Clinical study title:

Phase I-II clinical study to compare the safety and efficacy of Nanodrop® versus Systane® Balance in the treatment of patients with dry eye

Study number: SOPH176-1218/I-II

Protocol version: 1.0

Version date: 11 de diciembre de 2018

This protocol has been developed in accordance with the principles of the Declaration of Helsinki and will be carried out in accordance with Good Clinical Practices and in compliance with ICH guidelines and current local legislation.

Sponsor: Sophia Laboratories, SA de CV

Content

Index of tables, charts and figures

Study leaders

The administrative structure of the sponsoring party, corresponding to Laboratorios Sophia, SA de CV, is shown in ***Table 1Study leaders***

Function	Name/Contact	Membership ¥
Medical Director of the study	[redacted]	
Director of the study		
Clinical Team		
Clinical Team		
Clinical Team		

Employees of Laboratorios Sophia, SA de CV Av. Paseo del Norte No. 5255, Col. Guadalajara Technology Park, Guadalajara-Nogales Highway Km13.5 CP 45010 Zapopan, Jalisco, Mexico Tel +52 (33) 3000 4200

Table 1Study leaders

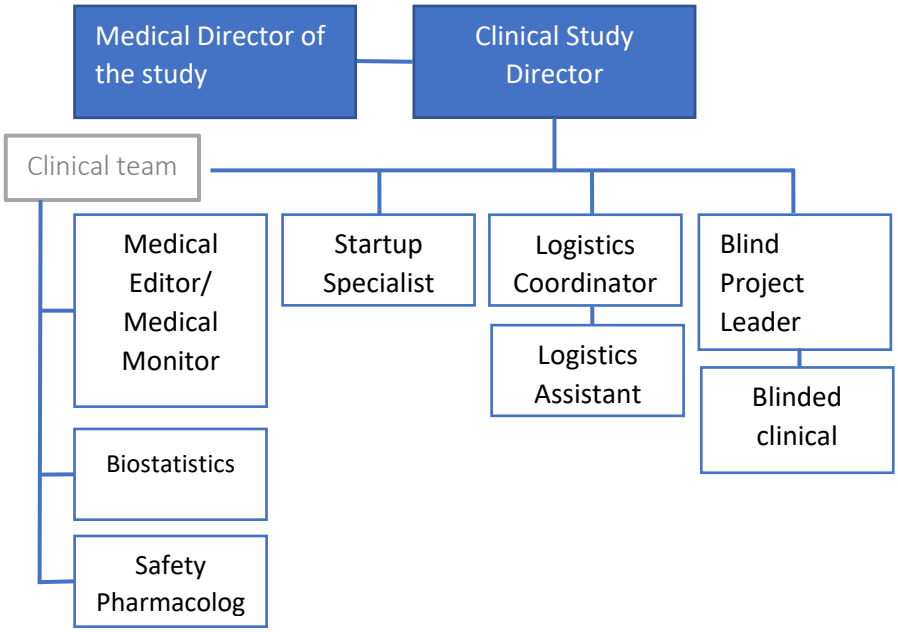


Figure 1Administrative structure

Signature page

From the sponsor

Name:	[redacted] **Signature**
Qualification: Medical Director of the study	[redacted] **Date**

Name:	[redacted] **Signature**
Qualification: Director of the study	[redacted] **Date**

Name:	[redacted] **Signature**
Qualification: Author of the protocol	[redacted] **Date**

Researcher Agreement

I agree to conduct this clinical study according to the design and guidelines of this protocol, adhering to the provisions of this protocol. I agree to conduct the study in accordance with accepted standards of Good Clinical Practice. I agree to report all information and data in accordance with the protocol, particularly any adverse events. I also agree to manage clinical supplies provided by the sponsor strictly in accordance with this protocol. I understand that the information that identifies me may be used by the sponsor. Because the information contained in this protocol and the Investigator's Manual is confidential, I understand that sharing it with any third party not involved in the approval, supervision, or conduct of the study is prohibited. I will ensure that necessary precautions are taken to protect the information from loss, inadvertent disclosure, or access by unauthorized parties.

Name:	
	Signature
Qualification:	
	Date
Name of the center:	
Geographic location (city/state/country)	

List of abbreviations

av	Visual acuity
CEI	Research Ethics Committee
COFEPRIS	Federal Commission for the Protection against Sanitary Risks
CTO	Ocular staining grading
IUD	Intrauterine device
EA	Adverse event
EEP	Punctate epithelial erosion
THAT	Ocular surface disease
FCI	Informed consent form
FDA	United States Food and Drug Administration
FERC	Electronic Case Report Form
IC	Confidence interval
IP	Principal Investigator
MAVC	Best corrected visual acuity
OD	Right eye
WHO	World Health Organization
YOU	Left eye
OSDI	Ocular Surface Disease Index
PNA	Unanticipated problems
PI	Research products
QID	Four times a day
RNEC	National Registry of Clinical Trials
SICCA	Sjögren's International Clinical Collaboration Alliance
TEA	Evaluation time
TFOS DEWS II	Tear Film and Ocular Surface Societies Dry Eye Workshop II
TRPL	Tear film break-up time
TRPL-NI	Non-invasive tear film break-up time

1. Summary

1.1 Synopsis

Title of the study: Phase I-II clinical study to compare the safety and efficacy of Nanodrop® versus Systane® Balance in the treatment of patients with dry eye	
Study number: SOPH176-1218/I-II	**Creation date:** December 11, 2018
Protocol version: 1.0	**Version date:** 11-dic.-18
Therapeutic indication: Lubricante ocular	**Use:** Ojo seco
Estimated duration of the study (from the first visit of the first patient to the preparation of the final report) **:** 11 meses	**Development phase:** I-II
Goals: <u>Security:</u> To evaluate the safety of ophthalmic application of Nanodrop® by quantifying the incidence of unexpected Adverse Events (AEs) related to the investigational product (IP). <u>Effectiveness:</u> To demonstrate the non-inferiority of Nanodrop® compared to Systane® Balance in the treatment efficacy of patients with dry eye, using the OSDI (Ocular Surface Disease Index) test score.	
Hypothesis: <u>Security:</u> H_0 =Nanodrop® is safe in its ophthalmic application, presenting an incidence of unexpected AEs, related to the PI less than 20% of the population of the Nanodrop® safety group H_1 = Nanodrop® is not safe for ophthalmic application, as it presents an incidence of unexpected AEs related to the PI greater than 20% of the population in the Nanodrop® safety group. <u>Effectiveness:</u> H_0 = Nanodrop® is inferior to Systane® Balance by more than 5 points on the OSDI test score $H_0: \mu_A - \mu_B > \delta$ H_1 = Nanodrop® is inferior to Systane® Balance by 5 points or less on the OSDI test score $H_1: \mu_A - \mu_B \leq \delta$	
Study design: Phase I-II, comparative, non-inferiority, active-controlled, parallel-group, double-blind, randomized clinical trial. Safety analysis is performed upon completion of the first 12 subjects in the Nanodrop® group. If fewer than 20% of unexpected adverse events (AEs) related to the investigational product occur, recruitment continues until the sample size for the efficacy analysis is complete.	
Number of subjects: n= 126 evaluable subjects 63 evaluable subjects per group (both eyes).	**Main inclusion criteria:** Diagnóstico de ojo seco
Selection criteria :	

Inclusion criteria:

- Have the ability to voluntarily grant signed informed consent
- Be able and willing to comply with scheduled visits, treatment plan, and other study procedures
- Be willing to modify your lifestyle activities. See 5.3 **Lifestyle considerations**
- Be of age
- Women of childbearing potential must ensure continued use (started ≥ 30 days prior to signing the Informed Consent Form [ICF]) of a hormonal contraceptive method or intrauterine device (IUD) during the study period.
- Present diagnosis of dry eye, defined by:
    - OSDI ≥ 13 points
      + 1 of the following:
        - Corneal staining with more than 5 sites
        - Conjunctival staining with more than 9 sites
        - Tear film break-up time < 10 seconds

Exclusion criteria:

- For women: be pregnant, breastfeeding, or planning to become pregnant during the study period.
- Having participated in another clinical research study ≤ 30 days prior to the screening visit.
- Having previously participated in this study.
- Present a Best Corrected Visual Acuity (BCVA) of 20/200 or worse in either eye.
- Present an additional ophthalmological diagnosis of:
    - Allergic, viral or bacterial conjunctivitis.
    - Anterior blepharitis.
    - Demodex.
    - Parasitic eye infections.
    - Unresolved ocular trauma.
    - Healing diseases of the ocular surface.
    - Corneal or conjunctival ulcers.
    - Filamentous keratitis.
    - Neurotrophic keratitis.
    - Bullous keratopathy.
    - Neoplastic diseases of the ocular surface or annexes.
    - Diseases with fibrovascular proliferations on the conjunctival and/or corneal surface.
    - Diseases of the retina and/or posterior segment that require treatment or threaten the visual prognosis.
    - Glaucoma.
- Have dry eye management that requires implementation of Stage 2 treatments from the TFO DEWS II (Tear Film and Ocular Surface Societies Dry Eye Workshop II) recommendations for the treatment and staged management of **Table 2**
- Have a history of drug addiction or dependence, currently or within the last two years prior to signing the FCI.
- Have a history of ocular surgical procedures within the last 3 months prior to signing the FCI.

- Wear soft or hard contact lenses. You may be admitted if you can discontinue use of contact lenses during the study. You must not wear contact lenses for 15 days prior to enrollment.
- Having another medical condition, acute or chronic, that, in the opinion of the investigator, could increase the risk associated with participation in the study or the administration of the investigational product, or that could interfere with the interpretation of the study results.
- Have a known hypersensitivity to the components of the investigational products.
- Be or have an immediate family member (e.g., spouse, parent/legal guardian, sibling, or child) who is an employee of the research site or the sponsor and who is directly involved in this study.

Research Products (RP):

Investigational product, dosage and route of administration:

- Nanodrop®. Propilenglicol 0.6%. Emulsión oftálmica.Laboratorios Sophia, S.A. de C.V.
- Posology:mínima a cumplir de 1 gota 4 veces al día, ambos ojos.
- Route of administration:Oftálmica.

Comparator product, dose and route of administration:

- Systane® Balance. Propilenglicol 0.6%. Emulsión oftálmica.Alcon Laboratories, Inc.
- Posology:mínima a cumplir de 1 gota 4 veces al día, ambos ojos.
- Route of administration:Oftálmica.

Duration of treatment:	**Approximate duration of the subject in the study:**
28 días	35days

Evaluation criteria:

Efficacy variables:

Primary efficacy outcome variable:

- OSDI Test Score (Evaluation Time [ET]: day 29).

Secondary efficacy outcome variables:

- Changes in best corrected visual acuity (BCVA) (TE: day 8 and 29).
- Changes in fluorescein tear film break-up time (TRPL) (TE: day 8 and 29)
- Corneal fluorescein staining changes (TE: day 8 and 29).
- Changes in conjunctival staining with lissamine green (TE: day 8 and 29).

Security variables:

Primary safety outcome variable:

- Incidence of unexpected AEs related to the PI (TE: day 8 and 29).

Secondary safety outcome variable:

- Incidence of expected AEs (TE day 8 and 29)

Exploratory outcome variable:

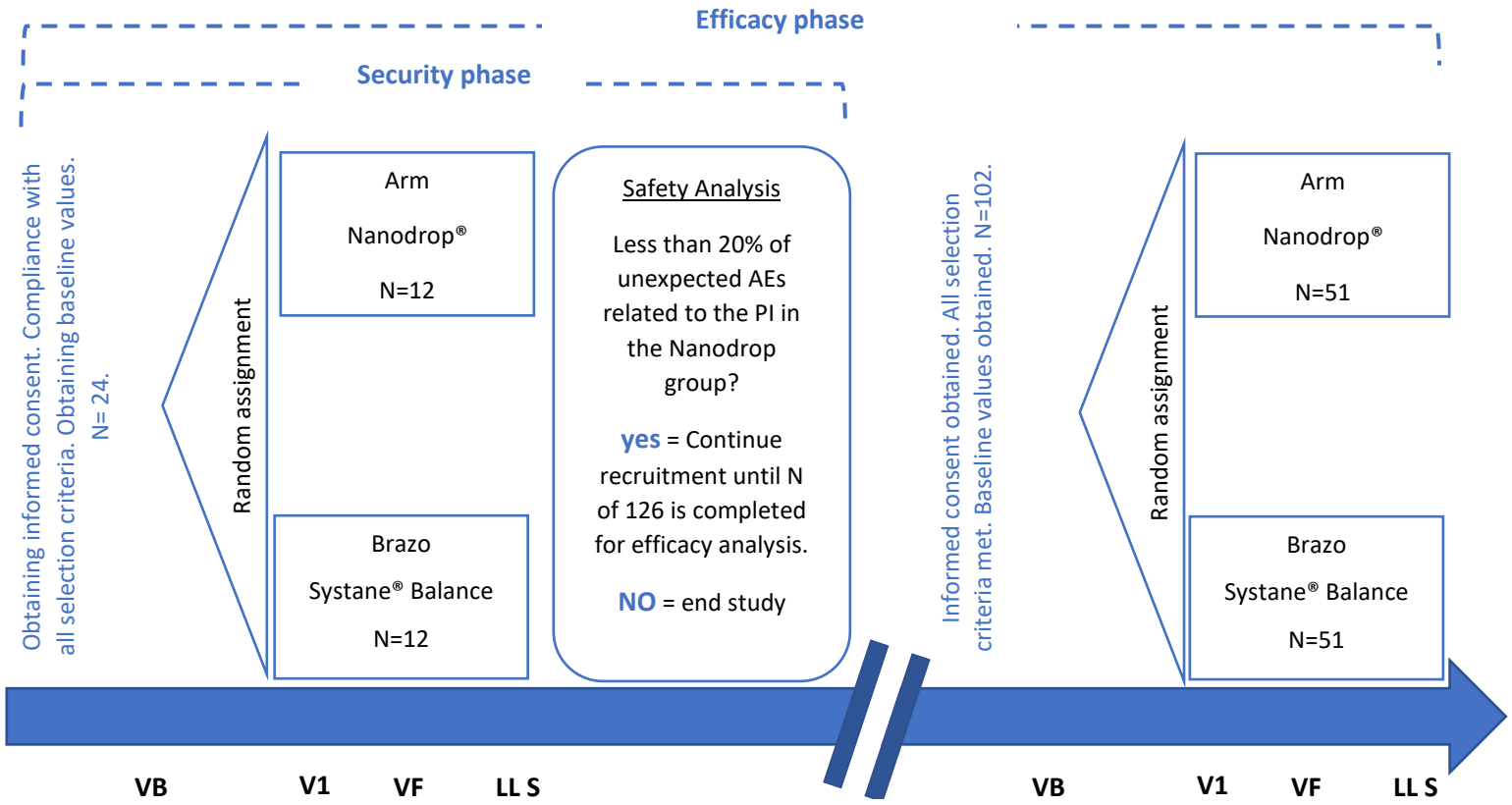
- Average number of applications performed daily

Statistical methodology:

Data will be expressed as measures of central tendency: mean, standard deviation, and ranges for quantitative variables. Qualitative variables will be presented as frequencies and percentages. Statistical analysis will be performed using the Student t test or the Mann-Whitney U test (if

applicable) for quantitative variables to determine the difference between groups. Differences between qualitative variables will be analyzed using the X^2 (Chi-square) test or Fisher's exact test. A 95% CI will be considered for non-inferiority criteria based on the study objectives, with an $\alpha < 0.05$ considered significant.

