FORMULATION DEVELOPMENT AND EVALUATION OF OPHTHALMIC SOLUTION OF TIMOLOL MALEATE 0.5%

Dissertation submitted to

THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY, CHENNAI-32

In partial fulfillment for the award of the degree of

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IN

PHARMACEUTICS

Submitted by

Register Number : 26111003

UNDER THE GUIDANCE OF

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CERTIFICATE

This is to certify that the dissertation work entitled **"FORMULATION DEVELOPMENT AND EVALUATION OF OPHTHALMIC SOLUTION OF TIMOLOL MALEATE 0.5%"** submitted to **THE TAMILNADU DR. M. G. R. MEDICAL UNIVERSITY, CHENNAI-32** for the award of the degree **Master of pharmacy in Pharmaceutics** is a bonafide research work done by **Register No: 26111003** under my Guidance in the Department of Pharmaceutics, C. L. Baid Metha College of Pharmacy, Chennai-600 097 during the academic year 2012-2013.

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January 23 2013

TO WHOMSOEVER IT MAY CONCERN

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During this period, we found him to be hardworking and committed and we wish him all the best in his future endeavors.

With Best Wishes.

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DECLARATION

I hereby declare that the thesis entitled **"FORMULATION DEVELOPMENT AND EVALUATION OF OPHTHALMIC SOLUTION OF TIMOLOL MALEATE 0.5%"** has been originally carried out by me under the supervision and guidance of **Chandrashekhar S. Kadam. M.Pharm.,** (Industrial guide) and **Mr. T. Udayakumar. M.pharm.,** (Institutional Guide) Asst.Professor, Department of Pharmaceutics, C.L.Baid Metha college of Pharmacy, Chennai-97 during the academic year 2012-2013.

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ABBREVIATIONS AND NOMENCLATURE

API	Active pharmaceutical Ingredient
BFS	Blow Fill Seal
BAK, BKC	Benzalkonium Chloride
BP	British Pharmacopoeia
FDA	Food and Drug Administration
GMP	Good Manufacturing Practices
HPLC	High performance liquid chromatography
IOP	Intra Ocular Pressure
JP	Japanese Pharmacopoeia
LDPE	Low Density Poly Ethylene
MOC	Material Of Construction or Content
NMT	Not More Than
Ph Eu	European Pharmacopoeia
RH	Relative Humidity
USP	United States Pharmacopoeia
UV	Ultra Violet
Cfu/ml	Colony forming unit per Millilitre
^{0}C	Celsius
gm	Gram
Hrs	Hours
Kg	Kilo gram
kGy	Kilogray
mg	Milli gram
Min	Minutes
mOsmol	Milli Osmoles
Mpa	Mega Pascal
Sec	Seconds
µl	Micro litre
µm	Micro meter

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CONTENTS

1. INTRODUCTION

Delivery of medication to the human eye is an integral part of medical treatment.[1] Ophthalmic drug delivery is one of the most interesting and challenging endeavors facing the pharmaceutical scientist. The anatomy, physiology, and biochemistry of the eye render this organ highly impervious to foreign substances. A significant challenge to the formulator is to circumvent the protective barriers of the eye without causing permanent tissue damage. Development of newer, more sensitive diagnostic techniques and novel therapeutic agents continue to provide ocular delivery systems with high therapeutic efficacy.[2]

Ophthalmic preparations are specialized dosage forms designed to be instilled onto the external surface of the eye (topical), administered inside (intraocular), adjacent to the eye (periocular) or used in conjunction with any special device.

The preparation may have any several purposes like therapeutic, prophylactic or palliative. The versatility of dosage form enables therapeutic agent to be suitable for function of preparation. Therapeutically active formulation may be designed to provide extended action for either convenience or reduction in dose frequency, improved bioavailability of an agent or improved delivery to target tissue. The residence time of an ocular preparation may range from few seconds (ophthalmic solutions) to hours (gel, ointments), to months or years (intra ocular or periocular dosage forms).

Ophthalmic preparations are similar to parentral dosage form in their requirements for sterility as well as consideration for osmotic pressure (tonicity), preservation, and tissue compatibility, avoidance of pyrogens and particulate matter and suitable packaging.

Widely used topical ophthalmic therapeutic dosage forms are solutions and suspensions. Ophthalmic solutions are most often multidose product containing suitable preservatives to meet compendial Preservative Efficacy Test (USP, BP, Ph Eu, and JP) requirements.

Drugs are administered to the eye for local effects such as bacterial infection, miosis, mydriasis, or to reduce intraocular pressure.[3]

➢Recent Trends in Ophthalmics

Various ophthalmic formulations include aqueous solution, aqueous suspension, ointments, and ocular inserts. Every ophthalmic product must be sterile in its final container to prevent the microbial contamination of the eye.

Ophthalmic Solutions:

By definition, it is common, ingredients are completely soluble such that dose uniformity is not an issue and there is no or very little physical interference with vision.

Advantages

The advantages of ophthalmic solutions includes

a. Easy manufacturing and low cost as compared to other dosage design.
b. Ophthalmic solutions have potentially better dose uniformity.
c. More ocular bioavailability.[4]

Qualities:

Ophthalmic solution must

a. Improve the ratio of local activity versus systemic effects.
b. Be easy to self-administer.
c. Not induce a foreign-body sensation, long-lasting blurring, or a very bad aftertaste.
d. Be sterilizable at industrial scale by a recognized process.
e. Not rely on "exotic" ingredients like new chemical entities or difficult-to-source excipients (unless this is a key element). Preferably, excipients should have a drug master file and history of safe use for humans.
f. Be compatible with an efficient antimicrobial preservative, or packaging.
g. Preferably be stored without specific conditions.

Gel forming Solutions:

Ophthalmic solutions usually aqueous based, which contain a polymer system that has low viscosity, aqueous in container and gels on contact with tear fluid.

- **Powders for Solutions:**

Drugs that have limited stability in aqueous solution can sometimes be prepared as sterile powder for reconstitution by pharmacist prior to dispensing. Reconstitution must be done with sterile vehicle.

- **Ophthalmic Suspension:**

Ophthalmic Suspensions are finely divided, relatively insoluble drug substances in an aqueous vehicle containing suitable suspending and dispersing agent.

Because of tendency of particles to be retained in cul-de-sac, the contact time and duration of action of suspension could be theoretically exceeds. The drug is absorbed from solution and the solution concentration is replenished from retained particles. Optimum activity should be result from optimum particle size.

- **Ophthalmic ointments: -**

Ophthalmic ointments are primarily anhydrous and contain mineral oil and white petrolatum as the base ingredients that can be varied in proportion to adjust consistency. In effort to maintain longer contact between drug and ophthalmic tissue, ointments have been used. Ophthalmic ointments tend to keep the drug in contact with eye longer than suspension and solution. Most ophthalmic ointments are mixture of mineral oil and white petrolatum and have melting point closed to body temperature. Sometime anhydrous Lanoline is used to take up an ingredient that was dissolved in small amount of water to affect dissolution. The aqueous solution incorporated into Lanoline and then the Lanoline is mixed with remaining ointment base ingredients.

Ointment must be non-irritating and free from grittiness so micronized form of the ingredients is required. The ointments are packaged in a sterile container such as an ointment tube.

Disadvantages

The disadvantages of ophthalmic ointments are

a. drug content non uniformity.
b. blurred vision due to sticky vehicle.
c. not removed easily by tear fluid.

d. only bedtime administration.[5]

The anhydrous nature of base enables use as carrier for moisture sensitive drugs.

- **Emulsions:**

Ophthalmic emulsions offers advantage of being able to deliver poorly water soluble drug in solubilised form as an eye drop. Emulsions are used to deliver drugs (cyclosporine) topically for treatment of chronic dry eye condition.[6]

- **Ocular Gel:**

Gel forming polymers such as carbomers have been used to develope aqueous semisolid dosage form which are packaged and administered same as ointments. eg.: Carbomer gel of Pilocarpine administered at bed time has been shown to prolong the intraocular pressure lowering effect in patient for up to 24 hours.[7]

- **Ocular inserts:**

Ocular inserts delivers the drug to eye by diffusional mechanism. Solid dosage form delivers an ophthalmic drug at a near constant known rate. The delivery of Pilocarpine by such an inserts was commercialized in 1975 (Ocusert Pilo) by Alza corporation. Ocusert is designed to be placed in a lower cul-de-sac to provide weekly dose.

Erodible inserts are developed (Lacrisert) for the treatment of dry eye. No preservative for unit dose required.[8]

- **Delivery route for ophthalmic solution:**

Conventionally, many ocular diseases are treated with either topical or systemic medications. Topical application of drug has remained the most preferred method due to ease of administration and low cost. Topical application is useful in the treatment of disorders affecting the anterior segment of the eye. Anatomical and physiological barriers hinder drugs from reaching posterior segment of eye mainly choroid and retina. A major fraction of drug following topical administration is lost by lacrimation, tear dilution, nasolacrimal drainage, and tear turnover. Such precorneal losses result in very low ocular bioavailability.

Typically, less than 5% of total administered dose reaches aqueous humor. So in order to maintain minimum inhibitory concentrations, the agents need to be frequently dosed.

Upon topical instillation drugs are absorbed by corneal route (cornea → aqueous humor → intra ocular tissue) or non corneal route (conjunctiva → sclera → choroid/ retinal epithelial pigment). The preferred route depends mainly on the corneal permeability of drug molecules. Unlike topical administration, systemic dosing helps in the treatment of disease affecting posterior segment of the eye. A major drawback associated with systemic administration is only 1-2% of administered drug reaches to vitreous cavity. Blood retinal barrier which is selectively permeable to more lipophillic molecules mainly governs the entry of drug molecules into posterior segment of the eye. This results in frequent administration of high amounts of drugs leading to systemic side effects.[9]

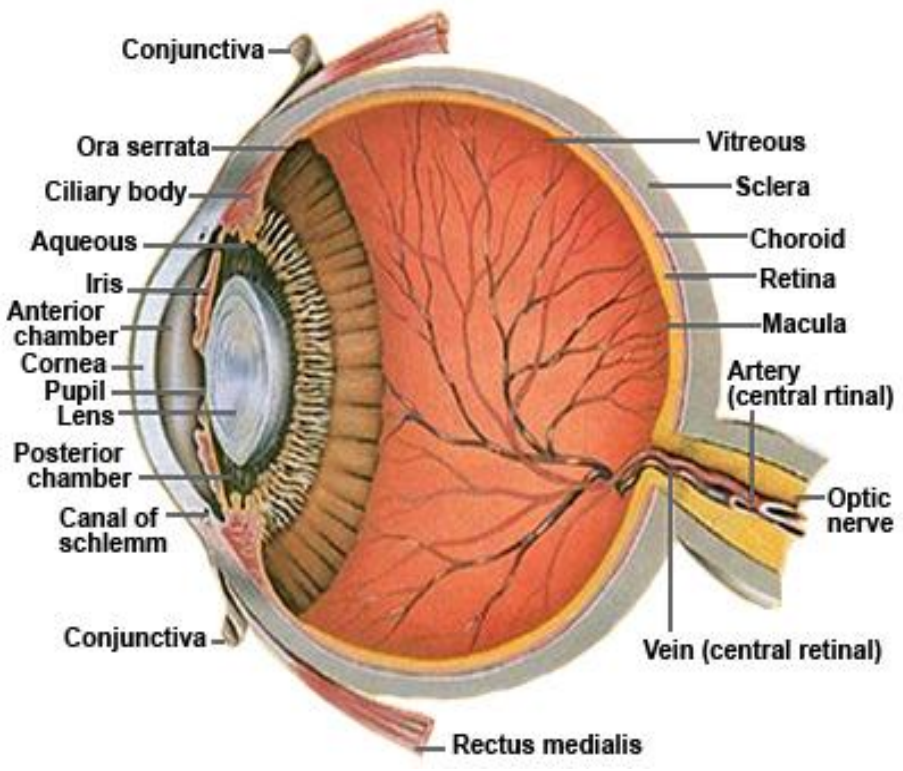


Figure 1: Human eye Anatomical structure

➢ **About drug**

Beta blockers (β-blockers, beta-adrenergic blocking agents, beta antagonists, beta-adrenergic antagonists, beta-adrenoreceptor antagonists, or beta adrenergic receptor antagonists) are a class of drugs.

Beta blockers target the beta receptor. Beta receptors are found on cells of the heart muscles, smooth muscles, airways, arteries, kidneys, and other tissues that are part of the sympathetic nervous system and lead to stress responses, especially when they are stimulated by epinephrine(adrenaline). Beta blockers interfere with the binding to the receptor of epinephrine and other stress hormones, and weaken the effects of stress hormones.

In 1962, Sir James W. Black found the first clinically significant beta blockers - propranolol and pronethalol; it revolutionized the medical management of angina pectoris and is considered by many to be one of the most important contributions to clinical medicine and pharmacology of the 20th century.[10]

First beta-blocker approved for topical use in treatment of glaucoma in the USA (1978). With monotherapy, it depresses Intra ocular pressure to 18-34% below baseline within first few treatments. In its ophthalmic form, Timolol is used to treat open-angle and occasionally secondary glaucoma by reducing aqueous humour production through blockage of the beta receptors on the ciliary epithelium.[11]

- **Classification of β- Blockers**[12]

The β- blockers are classified based on their selectivity towards the Adrenergic receptors. They are classified as

1.Non Selective agents

These are also called First Generation agents which include Propranolol, Pindolol, penbutlol, Timolol, and Sotalol etc,.. These are well absorbed orally and Effectively reduse the hyper tension at various organs.

2.β-1 selective agents

The second generation β- blockers or β-1 selective agents are cardio selective drugs and do not cause side effects in any site except the heart (site of mechanism). They are Atenolol, Betaxolol, Bisoprolol, Esmolol, and Metoprolol, etc,..

3. β-blockers with Vasodilator action

These Third generation β blockers lower the peripheral resistance either by β-2 receptor stimulation or by direct vasodilation. i.e Dilevalol.

The β-2 and β-3 selective agents have no significant clinical applications. Used in experimental purposes only.

- **GLAUCOMA :**

Glaucoma is an eye disease in which the optic nerve is damaged in a characteristic pattern. This can permanently damage vision in the affected eyes and lead to blindness if left untreated. It is normally associated with increased fluid pressure in the eye (aqueous humour). The term "ocular hypertension" is used for people with consistently raised intraocular pressure (IOP) without any associated optic nerve damage. Conversely, the term 'normal tension' or 'low tension' glaucoma is used for those with optic nerve damage and associated visual field loss, but normal or low IOP.

There are totally six types of Glaucoma

- Primary open angle Glaucoma
- Angle closure glaucoma
- Normal tension Glaucoma
- Pigmentary Glaucoma
- Secondary Glaucoma
- Congenital Glaucoma

In most cases, glaucoma is associated with higher-than-normal pressure inside the eye (ocular hypertension). If untreated or uncontrolled, glaucoma first causes peripheral vision loss and eventually can lead to blindness.

According to the American Academy of Ophthalmology (AAO), the most common type of glaucoma is called primary open-angle glaucoma which affects an estimated 2.2 million people in the United States, and that number is expected to increase to 3.3 million by 2020 as the U.S. population ages.

The Ophthalmic solution of timolol maleate is the best available formulation which is Primarily used for Chronic Open angle glaucoma.

➢ Formulation Parameters for Ophthalmic Solution

Ophthalmic solutions should be prepared and preserved according to whether they are to be used in surgical procedures, in clinic or office or by the patient at home.

There is an optimum pH level at which the solution of individual drugs should be buffered in order to obtain the maximum efficiency and stability. Deterioration of the drugs used is greatly diminished when they are dispensed at proper pH.

Preservative solutions in proper strength have been shown to be adequate for preservation of ophthalmic solutions.[13]

Important factors to be considered in formulating an ophthalmic solution include the following

- Clarity
- Sterility.
- Osmolarity.
- pH, buffering.
- Preservation.
- Solubility.
- Stability in appropriate vehicle.
- Viscosity.
- Suitable packaging and storage of finished product.[14]

- **Clarity:**

Ophthalmic solutions contain number of dissolved ingredients and are essentially free from foreign particles. Clarity of the solution may be enhanced by filtration. Ophthalmic solutions must be filtered in clean surroundings and laminar flow hood.

Both container and closure must be thoroughly clean sterile and non shedding, neither contributing particulate matter to the solution during prolong contact for the duration of shelf life. The European Pharmacopoeia describes visual clarity and recommended standard that can be used for clarity specification.

