Creation date : february de 2017

Clinical study of the efficacy of the ophthalmic solution of Pazufloxacin 0.6% (PRO-157) for the treatment of acute bacterial conjunctivitis, compared to the ophthalmic solution of Gatifloxacin 0.3%.

Protocol code : SOPH157-0217/III

Protocol version : 2

Date of the version : 19/01/2018

Registry : Pending

Sponsor : Sophia Laboratories, S.A. of C.V.

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1. Summary

<table>
<tr><td colspan="2">Title of the study:
Clinical study of the efficacy of the ophthalmic solution of Pazufloxacin 0.6% (PRO-157) for the treatment of acute bacterial conjunctivitis, compared to the ophthalmic solution of Gatifloxacin 0.3%.</td></tr>
<tr><td>Protocol code:SOPH157-0217/III</td><td>Creation date: 27/02/2017</td></tr>
<tr><td>Protocol version: 2.0</td><td>Date of the version: 19/01/2018</td></tr>
<tr><td colspan="2">Therapeutic indication:
Ophthalmic topical antibiotic for the treatment of bacterial conjunctivitis.</td></tr>
<tr><td>Study period:
18 months</td><td>Development phase:
III</td></tr>
<tr><td colspan="2">Goals:
To compare the efficacy of the ophthalmic solution of pazufloxacin 0.6%, against the ophthalmic solution of gatifloxacin 0.3%, in the treatment of acute bacterial conjunctivitis.</td></tr>
<tr><td colspan="2">Hypothesis:
The ophthalmic solution PRO-157 is not inferior in the treatment of bacterial conjunctivitis, compared to the ophthalmic solution of gatifloxacin 0.3%, by means of the clinical remission of the disease.</td></tr>
<tr><td colspan="2">Methodology:
Phase III clinical study of non-inferiority, multicenter, double-blind, with comparative group, of parallel groups and randomized.</td></tr>
<tr><td colspan="2">Number of patients:
160 patients, each one will provide an eye for efficacy analysis.
160 eyes divided into 2 groups (80 eyes per group).</td></tr>
<tr><td colspan="2">Diagnosis and main inclusion criteria:
Diagnosis: Acute bacterial conjunctivitis.
Main criteria:
- Informed consent
- Age ≥ 1 year
- Both genders
- Clinical picture of acute bacterial conjunctivitis defined by:
º Conjunctival secretion
º or bulbar conjunctival hyperemia</td></tr>
</table>

Test product, dose and route of administration:

• PRO-157. Pazufloxacin 0.6%. Prepared by Sophia Laboratories, S.A. of C.V., Zapopan, Jalisco, Mexico.

- Dosage: 1 drop, 3 times a day during the waking period in both eyes (at approximate intervals of 6 hours), for 7 days.
- Route of administration: topical ophthalmic

Treatment duration:

7 days

Reference product, dose and route of administration, batch:

1. Zymar®. Gatifloxacin 0.3%. Prepared by Allergan, S.A. of C.V.
   - Dosage: 1 drop, 3 times a day during the waking period in both eyes (at approximate intervals of 6 hours), for 7 days.
   - Route of administration: topical ophthalmic

Evaluation criteria:

Effectiveness:

• Primary

º Clinical remission of acute bacterial conjunctivitis on day 8, absence of:

- Conjunctival secretion
- Bulbar conjunctival hyperemia

• Secondary

o Bacterial eradication, on day 8, in the cultures of patients with positive basal cultures.

o Degree of conjunctival hyperemia during visits

o Degree of conjunctival secretion during visits

o Overall researcher's rating of clinical changes and symptoms during visits

Security:

- Adverse events (EA)

Statistical methodology:

The result of the continuous quantitative variables will be presented in measures of central tendency.

The normal distribution of the results will be obtained through the Kolmogorov-Smirnov and Shapiro-Wilks test. The statistical analysis of the continuous quantitative variables to find significant differences (p) will be in the analysis between groups: t test for independent groups.

2. Content.

Index of tables, figures and illustrations

3. Index of abbreviations.

AV	Visual acuity
BPC	Good clinical practices
IEC	Research Ethics Committee
CI	Informed Consent
CRF	Case Report Form (Case Report Form)
CV	Visual capacity
EA / EAS	Adverse event / serious adverse event
FDA	Food and Drug Administration (Food and Drug Administration)
ICH	International Conference on Harmonization (for its acronym in English International Conference on Harmonization**)**
IP	Principal investigator of the clinical study
PIO	intraocular pressure
VDF	Verification of source documents

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4. Administrative structure of the study.

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

Function	Contact/ name	Membership ¥
Medical responsible for the study	Dr. Leopoldo Martín Baiza Durán leopoldo.baiza@sophia.com.mx	Medical Director and Regulatory Affairs
Director of the study	QFB. Francisco García Velez francisco.garcia@sophia.com.mx	Clinical Operations Manager
Scientific Comittee	Dr. Oscar Olvera Montaño oscar.olvera@sophia.com.mx	Ophthalmologist Investigator
Scientific Comittee	Dr. en C. Patricia del Carmen Muñoz Villegas paticia.muñoz@sophia.com.mx	Biostatist
Scientific Comittee	Dr. en C. Ricardo Alonso Llamas ricardo.llamas@sophia.com.mx	Clinical Pharmacologist

¥ Employees of Sophia Laboratories, S.A. of C.V Av. Paseo del Norte No.5255, Col. Guadalajara Technology Park, Carretera Guadalajara-Nogales Km13.5 C.P. 45010 Zapopan, Jalisco, Mexico Tel +52 (33) 3000 4200

Table 1. Administrative structure.

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5. Introduction

5.1 Theoretical framework

Conjunctivitis, or inflammation of the conjunctiva, is a general term that refers to a group of diseases / disorders that mainly and primarily affect the conjunctiva, differentiating from those diseases in which the inflammation of the conjunctiva is secondary to other diseases. [1]

Conjunctivitis is a very common disease, which can affect any age group, presenting a classic semiology, more non-specific, such as conjunctival hyperemia, secretion, lachrymation, foreign body sensation, pain and pruritus among others. [1] It is estimated that acute conjunctivitis affects 6 million people annually in the United States of America. [2]
Due to its etiology, conjunctivitis is classified as infectious or non-infectious. Among the non-infectious are the allergic, the irritative (mechanical or toxic), the mediated by immunity and the neoplastic. The causes of infectious diseases include viruses and bacteria. [1]

The bacterial conjunctivitis is divided in turn, according to the course and severity, in hyperacute, acute and chronic. Hyperacute bacterial conjunctivitis is characterized by an abrupt onset, with purulent yellow-green discharge, profuse and thick, mixed ocular injection and chemosis, sometimes accompanied by the formation of an inflammatory membrane. [3] [4] Its most frequent etiological agent is Neisseria gonorrhoeae, and can cause corneal lesions, perforations and even endophthalmitis if not treated in a timely manner. [5]

In chronic bacterial conjunctivitis, the clinical picture lasts at least 4 weeks, with frequent relapses. Conjunctival hyperemia and secretion are mild. Coagulase positive and negative staphylococci are the most common organisms involved. [3]

Globally, the most common presentation of bacterial conjunctivitis is acute. Some variations in the presentation of cases of acute bacterial conjunctivitis have been described, showing a peak in the period from December to April in the northern hemisphere, corresponding to winter, while viral conjunctivitis has its peak in the summer months and conjunctivitis. allergic during the spring. [6] [7] [8] It is estimated that in developed countries, 1-4% of general medical consultations have the red eye as a reason for consultation, and that most of these are diagnosed as acute bacterial conjunctivitis. [9] [10] [11] [12] [13] In countries such as Norway, it has been suggested that in 30 out of every 1000 patients who attend a general medical consultation, there is a diagnostic impression of acute bacterial conjunctivitis, this being confirmed diagnosis in 2 of 3 cases. [5] Epidemiological data on bacterial conjunctivitis tend to differ among authors, due in large part to the fact that most cases of conjunctivitis are treated at the first level of medical care, and it has been suggested that general practitioners diagnose conjunctivitis bacterial. [5]

5.1.1 Etiology of bacterial conjunctivitis.

In healthy subjects, it is common for microorganisms to be isolated from their conjunctiva. The conjunctiva under normal circumstances presents a flora with bacterial colonization and transient or recurrent bacterial contamination. Colonization means the stable presence of microorganisms, in balance with host defense mechanisms; while the contamination represents microorganisms introduced from sources external to the conjunctiva. [14] Most of the microorganisms present, in a healthy conjunctiva, are due to transient or recurrent contamination, true colonization is more common in the palpebral margins. Given the right conditions, any microorganism can cause an

infection. A primary pathogen consistently produces infections, opportunists require that the individual's immunity be compromised, while most microorganisms act as incidental pathogens, that is, they replicate and cause disease when the host's defense mechanisms are affected they see compromised. [15]

In samples of healthy conjunctiva, it is common to isolate coagulase-negative staphylococci and corynebacteria, and it has been reported that in normal, non-inflamed conjunctiva, coagulase-positive staphylococci, streptococci, haemophilus, moraxella, and gram-negative bacilli have also been isolated, which are organisms traditionally pathogenic [16] [17] [4]

The most frequent causative agents of bacterial conjunctivitis are: S. aureus, S. epidermidis, H. influenzae, S. pneumoniae, S. viridians, Moraxella catarrhalis and gram-negative intestinal bacteria. [4] Varying in its presentation, according to climatic, social, hygienic and age conditions.

However, several studies have shown a high frequency of negative bacteriological cultures in patients with classical symptoms of acute bacterial conjunctivitis, reporting up to 22-68% of negative cultures. [18] [19] [20] [21] The variation in this frequency, has been tried to explain by the different interpretations that the authors give the results of the cultures.

5.1.2 Clinical picture of acute bacterial conjunctivitis

The symptoms of acute bacterial conjunctivitis can present a wide spectrum of signs and symptoms, and the severity of these. However, classically, the onset of bacterial conjunctivitis with a unilateral foreign body sensation, secretion and conjunctival hyperemia is described. Symptoms can become bilateral in one or two days. The secretion evolves to be more copious and purulent, the "sticky eyes" in the morning are a very common symptom. In some patients the condition is accompanied by pain or burning. Although these symptoms are generally accepted as typical of acute bacterial conjunctivitis, they have not been classified as diagnostic criteria based on evidence for the differentiation between bacterial and viral conjunctivitis. [22]

Conjunctival hyperemia is the rule, being more intense in the peripheral bulbar conjunctiva and less intense towards the limbus. In more severe cases, conjunctival hyperemia can be totally generalized. The secretion can occur throughout the palpebral fissure, or only in the cul-de-sac or as yellowish incrustations on the eyelid margin. [5]

In patients diagnosed with bacterial conjunctivitis, conjunctival hyperemia has been reported in all cases, secretion in 85-90% of cases, foreign body sensation in 90%, and 50% with various degrees of burning or pruritus.[23]

5.1.3 Treatment of acute bacterial conjunctivitis.

Acute bacterial conjunctivitis is a disease with a good prognosis and self-limiting in at least 60% of cases in non-immunocompromised adults. [11] The use of antibacterial therapy is associated with an earlier clinical and bacterial remission, compared with placebo on days 2 and 5 of treatment. These advantages over placebo remain on days 6 and 10, but tend to decrease with time extension. [1]

The choice of antibiotic is usually made empirically. Because a course of 5-7 days with broad spectrum antibiotic is sufficiently effective, you can choose the option that is most convenient; there is still no clinical evidence to suggest the superiority of any particular antibiotic. [1] [24]

Classically, chloramphenicol has been the most widely used antibiotic for the treatment of bacterial conjunctivitis, nevertheless, due to its adverse effects other safer alternatives have been preferred. Being drugs such as tobramycin and fluoroquinolones the most chosen as first-line drugs. The table shows the evidence of the use of topical antibiotics for the treatment of acute bacterial conjunctivitis. Sometimes an anti-inflammatory can be added to decrease symptoms more quickly. [1] [24]

Table 2. Antibiotic treatment of acute bacterial conjunctivitis.

Diagnosis	**Epidemiology**	Type of secretion	**Cause**	**Treatment**	**Level of evidence**
Acute bacterial conjunctivitis	135 cases per 10,000 hab.	Purulent	*S. aureus* *S. epidermidis* *H. influenza* *S. pneumoniae* *S. viridans* *Moraxella*	Aminoglycosides	
				Gentamicin	B
				Tobramycin	A
				Fluoroquinolones	
				Besifloxacin	A
				Ciprofloxacin	A
				Gatifloxacin	B
				Levofloxacin	B
				Moxifloxacin	A
				Ofloxacin	A
				Macrolides	
				Azithromycin	A
				Erythromycin	B
				Sulfonamides	
				Sulfacetamide	B

Modified from Azari AA. [25]

All the guidelines recommend a responsible and non-indiscriminate use of antibiotics and anti-inflammatories.