1.2 Study diagram



VB= Baseline visit
V1= Visit 1. Safety visit.
VF= Final visit. Assessment of final outcome variables.
Ll S= Safety call

1.3 Study schedule

PROCEDURES	VB D 1	V1 D 8 to ± 1	VF D 29 to +1	LLS D 35 ± 1
FCI Signature	X			
Medical record*	X			
Vital signs	X	X	X	
OSDI	X		X	
Evaluation of concomitant medications	X	X	X	
Urine pregnancy test	X		X	
MAVC	X	X	X	
Ocular surface stains	X	X	X	
TRPL	X	X	X	
Comprehensive ophthalmological evaluation	X	X	X	
Eligibility criteria	X			
PI Allocation	X			
EA Assessment	X	X	X	X
Delivery of the PI and start of intervention	X			
Delivery of patient materials and instructions for completion (Subject Diary)	X			
Subject Diary Evaluation		X	X	
Adherence assessment		X	X	
Subject continuity assessment		X	X	
Return of PI and subject diary			X	

*Includes somatometry and general physical examination

2. Introduction and Background

2.1 Theoretical framework

Dry eye is currently defined as a multifactorial ocular surface disease characterized by a loss of tear film homeostasis accompanied by ocular symptoms, in which ocular surface instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities play etiological roles. [1]

Dry eye is a common disease; the initial report of the TFOS DEWS I Epidemiology Subcommittee concluded that the worldwide prevalence of dry eye in individuals over 50 years of age was between 5% and 30%. [2]One of the main challenges facing epidemiological studies has been the lack of standardized definition and classification of dry eye. Despite the efforts of TFOS DEWS I and TFOS DEWS II to standardize these criteria, this has not yet been achieved. The TFOS DEWS II Epidemiology Subcommittee report indicated a prevalence of 5% to 50% in studies that included symptoms, with or without signs; for studies in which the diagnosis was based primarily on signs, the prevalence was even higher, reaching 75%. [3]

The incidence of the disease has been reported in few studies. The *Beaver Dam Eye Study* established, in a Caucasian population aged 48–91 years, that 13.3% (95% CI 12.0–14.7%) of individuals developed symptomatic dry eye at 5 years and 21.6% (95% CI 19.9–23.3%) at 10 years. Age is a risk factor for increased incidence with an odds ratio of 1.2x (1.1–1.3) per 10-year increase. [4]

Although there is no formal study on the prevalence of the disease in Latin American countries, various reports suggest a higher prevalence of severe symptoms and clinical diagnosis of dry eye in the Hispanic population compared to the Caucasian population.[5] [6]

The current definition of TFOS DEWS II does not require the presence of any specific sign for diagnosis; instead, it emphasizes tear film homeostasis. The tear film plays an essential role in lubricating and protecting the ocular surface, as well as maintaining a smooth refractive surface for optimal visual performance. [7]

Physiologically, homeostasis describes the state of equilibrium within the body with respect to its various functions and the chemical composition of fluids and tissues. When applied to dry eye, the concept recognizes the possibility of numerous changes that can occur in the tear film and ocular surface in response to one or more underlying causes of dry eye. [7, 8]Altered homeostasis is considered the unifying characteristic that describes the fundamental process in the development of dry eye.

The most current classification of dry eye attempts to eliminate any perception of exclusivity by indicating that the diagnoses of aqueous deficiency and evaporative dry eye exist as a continuum rather than as separate entities. Elements of each should be considered in diagnosis and treatment. [1]

This classification system incorporates assessment elements to clarify the diagnosis, from which various etiologies can be considered and appropriate treatment plans can be established. Appropriate treatment of ocular surface diseases (OSD) with a differential diagnosis that masks dry eye increases the chances of successful treatment and allows for the identification and appropriate management of any coexisting components of the condition attributable to dry eye.

Figure **Figure 2**incorporates a clinical decision algorithm based on current knowledge of dry eye pathophysiology **.**

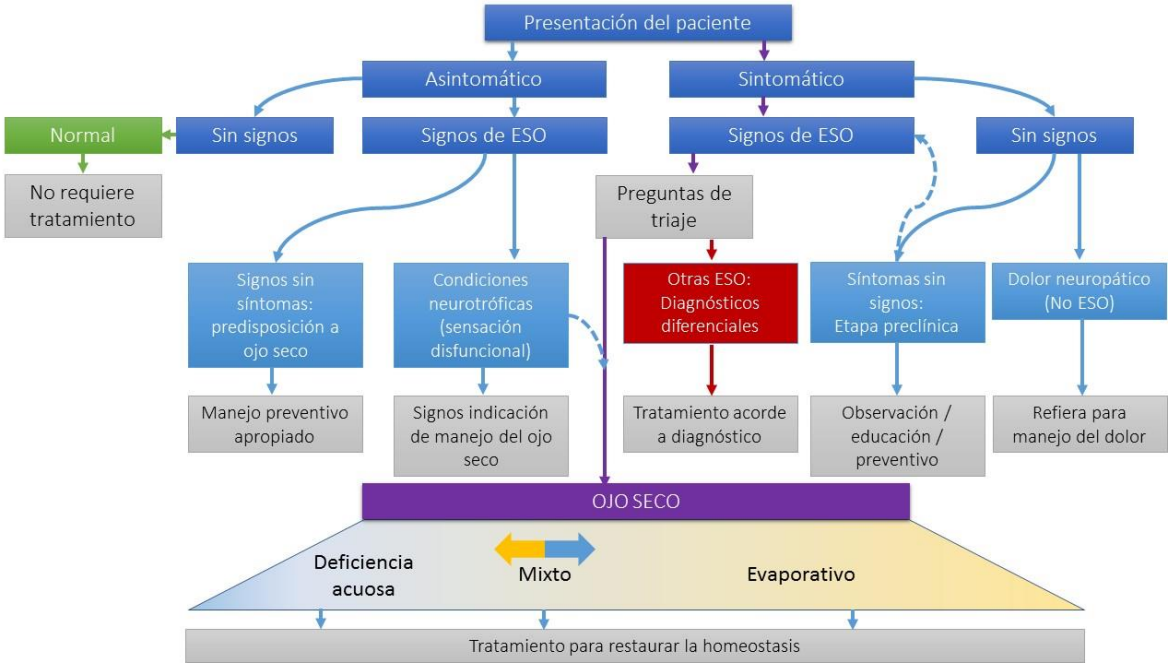


Figure 2Dry eye classification

Adapted from TFOS DEWS II Definition and Classification Report [1]

Tear replacement with ocular lubricants is traditionally considered a cornerstone of dry eye treatment, and numerous topical formulations are available. These non-pharmacological products are often referred to as "artificial tears," which, as their name suggests, attempt to replace and/or supplement the natural tear film. However, these products do not address the underlying pathophysiology of dry eye, and the mechanisms of any palliative action are generally poorly understood. [9]

Tear substitutes consist of various products, typically targeting one or more layers of the tear film. The wide variety of properties of these ocular lubricants has been described elsewhere. [10, 11, 12, 13, 14]

Ocular lubricants are largely considered safe, although some side effects have been reported, primarily blurred vision, varying levels of “ocular discomfort,” and foreign body [15]sensation . Relatively few randomized controlled trials have compared the relative superiority of one product versus another for the treatment of dry eye. [16]A recent Cochrane systematic review assessing the effect of ocular lubricants for the treatment of dry eye included 43 randomized controlled trials comparing various lubricant formulations with no treatment or placebo. [15]The primary outcome

measure was patient-reported symptoms. The authors reported that the overall quality of the evidence was low for the different tear supplement formulations compared in the review and concluded that, although artificial tears may be effective for the treatment of dry eye, there remains a need for future research to allow firm conclusions to be drawn about the effectiveness of ophthalmic lubricant formulations.

Dry eye management is complicated due to its multifactorial etiology. Developing the simple principle that "diagnosis precedes treatment" means that physicians must do their best to identify the degree to which OS contributes to their patients' presentation. This aspect of determining the main causative factors of dry eye is critical for proper management.

Figure **Figure 3a** diagnosis should be made and diagnostic testing performed to determine whether the patient actually has dry eye and whether they generally show more signs of dry eye. Once the diagnosis is confirmed, the severity of the disease, along with the determination of the etiological subtype, will allow for the development of an appropriate treatment plan.

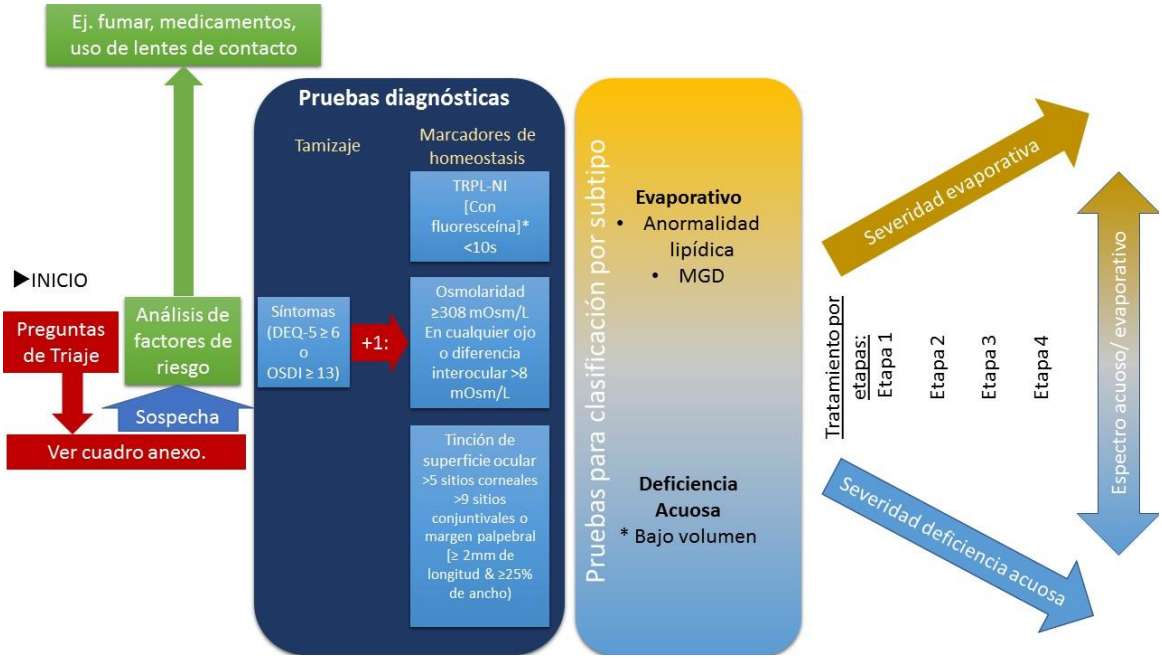


Figure 3Diagnosis and management of dry eye

*Fluorescein is used when TRPL-NI is unavailable. *If more than one homeostasis marker test is performed, the order should be: TRPL-NI, osmolarity, TRPL with fluorescein, and stains. Adapted from the TFOS DEWS II Management and Treatment Report

- How severe is the eye discomfort?
- Do you have dry mouth or inflammation of any glands?
- How long have your symptoms lasted, was there any triggering factor?
- Has your vision been affected, does it clear up when you blink?
- Are the symptoms or redness much worse in one eye than in the other?
- Have your eyes become itchy, swollen, crusty, or have they been draining?
- Do you wear contact lenses?
- Have you recently been diagnosed with any general illness, or are you taking any medication?

+ Detailed eye examination

Table 1Triage questions

Stage 1:

- Education according to the disease, its management, treatment and prognosis
- Modification of the local environment
- Education according to dietary modifications
- Identification and modification/elimination of offending topical and systemic medications
- Eye lubricants
- Eyelid hygiene and warm compresses

Stage 2:

If the above options are inadequate, consider the following:

- Treatment for Demodex
- Tear preservation
  - Tear duct occlusion
  - Wet chamber goggles
- Night treatments (e.g. ointments)
- Physical warm-up and meibomian gland expression
- Pulsed light therapy
- Prescription of:
  - Antibiotics or their combination with steroids on eyelid margins
  - Topical steroids (limited duration)
  - Topical secretagogues
  - Topical immunomodulators
  - Topical LFA-1 antagonists
  - Topical macrolides or tetracyclines

Stage 3:

- Oral secretagogues
- Autologous serum
- Therapeutic contact lenses

Stage 4:

- Topical steroids (extended duration)
- Amniotic membrane grafts
- Surgical occlusion of tear ducts
- Other surgical approaches.

Table 2Stages of dry eye treatment

Treatment algorithms are often constructed to recommend a sequence of treatments according to disease stage, but this is not possible for dry eye, as it is a complex condition that varies from patient to patient in both severity and character. However, with the intention of assisting ophthalmic physicians in creating a logical, evidence-based treatment approach, the treatment algorithm shown in **Table 2**. For patients who do not respond to a given level, or who show greater severity, the next higher level is recommended, and in some cases, the previous treatment may be continued in addition to any new treatments. In general, the approach is to start with low-risk, commonly available conventional treatments, such as ocular lubricants for early stages of the disease, and progress to more advanced treatments for more severe forms.

2.2 Background on the product under study

2.2.1 Pharmacology of the investigational product

Propylene glycol is considered a demulcent or humectant in ophthalmic lubricant formulations. These agents are used to lubricate the ocular surface, thereby helping to reduce associated discomfort and irritation.