Particulate matter consists of mobile randomly sourced extraneous substances, other than gas bubbles, that cannot be quantitated by chemical analysis because of the small amount of material they represent and because of their heterogeneous composition. The tests described in USP are physical tests performed for the purpose of enumerating extraneous particles within specific size ranges. Ophthalmic preparation that are suspension, emulsions, or gels are exempt from these requirements.

Light Obscuration and Microscopic procedure are specified in USP.[15]

Table 1: Light Obscuration Test Particle Count

Parameter	Diameter	
Particle size	≥10 µm	≥25 µm
Number of particle	50 per ml	5 per ml

Table 2: Microscopic Method Particle Count

Parameter	Diameter		
Particle size	≥10 µm	≥25 µm	≥50 µm
Number of particle	50 per ml	5 per ml	2 per ml

- **Sterility:**

Sterility is defined as absence of viable microbial contamination. Sterility is an absolute requirement of all ophthalmic formulation. Contaminated ophthalmic formulation may result in eye infection that could ultimately cause blindness, especially if Pseudomonas aeruginosa microbes are involved. Therefore, ophthalmic formulations must be prepared in Laminar flow hood using aseptic technique just same as intravenous formulations. The sterile formulations must be packed in sterile containers.

Products to be instilled into the eye, while not parenterals by definition have many similar and often identical characteristics. The formulation of stable therapeutically active ophthalmic preparation requires high purity of ingredients as well as chemical, physical (particles), and microbial contaminants.[16]

- **Osmolarity:**

Osmolarity is measure of osmoles of solute per liter of solution, while osmolality is measure of osmoles of solute per kilogram of solvent. Tonicity refers to the osmotic pressure exerted by salts in aqueous solution. Osmolarity and tonicity are not the same. The key difference between two is osmolarity is measure of all solutes in solution, whereas tonicity is measure of impermeable solute. Osmolarity compares the solute in two solutions, whereas tonicity compares the osmotic pressure gradient. An ophthalmic solution is isotonic with solution when the magnitude of colligative properties such as osmotic pressure, freezing point depression, boiling point elevation and vapour pressure is same. The derivatives of the terms: Iso osmotic, Hyper osmotic and Hypo osmotic should not be confused with Isotonic, Hypertonic and Hypotonic.[17]

Actuality, the external eye is much more tolerant of tonicity variation. Normal human plasma has an osmolality in the range of 285-295 milliosmol/ kg. Pharmaceutical solutions which have an osmolality higher than 600 milliosmol/ kg cause crenation (shrinking) of blood cells and significant pain. Whereas solutions which have osmolality less than about 150 milliosmol/ kg cause Haemolysis (rupture) of blood cells. Normal plasma osmolality is tightly controlled by homeostatic mechanisms in the body; a change of 3 milliosmol

represents a change from minimal to maximal ADH output. Thus, osmolality plays a role in formulation development.

Changes in osmolality can be used as guide to the breakdown of a substance in solution and is therefore used in stability testing.[18]

Osmolarity cannot be measured but it is calculated theoretically from the experimentally measured value of osmolality. Sometimes, osmolarity (mOsmol/L) is calculated theoretically from Molar concentration of dissolved solute and depends on dissociation constant of solution and deviation from ideality. The osmolality of a solution is commonly determined by the measurement of freezing point depression of solution.

Commonly used tonicity modifiers are Sodium chloride(0.9%), Boric acid (1.9%) and Dextrose (5%).[19]

- **pH and Buffering:**

The physiologic pH of blood and tears is approximately 7.4. Eye can tolerate preparation of pH as low as 3.5 and as high as 9.0. It is preferable to formulate as close to physiological pH values to minimize the pH induced lacrimation, eye irritation and discomfort.[20]

Another important consideration in selection of optimum pH formulation is the drug stability for pH sensitive drugs such as peptides and proteins. pH may affect the function of the other components in formulation. For example The antimicrobial preservative, parabens are inactivated at alkaline pH and more active as the pH becomes acidic.

A variety of regulatory approved buffers are available covering the useful pH range to maintain the pH of the formulation. For acidic pH adjustment, acetic acid /sodium citrate are often employed. For alkaline pH phosphate or borate buffer are frequently used.[18]

- **Preservation:**

Topically applied ophthalmic products, regardless of their use, usually contain water as one of primary component. This water provides a medium in which microorganisms can survive or grow. Other ingredients in these formulations can also create viable growth medium for these organisms.

Hence such formulations usually contain preservative system. Preservative system can be either a single agent or combination of agents.

An ideal preservative should have broad spectrum of activity against all types of microorganisms, including yeast, mold, fungi, gram positive and gram negative bacteria. The preservative is also ideally effective at low concentration to minimize expense, to avoid irritation and/or sensitization reaction. Agent should be stable over wide range of conditions including autoclaving temperature and pH range. Compatibility should establish with other component of preparation and with packaging system.[21]

1. Types of Preservatives:

There are several preservatives, but only a few are used frequently in topical ophthalmic preparation. (Table-3) Preservatives typically work by one of two basic mechanisms: they are either detergents or act through oxidative processes. Detergents (or, more specifically surfactants) act by dissolving or disrupting lipids. Detergent preservatives kill microorganisms by disrupting cell membranes and causing cell lyses. Example includes Benzalkonium chloride (BAK or sometimes BKC), polyquaternium-1 (PQ1), alcohol preservatives, and phenols.

Oxidative preservative cause oxidative reaction that disrupt cellular metabolism. Examples of oxidative preservative include thiomersal, sodium perborate, sorbic acid, and chlorhexidine.[22]

Table 3: Common Preservatives Used For Ophthalmic Solutions

Compound class	**Example**
Quaternary ammoniums	Benzalkonium chloride (BAK), Polyquaternium-1
Mercurials	Thiomersal, Phenyl mercuric nitrate, Phenyl mercuric acetate
Alcohols	Chlorbutanol, Benzyl alcohol
Carboxylic acid	Sorbic acid
Phenols	Methyl/propyl paraben
Amidines	Chlorhexidine
Other	Disodium EDTA

Specific chemicals which are toxic to micro-organisms may be formulated in products which are not self-preserving and for which contamination with undesirable organisms is possible in use e.g. multi-dose vials, eye drops, nasal sprays, and topicals. The effectiveness of preservation (continuance of antimicrobial effectiveness) may depend on several factors

2. Factors Affecting Preservative Activity :

- Concentration (Dilution or Loss)
- pH (Non-optimal range)
- Temperature (Non optimal range)
- Partitioning (Between aqueous and non-aqueous phases or between liquids/solids and headspace.)

Effect of Concentration (Dilution) :

Preservative dilution (loss) may be due to a number of factors

- Chemical degradation
- Biological degradation
- Diffusion of volatile components through the packaging e.g. phenyl ethanol
- Partitioning between the liquid and headspace phases
- Absorption by the packaging e.g. plastic containers, benzalkonium chloride
- Precipitation e.g. benzalkonium chloride with certain label adhesive components migrating through plastic bottles.

Investigations at the turn of the century demonstrated an exponential relationship between the rate of microbial death and the concentration of the antimicrobial agent, expressed as follows;

$$C_1^{\eta} t_1 = C_2^{\eta} t_2$$

$$\eta = \frac{log\ t2 - log\ t1}{log\ C1 - log\ C2}$$

C_1 and C_2 represent the two concentrations of the antimicrobial agent and t_1 and t_2 their respective times to achieve the same level of reduction in viable count. η is a measure of the effect of changes in the concentration (or dilution level) on the microbial death rate and is termed the *concentration exponent* or *dilution coefficient.*

In practical terms the activity of a compound with high η will be markedly decreased by dilution whilst a compound with low η will be less severely affected.

Table 4: Relation of Concentration and Activity of the Preservative

Concentration Exponent	Fraction of Activity Remaining After Dilution to One Half
1	½
2	¼
3	1/8
4	1/16
5	1/32
6	1/64
7	1/128
8	1/256

Preservative activity monitored by chemical assay may give very misleading data.

Table 5: Preservative Characteristics

Preservative agent	Concentration exponent
Benzalkonium chloride	3.5, 1.8(y), 9(m)
Benzyl alcohol	6.6, 4(y), 2(m)
Chlorocresol	8.3
Parabens	2.5
Phenol	5.8, 4(y), 4.3 (m)
Phenoxyethanol	9
Phenylethanol	5.6

Note: y = yeast, m= moulds, otherwise bacteria.

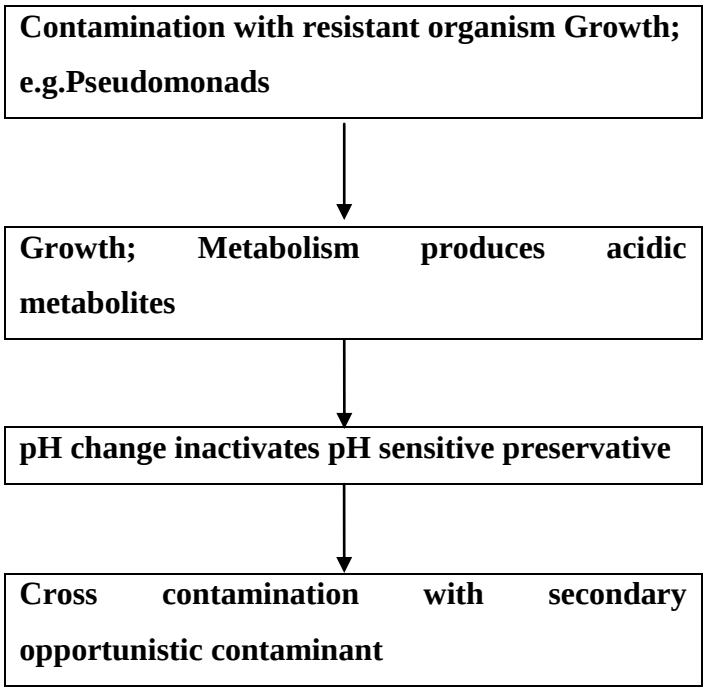
Effect of pH

The antimicrobial activity of many preservatives is strongly influenced by pH. The effect of pH on the antimicrobial activity is depicted in table 6.

Table 6: Effect of pH

Preservative agent	Optimal pH range
Benzalkonium chloride	4 – 10
Benzyl alcohol	2 – 5
Chlorocresol	< 8.5
Parabens	3 - 9.5
Phenol	<9
Phenoxyethanol	<10
Phenylethanol	<7

A change in pH can have a significant effect on preservative efficacy e.g. the activity of phenol can be reduced by a factor of 10 by a change of one pH unit. Changes in pH of products may be the result of ageing or by the metabolic activity of a micro-organism resistant to the preservative.



Effect of Temperature :

The effect of temperature on preservative activity may be quantified in terms of the Q10 value (the change in activity for a 10°C change in temperature). Activity usually increases with temperature however preservative agents respond differently.

Table 7: Effect of Temperature on Preservative Activity

Preservative agent	**Temperature coefficient Q_{10}**
Benzalkonium chloride	2.9-5.8
Benzyl alcohol	2.3-7.2
Chlorocresol	3-5
Phenol	5

Effect of Partitioning :

For multi-phase systems e.g. creams, emulsions etc. effective preservation is complex. Partitioning of the preservative between the aqueous and oil phases may result in ineffective concentrations in the water phase. Surface active agents may need to be employed. Partitioning may also be affected by the pH of the product and the degree of ionization of the preservative.

➢ **Benzalkonium Chloride :**

Benzalkonium chloride, a popular preservative for pharmaceutical products, is a complex mixture since the alkyl portion of the molecule is derived from natural sources. The chain lengths are principally C12 to C16, however the antimicrobial activity increases with the proportion of longer chain lengths. Unfortunately the tendency to adsorb to plastics also increases with chain length i.e. the most effective constituents of the mixture may be preferentially adsorbed.

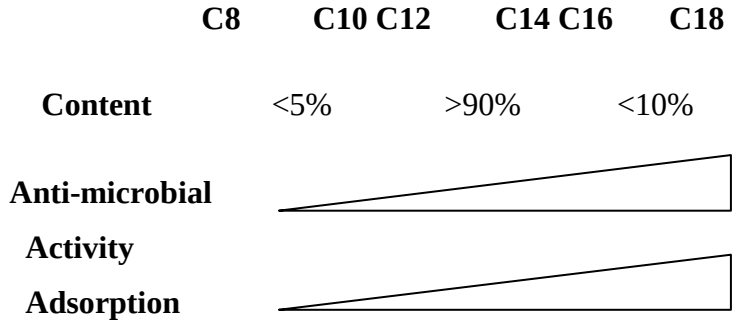


Fig 2: Adsorption of Benzalkonium chloride

Adsorption of benzalkonium chloride will therefore not only affect the available concentration in solution but will also affect the concentration distribution of the homologues. Both of these parameters will have significant microbiological effect even though chemical testing, which normally does not differentiate between homologues of BKC, may not reveal a significant change in total benzalkonium chloride.

- **Characteristics of Chemical Preservatives :**
  - *Range of antimicrobial activities*
  - *Structures, synonyms*
  - *Stability, compatibility*

Table 8: Range of Antimicrobial Activities

Preservative agent	Bacteria		Yeasts	Moulds
	Gram-positive	Gram-negative		
Benzalkonium chloride	+++	++	++	+
Benzyl alcohol	+++	+	+	+
Chlorocresol	+++	++	+	+
Parabens	++	+	++	++
Phenol	++	+	+	+
Phenoxyethanol	++	+++	+	+
Phenylethanol	++	+++	+	+

+++ Active

++ Moderately Active

+ Weakly Active.[23]

- **Viscosity :**

Viscosity measures the resistance of a solution to flow when a stress is applied. The viscosity of a solution is given in poise units. The unit centipoise (cp or the plural cps) is equal to 0.01 poise and is most often used in pharmaceutical applications. Compounds used to enhance viscosity are available in various grades such as 15 cps, 100 cps, etc. The grade number refers to the viscosities that result

when a fixed percentage aqueous solution is made. Generally the solutions are 1% or 2% and the viscosity is measured at 20^{o}C.

Viscosity enhancers are used in ophthalmic solutions to increase their viscosity. This enables the formulation to remain in the eye longer and gives more time for the drug to exert its therapeutic activity or undergo absorption. Commonly used viscosity enhancers and their maximum concentrations are given in the table below.

Table 9: List of Viscosity Enhancers

Viscosity Enhancer	Maximum Concentration (%)
Hydroxyethylcellulose	0.8
Hydroxypropylmethylcellulose	1.0
Methylcellulose	2.0
Polyvinyl alcohol	1.4
Polyvinylpyrrolidone	1.7

The most common viscosity desired in an ophthalmic solution is between 25 and 50 cps. The actual concentration of the enhancer required to produce that viscosity will depend on the grade of the enhancer. For example, if methylcelluse 25 cps is used, a 1% solution will create a viscosity of 25 cps. If methylcellulose 4000 cps is used, a 0.25% solution provides the desired viscosity. Standard references give tables of viscosities produced by percentage solutions and grades of ingredients.[24]

➢ **Sterilization of Ophthalmic Solution:**

- **Methods of sterilization of ophthalmic solution**

Ophthalmic solutions can be sterilized by both terminal sterilization and filtration sterilization. Probably ophthalmic solutions packaged in plastic container are sterilized by filtration method. Both the methods are elaborated as follows.

1. Terminal sterilization :

Wherever possible, a process in which the product is sterilized in its final container (terminal sterilization) is chosen.

I. **Steam sterilization :**

Sterilization by saturated steam under pressure is preferred, wherever applicable, especially for aqueous preparations. For this method of terminal sterilization the reference conditions for aqueous preparations are heating at a minimum of 121 °C for 15 min.

II. **Dry heat sterilization :**

Dry heat sterilization is carried out in an oven equipped with forced air circulation or other equipment specially designed for the purpose. For this method the reference conditions are a minimum of 160 °C for at least 2 hrs.

III. **Ionizing radiation sterilization :**

Sterilization by this method is achieved by exposure of the product to ionizing radiation in the form of gamma radiation from a suitable radio isotopic source (such as cobalt 60) or of a beam of electrons energized by a suitable electron accelerator. For this method of terminal sterilization the reference absorbed dose is 25 kGy.

IV. **Gas sterilization :**

This method of sterilization is only to be used where there is no suitable alternative. It is essential that penetration by gas and moisture into the material to be sterilized is ensured and that it is followed by a process of elimination of the gas under conditions that have been previously established to ensure that any residue of gas or its transformation products in the sterilized product is below the concentration that could give rise to toxic effects during use of the product.