5.2 Definition of the problem and fundamental reason

It is estimated that in the treatment of bacterial conjunctivitis an expense of $ 377 to $ 857 million dollars is made. [25] Without counting the socio-economic burden that the acute stage of the disease produces on the individual, reflecting with work or school absenteeism and a decrease in productivity.

Although it is accepted that acute bacterial conjunctivitis has a self-limiting course and a favorable prognosis, antibiotic treatment provides an earlier clinical remission of the disease and theoretically prevents the appearance of more serious complications such as keratitis.

Currently, there is a wide variety of antibiotics in ophthalmic solution, from different families, such as macrolides, aminoglycosides, sulfas and fluoroquinolones. Many of these medications are also used in more serious infections, so development of bacterial resistance is highly undesirable. [5]

The pazufloxacin, active ingredient of PRO-157, is a fourth-generation fluoroquinolone that has not been marketed for ophthalmic use. Currently, up to 26% resistance to the 4th generation quinolones (moxifloxacin, gatifloxacin, and levofloxacin) is reported in strains of methicillin-resistant S. epidermidis [26], so pazufloxacin represents a treatment option.

5.3 Background

Among the therapeutic options for bacterial conjunctivitis, there are quinolones. [27] The mesolate of pazufloxacin, is a member of this family, nevertheless, it differs in chemical structure since it was added an amino group in carbon 10, which increases its pharmacological potency and allows it to expand its antimicrobial spectrum. [28]

The pazufloxacin, is currently marketed in Japan in injectable form, for the treatment of respiratory infections with a broad spectrum and a powerful activity. [29] Sophia Laboratories, S.A of C.V has created a drug in ophthalmic presentation that contains pazufloxacin as an active ingredient, and its clinical development program has shown favorable results in phase I and phase II studies.

5.3.1 Pazufloxacin

The fourth generation fluoroquinolone pazufloxacin is a fused tricyclic quinolone, with a 1-aminocyclopropyl substitute at the C10 position, unique property of the molecule that contributes to the broad spectrum and potent activity of this drug. [30] Originally developed for systemic use in the treatment of respiratory tract infections. [29]

5.3.1.1 Pharmacodynamics

Pazufloxacin has demonstrated a multimodal mechanism of action and inhibits both the DNA gyrase enzyme and the topoisomerase IV enzyme, increasing the antibacterial spectrum. In addition, pazufloxacin has also shown antagonistic DNA actions; The mechanism of multimodal action is linked to the potential for the development of resistance in pazufloxacin and it has been confirmed that it is not affected by said mechanism. [28]

5.3.1.2 Eyeball pharmacokinetics.

Route of administration: Ophthalmic.

Release: immediate.

Absorption: At the ocular level it is easy to penetrate the cornea and concentrations of said quinolone have been detected in aqueous humor. One of the characteristics of this molecule is its ability to penetrate intracellularly, which favors its bactericidal action. [31]

Metabolism: The quinolones may undergo different metabolic reactions such as glucuronidation, hydroxylation and oxidation depending on the participating drug. In general terms, the metabolites have less antibacterial activity than the compounds of origin. [31]

Elimination: It is mostly eliminated by the kidney, by the mechanism of tubular secretion, some of the metabolites may undergo entero-hepatic circulation. [31][17]

5.3.1.3 Preclinical studies

Sophia Laboratories has carried out studies in animal models to evaluate the safety and bioavailability of pazufloxacin. Other studies have been reported in systemic application,

nevertheless we will only show those of ophthalmic route, for more information you can refer to the manual of the researcher of PRO-157.

In a study conducted in New Zealand albino rabbits, the safety and toxicity of PRO-157 after its ophthalmic application was evaluated, compared to the ophthalmic solutions of moxifloxacin 0.6% (Vigamoxi®, Alcon Laboratories) and gatifloxacin 0.3% (Zymar®, Allergan Inc.) The intervention consisted in the application of 1 drop in the bottom of the sac of both eyes, of the products under investigation, 4 times a day, for 30 days. Thirty rabbits were included in the study, 10 per group. Visits were made on days 2, 7, 10, 15, 20, 25 and 30; on day 31 the animals were sacrificed and the eyes were enucleated for the histopathological analysis of the cornea, conjunctiva and retina. The safety variables to be evaluated were conjunctival hyperemia, secretion, intraocular pressure, corneal staining (lysine and fluorescein green) and the presence of adverse events (AD). Only alterations in the stains were reported from day 10 in the groups of PRO-157 and moxifloxacin, nevertheless there were no statistically significant differences; no changes were found in the PIO. The toxicity was evaluated by histopathological analysis of cornea, conjunctiva and retina, which showed no alterations in any of the groups. The authors of the study concluded that the use of PRO-157 is equally safe and non-toxic, following their ophthalmic in albino rabbits New Zealand, than the use of the ophthalmic solutions of moxifloxacin 0.6% and gatifloxacin 0.3%. [32]

Sophia Laboratories, S.A. of C.V. also conducted a distribution study in the eye of albino New Zealand rabbits, with the PRO-157. Ninety-six rabbits were included in the study, which were divided into three groups corresponding to the investigational drugs: PRO-157 (n = 32), Vigamoxi® (n = 32) and Zymar® (n = 32). Each group was divided into 8 subgroups, which corresponded to the sampling time points (15min, 1h, 3h, 4h, 6h, 8h, 12h, and 24h). After the application of a dose of 30µl, of the corresponding investigational product, in bottom of bag, in both eyes, the rabbits were sacrificed at the determined point of time and samples of aqueous humor, conjunctiva and cornea were taken. These were later analyzed by high performance liquid chromatography. The results are shown in the figures: Figure 1, Figure 2 and Figure 3. [33]

An adequate penetration of PRO-157 was observed in conjunctiva, cornea and aqueous humor, after the application of a dose on the ocular surface. The Tmax was 15 minutes for cornea and conjunctiva, and 1 hour for aqueous humor. Showing behavior similar to that of the other fluoroquinolones evaluated.

Figure 1. Pharmacokinetic profile, in aqueous humor, of PRO-157, Vigamoxi and Zymar.

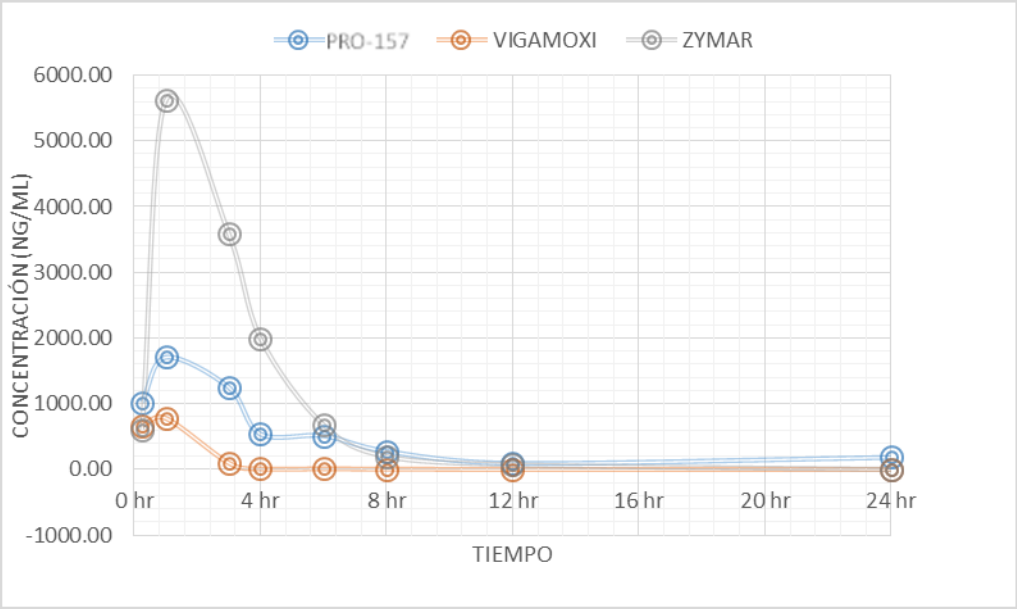


Figure 2. Pharmacokinetic profile, in cornea, of PRO-157, Vigamoxi and Zymar.

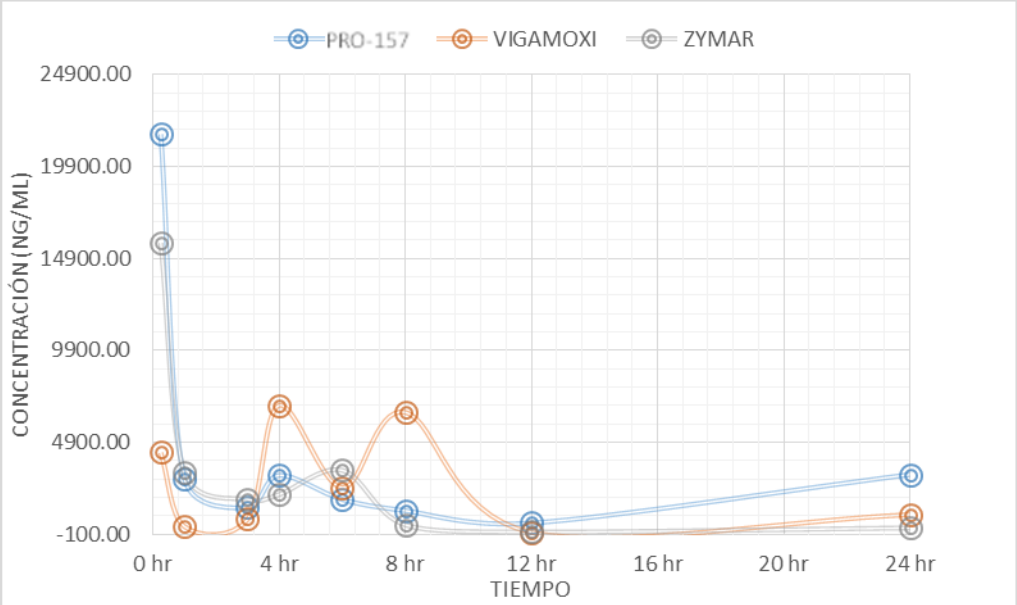
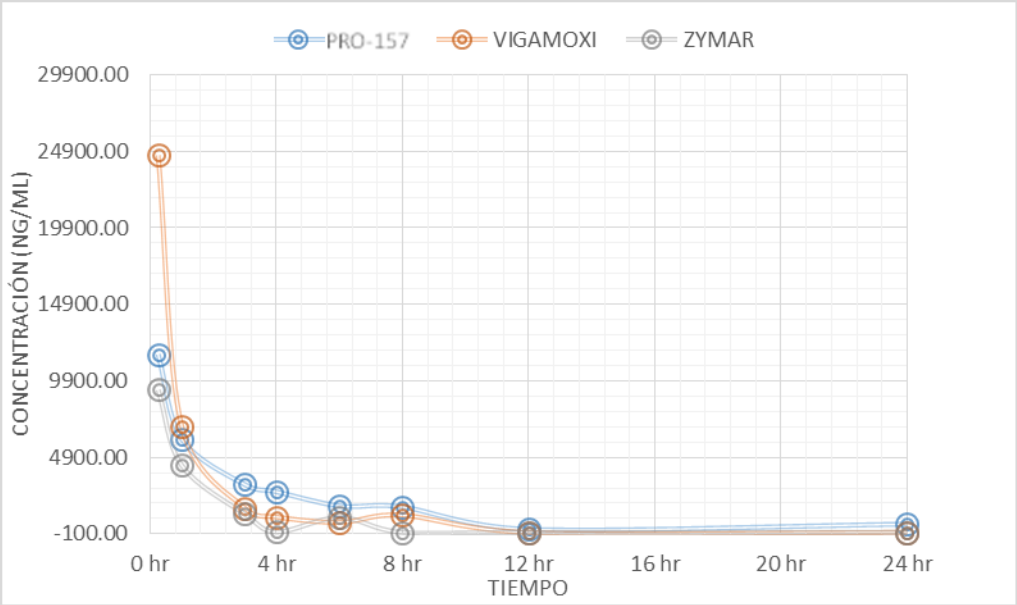


Figure 3. Pharmacokinetic profile, in conjunctiva, of PRO-157, Vigamoxi and Zymar.