Propylene glycol is a colorless, viscous, water-soluble polymer. Its chemical name is propane-1,2-diol (molecular formula: $C_3H_8O_2$), with a molecular weight of 76.1 g/mol. [17, 18]

The physical properties of propylene glycol in contact with the ocular surface modify tear viscosity. The lipid layer of the nanoemulsion acts as a surfactant, helping to spread nonpolar lipids into the aqueous component of the tear film. It thus provides a barrier between the two phases and provides a support structure for the nonpolar phase. The combination of these helps reduce tear film evaporation.[19]

Due to the nature of propylene glycol, a large molecule that does not penetrate the ocular barrier, the pharmacokinetic profile of propylene glycol for ophthalmic administration has not been described. However, considering the route of elimination through the nasolacrimal duct, this fraction could follow the profile described for oral administration.

2.2.2 Product efficacy under investigation

Propylene glycol's effectiveness as a humectant is based on its ability to retain water, up to three times its own weight, [20]combined with the previously mentioned characteristics conferred by its nanoemulsion formulation.

There is no information yet on the efficacy of Nanodrop®; however, propylene glycol has been included in formulations that have been studied in clinical trials.

2.2.3 Safety of the investigational product

The U.S. Food and Drug Administration (FDA) has determined that propylene glycol is “generally regarded as safe” (GRAS) for use in food, cosmetics, and drug products.

In addition, the FDA created a monograph in the late 1980s considering the wide variety of components present in ocular lubricants. The monograph determines that ophthalmic lubricants, in a dosage form suitable for ophthalmic application, are generally considered safe and effective if they contain the ingredients at the concentrations specified in the monograph. Propylene glycol is found in this monograph at concentrations of 0.2% to 1%. [21]

2.2.4 Summary of the pharmaceutical development of the investigational product

Nanodrop® was developed by Laboratorios Sophia, SA de CV. It has physicochemical characterization and an accelerated and long-term stability protocol. A preclinical safety and toxicity study was conducted in New Zealand albino rabbits, with 90-day QID application of Nanodrop®. Follow-up visits were conducted throughout the 90 days, with a final visit considered for outcome variables. Safety variables included conjunctival hyperemia, discharge, ocular surface staining, and

incidence of adverse events; toxicity variables were histopathological changes in the cornea and conjunctiva. Nanodrop® was considered safe for ophthalmic application.

2.3 Background on the research

2.3.1 From the research question

There is no prior clinical trial data on Nanodrop®. However, the efficacy and tolerability of Systane® Balance have been previously evaluated in three single-center clinical studies. [22, 23, 24]Systane® Balance is the comparator for the present study. It shares with Nanodrop® the use of 0.6% propylene glycol, both in ophthalmic emulsion dosage form. However, Nanodrop® is classified as a nanoemulsion.

In all three clinical studies of Systane® Balance, the endpoints evaluated were TRPL, comfort, blurred vision, and lipid layer thickness. Systane® Balance was well tolerated, and the final results were consistently better than baseline.

2.4 Risk benefit assessment

2.4.1 Known potential risks

Ocular lubricants are safe formulations. Propylene glycol has a known safety profile. The diagnostic tests considered in the study design are also considered safe.

The only anticipated side effects with ophthalmic applications are burning, a foreign body sensation, and blurred vision. These are mild and transient, lasting no more than one minute post-instillation.

2.4.2 Known potential benefits

Potential benefits include relief of symptoms associated with dry eye and the likely restoration of tear film homeostasis.

2.5 Problem statement

Although the pathogenesis of dry eye is multiple and its semiotics variable, the different treatment phases have a common denominator: the use of ocular lubricants. There is a wide variety of topical lubricants, with different moisturizing agents; however, there is no evidence that one is better than another.

While ocular lubricants have not been shown to be sufficient to completely resolve the ocular surface alteration and inflammation seen in patients with dry eye, they have been shown to be effective in providing protection to the ocular surface and reducing symptoms and clinical findings.

Nanodrop®'s formulation of 0.6% propylene glycol in a nanoemulsion could function as an effective lubricant that protects the ocular surface and reconstructs the tear film, thanks to its properties.

2.6 Justification

Patients with dry eye, regardless of its etiology and severity, will need to use ocular lubricants to reduce symptoms and improve their quality of life.

Ocular lubricants are the first-line treatment for ocular symptoms related to dry eye in self-reportedly healthy subjects, with a prevalence of 5% to 35% in those over 50 years of age. [25]If we add to this the number of patients who report occasional symptoms or symptoms that are dependent on a work or occupational situation and who will use them intermittently throughout their lives, the population spectrum that will have access to these medications is very broad. It is estimated that 50% of patients diagnosed with dry eye without concomitant diseases will use more than two types of ophthalmic solutions in 5 years of treatment.

Nanodrop® is an ophthalmic lubricant formulated as a nanoemulsion for which documentation of its safety and efficacy profile is required. It is the first emulsion lubricant in Laboratorios Sophia, SA de CV's ocular lubricant portfolio.

3. Objectives and hypotheses

3.1 Primary objectives

3.1.1 Primary security objective

To evaluate the safety of ophthalmic application of Nanodrop® by quantifying the incidence of unexpected Adverse Events (AEs).

3.1.2 Primary efficacy objective

To demonstrate the non-inferiority of Nanodrop® compared to Systane® Balance in the treatment efficacy of patients with dry eye, using the OSDI (Ocular Surface Disease Index) test score.

3.2 Secondary objectives

3.2.1 Secondary security objective

To compare the safety of ophthalmic application of Nanodrop® versus Systane® Balance by quantifying the incidence of expected adverse events.

3.2.2 Secondary efficacy objectives

To compare the efficacy of ophthalmic application of Nanodrop® versus Systane® Balance through changes in BCVA.

To compare the efficacy of ophthalmic application of Nanodrop® versus Systane® Balance by changes in conjunctival staining with lissamine green.

To compare the efficacy of ophthalmic application of Nanodrop® versus Systane® Balance using changes in corneal fluorescein staining.

To compare the efficacy of ophthalmic application of Nanodrop® versus Systane® Balance by means of changes in TRPL.

3.3 Exploratory objectives

Compare the average daily applications of Nanodrop® versus Systane® Balance.

3.4 Hypothesis

3.4.1 Security hypothesis

H_0 =Nanodrop® is safe in its ophthalmic application, presenting an incidence of unexpected AEs, related to the PI less than 20% of the population of the Nanodrop® safety group

H_1 = Nanodrop® is not safe for ophthalmic application, as it presents an incidence of unexpected AEs related to the PI greater than 20% of the population in the Nanodrop® safety group.

3.4.2 Efficacy hypothesis

H_0 = Nanodrop® is inferior to Systane® Balance by more than 5 points on the OSDI test score

$$H_0: \mu_A - \mu_B > \delta$$

H_1 = Nanodrop® is inferior to Systane® Balance by 5 points or less on the OSDI test score

$$H_1: \mu_A - \mu_B \leq \delta$$

4. Study design

4.1 Design Overview

Phase I-II, comparative, non-inferiority, active-controlled, parallel-group, double-blind, randomized clinical trial. Safety analysis is performed upon completion of the first 12 subjects in the Nanodrop® group. If fewer than 20% of unexpected AEs related to the investigational product occur, recruitment continues until the sample size for efficacy analysis is complete. (See pp. 14)

4.2 Justification of the study design

Propylene glycol, a component of Nanodrop®, is a compound classified by the FDA as GRAS for use in food, cosmetics, and drug products. The propylene glycol concentration falls within the specifications of the FDA monograph for ophthalmic lubricants considered safe.

Because of this, the design of a separate Phase I study is not considered necessary; the advantages of the proposed design include having data on the efficacy variables from all subjects included in the study for the efficacy analysis. The safety phase will allow for the identification of unforeseen risks related to the study's formulation or procedures without interfering with the efficacy analyses.

4.3 Expected duration

The total duration of the study, from the first patient visit to the final report, is estimated to be 11 months.

The planned recruitment period is 9 months. Considering the proposed sample size of 126 subjects, the average recruitment rate throughout the study should be no less than 0.5 subjects per day.

The approximate duration of each subject in the study is 35 days.

5. Study population

5.1 Eligibility criteria

5.1.1 Inclusion criteria

- Have the ability to voluntarily grant signed informed consent.
- Be able and willing to comply with scheduled visits, treatment plan, and other study procedures.
- Be willing to modify your lifestyle activities. See 5.3 **Lifestyle considerations.**
- Be of age.
- Women of childbearing potential must ensure continued use (started ≥ 30 days prior to signing the FCI) of a hormonal contraceptive method or IUD during the study period.
- Present diagnosis of dry eye, defined by:
    - OSDI ≥ 13 points
    - + 1 of the following:
        - Corneal staining with more than 5 sites
        - Conjunctival staining with more than 9 sites
        - Tear film break-up time < 10 seconds

5.1.2 Exclusion criteria

- For women: be pregnant, breastfeeding, or planning to become pregnant during the study period.
- Having participated in another clinical research study ≤ 30 days prior to the screening visit.
- Having previously participated in this study.
- Present a BCVA of 20/200 or worse in either eye.
- Present an additional ophthalmological diagnosis of:
    - Allergic, viral or bacterial conjunctivitis.
    - Anterior blepharitis.
    - Demodex.
    - Parasitic eye infections.
    - Unresolved ocular trauma.
    - Healing diseases of the ocular surface.
    - Corneal or conjunctival ulcers.
    - Filamentous keratitis.
    - Neurotrophic keratitis.
    - Bullous keratopathy.
    - Neoplastic diseases of the ocular surface or annexes.
    - Diseases with fibrovascular proliferations on the conjunctival and/or corneal surface.
    - Diseases of the retina and/or posterior segment that require treatment or threaten the visual prognosis.
    - Glaucoma.
- Have dry eye management that requires implementation of Stage 2 treatments in the DEWS II Staged Treatment and Management for Dry Eye Disease recommendations. See **Table 2**.

- Have a history of drug addiction or dependence, currently or within the last two years prior to signing the FCI.
- Have a history of ocular surgical procedures within the last 3 months prior to signing the FCI.
- Wear soft or hard contact lenses. You may be admitted if you can discontinue use of contact lenses during the study. You must not wear contact lenses for 15 days prior to enrollment.
- Having another medical condition, acute or chronic, that, in the opinion of the investigator, could increase the risk associated with participation in the study or the administration of the investigational product, or that could interfere with the interpretation of the study results.
- Have a known hypersensitivity to the components of the investigational products.
- Be or have an immediate family member (e.g., spouse, parent/legal guardian, sibling, or child) who is an employee of the research site or the sponsor and who is directly involved in this study.

5.2 Criteria for eliminating and replacing subjects

5.2.1 Elimination criteria

- Withdrawal of the FCI letter.
- Presentation of a serious adverse event, whether or not related to the investigational product, which, in the opinion of the PI and/or the sponsor, could affect the patient's ability to safely continue study procedures.
- Non-tolerability or hypersensitivity to any of the compounds used during the tests (fluorescein, lissamine green, tetracaine).
- Non-tolerability or hypersensitivity to any of the investigational drugs.

5.3.1 Substitution of subjects

The sponsor, with prior authorization from the research ethics committees, may decide to replace subjects who withdraw their FCI or those who are lost to follow-up, if it is necessary to balance the study groups so that they are evaluable, or to complete the minimum population to be evaluated for the efficacy analysis.

The results obtained from the replaced subjects will continue to be used for safety analysis.

5.3 Lifestyle considerations

For the study, participants may need to modify some lifestyle activities to accomplish the following:

- Refrain from smoking.
- Refrain from using electronic vaporizers.
- Avoid immersing yourself in water without eye protection (*goggles*).
- Avoid direct exposure to fans (including air conditioning vents) during activities that involve vision. 24 hours before your checkup visits.
- Maintain your sleep-wake cycle with the one you entered the study.

5.4 Scrutiny failures

A screening failure is defined as a participant who agrees to participate in the study, giving their consent, but who is not assigned to a treatment group; that is, they do not enter the study. The following information regarding screening failures must be reported, at a minimum:

- Demographic data.
- Details of the counting failure (specify whether due to eligibility criteria, which one, or some other reason for the failure).
- Presence of serious adverse events during the scrutiny.

This is necessary to comply with the CONSORT (*Consolidated Standards of Reporting Trials*) guidelines for publishing results or to respond to potential questions from regulatory authorities.

Subjects who do not meet the eligibility criteria for participation in the study due to a specific modifiable factor may be re-screened. Subjects in this situation must use the same initial screening number.

5.5 Recruitment and retention strategies

This is a Phase I-II study planned for six centers. The selected centers will be responsible for subject recruitment. Recruitment will be competitive, with a proposed initial recruitment of 21 subjects per center; however, this will be contingent on the performance of each center, so this number may vary by center.

The minimum expected recruitment rate per center is 0.2 subjects per day.

Subject participation in the study is approximately 35 days, during which time they are only required to attend two visits after the baseline visit, so retention issues are not anticipated. However, subjects will be eligible for travel and travel expenses to attend their visits. Other strategies to improve subject retention include, but are not limited to:

- Clearly communicate the importance of the study and the benefits the population will gain from its results.
- Make calls or send text messages to remind yourself of appointments or activities to do.
- Provide a printed calendar and ID card to remind you of upcoming appointments and activities, as well as their estimated duration.
- Offer flexible business hours.
- Systematic organization of the study procedures, so that the subject does not stay longer than necessary during his visit.
- Minimize subject wait times.

All materials to be delivered to the subject or recruitment strategies implemented by the Centers will be submitted for approval by the corresponding committees.

5.6 Procedure in case of loss of follow-up

For this protocol, loss to follow-up is defined as those subjects who were randomized, who at some point were active subjects in the study, but their final evaluation could not be completed.

If the participating subject does not attend their appointment, the research site will call to determine the reason and attempt to schedule a new appointment within the established window or an unscheduled appointment. If an appointment cannot be scheduled, the subject will be asked about the presence of adverse events and the reason for discontinuing the study, as minimum information.

A loss to follow-up <5% is not considered to represent a problem for the validity of the results obtained. [26, 27]

5.7 Subject identification

Study subjects will be identified by a number and the initials of their name.

The initials of the subject of study will be obtained starting with the first letter of the name, followed by the first letter of the first surname and the first letter of the second surname, obtaining a maximum of three letters. In case the person has two names or a compound surname, the first letter will always be used.

