Excelencia en oftálmicos

Study protocol:

SOPHMAN-0824/IV

Title: Clinical study of non-inferiority between Manzanilla Sophia® and Meticel Ofteno® 0.5% to provide a feeling of rest to the eyes.

Information about the molecule under study
Generic name: *Matricaria recutita* 0.025%
Distinctive name: Chamomile Sophia®
Indication: Cleaning and resting of the eyes.

Protocol Information
Study Phase: IV
Version: 2.0
Release Date: 21/Feb/25

This protocol has been carried out in accordance with the principles of the Declaration of Helsinki and will be carried out in compliance with the ICH guidelines and current local legislation.
Sponsor: Laboratorios Sophia, S.A. de C.V.

Intentionally blank page

Contents

Figure Index

Table of Contents

Responsible for the study

The administrative structure of the sponsoring party, corresponding to Laboratorios Sophia, S.A. de C.V., is shown in Table 1.

Table 1 Responsible for the study

Signature Pages

From the sponsor

Investigator Agreement

I agree to conduct this clinical study in accordance with the design and guidelines of this protocol, in accordance with the provisions of this protocol and in accordance with the accepted standards of Good Clinical Practice (GCP).

I agree to report all information or data in accordance with what is indicated in the protocol. I also agree to handle 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 is confidential, I understand that it is prohibited from sharing it with any third party, which is not involved in the approval, supervision, or conduct of the study. I will ensure that I take the necessary precautions to protect the information from loss, inadvertent disclosure, or access by unauthorized third parties.

Researcher's Name (printed or typed):	
	Signature
Title of the Researcher (in print or typescript):	
	Date
Name of the research center (printed or typed):	
Research Center Address (City/State/Country)	

List of abbreviations

GCP	Good Clinical Practices
REC	Research Ethics Committee
RC	Research Committee
COFEPRIS	Federal Commission for the Protection against Sanitary Risks
COX-2	Cyclooxygenase-2
IUD	Intrauterine device
EA	Adverse Event / Adverse Events
ERTV	Ethnopediatric relative therapeutic versatility *(ERTV*)
FC	Frequency *of Citation*
ICF	Informed consent form
eCRF	Electronic Case Report Form
ICAM-1	Interleukin adhesion molecule
ICF	Informant *Consensus Factor*
ICH	International *Council on Harmonisation*
IL	Interleukin
PI	Principal Investigator
ITT	Intent to Treat
LD50	Average lethal dose
LLS	Safety call
WHO	World Health Organization
WHO/PAHO	Regional Office of the Health Organization of the Americas
PGE2	Prostaglandin E2
RP	Research product
QID	Four times a day
RFC	Relative *Frequency of Citation*
RII	Relative *Importance Index*
NCTR	National Clinical Trials Registry
UV	Use Value
VCAM-1	Vascular adhesion molecule
VS	Scrutiny visit
FV	Final visit

Intentionally blank page

1. Summary

1.1 Synopsis

<table>
<tr><td colspan="2">Title of the study:
Clinical study of non-inferiority between Manzanilla Sophia® and Meticel Ofteno® 0.5% to provide a feeling of rest to the eyes.</td></tr>
<tr><td>Study Number:
SOPHMAN-0824/IV</td><td>Date of creation:
21/Feb/25</td></tr>
<tr><td>Protocol version:
2.0</td><td>Release Date:
21/Feb/25</td></tr>
<tr><td>Therapeutic indication:
Cleaning and resting of the eyes.</td><td>Application:
Eye strain</td></tr>
<tr><td>Estimated duration of the study (from the first visit of the first subject to the preparation of the final report):
10 months</td><td>Clinical Development Phase: Phase IV Clinical Study</td></tr>
<tr><td colspan="2">Objectives:
Primary Objective:
To demonstrate the non-inferiority of Manzanilla Sophia compared to® Meticel Ofteno® 0.5% in providing a feeling of rest to the eyes, by scoring an analogous visual test of eye strain.
Secondary objectives
Signs and symptoms evaluated:
Compare Manzanilla Sophia® vs. Meticel Ofteno® 0.5% in the incidence of red eye.
Compare Manzanilla Sophia® vs. Meticel Ofteno® 0.5% in the incidence of dry eye sensation.
Compare Manzanilla Sophia® vs. Meticel Ofteno® 0.5% in the incidence of eye irritation.
Compare Manzanilla Sophia® vs. Meticel Ofteno® 0.5% in the incidence of sleep sensation.
Compare Manzanilla Sophia® vs. Meticel Ofteno® 0.5% in the incidence of comfort with investigational product (IP) application.
Safety Assessment:
To compare the incidence of adverse events (AEs) related to interventions.</td></tr>
<tr><td colspan="2">Hypothesis:
H0= The Manzanilla Sophia® ophthalmic solution is inferior in its efficacy compared to its comparator, after 7 days of treatment, presenting a difference of less than or equal to 6 points (or the equivalent of 20%) in the final score of an analogous visual test of eye fatigue.
$H_0: p_A - p_B \leq \delta$</td></tr>
</table>

H1 = The Manzanilla Sophia® ophthalmic solution is not inferior in its efficacy compared to its comparator, after 7 days of treatment, presenting a difference of more than 6 points (or the equivalent of 20%) in the final score of an analogous visual test for eye fatigue.

$$H_1: p_A - p_B > \delta$$

Where δ = -10%. When the lower limit of the 95% confidence interval (95%CI) is not higher than δ, the non-inferiority of the investigational product (Manzanilla Sophia®) compared to the comparator (Meticel Ofteno®) is established.

Study Design:

Phase IV non-inferiority, open label, controlled, comparative, multicenter clinical study.

Number of subjects (planned and analyzed):

Number of planned subjects: 84 evaluable subjects per arm.
Total sample = 168 evaluable subjects.

Diagnosis and main inclusion criteria:

- Subjects with a history of eye strain, as defined for this protocol.

Selection criteria:

Inclusion criteria:

- Can voluntarily give their signed informed consent.
- Be able and willing to comply with scheduled visits, treatment plans, and other study procedures.
- Be of legal age.
- Women of childbearing potential who have not undergone bilateral tubal occlusion or hysterectomy should ensure continued use (initiated ≥ 30 days prior to signing the Informed Consent Form [ICF]) of the use of a hormonal contraceptive method or intrauterine device (IUD) during the study period.
- Present a score on the visual analog test of eye strain ≥ 3 in at least 4 of the questions included.

Exclusion criteria:

- Women who are pregnant, breastfeeding, or planning to become pregnant within the study period.
- Have participated in another clinical research study ≤ 30 days prior to the screening visit.
- Have previously participated in this study.
- Have a functional single eye.
- Have a history of drug addiction or drug dependence current or within the last two years prior to signing the FCI.
- Be a soft or hard contact lens wearer. You will be able to enter in case of suspending its use during the study, you must comply within 15 days without using the contact lens prior to your inclusion.
- Have known hypersensitivity to the components of the investigational products.

Research Product (PI):

Investigational product, dosage and route of administration:

- Manzanilla Sophia®. *Matricaria recutita* 0.025%. Ophthalmic solution. Laboratorios Sophia, S.A. de C.V., Zapopan, Jalisco, Mexico.
- Dosage: 1 drop 4 times a day (QID), with a minimum interval of 3 hours between applications.

- Route of administration: Ophthalmic

Reference products, dosage and route of administration:

- Meticel Ofteno® 0.5%. Hypromellose 0.5%. Ophthalmic solution. Laboratorios Sophia, S.A. de C.V., Zapopan, Jalisco, Mexico.
- Dosage: 1 drop 4 times a day (QID), with a minimum interval of 3 hours between applications.

Duration of treatment:	Duration of the subject in the study:
7 days	Up to 11 days

Evaluation criteria:

Primary outcome variables

- Score of the analog visual test of eye strain (TE: screening and final visits).

Secondary Variables

Signs and symptoms assessed

- Incidence of red eye (TE: counting and final visits).
- Incidence of dry eye sensation (ET: screening and final visits).
- Incidence of eye irritation sensation (ET: screening and final visits).
- Incidence of sleep sensation (TE: counting and final visits).
- Incidence of comfort with the application of the IP (TE: final visit).

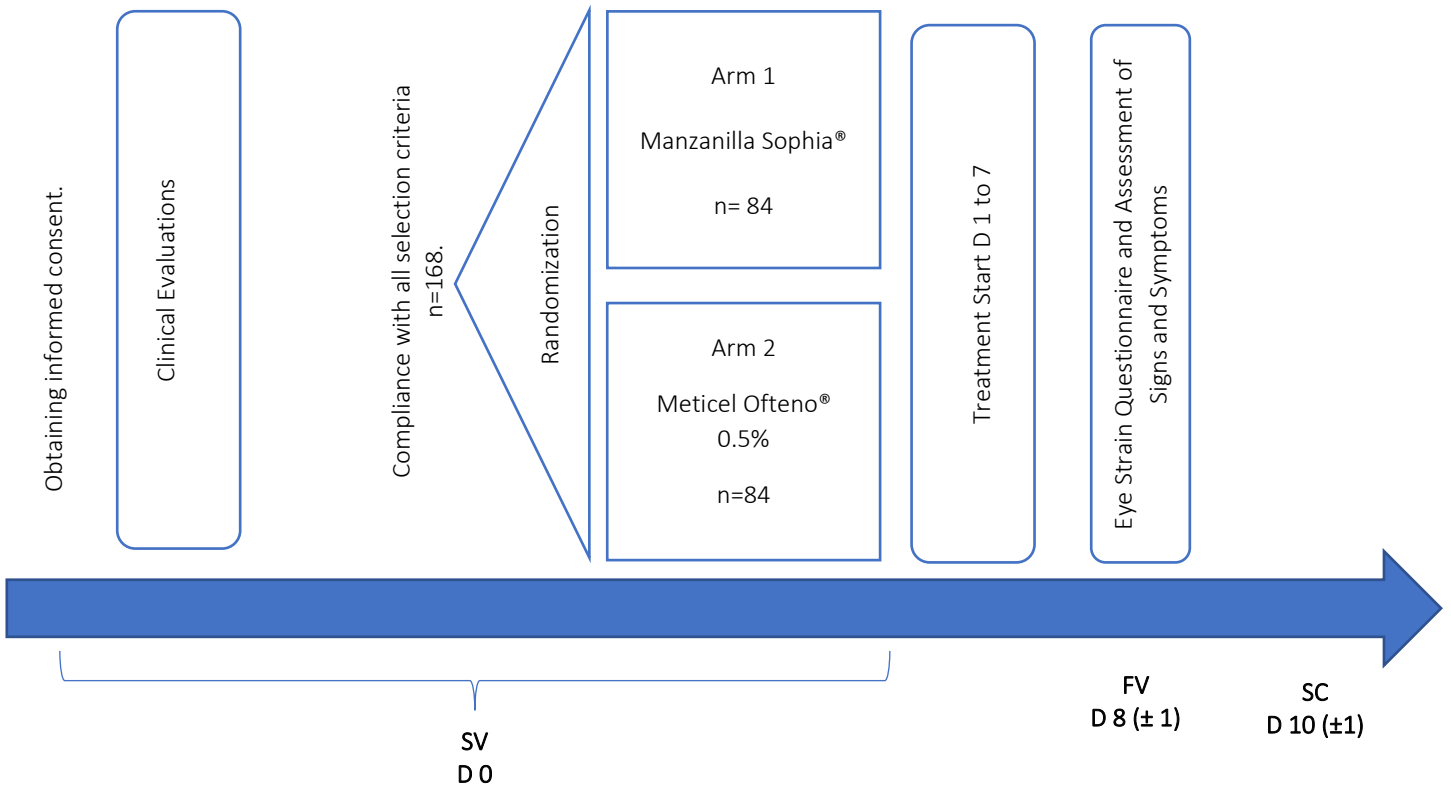
Security Assessment

- Incidence of intervention-related adverse events (AEs) (ET: screening, end, and safety call visits).

Statistical methodology

The analysis of the data collected in the study will be carried out by means of specialized statistical software (SPSS available version or R software). The data will be expressed with measures of central tendency and dispersion: mean, standard deviation and/or ranges for quantitative variables, and frequencies and/or percentages for qualitative variables. Due to the size of the selected samples, we can consider that they will be distributed normally. The Student's t-statistic will be used for independent groups, for discrete variables. The statistical analysis for the qualitative variables will be by means of the McNemar test for the dichotomous variables and the Pearson's X^2 or Fisher's exact statistic for expected values <5. A non-inferiority margin (δ) of -10% will be used for the primary efficacy endpoint, based on the primary endpoint of the study, when the lower limit of the 95% confidence interval is not higher than the non-inferiority margin (δ= -10) established in the protocol.

1.2 Study diagram



SV=Scrutiny visit
FV= Final visit.
SC= security call.

Figure 1. Study Diagram

1.3 Subject Timeline

The **screening visit (SV)** will correspond to an appointment that will be considered on day 0 for each subject.

The **final visit (FV)** will take place on day 8 of the study, in relation to the start of treatment, with a window period of ±1 DAY.

The **safety call (SC)** will be made 10 days after the start of treatment, with a window period of ±1 DAY.

Procedures	SV D 0	D 1 to 7	FV D 8 (±1)	SC D 10 (±1)
FCI Signature	X			
Medical History*	X			
Concomitant Drug Evaluation	X		X	
Urine pregnancy test	X		X	
Evaluation of ocular signs and symptoms	X		X	
Eye Strain Questionnaire	X		X	
Eligibility Criteria	X			
Evaluation of adverse events	X	X	X	X
Investigational Product (PI) Randomization	X			
Delivery of the PI	X			
Subject material delivery and filling instructions	X			
Intervention administration		X		
Adherence assessment			X	
Return / Evaluation of the subject's diary			X	
Return of PI			X	
*Includes physical examination, somatometry and taking of vital signs.				