5.3.1.4 Clinical safety studies.

Studies have been reported in systemic application, nevertheless we will only show those of ophthalmic route, for more information of clinical safety studies by other administration routes it can refer to the manual of the researcher of PRO-157.

In a phase I study, carried out in 30 healthy subjects, PRO-157 was applied 4 times a day (every 6 hours), for 10 days. There were 3 follow-up visits on days 2, 4 and 7, and a final visit on day 11. The primary safety variables were: visual acuity, ocular surface staining with fluorescein and lysamine green, PIO and presence of EA. The tolerability variables were burning, hyperemia, chemosis, foreign body sensation, lacrimation, pruritus and secretion. In the safety variables there were no changes between the baseline and the final visit, there were no adverse events. Regarding tolerability, burning was the most common symptom in all visits, reporting as mild in 48-70% of cases, the highest incidence of burning reported as severe was on day 2, with 27% of cases. [34]

5.3.1.5 Clinical efficacy studies.

Studies have been reported in systemic application, nevertheless we will only show those of ophthalmic route, for more information on efficacy clinical studies by other routes of administration, refer to the manual of the researcher of PRO-157.

In a phase II, multicentric, double masked, randomized and active control study, the efficacy, in the treatment of bacterial conjunctivitis, of the PRO-157 formulation (pazufloxacin 0.6%) was tested in 3 different posologies (twice per day, three times a day and 4 times a day), compared to the ophthalmic solutions of moxifloxacin 0.6% (Vigamoxi®, Alcon Laboratories) and gatifloxacin 0.3% (Zymar, Allergan Inc.) 3 times a day. A total of 300 eyes with a clinical diagnosis of bacterial conjunctivitis were included in the study. The intervention lasted 7 days. The efficacy variables were the clinical remission of the disease and bacterial eradication on day 7. The instillation of 1 drop 3 times a day of PRO-157 for 7 days, is the dose that showed greater efficacy in relation to the

comparators. Nevertheless, this difference did not have statistical significance among the groups based on bacterial eradication. From the clinical perspective, the main difference was observed in the decrease of conjunctival hyperemia and chemosis, which was absent at the end of the intervention in the group with the indicated posology. In the overall clinical efficacy a statistical difference was observed between the group that was applied 1 drop 3 times a day vs 1 drop 2 times a day. Regarding tolerability, the main symptoms were burning, foreign body sensation and pruritus; which are difficult to distinguish if they are due to the physico-chemical characteristics of the molecule or inherent to the chosen disease model. Nevertheless, no differences were observed in these parameters between the groups. [35]

5.3.1.6. Safety and tolerability.

Ophthalmic fluoroquinolones have a similar safety and tolerability profile, being generally well tolerated. Burning and discomfort post-stuttering is the most reported discomfort. With the use of PRO-157, the presence of white crystalline deposits, related to the use of other topical fluoroquinolones such as ciprofloxacin, has not been reported.

In the clinical studies carried out with PRO-157, conjunctival hyperemia and foreign body sensation have been reported after burning. Symptoms that have been reported as transient.

5.4 Justification

PRO-157 is a 0.6% pazufloxacin formulation in ophthalmic solution, developed by Sophia Laboratories, S.A. of C.V. which has demonstrated an acceptable safety profile in preclinical and clinical phase I studies, a bioavailability, in animal models, similar to that of other fourth generation fluoroquinolones and efficacy in the treatment of bacterial conjunctivitis in a phase II clinical study. With this background, a phase III clinical study is required to verify the efficacy profile of PRO-157.

Complying with the clinical development plan of a new fourth generation fluoroquinolone will allow Sophia Laboratories, S.A. of C.V. make available to physicians a safe and effective alternative for the treatment of ocular surface infections. In the context of increasing bacterial resistance, to the antibiotics currently available, this new option may prove valuable. Notwithstanding, because the present study aims to demonstrate the efficacy in the treatment of bacterial conjunctivitis, it would provide a foundation for future studies where the indication for more serious pathologies, such as keratitis and intraocular infections, can be extended; this based on the bioavailability behavior demonstrated in preclinical studies.

The outcome variable of primary outcome is the clinical remission of the disease. Understanding that in the clinical context of the treatment of this disease, empirical treatment with a broad-spectrum antibiotic is common, due to the nature of the disease (how quickly it can be resolved in a natural course); reserving the use of cultures for neonatal conjunctivitis, recurrent, resistant to initial treatment and in suspicion of gonococcal infection (bacterial conjunctivitis hyperacute) or chlamydia. [1] Given this context, resembling clinical practice, is that the present study is proposed. Notwithstanding, the crop is considered as a scientific support for diagnosis and bacterial eradication as a secondary outcome point.

5.5 Objectives and hypothesis.

5.5.1 General purpose.

To compare the efficacy of the ophthalmic solution of pazufloxacin 0.6%, against the ophthalmic solution of gatifloxacin 0.3%, in the treatment of acute bacterial conjunctivitis.

5.5.2 Specific objectives.

- To evaluate the efficacy of PRO-157 by the clinical remission of acute bacterial conjunctivitis on day 8, through the absence of conjunctival secretion and bulbar hyperemia.
- To evaluate the efficacy of PRO-157 by decreasing the degree of hyperemia and conjunctival secretion during visits.
- To evaluate the efficacy of the ophthalmic solution of gatifloxacin 0.3%, by means of the clinical remission of acute bacterial conjunctivitis on day 8, by means of the absence of conjunctival secretion and bulbar hyperemia.
- To evaluate the efficacy of the ophthalmic solution of gatifloxacin 0.3%, by decreasing the degree of hyperemia and conjunctival secretion during visits.

5.5.3 Secondary objectives.

- To evaluate the efficacy of PRO-157 by bacterial eradication with respect to baseline, on day 8.
- To assess the efficacy of PRO-157 by decreasing the researcher's overall rating of clinical changes and symptoms during visits.
- Evaluate the safety of PRO-157 through the presence of EA.
- To evaluate the efficacy of the ophthalmic solution of gatifloxacin 0.3%, by bacterial eradication with respect to the baseline on day 8.
- To evaluate the efficacy of the 0.3% gatifloxacin ophthalmic solution, by decreasing the researcher's overall rating of clinical changes and symptoms during visits.
- To evaluate the safety of the ophthalmic solution of gatifloxacin 0.3%, through the presence of EA.

5.5.4 Hypothesis.

H0I: The ophthalmic solution PRO-157 is not inferior in the treatment of bacterial conjunctivitis, compared to the ophthalmic solution of gatifloxacin 0.3%, by means of the clinical remission of the disease.

Ha: The ophthalmic solution PRO-157 is inferior in the treatment of bacterial conjunctivitis, compared to the ophthalmic solution of gatifloxacin 0.3%, by means of the clinical remission of the disease.

5.6 Design and plan of the study.

Phase III clinical study of noninferiority, multicenter, double blind, with comparative group, of parallel groups, with randomization. Patients with clinical diagnosis of acute bacterial conjunctivitis will participate, who will be randomly assigned to 2 intervention groups. One group will receive the study formulation PRO-157 and the other group will be exposed to gatifloxacin 0.3%.

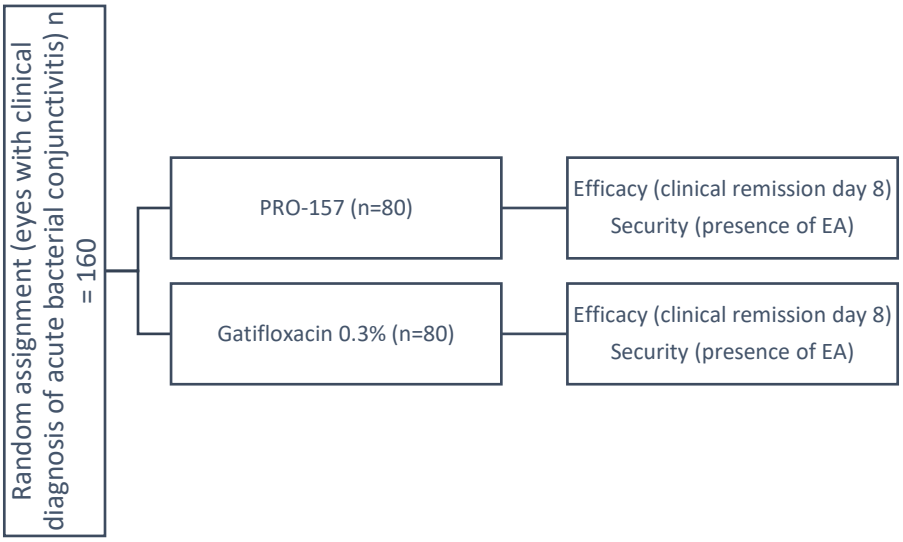


Figure 4. Study design.

5.6.1 Discussion of the study design.

In clinical research it is common to propose trials with the objective of evaluating the efficacy of a product under investigation against a reference medicine or active control, in order to compare the new product under investigation from a clinical or statistical point of view to a drug commercially available

The clinical trial is the ideal model to evaluate the effectiveness of two interventions, it allows obtaining the highest quality evidence among different types of research. The characteristics of randomization and double blind, allow to avoid biases (selection, evaluation, etc.) that can not be avoided with other models. Being controlled and parallel groups allows distinguishing the effects of interventions in isolation.

The proposed clinical trial aims to evaluate the efficacy and safety profile of a 0.6% pazufloxacin formulation and compare it against the efficacy and safety profile of the administration of a commercially available fluoroquinolone widely used for its known efficacy and safety profile.

6. Material and methods. Participants, interventions and variables.

6.1 Study Center.

The present study will be performed in ophthalmology offices duly equipped and registered for their proper functioning. According to the needs of the sponsor, these may be private or public, be attached to a hospital or clinic or be independent.

This is a multicentric study that is intended to be carried out in the Mexican Republic. It is foreseen, primarily to open centers in the cities of Guadalajara, Monterrey and Mexico City; not excluding, that according to the needs of the sponsor, other centers may be required within the Republic.

6.1.1 Organization of the center.

Each study center will have a principal investigator (IP). The IP is the ophthalmology specialist in the clinical study.

The IP is responsible for forming a multidisciplinary research team to carry out the clinical study according to protocol, under its scientific guidance. It is the prerogative of the IP the design of the organization of its center and the selection of the personnel that will perform the functions. Notwithstanding, the minimum organization of the research team requested by the sponsor requires the figure of sub-researcher, study coordinator and pharmacist.

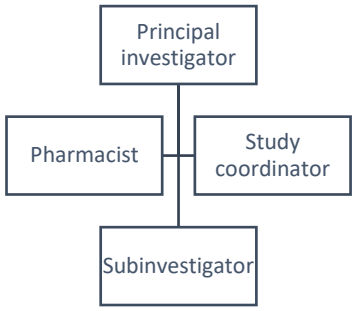


Figure 5. Minimum organization of the center

Any person to whom the IP designates, under his / her responsibility, a part of the follow-up of the study (co-investigator, sub-researcher, nurse, etc.) or a specific function of participation in the study (pharmacist, administrative assistant, study coordinator, etc.) It must appear in the "Delegation of Responsibilities" format.

The "Delegation of Responsibilities" and the "Organizational Chart of the Center" must be delivered to the sponsor before the start of the study and updated if the members or their responsibilities are changed.

6.1.2 Documentation to be delivered to the sponsor.

The IP must deliver to the sponsor, before the start of the study:

- Curriculum vitae updated, in Spanish, dated and signed (maximum 10 pages), of the IP and the staff that integrates its organizational chart of the center.

- Copy of IP academic certifications (degree certificate and specialty diploma in ophthalmology, federal professional certificates).

- Copy of academic certifications of the maximum degree obtained, from each one of the members of your research team, that cover their capacity to perform the delegated functions.

- Copy of operating notice or similar issued by corresponding regulatory entity (When applicable)

- Certificate of good clinical practice in force. If the issuing institution does not specify the validity period in the certificate, the date of issue of the certificate must not exceed one year

- Letter of declaration of interests of the IP and subinvestigador.

6.1.3 Closure of the center.

The closing of the center will be carried out once the last visit of the last included subject previously agreed between the sponsor and the IP has been made. The closing process will be according to the internal operating procedures of the sponsor.

It is the sponsor's prerogative to prematurely close a study center, it must inform the IP the reasons for the closure.

6.2 Eligibility criteria.

6.2.1 Inclusion criteria.

-Signed informed consent.

-Age ≥ 1 year.

-Both genders.