2. Filtration Sterilization :

Certain active ingredients and products that cannot be terminally sterilized may be subjected to a filtration procedure using a filter of a type that has been demonstrated to be satisfactory by means of a microbial challenge test using a

suitable test micro-organism. A suspension of Pseudomonas diminuta (ATCC 19146, NCIMB 11091, or CIP 103020) may be suitable. Most of the ophthalmic solutions are sterilized by filtration method.

Solutions are passed through a bacteria-retentive membrane with a nominal pore size of 0.22 µm or less or any other type of filter known to have equivalent properties of bacteria retention.[25] Factors that can affect filter performance generally include Viscosity and surface tension of the material to be filtered, pH, compatibility of the material or formulation components with the filter itself, pressures, flow rates, maximum use time, temperature, osmolality and the effects of hydraulic shock.

Filters are of two basic types Depth and Membrane Filter.

Depth Filter relies on combination of tortuous pathway and adsorption to retain particles or micro-organisms. They are made from material such as diatomaceous earth, inorganic fibers, natural fibers, and porcelain.

Membrane filters are made from cellulose ester derivatives. The advantages of membrane filter include no retention of product, no media migration, and efficiency independent flow-rate pressure differential. The major disadvantage of membrane filter is low capacity before clogging and need to prewash the filter to remove surfactant.[25]

- **Filter Integrity Test Methods** [26]

Integrity testing sterilizing filters is a fundamental requirement of critical process filtration applications. FDA Guidelines require integrity testing of filters used in the processing of sterile solutions such as large volume parenterals (LVPs) and small volume parenterals (SVPs).

- **Classifications of integrity testing :**

1. Destructive Testing

Destructive bacterial challenge testing in performed in accordance with ASTM F838-83 methodology. Destructive challenge testing is the best way to determine a sterilizing filter's ability to retain bacteria.

During the bacterial retention test, 0.22 µm filter discs and devices are challenged with a solution of culture medium containing bacteria (Brevundimonas diminuta ATCC 19146) at a minimum challenge of 10^7 per cm^2. The effluent is then passed through a second 0.45 µm assay filter disc that is placed on an agar plate and incubated.

2. Non-Destructive Testing :

Non-destructive testing may be done on filters before and after use. Integrity testing sterilizing filters before use monitors filter integrity prior to batch processing, preventing use of a non-integral filter. Integrity testing sterilizing filters after a batch has been filtered can detect if the integrity of the filter has been compromised during the process.

There are two types of non-destructive testing

I. Bubble point test.
II. Diffusion test.

Bubble point test :

The most widely used non-destructive integrity test is the bubble point test. Bubble point is based on the fact that liquid is held in the pores of the filter by surface tension and capillary forces. The minimum pressure required to force liquid out of the pores is a measure of the pore diameter.

$$P = 4k \cos\theta \, \sigma / d$$

P = bubble point pressure d = pore diameter

k = shape correction factor q = liquid-solid contact angle

s = surface tension

Diffusion Test :

At differential gas pressures below the bubble point, gas molecules migrate through the water-filled pores of a wetted membrane following Ficks Law of Diffusion. The gas diffusional flow rate for a filter is proportional to the differential pressure and the total surface area of the filter. At a pressure approximately 80% of the minimum bubble point, the gas which diffuses through the membrane is measured to determine a filter's integrity. The flow of gas is very low in small area filters, but it is significant in large area filters. Maximum diffusional flow specifications have been determined for specific membranes and devices and are used to predict bacterial retention test results.

$$DF = K (P1-P2) A P / L$$

Where:

K = Diffusivity/Solubility coefficient P1, P2 = Pressure difference across the system

P = Membrane porosity L = Effective path length

A = Membrane area DF = Diffusional Flow

Pressure Hold Testing

The Pressure Hold Test, also known as pressure decay or pressure drop test, is a variation of the diffusion test. In this test, a highly accurate gauge is used to monitor upstream pressure changes due to gas diffusion through the filter. Because there is no need to measure gas flow downstream of the filter, any risk to downstream sterility is eliminated. The pressure hold value is dependent on the

diffusional flow and upstream volume. It can be calculated using the following equation:

Pressure Hold = D (T) (Pa) / Vh = DP

Where:

D = Diffusion rate (cc/min) T = Time (minutes)

Pa = Atmosphere pressure (1 Atm. or 14.7 psi) Vh = Upstream volume of apparatus (cc)

DP = Pressure Drop (bar or psi)

➢ Packaging of Finished Product

Ophthalmic products must possess certain levels of stability and purity in order to be suitable for safe and efficacious administration to patients. Ophthalmic products are considered stable if the active ingredient can maintain its strength at the level specified on the label for the maximum anticipated shelf-life under given environmental conditions. An ophthalmic product is considered unstable when the active ingredient or excipients such as preservatives, loses sufficient potency to adversely affect the safety or efficacy of the drug or falls outside labeled specifications. A typical example of relatively unstable medicinal agents is prostaglandin. The potency of a drug product may decline over time during storage due to various reasons, such as degradation of the active ingredient, reaction of the active ingredient with excipients or container materials, or leaching of the active ingredient through the container wall or absorption of the active ingredient into the container wall.

In addition, many medicinal preparations contain preservatives, such as Chlorobutanol, Phenoxyethanol, Methyl, and Propyl parabens and Benzalkonium chloride, as certain concentrations, which enable storage of the medicinal preparations for periods of time up to 24 months or more. The preservatives may permeate the container wall upon storage, reducing the concentration in the preparation, and as a result their preservative value is diminished. Similarly, the purity of a medicinal preparation may also change during storage due to leaching of chemical or chemicals into the drug preparation from the container materials, from

the labels on the containers, or from the environment where the packaged ophthalmic product is stored. Thus, containers used for packaging medicinal preparations can significantly affect the stability and purity of the preparations.[27]

Containers commonly used for ophthalmic products include glass containers, and polyethylene containers. Glass containers and polyethylene containers are said to be superior in maintaining stability of ophthalmic preparations.[28]

- **Glass:**

The three types of glass recognized by USP for parenteral /ophthalmic use are

a. Type I: Borosilicate glass (highly resistant)

b. Type II: Treated soda lime glass

c. Type III: Soda lime glass

Type I is borosilicate and is the least reactive as measured by a standardized alkalinity test run on powdered (ground) samples. Type II and III glass are soda lime, with type II being surface treated with sulfate, sulfite, or sulfide to make it less reactive. Type I glass is, theoretically, the best all purpose glass for injectables, ophthalmics and should be the only glass that is used with alkaline products. However, it is significantly more expensive than types II and III. Type II glass is often used for solutions that remain below pH 7.0 during their shelf life, while type III glass can be used for dry powders that are reconstituted. The particular glass container intended for use must be an integral part of product stability program.

Amber glass containers are often used where the product is suspected of being a light sensitive. The amber color is imparted by addition of iron and manganese oxides, the cations of which are known to catalyze the oxidative reactions. Studies have shown that these ions are extracted from glass and that the decomposition rate of several drugs, thiomerosal, amitriptylene and L-ascorbic acid is enhanced in amber glass containers.[29]

- **Plastic:**

Since 1970, the use of glass containers has diminished dramatically. Plastic dropper bottle have been favored because they weight loss, are more resistant to shock and other mechanical influences, cost less and offer more design possibilities. Polyethylenes, that is, low density polyethylene with or without additives and

polypropylenes are the plastics required by the European Pharmacopoeia. Examples of additives for both polyethylene ad polypropylenes include antioxidants, stabilizers, and plasticizers, lubricants coloring matter and softening agents. However the United States Pharmacopoeia does not specify the types of plastic.

Plastic bottles are thin walled as compared to glass bottles (plastic: 0.70-1.19 mm; glass: 2.00-2.20 mm). The wall thickness and the density of the plastic material determine the flexibility (ability to deform), elasticity (ability to return to its original form after deformation), and stiffness (resistance to deformation) of the bottle. Dropper bottles are shaped with round or oval bases and usually contain volume of 3 to 15mL.

The disadvantage of polyethylene includes its permeability to vapors and gases, the adsorption and absorption of contents (e.g. preservatives like benzalkonium chloride and chlorobutanol). Polypropylene has poorer resistance to oxidation agents such as oxygen and acids which can lead to fissures and yellowing of plastic.[30]

- **Information concerning the plastic material used for the packaging purpose**

1. **General information :**

The following information should be provided for plastic materials used in the container, including those already described in the pharmacopoeia where the monographs authorize the use of several additives from which the manufacturer may choose one or several (within certain limits).

– The name and grade given by the manufacturer of the material.

– For ophthalmic and parenteral preparations, the name of the plastic manufacturer.

– The chemical name of the material.

– The chemical name(s) of any monomer used.

– The complete qualitative composition of the plastic material is required where an interaction between the container and the contents occurs. The qualitative composition covers all substances, including additives such as antioxidants, stabilizers, catalysts, plasticizers, lubricants, solvents and/or dyes (comprising the color index number and/or the EC number).

If the material has not been approved for use for packaging of food, toxicological data should be provided. In addition, toxicological information is

required for plastics normally approved for use in food packaging, if they are used for parenteral or ophthalmic medicinal products.

2. Technical information :

– Characteristics

Description of the material, its solubility in various solvents.

– Identification of the material generally by infrared absorption spectrophotometry, with indication of the position of characteristic absorption bands. The infrared spectrum of the reference material should be provided: other methods of identification may be appropriate.

– Identification of the main additives in particular those which are likely to migrate into the contents (such as antioxidants, plasticizers, catalysts, initiators, etc.... and, for PVC, phthalates, adipates and organic tin compounds).

– Identification of dyes by using chromatographic or any other appropriate method.

– Tests

• General tests

• Mechanical tests

• Physical tests: An extraction test should be performed where the plastic material is used as primary packaging material for liquid and semi-solid preparations.

The choice of solvent for this test depends on the composition of the product. The test should investigate the level of extractives (antioxidants, plasticizers…[31])

There are two types of plastic containers made up of low density polyethylene

1.BFS Containers

2. Three Piece Containers

- **BFS Containers**:[32]

These types of containers are manufactured by Blow-Fill-Seal (BFS) technology. Blow-Fill-Seal technology is an automated packaging process whereby plastic containers are blow–moulded, filled, and sealed in one continuous process protected operation. This technology was first invented by Rommelag in the 1960s. Development of bottle pack range of aseptic BFS machine in the 1970s extended its use to pharmaceutical applications.

By its very nature, the BFS process offers high level of quality assurance. By integrating container manufacture with product filling, BFS removes a major step from a production process, together with its associated risk. At the end of 2004, the US FDA defined for the first time regulatory requirements on the use of BFS technology. As a rule the filling process is considered to be a process of aseptic processing and is therefore subject to strict GMP requirements on these processes. Special attention needs to be paid some of the central requirements such as environmental monitoring, the absence of particles in the product and validation of the process by media fill.

Ophthalmic application:

Back in the 1970s, BFS system became established in the field of eye drops and ocular medicines for volumes of between approximately 0.3mL to 1mL as unit dose presentation, and between 5 ml to 15 ml for multidose applications. The BFS process is particularly suited to the filling of ophthalmic solutions; most of these products has been are heat labile and hence will not withstand terminal sterilization. The sterility assurance level of BFS process is extraordinary, and has been proven by millions of containers manufactured in media validation runs. The system requires little or no human intervention during the operation cycle, and consequently the major source of particulate matter- the human operator- is excluded as risk of contamination. The regulatory authorities have recognized the immense superiority of containers made with BFS technology and have stated that the application advantages are such that the choice of aseptic production supersedes that of terminal sterilization.

Blow-Fill-Seal (BFS) technology is recognized as an efficient, advanced aseptic processing technology for ophthalmic drops. It provides far higher levels of quality assurance, together with definite advantages compared with traditional aseptic filling techniques. The technology has been well established in the field of ophthalmic products for long period of time, and has shown an excellent record of successful launches of new products and designs of benefit to patient. Worldwide acceptance in the ophthalmic market has confirmed the Particular suitability of this form of packaging for ophthalmic applications.

Fig 3: BFS Container

- **Three Piece Containers:**

Three Piece Container name itself indicates system contains three components viz... body, nozzle, and cap. Out of which, body and nozzle are made up of various grades of polymers of low density polyethylene whereas caps are made up of high density polyethylene which results in a packaged ophthalmic products that is user-friendly for dispensing of the pharmaceutical preparation on a drop-by-drop basis.

Cap has two rings which have to break while opening the container. Intact rings work as the tamper proof evidence. The nozzle is punctured with the help of piercing spike inside the cap.

Variety of grades of polymers allow manufacturer to mould the container in various sizes (3ml-50ml) and shapes (oval, cylindrical, quadrangular, etc). Variety of polymers also provides a facility for opaque (for light sensitive drugs) containers which give good optical and chemical resistance. Three piece containers are robust in nature as their wall thickness is greater than BFS containers. This limits the reduction in the volume of liquid by evaporation at accelerated conditions applied during stability testing.

Fig 4: Three Piece Container

Polymers used in construction of body and cap are briefly described in table 10

Table 10: Various Polymers for Packaging Material of Ophthalmics[33]

Sl. No	Parameters	PE 1810 E	PE 1840 H	PE 3020 D	PE 3040 D	3220 D
1	Resin Type	Polyethylene, low density	Polyethylene, low density	Polyethylene, low density	Polyethylene, low density	Polyethylene, low density
2	Description	PE 1810 E is LDPE with good flexibility and delivered in pellet form. Target applications are small blow moulding and injection moulding of engineering parts and tubes as well as domestic wares.	PE 1840 H is a LDPE with a good flexibility and delivered in pellet form. It is designed for small blow moulding but also be used in film application and injection moulding. It can be used for medical devices and packaging of pharmaceuticals.	PE 3020 D is LDPE with high rigidity, good optical and chemical resistance. It is delivered in pellet form. It can be used in small blow moulding including packaging pharmaceuticals and injection moulding for medical devices, closures, and seals.	PE 3040 D is LDPE with high rigidity and good chemical resistance and it is delivered in pellet form. It is used in small blow moulding packaging pharmaceuticals and injection moulding for medical devices, closures, and seals.	PE 3220 D is LDPE with high rigidity and good chemical resistance and it is delivered in pellet form. It is used in packaging of pharmaceuticals in small blow moulding market.
3	Melt temperature	170 to 220^0 C	160 to 200^0 C	170 to 220^0 C	170 to 220^0 C	170 to 220^0 C
4	Density	0.920 g/cm^3	0.919 g/cm^3	0.927 g/cm^3	0.928 g/cm^3	0.930 g/cm^3
5	Melt Flow Rate	0.40 g/10 min (190^{0}C/2.16 Kg)	1.5 g/10 min (190^{0}C/2.16 Kg)	0.30 g/10 min (190^{0}C/2.16 Kg)	0.25 g/10 min (190^{0}C/2.16 Kg)	0.40 g/10 min (190^{0}C/2.16 Kg)
6	Tensile Modulus	200 Mpa (23^{0}C)	200 Mpa (23^{0}C)	300 Mpa (23^{0}C)	300 Mpa (23^{0}C)	430 Mpa (23^{0}C)

7	Softening temperature	92^0C	88^0C	102^0C	102^0C	110^0C
8	Melting temperature	108^0C	108^0C	114^0C	115^0C	117^0C
9	Density	>0.500	> 0.500	> 0.500	> 0.500	> 0.500
10	Melt Index	0.40g/10 min 190^0c/2.16 Kg	1.5g/10 min 190^0c/2.16 Kg	0.30 g/10 min 190^0c/2.16 Kg	0.25g/10 min 190^0c/2.16 Kg	0.40g/10 min 190^0c/2.16 Kg

- **Compatibility of the packaging material with drug product:**

Chemical incompatibility may arise between the packaging materials with drug product due to one of the following mechanisms.

1. Adsorption of the chemical entities onto the component surfaces. for e.g. EDTA and preservative like Benzalkonium chloride.
2. More volatile preservatives like chlorobutol show rapid loss through low density polyethylene by adsorption and surface evaporation.
3. Surface active ingredients which may be found in plastics may enter in to the product by dissolution and surface abrasion mechanisms.
4. In case of glass, detachment of spicules may occur when alkaline solution stored in soda glass.
5. Organoleptic changes may occur, caused by permission of volatile or odorous substances through the plastic material. [34]

- **Drug products packaged in impermeable containers**

Sensitivity to moisture or potential for solvent loss is not a concern for drug products packaged in impermeable containers that provide permanent barrier to passage of moisture or solvent. Thus, stability studies for product stored in a impermeable containers can be conducted under any controlled or ambient humidity condition.