Example:

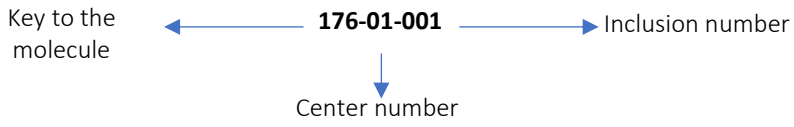
A. A rieh Daniel M ercado C arrizalez
   a. Initials: AMC

B. J uan D e la Torre O rozco
   b. Initials: JDO

During the screening phase, participants will be assigned a consecutive three-digit number. Once the subject has been selected, they will be assigned a number that will identify them throughout the study. This code will consist of eight numbers in the following order, from left to right:

- three digits of the molecule under study according to the name given by the sponsor.
- two digits corresponding to the research center number.
- three digits of the consecutive number assigned to its inclusion in the research center.

Example:



6. Investigational product

6.1 Managed Products

6.1.1 Investigational product

- Generic name: Propylene glycol
- Distinguishing name: Nanodrop® (PRO-176)
- Active ingredients: Propylene glycol 0.6%.
- Pharmaceutical form: Ophthalmic emulsion
- Presentation: multi-dose dropper bottle, 10 ml.
- Prepared by: Laboratorios Sophia, SA de CV
- Solution description: Homogeneous emulsion, free of visible particles.
- Packaging description: White bottle with a capacity of 10 mL, made of low-density polyethylene.

6.1.2 Reference product

- Generic name: Propylene glycol
- Distinguishing name: Systane® Balance
- Active ingredients: Propylene glycol 0.6%
- Pharmaceutical form: Ophthalmic emulsion
- Presentation: multi-dose dropper bottle, 10 ml.
- Manufactured by: Alcon Laboratories, Inc.

6.1.2.1 Justification of the reference product

Systane® Balance is a commercially available lubricant with a previously described safety and efficacy profile. It is a propylene glycol ophthalmic emulsion at the same concentration as Nanodrop®. Furthermore, it will be a direct commercial competitor.

6.1.3 Dosage of investigational products

The minimum dosage to follow is 1 drop, four times a day, in both eyes.

However, subjects may increase the number of applications, if they deem necessary, as many times per day as they deem necessary. Each application must be recorded in the "Subject Diary."

6.1.3.1 Justification of the dose

Ophthalmic lubricants are generally prescribed as needed (pro re nata [PRN]). [28]However, the study by Asbell et al. compared the efficacy of QID versus PRN dosing. Their results showed that, although there was no difference in clinical signs, the QID group had greater symptom improvement. [29]For this reason, the minimum dosage proposed for this study, and as the basis for calculating adherence, is 1 drop four times daily. However, the possibility of increasing this PRN dosage remains open to assess whether more applications are better for the patient.

6.2 Storage and handling of research products at the study center

Delivery will be made via a courier service contracted by the sponsor, specifically selected for this purpose, to the address of the research center in accordance with the study plan.

Reception will be carried out by the assigned research team staff. They must verify the condition of the primary packaging (box). If it shows alterations or defects in its integrity that, in their judgment, could have damaged the contents, they must report this to the sponsor. If the package shows no significant defects, they will proceed to open it.

Inside, you should locate the receipt acknowledgment document and the temperature *data logger* . You should check that the recorded temperature meets the specifications for its transport and storage. You will verify the contents (PI) with what is reported on the document. If the document matches the contents, you will sign the receipt and send it to the sponsor. If not, you will notify the sponsor.

Storage and safeguarding are the responsibility of the research center. The medication must be kept in a secure area with restricted access.

Storage temperature should be 2°C to 30°C.

The research center is required to record the temperature recorded in the *data logger* , using the designated format, every day while the protocol is in effect and has PIs. These data will be reviewed by the clinical monitor according to the *data logger records.*

In the event of material loss, this must be documented in the input and output log along with a clear description of the mechanism by which the loss occurred.

Upon completion of the protocol, all study materials will be retrieved by the sponsor as part of the closure audit. The final return of materials will be made by the principal investigator or the person designated by the principal investigator to return materials at the end of the study.

The sponsor reserves the right to initiate civil and criminal action against the principal investigator in the event of undocumented material missing at the end of the study.

6.3 Concomitant treatments and medications not authorized during the study

Subjects successfully enrolled in the study and who meet the eligibility criteria may continue systemic treatment for their underlying conditions. If they require the introduction of a new approved medication during the course of the study, they may do so. All concomitant medications used must be duly reported in the clinical record notes and in the corresponding section.

<u>Permitted medications:</u>

- Ophthalmic:

All permitted medications administered ophthally during the study must wait a minimum of 10 minutes after the last application of the study or reference treatments. This is to avoid treatment interactions with the tear film, based on the tear flow rate and physiological tear volume. [30]

  - Tetracaine 0.5%
  - Tropicamide 0.8% / Phenylephrine 5%

    - All topical antibiotics except tetracyclines
- Administered by a route other than ophthalmic:
    - Any medication whose effect may be susceptible to modifying any of the efficacy, safety or tolerability parameters of this research protocol must be notified to the clinical monitor or the sponsor's scientific committee to determine the appropriateness of the participant's admission, continuation or elimination as appropriate.
    - Women who enter using hormonal contraceptives must continue using them throughout the study.

Prohibited medications:

- Any medication with ophthalmic application that is not on the list of permitted medications
- Systemic steroids
- Immunomodulators
- Tetracyclines

6.4 Procedure for monitoring and measuring adherence

For more than four decades, numerous studies have been conducted on the appropriate way to measure and quantify medication adherence, but none have reached a consensus that could serve as the gold standard, both in cross-sectional and longitudinal studies. [31, 32, 33, 34, 35, 36, 37, 38]

There are different procedures for measuring adherence to pharmacological interventions. The most common procedure involves self-reports, which include patient interviews, questionnaires, and self-monitoring diaries. Their strengths are speed, flexibility, low cost, and ease of implementation; they have a high degree of specificity for nonadherence, but their sensitivity and reliability for adherence are low. [38, 39]

Biochemical measurement of the drug, or its metabolite, is one of the methods that best confirms drug use. However, in addition to being costly and impractical, it is of little use in ophthalmic applications, as peripheral concentrations may be undetectable; and samples from other tissues require more invasive methods that would be inadvisable. [38]

Medication counting is another way to measure adherence. Classically referred to as "pill counting," in ophthalmology it is translated as the weight of the bottle. This is a simple, inexpensive, and noninvasive method. The main disadvantages of this method are: 1. It cannot confirm medication application (it could have been intentionally dropped or instilled outside the eye), and 2. It depends on the subject bringing the medication back. [38, 39]

A multi-method approach to adherence measurement is recommended. Because there is no ideal adherence measure, it is appropriate to use more than one method when aiming to achieve results that resemble reality. Selecting two or more methods allows for balancing their strengths and weaknesses, thus more accurately capturing adherence levels. [37]

Adhesion assessment will be based on the bottle's weight and will be performed taking into account the following information: drop weight, initial container weight, final container weight, and the total number of applications. The following simplified formula will be used:

$$Ad = \frac{(P_i - P_f)100}{P_T}$$

Where:
Ad = adhesion
P_i = weight of the container delivered to the subject at the beginning
P_f = weight of the container returned by the subject
P_T = weight of the dosage indicated for the investigational products

$$P_T = (P_g)G$$

Where:
P_g = weight of a drop of the intervention, determined by the research and development department
G = number of applications indicated for the investigational products

Packaging that does not maintain its physical integrity will not be considered for the calculation of adhesion.

In cases where the container is not returned, or has not maintained its physical integrity, adherence will be measured using the subject's diary, as follows:

$$Ad = (A_r)100/A_i$$

Ad = Adhesion
A_r = Registered applications
A_i = Applications indicated for investigational products

The "indicated applications for investigational products" will correspond to a minimum dosage of 4 drops per day in each eye, i.e., 8 drops per day for 28 days (224). Because dosage increases are permitted, adherence rates greater than 100% may be observed.

The research center will designate a person responsible for monitoring adherence through a diary during visits. Measuring adherence through weight will be the sponsor's responsibility.

There is no standardized parameter for defining adequate adherence; it must be defined and outlined by the objectives of the particular research. [38]

For this study, a minimum adherence of 70% will be considered necessary to meet the research objectives. Therefore, subjects with less than 70% adherence will not be considered for the efficacy analysis; they will only be included in the safety analysis.

6.5 Strategies to improve adherence

1. The PI will sensitize the subject to the importance of correctly applying the PI to achieve the study objectives.
2. Direct questioning by the IP regarding the application of the PI.
3. Delivery of a printed calendar specifying the date of the visit and its activities.
4. Training in completing and reviewing the “Subject Diary.”
5. If deemed necessary, text messages may be sent as reminders. The content of these messages must be approved in advance by the IEC.

7. Methods and procedures of the study

7.1 From the research center

This study will be conducted at research centers previously evaluated by the sponsor. These centers will be institutions or establishments that conduct health research and comply with current regulations.

The research center will be responsible for forming a multidisciplinary research team to execute the clinical study according to the protocol. It is its prerogative to design the organization and select the personnel who will perform these functions. However, the sponsor requires that the Principal Investigator (PI) be a physician specialized in ophthalmology.

Any person assigned responsibility for part of the study monitoring (coinvestigator, sub-investigator, nurse, etc.) or a specific role in the study (pharmacist, administrative assistant, study coordinator, etc.) must be listed in the "Delegation of Responsibilities."

The competency and training of all individuals directly involved in study activities must be verified prior to the conduct of any protocol-related activities. This must be recorded, and documents constituting evidence of this competency and/or training must be retained in the study master file. The competency and training of personnel involved in the study, both at the central level and at the study sites, is the responsibility of the sponsor.

The sponsor must ensure that all study center personnel participating in the study are adequately trained in the study (research protocol, investigator's manual, amendments, standard operating procedures, etc.) and in ICH Good Clinical Practices prior to the start of their participation in the study. Training must be documented in writing and filed in the study's master file.

7.2 Clinical study registration

This clinical study will be registered by the sponsor in public clinical trial registries prior to its initiation (enrollment of the first patient): the National Registry of Clinical Trials (RNEC) of the Federal Commission for the Protection against Sanitary Risks (COFEPRIS) and on a WHO primary registry platform. WHO primary registries meet specific criteria regarding content, quality and validity, accessibility, unique identification, technical capacity, and administration. WHO primary registries meet the requirements of the International Committee of Medical Journal Editors (ICMJE).

7.3 Randomization and blinding

Subject randomization will be performed using a computerized allocation system. After signing the FCI, the patient will receive a patient number, which will be used to pseudonymize all information during collection and completely anonymize it during analysis.

The generation will be carried out by a third party authorized by Laboratorios Sophia, SA de CV, through its electronic system. The information corresponding to this third party will be found in the file.

Blinding will be performed by the subject, PI, and subinvestigator. Furthermore, the statistical analysis will be performed blinded for the final analysis. Blinding cannot be guaranteed for the subject.

Masking will be done through primary and secondary packaging.

They will be identified by identical labels. These, in compliance with current and applicable regulations, must contain at least:

- Name, address and telephone number of the sponsor.
- Pharmaceutical form and route of administration.
- Batch number.
- Legend “Exclusively for clinical studies”
- Expiration date.

7.3.1 Opening of the cecum

The cecum may be opened in the following cases:

1. Presence of a serious adverse event.
2. Safety alert for the use of the drugs under study.
3. In the event that the sponsor determines it for any security reason or other reason that it deems appropriate.
4. In the event that the regulatory authority or the ethics committee justifiably requests it and deems it necessary

7.4 Outcome variables

7.4.1 Primary outcome variables

- OSDI Test Score (TE: Day 29)
- Incidence of unexpected AEs related to the PI (TE: day 8 and 29)

7.4.2 Secondary outcome variables

- Changes in BCVA (TE: day 8 and 29)
- Changes in TRPL with fluorescein (TE: day 8 and 29)
- Corneal fluorescein staining changes (TE: day 8 and 29)
- Conjunctival staining changes with lissamine green (TE: day 8 and 29)
- Incidence of expected AEs (TE day 8 and 29)

7.4.3 Exploratory outcome variable

- Average number of applications performed daily

7.4.4 Definition of variables, methods and scales to be used for measurement

Variable	Guy	Unit (symbol)	Measurement method	Normal value	Evaluation time	Statistical test
Primary outcome						
OSDI	Discreet	Points (-)	Questionnaire	< 13	VB and VF	Mann-Whitney U
Unexpected EAs related to PI	Discreet	Number of cases (n)	Counting	0	VB, V1 and VF	Mann-Whitney U
Unexpected AEs related to the PI (bis)	Nominal categorical	Present/absent (-)	Observation	Absent	VB, V1 and VF	χ^2 or Fisher's exact test
Of secondary outcome						
MAVC	Discreet	Fraction (-)	Snellen chart	1	VB, V1 and VF	Mann-Whitney U
TRPL with fluorescein	Continue	Seconds (s)	Direct observation with slit lamp and blue filter	>10s	VB, V1 and VF	Mann-Whitney U
Corneal staining with fluorescein	Ordinal	Degrees (-)	Direct observation with a slit lamp on the Oxford scale	0	VB, V1 and VF	χ^2 or Fisher's exact test
Conjunctival staining with lissamine green	Ordinal	Degrees (-)	Direct observation with slit lamp and cobalt blue filter, Oxford scale graduation	0	VB, V1 and VF	χ^2 or Fisher's exact test
EA	Discreet	Number of cases (n)	Counting	0	VB, V1 and VF	Mann-Whitne U
EA (repeat)	Nominal categorical	Present/absent (-)	Observation	Absent	VB, V1 and VF	χ^2 or Fishe's exact
Exploratory						
Number of applications made daily	Discreet	Number of applications (n)	Count by Subject's Diary	>4	V1 and VF	Mann-Whitne U

Table 2Operational definition of variables

The variables, method, and scales for their measurement are described in detail below. They are listed in order according to **Table 2**

7.4.4.1 OSDI Score

The OSDI is a validated and reliable instrument for measuring dry eye severity. It has the psychometric properties necessary for use as an outcome variable in clinical studies. [40]It consists of a 3-question, 12-item questionnaire. Responses are based on frequency and offer 5 options, ranging from 4 (all the time) to 0 (none of the time). Questions 2 and 3 also contain the option NA. (See **15.1**.) The OSDI score is calculated based on the following formula:

$$OSDI = \frac{D \text{ x } 25}{E}$$

Where:
D = sum of the points of the questions answered
E = number of items answered, those answered with NA are not counted as answered

The OSDI score will be recorded on the Electronic Case Report Form (FERC)

Management as an AE: An increase of more than 40% from the baseline OSDI score should be reported and managed as an AE due to lack of efficacy.