Table 2 Subject Timeline

2. Introduction and Background

2.1 Theoretical framework

The benefits and importance of ethnomedicine can be expressed through several quantitative indices such as: informant consensus factor (ICF), *Informant Consensus Factor),* Usage Value *(*UV, for its acronym in English, *Use Value),* Frequency of Mention (FC)*, Frequency of Citation),* Relative Frequency of Mention (RFC) *Relative Frequency of Citation),* and Relative Importance Index (RI), *Relative Importance Index*). The homogeneity between the present and previous studies among indigenous communities was evaluated using the Jaccard index. [1]

In a study published by Faruque (2018), these parameters were evaluated in the most used plant species in a Bangladeshi community, in which a total of 159 species were recorded. Among the most used, in fourth place, was the *Matricaria chamomilla*, with a UV of 0.33. Fortini (2006) can also be cited, who registered this species with the third UV of a total of 106 species inventoried in this study carried out in Italy. [1, 2]

According to the World Health Organization, 80% of the world's population, especially those in developing countries, still resort primarily to traditional medicines. In addition, more than 50% of the origin of pharmaceutical drugs can be traced to ethnomedicine. [1]

Chamomile is well known among the medicinal plant species of the family *Asteraceae,* and it is even known as the "star among medicinal species". [3]

It is one of the most important medicinal plants natives to Eastern and Southern Europe. It also grows in Germany, Hungary, France, Russia, Yugoslavia and Brazil. It was introduced to India and can now be found there as well, as well as in Asia, North America and South America, among others. [3]

It has been used as an herbal remedy for thousands of years, from ancient Egypt through Greece and Rome, to the present day. Chamomile is included in the pharmacopoeia of 26 countries and is an ingredient in several traditional medicine preparations and phytopharmaceuticals. It is used to treat flatulence, colic, hysteria and intermittent fever; it is attributed uses as an anti-inflammatory and antiseptic, as well as antispasmodic. It contains flavonoids, coumarins and polyacetylenes. [3]

Most European ethnopharmacological knowledge has its origins in Greek and Roman cultures, influenced by Dioscorides, Pliny the Elder, Galen, Paracelsus, and Hippocrates. This knowledge, with some additions from the Middle East, Asia, America, etc., makes up the plants currently used with ethnopediatric significance in multiple countries of the world, including Romania. In a study carried out in this country by Petran, (2020) which covers the evaluation of more than a century of use of medicinal plants in that region, relative ethnopediatric therapeutic versatility (ERTV) was used *ethnopediatric relative therapeutic versatility*) expressed as a percentage and calculated based on the number of different uses described for each species of medicinal plant. Out of a total of 153 species, chamomile (*Chamomilla Fever*) reached third place along with 4 other plants, all with an ERTV of 71.42%. [4] Some of its main indications are abdominal pain, colic, cold, dermatological lesions, impetigo, pain in general, among others. Particularly for the treatment of colic in newborns, there is scientific evidence that supports this indication. In clinical studies carried out in adults, chamomile has also shown positive effects in the treatment of wounds, atopic dermatitis, migraine, sleep disturbances, etc. [4]

In Asian countries such as Japan (60 to 70% of allopathic doctors complement their treatments with traditional medicine) and China (40% of all treatments are based on traditional medicine) they rely heavily on this type of therapy.

Similarly, in Latin America, the use of traditional medicine continues to be used regularly by a large percentage of the population; 71% of Chileans and 40% of Colombians have used Traditional Medicine, according to the Regional Office of the World Health Organization of the Americas (WHO/PAHO).

Particularly in the North Peruvian markets, fifty-nine exotic species (15%) have been reported. However, among the species most found in inventories is the *Feverfew recutita* (chamomile) present in about 70% of stores. [5]

The *Matricaria chamomilla* It is indicated for diseases such as love disease, nerves, insomnia, wound inflammation, colic, stomach pain, bronchitis, etc. While the *Feverfew recutita* It is used to treat fright, wound infection, menstrual cramp, among others. [5]

On the other hand, within the information of the Andean Health Agency and its List of Common Medicinal Plants in the Andean Subregion, you can find the description of chamomile and its most common uses according to country: [6]

- Bolivia – Anti-inflammatory, antispasmodic and sedative
- Chile – Digestive disorders such as stomach pain, indigestion, chronic difficult digestion, and dyspepsia, colic, flatulence, diarrhea; urinary tract conditions such as cystitis; menstrual cramps and insomnia.
- Colombia – Adjuvant in the symptomatic treatment of digestive disorders and inflammations and irritations of the skin and mucous membranes.
- Ecuador – Anti-inflammatory, antispasmodic and sedative
- Peru – Antispasmodic, headache, colic, flu, loss of appetite, burns, etc.

The use of chamomile within traditional medicine has a long history. The first written report of it was found in Ancient Egypt, where chamomile flowers were used to relieve skin inflammation, to prevent dermatitis and as part of the ingredients of cosmetic preparations.

The use of traditional medicinal products can be a useful complement to pharmacotherapy and modern treatments. Its safety is based on the experience gained through many years of this traditional medicine.

Chamomile is a plant in the *Asteraceae* family that contains substances such as flavonoids, coumarins, and other compounds. These have shown an antiphlogistic effect, which is why chamomile has been used for the treatment of inflammatory processes of skin and mucosa of different etiology. In addition, chamomile essential oil has demonstrated fungistatic and fungicidal action against several dermatophytes. The antiseptic properties and anti-inflammatory effects of chamomile have been confirmed through scientific research.

According to the European Union, the herbal monograph of *Matricaria recutita* includes the following indications: gastrointestinal disorders such as inflammation and mild spasms, common cold, minor ulcers and inflammations of the mouth and throat, irritations of the skin and mucosa of the genital region, and inflammation of the skin, superficial wounds and small boils.

In an ethnopharmacology study conducted in a region of Serbia, chamomile is one of the ten best-known medicinal plants. It is used to relieve colds, stomach illnesses, throat infection, and to generate

mild sedation. It is also used for the management of eye inflammation, skin inflammation and infections, as well as in the treatment of wounds and burns. Out of a total of 631 people interviewed in the study published by Marković (2020), a total of 383 participants described chamomile as a medicinal species. The most frequent indications for this were: cold, stomach disorders, sore throat, gargle, sedation, cough, skin alterations; as well as eye washing (n = 24) and inflammation management (n = 16).

These results were like those obtained in other ethnobotanical studies in Serbia published as is the case of Stevanović et al (2014) and Jarić et al. (2017). In this study, similar indications were reported for chamomile, emphasizing its use as a treatment for skin and mucous membranes, burns, wounds, gastrointestinal spasms, bronchitis, fever, cold, cough, etc. [7]

Another example is the article on ethnobotany and uses of chamomile published by Bahmani et al (2015) in which the hygienic, pharmaceutical, cosmetic and nutritional properties of this medicinal plant are expressed. Some of the indications described for chamomile in this article are: eye discomfort, muscle aches, oral ulcers, to stimulate libido, fever, insomnia, as a sedative, as a stomach tonic, anorexia, enteritis, anemia, intestinal parasitosis, menstrual cramps, jaundice, skin alterations such as eczema, hives and itching, joint pain, alterations due to menopause, allergies, spasms, flatulence, among others. [8]

Eye strain can be defined in multiple ways and includes a variety of symptoms including: [9, 10, 11]

- Sore, burning, or itchy eyes
- Blurred or double vision
- Headache
- Increased sensitivity to light
- Redness or swelling of the eyes.
- A feeling of heaviness or heaviness in the eyes or eyelids.
- Sting eyes and the need to rub them.
- Gritty or foreign body sensation.
- Dry eyes or, on the contrary, excessive tearing.
- Dark circles and bags under the lower eyelid.
- Eye prickling and/or moderate pain.

- Neck, shoulder or back pain.
- Difficulty concentrating.
- Feeling dizzy and/or neck pain.

This type of condition is commonly associated with the use of screens and electronic equipment, and several instruments have been developed for the diagnosis and classification of the severity of this type of condition. Some of these include questionnaires such as the one created by Hayes et al., the Computer Vision Syndrome Self-Administered Questionnaire or CVS-Q, the CVSS17 scale to measure visual and ocular symptoms associated with the use of video terminals at work, and the Visual Fatigue Scale in which six items are evaluated using a Likert scale. In the latter, the following criteria are evaluated: difficulties in seeing, strange sensations around the eyes, feeling of eye fatigue, feeling of numbness, headache, feeling of dizziness when looking at the screen. [12]

2.2 Background on active ingredients

2.2.1.1 Pharmacology of chamomile

The inflammatory process is linked to the production of inflammatory mediators by macrophages and neutrophils, generating expression induced by enzymatic activity of cyclooxygenase-2 (COX-2). COX-2 promotes the synthesis of various inflammatory mediators, including prostaglandin E2 (PGE2). In eye tissue, the expressions of COX-2 and PGE2 are primarily responsible for inflammation; so that currently anti-inflammatories that block the expression of COX-2 are the most widely used. Chamomile has anti-inflammatory activity, attributed to the presence of flavonoids that, when acting topically, penetrate the epithelia and act as anti-inflammatories.[13] [13][13, 14] [15]

Sirvastava et al. demonstrated that the mechanism of action of chamomile in inhibiting PGE2 production is due to the suppression of cytokine gene expression, COX-2 and direct enzymatic inhibition, causing arrest in the inflammatory process and contributing to an anti-neoplastic and immunomodulatory effect.[15] [14]

Apigenin has a dose- and time-dependent reversible effect on adhesion protein expression and inhibits leukocyte adhesion on the endothelial surface. It inhibits prostaglandin-induced IL-1-α synthesis and the production of tumor necrosis factor-alpha (TNF-α) and interleukin (IL) 6 and 8, inhibits the gene expression of endothelial factor in human cells, blocks the generation of interleukin adhesion molecule (ICAM-1), vascular adhesion molecule (VCAM-1) and E-selectin mRNA, thus

generating inhibition in the transduction signals of cytokine-activating cells and blocking the inflammation mechanism. [14] [16, 17] [17]

Apigenin also decreases inflammation by acting directly by inhibiting myeloperoxidase. It can reduce edema when there is direct contact due to hypersensitivity. [17]

In histological studies, apigenin has been shown to reduce the inflammatory flow associated with the hypersensitivity response.

Further studies are needed to elucidate the molecular mechanism of flavonoids, to generate innovative therapeutic opportunities for the treatment of chronic and acute inflammatory diseases. [17]

Antimicrobial characteristics of chamomile have been reported attributed to its essential oils with bacteriostatic, bactericidal and fungicidal potential. Its effectiveness against gram-positive bacteria (*S. aureus*), gram negative (*E. coli*) and *C. albicans*. [17, 18, 19]

Flavonoids, the main components of chamomile, have also been shown to have a potent antioxidant effect and slow the growth of cancer cells by inhibiting tumor angiogenesis. There is substantial evidence on the suppressive activity that generates free radicals (oxidative stress) at the level of the skin, mucous membranes, proteins and deoxyribonucleic acid. One of the least known aspects of corneal pathophysiology is the toxic action of free radicals which, as intermediate products, are produced by different mechanisms in the course of inflammatory disease and during surgical procedures. The use of [20][21, 22, 23] [24]*M. Recutita* topically to reduce the damage generated in these scenarios.

Studies show that chamomile extract inhibits the growth of cancer cells. Another study shows that its daily consumption could be potentially useful in the prevention of hyperglycemia and complications caused by Diabetes Mellitus. [22] [23]

They have potential properties for the prevention and treatment of eye diseases, among which the most benefited would-be diseases with loss of nerve fibers. These properties are linked to the fact that flavonoids decrease death by oxidative stress and induce the activation and expression of phase II detoxifying proteins. These proteins include enzymes associated with glutathione biosynthesis and proteins sensitive to redox metabolism. It has also been shown that flavonoids can reduce endothelial cell proliferation, reduce inflammation and enhance neuronal regeneration. [20]

Inhibition by apigenin of protein kinase 2 decreases endothelial proliferation, endothelial survival and migration, and tubular formation in choroidal neovascularization. A decrease in stem cell hematopoiesis has also been observed in areas of retinal neovascularization. Apigenin has an inhibitory effect on angiogenesis, with encouraging results in choroidal neovascularization. [25] [26]

2.2.1.2 Pharmacokinetics

There is no information on the absorption, distribution, disposal of the *M. recutita*. However, the absorption of apigenin after oral administration in rats has been corroborated, with 51% being eliminated in the urine, and 12% in the feces. The blood elimination half-life of some components of [27] *M. recutita* it is 91.8 hours, but the biological half-life is 12 hours. [28]

In pharmacokinetic interactions, feverfew extracts diluted to 1-2% in ethanol inhibit 50% of human cytochrome 450 3A4, in vitro studies. Feverfew essential oil demonstrated its ability to inhibit cytochrome P450 (CYP1A2, CYP2C9, CYP2D6, CYP3A4). [29]

2.2.1.3 Toxicity

The acute toxicity of the extract of *M. recutita* It was evaluated using 2 groups of 12 Swiss female mice. Each group received a single oral dose of 720 or 440 mg/kg and was observed for 24 hours. None of the animals died, and there was no evidence of acute toxicity. [27]

In another acute toxicity study, *M. recutita* 10 rats were given a dose of 5 g/kg each. The rats were observed for 14 days. None of the animals died, and a lethal dose 50 (LD50) greater than 5 g/kg was reported. In another review, an LD50 was obtained with *M. recutita* between 8,560 mg/kg and 10,000 mg/kg in rats. [27]

Acute toxicity with oil *M. recutita* In leather, it was evaluated when applying 5g/kg in 6 rabbits. None of the animals died during the 14 days they were observed. But it was found mild redness of the skin in two rabbits, moderate in four; mild edema in two rabbits, and moderate in four. [27]

Repeated dose toxicity was studied for 14 days in rats, administering doses of 1, 2, 4, 8 g/kg of the extract of *M. recutita* dissolved in water. There was no evidence of toxicity, and all the animals remained physically active. [27]

The hepatic effects of the tea of *M. recutita* with repeated consumption. 5 female rats were used as the study animal model with free access only to water with tea at 2%, and another 5 female control

rats with free access to water without tea. After 4 weeks, the animals were euthanized. There were no differences in both groups regarding the weight of the internal organs, and the ratio between the weight of the liver and the body of the animal. [27]

2.3 Background on research

2.3.1 From the research question

Today, the use of traditional medicinal products continues to be a complement to pharmacotherapy and modern treatments. The use of chamomile in traditional medicine has a history of thousands of years around the world.

Manzanilla Sophia is indicated to relieve eye fatigue, however, many of the symptoms related or® associated with this fatigue can also improve with the use of eye lubricants. For this reason, this study is being developed to compare the effect of Manzanilla Sophia® versus Meticel Ofteno® (Hypromellose 0.5%) for the management of some symptoms of eye strain.

2.3.2 From the investigational product development phase

Manzanilla Sophia® is a product that is available for purchase without a prescription in Latin America (Bolivia. Colombia, Costa Rica, Dominican Republic, Ecuador, El Salvador, Guatemala, Haiti, Honduras, Mexico, Nicaragua, Panama, Peru) and the United States of America. Since it has been previously marketed for 23 years in Mexico, this study is considered a phase IV study.

2.3.3 Know potential risks

Very rarely, the fresh plant can cause contact dermatitis, conjunctival and eyelid hypersensitivity. On rare occasions when having contact with the oral mucosa (tea), angioedema, dyspnea, anaphylactic shock has been reported.

While there are reports of side effects, it is worth mentioning that the concentration used in Manzanilla Sophia® is 0.25 mg of chamomile extract per millimeter of vehicle, which means that it is minimum concentration and only a small fraction is absorbed by the mucosa. As with the rest of the topical ophthalmic application formulations, the possibility of ocular hyperemia, pruritus in the eye, eye irritation, dry eye, contact dermatitis, allergic conjunctivitis is anticipated. The procedures involved in the execution of this study are also safe and non-invasive, so no significant risk to the health and integrity of the participants is expected.