- **Drug products packaged in semi- permeable containers**

Aqueous –based products packaged in semi-permeable containers should be evaluated for potential water loss in addition to physical, chemical, biological, and microbiological stability. This evaluation can be carried out under low relative humidity. Ultimately, it should be demonstrated that aqueous base drug product stored in a semi-permeable containers can withstand low relative humidity environment.

2. LITERATURE REVIEW

1. **Mitchell h. Friedlaender** et al [35]**(2006)** evaluated the effect of dilution of benzalkonium chloride (BAK) on the surface of human eye by determining its concentration in the tear film after topical administration of Gatifloxacin ophthalmic solution 0.3% (preserved with 0.005%benzalkonium chloride). The purpose of this prospective clinical study was to test the hypothesis that BAK would be significantly diluted shortly after topical administration and would thereafter have little or no effect on enhancement of the antibiotic efficacy of commercial Gatifloxacin on the ocular surface. Investigators measured concentration of tear film BAK at successive time points after topical administration of commercial Gatifloxacin. Results showed rapid BAK dilution to 6.4 µg/ml, 3.2 µg/ml, and 1.4 µg/ml, below the detection limit, at 30 sec, 1 min, 3min, 5 min, and 20 min after instillation of single 35 µl drop of Gatifloxacin. Because of rapid dilution reduces the concentration of BAK to near zero in minute. And does not allow the time (1 hour) required for effective bacterial kill power. So BAK does not have significant effect on enhancement of antimicrobial efficacy of antibiotic on human ocular surface.
2. **Komei okabe** *et al* [36]**(2005)** investigated the effect and safety of Benzalkonium chloride (BAK) on transcleral drug delivery in rabbit after continuous intrascleral administration. In this study, to investigate the effect of BAK on transcleral permeability of betamethasone 21-phosphate (BP) in vitro, penetration of BP aqueous solution with or without BAK across the rabbit sclera was evaluated using two chamber Ussing apparatus. They found that BAK increases concentration of BP in the vitreous and retina-choroid compared with the control. In the in-vitro study BAK did not increase the scleral permeability of BP. The result of the study demonstrates that BAK may improve the ocular penetration of drug in a transcleral drug delivery system without producing toxic reaction.
3. **M.M.A.Al-Hiti** *et al* [37]**(1980)** evaluated the changes in preservative sensitivity for the USP antimicrobial agents' effectiveness test micro-organisms. Investigator designed chemically defined and semi-defined media for preservative efficacy testing micro-organisms designated by the USP, in

which the organisms went into the stationary phase of growth at an optical density of 1.0, because of depletion of single carbon, nitrogen, or phosphate source. *Aspergillus niger* was grown on solid media containing concentration of these nutrients which limits the development of sporulation density. The ability of micro-organism to survive and grow in the presence of chlorhexidine, benzalkonium chloride, and thiomersal varied markedly with the nutrition depletion of inocula. No universal pattern of sensitivity emerged among micro-organisms. Only *a. Niger* showed little overall change in preservative sensitivity. These results highlight the need to define more adequately growth media and conditions for the production of inocula for antimicrobial challenge test.

4. **Roswell R. Pfister** *et al* [38]**(1976)** studied the effects of ophthalmic drugs, vehicles, and preservatives on corneal epithelium by a scanning electron microscope on corneal surface. Of the preparations tested 0.3 % gentamicin caused many central cellular microvilli to stand up prominently. Moderate losses of peripheral microvilli, with mild superficial cellular desquamation was noted with 0.25 % phospholine iodide, 2 % pilocarpine, 2 % luorescein, and fluor-i-strip. The top layer of epithelial cells desquamated with 4 % cocaine or neopolycin treatment. The top two layers of cells were lost when 0.01 % Benzalkonium chloride was instilled. When cell death occurred severe membrane disruption was accompanied by loss of microvilli and rupture of intercellular tight junctions. These Studies show that the cytotoxicity of topical ocular preparations can be tested in an in vivo Model and evaluated by scanning electron microscopy.
5. **So-hyang Chung** *et.al* [39]**(2006)** studied the impact of short-term exposure of commercial eye drops preserved with benzalkonium chloride, a preservative used in many ophthalmic topical solutions, on precorneal mucin in human. They exposed the immortalized human corneal- limbal epithelial (HCLE) cells to eye drops containing BAK solutions at 0.0025% and 0.01% concentration for period of 15 minutes. Human corneal epithelium was acquired with keratectomy procedures after application of *Ocuflox* eye drops (0.3% Ofloxacin with 0.0025% BAK) for 1 week before surgery. The relative expression of the muc1and muc16 mucin gene was determined by conventional polymerase chain reaction. Human corneas exposed to 0.01%

BAK solutions were examined by transmission electron microscopy. The result showed that the expressions of muc1and muc16 were reduced after exposure to BAK in HCLE cells and human corneal epithelium. Transmission electron microscopy of the anterior corneal surface revealed fixation of the mucos layer after exposure to 0.01% BAK for 5 or 15 minutes; prolong exposure (60 min) to 0.01% BAK destroys the mucos layer. This study demonstrates that short–term exposure to BAK can alter the precorneal mucin.

6. **Michael J. Hogan (1949)** reviewed that pH of the solution influence the stability of the formulation considerably, since Blok found a 44% decomposition of 0.5% Atropine solution and 89% decomposition of a 1% homatropine solution in one month at pH 8.3. Deterioration was less than 20% at pH 6.8. In general, slightly acid solutions of ophthalmic drugs are more stable and effective. If they are too acid the free base is quite irritating.[40]

7. **Luc van Santvliet** *et al* **(2004)** reviewed the technical, pharmaceutical, and therapeutic aspects of eye drop formation and delivery. They discussed different types of containers and determinants of eye drop size. They concluded that dropper tips should deliver small-volume eye drops. The ideal dropper tip consist small diameter outer orifice with design clearly defining the surface area from which drop will fall. Surface tension reducing agents in formulation can reduce the drop size viscosity.[41]
8. **Mark B. Abelson** *et al* [42]**(2002)** reviewed Benzalkonium background. They stated that BAK 0.02% and 0.01% killed 100 percent of Pseudomonas aeruginosa cultures and inhibited growth of Staphylococcus aureus as evidenced by zones of growth inhibition in agar inoculated with S. aureus.[2] Additionally, BAK 0.1% and 0.05% killed all strains tested of methicillin-resistant S. aureus and methicillin-sensitive S. aureus within 20-40 seconds,[3] and had complete efficacy within 30 seconds in killing 100 isolates of Serratia marcescens, 103 of Klebsiella pneumoniae, 99 of Pseudomonas aeruginosa, 19 of Alcaligenes faecalis and 30 of A. xylosoxydans.
9. **Hourcade F. Sautov – Miranda V** *et al* [43]**(1997)** studied compatibility of granisetron towards glass and plastics and its stability under various storage

conditions.they evaluated the compatibility of graniusetron as an undiluted solution towards glass polypropylene and PVC containers over period of 15 days. the solution were exposed to various temperature and light. from the results obtained they concluded that undiluted granisetron was stable in polypropylene. in contrast variations were found in concentration diluted granisetron with 5 % glucose and 0.9% sodium chloride.

10. ***Beitz C., Bersch T***. *et al* [44]**(1999)** studied the compatibility of plastics with cytotoxic drug solutions. They compared the low density polyethylene (LDPE)containers with glass bottles and polyvinyl chloride (PVC). They concluded that investigated drugs were stable in all three containers with the best stability in glass bottles, followed by LDPE and PVC.

11. **Guidance for Industry (1999):** "Container Closure System for Packaging of Human Drugs and Biologics" in 1999. The guidance notes that packaging should be constructed of materials that will not leach harmful or undesirable amount of substances to which the patient will be exposed. For ophthalmic drug product comprehensive assessment involves two parts: an extraction study of packaging components to determine which chemical species may migrate into dosage form and toxicological evaluation of those substances that are extracted to determine the safe level of exposure via label route of administration.[45]

12. **Howard Schenker** *et al* [46]**(1999)** investigated the patient preference,efficacy, and compliance with Timolol Maleate Ophthalmic Gel-Forming Solution Versus Timolol Maleate Ophthalmic Solution for treating Open-Angle glaucoma. In this study total of 202 patients were selected and treated once daily with Timolol gel forming solution or twice daily with Timolol ophthalmic solution. This study demonstrated that patients preferred timolol gel once daily to timolol solution twice daily. study demonstrated more stinging with timolol solution and a higher percentage of blurred vision with timolol gel. Compliance was greater with timolol gel. This study confirms previous studies showing that timolol gel and timolol solution are equally effective in lowering IOP. The incidents of adverse drug reactions are less in the case of tiomol solution but however due to less dosing compliance was more with the Timolol gel solution.

13. **Masayo Higashiyama** *et al* [47]**(2003)** studied the Improvement of the ocular bioavailability of

    timolol by sorbic acid. The Timolol maleate in the presence of sorbic acid was tested against marketed formulations of Timolol ophthalmic solution (Timoptlo®), and Timolol gel forming solution (Timoptic-XE®). The comparison was done by various parameters like Effect of sorbic acid on the apparent lipophilicity of timolol, Evaluation of the effect of sorbic acid concentration on timolol bioavailability, Comparison of the influence of various timolol formulations on bioavailability, In vitro corneal penetration of timolol with sorbic acid. Results showed that The ocular bioavailability of timolol increased in sorbic acid solution due to ion pair formation. Its octanol/water partition coefficient also increased, suggesting the formation of a more lipophilic complex. The concentration of timolol in rabbit aqueous humor was Maximum in the presence of sorbic acid and the area under the curve were more than two-fold higher than those of Timoptol®, and similar in value to TIMOPTIC-XE®. The partition coefficient was also noted higer in the presence of sorbic acid.

14. **K. S. Rathore** *et al* [48]**(2011)** Developed an In-situ gel forming system of Timolol maleate by using poly acrylic acid as a gelling agent and hypromellose as viscolyzer. The developed formulation was therapeutically efficacious, non-irritant, stable and provided sustained release of the drug over a long period and shelf-life determined by Arrhenius equation was 1.6 years. Intra ocular pressure determined with Schiotz tonometer and eye irritation. study. conducted on albino rabbits by Draize technique. The developed system is concluded as viable alternative for conventional formulations.

15. **Hyun Jung Jung** *et al* [49]**(2012)** fabricated a contact lenses system by dispersing nanoparticles of PGT (propoxylated glyceryl triacylate) that contain a glaucoma drug timolol. The particles were loaded into prefabricated lenses by soaking the lenses in a solution of particles in ethanol. The particle loaded gels can release timolol in phosphate buffered saline (PBS) for about a month at room temperature. Preliminary animal

studies were conducted in Beagle dogs with lenses in which particles are loaded by soaking the lenses in ethanol show a reduction in IOP.

16. **Pfister** *et al* [50]**(1976)** found that BAK-containing preparations can cause severe plasma membrane disruptions and cell death in the cornea. Pfister et al. used scanning electron microscopy (SEM) to study the effect of topical drugs, vehicles, and preservatives (i.e. BAK) on the surface corneal epithelium. Treatment of the cornea with a 0.01 percent solution of BAK resulted in the top two layers of cells being desquamated. When cell death occurred, severe membrane disruption was accompanied by loss of microvilli and rupture of intercellular tight junctions. They concluded that frequent use of BAK-containing preparations can act as an iatrogenic impediment to the epithelial healing process and can shorten the tear film break up time.
17. **Burstein** *et al* [51]**(1977)** studied the effects of very low concentrations of preservatives (e.g. BAK, thimerosal and amphotericin B) on the cornea. Burstein et al. found that BAK, at a concentration as low as 0.01%, briefly increased ion transport, and then greatly decreased epithelial resistance with severe disruption of surface cell layers occurring simultaneously with the decrease in resistance. Burstein, found BAK causes a progressive increase in damage to corneal epithelial cells at concentrations between 0.001% and 0.01%, as determined by SEM.
18. **Solomun L** *et al* [52]**(2008)** investigated the impact of primary packaging material on the quality of parenteral products.they used the dual chamber vials made up of Type I borosilicate glass. they evaluated the compatibility of solutrion with glass container in view of *pH* shift.on the basis of results obtained they concluded that pH value of the reconstituted solution remains unchanged in samples tested both ex-tempore and and after in-use period of 48 hours.

3. AIM AND OBJECTIVES

Recently, ophthalmic drug delivery has become the standards in the modern pharmaceutical design and intensive research for achieving better drug product effectiveness, reliability, and safety. Topical medication to eye through eye drops will continue to account for the largest share (up to 90%) of drug delivery systems. The ophthalmic solution with minimum concentration of preservative preparation in an appropriate packaging material appears to be most attractive approach for the process development and scale-up point of view.

A first generation Beta blocker has found its applicability in treating Chronic Open angled Glaucoma and used widely in young as well as adults, commonly associated with multiple doses.

Ophthalmic medication stored in multiple dose containers is required by the U.S. Food and Drug Administration to contain a preservative so that patients are provided with microbe free medication. Benzalkonium chloride in concentrations from 0.1% to 0.0001% induced dose-dependent growth arrest and conjunctival epithelial cell death, either delayed or immediately after administration. In such case, a preservative Benzalkonium chloride must be used within reasonable bound. Benzalkonium chloride can provide more help than harm.

Therefore, the aim of the present study was to formulate a formulation for Timolol Maleate (0.5%) ophthalmic solution using different concentration of Benzalkonium chloride as preservative. While reducing the concentration of Benzalkonium chloride it must be keep in mind that added quantity of preservative must meet compendial requirement of ***Preservative Efficacy Testing***.

The present research work is also planned to provide the data about the selection of suitable primary packaging material for Timolol Maleate (0.5%) ophthalmic solution to achieve the better stability during the shelf life of the product. As there are several factors responsible for the incompatibility of packaging material with the product, most suitable packaging material must be selected.

The product will be evaluated for stability, potency, toxicity, and safety under the accelerated conditions of temperature and humidity.

➢ OBJECTIVES

The following objectives were thus framed for the present investigation

1. The designed optimized formulation with minimum concentration of Benzalkonium chloride will provide better therapeutic effect without the corneal irritation caused due the deposition of Benzalkonium chloride.

2. The optimized formulation with minimum concentration of Benzalkonium chloride, will meet the compendial requirement of preservative efficacy test, so that it will maintain the sterility of the product throughout the shelf life of product.

3. Effective antimicrobial activity without interference with the mechanism of action of the active ingredient.

4. As there will be no eye irritation and conjunctival epithelial cell death, optimized formulation will help to achieve the better patient compliance and improved therapeutics with reduced systemic side effects and toxicity in ocular bacterial infection.

5. The selected packaging material will provide the better stability at both room and elevated conditions of temperature and humidity.

6. No or minimum introduction of extractables and leachables in the product from the packaging material.

4. PLAN OF WORK

The present proposed research work was planned as per the following experimental protocol –

- **Phase I Study**

Step 1. Literature Survey; the various work carried out on this topic is reviewed.

Step 2. Procurement of drug, preservative, other ingredients and various types of containers required for the study.

Step 3. Physical characterization of drug sample includes description, identification, solubility, loss on drying, and assay by potentiometric titration.

- **Phase II Study**

Step 4. Development strategy

Step 4.1 Prototype formulation development: the excipients will scientifically identify based on their category

Step 4.2 Optimization of formula and process

Step 4.3 Development of manufacturing process

Step 4.4 Determination of Preservative Efficacy by microbial plate count method.

Step 4.5 Product analysis

Step 4.5.1 In Process Tests: pH, Leak test and Visual inspection for particulate matter.

Step 4.5.2 Finished product analysis: Appearance, assay, Osmolality and pH

➢ Phase III Study

Step 4.6 Stability and Container compatibility study.

Step 4.6.1 Charging of optimized batch in various packaging containers viz.. glass and plastic containers to stress condition of temperature and accelerated condition of temperature and humidity

Step 4.6.2 Analysis of product at every station of the stability: Appearance, Assay of active drug and preservative, Osmolality, pH, Drop size study, and Water loss study (for semi permeable containers only).

5.1. DRUG PROFILE

Drug used for the study is from Anti-hypertensive β- blocker category. Details of **Timolol maleate** are given are as follows.