7.4.4.2 Adverse events

As defined in section **8.2,** an EA It is any unfavorable medical occurrence in a subject to whom a PI is administered, regardless of the causal attribution.

The management of AEs will be carried out as described in the Adverse Events section.

The IP will record any AEs that the study subjects may present in the corresponding section of the FERC and will also include them in the clinical record.

For an adequate evaluation of AEs, in addition to the targeted questioning, a Comprehensive Ophthalmologic Evaluation must be performed at each visit. This evaluation consists of an ophthalmologic examination of the eyelids and adnexa, anterior segments, and posterior segments, which are performed during a routine ophthalmologic examination and are not specifically included in the study variables. The posterior pole evaluation may be performed with direct or indirect ophthalmoscopy, with or without pharmacological mydriasis, at the discretion of the PI. The fundus will be assessed for abnormalities that could alter the study results. IOP will be measured during this evaluation, using the PI's chosen instrument, and should be measured after the stain evaluation. The results of the evaluation will be recorded in the clinical record. Only findings that the PI considers to be AEs will be reported in the FERC.

The expected adverse effects associated with the use of PIs are: blurred vision, burning, eye irritation, foreign body sensation, and sticky eyelashes. They are expected to be transient, lasting no more than 30 seconds after PI instillation, and to be mild in intensity.

7.4.4.3 Best Corrected Visual Acuity

Visual acuity (VA) is a test of visual function. Spatial VA is the ability to distinguish separate elements of an object and identify them as a whole. It is quantified as the minimum angle of separation (located at the nodal point of the eye) between two objects that allows them to be perceived as separate objects.

Snellen notation is described as the distance at which the test is performed divided by the distance at which the letter vertically equals 5 minutes of arc. Thus, at 6 meters, a letter 6/6 (20/20) equals 5 minutes of arc, a letter 6/12 (20/40) equals 10 minutes, and a letter 6/60 (20/200) equals 50 minutes. The Snellen fraction can also be expressed as a decimal (i.e., 20/20 = 1 and 20/40 = 0.5). [41]

VA will be assessed at baseline, without refractive correction, using the Snellen chart. This chart will be placed in a location with adequate natural or artificial lighting and at a distance of 3 m from the subject being assessed. Visual acuity will be measured in each eye, starting with the right eye (RE), asking the subject to keep both eyes open and using an occluder to cover the left eye (LE); the subject will read aloud the lines indicated by the evaluator. The evaluator will record the smallest line of letters visible as the RE VA in the clinical record. The LE is then assessed using the same method.

The subject's objective and subjective refractive correction will then be performed. The subjective refraction result will be reported as BCVA, recorded as a fraction in the clinical record and on the FERC, and recorded as a decimal on the FERC. By definition, BCVA cannot be less than VA.

Management as AE: A decrease of more than 2 lines on the Snellen chart should be reported and managed as an AE.

7.4.4.4 Tear film breakup time

One of the first aspects of the tear film to change when there is a change in the ocular surface is its stability. In general, if the corneal or conjunctival surface is damaged, it is unlikely that a stable tear film can be maintained.

The most common method for assessing tear film stability is fluorescein-based TRPL. Once fluorescein has been instilled (see **7.4.4.5**), the patient is asked to hold their breath after blinking 1 to 2 times using the cobalt blue filter. The fluorescein-stained precorneal layer will shift to less fluorescent or nonfluorescent regions. The time elapsed from the last blink to the appearance of these regions is the TRPL. It is reported in seconds in the clinical record and on the FERC.

Management as an AE: A decrease in TRPL of more than 40% from baseline should be reported and managed as an AE due to lack of efficacy.

7.4.4.5 Corneal staining with fluorescein

A drop of topical anesthetic is instilled into the conjunctival fornix. A second drop is then applied to the tip of the fluorescein strip, allowing it to sit on the strip for 5 seconds to elute the dye, shaking off the excess at the end. A small amount of contact is made between the strip and the conjunctiva in the temporal fornix while the patient looks upward, avoiding damaging the conjunctiva. After assessing the TRPL, 4 to 8 minutes after instillation of the dye, the corneal fluorescein staining is assessed using a slit lamp with a cobalt blue filter. It is graded according to the Sjögren's Clinical Collaboration Alliance (SICCA) Ocular Staining Grading (OSG). [42]

According to the CTO, grade 0 corresponds to the absence of punctate epithelial erosions (PEEs); grade 1 is defined as the presence of 1-5 PEEs; grade 2 corresponds to 6-30 PEEs; and >30 PEEs will be classified as grade 3. An additional grading point will be added if: 1) PEEs with a diameter of 4 mm are present in the central portion of the cornea; 2) filaments are observed; and 3) confluent staining patches, including linear staining, are observed. [42]See **Figure 4**

The IP will record in the file and with the FERC the grades awarded for corneal staining of OD and OS, respectively. The maximum grade per eye is 6.

Management as AE: Corneal staining that is greater than that recorded as baseline will be considered AE due to lack of efficacy.

7.4.4.6 Conjunctival staining with lissamine green

After the fluorescein examination, a drop of saline solution will be applied to the tip of the lissamine green strip, allowing it to sit on the strip for 5 seconds to elute the dye. A drop from the strip is instilled into the temporal fornix while the patient looks up, without damaging the conjunctiva. The patient may be asked to blink repeatedly to avoid accumulation in the conjunctival folds. The examination should be performed between 1 and 4 minutes after instillation through a neutral density filter or a red-free filter. It will be graded according to the SICCA CTO. [42]

In CTO, grade 0 is defined as the presence of 0 to 9 lissamine green staining points in the interpalpebral bulbar conjunctiva (scoring the temporal and nasal portions separately); grade 1 is defined by the presence of 10 to 32 points; grade 2 by 33 to 100; and grade 3 by >100 points. Because of the difficulty in counting individual points in a moving eye, any area ≥4 mm ^{2} of confluent points is considered >100 points [42]. See **Figure 4**

The IP will record, in the file and with the FERC, the sum of the grades awarded to the temporal and nasal portions for OD and OS, respectively. The maximum grade per eye is 6.

Management as AE: Conjunctival stains that obtain scores ≥ 3 and that are higher than the baseline score will be considered AE due to lack of efficacy. For example: Subject X obtains a baseline score of 1, and at a follow-up visit, his staining is rated as 2. This does not need to be managed as AE. If he obtains a score of 3 or higher, he should be managed as AE.

7.4.4.7 Number of applications made daily

According to the PI dosing schedule, subjects may increase the number of daily instillations. They should not decrease the number to four per eye per day.

An instillation is considered separate when there are more than 10 minutes between each instillation. Similarly, if two or more drops are applied to a single eye during a single instillation, it will be considered one instillation. The subject must record each instillation performed during the day in the Subject Diary. The PI will tally the number of applications recorded in the diary and divide it by the number of days of treatment. This average will be recorded in the FERC.

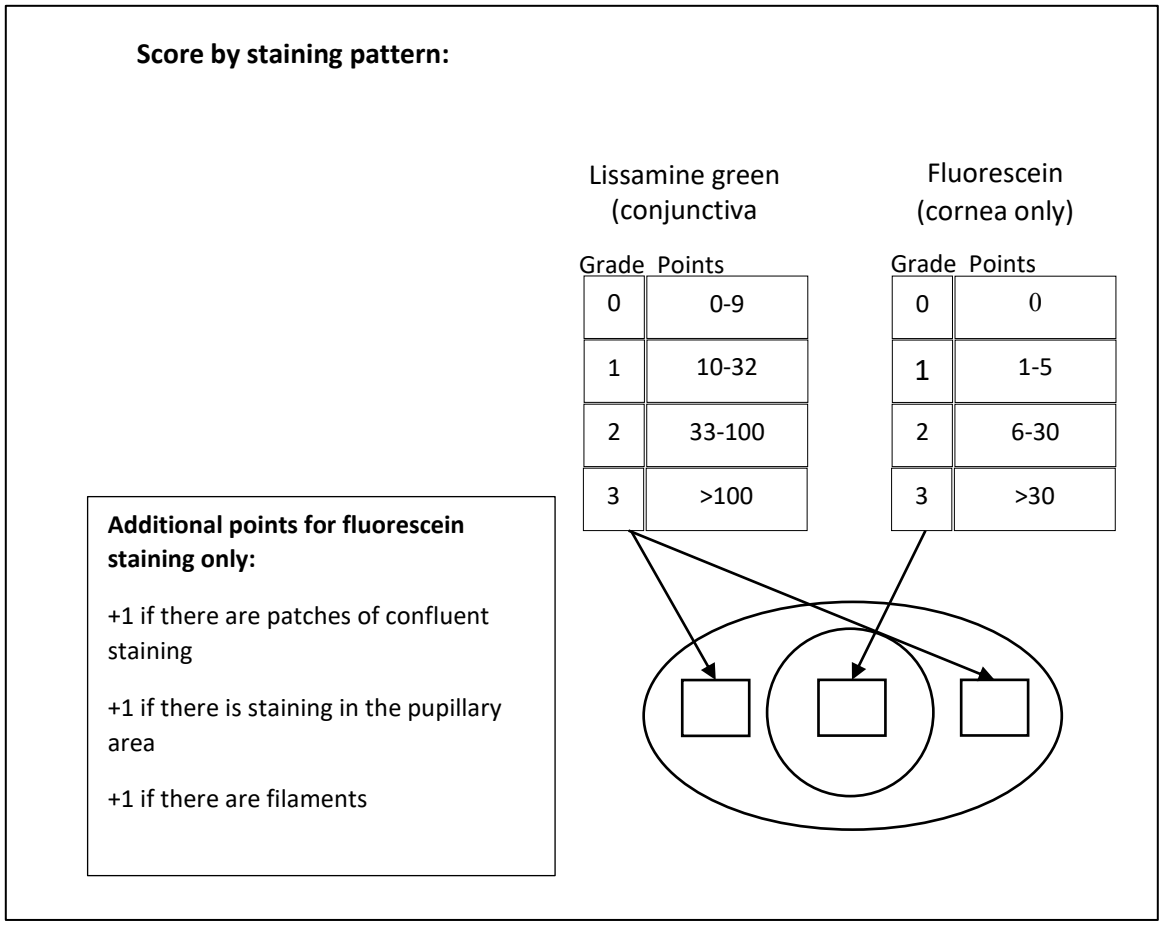


Figure 4SICCA Ocular Staining Rating
Modified from Whitcher et al. [42]

7.5 Program of visits and activities of the study

7.5.1 Description of activities per visit

The procedures are listed in the order in which they are suggested to be performed, trying to maintain the consistency of the evaluations and, as far as possible, from the least invasive to the most invasive.

7.5.1.1 Baseline Visit

- Signature of the ICF : refers to the signing of the written informed consent document. Without informed consent, none of the study procedures can be performed.
- Medical record: refers to the technical, clinical, and legal document that chronologically records the patient's health conditions, medical procedures, and other procedures performed on the patient. It includes anthropometric measurements, anamnesis, and a comprehensive ophthalmologic examination that allows determining the patient's eligibility, i.e., evaluation of both eyes and ocular adnexa, slit-lamp examination of the ocular surface and anterior segment, and fundoscopy. If the patient is taken from the study

center's established population base, the existing medical record may be used; only one update is required .

- Vital signs: This refers to heart rate, respiratory rate, blood pressure, and temperature. This information should be recorded in the patient's medical history and progress notes.
- OSDI Score: See 7.4.4.1
- Evaluation of concomitant medications: refers to the IP questioning the subject, inquiring about the use of medications.
- Urine pregnancy test: This refers to the performance of a rapid pregnancy test in all women of childbearing age who wish to enter the study. By childbearing age, we mean women who have experienced menarche but have not yet experienced menopause. Menopause is defined as 12 months since the last menstrual period in women over 40 years of age, or those who have undergone a hysterectomy or bilateral oophorectomy. Women of childbearing age using contraceptive methods, including bilateral tubal obstruction, must undergo a pregnancy test. This test will be performed by the PI or a designated team member according to the instructions on the device provided by the sponsor.
- MAVC: Ver 7.4.4.3
- TRPL: See 7.4.4.4
- Ocular surface stains: See 7.4.4.5and 7.4.4.6
- Ophthalmological evaluation: See 7.4.4.2
- Eligibility criteria: Refers to the PI's review, which verifies that the subject can be included in the study if they meet the inclusion criteria and do not meet the exclusion criteria. See 5.1
- PI Allocation: This refers to determining the intervention the patient will follow during the study. This assignment will be carried out according to section 7.3. This assignment will be made at the baseline visit (day 1).
- Delivery of the PI and start of intervention: Refers to the delivery of the PI to the study patient by the research center. See
- EA Assessment: See 7.4.4.2
- Delivery of patient materials and instructions for completion: Refers to the IP providing the subject with the patient diary, identification card, and adherence-enhancing calendar. The assigned staff will provide the subject with pre-training on how to complete the diary.

7.5.1.2 Visit 1

- Vital signs: See above
- Evaluation of concomitant medications: See above
- MAVC: Ver 7.4.4.3
- TRPL: See 7.4.4.4
- Ocular surface stains: See 7.4.4.5and 7.4.4.6
- Ophthalmological evaluation: See 7.4.4.2
- EA Assessment: See 7.4.4.2
- Subject Diary Assessment: This refers to the review of the Subject Diary instrument by the PI or designated facility staff; it will be reviewed for correct completion, as well as the patient's comments. The PI may question these comments for AEs. At the PI's discretion, the comments may or may not be reported as AEs.

- Adherence assessment: Adherence will be calculated by recording applications in the subject's diary. Adherence will be calculated based on a dosage of 4 applications per day. In addition, the average number of daily applications will be recorded according to section 7.4.4.7
- Subject Continuance Assessment: Refers to the PI's determination of the subject's desire to continue participating in the study.

7.5.1.3 Final Visit

- Vital signs: See above
- OSDI Score: See 7.4.4.1
- Evaluation of concomitant medications: See above7.5.1.1
- Urine pregnancy test: See above7.5.1.1
- MAVC: Ver 7.4.4.3
- TRPL: See 7.4.4.4
- Ocular surface stains: See 7.4.4.5and 7.4.4.6
- Ophthalmological evaluation: See 7.4.4.2
- EA Assessment: See 7.4.4.2
- Subject Diary Assessment: See más atrás 7.5.1.1
- Adherence assessment: See más atrás 7.5.1.1
- Subject continuity assessment: See más atrás 7.5.1.1
- Return of PI and Subject Diary: refers to the return by the subject of the PI and the subject diary to the research center 7.5.1.1 .