-Clinical picture of acute bacterial conjunctivitis defined by:

- º Conjunctival secretion
- º Conjunctival bulbar hiperemia

6.2.2 Exclusion criteria.

6.2.2.1 General criteria.

I. Pregnant women, lactating or planning to become pregnant.
II. Women of reproductive age and who do not have a hormonal contraceptive method, intrauterine device or bilateral tubal obstruction.
III. Participation in another clinical research study ≤ 30 days before the baseline visit.
IV. Previous participation in this same study.
V. That they can not comply with their attendance at appointments or with all the requirements of the protocol.

6.2.2.2 Medical and therapeutic criteria.

I. Single eye
II. Presence of corneal abrasion or corneal ulceration in the study eye.
III. History Users of contact lenses who are not willing to suspend their use during the study.
IV. Users of any formulation with ophthalmic application, including lubricants, that can not, or do not want to suspend it during the study.
V. Antecedents of eye surgery 6 weeks prior to study entry.
VI. Viral or allergic conjunctivitis.
VII. Active uveitis.
VIII. Active ulcerative keratitis.
IX. Recurrent corneal erosion syndrome
X. Antecedent of hypersensitivity or allergy to fluoroquinolones.

6.2.3 Elimination criteria.

I. Subject's decision. The subject may decide unilaterally to withdraw from the study at any time.
II. Pregnancy.
III. Presence of a serious adverse event (EAS), including the presence of hypersensitivity.
IV. Investigator's decision:

a. Due to the presence of an EA that, although not serious, at the discretion of the IP merits the prescription of a medicine not authorized in the protocol.

V. Any deviation to the protocol that affects the security of the subject.

6.2.4 Identification of the subject.

The patients of the study will be identified by a number and the initials of their name.

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

Example*:*

1. Adolfo Daniel Mercado Carrizales

a. a. Initials: AMC

2. Juan De la Torre Orozco

a. Initials: JDO

3. Luis Carlos Pérez-Gómez Ramírez

a. Initials LPR

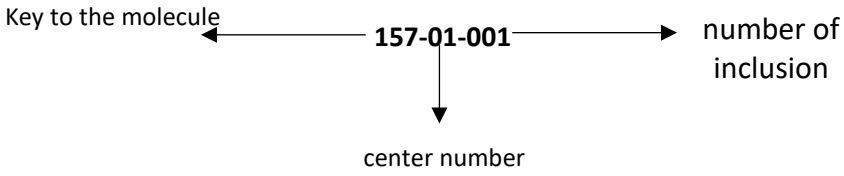
In the counting stage, the participant number will be assigned consecutively, using 3 consecutive digits. Once the subject has been selected, he will be assigned a number with which he will be identified throughout the study. Said code will be composed of eight numbers in the following order from left to right:

• Three digits of the molecule under study according to the denomination by the sponsor.

• Two digits corresponding to the research center number

• Three digits of the number consecutive to its inclusion assigned in the research center.

Example*:*



6.3 Intervention.

6.3.1 Managed treatments.

The treatments will be administered three times a day, at approximate intervals of 6 hours during the waking period, for 7 days. The interventions will be applied in both eyes. Independently that only one eye will be provided for the efficacy analysis.

6.3.1.1 Treatment in study.

- PRO-157
  - Active ingredients: Pazufloxacin 0.6%
  - Pharmaceutical form: ophthalmic solution.
  - Prepared by: Sophia Laboratories, S.A. of C.V.
  - Dosage: 1 drop 3 times a day for 7 days both eyes

- Description of the solution: transparent colorless solution.
- Description of container: sterile multi-dose bottle.
- The product has certificates of sterility and stability, which are in the master folder of the study, and its characteristics are included in the researcher's manual.

Table 3. Quali-quantitative formulation of PRO-157.

Active principle	**Quantity mg / dL**	**%**	**Function**
Pazufloxacin	6	0.6	Antibiotic
Additives	**Quantity**	**%**	**Function**
Sodium chloride	Not shown	Not shown	Osmotic agent
Disodium edetate dihydrate	Not shown	Not shown	Chelating agent
Water for cbp injectable preparation (1)	Not shown	Not shown	Vehicle

Qualitative composition of the formulation PRO-157. The agents that constitute the active substance and the additives are shown. Pharmaceutical form: emulsion. (1) How much is enough to.

6.3.1.2 Reference treatment.

- Zymar®
  - Active ingredients: Gatifloxacin 0.3%
  - Pharmaceutical form: Ophthalmic solution
  - Prepared by: Allergan, S.A. of C.V.
  - Dosage: 1 drop 3 times a day for 7 days both eyes.
  - Description of the solution: transparent, crystalline solution.
  - Description of the container: multi-dose dropper bottle.
  - The data on characterization and stability of the reference product will be supported by its batch number and the manufacturer's insert.

6.3.2 Strategies to improve adherence and procedure to monitor adherence.

Strategies:

- • At each visit, the IP reaffirms in the research subject the importance of following the indicated regimen and will ask if it has had any inconvenience in following the indications, if necessary it will retrain the subject in the application of the medications.
- • In case the IP considers it necessary, the subjects that have an email, the IP or the person designated for this, will send them emails to remember the adherence to the treatment and the importance of it. The content of the emails will be previously submitted to the research ethics committee for approval.
- • By means of the revision of the daily tool of the subject.

Procedure to monitor adherence:

- • The evaluation of the adherence by means of the diary of the subject will be carried out in the following way:

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

Where:
Ad = Adherence
Ar = registered applications.
Ai = Indicated applications.

Only subjects with a minimum adherence of 80% by means of the subject's daily tool will be considered for efficacy analysis.

6.3.3 Treatments and concomitant interventions allowed and prohibited before and during the study.

The subjects admitted correctly to the study, who meet the eligibility criteria, may continue with the systemic treatment of the underlying diseases. If during the development of the study they require the implementation of a new permitted medication, they may do so. All concomitant medications used must be duly reported in the notes of the clinical file and in the corresponding section.

Allowed medications:

• Ophthalmic:

All permitted medications applied via ophthalmic during the study, must wait a minimum period of 10 minutes from the last application of the treatments under study or reference. The above in order to avoid the interaction of treatments in the tear film, based on the tear flow index and the physiological tear volume. [36]

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

• Administered by a route other than ophthalmic:

o Medicines whose effect may be susceptible of modifying any of the parameters of efficacy, safety or tolerability of this research protocol must notify their clinical monitor or the scientific committee of the sponsor to judge the convenience of entry, continuation or elimination of the participant according to correspond

Any medication allowed that is used, besides appearing in the clinical note, must be registered in the section of concomitant medications in the CRF.

Prohibited drugs:

- - Any medication with ophthalmic application that is not on the list of allowed medications. The rest period prior to entering the study is according to the table.

Table 4. Rest period of medications with ophthalmic application.

Type of medication	Example	Rest period
Ophthalmic anti-inflammatories (Steroids and NSAIDs)	Diclofenac Dexamethasone	48 hours
Ophthalmic antibiotics	Ciprofloxacin Tobramycin	24 hours
Any other ophthalmic medication, including lubricants	Hipromellose Timolol	2 hours

6.3.4 Treatment management.

The treatments will be provided by Sophia Laboratories, S.A. of C.V., for each research center. They will be labeled and reconciled. The handling of the treatment will be under the responsibility of the IP or a designated member of his team.

6.3.4.1 Delivery and reception.

The sponsor will be responsible for delivering the study treatments at the research center according to internal procedures. The delivery will be made in closed boxes by means of a courier service or directly by the sponsor's staff to the address of the research center according to the study plan.

The reception will be exclusively carried out by the team of the research center, by the pharmacist. You should check the good condition of the primary packaging (box). In the event that it shows alterations or defects in its integrity that from its judgment could have damaged the content, it should 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 logger (data logger) of temperature and humidity. You should check that the registered temperature and humidity comply with the specifications for transport and shelter (see 6.3.4.2 Storage). Subsequently verify the content (treatments) with what is reported in the document. In case the document corresponds to the content, it will sign the receipt and send it to the sponsor. Otherwise, notify the sponsor.

In the study center, the pharmacist will deliver the corresponding treatment to the subjects admitted. The center must register the medicine delivered.

6.3.4.2 Storage .

The medication must be stored in a secure area with restricted access. Storage should be at room temperature to no more than 30 ° Celsius.

The research center has the obligation to record, in the format designated by the sponsor, the temperature and humidity registered in the data logger. This record should include the current temperature and humidity, as well as the minimum and maximum of each of these. It must be done at least once a day, on business days.

Said data will be compared by the clinical monitor according to the registration in the data logger.

6.3.4.3 Return .

The research subjects will return, to the personnel indicated by the IP in the center, their treatments in the final visit.

The refund will be made by the research center when the sponsor indicates it. Prior to the return the research center must make a count of the assigned medication and the remaining medication, with the aim of creating an inventory which serves for the final filling of the medication return form.

6.4 Outcome variables.

6.4.1 Security variables.

- Adverse events

6.4.2 Efficacy variables.

6.4.2.1 Primary outcome variables.

• Clinical remission of acute bacterial conjunctivitis on day 8, defined by the absence of:
- Conjunctival secretion
- Bulbar conjunctival hyperemia.

6.4.2.2 Secondary outcome variables.

• Bacterial eradication, on day 8, in the cultures of patients with positive basal cultures. Bacterial eradication will be defined as the absence of bacterial species that were present in the culture of the baseline visit.
• Degree of conjunctival hyperemia during visits
• Degree of conjunctival secretion during visits
• Overall investigator rating of clinical changes and symptoms during visits.

6.4.3 Methods and scales to be used for the measurement of the variables.

The variables and their units of measurement are included in **Table 5 Method of measuring variables .**

Variable	Unity	Symbol	Type	Method of measurement
Overall rating	Degrees	---	C. Ordinal	Direct observation and interrogation
Bacterial eradication	Colony forming units	UFC	C. Continuous	Secretion culture
Adverse events	Number of cases	N	C. Discrete	Count
Conjunctival bulbar hyperemia	Degrees	---	C. Ordinal	Direct observation (Biomicroscopy)
Clinical referral	If not	---	Qualitative rated	Direct observation (Biomicroscopy)

Variable	Unity	Symbol	Type	Method of measurement
Conjunctival secretion	Degrees	---	C. Ordinal	Direct observation (Biomicroscopy)

Table 5 Method of measuring variables.

The following describes the methods and scales that will be used for the measurement of the variables, which are in strict alphabetical order of the procedure:

6.4.3.1 Biomicroscopy

It is the procedure by which, with the use of the slit lamp or biomicroscope, the ocular structures are evaluated thanks to the characteristics of the light source and the optical power of the instrument. A full assessment of the previous segment will be made, which will be recorded in the clinical file.

By means of biomicroscopy **bulbar conjunctival hyperemia, conjunctival secretion will be evaluated and will serve for the evaluation of adverse events and the global qualification**.

Conjunctival hyperemia is defined as the simplest reaction of the conjunctiva to a stimulus, a red appearance secondary to the vasodilation of the conjunctival vessels of variable intensity. He will graduate using the Efron scale. [37] The number will be reported in the CRF, according to the rating granted, in each of the visits. (**See Illustration 1**)

Illustration 1. Efron scale for conjunctival hyperemia.

The ocular secretion will be classified as 0 = absent, 1 = mild, 2 = moderate and 3 = severe. The number will be reported in the CRF, according to the rating granted in each of the visits.

The clinical remission in the visits will be evaluated as "Yes" or "No", to report "Yes" it must have a grade of "0" in conjunctival hyperemia and "0" in secretion; otherwise, you must report as "No".

6.4.3.2 Global qualification of the investigator.

The global qualification of the investigator constitutes the integral judgment of the clinical picture of the subject, including signs and symptoms, after a routine ophthalmological evaluation and interrogation. It will be classified 0 = cure, 1 = improvement, 2 = no changes compared to before starting treatment, 3 = worsened. It will be registered in the CRF in each of the follow-up visits.

6.4.3.3 Evaluation of adverse events.

The evaluation of adverse events requires a questioning conducted by the IP and the appropriate exploratory techniques for its detection.

The IP will register in the corresponding section of the CRF the EA that the subjects of the study will present.

The management of the EAs will be done according to what is described in section 9.3 Adverse events.

6.4.3.4 Sampling of conjunctival secretion for bacterial culture.

The microbiological study of body tissue and fluid samples allows to establish the etiological diagnosis of different infectious diseases. For this reason it is important to guarantee the quality of the sample and the information that must accompany it during the process that begins in the pre-analysis phase, which includes preparation, procurement and transportation, which concludes in the analysis of the sample.

The conjunctival secretion sample will be taken prior to the instillation of any medication (eg tetracaine, tropicamide / phenylephrine) or staining (fluorescein, lysine green) required for ophthalmologic evaluation.