- **Molecular Structure :**

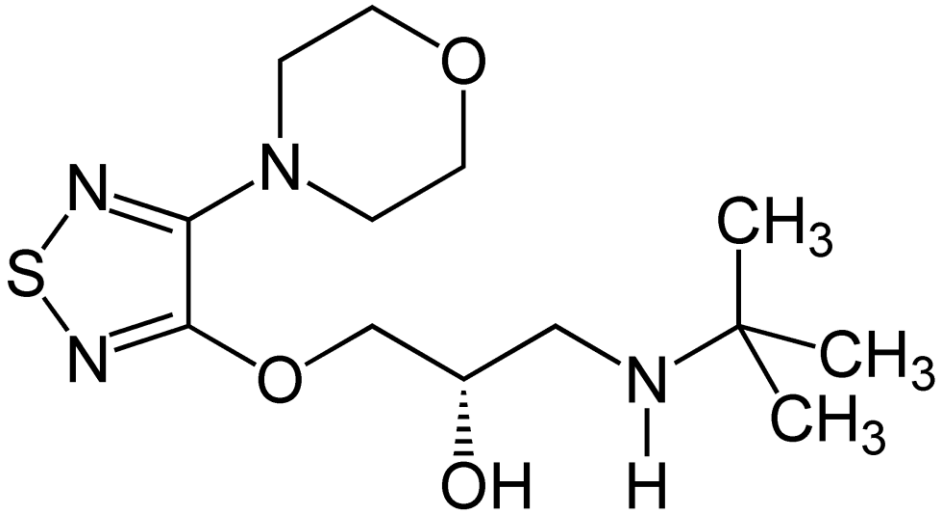


Fig 5: Molecular structure of Timolol

2-Propanol, 1-[(1,1-dimethylethyl)amino]-3-[[4-(4-morpholinyl)-1,2,5-thiadiazol-3-yl]oxy]-, (*S*)-, (*Z*)-2-butenedioate (1:1) (salt).

- **Monograph**[53] **:**

Molecular weight	: 432.50
Molecular formula	: $C_{13}H_{24}N_4O_3S \bullet C_4H_4O_4$
Description	: It is a white, odorless, crystalline powder.
Solubility	: Soluble in water, methanol, and alcohol.
Category	: β- adrenergic blocking agent..
Storage	: Store in well-closed, light resistant container.

- **Therapeutic uses[54] :**

Treatment of hypertension, alone or in combination with other agents; reduction of risk of reinfarction post-MI; migraine prophylaxis; treatment of elevated IOP in chronic open-angle glaucoma, ocular hypertension, aphakic glaucoma patients, patients with secondary glaucoma, and in patients with elevated Intra Ocular Pressure(IOP) who need ocular pressure lowering.

- **Pharmacology :**

Timolol maleate is a β1 and β2 (non-selective) adrenergic receptor blocking agent that does not have significant intrinsic sympathomimetic, direct myocardial depressant, or local anesthetic (membrane-stabilizing) activity.

β -adrenergic receptor blockade reduces cardiac output in both healthy subjects and patients with heart disease. In patients with severe impairment of myocardial function, beta-adrenergic receptor blockade may inhibit the stimulatory effect of the sympathetic nervous system necessary to maintain adequate cardiac function.

β -adrenergic receptor blockade in the bronchi and bronchioles results in increased airway resistance from unopposed parasympathetic activity. Such an effect in patients with asthma or other bronchospastic conditions is potentially dangerous.

Timolol maleate ophthalmic solution, when applied topically on the eye, has the action of reducing elevated as well as normal intraocular pressure, whether or not accompanied by glaucoma. Elevated intraocular pressure is a major risk factor in the pathogenesis of glaucomatous visual field loss. The higher the level of intraocular pressure, the greater the likelihood of glaucomatous visual field loss and optic nerve damage.

The onset of reduction in intraocular pressure following administration of timolol maleate ophthalmic solution can usually be detected within one-half hour after a single dose. The maximum effect usually occurs in one to two hours and significant lowering of intraocular pressure can be maintained for periods as long as

24 hours with a single dose. Repeated observations over a period of one year indicate that the intraocular pressure-lowering effect of timolol is well maintained.

The precise mechanism of the ocular hypotensive action of timolol is not clearly established at this time. Tonography and fluorophotometry studies in man suggest that its predominant action may be related to reduced aqueous formation. However, in some studies a slight increase in outflow facility was also observed.

- **Pharmacokinetics of drug :**

Absorption :

Timolol is rapidly and about 90% absorbed following oral administration. T_{max} is approximately 1 to 2 hrs.

Distribution :

Timolol is not extensively bound to plasma proteins.

Metabolism :

Timolol undergoes approximately 50% first-pass metabolism.

Elimination :

Timolol $t_{½}$ is approximately 4 hrs.

In a study of plasma drug concentration in six subjects, the systemic exposure to timolol was determined following twice daily administration of timolol maleate ophthalmic solution, 0.5%. The mean peak plasma concentration following morning dosing was 0.46 ng/mL and following afternoon dosing was 0.35 ng/mL.

- **Adverse reactions :**

Timolol maleate has good safety records. As with many topically applied ophthalmic drugs, this drug also absorbed systemically. The Adverse reactions generally produced

Gastro Intestinal : Abdominal pain, nausia, diarrhea.

CNS : Dizziness, depression, lethargy, headach, insomnia.

Respiratory : Wheezing, cough, breathing difficulties in asthmatic patients

Metabolic : Alteration of glucose metabolism, increased uric acid.

Micellaneous : joint pains, muscle cramps.

Timolol is contraindicated in the patients with

- Bronchial asthma
- History of bronchial asthma
- Severe chronic obstructive pulmonary disease
- Sinus bradycardia
- Antiventricular blockade
- Cardiogenic shock
- Hypersensitivity to the product.

- **Dosage forms :**

1. Topical ophthalmic solutions : 0.25%, 0.5%
2. Tablets : 5mg, 10mg, 20mg
3. Intravenous injections : 0.5%

5.2. EXCIPIENTS REVIEW

The various excipients used in the Formulation are Benzalkonium chloride, Dibasic Sodium Phosphate, Monobasic Sodium phosphate, Sodium Hydroxide.

I. BENZALKONIUM CHLORIDE [55]

- **Nonproprietary Names :**

- BP: Benzalkonium chloride
- JP: Benzalkonium chloride
- PhEur: Benzalkonii chloridum
- USPNF: Benzalkonium chloride

- **Synonyms :**

Alkyl benzyl dimethyl ammonium chloride; alkyl dimethyl benzyl ammonium chloride; BKC; Hyamine 3500; Pentonium; Zephiran.

- **Chemical Name and CAS Registry Number :**

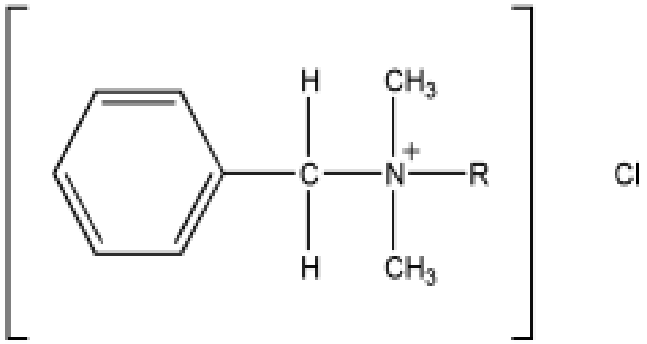
Alkyl dimethyl (phenyl methyl) ammonium chloride [8001-54-5]

- **Empirical Formula :**

$[C_6H_5CH_2N(CH_3)_2R]Cl.$

- **Molecular Weight :** 360.

- **Structural formula :**



R = mixture of alkyls: *n*-C_8H_{17} to *n*-$C_{18}H_{37}$; mainly *n*-$C_{12}H_{25}$ (dodecyl), *n*-$C_{14}H_{29}$ (tetradecyl), and *n*-$C_{16}H_{33}$ (hexadecyl).

- **Functional Category :**

Antimicrobial preservative; antiseptic; disinfectant; solubilizing agent; wetting agent.

- **Applications in Pharmaceutical Formulation or Technology**

Benzalkonium chloride is a quaternary ammonium compound used in pharmaceutical formulations as an antimicrobial preservative. In ophthalmic preparations, benzalkonium chloride is one of the most widely used preservatives (0.01–0.02% w/v) Often it is used in combination with other preservatives or excipients, particularly 0.1% w/v disodium edetate, to enhance its antimicrobial activity against strains of Pseudomonas.

Benzalkonium chloride (0.002–0.02% w/v) in nasal and otic formulations, (0.01% w/v) small-volume parenteral products and cosmetics is used.

- **Description :**

Benzalkonium chloride occurs as a white or yellowish-white amorphous powder, a thick gel, or gelatinous flakes. It is hygroscopic, soapy to the touch, and has a mild aromatic odor and very bitter taste.

- **Typical Properties**

- **Antimicrobial activity :**

Benzalkonium chloride solutions are active against a wide range of bacteria, yeasts, and fungi. Activity is more marked against Gram-positive than Gram-negative bacteria. The antimicrobial activity of benzalkonium chloride is significantly dependent upon the alkyl composition of the homolog mixture. However, combined with disodium edetate (0.01–0.1% w/v), the activity against Pseudomonas aeruginosa is increased. Inhibitory activity increases with pH, although antimicrobial activity occurs at pH 4–10.

- **Acidity/alkalinity:** pH = 5–8 for a 10% w/v aqueous solution.
- **Density:** ≈0.98 g/cm^3 at 20°C
- **Melting point:** ≈40°C

- **Partition coefficients:**

The octanol : water partition coefficient varies with the alkyl chain length of the homolog; 9.98 for C_{12}, 32.9 for C_{14}, and 82.5 for C_{16}.

- **Solubility:**

Practically insoluble in ether; very soluble in acetone, ethanol (95%), methanol, propanol, and water. Aqueous solutions of benzalkonium chloride foam when shaken.

➢ **Stability and Storage Conditions :**

Benzalkonium chloride is hygroscopic and may be affected by light, air, and metals.

➢ **Incompatibilities :**

Incompatible with aluminum, anionic surfactants, some rubber mixes, and some plastic mixes. Benzalkonium chloride has been shown to be adsorbed to various filtering membranes, especially those that are hydrophobic or anionic.

➢ **Safety :**

Benzalkonium chloride is usually nonirritating, nonsensitizing, and is well tolerated in the dilutions normally employed on the skin and mucous membranes. However, benzalkonium chloride has been associated with adverse effects when used in some pharmaceutical formulations. Ototoxicity can occur when benzalkonium chloride is applied to the ear and prolonged contact with the skin can occasionally cause irritation and hypersensitivity. Benzalkonium chloride is also known to cause bronchoconstriction in some asthmatics when used in nebulizer solutions.

Toxicity experiments with rabbits have shown benzalkonium chloride to be harmful to the eye in concentrations higher than that normally used as a

preservative. However, the human eye appears to be less affected than the rabbit eye and many ophthalmic products
have been formulated with benzalkonium chloride 0.01% w/v as the preservative.

➢ **Regulatory Status :**

Included in the FDA Inactive Ingredients Guide (inhalations, IM injections, nasal, ophthalmic, otic, and topical preparations). Included in nonparenteral medicines licensed in the UK. It is also included in the Canadian List of Acceptable Non-medicinal Ingredients.

II. DIBASIC SODIUM PHOSPHATE[56]

➢ **Nonproprietary Names :**

- BP: Anhydrous Disodium Hydrogen Phosphate
  Disodium Hydrogen Phosphate Dihydrate
  Disodium Hydrogen Phosphate Dodecahydrate
- JP: Dibasic Sodium Phosphate Hydrate
- PhEur: Disodium Phosphate, Anhydrous
  Disodium Phosphate Dihydrate
  Disodium Phosphate Dodecahydrate
- USP: Dibasic Sodium Phosphate

➢ **Synonyms :**

Dinatrii phosphas anhydricus; dinatrii phosphas dihydricus; dinatrii phosphas dodecahydricus; disodium hydrogen phosphate; disodium phosphate; E339; phosphoric acid, disodium salt; secondary sodium phosphate; sodium orthophosphate.

➢ **Chemical Name and CAS Registry Number :**

Anhydrous dibasic sodium phosphate [7558-79-4]
Dibasic sodium phosphate dihydrate [10028-24-7]
Dibasic sodium phosphate dodecahydrate [10039-32-4]
Dibasic sodium phosphate heptahydrate [7782-85-6]

Dibasic sodium phosphate hydrate [10140-65-5]

Dibasic sodium phosphate monohydrate [118830-14-1**]**

- **Empirical Formula and Molecular Weight :**

Na_2HPO_4 141.96 (for Anhydrate)

$Na_2HPO_4\_H_2O$ 159.94 (for Monohydrate)

$Na_2HPO_4\_2H_2O$ 177.98 (for Dihydrate)

$Na2HPO_4\_7H_2O$ 268.03 (for Heptahydrate)

Na2HPO4_12H2O 358.08 (for Dodecahydrate)

- **Functional Category :**

Buffering agent; sequestering agent.

- **Applications in Pharmaceutical Formulation or Technology**

Dibasic sodium phosphate is used in a wide variety of pharmaceutical formulations as a buffering agent and as a sequestering agent. Therapeutically, dibasic sodium phosphate is used as a mild laxative and in the treatment of hypophosphatemia. Dibasic sodium phosphate is also used in food products; for example as an emulsifier in processed cheese.

- **Description :**

The USP 32 states that dibasic sodium phosphate is dried or contains, 1, 2, 7, or 12 molecules of water of hydration.

Anhydrous dibasic sodium phosphate occurs as a white powder. The dihydrate occurs as white or almost white, odorless crystals. The heptahydrate occurs as colorless crystals or as a white granular or caked salt that effloresces in warm, dry air. The dodecahydrate occurs as strongly efflorescent, colorless or transparent crystals.

- **Typical Properties**

Table 11: Typical Properties of Dibasic Sodium phosphate

Acidity/alkalinity	pH = 9.1 for a 1% w/v aqueous solution of the anhydrous material at 258°C. A saturated aqueous solution of the dodecahydrate has a pH of about 9.5
Ionization constants	PKa1 = 2.15 at 258°C; pKa2 = 7.20 at 258°C; pKa3 = 12.38 at 258°C.
Moisture content	The anhydrous form is hygroscopic and will absorb up to 7 moles of water on exposure to air, whereas the heptahydrate is stable in air.
Solubility	Very soluble in water, more so in hot or boiling water; practically insoluble in ethanol (95%). The anhydrous material is soluble 1 in 8 parts of water, the heptahydrate 1 in 4 parts of water, and the dodecahydrate 1 in 3 parts of water
Osmolarity	A 2.23% w/v aqueous solution of the dihydrate is isoosmotic with serum; a 4.45% w/v aqueous solution of the dodecahydrate is isoosmotic with serum.

- **Stability and Storage Conditions :**

The anhydrous form of dibasic sodium phosphate is hygroscopic. When heated to 408°C, the dodecahydrate fuses; at 1008°C it loses its water of crystallization; and at a dull-red heat (about 2408°C) it is converted into the pyrophosphate, $Na_4P_2O_7$. Aqueous solutions of dibasic sodium phosphate are stable and may be sterilized by autoclaving. The bulk material should be stored in an airtight container, in a cool, dry place.

- **Incompatibilities :**

Dibasic sodium phosphate is incompatible with alkaloids, antipyrine, chloral hydrate, lead acetate, pyrogallol, resorcinol and calcium gluconate, and ciprofloxacin. Interaction between calcium and phosphate, leading to the formation of insoluble calcium–phosphate precipitates, is possible in parenteral admixtures.

- **Safety :**

Dibasic sodium phosphate is widely used as an excipient in parenteral, oral, and topical pharmaceutical formulations. Phosphate occurs extensively in the body and is involved in many physiological processes since it is the principal anion of intracellular fluid. Excessive administration through oral route may cause some side effects like Diarrhea, nausea and vomiting etc,.. But the level of Dibasic sodium phosphate used in the pharmaceutical formulations is not usually produse side effects.

- **Regulatory Status :**

GRAS listed. Accepted in Europe for use as a food additive. Included in the FDA Inactive Ingredients Database (injections; infusions; nasal, ophthalmic, oral, otic, topical, and vaginal preparations). Included in nonparenteral and parenteral medicines licensed in the UK. Included in the Canadian List of Acceptable Non-medicinal Ingredients.

III. MONOBASIC SODIUM PHOSPHATE[57]

- **Nonproprietary Names :**

- BP: Anhydrous Sodium Dihydrogen Phosphate
  Sodium Dihydrogen Phosphate Monohydrate
  Sodium Dihydrogen Phosphate Dihydrate
- PhEur: Sodium Dihydrogen Phosphate Dihydrate
- USP: Monobasic Sodium Phosphate

- **Synonyms :**

Acid sodium phosphate; E339; Kalipol 32; monosodium orthophosphate; monosodium phosphate; natrii dihydrogenophosphas dihydricus; phosphoric acid, monosodium salt; primary sodium phosphate; sodium biphosphate; sodium dihydrogen orthophosphate; sodium dihydrogen phosphate.