2.3.4 Known potential benefits

Chamomile has been used for thousands of years around the world and for multiple indications. Its anti-inflammatory properties have been shown to be useful in the management of alterations of various organs and systems, including eye conditions. It is expected that the discomfort related to eye strain will decrease with the use of this product, improving the quality of life of the subjects included in this protocol.[7, 8]

2.4 Problem statement

Eye fatigue has previously been linked to prolonged use of electronic equipment. Some studies have even estimated that 90% of workers who use the computer more than 3 hours a day have some related eye alteration. [30]

Symptoms related to eye strain such as sore, burning or itchy eyes, blurred vision, headache, sensitivity to light, swelling of the eyes, feeling of heaviness, etc., are frequent and potentially decrease the quality of life of those who suffer from them. To relieve these discomforts, the use of alternative medicine is common, as in the case of chamomile.

2.5 Justification

Eye fatigue is a non-specific condition that includes both systemic and ocular surface-related symptoms that decrease the quality of life of those who suffer from it. Chamomile has been used as an alternative medicine by multiple societies around the world, one of its indications being the management of eye discomfort coined to different causes. [31] [32] Manzanilla Sophia® is indicated to relieve eye fatigue and has been marketed for more than 20 years in Mexico, without known safety alerts. [1] [2]Padilla-Morones et al. previously reported the efficacy and tolerability of a chamomile ophthalmic solution to induce a sensation of eye rest, in healthy subjects, when compared to placebo. However, the results on the efficacy of chamomile in the population of interest are inconclusive. In addition, to our knowledge, there are no studies comparing the effectiveness of chamomile with that of eye lubricants, which are also an alternative for the management of some symptoms of eye fatigue.[33]

Objectives and hypotheses

2.6 Primary Objective

To demonstrate the non-inferiority of Manzanilla Sophia compared to® Meticel Ofteno® 0.5% in providing a feeling of rest to the eyes, by scoring an analogous visual test of eye strain.

2.7 Signs and symptoms evaluated:

- Compare Manzanilla Sophia® vs. Meticel Ofteno® 0.5% in the incidence of red eye.
- Compare Manzanilla Sophia® vs. Meticel Ofteno® 0.5% in the incidence of dry eye sensation.
- Compare Manzanilla Sophia® vs. Meticel Ofteno® 0.5% in the incidence of eye irritation.
- Compare Manzanilla Sophia® vs. Meticel Ofteno® 0.5% in the incidence of eye discharge (sleep) sensation.
- Compare Manzanilla Sophia® vs. Meticel Ofteno® 0.5% in the incidence of comfort with investigational product (IP) application.

2.8 Security Assessment

- Incidence of intervention-related adverse events (AEs) (ET: screening, end, and safety call visits).

2.9 Hypothesis

H_0 = The Manzanilla Sophia® ophthalmic solution is inferior in its efficacy compared to its comparator, after 7 days of treatment, presenting a difference greater than or equal to 6 points (or the equivalent of 20%) in the final score of an analogous visual test for eye fatigue.

$$H_0: p_A - p_B \leq \delta$$

H_1 = The Manzanilla Sophia® ophthalmic solution is not inferior in its efficacy compared to its comparator, after 7 days of treatment, presenting a difference of more than 6 points (or the equivalent of 20%) in the final score of an analogous visual test for eye fatigue.

$$H_1: p_A - p_B > \delta$$

Where δ is the margin of non-inferiority. When $\delta < 0$, the rejection of the null hypothesis indicates the non-inferiority of the product under investigation (Manzanilla Sophia®) compared to the comparator (Meticel Ofteno®).

Where:

pA = Proportion of subjects assigned to Manzanilla Sophia® who has a difference of more than 6 points in the score of an analogous visual test of eye strain.

PB = Proportion of subjects assigned to Meticel Ofteno® who have a difference greater than 6 points in the score of a visual analog test for eye strain

δ = -10%

The decision rule for determining non-inferiority should be:

When the lower limit of the 95% confidence interval (95%CI) is not higher than the non-inferiority margin (δ= -10) established in the protocol.

$$IC = p_1 - p_2 \pm Z_{1-\alpha/2}\sqrt{(\frac{p_1(1-p_1)}{n_1} + \frac{p_2(1-p_2)}{n_2})}$$

Where:

p_1 and p_2= are the observed proportions

n_1 y n_2 = sample size of each group

3. Study design

3.1 Design Overview

Phase IV non-inferiority, open label, controlled, comparative, multicenter clinical study.

3.2 Rationale for study design

It is a previously marketed research product whose efficacy will be evaluated through the analysis of specific variables related to a defined clinical picture. In addition, it will be compared with another widely used reference product with proven efficacy for the management of some of the symptoms described in the profile of subjects to be analyzed in this study.

3.3 Expected duration

The total duration of the study, from the first visit of the first subject to the preparation of the final report, is estimated to be 10 months.

The approximate duration of each subject in the study is up to 11 days.

4. Study Population

4.1 Eligibility Criteria

4.1.1 Inclusion criteria

- Can voluntarily give their signed informed consent.
- Be able and willing to comply with scheduled visits, treatment plan, and other study procedures.
- Be of legal age.
- Women of childbearing potential who have not undergone bilateral tubal occlusion or hysterectomy should ensure continued use (initiated ≥ 30 days prior to signing the Informed Consent Form [ICF]) of use of a hormonal contraceptive method or intrauterine device (IUD) during the study period.
- Present a score on the visual analog test of eye strain ≥ 3 in at least 4 of the questions included.

4.1.2 Exclusion Criteria

- Women who are pregnant, breastfeeding, or planning to become pregnant within the study period.
- Have participated in another clinical research study ≤ 30 days prior to the screening visit.
- Have previously participated in this study.
- Have a functional single eye.
- Have a history of drug addiction or drug dependence current or within the last two years prior to signing the FCI.
- Be a soft or hard contact lens wearer. You will be able to enter in case of suspending its use during the study, you must comply within 15 days without using the contact lens prior to your inclusion.
- Have known hypersensitivity to the components of the investigational products.

4.2 Elimination criteria

– Withdrawal of the informed consent form.

- Presentation of a serious complication related or not to the investigational product, which at the discretion of the PI and/or the sponsor could affect the subject's ability to continue with the study procedures safely.
- No tolerability or hypersensitivity to any of the investigational products.

4.3 Substitution of subjects

The sponsor, with prior authorization from the Research Ethics Committees, may decide to replace the subjects who withdraw their FCI or those who present loss of follow-up, or in case it is necessary to balance the groups of studies so that they are evaluable.

The substitution of subjects from 10% of losses was estimated based on the discontinuation rates observed in different clinical studies in ophthalmology, where losses ranging from 10% to 30% were presented. In addition, it has been described that a loss of 5% or less is usually irrelevant, while a loss of 20% or greater can compromise the validity of the study, by generating possible biases. Therefore, if the proportion of subjects who discontinue the study is greater than 10%, they will be replaced to ensure the validity of the efficacy analysis, forming part of the safety analysis. [34, 35, 36, 37][38, 39]

The primary efficacy analysis will be performed in both the per-protocol (PP) and intention-to-treat (ITT) populations. The first is defined as all those subjects who conclude their participation in the protocol without deviations greater than it. While the ITT population refers to all those subjects who completed their participation in the study (follow-up), but who presented deviations from the protocol, this based on the recommendations of the ICH E9 guidelines for non-inferiority studies. [40]

4.4 Counting failures

A screening failure is defined as those participants who agree to participate in the study, giving their consent, but who are not assigned to a treatment group, i.e., do not enter the study. It is necessary to report at least the following information on counting failures:

- Demographics.
- Details of the failure to count (specify whether it is due to eligibility criteria, which one, or some other reason for the failure).
- Presence of serious adverse events during the count.

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

Subjects who do not meet the eligibility criteria to participate in the study due to a specific modifiable factor may re-participate in the scrutiny. The subjects in this case must use the same initial count number.

4.5 Procedure in case of loss of follow-up

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

If the participating subject does not attend their appointment, the research center must make a call to find out the reason and will try to make a new appointment within the established window period or an unscheduled appointment.

A loss of follow-up <10% is considered not to be a problem for the validity of the results obtained based on the stipulations of the sample size. View section **9.2 Sample size estimation.**

According to the estimate of the sample size to meet the objective of the study, 84 evaluable subjects are required per arm. If this number is not met due to a loss of subjects greater than 10% contemplated in this protocol (loss of follow-up or withdrawal from the FCI), the sponsor may replace these subjects.

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

4.6 Identification of the subject

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

The initials of the study subject 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 compound surname, the first letter will always be used.

Example:

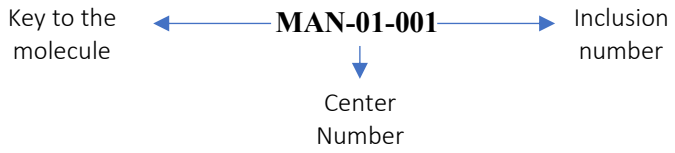
A. Arieh Daniel Mercado Carrizalez B. Juan De la Torre Orozco

a. Initials: AMC b. Initials: JDO

At the counting visit, the participant number will be assigned consecutively, using 3 consecutive digits. Once the subject has been selected, they will be assigned a number with which they will be identified throughout the study. This code will be made up of eight numbers in the following order from left to right:

- three digits of the molecule under study according to the name by the sponsor.
- Two digits correspond to the research center number.
- three digits of the number following their inclusion assigned in the research center.

Example:



The subjects' information will be handled confidentially by the sponsor using only the codes assigned to them. The research site will be responsible for safeguarding the information corresponding to the identity of the subjects.

5. Investigational Product

5.1 Managed Products

5.1.1 Investigational Product

- Generic name: *Matricaria recutita*
- Distinctive name: Manzanilla Sophia
- Active ingredients: *Matricaria recutita* 0.025%
- Pharmaceutical form: Ophthalmic solution.
- Presentation: multi-dose dropper bottle, 15 mL.
- Prepared by: Laboratorios Sophia, S.A. de C.V. Zapopan, Jalisco, Mexico.
- Solution description: Transparent solution, free of visible particles.
- Packaging description: White low-density polyethylene bottle filled to 15 mL.

5.1.2 Reference product

- Generic name: Hypromellose
- Distinctive name: Meticel Ofteno®
- Active ingredients: Hypromellose 0.5%.
- Pharmaceutical form: Ophthalmic solution.
- Presentation: multi-dose dropper bottle, 10 mL.
- Prepared by: Laboratorios Sophia, S.A. de C.V. Zapopan, Jalisco, Mexico.
- Solution description: Transparent solution, free of visible particles.
- Packaging description: White low-density polyethylene bottle filled to 10 mL.

5.1.2.1 Justification of the reference product

Meticel Ofteno® is a widely known eye lubricant that contains Hypromellose. This compound has been used as an artificial tear for more than five decades [41]

Its profile is safe and has been proven effective in managing some of the symptoms of ocular surface irritation, [42, 43, 44] such as those that occur in the picture of eye fatigue. This is why it was chosen as a reference product to evaluate and compare the effects of Chamomile Sophia® on eye strain.

5.1.3 Dosage of investigational product

One QID drop, with a minimum interval of 3 hours between applications, for 7 days.

5.1.3.1 Dose Justification

The usual dosage of the investigational product, Manzanilla Sophia®, is every 6 hours, i.e. four times a day. Similarly, the use of the reference product Meticel Ofteno® usually corresponds to the same dose, so it is decided to use a QID application frequency to emulate the usual use of both products, which have already been previously mentioned have been marketed for several decades with this indication.

The requirement to wait at least three hours between each application is added to distribute the dosage throughout the day.

5.2 Storage and handling of research products at the study site

The delivery will be made by means of a courier service contracted by the sponsor, expressly selected for this purpose, to the address of the research center according to the study plan.

The reception will be carried out by the assigned personnel of the research team. You will need to check that the primary packaging (box) is in good condition. If you show alterations or defects in its integrity that in your judgment could have damaged the content, you must report it to the sponsor. If the package does not show significant defects, it will proceed to open it.

Inside you must locate the acknowledgment document and the temperature *logger*. You must check that the temperature recorded complies with what is specified for transport and safekeeping. It will verify the content (PI) with what is reported in the document. In case the document corresponds to the content, you will sign the receipt and send it to the sponsor. Otherwise, it will notify the sponsor.

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

The storage temperature should be less than 30°C. This limit is stipulated as described in the information of the investigational product and the reference product.

The research center is obliged to record, in the designated format, the temperature recorded in the *data logger*, every day while the protocol is in force and has PIs. This data will be reviewed by the clinical monitor according to the record in the data logger.

In the event of loss of material, it must be documented in the logbook of inputs and outputs along with a clear description of the mechanism by which the loss occurred.

Upon completion of the protocol, all study material will be retrieved by the sponsor as part of the closing visit. The final delivery of material will be made by the principal investigator (PI) or the person designated by him to deliver material at the end of the study.

The sponsor reserves the right to initiate civil and criminal action against the PI in the event of a lack of undocumented material at the conclusion of the study.

5.3 Concomitant treatments and medications not approved during the study

The use of concomitant medications by ophthalmic administration will not be allowed during the intervention period. The use of systemic medicinal products will be allowed, including homeopathic and/or herbal treatments that the subject requires for other conditions that may occur, as well as the use of hormonal contraceptives in women of childbearing age.

5.4 Procedure for monitoring and measuring adherence

For more than four decades, numerous investigations have been conducted in the appropriate way to measure and quantify medication adherence, however, none has reached a consensus to stand as the gold standard, both in cross-sectional and longitudinal studies. [38] [39] [40] [41] [42] [43] [44] [45]

There are different procedures for measuring the adherence of interventions. The most common procedure includes self-reports, which include interviews with the subject, questionnaires and self-monitoring diaries. Its strengths are speed, flexibility, low cost and ease of implementation; They have a high degree of specificity for non-adherence, however, the sensitivity and reliability for adherence is low. [45] [46]

Product counting is another way to measure stickiness. Classically referred to as "pill counting," in ophthalmology it translates to the weight of the bottle. This is a simple, inexpensive, and non-invasive method. The main disadvantages of this method are: 1. The application of the product cannot be

confirmed (it may have been intentionally pulled or instilled out of the eye) and 2. It depends on the subject bringing back the product. [45] [46]

A multi-procedure approach to measuring adherence is recommended. Because there is no measurement of ideal adherence, it is appropriate to use more than one method when trying to achieve results that resemble reality. Selecting two or more methods allows their strengths and weaknesses to be compensated, to more accurately capture adherence levels. [44] There is no standardized parameter to define adequate adherence, it must be defined and delineated by the objectives of the research. [45]

Considering all the antecedents presented above, for this study adherence will be measured by the weight of the bottle and the record of applications of the subject's diary. A subject will be considered to comply with the required adherence if there is a positive record of applications in the subject's diary of at least 80%; as well as at least 65% of the decrease in the expected weight of the bottle used.

The evaluation of adhesion by means of the weight of the bottle will be carried out as follows, considering the following information: the weight of the drop, the initial weight of the container, the final weight of the container and the calculation of the total applications. The following simplified formula shall be used:

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

Where:

Ad = adherence

P_i = weight of the container delivered to the subject at the beginning

P_f = weight of container returned by subject

P_T = weight of the dosage indicated for the intervention

$$P_T = (P_g)G$$

Where:

P_g= weight of one drop of the intervention, determined by the research and development department

G = number of applications indicated for the intervention

The adherence calculation according to the subject's diary will be evaluated as follows:

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

Where:

Ad = Adherence

Ar = Registered Applications

Ai = Applications indicated for the intervention

The research center will designate a person in charge of monitoring adherence through the diary, during the visits. Measuring grip by weight will be the responsibility of the sponsor.