You will use the sample taking kit delivered by the sponsor for this purpose. The kit will consist, as a minimum, of two test tubes and two sterile swabs. Each tube will have a label with the following minimum information:

- Study number: SOPH157-0217 / III
- Initials of the subject
- Subject number
- Visit
- Right Eye or Left Eye (as the case may be)

Use gloves for this procedure. Take a sample with the swab, use a swab per eye. Rotate the swab in the conjunctival cul-de-sac while moving it from the medial to the lateral zone (see Illustration 2). Place the swab in the transport medium (sterile tube with screw cap or rubber stopper). Fill in the required information on the label of each tube. And perform the same procedure on the contralateral eye.

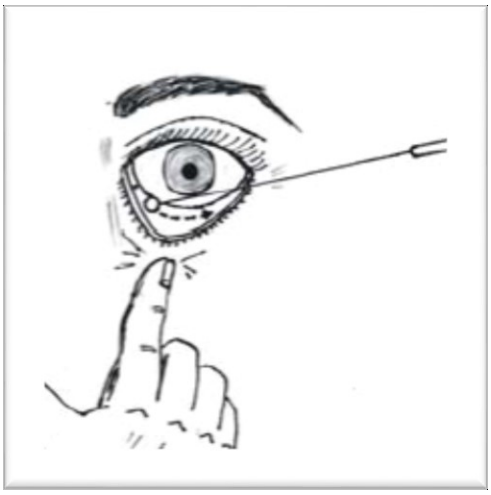


Illustration 2. Conjunctival secretion sampling.

Fill the requisition form delivered by the sponsor and contact the authorized third party for the analysis of the samples, to schedule the collection. The sample should be kept at room temperature.

In the CRF it will register as done, in each one of the eyes. The results will be delivered by the third party authorized to the IP and the sponsor.

Once the result of the basal crop has been obtained, the center will register this result in the CRF. When you obtain the results of the final crop you will register them in the CRF. And it will mark in the box of the CRF the question ¿Bacterial Eradication? If not. The absence of bacterial species that were present in the culture of the baseline visit is considered bacterial eradication.

The authorized third party will be selected by the sponsor and the relevant information will be shared with the centers, which will be in the master folder of the study.

6.4.4 Time of the measurement of variables.

- **Measurements in the baseline visit**
- Bulbar conjunctival hyperemia
- Conjunctival secretion
- Sampling of conjunctival secretion
- **Measurements in visit 1**
- Overall rating
- Adverse events
- Bulbar conjunctival hyperemia
- Clinical referral
- Conjunctival secretion
- **Final visit measurements**
- Overall rating
- Adverse events
- Bulbar conjunctival hyperemia
- Clinical referral
- Conjunctival secretion
- Sampling of conjunctival secretion
- **Security call measurements**
- Evaluation of adverse events

6.5 Schedule and study diagram.

The baseline visit will be considered on day 1 of the study, the IP will indicate the subject to start the application of the products under investigation that same day. If necessary, adjust the scheme so that it complies with the 3 applications of day 1; nevertheless, in the event that during the opening hours it is not feasible to adjust the 3 applications, the IP will indicate the application of at least 2 instillations, with a minimum of 3 hours between them.

Visit 1 will be performed in relation to the start of treatment on day 5 with a window period of ± 1 day. The **final visit** will be made on day 8, with a window period of + 1 day. The **security call** will be made on day 15, with a window period of ± 2 days.

6.5.1 Timeline

Process	B	1	Final	LL. S.
	1	5 ± 1	8 + 1	15 ± 2
Eligibility criteria	X			
Informed consent signature	X			
Pregnancy test (if applicable)	X		X	
General and ophthalmological clinical history	X			
Comprehensive ophthalmologic exploration	X	X	X	
Assignment of subject code	X			
Assignment to treatment group	X			
Delivery of medication and start of instillation	X			
Sampling of conjunctival secretion	X		X	
Biomicroscopy	X	X	X	
Global qualification of the researcher		X	X	
Evaluation of concomitant medications	X	X	X	
Delivery of the subject's diary	X			
Evaluation of adverse events		X	X	X
Continuity evaluation		X	X	
Adherence evaluation (per subject's diary)		X	X	
Return of the medication			X	
Returning the subject's journal			X	

6.5.2 Procedures to be performed per visit.

Next, the procedures that will be carried out in each visit are listed, as well as a brief description of these.

6.5.2.1 Basal visit.

- Eligibility criteria: refers to the review by the IP, where it states that the subject can be included in the study by meeting the inclusion criteria and not meeting the exclusion criteria. See 6.2 Eligibility criteria. The IP selects the eye that will be included for the efficacy analysis, indicating it in the clinical note and in the CRF.
- Signature of informed consent: refers to the signing of the written informed consent document. See 10.3 Consent
- Pregnancy test: refers to the performance of a rapid pregnancy test in all women of age who wish to enter the study. By fertile age we understand women who have not had their menopause, defined as 12 months since the last menstrual period in women over 40 years of age, or who have undergone a bilateral hysterectomy or oophorectomy. Women of childbearing age with contraceptive methods including bilateral tubal obstruction should be tested for pregnancy. This test will be performed by the IP or the designated team person according to the instructions of the device delivered by the sponsor.
- General and ophthalmological clinical history: refers to the technical, clinical and legal document in which the patient's health conditions, medical acts and other procedures performed on the patient are recorded chronologically. It includes the anamnesis and comprehensive ophthalmological exploration that allows to discern the patient's eligibility. If the patient is taken from the established consultation of the study center, he / she will be able to use the existing clinical history, only having to perform an update.
- Conjunctival secretion sampling: see section 6.4.3.4 Conjunctival secretion sampling for bacterial culture.
- Comprehensive ophthalmological exploration: it refers to the evaluation of the ophthalmic structures of the subject, eyelids and appendices, ocular surface, anterior segment and posterior segment; not considered within the outcome variables. This evaluation has the purpose of identifying alterations that may interfere with the course of the investigation or identify adverse events. This scan will be recorded in the clinical file, in the CRF only what is considered an adverse event will be reported.
- Assignment of the subject's code: It refers to granting the number that will identify the patient throughout the study. This will be done according to section 6.2.4 Identification of the subject.
- Assignment to the treatment group: It refers to determining the treatment that the patient will follow during the study. It will be done according to section 7. Methods. Assignment of the intervention.
- Delivery of medication: This refers to the delivery of the medication to the patient of the study, by the research center. It will be done according to sections 6.3.1 Managed treatments and 6.3.4.1 Delivery and reception. After each medication delivery, training or re-training of the application of the study medication will be carried out.
- Biomicroscopy: see 6.4.3.1 Biomicroscopy

- Evaluation of concomitant medications: it refers to the interrogation that the IP of the medications that the patient uses, before, during and after the study. They must be registered in the corresponding section in the CRF, including name of active substance,

posology and duration. See: 6.3.3 Treatments and concomitant interventions allowed and prohibited before and during the study.

- Subject's journal delivery: refers to the delivery by the IP, of the subject's daily instrument, to the subject.

6.5.2.2 Visit 1

- Comprehensive ophthalmological exploration: see 6.5.2.1 Baseline visit.
- Biomicroscopy: see 6.4.3.1 Biomicroscopy
- Global qualification of the researcher: see 6.4.3.2 Global qualification of the researcher
- Evaluation of concomitant medications: see 6.5.2.1 Baseline visit.
- Assessment of adverse events: see 6.4.3.3 Evaluation of adverse events
- Continuity evaluation: refers to the determination by the PI and desire of the subject to continue with their participation in the study.
- Evaluation of the adherence: refers to the review of the diary of the subject to evaluate the registration of drug application.
- Global qualification of the researcher: see 6.4.3.2 Global qualification of the researcher

6.5.2.4 Final Visit

- Pregnancy test: see 6.5.2.1 Baseline visit.
- Conjunctival secretion sampling: see section 6.4.3.4 Conjunctival secretion sampling for bacterial culture
- Ophthalmological examination: see 6.5.2.1 Baseline visit.
- Biomicroscopy: see 6.4.3.1 Biomicroscopy
- Global qualification of the researcher: see 6.4.3.2 Global qualification of the researcher
- Evaluation of concomitant medications: see 6.5.2.1 Baseline visit.
- Assessment of adverse events: see 6.4.3.3 Evaluation of adverse events
- Continuity evaluation: see 6.5.2.2 Visit 1
- Adherence evaluation: see 6.5.2.2 Visit 1
- Global qualification of the researcher: see 6.4.3.2 Global qualification of the researcher
- Return of the medication: refers to the delivery, by the subject, of the excess medication to the research center.
- Return of the subject's diary: refers to the delivery, by the subject, of the tool "subject's diary" to the research center.

6.5.2.5 Security call:

- Assessment of adverse events: see 6.4.3.3 Evaluation of adverse events.

6.5.3 Diagram of the study.

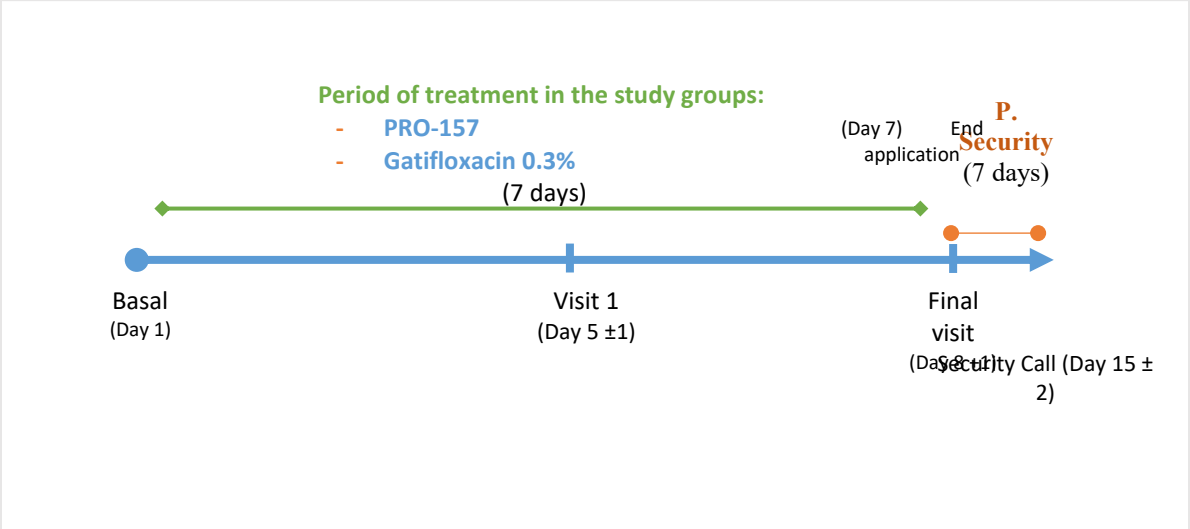


Figure 6. Diagram of the study.

6.6 Sample size.

The sample size calculated for the present study is 160 eyes. They plan to recruit 160 subjects, each one will provide an eye for the study, for the analysis of efficacy.

Divided into two intervention groups, each group will be comprised of 80 eyes.

6.6.1 Calculation of the sample size.

The sample size was calculated using the formula for variables of two proportions, likewise the data was entered into the online calculator, software developed in: www.powerandsamplesize.com based on Chow S et al. [39]

Based on the final report of the Phase II clinical study, and the difference found between the intervention of PRO-157, three times a day, against gatifloxacin 0.3%, the calculation of the sample size was made.

The dosage that showed the greatest efficacy was the instillation of 1 drop three times a day. A cure percentage of 88% was observed with the mentioned group. The group that received Gatifloxacin reported a cure rate of 94%. Nevertheless, there was no statistical difference between the groups. [35]

The sample size was calculated considering the clinical cure rate of 0.88 submitted to the treatment with PRO-157 1 drop three times a day compared to a percentage of 0.94 of the group with Gatifloxacin. It is expected that at least a healing difference of 15% will be present to demonstrate superiority in the upper and lower limits, in order to demonstrate non-inferiority.

The formula for non-inferiority or superiority was used comparing two proportions1 with a Power (1-β): 90% and an error type 1, α: 5%. Margin or limit, δ: 15%.

$$n = p(1-p)\left(\frac{Z_{1-\alpha} + Z_{1-\beta}}{p - p_0 - \delta}\right)^2$$

The result is **112 eyes with acute bacterial conjunctivitis**. Considering an increase of 30% (15) due to the probable losses and a balance between the groups, the final **sample size is 160 eyes**.

The intervention will be applied in both eyes, however each subject will only provide one eye for efficacy analysis.

6.7 Recruitment .

The subjects will be recruited from the IP external consultation or referenced from first level centers. Recruitment will be competitive for all centers.