7.5.2 Unscheduled follow-up visits

At the request of the patient or any other individual involved in the study, unscheduled follow-up visits may be conducted to report adverse events. During these visits, all relevant data on reported adverse events must be collected, and an appropriate management plan must be established, if applicable.

7.6 Data collection

7.6.1 Source documents

Source documents are all written or printed records derived from automated processes (for example, printouts of laboratory results issued by automated analysis equipment) where information is first recorded and which become part of the patient's permanent record. Examples of source documents include medical records, clinical progress notes, laboratory reports, office study reports, nursing notes, follow-up notes, surgical records, etc.

Researchers from participating centers and institutions are obliged to accept the monitoring of information related to the study, audits, review by ethics and research committees, and inspections by the health authority. This obligation implies direct access to source documents.

7.6.2 Electronic forms of data collection

All protocol-related data will be captured via an electronic case report form (ECF) by research team personnel. Protocol-related data should NOT be captured directly into the ECF; rather, they should

be transcribed from the corresponding source document. This procedure allows for monitoring to verify the information captured in the ECF. It is the investigator's responsibility to ensure the information is transcribed to the ECF correctly, completely, and in a timely manner. It is understood that all ECF forms captured and submitted for data analysis are approved by the investigator.

7.6.3 Archive

The data collected in this database is anonymous (only the patient number is stored along with other relevant information). The software used for data capture and storage meets the traceability requirements necessary for conducting clinical studies. The collected data will be stored by the sponsor or designated clinical research organization for a period of 10 years. Records of patient number assignment will remain at the participating institutions under the care of the PI or their team and must be safeguarded for at least 5 years.

8. Evaluation and management of adverse events

8.1 Regulation and standards on adverse events

The registration and reporting of adverse events will be carried out in accordance with the guidelines established in NOM-220-SSA1-2016, which is in accordance with the international ICH E6 guidelines.

8.2 Definition of adverse event

According to the International Conference on Harmonization (ICH), an adverse event (AE) is any unfavorable medical occurrence in a patient undergoing clinical research who is administered a pharmaceutical product, regardless of causal attribution.

Therefore, an AE may be any of the following: any unfavorable, unintended disease, symptom, or sign (including an abnormal laboratory finding) that is temporally related to the use of a medical product, whether or not considered to be related to that product; any new disease or exacerbation of an existing disease (worsening of the nature, frequency, or severity of a known condition); relapse of an intermittent medical condition (e.g., headache) not present at baseline; any deterioration in a laboratory value or other clinical test (e.g., electrocardiogram [ECG], x-ray) that is related to the symptoms or that results in a change in study or concomitant treatment or discontinuation of study drug.

8.3 Definitions relevant to the classification of adverse events

Severity (serious/non-serious), also called seriousness (serious/non-serious). A serious event is defined as any event that: results in death, threatens life, requires hospitalization or prolongs hospitalization, causes permanent or significant disability or incapacity, causes abnormalities or malformations in the newborn, or other medically significant conditions.

Severity (mild, moderate, or severe). Mild conditions present with minimal symptoms and do not require treatment or discontinuation of medication; moderate conditions interfere with normal activities without threatening the patient's life, require treatment, and may or may not require discontinuation of medication; severe conditions interfere with normal activities and require pharmacological treatment and discontinuation of medication.

Causality. This is the relationship assigned between the drug and the adverse event: certainly caused by the drug, there is clear evidence of causality, i.e. the adverse event reappears with the administration of the drug; probably caused by the drug, there is a high suspicion of causality but no direct evidence is available or it is considered unnecessary or dangerous, i.e. the reaction disappears when the drug is discontinued; possibly caused by the drug, there is additional information suggesting that the cause may be due to another drug or disease; unlikely to be caused

by the drug, there is a clear explanation for the origin due to the underlying disease or the use of another drug; conditional, there is a lack of data to issue a clear causality; unclassifiable, those for which, once all possible information on the adverse event has been obtained, it remains unclassifiable.

8.4 Researcher Responsibilities

The investigator is responsible for verifying the AE by conducting a questioning, reviewing the information recorded in the subject's diary, conducting a relevant physical examination, assessing progress, and providing appropriate medical and pharmacological management. The investigator is also responsible for monitoring the AE until it is resolved or resolved, and the patient is discharged, following the definitions established in national and international regulations.[43] [44] [45]

In the event of an AE or any event that puts the health and well-being of the subjects at risk, appropriate medical care will be provided, either at the research center or by referring the subject to the highest-resolution hospital with which the research center has a medical care agreement. The PI will notify the sponsor's clinical monitor, in accordance with the timeframes established in national and international regulations. In the case of serious adverse events, the PI will notify the sponsor and record the corresponding information in the FERC, and in turn, will inform the IEC and the IC.

The attention of the AE will be carried out according to the event attention diagram (see

Figure 5Adverse event care).

The final report prepared by the Clinical Team of the Clinical Operations Department of Laboratorios Sophia, SA de CV, will include adverse event reporting in compliance with current national and international regulations. [44] [43]

If the research subject develops a chronic adverse event during their participation in the study, such as diabetes or arterial hypertension, they will be referred to a healthcare professional for chronic treatment. Follow-up and termination of participation will be in accordance with ICH guidelines.

8.4.1 Recording of adverse events in the electronic case report form

The EA registry considers:

- Subject identification information such as: subject number, age, sex, and if applicable, specify the eye.
- Information about the causality of the AE, its relationship to the PI, or to another study-related drug, as appropriate.
- Information on important dates:
    - Date on which the EA occurs
    - Date on which the IP is informed of the same
    - Date of resolution or outcome, as applicable.
- Information on diagnosis and clinical management.

- PIs is detected , it must be reported as a serious adverse event within the timeframe stipulated by current regulations. The concomitant medications should include the therapy used for the pharmacological management of the adverse event.
- Establish the outcome or resolution of the event:
    - Recovered/resolved without sequelae
    - Recovered/resolved with sequelae
    - Not recovered/Not resolved
    - Patient who died due to AE
    - Patient who died and it is judged that the drug may have contributed
    - Patient who died and this was not related to the product or drug under investigation,
    - A stranger
- Information about the investigational product or medicinal product or the medicinal product associated with the AE, ADR or SRAM. As applicable, information concerning the generic name, distinctive name or code of the PI and/or investigational medicinal product must be recorded, as appropriate according to the methodological design of the study. This is relevant in the case of blinded studies or those where placebo is used as comparators, since there are circumstances that justify opening the blind to determine whether the adverse event can be attributable to the active agent, the combination of active agents, or to the pharmacologically inert substance(s), such as the vehicle or additives, as appropriate to the phase of clinical research in which the development of the medicinal product is located. It will also be necessary to record data concerning the batch number, manufacturing laboratory, expiration date, dose, route of administration, start and end dates of administration and/or consumption, reason for the prescription; depending on whether it is a product or medication under investigation (protocol in which the patient is currently participating) or a medication that the subject under investigation consumes for the treatment of concomitant underlying diseases or uses for the management of some temporary sign or symptom that does not correspond to the Natural History of the pathology that motivated his/her entry into the research protocol.
- Indicate the withdrawal or continuation of the medication, as appropriate. Indicate whether the adverse event disappears upon withdrawal of the PI, investigational medication, or suspected medication (of causing the event). Also indicate whether a dose adjustment is made, if the event changes in intensity or severity, and if the reaction persists. It is important to indicate whether the AE reappears in subjects who are re-exposed to the medication after having been previously discontinued.
- Information regarding concomitant pharmacotherapy. Indicate the generic name, dose, route of administration, start and end dates, and the reason for the prescription, regardless of whether it is in accordance with the prescribing information or the data sheet or if it is used outside of the regulations or as authorized by the local, national, or international regulatory body.
- Information on relevant clinical history. The analysis of the AE considers the information previously described. However, the clinical context in which the adverse event occurs in the participants in the clinical research protocol is of particular interest. Therefore, information about previous conditions, hypersensitivity or allergy symptoms, previous surgical

procedures, laboratory tests or imaging examinations the participant has undergone, etc., that the researcher deems appropriate may be mentioned.

8.4.2 Monitoring of adverse events

The IP will provide care and follow-up to the AE presented by the participant until its outcome, in accordance with the provisions of the following section.

8.4.3 Procedures for a serious adverse event

The EA care process considers the following stages:

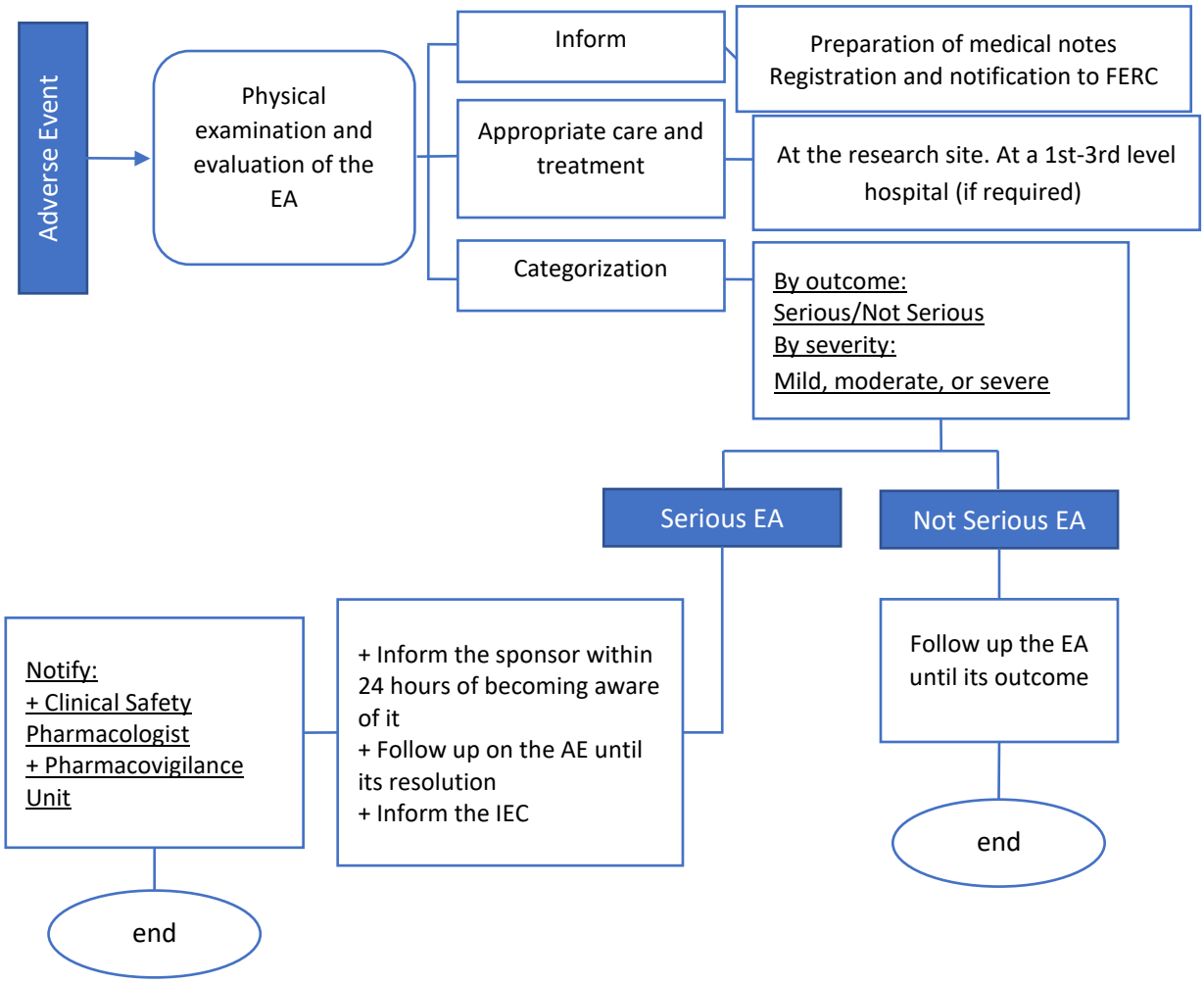


Figure 5Adverse event care

During the development and conduct of this study, undesirable adverse events or adverse reactions of medical significance may occur in the research subject, which are not necessarily causally related to the investigational medicinal products. These adverse events may occur during the use of investigational medicinal products at doses authorized for human use by a local, national, or

international regulatory body. However, it may be suspected that the investigational medicinal product or the investigational medicinal product may cause some undesirable clinical manifestation. AEs, ADRs, or SARs associated with one or more medicinal products may occur during the systematic evaluation of participants (on the days when clinical reviews are scheduled, according to the activity schedule) or suddenly, such that:

1. The researcher should be the first person to whom the patient notifies that he or she has developed or experienced any harmful clinical phenomenon during his or her participation in this study.
2. Based on their clinical judgment, the principal investigator will determine the appropriate treatment for the adverse event/reaction based on the relevant physical examination, history, etc., as well as the analysis of information available in the medical literature and the information contained in the investigator's manual, Prescribing Information, or the comparator drug's data sheet.
3. This care may be provided at the research center or at the hospital with the highest capacity for treatment. Thus, if the patient is referred by the PI to a hospital, they will receive care through a referral system. The referral may be through a card identifying the subject as a study participant and linking them to the pre-established agreement with the institution, or through a referral medical note issued by the PI. Laboratorios Sophia, SA de CV, will pay the costs for medical care for participating patients when the adverse event is associated with or related to the PI or investigational drug.
4. Taking into account the clinical information collected, either during the care provided at the research center or provided by the treating physician(s) at the hospital, the IP will record the AE in his/her clinical note, stating the seriousness, intensity (mild, moderate, or severe), and relationship with the product or drug under investigation.
5. The PI must migrate the relevant data to the FERC and its respective adverse event section. In the case of serious adverse events, the clinical monitor of the study must be notified within 24 hours of becoming aware of them, so that they can then inform the Clinical Team and the UTFLS, and subsequently inform the IEC. Regarding non-serious adverse events, these will be recorded and appropriately addressed, and the corresponding regulatory body will be informed about the safety profile of the PI or investigational drug in the final clinical trial report.

Recording the outcome of the AE depends substantially on the PI's follow-up of the subject, as most adverse events (see the safety profile section in section 5.3 and the investigator's manual) are expected to be ophthalmic in nature; however, systemic alterations may occur. Therefore, at the investigator's discretion, the participant's withdrawal or continuation will be considered.