In cases where the container is not returned, or it has not preserved its physical integrity, adherence will only be measured through the subject's diary.

5.5 Strategies to improve adherence

- The PI will sensitize the subject to importance, to achieve the objectives of the study, of the correct application of the RP.
- Direct questioning by the PI about the application of RP.
- Delivery of a printed calendar (my calendar for study) specifying the date of the visit and its activities.
- Training in the completion and revision of the subject's diary.

6. Study methods and procedures

6.1 From the research center

This study will be carried out at the research center previously evaluated by the sponsor. This center will be an institution or establishment where health research is carried out that complies 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 their prerogative to design the organization and select the personnel who will perform the functions. However, it is the sponsor's need for the Principal Investigator (PI) to be a surgeon and midwife.

Any person who is designated, under their responsibility, a part of the study monitoring (co-investigator, sub-investigator, nurse, etc.) or a specific function of participation in the study (pharmacist, administrative assistant, study coordinator, etc.) must appear in the "Delegation of Responsibilities".

The competence and training of any person who has direct participation in the activities of the study must be verified prior to the performance of any activity related to the protocol. The above must be recorded and the documents that constitute evidence of this competence and/or training must be kept in the master file of the study. The competence and training of the personnel who have functions in the study, both at the central level and in the research center, is the responsibility of the researcher.

The sponsor must ensure that all research site staff participating in the study are adequately trained on the study (research protocol, prescribing information and/or Investigator's Manual amendments, etc.) and on the ICH (International Council for Harmonization) Good Clinical Practice (GCP). *International Council for Harmonization*), before the start of their participation in it. Training must be recorded in writing, and those records must be filed in the master record of the study.

6.2 Clinical Study Registry

This clinical study will be registered by the sponsor in public clinical trial registries before its start (inclusion of the first subject): National Registry of Clinical Trials (NRCT) of the Federal Commission

for the Protection against Sanitary Risks (COFEPRIS) and in a platform of primary registries of the World Health Organization (WHO). WHO primary registries meet specific criteria for 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).

6.3 Randomization

Randomization of subjects will be carried out using a computer-based assignment system. After signing up for the FCI, the subject will receive a subject number with which all their information will be encoded pseudonymously during collection and completely anonymized during analysis.

The containers will be identified by means of labels, which, in accordance with current and applicable regulations, must contain at least:

1. Sponsor's name, address, and phone number.
2. Dosage form and route of administration.
3. Batch number.
4. Legend "For clinical studies only"
5. Expiration date.

6.4 Outcome variables

6.4.1 Primary outcome variables

- Score of the visual analogue eye strain test (TE: visits, scrutiny and final).

6.4.2 Signs and symptoms assessed

- Incidence of red eye (TE: counting and final visits).
- Incidence of dry eye sensation (ET: screening and final visits).
- Incidence of sensation of eye irritation (ET: screening and final visits).
- Incidence of the sensation of sleep (TE: counting and final visits).
- Incidence of comfort with the application of the PI (TE: final visit).

6.4.3 Security Assessment

- Incidence of intervention-related adverse events (AEs) (ET: screening, end, and safety call visits).

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

Variable	Conceptual Definition	Operational Definition	Measurement Type	Normal value	Statistical test
Eye Strain Analog Visual Test Score **Proportion of subjects with a change ≥ 6 points (or 20%) from baseline**	The analogue visual test is a questionnaire designed to establish the degree of eye fatigue according to its symptoms	The evaluator will apply the questionnaire to the subject and allow the subject to answer it calmly without any pressure and/or coercion.See annex15.1 Visual analogue scale of eye strain	Discrete quantitative Qualitative dichotomous	6 – 30 ≥ 50%	• Student's t-test • McNemar Test
Incidence of red eye	It is defined as the simplest reaction of the conjunctiva to a stimulus, a red appearance secondary to the vasodilation of the conjunctiva vessels of variable intensity is appreciated.	Direct observation.	Qualitative dichotomous	Present/absent	• McNemar test or Pearson X2 when applicable.
Incidence of dry eye sensation	It is defined as the subjective sensation of dry eye.	The subject will be questioned directly.	Qualitative dichotomous	Present/absent	• McNemar test or Pearson X2 when applicable.
Incidence of sensation of eye irritation	It is defined as the subjective sensation of eye irritation.	The subject will be questioned directly.	Qualitative dichotomous	Present/absent	• McNemar test or Pearson X2 when applicable.
Incidence of the sensation of eye discharge (sleep)	It is defined as the subjective sensation of the presence of tears or eye discharge.	The subject will be questioned directly.	Qualitative dichotomous	Present/absent	• McNemar test or Pearson X2 when applicable.
Incidence of comfort with the application of PI	It is defined as the subjective feeling of comfort after the application of the IP.	The subject will be questioned directly.	Qualitative dichotomous	Present/absent	• McNemar test or Pearson X2 when applicable.
Incidence of intervention-related adverse events (AEs)	Any adverse medical event that occurs in the clinical research subject to whom the PI was administered that has a certain causal relationship to the PI and is not expected	AEs in which the PI suspects a causal relationship with the PI	Discrete quantitative Categorical qualitative	• Incidence • Seriousness (severity) • Causation	• Student's t-test • Binomial X2

Table 3 Operational definition of variables

6.4.4.1 Visual analogue test for eye strain

Based on other tools previously used for the assessment of eye fatigue, the visual analog test for eye fatigue used in this protocol is adapted and created.

This test includes the following questions:

- Have you had a headache?
- Have you had eye strain?
- Do you have gritty sensation in your eyes?
- Have your eyes been itchy?
- How would you describe your clarity of seeing?
- Do you feel any discomfort when you fix your eyes?

These will be answered according to a Likert scale of 1-5. See Annex 15.1 Visual analogue scale of eye strain . This is a self-administered questionnaire which will be answered by the subject, who will receive the instructions for its completion by the PI.

In the electronic case report form (eCRF), the answer to each of the questions (subtotal) will be recorded, on a scale between 1 and 5; as well as the sum of the answer to each of the questions (total), on a scale of between 6 and 30.

6.4.4.2 Red eye

Red eye is a sign that usually represents a reaction of the conjunctiva to a stimulus, this red appearance is seen secondary to vasodilation of variable intensity of the conjunctival vessels. Their presence/absence will be evaluated according to the direct observation of the investigator.

The presence of severe red eye, according to the criteria of the PI, must be reported.

Management as AE: severe cases of red eye (not present in baseline evaluations, and that interfere with usual activities, require pharmacological treatment and discontinuation of medication), and/or that present worsening compared to baseline characteristics, according to the PI's criteria, will be considered as AEs. Cases of red eye (both those observed during visits and those reported in the subject's diary) that do not meet these specifications should not be entered as AEs.

6.4.4.3 Feeling of dry eyes, feeling of eye irritation, feeling of tears and feeling of comfort with the application of PI

These symptoms will be questioned on the subject by the principal investigator, and the response will be reflected as presence/absence for each one.

Management as AE: cases of dry eyes, sensation of eye irritation and sensation of sleep considered severe (not present in baseline evaluations, and that interfere with usual activities, that require pharmacological treatment and discontinuation of the medication), and/or that present worsening compared to baseline characteristics, will be considered as AEs. according to the criteria of the PI. Cases of dry eyes, sensation of eye irritation, and sensation of tearfulness (both those reported during visits and those reported in the subject's diary) that do not meet these specifications should not be entered as AEs.

6.4.4.4 Adverse events

As described in the Evaluation and management of adverse events An adverse event is defined as any unfavorable medical occurrence in a subject to whom an investigational product is administered, regardless of causal attribution.

Adverse event management shall be carried out as described in section 8. Evaluation and management of adverse events and incidents.

The Principal Investigator will record in the corresponding section of the FRCE the adverse events that the study subjects may present, in addition to referring them in the clinical record.

Adverse events that have been reported with the use of chamomile include allergic conjunctivitis, angioedema, contact dermatitis, ocular hyperemia, pruritus in the eye, eye irritation, dry eye. . [45] [46] [47] [48] [46][47][48][49] [50][51][52][53][54] [55]

6.5 Study visits and activities program

6.5.1 Description of activities per visit

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

6.5.1.1 Scrutiny Visit

- FCI signature refers to the signing of the written informed consent document. Without obtaining informed consent, it is not possible to perform any of the study procedures.
- Clinical history: refers to the technical, clinical, and legal document in which the subject's health conditions, medical acts, and other procedures performed on the subject are chronologically recorded. It includes physical examination, somatometry and taking vital signs.
- Evaluation of concomitant medications refers to the interrogation by the PI of the subject, inquiring about the use of medications.
- Urine pregnancy test: This refers to performing a rapid pregnancy test on all women of childbearing potential who wish to enter the study. By fertile age we mean women who have had their menarche and have not presented their menopause. Menopause is defined as 12 months from the last menstruation in women over 40 years of age, or who have had a hysterectomy or bilateral oophorectomy. Women of childbearing potential with contraception, including bilateral tubal obstruction (tubal ligation), should be tested for pregnancy. This test will be performed by the IP or designated team person in accordance with the instructions on the device provided by the sponsor.
- Evaluation of ocular signs and symptoms: It refers to the evaluation of red eye by direct observation by the PI, as well as questioning about the rest of the symptoms to be evaluated and their registration as presence/absence. View sections 6.4.4.2 Red eye and 6.4.4.3 Feeling of dry eyes, feeling of eye irritation, feeling of tears and feeling of comfort with the application of PI.
- Eye Strain Questionnaire: This refers to the answer to the visual analogue questionnaire for eye strain. View 15.1 Visual analogue scale of eye strain
- Eligibility criteria: refers to the review by the PI, where it is found that the subject can be included in the study by meeting the inclusion criteria and not meeting the exclusion criteria.
- Evaluation of adverse events: See management point *as AEs*: cases of red eye, dry eyes, sensation of eye irritation and sensation of tears considered severe (not present in baseline evaluations, and that interfere with usual activities, that require pharmacological treatment and discontinuation of the medication) will be considered as AEs), and/or that present worsening compared to baseline characteristics, according to the PI criteria. Cases (both

those reported during visits and those reported in the subject's k) that do not meet these specifications should not be entered as AEs.

- IP randomization: refers to determining the intervention that the subject will follow during the study. This assignment will be made at the counting visit (day 0). View section **6.3 Randomization**
- Delivery of the PI and initiation of intervention refers to the delivery of the PI to the subject of the study, by the research center.
- Delivery of Subject Material and Filling Instructions: Refers to the PI's delivery to the subject of the subject's diary, ID card, and calendar (my calendar for the study). The personnel assigned by the research center will carry out prior training on the subject, on the completion of the diary.

6.5.1.2 Final Visit

- Evaluation of concomitant medications: View section **6.5.1.1 Scrutiny Visit**.
- Urine pregnancy test: View section t**6.5.1.1 Scrutiny Visit.**
- Evaluation of signs and symptoms: View section **6.5.1.1 Scrutiny Visit.**
- Eye Strain Questionnaire: View section **6.5.1.1 Scrutiny Visit.**
- Evaluation of adverse events: See management point *as AEs*: cases of red eye, dry eyes, sensation of eye irritation and sensation of tears considered severe (not present in baseline evaluations, and that interfere with usual activities, that require pharmacological treatment and discontinuation of the medication) will be considered as AEs), and/or that present worsening compared to baseline characteristics, according to the PI criteria. Cases (both those reported during visits and those reported in the subject's diary) that do not meet these specifications should not be entered into as AEs.
- Subject's Journal Evaluation: View section **6.5.1.1 Scrutiny Visit**.
- Assessment of adherence: View Point **5.4 Procedure for monitoring and measuring adherence.**
- Return of PI and Subject's Diary: refers to the subject's return of the PI and the subject's diary to the research center.

6.5.1.3 Safety call

- Evaluation of adverse events: See management point *as AEs*: cases of red eye, dry eyes, sensation of eye irritation and sensation of tears considered severe (not present in baseline evaluations, and that interfere with usual activities, that require pharmacological treatment and discontinuation of the medication) will be considered as AEs), and/or that present worsening compared to baseline characteristics, according to the PI criteria. Cases (both those reported during visits and those reported in the subject's diary) that do not meet these specifications should not be entered into as AEs.

6.5.2 Unscheduled follow-up visits

At the request of the subject or any other individual connected to the study, unscheduled follow-up visits may be conducted.

6.6 Data collection

6.6.1 Source documents

Source documents are all written or printed records derived from automated processes (e.g., printouts of laboratory results issued by automated analysis equipment) where the information is recorded for the first time and which is part of the permanent records of the subject's history. Examples of source documents are medical history, clinical evolution notes, laboratory reports, cabinet study reports, nursing notes, follow-up notes, surgery records, etc.

The PI is obliged to accept the monitoring of the information related to the study, audits, review by ethics and research committees, and inspections by the health authority, this obligation implies direct access to the source documents.

6.6.2 Electronic forms of data collection

All data related to the protocol will be captured through an eCRF by the research team staff. The data related to the protocol should NOT be captured directly in the eCRF but should be transcribed from the corresponding source document. This procedure allows monitoring to verify the information captured in the FRCEs. It is the responsibility of the researcher to ensure that the information is transcribed to the FRCEs in a correct, complete, and timely manner. It is understood that all eCRF captured and submitted for data analysis are approved by the Investigator.

6.6.3 Archive

The data collected in this database is anonymous (it only stores the subject number together with other information of interest). The program used for data capture and storage covers the traceability requirements necessary for the execution of clinical studies. The data collected will be stored by the sponsor or the clinical research organization designated for this purpose and its storage will have a duration of 10 years. The records of assignments of subject number will remain in the participating institutions in charge of the PI or his work team and must be kept for at least 5 years.