- **Chemical Name and CAS Registry Number :**

  Anhydrous monobasic sodium phosphate [7558-80-7]

  Monobasic sodium phosphate monohydrate [10049-21-5]

  Monobasic sodium phosphate dihydrate [13472-35-0]

- **Empirical Formula and Molecular Weight :**

NaH_2PO_4	119.98 (for Anhydrate)
$NaH_2PO_4\_H_2O$	137.99 (for Monohydrate)
$NaH_2PO_4\_2H_2O$	156.01 (for Dihydrate)

- **Functional Category :**

  Buffering agent; sequestering agent; emulsifying agent

- **Applications in Pharmaceutical Formulation or Technology**

Monobasic sodium phosphate is used in a wide variety of pharmaceutical formulations as a buffering agent and as a sequestering agent. Therapeutically, monobasic sodium phosphate is used as a mild saline laxative and in the treatment of hypophosphatemia.

Monobasic sodium phosphate is also used in food products, for example, in baking powders, and as a dry acidulant and sequestrant.

- **Description :**

The USP 32 states that monobasic sodium phosphate contains one or two molecules of water of hydration or is anhydrous.

The hydrated forms of monobasic sodium phosphate occur as odorless, colorless or white, slightly deliquescent crystals. The anhydrous form occurs as a white crystalline powder or granules.

Table 12: Typical Properties of Monobasic Sodium phosphate

Acidity/alkalinity	pH = 4.1–4.5 for a 5% w/v aqueous solution of the monohydrate at 258°C.
Density	1.915 g/cm3 for the dihydrate.
Dissociation constant	pKa = 2.15 at 258°C
Solubility	Soluble 1 in 1 of water; very slightly soluble in ethanol(95%).

- **Stability and Storage Conditions :**

Monobasic sodium phosphate is chemically stable, although it is slightly deliquescent. On heating at 1008°C, the dihydrate loses all of its water of crystallization. On further heating, it melts with decomposition at 2058°C, forming sodium hydrogen pyrophosphate, $Na_2H_2P_2O_7$. At 2508°C it leaves a final residue of sodium metaphosphate, $NaPO_3$.

Aqueous solutions are stable and may be sterilized by autoclaving. Monobasic sodium phosphate should be stored in an airtight container in a cool, dry place.

- **Incompatibilities :**

Monobasic sodium phosphate is an acid salt and is therefore generally incompatible with alkaline materials and carbonates; aqueous solutions of monobasic sodium phosphate are acidic and will cause carbonates to effervesce.

Monobasic sodium phosphate should not be administered concomitantly with aluminum, calcium, or magnesium salts since they bind phosphate and could impair its absorption from the gastrointestinal tract. Interaction between calcium and phosphate, leading to the formation of insoluble calcium phosphate precipitates, is possible in parenteral admixtures.

- **Regulatory Status :**

GRAS listed. Included in the FDA Inactive Ingredients Guide (inhalations; injections; ophthalmic preparations; oral capsules, solutions, suspensions, syrups, and tablets; rectal topical, and vaginal preparations). Included in nonparenteral and parenteral medicines licensed in the UK. Included in the Canadian List of Acceptable Non-medicinal Ingredients.

IV. SODIUM HYDROXIDE[58]

- **Nonproprietary Names :**
  - BP: Sodium hydroxide
  - JP: Sodium hydroxide
  - PhEur: Natrii hydroxidum
  - USPNF: Sodium hydroxide
- **Synonyms :**

  Caustic soda; E524; lye; soda lye; sodium hydrate.
- **Chemical Name and CAS Registry Number:**

  Sodium hydroxide [1310-73-2]
- **Empirical Formula and Molecular Weight:** NaOH; 40.00
- **Structural Formula:** NaOH
- **Functional Category :** Alkalizing agent; buffering agent.
- **Applications in Pharmaceutical Formulation or Technology**

Sodium hydroxide is widely used in pharmaceutical formulations to adjust the pH of solutions. It can also be used to react with weak acids to form salts.

- **Description :**

Sodium hydroxide occurs as a white or nearly white fused mass. It is available in small pellets.Sodium hydroxide is very deliquescent and on exposure to air it rapidly absorbs carbon dioxide and water.

- **Typical Properties :**
  - **Acidity/alkalinity:**

    pH ≈ 12 (0.05% w/w aqueous solution);
    pH ≈ 13 (0.5% w/w aqueous solution);
    pH ≈ 14 (5% w/w aqueous solution).
  - **Melting point:** 318°C

- **Solubility:** Solubility of the sodium hydroxide in various solvents is given the Table 13.

Table 13: Solubility of Sodium hydroxide.

Solvent	Solubility at 20°C
Ethanol	1 in 7.2
Ether	Practically insoluble
Glycerin	Soluble
Methanol	1 in 4.2
Water	1 in 0.9

➢ **Stability and Storage Conditions :**

Sodium hydroxide should be stored in an airtight nonmetallic container in a cool, dry place. When exposed to air, sodium hydroxide rapidly absorbs moisture and liquefies.

➢ **Incompatibilities :**

Sodium hydroxide is a strong base and is incompatible with any compound that readily undergoes hydrolysis or oxidation.

➢ **Regulatory Status :**

GRAS listed. Accepted for use as a food additive in Europe. Included in the FDA Inactive Ingredients Guide (dental preparations; injections; inhalations; nasal, ophthalmic, oral, otic, rectal, topical, and vaginal preparations). Included in nonparenteral and parenteral medicines licensed in the UK. Included in the Canadian List of Acceptable Non-medicinal Ingredients.

6.1. MATERIAL AND INSTRUMENTS

Drug, excipients and material used in the experiment are listed in Table 14

Table 14: List of Material used

S. No.	Name of Material	Manufacturer
1	Timolol Maleate USP	Syn-tech pharma , Taiwan
2	Benzalkonium chloride USP	Merck Ltd. Germany
3	Dibasic Sodium phosphate USP	Merck Ltd. Germany
4	Monobasic Sodium Phosphate USP	Merck Ltd. Germany
5	Sodium hydroxide USP	Merck Ltd. Germany
6	Water for Injection	In House
7	Growth Media	High Media, Mumbai
8	Neutralizer media	High Media, Mumbai
9	Three piece containers	Rexam Packagings, Bangalore
10	BFS containers	Borealis Pvt ltd, Mumbai
11	Glass containers	Matri Mirra, Hyderabad

Instruments and equipments used in experimental work are listed in Table 15.

Table 15: List of Instruments used

S. no.	Instrument	Manufacturer
1	Digital Potentiometer	Meter Toledo DL-50, USA
2	Digital Polarimeter	Rudolph Research Analytical Autopol®IV, USA
3	UV- Spectrophotometer	1800,Pharmaspec, Shimadzu,Japan
4	Balance	CPA224S, Sartorius, Bangalore
5	Magnetic stirrer	Remi equipments, Bangalore
6	pH meter	Cyberscan®510^{PC} Eutech, Japan
7	Osmometer	Model-3320, Advanced instruments INC, USA
8	PVDF Filter	Sartorius, Bangalore
9	Laminar flow clean air station	Model no. 1500C-48-24-24 Klenz Pvt.Ltd, Mumbai
10	Digital colony counter	Servewell instruments, Pvt. Ltd, Bangalore
11	HPLC	Shimadzu Prominence LC-2010 CHT model, Japan
12	Stability chambers	Newtronics company Pvt.Ltd. Mumbai
13	Hot air oven	Alpha scientific, Bangalore
14	Incubator	Servewell instruments, Pvt. Ltd, Bangalore

6.2. EXPERIMENTAL WORK

➢ PREFORMULATION STUDIES[59]

The Preformulation studies like Physical characterization and Analytical characterization of drug sample including description, identification, melting point, solubility, loss on drying, and assay by potentiometric titration method were performed.

- **PHYSICAL CHARACTERIZATION OF DRUG SAMPLE**

Timolol Maleate was supplied by Syn-tech pharma, Taiwan and was characterized for its identification and authenticity.

The drug was physically characterized according to following methods-

1. **Description :**

The received sample Timolol Maleate was subjected to the following tests for its characterization:

- **Nature of drug sample**

The drug sample was observed visually and viewed under the Compound microscope for the determination of its nature and then the results were compared with the official books and British Pharmacopoeia and Ph Eur 2006.

- **Color of drug sample**

The drug sample was viewed visually for the determination of its color and then the results were compared with the British Pharmacopoeia and Ph Eur 2006

2. Loss on drying :

Loss on drying was performed for the sample of Timolol maleate according to method specified in British Pharmacopoeia and Ph Eur. It was determined on 1.000 g of Timolol sample by drying at 100^0C-105^0C for 4 hours. The results were then compared with those given in the official books and British Pharmacopoeia and Ph Eur 2006.

3. Solubility:

The solubility of the Timolol maleate sample was carried out in different aqueous and organic solvents like water, glacial acetic acid, methylene chloride, Chloroform and methanol according to British Pharmacopoeia. The results were

then compared with those given in the official books and British Pharmacopoeia and Ph Eur 2006

- **ANALYTICAL CHARACTERIZATION OF DRUG SAMPLE**[59]

1. Absorbance:

Dissolve 0.5 g of Timolol maleate in 0.12 N *Hydrochloric acid* and dilute to 25 μL with the same solvent. The absorbance of the solution measured at 294 nm, is not greater than 0.3 on dried basis.

2. Optical Rotation:

Dissolve 50 mg per ml in 0.1 N *Hydrochloric acid*. The angle of optical rotation must be between -11.7^0 and -12.5^0 (λ= 405 nm).

3. Assay:

Assay was performed using digital potentiometer. About 800 mg of Timolol maleate was accurately weighed, and was transferred to 400-mL beaker, 90 ml of Glacial acetic anhydride was added, and stirred to dissolve. Titration was performed with 0.1 N Perchloric acid. The end point was determined potentiometrically, using a glass silver electrode system. The first two inflection points were used. A blank determination was performed. Each ml of perchloric acid is equivalent to 43.25 mg of $C_{13}H_{24}N_4O_3S.C_4H_4O_4$

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- **FORMULATION STUDIES**

- **Development Strategy :**

Development of Timolol Maleate ophthalmic solution is divided in to two phases as follows.

1. Prototype Formulation Development :

The following excipients were scientifically identified based on their functional. The rational for selecting the excipients is given below.

Table 16: Prototype Formulation

S. No.	Name of the Excipients	Category	Uses
1.	Benzalkonium chloride	Preservative	Benzalkonium chloride prevents bacterial and fungal contamination of the product during its shelf life.
2.	Dibasic Sodium Phosphate	Buffering agent, Sequestering agent	Buffering agent and electrolyte replenisher, when combined with other phosphates
3	Monobasic Sodium Phosphate	Buffering agent, Sequestering agent, Emulsifying agent	Buffering agent and electrolyte replenisher, when combined with other phosphates
4	Sodium hydroxide	Alkali	For pH adjustment

2. Process Development

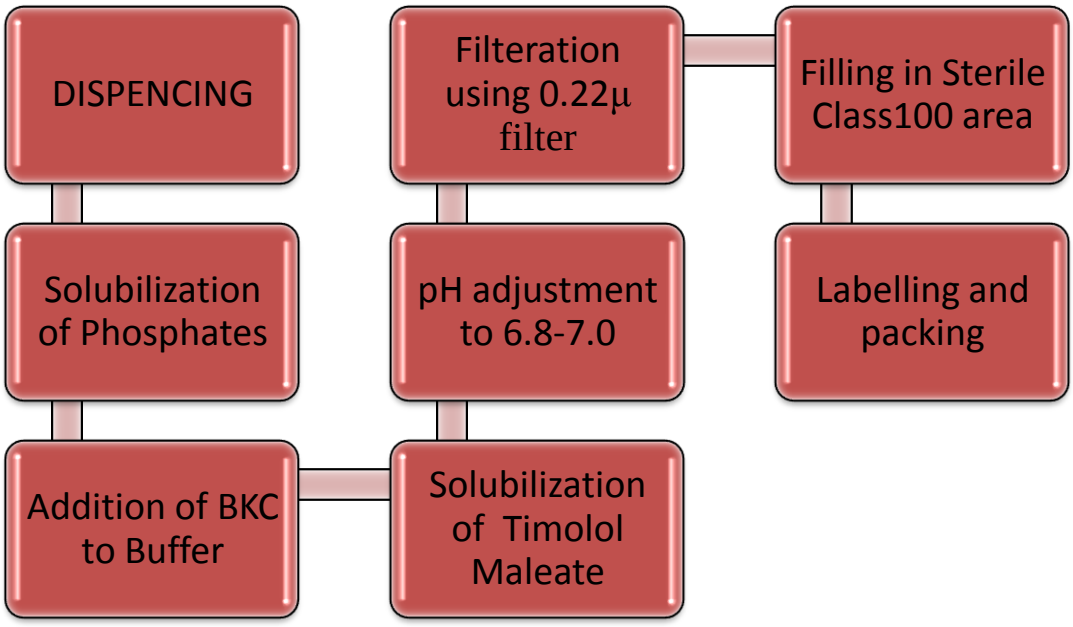


Fig 6: Manufacturing Process Flow Chart

3. Manufacturing Process :

1. Dibasic Sodium Phosphate and Monobasic Sodium Phosphate were dissolved in water for injection by the slow addition. pH was adjusted to the 6.8-7.0 using 5% sodium hydroxide.
2. To this container with buffer solution of phosphates, Benzalkonium chloride was added with continuous stirring.
3. After solubilizing the excipients, to this solution the Timolol maleate USP was added and mixed vigorously until it dissolved in the solution.
4. The solution pH was adjusted to the 6.8-7.0 using 5% Sodium hydroxide solution.
5. The volume was made with water for injection.
6. Bulk Timolol maleate solution was filtered through 0.22 µ PVDF sterilizing grade filter.
7. Solution was filled into two types of LDPE containers and glass containers in sterile area (Class 100) under Laminar Air Flow. LDPE containers were pre-sterilized with gamma radiation. Glass containers were washed with filtered water for injection and were sterilized by Dry Heat Sterilization (160°C for 2 hours).
8. Both filled LDPE and Glass vials were inspected individually against black and white surface for particulate matter.

Above procedure was followed for all the six batches each of 500 ml.

4. FORMULATION DESIGN

The proposed formula was optimized by varying the concentration of Benzalkonium chloride. The quantities of Timolol maleate and other excipients were kept constant. As the aim of the present study was to optimize the concentration of BKC in formulation for Timolol maleate (0.5%) ophthalmic solution.

Batches were planned by taking different concentrations viz.0.0 % v/v, 0.01%,0.012%, 0.016%, and 0.02% ,0.024 % v/v of BKC, Timolol maleate 0.5%, Dibasic Sodium Phosphate, Monobasic Sodium Phosphate and Sodium hydroxide to adjust pH between 6.8 and 7.0 and volume was made up by water for injection.

Table 17: Composition of Timolol Maleate Ophthalmic Solution.

Name of ingredients	Formulation Batches					
	OPT/TIM/ T-001	OPT/TIM/ T-002	OPT/TIM/ T-003	OPT/TIM/ T-004	OPT/TIM/ T-005	OPT/TIM/ T-006
Timolol maleate	6.8 mg/ml	6.8 mg/ml	6.8 mg/ml	6.8 mg/ml	6.8 mg/ml	6.8 mg/ml
Benzalkonium chloride*	0.0%v/v	0.01%v/v	0.012% v/v	0.016% v/v	0.02% v/v	0.024% v/v
Dibasic Sodium Phosphate	30.42 mg/ml	30.42 mg/ml	30.42 mg/ml	30.42 mg/ml	30.42 mg/ml	30.42 mg/ml
Monobasic Sodium Phosphate	6.10 mg/ml	6.10 mg/ml	6.10 mg/ml	6.10 mg/ml	6.10 mg/ml	6.10 mg/ml
Sodium hydroxide	QS to adjust pH	QS to adjust pH	QS to adjust pH	QS to adjust pH	QS to adjust pH	QS to adjust pH
Water for injection	QS	QS	QS	QS	QS	QS

➢ **Preservative Efficacy Test for Timolol maleate0.5% Ophthalmic Solution**[60]

All useful antimicrobial agents are toxic substances. For maximum protection of patients, the concentration of preservative shown to be effective in the final packaged product should be below a level that may be toxic to human being.