8.4.4 Assessment of causality

Causality assessment is the methodology used to estimate the probability of attributing an observed adverse event to a medication. It considers probabilistic categories according to the available evidence and the quality of the information, based on national pharmacovigilance regulations. [43]

The Pharmacovigilance and Technovigilance Unit of Laboratorios Sophia (UFTLS) can use the Karch and Lasagna algorithm, modified by Naranjo, referred to by Aramendi I, as a tool to facilitate the probabilistic categorization of causality. This algorithm scores different items, which allow a value to be assigned to the cause-effect relationship between the administration of the medication and the adverse reaction. [46]See **Table 3Karch and Lasagna algorithm modified by Naranjo**.

No.	Reagent	Score	
		Yeah	**No**
1.	There are conclusive previous reports on adverse drug reaction, adverse event or suspected adverse drug reaction	+1	0
2.	The adverse event occurred when the suspected drug was administered	+2	-1
3.	The adverse drug reaction, adverse event, or suspected adverse drug reaction improved upon discontinuation or administration of a specific antagonist	+1	0
4.	The adverse drug reaction/adverse event/suspected adverse drug reaction recurred upon administration of the investigational drug/investigational product/investigational drug	+2	-1
5.	There are alternative causes that can provoke this reaction.	-1	+2
6.	The adverse reaction/adverse event/suspected adverse drug reaction occurred after placebo administration	-1	+1
7.	The drug was determined in blood or other fluids in toxic concentrations	+1	0
8.	The intensity of the adverse reaction/adverse event/suspected adverse drug reaction was greater with higher doses or less with lower doses	+1	0
9.	The patient has had similar reactions to the investigational drug/product or investigational drug in the past	+1	0
10.	The adverse reaction/adverse event/suspected adverse drug reaction was confirmed with some objective evidence	+1	0
Total score		summation	
	Probabilistic category based on the score obtained		
Yo	The causal relationship is verified	≥ ,9	
II	The ADR is likely due to the investigational drug or product	5 to 8	
III	The ADR may be due to the investigational drug or product	1 to 4	
IV	The causal relationship is doubtful	0	

Each reagent receives a defined score and the final sum allows estimating the probabilistic category of the cause-effect relationship between the administration of the investigational product and the adverse reaction, adverse event or suspected adverse reaction.

Table 3Karch and Lasagna algorithm modified by Naranjo

Thus, the degree of certainty required to establish the PI as the causal agent of the harmful phenomenon occurring to the subject of the clinical study is determined. This can also be indicated directly by the PI based on their clinical experience or through the voluntary application of the aforementioned tool. However , it is important that the researcher and the UFTLS consider the following arguments in favor of a causal relationship:

a) Strength of association, which refers to the number of cases in relation to those exposed.
b) The consistency of the data, that is, the presence of a common characteristic or pattern.
c) The exposure-effect pattern, which determines the relationship with the site of onset, time, dose and reversibility after suppression.

d) Biological plausibility, which refers to the possible pharmacological or pathophysiological mechanisms involved in the development or presentation of the adverse event.
e) Experimental findings, for example, the appearance of anomalous metabolites or high levels of drug or its biotransformation product.
f) Analogy, which refers to the experience acquired with other related drugs, adverse reactions frequently produced by the same family of pharmacological agents.
g) Nature and characteristics of the data, i.e. objectivity, accuracy and validity of the relevant documentation. [47]

8.5 Unanticipated problems

Unanticipated problems (ANP) are considered situations that pose risks to the participating subjects, generally any incident, experience or result that meets all of the following criteria:

- Unexpected in terms of its nature, severity, or frequency in relation to: 1) study-related documents such as the investigator's manual, study protocol, and informed consent form; and 2) the characteristics of the study population.
- Related or possibly related to your participation in the study (possibly related means that there is a reasonable possibility that the incident or results were caused by study procedures).
- Indication that the research places participants at greater risk of harm (including physical, psychological, economic, or social) than previously recognized.

8.5.1 PNAs Report

The PI will be responsible for reporting PNAs to the sponsor, the IC, and the IEC. The report should contain the following information:

- Study identification: protocol title and number, name of the PI and, where applicable, the center.
- Detailed description of the event, incident, experience or outcome.
- Explanation, justification of the reasons why the incident represents a PNA.
- Description of changes to the protocol or corrective actions taken or proposed in response to the NAP.

PNAs that are EAS must be reported to the IEC and the sponsor within the first 24 hours of the IP becoming aware of them.

Any other PNA will be reported to the IEC and the sponsor within the first 5 business days after the IP becomes aware of it.

9. Study monitoring

The study sponsor is responsible for monitoring the study. Monitoring activities include, but are not limited to: general safety monitoring, general study quality monitoring, study site monitoring, adverse event detection monitoring, reporting and follow-up, monitoring to resolve data entry discrepancies, etc.

Responsibility for monitoring activities and ultimate responsibility for monitoring rests with the sponsor.

The details of the monitoring activities are specified in a separate document from this protocol in a Monitoring Plan.

9.1 Monitoring of study centers

The research centers participating in the study will be monitored. At least one initial visit and one closing visit must be conducted for each center, although one or more follow-up visits may be required between these two mandatory visits.

The initial visit must be conducted before the first participant is enrolled at that center. During this visit, the monitor will verify that the materials to be used during the study have been received and that the personnel involved in study activities have been trained in the study. The monitor will also verify compliance with applicable regulatory requirements and standard operating procedures.

At the follow-up visit(s), the monitor will review the study documents to confirm that the research protocol and applicable standard operating procedures are being followed, that data entry is complete and timely, and that adverse event reporting is being conducted appropriately. At each visit, the monitor will discuss the findings with the investigator, and the investigator will determine the appropriate actions to be taken.

The closing visit will take place at the end of the study, once the last participant at the site has been discharged from follow-up. During this visit, the monitor will verify that the site has all the necessary documentation for archiving, that all biological samples have been sent for analysis, that all study drug (used and unused) has been recovered and sent to the sponsor, and that all unused materials have been retrieved.

Details of monitoring are set out in the relevant plan.

9.2 Audit and quality control

To ensure compliance with GCPs and all applicable regulatory requirements, Laboratorios Sophia, SA de CV may conduct quality assurance audits. Regulatory agencies may also conduct a regulatory inspection of this study.

Details of the audit process are set out separately in an Audit Plan.

9.2.1 Pre-study audit

The study centers included in the study will be subject to a feasibility visit prior to center selection, where they will be verified to meet the minimum requirements indicated by the sponsor.

9.2.2 Audit during the conduct of the study

They may take place at any time before, during, or after the conclusion of the study. If an audit or inspection is conducted, the investigator and the institution must agree to allow the auditor/inspector direct access to all relevant documents and must allocate their time and staff time to the auditor/inspector to discuss the findings and any pertinent issues. If the audit has not been scheduled by the sponsor, the center must notify Laboratorios Sophia, SA de CV immediately.

10. Statistical analysis

10.1 Data analysis

10.1.1 Statistical analysis

Statistical analysis will be performed by staff of Laboratorios Sophia, SA de CV. The statistical program SPSS version 19.0 (IBM Corporation, Armonk, NY, USA) will be used.

The designated personnel will be blinded to the intervention groups. Coding will be performed using consecutive numbers for each intervention group.

The data will be collected and organized in an Excel spreadsheet (Microsoft® Office). The data will then be exported to the SPSS software platform. The variables will be categorized according to their nature (see Table 2).

10.1.2 Data interpretation

The Kolmogorov-Smirnov and Shapiro-Wilk tests will be performed, as applicable, to determine whether the distribution is normal in the results obtained in each study group.

The results of continuous quantitative variables will be presented in measures of central tendency: mean, standard deviation and ranges.

The statistical analysis of continuous quantitative variables to find significant differences ($p<0.05$) will be as follows:

- Intra-group analysis: will be determined using the Wilcoxon rank test for quantitative variables.

- Between-group analysis: Differences between groups will be analyzed using the Student t-test or the Mann-Whitney U statistic if applicable (it will be used to test whether a group of data comes from the same population).

The level of difference considered significant will be an alpha (α) of 0.05 or less. A 95% CI will be considered for noninferiority criteria.

The results of the nominal and ordinal qualitative variables will be presented in frequencies, proportions and percentages.

Statistical analysis to identify significant differences in qualitative variables will be performed by creating 2x2 contingency tables and will be carried out as follows:

- Intra-group difference: McNemar's test. This test is applied to 2x2 contingency tables with a dichotomous trait, with matched pairs of subjects, to determine whether the marginal frequencies of the row and column are equal (marginal homogeneity).

- Difference between groups: Pearson's Chi-square test (X^2) or Fisher's exact test for expected values less than 5.

The level of difference to consider significance will be an alpha (α) of 0.05 or less.

For adverse event reporting, all eyes of participants randomized to an intervention group after the baseline visit will be considered. Results will be expressed as the number of subjects.

The final results report will be displayed in tables or graphs, as appropriate.

10.1.3 Procedure for handling missing data

Safety: The safety assessment will include in the analysis all subjects (both eyes) who have been exposed at least once to any of the interventions. Subjects enrolled in the efficacy phase will be included in the safety analysis for this phase, regardless of the visit at which they were eliminated from the study (ITT; intention-to-treat population).

Efficacy: Subjects who meet a minimum adherence of 70% will be included in the statistical analysis to meet the study objective, based on the weight of the PI. In cases where the container is not returned or has not been physically intact, adherence will be measured using the subject's diary.

10.1.4 Deviations from the statistical analysis plan

According to the sample size calculation to meet the efficacy objective, 102 evaluable subjects (51 subjects per arm) are required. If this number is not met due to a loss of subjects exceeding the 20% threshold established in this protocol (loss to follow-up or withdrawal from ICF), as mentioned in section 5.3.1, in order to balance the treatment groups, the sponsor may substitute these subjects.

The results obtained from the replaced subjects will continue to be used for safety analysis and will be part of the ITT.

10.1.5 Subjects included in the analysis

A preliminary safety analysis will be performed upon completion of follow-up of subject 12 in the Nanodrop® group (Phase I).

If fewer than 20% of unexpected drug-related AEs occur in the Nanodrop® group, enrollment for the efficacy analysis (Phase II) will be completed. Otherwise, the study will be terminated.

For phase II of the study, those subjects who meet a minimum adherence of 70% will be included in the statistical analysis to meet the study objective (PP; per-protocol population).

10.2 Sample size calculation

10.2.1 Number of subjects calculated

n= 126 evaluable subjects (both eyes)

63 subjects per arm.

An estimated 63 subjects (both eyes) are enrolled per treatment arm. During the safety phase, 24 subjects (12 from each group) will be recruited, and 102 will be recruited during the final phase, completing the sample of 126 required for the efficacy analysis.

10.2.2 Justification of the sample calculation

For the sample size calculation, studies with ocular lubricants in patients with dry eye were considered, in which changes in the OSDI score were the primary outcome variable.

Nanodrop® is expected to be non-inferior to its comparator (Systane® Balance), based on the following working hypotheses:

$$H_0: \varepsilon > \delta$$

$$H_0: \varepsilon \leq \delta$$

Where $\mu_A - \mu_B = \varepsilon$ is the true difference in means between the test treatment (PRO-176) and the active control (Systane® Balance). Where δ is the non-inferiority margin, and the ratio of the sample sizes between the two groups is given by:

$$k = \frac{n_A}{n_B}$$

Calculations were performed using non-inferiority equations for two means and for statistical power. [48, 49]

$$n_A = kn_B \; y \; n_B = \left(1 + \frac{1}{k}\right)\left(\sigma \frac{z_{1-\alpha} + z_{1-\beta}}{\mu_A - \mu_B - \delta}\right)^2$$

$$1 - \beta = \theta(z - z_{1-\alpha}) + \theta(-z - z_{1-\alpha}) \;\; ,z = \frac{\pi_A - \pi_B - \delta}{\sigma\sqrt{\frac{1}{n_A} + \frac{1}{n_B}}}$$

Where; σ is the standard deviation, $k = n_A / n_B$ is the coincidence ratio, θ is the standard Normal distribution function, α is the type I error (confidence interval), β is the type II error (meaning that 1-β corresponds to the power) and δ is the test margin.

With a power of 80% (β=0.20), a significance level of 0.05 (α) and a non-inferiority margin (δ) of -5 points on the OSDI score.

The sample size calculation was performed considering a reduction in the OSDI score of 17.6 points for the placebo group at their final visit versus a reduction of 16.5 points for the treated group. Considering a σ of 17.1, a similar reduction in the OSDI score is expected for both treatments, with no statistically significant differences at their final visit. [50, 51, 52, 53]

11. Ethical considerations

11.1 Approval of the committees

This study will be conducted in accordance with the standards of the Declaration of Helsinki, World Medical Association 2013. Nuremberg Code; Nuremberg Judgment by the International Tribunal of Nuremberg, 1947. Belmont Report, National Commission for the Protection of Subjects of Biomedical Research and Conduct, 1979. It will be conducted in accordance with the scientific and technical requirements necessary for the registration of medicines for human use of the International Conference on Harmonization (ICH) Guide to Good Clinical Practice. International Ethical Guidelines for Biomedical Research Involving Human Subjects of the Council for International Organizations of Medical Sciences (CIOMS, 2002). International Ethical Guidelines for Epidemiological Studies of the Council for International Organizations of Medical Sciences (CIOMS, 2008). The Research Ethics Committee and the Research Committee will evaluate the protocol before conducting the study and will issue their approval or any possible modifications for its implementation. These Committees must be notified of any significant changes to the protocol. In addition to the above, the current regulations of the regulatory authority must also be complied with.

The sponsor's authorized personnel will submit the essential documentation of the research project for evaluation by the Research Ethics Committees, Research Committees, and when applicable, to the Biosafety Committee: research protocol, informed consent form, researcher's manual, patient materials, as well as other additionally requested documents, in accordance with the local, national or international requirements applicable by regulatory entities.

The study will not be initiated at the research center without the confidentiality agreements and financial proposals from each of the principal investigators, duly signed, and without having previously obtained the favorable opinion and/or approval of the corresponding Research Ethics Committees, Research Committees, and, where applicable, the Biosafety Committee.

The study will not begin without meeting the relevant local, national, or international regulatory requirements and obtaining the appropriate health authorization.

The study is considered as research with greater than minimum risk, in accordance with the Regulations of the General Health Law on Health Research, Second Title, Chapter I, Article 17, Section III, published in the Official Gazette on January 6, 1987 [3]

11.2 Amendments to the protocol

The amendment process will be relevant when there is a need to make any changes to a document that is part of the research project or protocol, due to changes in the methodological structure, replacement of the principal investigator, or the identification of risks to the research subjects. Documents that may be amended include: the protocol, informed consent letter, researcher's manual, patient documents, measurement scales, and activity schedule.