7. Evaluation and management of adverse events

7.1 Regulation and regulations 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 and the international guidelines ICH E6. [56][57][58][59]

7.2 Definition of Adverse Event

According to the International Council on Harmonization (ICH), an adverse event (AE) is any unfavorable medical appearance in a clinical investigational subject who is administered a pharmaceutical product, regardless of causal attribution. [57] [58] [59]

Therefore, an AE can be any of the following: any unfavorable and unintentional disease, symptom, or sign (including an abnormal laboratory finding) that is temporally related to the use of a medical product, whether or not it is considered related to such a product; any new illness 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 symptoms or that results in a change in study treatment or concomitant treatment or discontinuation of study medication. [57] [58] [59]

As defined in the previous paragraph, an adverse event is defined as any event that occurs during treatment with a drug or device. However, the definition can also be applied as any unwanted event that occurs during a clinical trial, including behavioral disorders. [59]

7.3 Use of adverse events as study safety variable

Measuring the safety of the use of Chamomile Sophia® is paramount to the study, therefore, it is considered important to report any unwanted manifestation or disease that occurs during the study, regardless of whether the manifestation is related to the treatment under investigation. [59]

7.4 Definitions relevant to the classification of adverse events

Severity (serious/non-serious), also called seriousness (serious/non-serious). Serious or serious is defined as any event that: results in death, threatens life, requires hospitalization or prolongs hospitalization, is a cause of permanent or significant disability or disability, is the cause of alterations or malformations in the newborn, other medically important conditions (Medically important event or reaction: That clinical manifestation or adverse event that in the opinion of the doctor may not be immediately life-threatening, result in death or hospitalization, but which could endanger the subject or require medical intervention to prevent the occurrence of any of the criteria listed in the definition of serious adverse reaction). [60]

Severity (mild, moderate, or severe). Mild is those that present with minimal symptoms, do not require treatment or suspension of the medication; moderate, when they interfere with usual activities, without threatening the subject's life, require treatment and may or may not require discontinuation of the medication; severe, those that interfere with usual activities and require pharmacological treatment and discontinuation of the medication. [56][57][58]

Causality. It is the relationship that is assigned between the pharmaceutical product and the adverse event: certainly caused by the pharmaceutical product, there is clear evidence of causality, i.e. the adverse event reappears with the administration of the pharmaceutical product; probably caused by the pharmaceutical product, 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 pharmaceutical product is discontinued; possibly caused by the pharmaceutical, there is additional information to suggest that the cause may be due to another pharmaceuticals or disease; unlikely to be caused by the pharmaceutical product, there is a clear explanation of the origin due to the underlying disease or the use of another pharmaceutical product; conditional, there is a lack of data to issue a clear causality; non-classifiable, those for which once all possible information has been obtained about the adverse event, it remains unclassifiable. [56] [57] [58] [59]

7.5 Investigator Responsibilities

It is the responsibility of the Investigator to verify AEs through questioning, pertinent physical examination, assessment of evolution, as well as appropriate medical and pharmacological management; as well as to follow up until the resolution or outcome and definitive discharge of the AE, following the definitions determined in national and international regulations. [56] [57] [58]

In the event of an AE or any event that puts the health and well-being of the subjects at risk, pertinent medical care will be provided, either at the research center or will be referred to the Hospital Center with the highest resolution power with which the research center has a medical care agreement. The PI will notify the sponsor's clinical monitor, according to the times established in national and international regulations. In the case of serious adverse events, it will notify the sponsor and record the corresponding information in the FRCE and in turn, inform the IRB and IC.

The attention of the AEs will be carried out according to the event care diagram (see Figure 2. Adverse Event Care).

The sponsor's final report will include the report of adverse events in compliance with current national and international regulations. [56] [57]

If the research subject debuts during their participation in the study with any chronic adverse event, such as diabetes or systemic arterial hypertension, they will be referred to the competent health professional for chronic treatment. The follow-up and termination of their participation will be in accordance with the stipulations of NOM-012-SSA3-2012. [56]

7.5.1 Record of adverse events in the electronic case report form

The adverse event registry considers:

- Subject identification information such as subject number, age, gender, and if applicable specify the eye.
- Information about the causality of the adverse event, its relationship to investigational products, or to another drug related to the study, as appropriate.
- Important date information:
    - Date on which the adverse event occurs.
    - Date on which the Principal Investigator becomes aware of it.
    - Date of resolution or outcome, as applicable.
- Information on diagnosis and clinical management.
- Record the outcome or resolution of the event:
    - Retrieved/Resolved
    - Recovered/Resolved with sequelae
    - Recovering/Resolving

- Not Recovered/Unresolved
- Fatal
- Unknown

- Information about the investigational product or product associated with the Adverse Event, Incident, Adverse Incident, AMR or SRAM must be recorded. The information essential for registration is the generic name, distinctive name or code of the investigational product or of the product associated with the undesirable clinical manifestation; it will also be necessary to enter the data concerning the batch number, manufacturing laboratory, expiration date, dose, route of administration, start and end dates of administration and/or consumption, reason for prescription; according to whether it is an investigational product or drug (protocol in which the subject currently participates) or whether it is a drug that the research subject consumes for the treatment of underlying concomitant diseases or uses for the management of some transitory sign or symptom that does not correspond to the Natural History of the pathology that motivated its entry into the research protocol.

- Indicate whether the removal of the suspected product (of causing the event) eliminates the adverse event. Also indicate if a dose adjustment is made, if the event changes in terms of intensity or seriousness, persistence of the reaction. It is important to indicate whether in those subjects who are exposed again to the product, which had previously been suspended, the AD reappears.

- Information regarding concomitant pharmacotherapy. Indicate the generic name, the dose, the route of administration, start and end dates of its use, as well as the reason for the prescription, regardless of whether it is in accordance with the prescribing information or technical data sheet or is used outside the regulations or what has been authorized by the local, national or international regulatory authority.

- Relevant medical history information. The analysis of the AE considers the information previously narrated, despite the clinical context in which this harmful phenomenon occurs in the participants of the clinical research protocol, is of special interest, so the information about previous conditions, hypersensitivity or allergy phenomena, previous surgical procedures, laboratory analyses or cabinet examinations that have been performed on the participant, etc., that the researcher deems it appropriate to mention may do so.

7.5.2 Adverse Event Tracking

The Principal Investigator will provide care and follow-up to the adverse event presented by the participant until its outcome, according to what is referred to in the following section.

7.5.2.1 Diagnosis against signs or symptoms

Whenever possible, an AE should be assessed/reported as a diagnosis and not as a sign or symptom (e.g., liver failure or hepatitis rather than jaundice, asterixis, and elevated transaminases). If a diagnosis cannot be made from the signs or symptoms, then each sign or symptom should be recorded as an AE. If a diagnosis is made later, all AEs reported as signs or symptoms should be nullified and replaced by a single adverse event based on the diagnosis, with an onset date that corresponds to the onset date of the first symptom or sign of the eventual diagnosis.

7.5.2.2 Adverse events secondary to other adverse events

In general, AEs secondary to other AEs (cascading events or sequelae) should be identified according to the primary cause.

All EAs must register separately if there are concerns regarding the association of events.

7.5.2.3 Persistent or recurrent adverse events

A persistent adverse event is one that spread continuously without resolution between different points of assessment of the subject. Such events only need to be registered once in the eCRF. The initial severity (intensity or degree) of the event will be recorded at the time of the first AD record. If a persistent AE becomes acute, the maximum severity should be recorded in the appropriate section of the eCRF. If the event becomes a serious event, it must be reported no later than 24 hours after the knowledge of the change in the status of the event. The eCRF section should be updated to reflect serious status and will record the date on which the event became serious, thus completing all relevant serious EA reporting data.

A recurrent AE is one that resolves between assessment points and subsequently reappears. Such events should only be recorded once in the eCRF if they are of the same nature, intensity, association with the product of the investigation and/or cause of the AE, with recurrence and duration being recorded within the narrative of the event or in the corresponding sections.

If AEs are different in nature, in association with the product under investigation and/or cause of the AE, they must be registered individually in the eCRF.

7.5.2.4 Abnormal vital signs

Not all abnormal vital signs qualify as AE. For an abnormal vital sign to be reported as an AD, it must meet any of the following criteria:

- Be accompanied by clinical symptoms. It results in a change in treatment (dose modification, treatment interruption, etc.).
- The report on abnormal values should focus on obtaining a diagnosis and not just a description of abnormality.

7.5.2.5 Death

Death should be considered an outcome and not an event. The event or condition that caused or contributed to the fatal outcome should be recorded as the AE in the eCRF section.

7.5.2.6 Pre-existing medical conditions

A pre-existing medical condition should be recorded as AE only if the frequency, severity, or characteristics of the condition worsen during the study.

7.5.2.7 Hospitalization or prolonged hospitalization

Any AE that results in hospitalization or prolongation of hospitalization must be documented or reported as a serious AE with the following exceptions:

Hospitalization for pre-existing conditions if the following are met:

Hospitalization was planned before the study.

7.5.2.8 Pregnancies, miscarriages, and birth defects

Fertile women should contact their doctor to report any suspected pregnancy during the study. A pregnancy report must be issued, and the sponsor must be informed immediately. Subject monitoring should continue until the outcome of pregnancy and for 6 months after the birth of the product. Pregnancy is not by itself an AE. [61]

Any abortion should be classified as a serious AE.

Any birth defect or birth defect in a product from a woman who received the study drug should be classified as a serious AE.

7.5.3 Procedures for a serious adverse event

The process of attention to the adverse event considers the following stages:

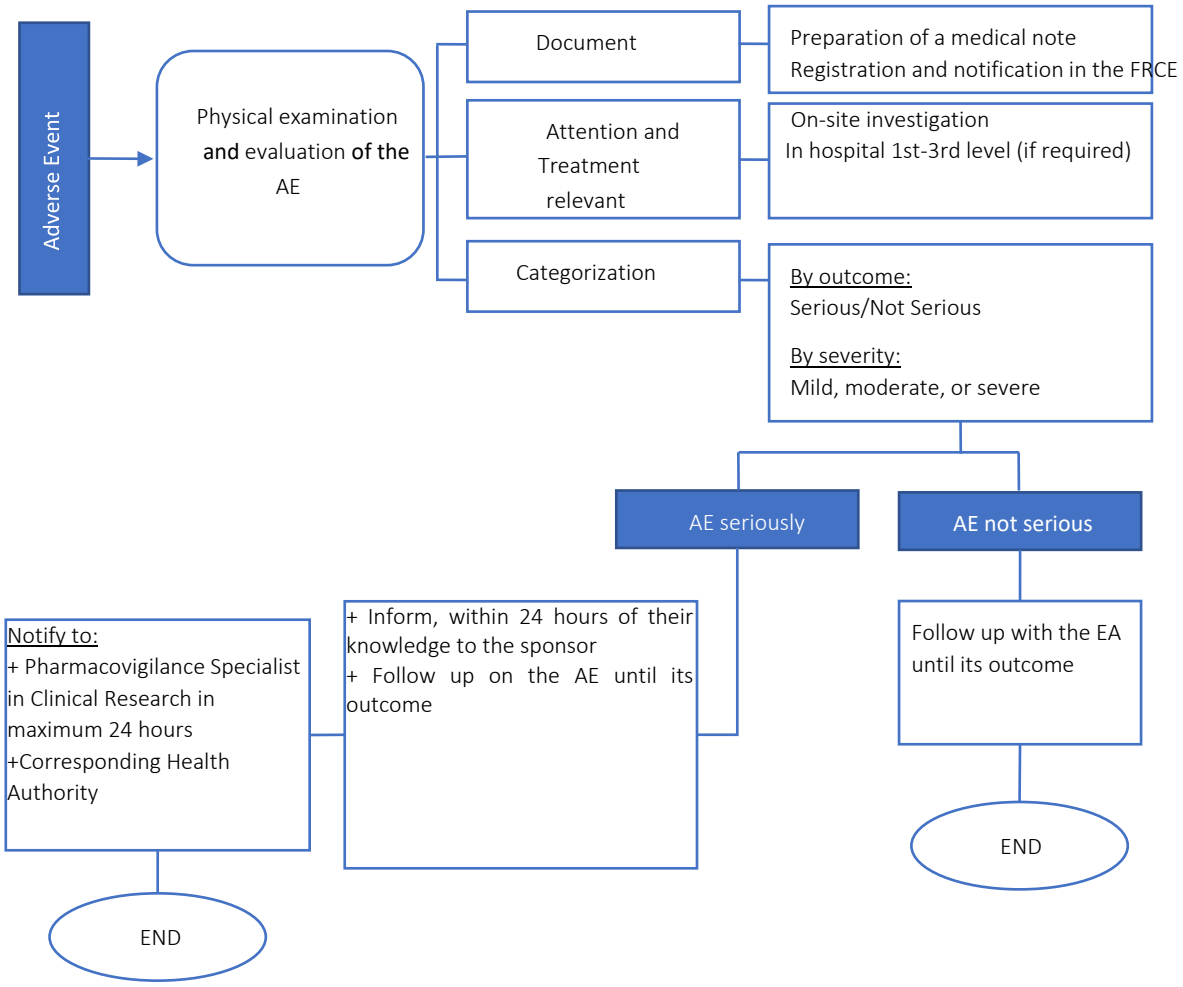


Figure 2 Adverse Event Care

During the development and conduct of this study, undesirable harmful events or adverse reactions/incidents, of medical implication, may occur in the research subject, which do not necessarily have a causal relationship with the investigational products. These harmful phenomena can occur during the use of investigational pharmaceutical products at doses authorized for use in humans by a local, national or international regulatory authority. However, it may be suspected that the investigational product causes some unwanted clinical manifestation. AEs, Incidents, Adverse Incidents, ADRs or SRAMs to one or more pharmaceutical products can occur during the systematic

evaluation of the participants (on the days when the clinical review is scheduled, according to the schedule of activities) or suddenly, in such a way that:

1. The investigator must be the first person to whom the subject notifies that he or she has developed or presented any harmful phenomenon of a clinical nature during his or her participation in this study.

2. According to his clinical judgment, Based on the pertinent physical examination, interrogation, etc., as well as the analysis of the information available in the medical literature and what is referred to in the Investigator's Manual, Information to prescribe or Summary of the Comparator Drug, the principal investigator determines the pertinent care of the harmful event/reaction.

3. Such care can be in the research center or in the hospital with the greatest resolution power. In such a way that, if the subject is sent by the PI to a hospital, he or she attends through a referral system. The reference can be with a card that identifies the subject as a study participant and links him or her to the pre-established agreement with the institution, or through a medical reference note issued by the Principal Investigator. Laboratorios Sophia, S.A. de C.V., will pay the expenses for the medical care of the participating subject, when the adverse event is associated with or related to the product under investigation.

4. Taking the clinical information collected, either during the care provided at the research center or that provided by the treating physician(s) in the hospital, the PI will record the AE in its clinical note, stating the seriousness, intensity (mild, moderate or severe) and relationship with the investigational product.

5. The PI must migrate the relevant data to the eCRF and its respective adverse event section. By, in cases of serious adverse events, the clinical monitor of the study must be notified within 24 hours after learning of it, so that in turn it informs the Clinical Team and the Pharmacovigilance and Technovigilance Unit of Sophia Laboratories (UFTLS), and that the CEI/CI is subsequently notified. Non-serious adverse events will be recorded and treated appropriately, and the safety profile of the investigational drug or drug will be reported to the appropriate regulatory authority in the final report of the clinical trial.

The recording of the outcome of the AE depends substantially on the follow-up that the Principal Investigator performs on the subject, since it is expected that most of the harmful phenomena (Consult the information to prescribe (PPI) and/or the Investigator's Manual (as appropriate) are ophthalmic in nature, however, systemic alterations may exist. Therefore, in the opinion of the researcher, the withdrawal of the participant or their permanence will be considered.