7. Methods Assignment of the intervention.

7.1 Generation of the allocation sequence.

2 strata corresponding to the intervention groups will be used, which will be balanced for each research center. The allocation will be 1: 1. The generation will be done through an electronic system validated by an external provider, previously evaluated and authorized by Sophia Laboratories S.A. of C.V. The information corresponding to this provider will be found in the master folder of the study.

7.2 Blinding mechanism.

Blinding will be performed by the personnel indicated by the Clinical Operations Management of Sophia Laboratories, S.A. of C.V. The vials of both interventions will be re-labeled and the secondary packaging will be masked.

7.3 Implementation.

The sequence will be generated by means of an electronic randomisation system. Said system will be hired by Sophia Laboratories, S.A. of C.V. to a third party. The information corresponding to this third party will be found in the master folder of the study.

7.4 Blinding and masking.

The blinding will correspond to the research subject and the principal investigator. In addition, the statistical analysis will be carried out in a blinded manner in the case of a partial and final analysis.
The masking will be done using boxes in the primary packaging identical in the two groups and relabelling the bottles of both interventions.
Blinding for the research subject and the researcher will be done by replacing the commercial labels in the case of the comparator in the bottles and the use of identical labels that contain the assignment number.

7.4.1 Opening of blinding.

Blinding may be opened in the following cases:

1. Presence of a serious adverse event.
2. Safety alarm due to the use of the drugs under study.
3. In case the sponsor determines it for any security reason or other reason that it considers pertinent.

8. Methods Collection, administration and data analysis.

8.1 Methods of data collection.

A clinical monitor will be assigned to each research center, which will be authorized to monitor, review, procure and ensure that the quality of the information obtained from the participants is reliable and trustworthy. Each monitor will schedule periodic visits to the research centers in order to review the source documents and corroborate the information captured in the CRF. All clinical monitors will be trained in relation to the information of the study protocol (objective, visits, procedures, range of accepted values, etc.). In the event that the data are not identical between the two registers, the clinical monitor will generate a discrepancy, which must be resolved by the research center in time that the sponsor deems reasonable to meet the objectives of the clinical study. The correction of the discrepancies will be made according to the Good Documentation Practices.

The data registered in the CRF will be reviewed by personnel of Sophia Laboratories, trained in the ophthalmological, clinical and pharmacological area, which will be able to generate discrepancies in the event that the data do not comply with the stipulations of the research protocol. Or put the participants at risk.

Once all discrepancies generated by the team of clinical monitors and clinical staff have been resolved, the data will be downloaded into an electronic database (Excel Sheet) by personnel designated by the sponsor. A new review of the data will be carried out to corroborate the fidelity of the same and new discrepancies may be generated in case it was considered.

The database generated will be safeguarded by the sponsor and will only have personal access designated by the same.

8.1.1 Strategies to complete the follow-up.

- • You will be clearly informed of the importance of the study and the benefits that the population will obtain from the results of the study.
- • Transportation assistance will be provided in order for the participant to attend their visits.
- • A printed calendar will be provided with the objective of reminding the participant of their appointments and the activities that will be carried out, in addition to the estimated duration of the same.
- • In case the participant does not attend his appointment, the research center must make a call to know the reason and try to arrange a new appointment within the established window period or an unscheduled appointment.
- • In case it is not possible to make an appointment, it will be asked about the presence of adverse events and the reason for leaving the study, such as minimum data.

8.2 Data management.

The subject's medical record (including clinical notes, test results, etc.), as well as the subject's diary, are considered source data.

The IP or the designated person of your team will fill out the Case Report Format (CRF) as well as all other documents provided by the sponsor (for example: documents related to the management of the treatment).

An electronic CRF was designed to record the data that are required in the protocol and that the researcher collects in each of the visits.

In the case of self-assessment questionnaires, it is not permissible for the principal investigator or person responsible for filling in to modify what was written by the subject of the study.

The data capture in the investigator's site will be done by the investigator or the designated person of his team after performing the Medical File. The researcher or a designated person of your team will be trained in filling the CRF.

All corrections to the CRF data should be made by the investigator or the designated person of your team in accordance with the instructions provided.

To ensure the confidentiality and security of the data, user names and access codes will be used to restrict access to the system only to authorized personnel.

The monitor must ensure that all the data has been filled in the CRF. After comparing the data against the source documents, the monitor will ask the researcher to make the necessary correction / clarification, so that they are answered and closed as quickly as possible.

The Scientific Committee of Sophia Laboratories S.A. of C.V. will give the latest medical-scientific review, and will set the standard for freezing the database.

8.3 Statistical methodology.

8.3.1 Analysis of primary and secondary outcome variables.

The statistical analysis will be carried out by personnel of Sophia Laboratories. The statistical program SPSS version 19 will be used.

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

The data will be collected and sorted in an excel sheet. Later they will be exported to the platform of the SPSS program. The variables will be categorized according to their nature.

The result of the continuous quantitative variables will be presented in measures of central tendency: mean, standard deviation and ranges. See **Table 5 Variable measurement method**. The total size of the samples will consider an eye as a case for the evaluation of efficacy. Paired samples will be considered in cases with visits before and after the intervention.

The normal distribution of results will be obtained through the Kolmogorov-Smirnov and Shapiro-Wilks test, as applicable.

The statistical analysis of the continuous quantitative variables to find significant differences (p) will be the following:

- Inter-group analysis: t test for paired samples.

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

The result of nominal and ordinal qualitative variables will be presented in frequencies, proportions and percentages. See **Table 5 Method for measuring variables** .

The statistical analysis to identify significant differences of the qualitative variables will be done by creating 2x2 contingency tables and will be done as follows:

- Difference between groups: χ2 test (Chi-square) of Pearson or Fisher's exact in expected values less than 5.

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

For the reporting of adverse events all participating subjects who were randomly assigned to an intervention group will be considered. The results will be expressed in number of subjects.

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

In case of loss of cases (data), they will not be replaced by any situation to complete the sample size.

According to the data obtained, the sponsor will decide to perform a subanalysis by age groups, which will have no influence on the final result.

8.3.2 Additional analyzes.

An internal analysis will be made to know the changes that occur between each visit. It is the sponsor's prerogative to request an additional analysis of the data, during the conduction of the study or at the end of this.

8.3.3 Population analysis and management of missing data.

The effectiveness analysis will consider those cases in which the measurements of the baseline and final visit are met. Those subjects who do not comply with any of these measurements will not be integrated into the final database to evaluate the effectiveness. Nevertheless, an intention to treat analysis (ITT) will be carried out.

The safety assessment will include in the analysis all those subjects (considering one eye or both) who have been exposed at least once to any of the interventions regardless of the visit in which they were eliminated from the study.

9. Methods Monitoring.

9.1 Data monitoring.

Monitoring visits by a site monitor from Sophia Laboratories, S.A. of C.V. are intended to confirm that the studies sponsored by Sophia Laboratories, S.A. of C.V. they are conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and that are consistent with Good Clinical Practices and with the applicable regulatory requirements (verifying a continuous compliance with the protocol, amendment or amendments, reviewing accounting records of the product under investigation, verifying that the personnel of the site and the facilities remain adequate to carry out the study).

The researcher must ensure that they have sufficient time, space and qualified personnel for the monitoring visits.

In order to carry out the monitoring review, it is mandatory to provide direct access to all source data and those related to the study site. The monitor will conduct a review of the CRF and a

Verification of Source Documents (VDF). By VDF means the verification of the records in the CRF through its comparison with the source data that the researcher will make available for this purpose.

Regarding the CRF, the monitor will mark the screens completed and approved at each visit.

In accordance with the applicable regulations, Good Clinical Practices, and the procedures of Sophia Laboratories, S.A. of C.V. The monitors of Sophia Laboratories, S.A. of C.V. will contact the site before the start of the study to review the protocol, the regulatory and ethical requirements of Sophia Laboratories, S.A. of C.V. with the staff of the site. When reviewing the procedures for data collection, the conversation will also include the identification, agreement and documentation of the individual data for which the records in the CRF serve as source documents.

Sophia Laboratories, S.A. of C.V. monitor the study to verify, among other things, that:

- The data is authentic, correct and complete.
- The safety and rights of the subjects are being protected.
- The study is being conducted in accordance with the currently approved protocol, any other study agreement, Good Clinical Practices and all applicable regulatory requirements.

The investigator and the head of the medical institution (when applicable) agree to allow the monitor to have direct access to all relevant documents.
Study monitoring visits will be conducted at regular intervals, depending on the recruitment rate, under the arrangements between the investigator and the sponsor. All information related to these visits will be handled as strictly confidential.

Upon completing or discontinuing the study prematurely, the monitor will carry out site closure activities with the investigator or site staff, as appropriate, in accordance with applicable regulations, Good Clinical Practices, and Sophia Laboratories SA of C.V. procedures.

After the study is closed, the researcher must keep all study records on the site in a safe place. Records should be maintained to allow easy and timely recovery, when necessary (for example, in an audit or inspection). Sophia Laboratories, S.A. of C.V. will inform the investigator / institution the period of time they will have to retain these records, in order to comply with all applicable regulatory requirements. However, the investigator / institution must seek the written approval of the sponsor before proceeding to the elimination of these records. The minimum retention time will satisfy the most stringent standard applicable to that site for the study, in accordance with the provisions of the PCBs, any institutional requirements or the applicable laws or regulations, or the standards / procedures of Sophia Laboratories, S.A. of C.V.

The researcher / institution must notify Sophia Laboratories, S.A. of C.V. Of any change in file arrangements including, without limitation, the following: file in an off-site facility, ownership transfer of records in the event the investigator leaves the site.

9.2 Preliminary analysis and early termination of the study.

The partial analysis will allow the sponsor to make a decision about the early termination of the study in the event that the safety of the participants is compromised.

The early termination of the study will be considered in the following cases:

1. Presence of serious adverse events in more than 5% of the participants in each intervention group.
2. The competent authority considers it for security alerts.
3. The Sponsor determined it for his convenience or eventualities such as: economic support, manufacturing errors, etc.
4. Lack of recruitment as expected.

In case the decision is the early termination of the clinical study, all the research centers will be informed within the first 24 hours by the available communication channels. Likewise, the corresponding authority in each country will be informed (if applicable) and the Ethics Committees involved.

Each research center has the obligation to inform the subjects that participate in the clinical study in a period no longer than 24 hours, after receiving the information from the sponsor. You must inform all the subjects involved in any phase of the study.

The result of the preliminary evaluation will be in charge of the Clinical Operations Management and the Medical Management of Sophia Laboratories, S.A. of C.V., which will have the faculty to determine the fate of the present protocol, as they deem convenient.

9.3 Adverse events.

9.3.1 Responsibilities of the Investigator.

Perform the verification of adverse events through questioning, relevant physical examination, assessment of evolution, as well as adequate medical and pharmacological management, resolution or outcome and final discharge following the definitions determined in national and international regulations. [40] [41] [42]

In case of adverse events or any event that puts the health and well-being of the patients at risk, appropriate medical attention will be provided, either at the research site or will be referred to the Hospital Center with greater resolving power with which the research site and / or researcher have medical care agreement. The researcher will notify the clinical monitor of the sponsor, according to the times established in the national and international regulations. In the case of serious adverse events, notify the sponsor and record the corresponding information in the case report form and in turn inform the Research Ethics Committee, the Research Committee.

The attention of the adverse events will be made according to the diagram of attention of the event (**see Figure 7. Attention of the adverse event**)

In the final report to be drafted by the Scientific Committee of the Department of Clinical Operations of Sophia Laboratories, S.A. of C.V., will include the report of adverse events in compliance with current national and international regulations. [40] [41]

9.3.1.1 Record of adverse events in the Case Report Form.

The registry of adverse events considers the information concerning the identification data of the participating patient as code, age, sex, left eye, right eye.

Information about the type of adverse event, adverse reaction or suspected adverse reaction to the product under investigation or to the study medication, as appropriate. The date on which the adverse event occurs is reported, as well as in which the Investigator is aware of it, date of resolution or outcome, as applicable. The clinical diagnosis is indicated. If a lack of therapeutic response is detected to the investigational product and / or investigational medication, it should be reported as an adverse reaction. Include in concomitant medications the therapy used for the pharmacological management of the adverse event, suspected adverse reaction, adverse reaction. Record the outcome or resolution of the event: patient recovered without sequelae, with sequelae, not recovered. Patient who presented death due to adverse reaction / adverse event, patient who presented death and it is judged that the drug could have contributed, patient who presented death and this is not related to the investigational product or drug, or indicate that it was not knows what the consequence of the event is.

