. Patient evaluations will be performed as described in the study protocol. All information generated by the patient evaluation will be recorded using eCRFs with access provided by the Sponsor or Sponsor designee.

Investigator(s) will not deviate from the study protocol without prior approval of the Sponsor, or Sponsor designee, unless the protection of health, safety or welfare of study subjects requires prompt action.

5. Record Retention

The Investigator shall maintain all patient records for at least 2 years after completion of the study.

6. Investigational Material Accountability

The Investigator must maintain accurate records of the receipt of all investigational material shipped by the Sponsor, or Sponsor designee, including the date, and identification numbers of the product received. In addition, accurate records must be kept on the amount and date that investigational material was dispensed or returned to the Sponsor. The Investigator must assure that study supplies be dispensed only to subjects enrolled in the study per study inclusion / exclusion criteria and under the direct supervision of the Investigator or his/her Co-Investigators.

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Records of all investigational supplies received, used and returned must be kept by the Investigator. All unused investigational supplies must be returned to the Sponsor, or Sponsor designee, as soon as practical upon completion of the trial. Investigational material accounting procedures must be completed before the study is considered terminated.

APPENDIX 6: WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI

Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the:
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
52nd WMA General Assembly, Edinburgh, Scotland, October 2000
53th WMA General Assembly, Washington 2002 (Note of Clarification on paragraph 29 added)
55th WMA General Assembly, Tokyo 2004 (Note of Clarification on Paragraph 30 added)
59th WMA General Assembly, Seoul, October 2008

A. INTRODUCTION

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data. The Declaration is intended to be read as a whole and each of its constituent paragraphs should not be applied without consideration of all other relevant paragraphs.
2. Although the Declaration is addressed primarily to physicians, the WMA encourages other participants in medical research involving human subjects to adopt these principles.
3. It is the duty of the physician to promote and safeguard the health of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfillment of this duty.
4. The Declaration of Geneva of the WMA binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act in the patient's best interest when providing medical care."
5. Medical progress is based on research that ultimately must include studies involving human subjects. Populations that are underrepresented in medical research should be provided appropriate access to participation in research.
6. In medical research involving human subjects, the well-being of the individual research subject must take precedence over all other interests.
7. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best current interventions must be

evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.

8. In medical practice and in medical research, most interventions involve risks and burdens.
9. Medical research is subject to ethical standards that promote respect for all human subjects and protect their health and rights. Some research populations are particularly vulnerable and need special protection. These include those who cannot give or refuse consent for themselves and those who may be vulnerable to coercion or undue influence.
10. Physicians should consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

B. PRINCIPLES FOR ALL MEDICAL RESEARCH

11. It is the duty of physicians who participate in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects.
12. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.
13. Appropriate caution must be exercised in the conduct of medical research that may harm the environment.
14. The design and performance of each research study involving human subjects must be clearly described in a research protocol. The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, institutional affiliations, other potential conflicts of interest, incentives for subjects and provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study. The protocol should describe arrangements for post-study access by study subjects to interventions identified as beneficial in the study or access to other appropriate care or benefits.
15. The research protocol must be submitted for consideration, comment, guidance and approval to a research ethics committee before the study begins. This committee must be independent of the researcher, the sponsor and any other undue influence. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate

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any of the protections for research subjects set forth in this Declaration. The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious AEs. No change to the protocol may be made without consideration and approval by the committee.