7.5.4 Causation assessment

Causality assessment is the methodology used to estimate the probability of attributing the observed adverse event to a pharmaceutical product. It considers probabilistic categories according to the available evidence and the quality of the information, based on the national pharmacovigilance and technovigilance regulations, the World Health Organization and the Uppsala Monitoring Center. [56][62]

An adverse event may or may not be related to the clinical trial. A causal relationship means that the intervention caused (or is reasonably likely to have caused) the adverse event. This usually implies a relationship between the time of the intervention and the adverse event (e.g., the adverse event occurred shortly after the subject under investigation received the intervention). [59]

For all adverse events, the Principal Investigator is responsible for examining and evaluating the subject to determine the association of the event with the clinical study and the intervention, whether related to the experimental treatment, concomitant, surgical procedure, or diagnostic procedures performed during the study. [59]

Accepting that the adverse event is related to the clinical study requires a plausible mechanism of action, that is, that there is a logical sequence between the event that occurred and the intervention that caused it. In some cases, it is helpful to know the opinion of other doctors directly or indirectly involved in the research; as well as whether the subject considers that a relationship exists or not. [59]

UFTLS may employ the causation categories described by *the Uppsala Monitoring Centre*, to categorize the likelihood of adverse event to the investigational product or concomitant treatments or treatments used during visits: [56] [62]

- Definitive (certain): a clinical event, including alterations in laboratory tests, that manifests itself with a plausible temporal sequence in relation to the administration of the drug, and

that cannot be explained by concurrent disease, or by other drugs or substances. The response to drug withdrawal (withdrawal) must be clinically plausible. The event must be definitive from a pharmacological or phenomenological point of view, using, if necessary, a conclusive re-exposure (challenge) procedure. [56] [62]

- Probable: A clinical event, including alterations in laboratory tests, that manifests itself with a reasonable time sequence in relation to the administration of the drug, that is unlikely to be attributed to concurrent disease, or to other drugs or substances, and that a clinically reasonable response is presented upon withdrawal. It is not necessary to have re-exposure (challenge) information to assign this definition [56] [62]
- Possible: A clinical event, including alterations in laboratory tests, that manifests itself with a reasonable time sequence in relation to the administration of the medicinal product, but which can also be explained by concurrent disease, or by other drugs or substances. Information regarding the withdrawal of the drug may be missing or unclear. [56] [62]
- Unlikely: A clinical event, including alterations in laboratory tests, that manifests itself with an unlikely temporal sequence in relation to the administration of the drug, and that can be more plausibly explained by concurrent disease, or by other drugs or substances. [56] [62]
- Conditional/Unclassified: A clinical event, including alterations in laboratory tests, reported as an adverse reaction, for which further data are essential for appropriate evaluation, or additional data are under review. [56] [62]
- Non-assessable/Unclassifiable: A notification that suggests an adverse reaction but cannot be judged because the information is insufficient or contradictory, and that cannot be verified or completed in its data. [56] [62]

Thus, the degree of certainty to establish the investigational product as the causal agent of the harmful phenomenon that occurs to the subject of the clinical study, can be indicated directly by the Principal Investigator based on his clinical experience or by applying the categories of causality described by *the Uppsala Monitoring Centre*. It is important that the researcher and the UFTLS consider the following arguments in favor of the causal relationship:

a) Strength of association, which refers to the number of cases in relation to those exposed.

b) The consistency of the data, i.e. the presence of a common characteristic or pattern.

c) The exposure-effect pattern, which determines the relationship with the site of appearance, time, dose and reversibility after deletion.

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, e.g., the appearance of abnormal metabolites or high levels of the drug or its biotransformation product.

f) Analogy, which refers to the experience gained 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. [63]

8. Study Monitoring

The sponsor will hire an external provider, who will be responsible for monitoring the study. Monitoring activities include, but are not limited to general safety monitoring, general study quality monitoring, monitoring by study site, monitoring for resolution of discrepancies in data capture, etc.

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

8.1 Study Center Monitoring

The research center participating in the study will be monitored. At least one baseline visit, and one closure visit should be carried out, which does not exclude one or more follow-up visits between these two mandatory visits.

The initial visit must be carried out before the inclusion of the first participant in that center; In it, the monitor will verify that the material to be used during the study has been received and that the personnel who will participate in the study activities have been trained on the study, as well as verify that the regulatory requirements are met.

At the follow-up visit(s), the monitor will conduct a review of the study documents to confirm that: the investigation protocol has been followed, the applicable standard operating procedures have been followed, and that the data completion has been complete and timely. During this visit, the monitor will discuss the findings with the researcher and define the actions that should be taken.

The closure visit will take place at the end of the study, once the last site participant has been discharged from follow-up. On this visit the monitor will verify that the site has all the necessary documents for archiving, that all research products (used and unused) have been recovered and sent to the sponsor, and that all unused material has been recovered.

Details on monitoring are set out in the corresponding plan.

8.2 Audit and quality control

To ensure compliance with GCPs and all applicable regulatory requirements, Laboratorios Sophia, S.A. de C.V., may conduct quality assurance audits. Regulatory agencies could also conduct a regulatory verification of this study.

9. Statistical analysis

9.1 Data analysis

The statistical analysis will be carried out by personnel of Laboratorios Sophia, S.A. de C.V. Specialized statistical software (SPSS version available [IBM Corporation, Armonk, NY, USA] or R [*The R Foundation for Statistical Computing*; http://www.R-project.org]), for Windows. The coding will be done using consecutive numbers. The data will be collected and sorted into an Excel spreadsheet (Microsoft® Office). The data will then be exported to the selected statistical software platform.

Due to the size of the selected samples (n=84 per treatment group), we can consider that they will be distributed normally. The variables will be categorized according to their nature. View section 6.4 Outcome variables.

9.1.1 Data interpretation

The results of discrete quantitative variables (visual analogue test for eye strain) will be presented with measures of central tendency and dispersion: mean, standard deviation and/or ranges.

- Intergroup analysis: Differences between groups will be analyzed using Student's t-statistics for independent groups.

Proportion of subjects with a change ≥ 6 points (or 20%) from their baseline:

After calculating the score in the visual analog test of eye strain, the proportion of subjects in each group who presented a change ≥ 6 points (or 20%) with respect to their initial score will be estimated.

A non-inferiority margin (δ) of -10% was established for the primary efficacy endpoint, based on the primary endpoint of the study. According to the FDA [64] and the EMA [65], The determination of non-inferiority will be made using the 95% confidence interval (95% CI). When the lower limit of the 95% CI is not higher than the margin of non-inferiority (δ= -10) established in the protocol.

The following equation will be used to calculate the 95% CI for two proportions:

$$IC = p_1 - p_2 \pm Z_{1-\alpha/2}\sqrt{(\frac{p_1(1-p_1)}{n_1} + \frac{p_2(1-p_2)}{n_2})}$$

Where:

p_1 and p_2= are the observed proportions

n_1 y n_2 = sample size of each group

Under the alternative hypothesis of, the power of the above test is approximately:$\hat{p}_A - \hat{p}_B > \delta$

$$\Phi\left(\frac{\hat{p}_A - \hat{p}_B - \delta}{\sqrt{\frac{p_A(1-p_A)}{n_A} + \frac{p_2(1-p_B)}{n_B}}} - z_\alpha\right)$$

As a result, the sample size needed to achieve a power of 1−β can be obtained by solving:

$$\frac{\hat{p}_A - \hat{p}_B - \delta}{\sqrt{\frac{p_A(1-p_A)}{n_A} + \frac{p_B(1-p_B)}{n_B}}} - z_\alpha = z_\beta,$$

Leading to:

$$n_A = kn_B$$

$$n_B = \frac{(z_\alpha + z_\beta)^2}{(\hat{p}_A - \hat{p}_B - \delta)^2}\left[\frac{p_A(1-p_A)}{k} + p_B(1-p_B)\right]$$

Where:

p_A = Proportion of subjects assigned to Manzanilla Sophia® who presents a difference of more than 6 points in the score of an analogous visual test of eye strain.

p_B = Proportion of subjects assigned to Meticel Ofteno® who have a difference greater than 6 points in the score of an analog visual test of eye strain

δ = -10%

The result of the nominal and dichotomous qualitative variables will be presented in frequencies, proportions and/or percentages.

The statistical analysis to identify significant differences in the qualitative variables will be carried out by creating contingency tables p x q as follows:

- Intra-group difference: McNemar test. This is applied to 2×2 contingency tables with a dichotomous feature, with pairs of paired subjects, to determine if the marginal frequencies of row and column are equal (marginal homogeneity) [7].
- Difference between groups: Pearson's X2 (Chi-square) test or Fisher's exact test at expected values less than 5.

The level of difference to consider significance will be a value of $p \leq 0.05$.

For the reporting of adverse events, all subjects who were randomly assigned to an intervention group after baseline visit (ITT) will be considered.

The final report of the results will be shown in tables or graphs, as appropriate.

Variable Type	Variable	A1	B1	B2	C1	D1	E1
Background							
A1	Demographics	D					
Scrutiny/selection							
B1	Medical history	D	D				
B2	Eligibility Criteria	D	D	T			
Effectiveness							
C1	Eye strain			TB	B		
Secondary outcome							
D1	Signs and symptoms		D	DT	B		
Security							
E1	Incidence of AD					TB	TB
AEs, adverse events; B, bivariate analysis, D, descriptive statistics; T, contingency table p x q.							

Table 4 Triangulation of concepts

9.1.2 Procedure for handling missing data

The safety assessment will include in the analysis all those subjects (both eyes) who have been exposed at least once to the intervention, regardless of the visit in which they have been eliminated from the study (safety population), for this study the imputation of missing data is not contemplated.

9.1.3 Deviations from the statistical analysis plan

According to the estimate of the sample size to meet the objective of the study, 84 evaluable subjects are required per arm. If this number is not met due to a loss of subjects greater than 10% contemplated in this protocol (loss of follow-up or withdrawal of FCI), the sponsor may replace these subjects.

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

The primary efficacy analysis will be performed in both the PP population and the ITT population, based on the recommendations of the ICH E9 guidelines for non-inferiority studies. [40]

9.1.4 Subjects included in the analysis

Those subjects who meet an adherence ≥65% of the expected weight of the PI and ≥80% of expected applications in the subject's diary, will be included in the statistical analysis to meet the objective of the study. If it is not possible to assess the weight of the IP, only the subject's diary will be considered.

9.2 Sample size estimation

Eye fatigue is a non-specific condition that includes both systemic and ocular surface-related symptoms that decrease the quality of life of those who suffer from it. Manzanilla Sophia® is indicated to relieve eye fatigue. It has been marketed for more than 20 years in Mexico, with no known safety alerts. Some symptoms of eye fatigue can also be treated with eye lubricants, such as Meticel Ofteno® (Hypromellose 0.5%), and to our knowledge there are no studies comparing the efficacy of Chamomile versus that of a lubricant in subjects suffering from eye fatigue.[46][66]

The sample size calculation used the normal approximation to the binomial distribution. Whereas, if one or both proportions of the sample were close to 0 or 1, the approximation would not be valid and an alternative method for the calculation of sample size should be considered.

The following aspects were considered for the estimation of the sample size:

- The primary endpoint of the study (visual analog eye strain test score)
- Investigational product and comparator safety
- Subjects with normal approximation to the binomial distribution
- A conservative expected success ratio was assumed over the primary variable.

Expected ratio:

Sample proportions are what is expected in the results. They are often determined using results from previous studies or pilots. When the values are inconclusive or unknown, it is suggested to use proportions close to 50%, which is conservative and provides large sample sizes. [46] [66][67][46]

Manzanilla Sophia® is indicated to relieve eye fatigue. It has been marketed for more than 20 years in Mexico, during which time a total of 21 suspected adverse drug reactions with different causality have been reported. Of these, 11 have been classified, according to MedDRA, as eye disorders and 4

as general disorders and alterations in the place of administration; eye irritation being the most frequent. Of these suspicions, 6 have been true, 4 were probable, and 1 was doubtful. The EMA, through the HMPC, through a monograph of medicinal plants on chamomile[46] [66] [68], While it mentions undesirable effects, mainly severe allergic reactions after mucosal contact with liquid chamomile preparations, it also mentions that the frequency of these reactions is unknown. The HMPC concludes about the use of chamomile that although there is not enough evidence from clinical studies, the effectiveness of this plant is plausible and there is evidence that it has been used safely for at least 30 years (and at least 15 years in the United States), mainly because it does not require medical supervision.

Some symptoms of eye strain can also be treated with eye lubricants, such as Meticel Ofteno® (Hypromellose 0.5%). McCann et al., compared the efficacy of sodium hyaluronate, Hypromellose and an emulsion in the treatment of dry eye. The severity and frequency of dry eye symptoms were assessed using a questionnaire at 30 and 90 days after treatment. For the Hypromellose group, a reduction in symptom frequency was observed in 14 of 25 subjects (56%), while in severity a reduction was observed in 13 subjects (52%). Tauber evaluated the efficacy, tolerability and comfort of a 0.3% Hypromellose eye gel for the treatment of moderate to severe dry eye in 40 patients, who were chosen pragmatically to provide a reasonable estimate of the clinical outcome, it was observed that the number of patients reported improved ocular comfort at 4 weeks was 37.8%, while the proportion of patients who reported a slight improvement at 4 weeks was 40.5%. [69] [70]

To our knowledge, there are no clinical studies comparing the efficacy of Chamomile versus that of a lubricant in subjects suffering from eye strain. However, there are studies comparing the effectiveness of Hypromellose and other lubricants. Taking the data from these studies as a reference, a success rate of 50% is established. That it has been suggested to use this proportion size when the values are unknown or inconclusive, as it is a conservative value and provides sample sizes large enough to obtain reliable results [71][72].

The statistical software R (*The R Foundation for Statistical Computing*; http://www.R-project.org), for Windows, considering a conservative 50% success rate, and considering a margin of non-inferiority of -10% between both treatments. [71]

The Committee for Medicinal Products (HMPC) of the European Medicines Agency (EMA) mentions about the use of chamomile that there is not enough evidence derived from clinical studies, however,

the effectiveness of this plant is plausible and there is evidence that it has been used safely for at least 30 years (and at least 15 years in the United States). mainly because it does not require medical supervision. [68]

For its part, Hypromellose (0.3%) has been proposed by the World Health Organization to the WHO Expert Committee on the Selection and Use of Essential Medicines to be included as a representative of the pharmacological class of artificial tears for the treatment of dry eye, which is why more evidence is found derived from clinical studies. McCann et al. compared the efficacy of Hypromellose (n=25) with an emulsion (new treatment [n=24]) and with sodium hyaluronate (n=24). He observed that in the group treated with the emulsion there was a reduction in the frequency of symptoms in 83% subjects, while 79% reported a reduction in severity. In the group treated with sodium hyaluronate, 50% reported a reduction in symptom frequency and 54% a reduction in severity. For the group treated with Hypromellose, 56% and 52% subjects reported having a reduction in frequency and severity, respectively. For his part, Tauber reported that the number of patients who reported having better eye comfort compared to baseline at 4 weeks after treatment was 37.8%, while those who reported having a slight improvement in eye comfort at 4 weeks was 40.5%.[73] [69] [70]

Considering the difference between sodium hyaluronate and Hypromellose in the reduction of symptom severity and the difference in the improvement of eye comfort in 4 weeks of treatment, the decision was made to propose a non-inferiority margin of -10%. This is because a small value is often chosen that would constitute a clinically important effect and is more conservative. The above with the intention of avoiding the acceptance of inferior treatments in case of establishing a high margin, therefore, the sample size of non-inferiority studies is usually large as in this case. The choice of the non-inferiority margin must consider the uncertainty of the difference between the reference treatment. [74][72]

Different simulations were carried out, adjusting the success rate for the PI as shown in the following table. All calculations considered a α (Type I error) of 5% and a statistical power (1-β, where β is the Type II error) of 80%.