Consign information about the product or drug under investigation or the drug associated with the adverse event, adverse reaction or suspected adverse reaction. As applicable, information concerning generic denomination, distinctive denomination or product code in research and / or investigational medication should be recorded, as appropriate according to the methodological design of the study, this is relevant in the case of blinded studies or those where they use placebo as comparators, since there are circumstances that justify opening the cecum to determine if the adverse event, the adverse reaction or suspected adverse reaction may be attributable to the active agent, the combination of active agents, or the substance (s). s) pharmacologically inert (s), such as vehicles or additives, as appropriate to the clinical research phase in which the development of the drug is located.

It will also be necessary to record the data concerning a) batch number, b) manufacturer laboratory, c) expiration date, d) dosage, e) route of administration, f) start dates and g) term of administration and / or consumption , reason for the prescription; according to whether it is a product or investigational medicine (protocol in which the patient currently participates) or is a medicine that the subject under investigation consumes for the treatment of basic concomitant diseases or used for the management of any sign or transient symptom that does not correspond to the Natural History of the pathology that motivated its entry into the research protocol.

Record the withdrawal or maintenance of the medication, investigational product or investigational medication, as appropriate. Indicate if the adverse event disappears when the investigational product or investigational medication or suspicious medication is removed (to provoke the 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 that in those patients who are exposed again to the investigational product, investigational medication or medication, which had previously been suspended, if the adverse reaction or adverse event reappears.

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 if it is consistent with the information to prescribe or technical data sheet or is used outside the regulations or of what the local, national or international regulatory entity has authorized.

Concerning the relevant clinical antecedents. The analysis of the adverse event, adverse reaction or suspicion of adverse reaction considers the information previously reported, notwithstanding the clinical context in which said harmful phenomenon occurs in the participants of the clinical research protocol, it is of special interest, so that the information about previous ailments, hypersensitivity or allergy phenomena, previous surgical procedures, laboratory analysis or cabinet exams that have been practiced on the participant, etc.., that the researcher deems convenient to mention may do

so. If you have enough space in the case report format, you can complement the information in your clinical note of the clinical file.

9.3.1.2 Follow up of adverse events.

The IP will provide the attention and guidance of the EA that the participant presents until the end of the same, according to what is referred to the following section.

9.3.1.3 Procedures for a serious adverse event.

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

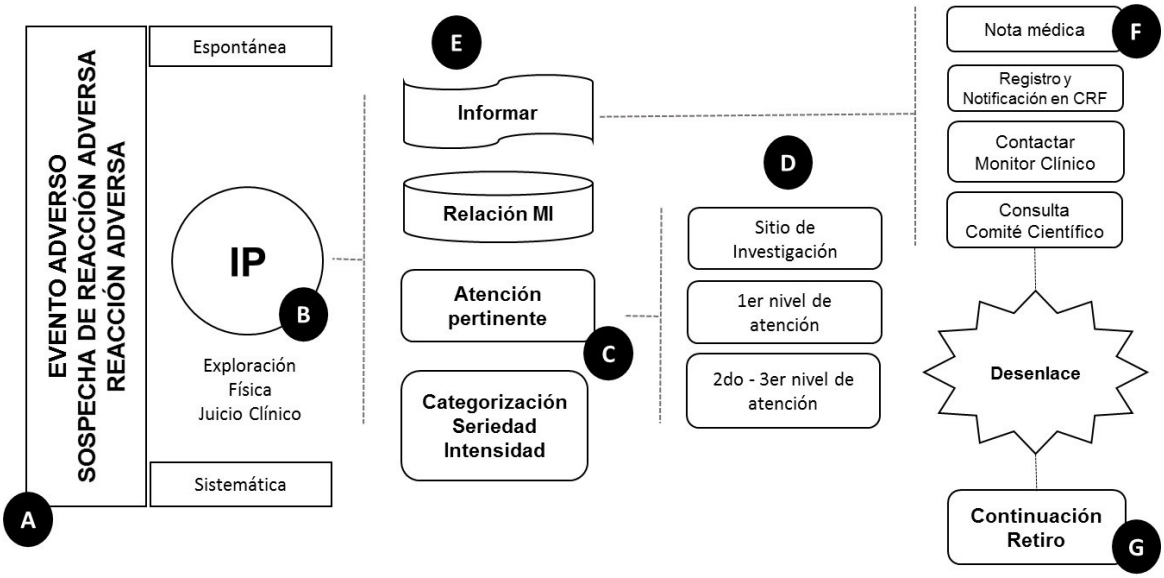


Figure 7. Adverse event attention.

A. During the development and conduct of the present clinical investigation, undesirable damaging events or adverse reactions, of medical involvement, which do not necessarily have a causal relationship with the investigational product or investigational drug, may occur in the participant patient. These harmful phenomena can occur during the use of investigational drugs, unintentionally, at doses authorized for use in humans; by a local, national or international regulatory entity, whether for prophylaxis, diagnosis, treatment or for the modification of some physiological process. However, it can be suspected that the investigational product or the investigational drug or the placebo cause some unwanted clinical manifestation. Adverse events, adverse reactions or suspected adverse reactions to one or several medications 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, as such way that,
B. The investigator must be the first person to whom the patient notifies that they have developed or presented a harmful clinical phenomenon during their participation in this research protocol.
C. According to their clinical judgment; on the basis of the pertinent physical examination, interrogation, etc., as well as the analysis of the information available in the medical literature and that referred to in the investigator's manual, information to prescribe or technical data sheet of the comparator drug, the principal investigator determines the relevant attention of the event / harmful reaction; either:

D. In the research site or in the hospital with the greatest resolving power (1st, 2nd or 3rd level of medical attention). In such a way that, in case the patient is sent by the Investigator to a hospital, he / she attends by means of a reference system, it can be with an identification card that the patient belongs to the present investigation and there is an official number or folio, which pertains to the emergency care agreement with the health institution with the greatest resolving power, or a medical reference note issued by the principal investigator, so that appropriate care is given to the participating patient. It should be noted that the Study Sponsor, Sophia Laboratories, S.A. of C.V., will pay the expenses for the medical care of the participating patient, only if the adverse event, adverse reaction or suspected adverse reaction to medication is associated or found in relation to the investigational product or investigational drug.
E. Taking the clinical information gathered, either during the care provided in the research site or provided by the treating physician (s) in the hospital, the principal investigator records the adverse event, suspected adverse reaction or adverse reaction to medication in your clinical note of the clinical file, indicating the seriousness, intensity (mild, moderate or severe), relationship with the product or investigational medicine, as well as:
F. The migration of the relevant data to the case report format and to its respective adverse event section; noting the pertinent information, already referred to in section 9.3.1.1., this in virtue of the fact that in cases of serious adverse events, which must be notified in less than 24 hours after the moment in which the principal investigator has knowledge of the same, the clinical monitor of the study is informed, so that in turn he / she informs the Scientific Committee and the Pharmacovigilance Department of the sponsor and later he / she informs the Research Ethics Committee. Regarding non-serious adverse events, these will be recorded and adequately addressed and the corresponding regulatory entity will be informed about the safety profile of the product under investigation or investigational medication in the final report of the clinical trial.

The record of the outcome of the adverse event, suspicion of adverse reaction or adverse reaction to medication depends substantially on the follow-up that the principal investigator makes to the participant, since most of the harmful phenomena are expected, consult section of the safety profile in number 5.3 and in the researcher's manual, they are ophthalmic in nature, however there may be systemic alterations. Therefore, in the opinion of the researcher, the withdrawal of the participant or his / her permanence will be considered, according to the stipulations of section 6.2.3 Elimination criteria of this research protocol.

9.3.1.4 Causality evaluation.

The assessment of the causality, the methodology used to estimate the probability of attributing to a drug, investigational drug or investigational product the adverse reaction, the suspicion of the same or the observed adverse event, considers probabilistic categories, according to the evidence available and the quality of information, based on national pharmacovigilance regulations. [40] As a tool to facilitate the probabilistic categorization of causality, the principal investigator can use the algorithm of Karch and Lasagna modified by Naranjo referred to by Aramendi I, 2011 in which

different items are qualified which allow assigning a value to the relationship cause-effect between the administration of the drug and the adverse reaction. [43]

Table 6. Algorithm of Karch and Lasagna modified by Naranjo.

No.	Reagents	Yes	No
1.	There are previous conclusive reports about the adverse drug reaction, adverse event or suspected adverse drug reaction	+1	0
2.	The adverse event appeared when the suspected drug was administered	+2	-1
3.	Adverse reaction to medication, adverse event or suspected adverse drug reaction improved upon discontinuation or administration of a specific antagonist	+1	0
4.	Adverse reaction to medication / adverse event / suspected adverse drug reaction reappeared when administering the drug / investigational product / investigational medication	+2	-1
5.	There are alternative causes that may cause this reaction	-1	+2
6.	Adverse reaction / adverse event / suspected adverse drug reaction occurred after placebo administration	-1	+1
7.	The drug was determined in blood or other liquids in toxic concentrations	+1	0
8.	The intensity of the adverse reaction / adverse event / suspected adverse drug reaction was higher with higher doses or lower with lower doses	+1	0
9.	The patient has had similar reactions with the drug / product under investigation or investigational medication, in the past	+1	0
10.	Adverse reaction / adverse event / suspected adverse reaction to medication was confirmed with some objective evidence	+1	0
	Total score	summation	
	Probabilistic category based on the score obtained		
I	The causal relationship is checked	≥,9	
II	It is likely that ADR is due to the drug or product under investigation	5 a 8	
III	It is possible that the RAM is due to the drug or product under investigation	1 a 4	
IV	The causal relationship is doubtful	0	

The reagents considered by the algorithm of Karch and Lasagna modified by Naranjo where each one receives a defined score are shown and the final summation allows estimating the probabilistic category of the cause-effect relationship between the administration of the drug / product in research / investigational medicine and the adverse reaction, adverse event or suspected adverse reaction. Consider that if the information is not available, a score equal to zero is recorded.

In such a way that the degree of certainty to establish the investigational product or investigational medication (as appropriate) as the causal agent of the harmful phenomenon that befalls the participating patient, can be directly indicated by the principal investigator based on his or her clinical experience or well through the voluntary application of the tool mentioned previously. Notwithstanding, it is important that the researcher take into account the following arguments in favor of the causal relationship: the strength of association that refers to the number of cases in relation to those exposed. The consistency of the data, ie the presence of a common characteristic or pattern. The exposure-effect pattern: which determines the relationship with the site of

appearance, time, dose and reversibility after suppression. The biological plausibility: that refers to the possible pharmacological or physiopathological mechanisms involved in the development or presentation of the adverse event. Experimental findings: for example the appearance of anomalous metabolites or high levels of drug or the product of its biotransformation. Analogy: experience acquired with other related drugs, adverse reactions frequently produced by the same family of pharmacological agents. Nature and characteristics of the data: objectivity, accuracy and validity of the relevant documentation. [44]

9.3.2 Responsibilities of the sponsor.

The sponsor will be responsible, and will cover the expenses derived from the medical attention to adverse events related to the product under investigation.

9.4 Audit

To guarantee compliance with the PCBs and with all applicable regulatory requirements, Sophia Laboratories S.A. of C.V. could carry out a quality assurance audit. Regulatory agencies could also carry out a regulatory inspection of this study.

9.4.1 Pre-study audit.

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

9.4.2 Audit / Inspection during the conduction of the study.

They may take place at any time before, during or after the conclusion of the study. If an audit or inspection is performed, the investigator and the institution should agree to allow the auditor / inspector direct access to all relevant documents, and will allocate their time and that of their staff to the auditor / inspector to discuss the findings and any relevant problems.

10. Ethical considerations.

10.1 Approval of the committees.

The present study will be conducted according to the standards of the Declaration of Helsinki, World Medical Association 2013. Nuremberg Code; Nuremberg Trial by the International Court of Nuremberg, 1947. Belmont Report, National Commission for the Protection of Subjects of Biomedical Research and Conduct, 1979.

It will be conducted in accordance with the scientific and technical requirements necessary for the registration of medicines for human use of the International Conference on Harmonization (The International Council for Harmonization, ICH for its acronym in English) Guide to Good Clinical Practices. International Ethical Guidelines for Biomedical Research in Human Beings of the Council for International Organizations of Medical Sciences (Council for International Organizations of Medical Sciences, CIOMS, 2002). International Ethical Guidelines for Epidemiological Studies of the Council for International Organizations of Medical Sciences (Council for International Organizations of Medical Sciences, CIOMS, 2008).