The concentration of an added antimicrobial preservative can be kept at minimum if active ingredients of the formulation possess an intrinsic antimicrobial activity.

Sample from all six batches were subjected to preservative efficacy test of Benzalkonium chloride in Timolol maleate 0.5% ophthalmic solution. The most stringent criteria of British pharmacopoeia was followed for experiment.

Test organisms:

Following micro-organisms supplied by *National Chemical Laboratory, Pune* were used for the PET of Benzalkonium chloride. i) Candida albicans ATCC 10231 ii) Aspergillus niger ATCC 16404 iii) Escherichia coli ATCC 8739 iv) Pseudomonas aeruginosa ATCC 9027 v) Staphylococcus aureus ATCC 6538 **Media**: Media for experiment were procured from *HIGH Media Mumbai* i) Soybean – Casein Digest Broth ii) Soybean – Casein Digest Agar iii) Sabouraude - Dextrose Agar iv) Sabouraude – Dextrose Broth.

Test Procedure for Anti-Infective Effectiveness

1. The product had been transferred to five sterile, capped bacteriological containers.
2. Each container was inoculated with one of the prepared and standardized inoculums and mixed.
3. The volume of suspension inoculum was between 0.5% and 1.0% of the volume of product.
4. The concentration of test microorganism added to the product was such that the final concentration of the test preparation after inoculation was between 1×10^5 and 1×10^6 cfu/ml of the product.
5. Sample was incubated at 22.5 ± 25^0C. The initial concentration of viable microorganisms was determined by plate count method.
6. Each container was sampled at intervals of 0 hrs, 6hrs, 24 hrs, 7days, 14 days, 21 days, and 28days for different microorganisms.
7. Sample was extracted into neutralizing fluid to inactivate residual preservative
8. Using the calculated concentration of cfu/ml present at the initial of the test, calculate the change in $\log_{10}$ values of the concentration (cfu/ml) for each micro-organism at the applicable test intervals, and express the changes in terms of log reduction.
9. Microbial Count (cfu/ml) of Product was calculated by using following formula

$$\text{cfu/ml of Product} = \frac{\text{Ml of the inoculum of the product} \times \text{cfu/ml of inoculum}}{\text{Volume of reconstituted product}}$$

Log reduction = Log of initial count – Log of final count

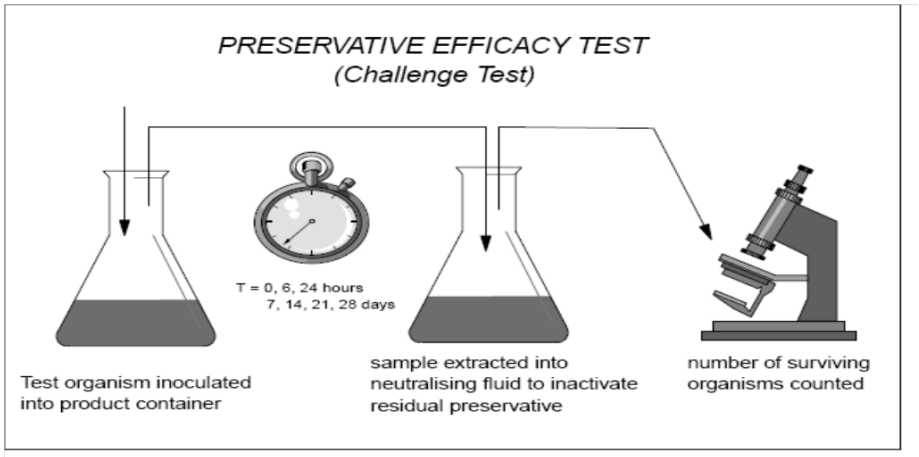


Fig7:

Preservative Efficacy Test procedure.

Table 18: Criteria of log reduction for ophthalmic solution as per BP

S. No.	Microorganisms	Acceptance Criteria
1	Bacteria	Bacteria reduced by 2 logs at 6 hours and 3 logs at 24 hours with no recovery at 28 days.
2	Fungi	Fungi reduced by 2 logs at 7 days with no increase at 28 days.

After performing the preservative efficacy test for all the six batches, the batch of optimized formula was subjected to initial analysis. The optimized batch was filled aseptically in three different types of containers viz. Three piece container, BFS container, and amber colored glass container.

➢ **Stability and Container Compatibility Study**[61] **:**

Batch of Timolol maleate 0.5% ophthalmic solution was optimized from the results of PET was filled in to Glass container and LDPE container viz. Three Piece and BFS containers. The material of construction used for containers are as follows

1. Three piece containers : PE 1840 H
2. BFS containers : PE 3020 D
3. Glass containers : Type I: Borosilicate glass

Above mentioned PE 1840 H and PE 3020 D are the grades of low density polyethylene. Polyethylene is a long chain polymer of repeating groups, each connected to two hydrogen atoms. The individual molecules are very long with a carbon"backbone"formed by the carbon atoms connecting to each other. The polymer contains millions of these long molecular chains, each hopelessly entangled with all of its neighbors. The strength of the molded part lies in the complexity of that entanglement. When cross linking occurs, the molecular weight increases with resulting improvement of the physical properties of the polyethylene.

Cross linking polyethylene compounds contain chemical agents designed to create a molecular change during the molding process, which results in the polymer molecules becoming interlocked (Cross linked) with each other. Other polyethylene resins bond with each other during the molding process by surface attachment only, while Crosslink creates a chemical interlocking bond between the molecules that is "in a sense" one giant molecule.

- **Stability :**

The design of the formal stability studies for the drug product should be based on knowledge of the behavior and properties of drug substance and from stability studies on the drug substances. The likely changes on storage and the rationale attributes to be tested in formal stability studies should be stated.

Table 19: Stability Protocol

PRODUCT	**TIMOLOL MALEATE OPHTHALMIC SOLUTION 0.5% w/v**						**Pack: 3-Piece, BFS, and Amber colored glass vials**
Condition	Time points	Analytical parameters					Number of samples
		Description, Assay	Preservative content	Osmolality	pH	Drop size study	Water Loss study	
Initial	-	4	2	1	2	3	5*	12 + 05*
60°C (stress testing)	1week	4	2	1	2	-	-	09
	2 week	4	2	1	2	-		09
40°C± 2°C/ NMT 25 % R H (accelerated testing)	1 month	4	2	1	2	3	#	12
	2 month	4	2	1	2	3	#	12
	3 month	4	2	1	2	3	#	12
Total								71

* The containers should be weighed individually before charging.

Parameter repeated at every station

- **Specifications**

Stability studies should include testing of those attributes of the drug product that are susceptible to change during storage and are likely to influence quality, safety, and / or efficacy. The testing should cover, as appropriate, the physical, chemical, biological and microbiological attributes, preservative content and functionality tests. Analytical procedures should be fully validated and stability indicating.

- **Testing frequency**

At the accelerated storage condition, a minimum of three time points, including the initial and final time points (e.g., 0, 3, and 6 months) from a 6 month study is recommended.

- **Storage condition for Stability/Container compatibility**

In general drug product should be evaluated under storage conditions (with appropriate tolerances) that it's thermal stability and, if applicable, its sensitivity to moisture or potential for solvent loss. Accelerated condition for semi-permeable

containers (40ºC ± 2ºC/ NMT 25% RH) and for glass containers (40ºC ± 2ºC/ 75% ± 5% RH) were chosen for storage for stability/compatibility study.

➢ **Product Analysis**

The product analysis was done as per the stability protocol. While manufacturing the in-process tests such as clarity, particulate matter, pH, were performed. Finished product tests include appearance, assay of API and preservative, osmolality, pH, drop size study, and water loss study at every time point of the stability.

- **Finished Product Analysis**

Finished product must be analyzed before the stability charging. These tests were performed initially and at every time point of the stability. All the analytical procedures were validated.

ANALYTICAL PARAMETERS TO BE TESTED ARE AS FOLLOWS.

1. Appearance:

Sample under test was inspected visually for color and clarity.

2. Assay of Timolol maleate:

USP recommends high performance liquid chromatography for assay of Timolol maleate 0.5% ophthalmic solution**.**

i. Mobile Phase:

A filtered and degassed mixture of Buffer solution(pH 2.8) and acetonitrile, (65:35) was prepared. The pH was adjusted to 2.50 ± 0.05 with 1M $HClO_4$ or 1N sodium hydroxide and was filtered through 0.45 µm filter.

ii. Resolution solution:

No separate resolution solution is required. The mobile phase itself acts as resolution solution.

iii. Standard preparation:

Accurately weighed quantity of USP Timolol maleate was quantitatively dissolved in mixture of pH 2.8 buffer and acetonitrile (1:2 ratio) to obtain a solution having a known concentration of about 0.06 mg per ml.

iv. Assay preparation:

Accurately weighed measured volume of ophthalmic solution, equivalent to 5 mg of Timolol maleate, to a 50 ml volumetric flask, dilute with solution of pH 2.8 buffer and acetonitrile (1:2 ratio) to volume and mixed.

Table 20: Chromatographic conditions for Timolol maleate assay

Column	150 mm x 4.6 mm; 3µ, Intersil ODS
Flow rate	1.5 ml/min
Detector wave length	295 nm
Column temperature	35º C
Injection volume	20 µl
Run time	20 minutes
Diluent	pH 2.8 buffer and acetonitrile (1:2 ratio)

v. Procedure:

Equal volumes of the (about 20µl) of the standard preparation and assay preparation was injected separately into the chromatographic system, chromatograms were recorded, and the areas for major peaks were measured. Percentage assay of Timolol maleate was calculated by formula

$$\frac{AI}{AS} \times \frac{WS}{100} \times \frac{5}{50} \times \frac{50}{WT} \times \frac{P}{100} \times \frac{100}{LC} = \text{---------- \% w/v}$$

AI : Area of Timolol maleate sample peak in sample preparation.

AS : Average area of Timolol maleate standard

WS : Weight of Timolol maleate standard taken (in grams)

WT : Weight of Timolol maleate sample taken (in grams)

P : Potency of Timolol maleate sample on as is basis[62]

3. Assay of Benzalkonium chloride :

Assay of preservative Benzalkonium chloride was performed at every station of stability by High performance liquid chromatography method as follows.

i. Buffer:

Accurately 9.0 ml of perchloric acid was pippeted out and diluted to 1000 ml of milli Q water.

ii. Mobile phase:

Buffer and Acetonitrile was mixed in the ratio of 35 : 65.The pH was adjusted to 2.50 ± 0.05 with 1M $HClO_4$ or 1N Sodium hydroxide and was filtered through 0.45 µm filter.

iii. Standard Solution:

Equivalent to 50 mg of Benzalkonium chloride standard was accurately weighed into 100 ml volumetric flask; 35 ml of Mobile phase was added and dissolved. The volume was made with the Mobile phase.

5.0 ml of the above solution was transferred in to 100 ml volumetric flask and volume was made with the Mobile phase. The solution was filtered through the 0.45 µm Nylon filter.

iv. Test solution:

The contents of 2 containers of Timolol maleate eye drop were mixed. 5.0ml of Timolol maleate ophthalmic solution was transferred in to a clean and dry 20 ml volumetric flask and sample weight was recorded. Add 10.0 ml of diluent, shaked well. The volume was made with the Mobile phase and the solution was filtered through the 0.45 µm Nylon filter.

The chromatographic conditions for Benzalkonium chloride assay are listed in the table 21

Table 21: Chromatographic conditions for Benzalkonium chloride assay

Column	Hypersil BDS C8-150 x 4.6 mm, 5µ
Flow rate	1.3 ml/min
Detector wave length	219nm
Column temperature	25°C
Injection volume	20 µl
Run time	20 min
Diluent:	mobile phase as diluents

v. Procedure:

20 µl of blank (single), standard solution (5 replicates) and test solution (2 replicates) were injected separately. Chromatograms were recorded. Amount of Benzalkonium chloride was calculated using the formula.

i. The tailing factor of Benzalkonium chloride NLT 2.0

ii. The RSD of 5 replicate injections of standard NMT 2.0%

$$\frac{AT}{AS} \times \frac{WS}{100} \times \frac{5}{100} \times \frac{20}{WT} \times P \times Wt/ml \times 100 = \text{---------- \% w/v}$$

Where,

AT: Area Benzalkonium chloride (BKC 1 + BKC 2) peak in sample preparation.

AS: Average area of standard (BKC 1 + BKC 2).

WS: Weight of standard taken (in grams)

WT: Weight of Benzalkonium chloride taken (in grams)

P: Potency of Benzalkonium chloride on as is basis[63]

4. pH:

Precalibrated digital pH meter was used for the pH measurement of Timolol maleate ophthalmic solution.

Procedure:

1. The equipment was switched on by pressing the ON/OFF key. Display showed main screen after initial checks.
2. Electrode was rinsed with distilled water and blotted dry with tissue paper
3. Electrode and temperature probe were dipped in sample solution.
4. By pressing 'MODE' key pH was selected.

5. ‘MEAS’ was selected to check the pH.
6. After stabilization reading for pH was noted.
7. Again glass electrode was flushed with water and wiped out. it was placed back in bottle congaing buffer solution.

5. Osmolality:

Precalibrated digital osmometer was used for the osmolarity measurement of Timolol maleate ophthalmic solution.

Procedure:

1. Instrument was switched on and waited till initialization completed.
2. ‘START’ key was pressed and waited till Running Diagnostic completed.
3. 250 µl of the sample was pipette out in sample tube with help of micro pipette and micro-tip.
4. The probe and stir/freeze wire was cleaned and wiped.
5. ‘START’ key was pressed and data for sample number was entered. Again ‘START’ key was pressed
6. Note the reading for osmolality in mOsmol on digital display.
7. Empty sample tube was leaved in freezing chamber to avoid deposition of debris.

6. Drop size:

Drop size of instilled drop is the function of the amount of drug delivered to the eye per instillation. It is reported that that average drop size of many commercially available topical medication is actually 39 µl with range of 25.1 µl to 56.4 µl[64].

Procedure

1. Container was punctured with piercing cap.
2. Container was held with thumb and index finger in inverted position at an angle of 90^0.
3. Pressure was applied to the container; separate drops will come out of the nozzle.
4. Weight of 10 drops was taken with precision scales.
5. Average was calculated for weight of single drop as follows

Weight of each drop (drop size) = Weight of 10 drops/10

6. In same way, procedure was repeated for three times. From these three observations, average volume of the drop was calculated.

7. Water loss:

1. Five Semi-permeable containers of each type, LDPE Three piece and BFS, were charged to accelerated condition of temperature and humidity(40ºC ± 2ºC/ 25% ± 5% RH) with proper labeling on each container.
2. Before charging, each container was weighed individually for initial weights.
3. At every station of stability, each container was individually weighed and noted.
4. Percentage water loss was calculated by using formula -

$$\% \text{ Water Loss} = \frac{(\text{Initial weight of container} - \text{final weight of container}) \times 100}{\text{Initial weight of container}}$$

7. RESULTS AND DISCUSSIONS

➢ PREFORMULATION STUDIES:

The Preformulation studies like Physical characterization and Analytical characterization of drug sample including description, identification, melting point, solubility, loss on drying, and assay by potentiometric titration method were performed.

• PHYSICAL CHARACTERIZATION OF DRUG SAMPLE

Timolol Maleate was supplied by Syn-tech pharma, Taiwan, was found to comply for its identification and authenticity as per certificate of analysis provided by supplier.

The drug was physically characterized according to following methods-

1. Description :

The received sample of Timolol maleate after visual observation and under compound microscope it was found to show the following characteristics and these are acceptable according to British Pharmacopoeia and Ph Eur 2007.

a) Nature of drug sample: Crystalline powder

b) Color of drug sample: White

2. Loss on Drying:

After drying 1.0 g of sample at 100^0 C for 4 hours in hot air oven. It was found that weight of sample reduced to 0.82 g. Sample passed the criteria for loss on drying. (NMT 0.5%).

3. Solubility:

It was found that drug was readily soluble in water for injection , slightly soluble to soluble in Chloroform, slightly soluble in methanol. Timolol maleate got solubilised in water for injection at pH 6.8.

• ANALYTICAL CHARACTERIZATION

1. Absorbance:

The absorbance of the 0.5 g of Timolol maleate dissolved in 0.1M HCl solution measured at 294 nm was found to be 0.27 using UV – visible spectrophotometer.

2. Optical Rotation:

After dissolving the drug sample in given proportion of 0.1N *hydrochloric acid* the angle of optical rotation was found to be -11.9^0.