PA	nA	Estimated power	Expected losses	Sample Size
0.5	310	0.8	10%	682
0.51	256	0.8	10%	564
0.52	215	0.8	10%	474
0.53	183	0.8	10%	402
0.54	158	0.8	10%	348
0.55	137	0.8	10%	302
0.56	120	0.8	10%	264
0.57	106	0.8	10%	234
0.58	95	0.8	10%	210
0.59	85	0.8	10%	188
0.60	76	0.8	10%	168
0.61	69	0.8	10%	152
0.62	63	0.8	10%	138
0.63	57	0.8	10%	126
0.64	52	0.8	10%	114
0.65	48	0.8	10%	106
0.66	44	0.8	10%	96
0.67	40	0.8	10%	88
0.68	37	0.8	10%	82
0.69	35	0.8	10%	78
0.70	32	0.8	10%	70

Based on the above, it was estimated that a sample of 76 subjects per treatment arm is sufficient to verify the non-inferiority of the PI with respect to the comparator, considering p_A = 60% and p_B = 50% as success proportions. Considering a 10% dropout rate due to loss, a sample of 168 subjects (84 subjects per treatment group) would be sufficient to meet the primary objective of the present study.

10. Ethical considerations

10.1 Approval of the committees

The present study will be conducted in accordance with the standards of the Declaration of Helsinki, World Medical Association 2013. Nuremberg Code; Nuremberg Trial by the International Tribunal of Nuremberg, 1947. Belmont Report, National Commission for the Protection of Biomedical and Behavioral Research Subjects, 1979. It will be conducted in accordance with the scientific and technical requirements necessary for the registration of medicines for human use of the ICH, GCP Guide. Council for International *Organizations of Medical Sciences* (CIOMS, 2016) International Ethical Guidelines for Biomedical Research in Human Subjects. The Research Ethics Committee (REC) and the Research Committee (IC) will evaluate the protocol before conducting the study and will issue their approval or possible modifications for its performance, these Committees must be notified of any significant changes to the protocol. In addition to the above, the current regulations of the regulatory authority will also be complied with.

Personnel authorized by the sponsor will submit to evaluation by the Research Ethics Committees, Research Committees, and when applicable to the Biosafety Committee the essential documentation of the research project: research protocol, Informed Consent Form, Information to Prescribe and/or Investigator's Manual (as applicable), subject diary, calendar and identification card, as well as other documents additionally requested, in accordance with the local, national or international requirements applicable by the regulatory entities.

The study will not be initiated at the research center if the confidentiality agreements and economic proposal of the principal investigator are not in place, duly signed and without having previously obtained the favorable opinion and/or approval of the Research Ethics Committees, Research Committees, and when applicable by the Biosafety Committee. corresponding.

The study will not begin without having complied with the relevant local, national or international regulatory requirements and without having the corresponding health authorization.

The study is considered as research with greater than the minimum risk, in accordance with the Regulations of the General Health Law on Health Research, Title Two, Chapter I, Article 17, Section III, last updated in the Official Gazette of the Federation on April 2, 2014.

10.2 Protocol Amendments

The amendment procedure will be pertinent when there is a need to make any change to a document that is part of the research project or protocol, derived from variations in the methodological structure, substitution of the principal investigator or in the face of the identification of risks in the subjects who will participate in the research. The documents subject to amendment will be Protocol, Informed Consent Form, Information to Prescribe and/or Investigator's Manual (as appropriate), documents for the subject, measurement scales and schedule of activities.

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, where 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 confer an additional or different risk on the subjects must be approved by the Committees. It is the investigator's responsibility to act in situations that require immediate action to avoid unnecessary harm to study participants.

The principal investigator is responsible for communicating to the Research Ethics Committee any amendments to the protocol that may eventually affect the rights, safety, or welfare of research participants. Likewise, it must inform us of any situation or new knowledge that will show a greater risk for the participants, the premature termination or suspension of the study, the reasons and the results obtained so far. It must also report on the conclusion of the study upon completion of the research protocol.

10.3 Early study termination

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

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

1. The regulatory authority (COFEPRIS) considered it for security alerts.

2. The Sponsor determined it for its convenience or eventualities such as financial support, manufacturing errors, etc.
3. Lower recruitment than stipulated.
4. Identification of unexpected risks to participants, which are significant or unacceptable.
5. Obtaining new relevant safety information.
6. Insufficient adherence to the requirements of the protocol.
7. The data obtained is not assessable or is not sufficiently complete.
8. Determination that the primary objective has been achieved.
9. Determination of futility.

In the event of suspension, the study may be resumed once the situations that led to the suspension have been corrected; if this justification is sufficient for the sponsor, CI, CEI and regulatory authorities.

10.4 Informed consent

The FCI contains complete and understandable information about the study and the product under investigation, in accordance with the applicable regulations in force and the GCP.

The FCI will be considered as 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 are submitted to the appropriate approvals (the same ones to which the original Informed Consent Form was submitted) and that the most current version authorized by COFEPRIS is the one presented to the subjects.

10.4.1 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 Guide to GCPs and will follow all applicable laws and regulations.

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

This information will be in a language understandable to the subject, it will be explained to the subject that he or she has the right to interrupt his or her participation in the study at any stage, without this affecting the relationship with the researcher and/or his or her future assistance. Informed consent

will be put to the consideration of the potential participant; He must have enough time to analyze each one of the aspects mentioned above and in case he has any doubts, it will be clarified by the person in charge of obtaining the informed consent.

Once the participant agrees to participate in the study, he/she must sign and date the Informed Consent Form in the presence of two witnesses who may or may not be related to the study subject, who will participate during the informed consent process and sign guarantee that the process was carried out prior to any study procedure. that the information of the study was clearly explained and doubts were clarified if any.

The PI or designee will also be required to sign and date this consent.

The FCI must be signed in duplicate by all those involved, 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 delegated personnel must document the process of obtaining the Informed Consent by means of a detailed medical note, specifying the signed version, the date on which the document was signed and how the process was carried out.

10.4.2 Special considerations

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

10.4.3 Modifications to informed consent

Any changes to the FCI constitute an amendment to this document and must be submitted for approval to the Research Ethics Committee and to COFEPRIS.

Such amendments may be implemented only after obtaining the written approval of the Research Ethics Committee and the Regulatory Entity, except for an amendment that is required to eliminate an immediate danger to the subjects.

A process of re-consent for each subject affected by the amendment must be carried out under the same conditions as those described above, to communicate the new information contained in the document to them in a timely manner. The subject will be given a signed original of the amendment, and the researcher will keep the second original.

10.5 Confidentiality

All documents and information provided to the research center by the sponsor are strictly confidential. PI expressly agrees that data about your professional and clinical experience, provided to the sponsor on paper and stored in electronic form, is solely for use related to your activities with the clinical trial sponsor, in accordance with the GCP.

The investigator 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 if the confidential information has not been disclosed to the public 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 trial protocol, as well as any information taken from it, should not be disclosed to other parties without the written permission of the sponsor.

The researcher shall not disclose any information without the prior written consent of Laboratorios Sophia, S.A. de C.V., except to the representatives of the Competent Authorities, and only at their request. In the latter case, the investigator undertakes to inform Laboratorios Sophia, S.A. de C.V. before disclosing the information to these authorities.

The investigator will fill out and maintain a subject selection record, as well as the identification and enrollment list of each of the subjects participating in the study. The investigator agrees to give on-site access to the auditor and/or representatives of the Competent Authorities. The information will be processed in compliance with professional secrecy.

All FRCE and communications related to study subjects will identify them only by the study subject identification number. The information collected in this study will be exchanged between the sponsor and the research center and must be treated confidentially. The Health Authority, the CEI, the IC, the sponsor, the monitors/auditors and third-party auditors will be the only bodies authorized to review the study documentation. If publications arise from this research project, in no case will they contain information on the identification of the study subjects. If the results of the study are published, no personal information on the subjects will be revealed.

The protection of personal data will be carried out in accordance with the corresponding regulations in force.

10.6 Conflict of interest

The independence of the conduct of the study and its results from any current or perceived external influences is critical. For this reason, any current conflict of interest of any person who has a role in design, conduct, analysis, publication or any aspect of this study will be declared. Furthermore, those who have a perceived conflict of interest will be asked to handle it in a manner appropriate to their participation in the study.

10.6.1 Declaration of interests

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

10.7 Access to information

The final database of the study will be the property of Laboratorios Sophia, S.A. de C.V., and its access will be restricted. The PI will not have access to it, unless it has prior written authorization from the sponsor.

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

10.8 Ancillary and post-study care

The sponsor will not extend care to the research subject.

11. Biosecurity aspects

NO BIOSECURITY IMPLICATIONS

This protocol, entitled: "Clinical study of non-inferiority between Chamomile Sophia® and Meticel Ofteno® 0.5% to provide a feeling of rest to the eyes.", and number: SOPHMAN-0824/IV HAS NO BIOSAFETY IMPLICATIONS, since infectious-contagious biological material will NOT be used; pathogenic strains of bacteria or parasites; viruses of any kind; radioactive material of any kind; genetically modified animals and/or cells and/or plants; toxic, dangerous or explosive substances; any other material that puts the health or physical integrity of the research center's staff or subjects at risk or affects the environment. It is also declared that this project will not carry out cell, tissue or organ transplant procedures, or cell therapy, nor will laboratories, farm or wildlife animals be used.

12. Publication Policy

12.1 Final Report

Once the statistical analysis is completed, the final report will be written with the results obtained, by the Clinical Team of the Regional Management Department of Medical Affairs of Laboratorios Sophia, S.A. de C.V. This report will be prepared following the recommendations of the E3 Step 4 Guide to ICH.

12.2 Communication of results

Regardless of the results of the study, Laboratorios Sophia, S.A. de C.V., is committed to communicating the final report of the study to the principal investigators and COFEPRIS. These results will also be shared with the research committee and the IRC. It will be the responsibility of the PI to communicate with the subjects.

Laboratorios Sophia, S.A. de C.V., will always maintain the rights over the publication and disclosure of the information contained.

12.3 Publication of the results

Laboratorios Sophia, S.A. de C.V., acting as the sponsor of the study, assumes full responsibility for its function and retains exclusive ownership rights over the results of the study, which it may use in any way it deems appropriate.

The PI undertakes not to publish or communicate data collected from the study, unless there is the prior written agreement of Laboratorios Sophia, S.A. de C.V. Any manuscript derived from the data obtained with this protocol must be reviewed by the sponsor before any attempt to submit it for publication in any scientific journal or congress

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

The assignment of authorship of publications, which is the responsibility of the sponsor, will be the prerogative of the latter. However, the express authorization of the people who are invited to participate as authors must be obtained. Authors have the right to review the manuscript prior to its publication, as well as to issue comments and suggestions in this regard, such comments must be delivered within the first 15 calendar days from the date on which the project is received.

13. Financing and insurance

13.1 Compensation to Study Participants

Subjects who participate in the study will not receive financial compensation for their participation in the study. However, the subjects will receive financial support for travel expenses on each scheduled visit to which they attend punctually. Such support, as well as the amount, will be specified in the Informed Consent Form.

13.2 Study Insurance

In accordance with current local regulations, Laboratorios Sophia S.A. de C.V., has a specific financial fund, to be able to fulfill the responsibility of providing the medical treatment and compensation to which a subject would be legally entitled, in the case of damages directly caused by this research.

In the event of a medical emergency, the research center must have personnel, material, equipment and procedures for its immediate management.

14. References

[1] M. O. Faruque, S. B. Uddin, J. W. Barlow, H. Sheng, D. Shuang, C. Qian, L. Xiaohua, and H. Xuebo, "Quantitative Ethnobotany of Medicinal Plants Used by Indigenous Communities in the Bandarban District of Bangladesh," *Frontiers in Pharmacology,* vol. 9, no. 40, 2018.

[2] P. Fortini, P. Di Marzio, P. M. Guarrera and M. Iorizz, «Ethnobotanical study on the medicinal plants in the Mainarde Mountains (central-southern Apennine Italy),» *Journal of Ethnopharmacology,* 2016.

[3] O. Singh, Z. Khanam, N. Misra, and M. K. Srivastava, "Chamomile (Matricaria chamomilla L.): An overview," *Pharmacogn Rev.,* vol. 5, no. 9, p. 82–95, 2011.

[4] M. Petran, D. Dragos and M. Gil, "Historical ethnobotanical review of medicinal plants used to treat children diseases in Romania (1860s–1970s)," *Journal of Ethnobiology and Ethnomedicine,* vol. 16, no. 15, 2020.

[5] R. W. Bussmann and D. Sharon, Medicinal Plants of the Andes and the Amazon - The Magical and Medicinal Flora of Northern Peru, Trujillo, Peru.: William L. Brown Center – Missouri Botanical Garden, 2015.

[6] J.-P. Le Loć h, List of Common Medicinal Plants in the Andean Subregion Proposals for their Integration into Health Systems, Lima, Peru: Andean Health Organization – Hipólito Unanue Agreement, 2014.

[7] M. Marković, D. Pljevljakušić, K. Kojičić and S. Cupara, "Ethnopharmacological application of chamomile (Matricaria chamomilla L.) in the Pirot County of Southeastern Serbia," *Arh. farm. 2020,* vol. 70, p. 238 – 247, 2020.

[8] M. Bahmani, K. Saki, H. Golshahi, M. Rafieian-Kopaei, N. Abdali, A. Adineh, F. Namdari and F. Bahmani, «Ethnobotanical and therapeutic uses of camomille,» *Journal of Chemical and Pharmaceutical Research,* vol. 7, no. 1, pp. 640-645, 2015.

[9] Mayo Clinic, "Eye Fatigue," 28 08 2020. [Online]. Available: https://www.mayoclinic.org/es-es/diseases-conditions/eyestrain/symptoms-causes/syc-20372397. [Accessed 30 09 2022].

[10] Clinica Bavaria, "Tired eyes: what is eye fatigue or asthenopia," 14 09 2021. [Online]. Available: https://www.clinicabaviera.com/blog/bye-bye-gafasconoce-tus-ojosconoce-los-sintomas-de-los-ojos-cansados/. [Accessed 30 09 2022].

[11] C. Vimont, "American Academy of Ophthalmology. Eye Strain: How to Prevent Tired Eyes," 26 02 2020. [Online]. Available: https://www.aao.org/eye-health/diseases/what-is-eye-strain. [Accessed 30 09 2022].

[12] A. L. Sheppard and J. S. Wolffsohn, "Digital eye strain: prevalence, measurement and amelioration," *BMJ Open Ophthalmology,* vol. 3, no. e000146, 2018.