The Research Ethics Committee and the Research Committee will evaluate the protocol before conducting the study and will issue their approval or possible modifications for its realization, these Committees should be notified of any significant changes to the protocol. In addition to the above,

the current regulations issued by the Ministry of Health will also be complied with. General Health Law, NOM 012 Official Mexican Standard NOM-012-SSA3-2012, which establishes the criteria for the execution of research projects for human health. The study is considered as an investigation with a risk greater than the minimum according to the Regulation of the General Health Law on Health Research, Title Two, Chapter I, Article 17, Category III, published in the Official Gazette on 6 January 1987.

The principal investigators or study coordinators or personnel authorized by the sponsor will be evaluated by the Research Ethics Committees, Research Committees, and when applying to the Biosafety Committee the essential documentation of the research project: research protocol, letter of informed consent, researcher's manual, subject's diary, as well as those requested, in addition, according to local, national or international requirements applicable by regulatory entities.

The study will not start in the research site if you do not have the confidentiality agreements and economic proposal of each of the principal investigators, duly signed and without having previously obtained the favorable opinion and / or the approval of the Committees of Ethics in Research, Research Committees, and when applicable by the Biosafety Committee, corresponding.

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

10.2 Amendments to the protocol.

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 that affects driving and results, substitution of the principal investigator or identification of risks in the research subjects. The documents susceptible of amendment will be: protocol, letter of informed consent, researcher's manual, documents for the patient, scales of measurement 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 when applicable, by the Committee of Inquiry. Biosafety, (entities that issued the initial favorable opinion for the conduct of the investigation) will be sent (s) for authorization by the relevant regulatory entity.

Amendments that substantially modify the protocol or confer an additional or different risk to the research subjects must be approved by the Committee. It is the investigator's responsibility to take action in situations that require immediate action to avoid unnecessary harm to study participants.

The principal investigator has the responsibility to inform the Research Ethics Committee of any amendment to the protocol that could eventually affect the rights, safety or welfare of the research participants. Likewise, he must know any situation or new knowledge that shows a greater risk for the participants, the termination or premature suspension of the study, the reasons and the results

obtained up to that moment. You must also inform about the conclusion of the study, when completing the research protocol.

The list of amendments, and in the necessary cases, the relation of the issuance of errata, will be referred to in the final report of the investigation.

10.3 Consent

10.3.1 Obtaining

Informed consent must be obtained before the subject undergoes any procedure indicated in the protocol.

The written consent documents will incorporate the elements of informed consent described in the Declaration of Helsinki and the ICH Guide to Good Clinical Practices and will be in compliance with all applicable laws and regulations.

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

This information will be with a language understandable to the subject, it will be explained to the subject that has the right to interrupt their participation in the study at any stage, without affecting the relationship with the researcher and / or their future assistance. The informed consent will be put to the consideration of the possible participant; This must have enough time to analyze each and every one of the aspects mentioned above and if there is any doubt this 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 letter in the presence of two witnesses who have or are not related to the subject of study, who will participate during the informed consent process and will sign endorse that the process was carried out prior to any study procedure, that the information of the study was clearly explained and doubts were clarified in case of existing.

If a subject is illiterate, the acceptance will be with their fingerprint, and in the event that the subject is not able to grant an informed written consent, a representative of the "legally authorized" subject can provide such consent. The subject in accordance with applicable laws and regulations.

The IP must also sign and date this consent.

The informed consent must be signed in duplicate by all involved, and two witnesses, one copy will be filed in the file of the subject and the other will be delivered to the participant. The PI must document in the patient's medical history, the date on which he signed the informed consent.

10.3.2 Special considerations.

In the case of minors (<18 years) it will be necessary to obtain the informed consent of the parents; and for minors, over 7 years of age or who, in the opinion of the researcher, show sufficient understanding, they must be accompanied by the informed consent.

10.3.2.1 Informed consent of parents.

The parents of minors, who can potentially be included in the study, must give their informed consent for the participation of their children. This will be documented in the "Informed Consent for Parents", which must include the signature of both parents. If the document does not have the signature of both parents, it will be considered invalid; only the signature of a single parent will be allowed in the following cases:

- In the case in which the father or mother does not share parental authority with their counterpart

- In the case in which the minor has been registered with only one parent

- In the case in which the minor is an orphan of a father or mother.

10.3.2.2 Informed consent.

Minors over the age of 7 must give their consent to participate in the study, which must be registered with their signature on the document "informed consent". If the researcher deems it advisable to include the consent of a child under 7 years of age, since he considers that he has sufficient understanding, he may attach it. Nevertheless, it will be obligatory for all those over 7 years of age to have their consent, together with the consent of their parents.

10.3.3 Modification to informed consent.

Any change to "informed consent", "informed consent for parents" and "informed consent" constitutes an amendment to these documents and must be presented for approval before the Ethics in Research Committees, and if applicable before the Competent Authorities.

The amendment will include a copy of the new version in the language or languages of the country.

Such amendments may be implemented only after having obtained the written approval of the corresponding committees and the Regulatory Entity (as applicable), with the exception of an amendment that is required to eliminate an immediate danger for the subjects of the study.

Each subject affected by the amendment must complete, date and sign two originals of the new version. The subject will be given a signed original of the amendment and the researcher will keep the second original.

10.4 Confidentiality

All documents and information provided to the researcher by the sponsor are strictly confidential. The researcher expressly agrees that the data on their professional and clinical experience, provided to the sponsor on paper and stored electronically or digitally, are only for use related to their activities with the sponsor of clinical studies, in accordance with Good Clinical Practices. The researcher accepts that he / she and the members of his team will use the information only within the framework of this study, to carry out the protocol. This agreement is mandatory as long as the confidential information has not been disclosed to the public by the sponsor. The protocol of the clinical study provided to the researcher may be used by him and by his colleagues to obtain the informed consent of the subjects for the study. The clinical trial protocol, like any information taken from it, should not be disclosed to other parties without the written authorization of the sponsor.

The researcher will not reveal any information without the prior written consent of Sophia Laboratories, S.A. of C.V., except to the representatives of the Competent Authorities, and only by request of the same. In the latter case, the researcher undertakes to inform Sophia Laboratories S.A. of C.V. before revealing the information to these authorities. The researcher will fill out and maintain a record of the subjects' selection, as well as the identification and enrollment list of each of the subjects participating in the study. The researcher agrees to give on-site access to the auditor

and / or the representatives of the Competent Authorities. The information will be treated in compliance with professional secrecy.

10.5 Deviations

A deviation is any alteration in the procedures and activities described in the research protocol approved by the committees and regulatory authorities. They may be the product of modifications or omissions, which may compromise the safety of the participants or the quality of the data generated.

Major deviation / violation: is one that impacts one or more of the following aspects:
- Subject security
- Alteration of the risk-benefit balance
- Commit the integrity of the study data
- It affects the voluntariness of the subject in the participation of the study.

The list of examples cited below serves the purpose of guidance, but does not cover all possible cases, so it is not limiting:

I. **In relation to informed consent:** 1) that informed consent has been taken by an unauthorized person to do so, 2) that the subject under investigation signs a version of informed consent not approved by the committees and regulatory entity, 3) that perform a study procedure prior to signing informed consent.
II. **Regarding the inclusion / exclusion criteria:** 1) enroll subjects who do not meet all the inclusion criteria and / or meet any exclusion criteria, 2) enroll defined subjects as part of the so-called vulnerable population: children, pregnant women, prisoners, without prior approval for such group; 3) Enroll patients before the start or after the end of the study.
III. **In relation to the medication of the study**: error in the delivery or dosage of the same.
IV. **In relation to concomitant medication**: use of prohibited medication.
V. **In relation to the study procedures**: that those that, in the opinion of the principal investigator, compromise the safety of the research subject are not carried out.
VI. **In relation to the reporting of serious adverse events**: those that are reported outside the time stipulated by the committees.

Minor deviation: is that which does not impact on the safety of the subject, does not alter the risk-benefit balance, does not compromise the integrity of the study data or does not affect the subject's willingness to participate in the study.

The list of examples cited below serves the purpose of guidance, but does not cover all possible cases, so it is not limiting:

I. Oblivion in the taking of the study medication.
II. Lack of return of study medication by the subject.
III. Visits of the research subject carried out outside the window.

10.5.1 Management of deviations.

All deviations must be reported by the IP to the sponsor and the corresponding committees.

At your discretion, and depending on the severity of the deviation, the sponsor and the corresponding committees may:

- Request more information.
- Citing the principal investigator and / or the members of his team.
- Temporarily suspend the researcher for present and / or future investigations until the situation is resolved and / or considers the explanations given by the person (s) responsible for the deviation satisfactory.

Conduct an audit for cause.

10.6 Declaration of interests.

The researchers who collaborate in the present study (principal investigator, subinvestigator) commit themselves to carry out, prior to the beginning of the study, a statement of financial interests, as well as conflict of interest.

10.7 Access to information.

The final database of the study will be owned by Sophia Laboratories, S.A. of C.V. and your access will be restricted. The IP will not have access to it, unless it has prior written authorization from the sponsor.

10.8 Auxiliary care and after the end of the study.

The care of the EAs will be done according to section 9.3 Adverse events.

10.9 Biosecurity aspects.

Inappropriate manipulation of the conjunctival secretion sample can become a source of biological risk for people who are in contact or for the environment. Therefore, for the protocol titled: "Clinical study of the efficacy of the ophthalmic solution of Pazufloxacin 0.6% (PRO-157) for the treatment of acute bacterial conjunctivitis, compared to the ophthalmic solution of Gatifloxacin 0.3%." And number: SOPH157-0217 / III, is urged to take **BASIC BIOSECURITY MEASURES**, due to the handling of infectious-contagious biological material of relative danger.

Use the necessary personal protection elements to avoid exposure with biological risk:

• Gloves.

• Coat.

• Leak-proof and easy-to-seal container.

In case of accident with biological risk, it is recommended to clean with soap and water from the area of the exposed body, not touch your own eyes until you have thoroughly cleaned the exposed area or follow the recommendations of the institutional guide of the work accident with biological risk.

10.10 Final report and publication of results.

10.10.1 Final report.

Once the statistical analysis is finished, a final report will be drafted with the results obtained, in charge of the Scientific Committee of the Department of Clinical Operations of Sophia Laboratories, S.A. of C.V. Said report will be prepared following the recommendations of the E3 Step 4 Guide of the ICH.

10.10.2 Communication of results.

Sponsor's plan to communicate the results of the study to researchers, participants and regulatory entities.

Regardless of the results in the study, Sophia Laboratories, S.A. of C.V., is committed to communicating the final report of the study to the principal investigators and to the regulatory entities corresponding to the countries with participating research centers. Maintaining at all times the rights on the publication and dissemination of the information contained.

10.10.3 Publication of the results

Sophia Laboratories, S.A. 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 may be used in the manner it deems appropriate.

Because the study is multicentric, the first publication should be made only with data collected from several centers and analyzed under the responsibility of Sophia Laboratories S.A. of C.V. The PI undertakes not to publish or communicate data collected only in a center or in part of the centers before the publication of the full results of the study, unless prior written agreement is given by Sophia Laboratories S.A. of C.V.

Any publication and / or communication project related to the study and / or the results obtained during the study or after the completion of the study will be presented to participating medical researchers at least 30 days in the case of a publication and 15 days in the case of a summary, before the scheduled date for the communication and / or presentation of a publication. He or the medical researchers will comment on the project within 15 days in the case of a publication and 7 days in the case of a summary, from the date on which the project is received.

Nevertheless, in case the sponsor is in the process of submitting 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.

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12. Signature page

12.1 Signatures of the sponsor's representatives

First name:	
Dr. Leopoldo Martín Baiza Durán	
Title:	Date
Medical responsible for the study	Firm

First name:	
QFB. Francisco García Vélez	
Title:	Date
Director of the study	Firm

First name:	
Dr. Oscar Olvera Montaño	
Title:	Date
Protocol author	Firm

12.2 Investigator

I agree to conduct this clinical study according to the design and guidelines of this protocol, abiding by the provisions of this protocol. I agree to conduct the study in accordance with the accepted standards of Good Clinical Practices. I agree to report all information or data in accordance with the provisions of the protocol, in particular, any adverse event. Also, I agree to handle the clinical supplies, provided by the sponsor, strictly in accordance with this protocol. I understand that the information that identifies me may be used by the sponsor. Because the information contained in this protocol and the Investigator's Manual is confidential, I understand that it is prohibited to share it with any third party, who is not involved in the approval, supervision or conduct of the study. I will make sure to take the necessary precautions to protect loss information, inadvertent disclosure or access by unauthorized third parties.

Name:	
	Firm
Title:	
	Date