16. Medical research involving human subjects must be conducted only by individuals with the appropriate scientific training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional. The responsibility for the protection of research subjects must always rest with the physician or other health care professional and never the research subjects, even though they have given consent.
17. Medical research involving a disadvantaged or vulnerable population or community is only justified if the research is responsive to the health needs and priorities of this population or community and if there is a reasonable likelihood that this population or community stands to benefit from the results of the research.
18. Every medical research study involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and communities involved in the research in comparison with foreseeable benefits to them and to other individuals or communities affected by the condition under investigation.
19. Every clinical trial must be registered in a publicly accessible database before recruitment of the first subject.
20. Physicians may not participate in a research study involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians must immediately stop a study when the risks are found to outweigh the potential benefits or when there is conclusive proof of positive and beneficial results.
21. Medical research involving human subjects may only be conducted if the importance of the objective outweighs the inherent risks and burdens to the research subjects.
22. Participation by competent individuals as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no competent individual may be enrolled in a research study unless he or she freely agrees.
23. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information and to minimize the impact of the study on their physical, mental and social integrity.
24. In medical research involving competent human subjects, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the

discomfort it may entail, and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information. After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

25. For medical research using identifiable human material or data, physicians must normally seek consent for the collection, analysis, storage and/or reuse. There may be situations where consent would be impossible or impractical to obtain for such research or would pose a threat to the validity of the research. In such situations the research may be done only after consideration and approval of a research ethics committee.
26. When seeking informed consent for participation in a research study the physician should be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent should be sought by an appropriately qualified individual who is completely independent of this relationship.
27. For a potential research subject who is incompetent, the physician must seek informed consent from the legally authorized representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the population represented by the potential subject, the research cannot instead be performed with competent persons, and the research entails only minimal risk and minimal burden.
28. When a potential research subject who is deemed incompetent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorized representative. The potential subject's dissent should be respected.
29. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research population. In such circumstances the physician should seek informed consent from the legally authorized representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research should be obtained as soon as possible from the subject or a legally authorized representative.
30. Authors, editors and publishers all have ethical obligations with regard to the publication of the results of research. Authors have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. They should

adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results should be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest should be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

31. The physician may combine medical research with medical care only to the extent that the research is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.
32. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best current proven intervention, except in the following circumstances:
 - The use of placebo, or no treatment, is acceptable in studies where no current proven intervention exists; or
 - Where for compelling and scientifically sound methodological reasons the use of placebo is necessary to determine the efficacy or safety of an intervention and the patients who receive placebo or no treatment will not be subject to any risk of serious or irreversible harm. Extreme care must be taken to avoid abuse of this option.
33. At the conclusion of the study, patients entered into the study are entitled to be informed about the outcome of the study and to share any benefits that result from it, for example, access to interventions identified as beneficial in the study or to other appropriate care or benefits.
34. The physician must fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never interfere with the patient-physician relationship.
35. In the treatment of a patient, where proven interventions do not exist or have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorized representative, may use an unproven intervention if in the physician's judgment it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, this intervention should be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information should be recorded and, where appropriate, made publicly available.

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VERSION B**

**A SINGLE-CENTER, ASSESSOR-MASKED, ACTIVE-CONTROLLED, PHASE 4 STUDY
EVALUATING SPEED OF PUPIL DILATION WITH THE MICRO-ARRAY PRINT (MAP)
DISPENSER WHEN COMPARING 2 DOSING REGIMENS OF TROPICAMIDE-
PHENYLEPHRINE FIXED COMBINATION OPHTHALMIC SOLUTION
(THE SPEED STUDY)**

SPONSOR:

**EYENOVIA, INC.
295 MADISON AVENUE, SUITE 2400
NEW YORK NY 10017
USA
PHONE: 917-289-1117**

I have read this protocol in its entirety. I agree to:

- Implement and conduct this study in compliance with this study protocol; conditions of approval imposed by my reviewing Ethics Committee (EC); Good Clinical Practice (GCP); and any other applicable laws and regulations.
- Maintain all study-related information supplied by Eyenovia in a confidential manner.

Protocol Amendment

Modification of the study protocol is prohibited without prior written agreement in the form of a protocol amendment. All amendments will be created by the Sponsor and must be approved by the reviewing EC prior to implementation at your site, except when required to mitigate immediate safety risks or when the changes involve only logistical or administrative revisions.

Print Name of Investigator

Signature

Date