[13] M. Ahuja, A. S. Dhake, S. K. Sharma, and D. K. Majumdar, "Topical ocular delivery of NSAIDs.," *AAPS J,* vol. 10, no. 2, pp. 229-41, 2008.

[14] J. K. Srivastava, M. Pandey and S. Gupta, «Chamomile, a novel and selective Cox-2 inhibitor with anti-inflammatory activity.,» *Life Sci.,* vol. 85, p. 663–669, 2009.

[15] A. Al-Achi, "Anti-inflammatory Herbs," *Pharmacist,* vol. 29, no. 3, 2004.

[16] J. K. Srivastava, E. Shankar, and S. Gupta, "Chamomile: An herbal medicine of the past with bright future.," *Mol Med Report.,* vol. 3, no. 6, pp. 895-901, 2010.

[17] M. E. Gerritsen, W. W. Carley, G. E. Ranges, C. P. Shen, S. A. Phan, G. F. Ligon, and C. A. Perry, "Flavinoids Inhibit Cytokine-Induced Endothelial Cell Adhesion Protein Gene Expression," *Am J. Phatol,* vol. 147, pp. 278-292., 1995.

[18] M. Sokovic, J. Glamočlija , P. D. Marin, D. Brkić and L. J. van Griensven, «Antibacterial effects of the essential oils of commonly consumed medicinal herbs using an in vitro model.,» *Molecules,* vol. 15, no. 11, pp. 7532-46, 2010.

[19] M. E. Aggag and R. T. Yousef, "Study of antimicrobial activity of chamomile oil," *Planta Med.,* vol. 22, no. 2, pp. 140-4, 1972.

[20] P. Maher and A. Hanneken, "Flavonoids Protect Retinal Ganglion Cells from Oxidative Stress-Induced Death.," *Invest Ophthalmol Vis Sci,* vol. 46, pp. 4796-4803, 2005.

[21] PubChem. National Center for Biotechnology Information, U.S. National Library of Medicine, "Levomenol," [Online]. Available: https://pubchem.ncbi.nlm.nih.gov/compound/442343#section=Information-Sources. [Accessed 05-09-2016].

[22] D. L. McKay and J. B. Blumberg, "A review of the bioactivity and potential health benefits of chamomile tea (Matricaria recutita L.).," *Phytother Res.,* vol. 20, no. 7, pp. 519-30, 2006.

[23] A. Kato, Y. Minoshima, J. Yamamoto, I. Adachi, A. A. Watson, and R. J. Nash, "Protective Effects of Dietary Chamomile Tea on Diabetic Complications.," *J. Agric Food Chem.,* vol. 56, no. 17, pp. 8206-11, 2008.

[24] N. Murano, M. Ishizaki, S. Sato, Y. Fukuda and H. Takahashi, «Corneal endothelial cell damage by free radicals associated with ultrasound oscillation.,» *Arch Ophthalmol.,* vol. 126, nº 6, pp. 816-21., 2008.

[25] A. A. Kramerow, M. Saghizadeh, S. Caballero, L. C. Shaw, S. L. Calzi, M. Bretner, M. Montenarh, L. A. Pinna, M. B. Grant, and A. V. Ljubimov, "Inhibition of protein kinase CK2 suppresses angiogenesis and hematopoietic stem cell recruitment to retinal neovascularization sites.," *Mol Cell Biochem,* vol. 316, no. 1-2, pp. 177-186, 2008.

[26] G. C. Chiou. and Y. Zou, "Apigenin Inhibits Laser-Induced Choroidal Neovascularization and Regulates Endothelial Cell Function.," *Journal of Ocular Pharmacology and Therapeutics.,* vol. 22, no. 6, pp. 425-430., 2006.

[27] Cosmetic Ingredient Review Expert Panel, "Safety Assessment of Chamomilla Recutita-Derived Ingredients as Used in Cosmetics.," [Online]. Available: http://www.cir-safety.org/sites/default/files/chamom122013final.pdf. [Accessed 05-09-2016].

[28] Pubchem. National Center for Biotechnology Information, U.S. National Library of Medicine., "520-36-5.," [Online]. Available: https://pubchem.ncbi.nlm.nih.gov/compound/apigenin#section=Identification. [Accessed 05-09-2016].

[29] European Medicines Agency., «Assessment reporto n Matricaria recutita L., flos and Matricaria recutita L., aetheroleum.,» 06 2014. [Online]. Available: http://www.ema.europa.eu/docs/en_GB/document_library/Herbal_-_HMPC_assessment_report/2014/07/WC500170079.pdf . [Accessed 05-09-2016].

[30] A. Prado-Montes, Á. Morales-Caballero and J. N. Molle-Cassia, «Ocular Fatigue Syndrome and its relationship with the work environment,» *Med. segur. trab.,* vol. 63, nº 249, 2017.

[31] American Academy of Ophthalmology, "Eye Strain: How to Prevent Tired Eyes," 05 05 2023. [Online]. Available: https://www.aao.org/eye-health/diseases/what-is-eye-strain. [Accessed 22 04 2024].

[32] A. El Mihyaoui, J. Esteves da Silva, S. Charfi, M. Candela Castillo, A. Lamarti and M. Arnao, «Chamomile (Matricaria chamomilla L.): A Review of Ethnomedicinal Use, Phytochemistry and Pharmacological Uses,» *Life (Basel),* vol. 12, no. 4, pp. 1 - 41, 2022.

[33] M. Padilla-Morones and E. López-Chávez, «Efficacy, safety and tolerance of a new chamomile ophthalmic solution,» *Pharmacotherapy,* vol. 22, nº 2, pp. 256 - 264, 2002.

[34] L. Baiza-Durán, P. Muñoz-Villegas, A. Sánchez-Ríos, and O. Olvera-Montaño, "Efficacy and Safety of an Ophthalmic DMPC-Based Nanoemulsion in Patients with Dry Eye Disease: A Phase I/II Randomized Clinical Trial," *Journal of Ophthalmology,* pp. 1 - 9, 2023.

[35] T. Rosenblatt, N. Reyess, A. Al-Moujahed, R. Khurana, and P. Mruthyunjaya, "Discontinuation and loss to follow-up in clinical trials of intravitreal anti-vascular endothelial growth factor injections," *Graefe's Archive for Clinical and Experimental Ophthalmology,* vol. 260, pp. 93 - 100, 2022.

[36] S. Sze-Yee Lee, G. Lingham, M. Blaszkowska, and P. Sanfilippo, "Low-concentration atropine eyedrops for myopia control in a multi-racial cohort of Australian children: A randomised clinical trial," *Clinical & Experimental Ophthalmology,* vol. 50, no. 9, pp. 1001 - 1012, 2022.

[37] S. Belalcázar-Rey, V. Sánchez-Huerta, J. Ochoa-Tabares and S. Altamirano-Vallejo, «Efficacy and Safety of Sodium Hyaluronate/Chondroitin Sulfate Preservative-free Ophthalmic Solution in the Treatment of Dry Eye: A Clinical Trial,» *Current Eye Research,* vol. 46, no. 7, pp. 919 - 929, 2020.

[38] J. Dettori, "Loss to follow-up," *Evidence-Based Spine-Care Journal,* pp. 7 - 10, 2011.

[39] J. Dumville, D. Torgerson, and C. Hewitt, "Reporting attririon in randomised controlled trials," *BMJ,* vol. 332, pp. 969 - 971, 2006.

[40] ICH E9 Expert Working Group, "Statistical Principles for Clinical Trials: ICH Harmonized Tripartite Guideline," *Stat Med,* vol. 18, pp. 1905 - 1942, 1999.

[41] DrugBank online, "Hypromellose," 04 10 2022. [Online]. Available: https://go.drugbank.com/drugs/DB11075. [Accessed 05 10 2022].

[42] J. Tauber, «Efficacy, tolerability and comfort of a 0.3% hypromellose gel ophthalmic lubricant in the treatment of patients with moderate to severe dry eye syndrome,» *Current Medical Research and Opinion®,* vol. 23, no. 11, 2007.

[43] A. Çömez, H. Tufa, Ö. Kocabıyık and B. Gencer, «Effects of Lubricating Agents with Different Osmolalities on Tear Osmolarity and Other Tear Function Tests in Patients with Dry Eye,» *Current Eye Research,* vol. 38, no. 11, p. 1095–1103, 2013.

[44] A. Pucker and J. Nichols, "Over the counter (OTC) artificial tear drops for dry eye syndrome (Review)," *Cochrane Database of Systematic Reviews 2016, Issue 2. Art. No.: CD009729..*

[45] R. M. Santaella and F. W. Fraunfelder, «Ocular Adverse Effects Associated with Systemic Medications. Recognition and Management," *Drugs,* p. 90, 2007.

[46] F. W. Fraunfelder, 'Corneal Toxicity From Topical Ocular and Systemic Medications,' *Cornea,* vol. 25, no. 10, p. 1136, December 2006.

[47] W. Johnson Jr, I. Boyer, W. F. Bergfeld, D. V. Belsito, R. A. Hill, C. D. Klaassen, D. C. Liebler, J. G. Marks Jr, R. C. Shank, T. J. Slaga, P. W. Snyder, L. J. Gill, and B. Heldreth, "Amended Safety Assessment of Chamomilla," *International Journal of Toxicology,* vol. 37, pp. 68F, 71S, 2018.

[48] C. Andres, W. C. Chen, M. Ollert, M. Mempe, U. Darsow, and J. Ring, "Anaphylactic Reaction to Camomile Tea," *Allergology International,* vol. 58, no. 1, pp. 135, 136, 2009.

[49] M. RODRIGUEZ-SERNA, J. M. SANCHEZ-MOTILLA, R. R. and A. ALIAGA, 'Allergic and systemic contact dermatitis from Matricaria chamomilla tea,', *Contact Dermatitis,* p. 1, 1998.

[50] N. REIDER, N. SEPP, P. FRITSCH, G. WEINLICH, and E. JENSEN-JAROLIM, "Anaphylaxis to camomile: clinical features and allergen cross-reactivity," *Clinical and Experimental Allergy,* vol. 30, pp. 1437, 1441, 2000.

[51] E. Paulsen, "Systemic allergic dermatitis caused by sesquiterpene," *Contact Dermatitis,* p. 1, 2016.

[52] E. Paulsen, «Contact sensitization from Compositae-containing herbal remedies and cosmetics,» *Contact Dermatitis,* nº ISSN 0105-1873, p. 194, 2002.

[53] J. Hong, "Allergy to ophthalmic preservatives," *Current Opinion in Allergy and Clinical,* vol. 9, p. 1447, 2009.

[54] M. H. Goldstein, "Ocular benzalkonium chloride exposure: problems and solutions," *EYE,* vol. 36, p. 361, 2022.

[55] C. Baudouin, "Preservatives in eyedrops: The good, the bad and the ugly," *Progress in Retinal and Eye Research,* vol. 29, p. 313, 2010.

[56] N. O. Mexicana, *NOM-220-SSA1-2016, Facilities and Operations of pharmacovigilance.,* CDMX: Official Gazette of the Federation. , 2017.

[57] International Conference on Harmanisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, 'Clinical Safety Data Management: Definitions and Standards for Expedited Reporting E2A,' ICH Harmonised Tripartite Guideline, 1994.

[58] International Conference on Harmonisation of Technical Requierements for Registration of Pharmaceuticals for Human Use, 'General Considerations for Clinical Trials,' ICH Topic E8, 1998.

[59] Good Clinical Practice, "Participant Safety & Adverse Events," gcp.nidatraining.org.

[60] Ministry of Health Mexico, "NORMA Oficial Mexicana NOM-220-SSA1-2016, Instalación y operación de la farmacovigilance.," *Official Gazette,* 2016.

[61] S. d. G. -. D. O. d. l. Federation, "NORMA Oficial Mexicana NOM-220-SSA1-2016, Instalación y operación de la farmacovigil.," 19 July 2017. [Online]. Available: https://dof.gob.mx/nota_detalle.php?codigo=5490830&fecha=19/07/2017#gsc.tab=0. [Accessed 01 February 2023].

[62] The Uppsala Monitoring Centre, "Monitoring the Safety of Medicines," World Health Organization, 2001.

[63] R. H. Meyboom, A. C. Egberts, I. R. Edwards, Y. A. Hekster, F. H. de Koning, and F. W. Gribnau, "Principles of signal detection in pharmacovigilance," *Drug Safety,* vol. 16, pp. 355-365, 2997.

[64] Food and Drug Administration, FDA, "Non-Inferiority Clinical Trials to Establish Effectiveness," 2016. [Online]. Available: https://www.ema.europa.eu/en/choice-non-inferiority-margin-scientific-guideline.

[65] European Medicines Agency, EMA, 'Guideline on the choice of the non-inferiority margin,' 2005. [Online].

[66] A. Salazar-Gómez, A. Velo-Silvestre, A. Alonso-Castro, and L. Hernández-Zimbrón, "Medicinal Plants Used for Eye Conditions in Mexico—A Review," *Pharmaceuticals,* vol. 16, no. 10, p. 1432, 2023.

[67] H. Pult, F. Gill, and B. Riede-Pult, "Effect of three different liposomal eye sprays on ocular comfort and tear film," *Contact Lens and Anterior Eye,* vol. 35, no. 5, pp. 203 - 207, 2012.

[68] E. M. A. EMA, "European Medicines Agency," 2015. [Online]. Available: https://www.ema.europa.eu/en/medicines/herbal/matricariae-aetheroleum.

[69] T. A. P. E. P. V. McCann LC, «Effectiveness of artificial tears in the management of evaporative dry eye.,» *Cornea,* pp. Jan; 31(1):1-5, 2012.

[70] T. J., «Efficacy, tolerability and comfort of a 0.3% hypromellose gel ophthalmic lubricant in the treatment of patients with moderate to severe dry eye syndrome.,» *Curr Med Res Opin.,* pp. Nov; 23(11):2629-36., 2007.

[71] H. W. and S. Chow, "Sample Size Calculation for Comparing Proportions," *Wiley Encyclopedia of Clinical,* 2007.

[72] W. L. R. D. Honório HM, « Non-inferiority clinical trials: importance and applications in health sciences. . . .,» *Braz Oral Res,* p. 2020; 34 Suppl 2:e072, 2020.

[73] World Health Organization (WHO), «Submission for the 2023 Meeting of the WHO Expert: Inclusion of Hypromellose as a Representative of a Pharmacological,» 2023.

[74] E. D. P. S. E. S. A. D. Piaggio G and C. Group., « Reporting of noninferiority and equivalence randomized trials: extension of the CONSORT 2010 statement.,» *JAMA. ,* pp. Dec 26; 308(24):2594-604. , 2012.

15. Appendices

15.1 Visual analogue scale of eye strain

Choose the option that most closely resembles how you usually feel

Subject number					
Visit (screening or final)					
Date					
	1	2	3	4	5
Have you had a headache?					
Have you had eye strain?					
Do you have gritty sensation in your eyes?					
Have your eyes been itchy?					
How would you describe your clarity of seeing?					
Do you feel any discomfort when you fix your eyes?					
Subtotal					
Total					