Any amendment must be approved by the sponsor and/or the principal investigator. The amended document(s), once reviewed and approved by the Research Ethics Committee and the Research Committee or, when applicable, by the Biosafety Committee (entities that issued the initial favorable opinion for the conduct of the research), will be sent for authorization by the relevant regulatory entity.

Amendments that substantially modify the protocol or impose additional or different risks on research subjects must be approved by the aforementioned Committees. It is the investigator's responsibility to take measures in situations requiring immediate action to prevent unnecessary harm to study participants.

The principal investigator is responsible for communicating to the Research Ethics Committee any amendments to the protocol that could affect the rights, safety, or well-being of the research participants. Likewise, they must report any situation or new knowledge that indicates an increased risk to participants, the premature termination or suspension of the study, the reasons for this, and the results obtained to that point. They must also report the conclusion of the study upon completion of the research protocol.

11.3 Early termination of study

The study may be temporarily suspended or terminated prematurely if there is sufficiently reasonable cause. Written notification documenting the reason for the suspension or early termination must be provided by the party executing the suspension. The PI must promptly inform the study participants, the IC, and the IRB, providing the reasons.

Situations in which suspension or early termination of the study will be considered include, but are not limited to:

1. The presence of serious adverse events in more than 10% of participants in a study group.
2. The regulatory authority (COFEPRIS) considered it due to security alerts.
3. The Sponsor determines this for its convenience or eventualities such as: financial support, manufacturing errors, etc.
4. The determination of unexpected risks to participants that are significant or unacceptable.
5. Obtaining new relevant safety information.
6. Insufficient adherence to protocol requirements.
7. The data obtained are not evaluable or are not sufficiently complete.
8. The determination that the primary objective has been achieved.
9. The determination of futility.

In the event of suspension, the study may be resumed once the situations that led to the suspension have been resolved, provided this justification is sufficient for the sponsor, IC, IEC, and regulatory authorities.

11.4 Informed consent

The informed consent letter contains complete and understandable information about the study and the investigational product, in accordance with current applicable regulations and Good Clinical Practices.

The informed consent letter will be considered a source document and will be filed as such. The site's principal investigator is responsible for ensuring that all new versions of the informed consent form undergo the appropriate approvals (the same ones that the original informed consent form underwent) and that the most current approved version is presented to the study subjects.

11.4.1 Obtaining

Informed consent must be obtained before the subject undergoes any procedure indicated in the protocol. For this purpose, the informed consent form must be signed.

Written consent documents will incorporate the elements of informed consent described in the Declaration of Helsinki and the ICH Guidelines for Good Clinical Practice and will be in compliance with all applicable laws and regulations.

The IP will provide the potential participant with all information regarding the characteristics of the study, its potential benefits, risks, objectives, and procedures.

This information will be provided in a language the subject understands. The subject will be explained that they have the right to discontinue their participation in the study at any stage, without affecting their relationship with the researcher and/or their future participation. Informed consent will be presented to the potential participant; they must have sufficient time to review each and every aspect mentioned above. Any questions they may have will be clarified by the person responsible for obtaining informed consent.

Once the participant agrees to participate in the study, he or she must sign and date the informed consent letter in the presence of two witnesses, whether or not related to the study subject. These witnesses will participate in the informed consent process and sign, confirming that the process was carried out prior to any study procedure, that the study information was clearly explained, and that any questions were clarified.

In the event that a subject is illiterate, acceptance will be with his or her fingerprint, and in the event that the subject is not capable of providing adequate written informed consent, a “legally authorized” representative of the subject may provide such consent for the subject in accordance with applicable laws and regulations.

The IP must also sign and date this consent.

Informed consent must be signed in duplicate by all participants and two witnesses. One copy will be filed in the researcher's folder and the other will be given to the participant. The PI or designated staff member must document the process of obtaining informed consent through a detailed medical note, specifying the signed version, the date the document was signed, and how the process was carried out.

11.4.2 Special considerations

The procedures that will be performed during the conduct of the study do not pose any additional risk that should be considered apart from the procedures listed in the informed consent.

11.4.3 Modifications to informed consent

Any change to the “informed consent” constitutes an amendment to this document and must be submitted for approval to the Research Ethics Committees and, if applicable, to the Competent Authorities.

Such amendments may be implemented only after obtaining written approval from the Research Ethics Committee and the Regulatory Body (as applicable), except for an amendment that is required to eliminate an immediate danger to the study subjects.

A re-consent process must be conducted for each subject affected by the amendment under the same conditions as those described above (see section 12.4.1) to ensure that the subject is promptly informed of the new information contained in the document. The subject will be given a signed original of the amendment, and the researcher will retain the second original.

11.5 Confidentiality

All documents and information provided to the research center by the sponsor are strictly confidential. The PI expressly agrees that the data regarding his or her professional and clinical experience, provided to the sponsor in paper form and stored electronically, are solely for use in connection with his or her activities with the clinical study sponsor, in accordance with Good Clinical Practice.

The researcher agrees that he and his team members will use the information only within the framework of this study, to carry out the protocol. This agreement is binding as long as the confidential information has not been publicly disclosed by the sponsor.

The clinical study protocol provided to the investigator may be used by the investigator and his or her team to obtain informed consent from the subjects for the study. The clinical study protocol, as well as any information derived from it, must not be disclosed to other parties without the sponsor's written authorization.

The researcher will not disclose any information without the prior written consent of Laboratorios Sophia, SA de CV, except to representatives of the Competent Authorities, and only at their request. In the latter case, the researcher is obligated to inform Laboratorios Sophia, SA de CV before disclosing the information to these authorities.

The researcher will complete and maintain a subject selection record, as well as the identification and enrollment list of each subject participating in the study. The researcher agrees to grant on-site access to the auditor and/or representatives of the Competent Authorities. The information will be treated in compliance with professional secrecy.

All FERCs and communications related to study subjects will identify them only by their study subject identification number. The information collected in this study will be exchanged between the

sponsor and the research site and must be treated confidentially. The Health Authority, the IRB, the IC, the sponsor, the monitors/auditors, and third-party auditors will be the only bodies authorized to review study documentation. If publications arise from this research project, under no circumstances will they contain information about the identification of study subjects. If the study results are published, no personal information about the study subjects will be revealed.

The protection of personal data will be in accordance with the corresponding current regulations.

11.6 Conflict of interest

The independence of the study's conduct and results from any actual or perceived external influences is critical. Therefore, any current conflict of interest of any person playing a role in the design, conduct, analysis, publication, or any other aspect of this study will be declared. Furthermore, those with a perceived conflict of interest will be asked to manage it in a manner appropriate to their participation in the study.

11.6.1 Declaration of interests

The IP undertakes to make a declaration of financial interests, as well as conflict of interests prior to the start of the study.

11.7 Access to information

The final study database will be the property of Laboratorios Sophia, SA de CV, and access to it will be restricted. The PI will not have access to it except with prior written authorization from the sponsor.

Any information obtained that is relevant to the safety of the subjects participating in the study must be immediately shared with the research center, so that the study subjects can be notified.

11.8 Ancillary and post-study care

Once the study is completed and adverse events are closed according to section 8, the sponsor will not extend care to the research subject.

12. Biosecurity aspects

NO BIOSECURITY IMPLICATIONS

This protocol, with title: " Phase I-II clinical study to compare the safety and efficacy of Nanodrop® versus Systane® Balance in the treatment of patients with dry eye", and number: SOPH176-1218/I-IIDOES NOT HAVE BIOSECURITY IMPLICATIONS, since infectious-contagious biological material will NOT be used; pathogenic strains of bacteria or parasites; viruses of any type; radioactive material of any type; genetically modified animals and/or cells and/or plants; toxic, hazardous or explosive substances; any other material that puts the health or physical integrity of the research center staff or research subjects at risk or affects the environment. Likewise, it is declared that no cell, tissue or organ transplant procedures, or cell therapy will be carried out in this project, nor will laboratory, farm or wildlife animals be used.

13. Publication Policy

13.1 Final report

Once the statistical analysis is completed, the final report will be written with the results obtained, by the Clinical Team of the Clinical Operations Department of Laboratorios Sophia, SA de CV. This report will be prepared following the recommendations of the ICH E3 *Step 4 Guide.*

13.2 Communication of results

Regardless of the results of the study, Laboratorios Sophia, SA de CV, is committed to communicating the final study report to the principal investigators and COFEPRIS. These results will also be shared with the research committee and the IEC. The PI will be responsible for communicating the results to the research subjects.

Laboratorios Sophia, SA de CV will retain at all times the rights to the publication and dissemination of the information contained herein.

13.3 Publication of the results

Laboratorios Sophia, SA de CV, acting as the sponsor of the study, assumes full responsibility for its role and retains exclusive ownership rights to the study results, which it may use as it sees fit.

The PI agrees not to publish or communicate data collected from the study, unless prior written agreement is obtained from Laboratorios Sophia, SA de CV. Any manuscript derived from the data obtained with this protocol must be submitted for review by the sponsor before any attempt to submit it for publication in any journal or scientific conference.

However, if the sponsor is in the process of filing a patent application on the results of the study, the sponsor may delay publication or communication of the results of the study until the date of registration or when it deems appropriate.

Authorship assignments for publications are the sponsor's responsibility. However, express authorization from those invited to participate as authors is required. Authors have the right to review the manuscript prior to publication, as well as to provide comments and suggestions. Such comments must be submitted within the first 15 calendar days of receipt of the project.

14. Financing and insurance

14.1 Compensation to study participants

Subjects participating in the study will not receive financial compensation for their participation. However, randomized subjects will receive financial support in the form of travel expenses for each scheduled visit they attend on time. This support, as well as the amount, will be specified in the informed consent form.

14.2 Insurance for study participants

Subjects participating in the study will sign the informed consent form, which specifies that Laboratorios Sophia, SA de CV agrees to pay for immediate treatment resulting from injuries or illnesses caused by the investigational products until resolved, in accordance with medical judgment.

All study participants will be covered by a liability insurance policy contracted by Laboratorios Sophia, SA de CV. Information on the policy will be made available to the research centers. In the event of a medical emergency, the research center must have the personnel, materials, equipment, and procedures in place for immediate management.

15. Annexes

15.1 OSDI

OSDI (Ocular Surface Disease Index)

Ficha de identificación

No. de estudio: SOPH176-1218/I-II **Fecha:** / /

Iniciales del sujeto: _____ **No. de sujeto:** 176-_____-_____

Indicaciones:

El test OSDI es un test sencillo creado para establecer una gravedad/ clasificación del ojo seco según su sintomatología. Conteste a las siguientes preguntas marcando la casilla que mejor represente su respuesta:

Durante la última semana,	Frecuencia				
¿Ha experimentado alguna de las siguientes alteraciones?	**En todo momento**	**Casi en todo momento**	**El 50% del tiempo**	**Casi en ningún momento**	**En ningún momento**
1. Sensibilidad a la luz	4	3	2	1	0
2. Sensación de arenilla en los ojos	4	3	2	1	0
3. Dolor de ojos	4	3	2	1	0
4. Visión borrosa	4	3	2	1	0
5. Mala visión	4	3	2	1	0

Subtotal de las celdas contestadas (A)

Durante la última semana,	Frecuencia					
¿Ha tenido problemas en los ojos que le han limitado a realizar estas acciones?	**En todo momento**	**Casi en todo momento**	**El 50% del tiempo**	**Casi en ningún momento**	**En ningún momento**	**NA**
6. Leer	4	3	2	1	0	NA
7. Conducir de noche	4	3	2	1	0	NA
8. Trabajar con computadora o un cajero automático	4	3	2	1	0	NA
9. Ver televisión	4	3	2	1	0	NA

Subtotal de las celdas contestadas (B)

Durante la última semana,	Frecuencia					
¿Ha sentido incomodidad en los ojos con alguna de las siguientes situaciones?	**En todo momento**	**Casi en todo momento**	**El 50% del tiempo**	**Casi en ningún momento**	**En ningún momento**	**NA**
10. Viento	4	3	2	1	0	NA
11. Lugares con baja humedad (secos)	4	3	2	1	0	NA
12. Zonas con aire acondicionado	4	3	2	1	0	NA

Subtotal de las celdas contestadas (C)

Suma de A+B+C = (D)

Número de reactivos contestados, no incluya las respuestas como NA (E)

15.2 SICCA CTO format

Formato de Calificación de Tinción Ocular del SICCA

Ficha de identificación

No. de estudio: SOPH176-1218/I-II **Fecha:** / /

Iniciales del sujeto: _____ **No. de sujeto:** 176-_____-_____

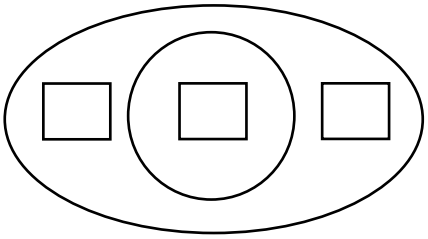
OD

Verde lisamina (solo conjuntiva)

Grado	Puntos
0	0-9
1	10-32
2	33-100
3	>100

Fluoresceína (solo córnea)

Grado	Puntos
0	0
1	1-5
2	6-30
3	>30



Puntos adicionales para la tinción de fluoresceína solamente:

+1 si hay parches de tinción confluente ☐

+1 si hay tinción en el área pupilar ☐

+1 si hay filamentos ☐

Puntaje Córnea:_____

Puntaje Conjuntiva:_____

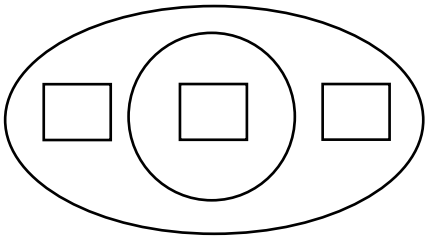
OS

Verde lisamina (solo conjuntiva)

Grado	Puntos
0	0-9
1	10-32
2	33-100
3	>100

Fluoresceína (solo córnea)

Grado	Puntos
0	0
1	1-5
2	6-30
3	>30



Puntos adicionales para la tinción de fluoresceína solamente:

+1 si hay parches de tinción confluente ☐

+1 si hay tinción en el área pupilar ☐

+1 si hay filamentos ☐

Puntaje Córnea:_____

Puntaje Conjuntiva:_____

Versión 1.0 07/Feb/19

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