3. Assay:

After performing assay of Timolol maleate by potentiometrically by using the digital potentiometer, assay of Timolol maleate sample was found to be 98.603%. Percentage purity of Timolol maleate must be calculated for the addition of exact quantity to achieve the concentration of 5 mg/ml in ophthalmic solution.

After designing of prototype formulation, six different batches with different concentration of Benzalkonium chloride were taken. All these six batches were subjected to the Preservative efficacy test as per the BP.

➢ **Results of Preservative Efficacy Test:**

Log reduction for each type of micro-organism was calculated using the given formula. Table showing Log reductions in microbial growth are given below.

- **Batch No. OPT/TIM/T-001** (BAK – 0.0% v/v)

Log reduction at 6^{th} hours

Escherichia coli	02×10^5-01×10^5	5.3-5.0	0 log reduction
Pseudomonas aeruginosa	02×10^5-01×10^5	5.3-5.0	0 log reduction
Staphylococcus aureus	03×10^5-02×10^5	5.4-5.3	0 log reduction
Candida albicans	01×10^6 - 01×10^6	6.0 – 6.0	0 log reduction
Aspergillus niger	03×10^5 -2×10^5	5.4 -5.3	0 log reduction

Log reduction at 24^{th} hours

Escherichia coli	02×10^5-01×10^5	5.3-5	0 log reduction
Pseudomonas aeruginosa	02×10^5-01×10^5	5.3-5	0 log reduction
Staphylococcus aureus	03×10^5 - 01×10^5	5.4 -5.0	0 log reduction
Candida albicans	5.3 -4.3	6.0 – 6.0	0 log reduction
Aspergillus niger	03×10^5 - 03×10^5	5.4 -5.4	0 log reduction

Log reduction at 7^{th} day

Escherichia coli	02×10^5-03×10^4	5.3 -4.4	1 log reduction
Pseudomonas aeruginosa	02×10^5- 02×10^4	5.3 -4.3	1 log reduction
Staphylococcus aureus	03×10^5 – 01×10^4	5.3 -4.0	1 log reduction
Candida albicans	5.3 - 4.3	6.0 – 6.0	0 log reduction
Aspergillus niger	03×10^5 - 03×10^5	5.4 -5.4	0 log reduction

Log reduction at 14th day

Escherichia coli	02×10^5 - 02×10^4	5.3 -4.3	1 log reduction
Pseudomonas aeruginosa	$02 \times 10^{5-}$ 02×10^4	5.3 -4.3	1 log reduction
Staphylococcus aureus	03×10^5 – 01×10^5	5.4-4.0	1 log reduction
Candida albicans	01×10^6 - 01×10^6	6.0 – 6.0	0 log reduction
Aspergillus niger	03×10^5 – 02×10^5	5.4-5.3	0 log reduction

Log reduction at 21th day

Escherichia coli	02×10^5 - 02×10^4	5.3 -4.3	1 log reduction
Pseudomonas aeruginosa	02×10^5 - 01×10^4	5.3 -4.0	1 log reduction
Staphylococcus aureus	03×10^5 – 01×10^4	5.4-4.0	1 log reduction
Candida albicans	01×10^6 - 01×10^6	6.0 – 6.0	0 log reduction
Aspergillus niger	03×10^5 – 02×10^5	5.4-5.3	0 log reduction

Log reduction at 28th day

Escherichia coli	02×10^5 - 01×10^4	5.3 -4.0	1 log reduction
Pseudomonas aeruginosa	02×10^5 - 01×10^4	5.3 -4.0	1 log reduction
Staphylococcus aureus	03×10^5 – 01×10^4	5.4-4.0	1 log reduction
Candida albicans	01×10^6 - 02×10^5	6.0 – 5.3	1 log reduction
Aspergillus niger	03×10^5 – 01×10^4	5.4-4.0	1 log reduction

- **Batch No. OPT/TIM/ T-002** (BAK – 0.01% v/v)

Log reduction at 6th hours

Escherichia coli	01×10^5 – 2×10^4	5.0 - 4.3	1 Log reduction
Pseudomonas aeruginosa	02×10^5 - 2×10^4	5.3 - 4.3	1 Log reduction
Staphylococcus aureus	02×10^5 - 2×10^4	5.3 - 4.3	1 Log reduction
Candida albicans	01×10^6 - 01×10^6	6.0 - 6.0	0 Log reduction
Aspergillus niger	03×10^5 - 01×10^5	5.4 – 5.0	0 Log reduction

Log reduction at 24th hours

Escherichia coli	01×10^5 – 3×10^3	5.0 - 3.0	2 Log reduction
Pseudomonas aeruginosa	02×10^5 - 1×10^3	5.3 – 3.0	2 Log reduction
Staphylococcus aureus	02×10^5 - 2×10^3	5.3 - 3.3	2 Log reduction
Candida albicans	01×10^6 - 01×10^6	6.0 – 6.0	0 Log reduction
Aspergillus niger	03×10^5 - 01×10^5	5.4 – 5.0	0 Log reduction

Log reduction at 7th day

Escherichia coli	01×10^5 – NIL	5.0 - NIL	5 Log reduction
Pseudomonas aeruginosa	02×10^5 – NIL	5.3 - NIL	5 Log reduction
Staphylococcus aureus	02×10^5 – NIL	5.3 - NIL	5 Log reduction
Candida albicans	01×10^6 - 02×10^5	6.0 – 5.3	1 Log reduction
Aspergillus niger	03×10^5 - 02×10^4	5.4 – 4.3	1 Log reduction

Log reduction at 14th day

Escherichia coli	01×10^5 – NIL	5.0 - NIL	5 Log reduction
Pseudomonas aeruginosa	02×10^5 - NIL	5.3 - NIL	5 Log reduction
Staphylococcus aureus	02×10^5 - NIL	5.3 - NIL	5 Log reduction
Candida albicans	01×10^6 - 02×10^5	6.0 – 5.3	1 Log reduction
Aspergillus niger	03×10^5 - 02×0^4	5.4 – 4.3	1 Log reduction

Log reduction at 21th day

Escherichia coli	01×10^5 – NIL	5.0 - NIL	5 Log reduction
Pseudomonas aeruginosa	02×10^5 - NIL	5.3 - NIL	5 Log reduction
Staphylococcus aureus	02×10^5 - NIL	5.3 - NIL	5 Log reduction
Candida albicans	01×10^6 - 02×10^5	6 – 5.3	1 Log reduction
Aspergillus niger	03×10^5 - 02×10^4	5.4 – 4.3	1 Log reduction

Log reduction at 28th day

Escherichia coli	01×10^5 – NIL	5.0 - NIL	5 Log reduction
Pseudomonas aeruginosa	02×10^5 - NIL	5.3 - NIL	5 Log reduction
Staphylococcus aureus	02×10^5 - NIL	5.3 - NIL	5 Log reduction
Candida albicans	01×10^6 - 02×0^5	6 – 5.3	1 Log reduction
Aspergillus niger	03×10^5 - 02×0^4	5.4 – 4.3	1 Log reduction

- **Batch No. OPT/TIM/T-003** (BAK – 0.012% v/v)

Log reduction at 6th hours

Escherichia coli	02×10^5 -03×10^3	5.3 -3.3	2 Log reduction
Pseudomonas aeruginosa	01×10^5 -01×10^3	5.0 – 3.0	2 Log reduction
Staphylococcus aureus	03×10^5 - 01×10^3	5.4 – 3.0	2 Log reduction
Candida albicans	01×10^6 - 01×10^6	6.0 -6.0	0 Log reduction
Aspergillus niger	03×10^5 - 01×10^5	5.4 – 5.0	0 Log reduction

Log reduction at 24th hours

Escherichia coli	$02 \times 10^5 – 4 \times 10^2$	5.3 - 2.6	3 Log reduction
Pseudomonas aeruginosa	01×10^5 - 2×10^5	5.0 – 2.3	3 Log reduction
Staphylococcus aureus	03×10^5 - 4×10^2	5.4 - 2.6	3 Log reduction
Candida albicans	01×10^6 - 01×0^6	6 – 6	0 Log reduction
Aspergillus niger	$03 \times 10^5 – 2 \times 10^5$	5.4 - 5.0	0 Log reduction

Log reduction at 7th day

Escherichia coli	02×10^5 – NIL	5.3 - NIL	5 Log reduction
Pseudomonas aeruginosa	01×10^5 – NIL	5.0 - NIL	5 Log reduction
Staphylococcus aureus	03×10^5 – NIL	5.4 - NIL	5 Log reduction
Candida albicans	01×10^6 - 01×0^5	6.0 – 5.0	1 Log reduction
Aspergillus niger	03×10^5 - 02×10^4	5.4 – 4.3	1 Log reduction

Log reduction at 14th day

Escherichia coli	02×10^5 – NIL	5.3 - NIL	5 Log reduction
Pseudomonas aeruginosa	01×10^5 – NIL	5.0 - NIL	5 Log reduction
Staphylococcus aureus	03×10^5 – NIL	5.4 - NIL	5 Log reduction
Candida albicans	01×10^6 - 01×0^5	6.0 – 5.0	1 Log reduction
Aspergillus niger	03×10^5 - 02×10^5	5.4 – 4.4	1 Log reduction

Log reduction at 21th day

Escherichia coli	02×10^5 – NIL	5.3 – NIL	5 Log reduction
Pseudomonas aeruginosa	01×10^5 – NIL	5.0 – NIL	5 Log reduction
Staphylococcus aureus	03×10^5 – NIL	5.4 – NIL	5 Log reduction
Candida albicans	01×10^6 - 01×0^5	6.0 – 5.0	1 Log reduction
Aspergillus niger	03×10^5 - 02×10^4	5.4 – 4.3	1 Log reduction

Log reduction at 28th day

Escherichia coli	02×10^5 – NIL	5.3 – NIL	5 Log reduction
Pseudomonas aeruginosa	01×10^5 – NIL	5.0 – NIL	5 Log reduction
Staphylococcus aureus	03×10^5 – NIL	5.4 – NIL	5 Log reduction
Candida albicans	01×10^6 - 01×0^5	6.0 – 5.0	1 Log reduction
Aspergillus niger	03×10^5 - 01×10^4	5.4 – 4..0	1 Log reduction

- **Batch No. OPT/TIM/T-004**(BAK – 0.016% v/v)

Log reduction at 6th hours

Escherichia coli	$01 \times 10^5 – 2 \times 10^2$	5.0 - 2.3	3 Log reduction
Pseudomonas aeruginosa	$01 \times 10^5 - 2 \times 10^2$	5.0 - 2.3	3 Log reduction
Staphylococcus aureus	$02 \times 10^5 - 1 \times 10^3$	5.3 – 3.0	2 Log reduction
Candida albicans	$01 \times 10^6 - 01 \times 10^6$	6.0 – 6.0	0 Log reduction
Aspergillus niger	$02 \times 10^5 - 02 \times 0^5$	5.3 – 5.3	0 Log reduction

Log reduction at 24th hours

Escherichia coli	$01 \times 10^5 – 2 \times 10^2$	5.0 - 2.3	3 Log reduction
Pseudomonas aeruginosa	$01 \times 10^5 - 2 \times 10^2$	5.0 - 2.3	3 Log reduction
Staphylococcus aureus	$02 \times 10^5 - 1 \times 10^3$	5.3 - 3	2 Log reduction
Candida albicans	$01 \times 10^6 - 02 \times 10^5$	6.0 – 5.3	1 Log reduction
Aspergillus niger	$02 \times 10^4 - 02 \times 10^4$	5.3 – 4.3	1 Log reduction

Log reduction at 7th day

Escherichia coli	01×10^5 – NIL	5.0 - NIL	5 Log reduction
Pseudomonas aeruginosa	01×10^5 - NIL	5.0 - NIL	5 Log reduction
Staphylococcus aureus	02×10^5 - NIL	5.3 - NIL	5 Log reduction
Candida albicans	$01\times 10^6 - 01 \times 10^5$	6.0 – 5.0	1 Log reduction
Aspergillus niger	$02\times 10^5 - 02 \times 10^4$	5.3 – 4.3	1 Log reduction

Log reduction at 14th day

Escherichia coli	01×10^5 – NIL	5.0 - NIL	5 Log reduction
Pseudomonas aeruginosa	02×10^5 - NIL	5.3 - NIL	5 Log reduction
Staphylococcus aureus	02×10^5 - NIL	5.3 - NIL	5 Log reduction
Candida albicans	$01 \times 10^6 - 01 \times 10^4$	6.0 – 4.0	2 Log reduction
Aspergillus niger	$02 \times 10^5 - 01 \times 10^3$	5.3 – 3.0	2 Log reduction

Log reduction at 21th day

Escherichia coli	01×10^5 – NIL	5.0 - NIL	5 Log reduction
Pseudomonas aeruginosa	02×10^5 - NIL	5.3 - NIL	5 Log reduction
Staphylococcus aureus	02×10^5 - NIL	5.3 - NIL	5 Log reduction
Candida albicans	$01 \times 10^6 - 01 \times 10^4$	6.0 – 4.0	2 Log reduction
Aspergillus niger	$02 \times 10^5 - 01 \times 10^3$	5.3 – 3.0	2 Log reduction

Log reduction at 28th day

Escherichia coli	01×10^5 – NIL	5.0 - NIL	5 Log reduction
Pseudomonas aeruginosa	02×10^5 – NIL	5.3 - NIL	5 Log reduction
Staphylococcus aureus	02×10^5 – NIL	5.3 - NIL	5 Log reduction
Candida albicans	01×10^6 - 01×10^4	6.0 – 4.0	2 Log reduction
Aspergillus niger	02×10^5 - 01×10^3	5.3 – 3.0	2 Log reduction

- **Batch No. OPT/TIM/T-005** (BAK – 0.02% v/v)

Log reduction at 6th hours

Escherichia coli	01×10^5 – 1×10^3	5.0 – 3.0	2 Log reduction
Pseudomonas aeruginosa	01×10^5 - 1×10^3	5.0 – 3.0	2 Log reduction
Staphylococcus aureus	02×10^5 - 1×10^3	5.3 – 3.0	2 Log reduction
Candida albicans	01×10^6 - 01×0^6	6.0– 6.0	0 Log reduction
Aspergillus niger	04×10^5 - 02×0^5	5.6 – 5.3	0 Log reduction

Log reduction at 24th hours

Escherichia coli	01×10^5 – NIL	5.0 - NIL	5 Log reduction
Pseudomonas aeruginosa	01×10^5 - NIL	5.0 - NIL	5 Log reduction
Staphylococcus aureus	02×10^5 - NIL	5.3 - NIL	5 Log reduction
Candida albicans	01×10^6 - 01×10^5	6.0 – 5.0	1 Log reduction
Aspergillus niger	04×10^5 - 01×10^4	5.6 – 4.0	1 Log reduction

Log reduction at 7th day

Escherichia coli	01×10^5 – NIL	5.0 - NIL	5 Log reduction
Pseudomonas aeruginosa	01×10^5 - NIL	5.0 - NIL	5 Log reduction
Staphylococcus aureus	02×10^5 - NIL	5.3 - NIL	5 Log reduction
Candida albicans	01×10^6 - 02×10^4	6.0 – 4.3	2 Log reduction
Aspergillus niger	04×10^5 - 02×10^4	5.6 – 4.3	2 Log reduction

Log reduction at 14th day

Escherichia coli	01×10^5 – NIL	5.0 – NIL	5 Log reduction
Pseudomonas aeruginosa	02×10^5 - NIL	5.3 – NIL	5 Log reduction
Staphylococcus aureus	02×10^5 - NIL	5.3 – NIL	5 Log reduction
Candida albicans	01×10^6 - 02×10^3	6.0 – 3.3	3 Log reduction
Aspergillus niger	04×10^5 - 03×10^3	5.6 – 3.3	3 Log reduction

Log reduction at 21th day

Escherichia coli	01×10^5 – NIL	5.0 - NIL	5 Log reduction
Pseudomonas aeruginosa	02×10^5 - NIL	5.3 - NIL	5 Log reduction
Staphylococcus aureus	02×10^5 - NIL	5.3 - NIL	5 Log reduction
Candida albicans	$01 \times 10^6 – 1 \times 10^2$	6.0 – 2.0	4 Log reduction
Aspergillus niger	04×10^5 - 01×10^2	5.6 – 2.0	4 Log reduction

Log reduction at 28th day

Escherichia coli	01×10^5 – NIL	5.0 - NIL	5 Log reduction
Pseudomonas aeruginosa	02×10^5 - NIL	5.3 - NIL	5 Log reduction
Staphylococcus aureus	02×10^5 - NIL	5.3 - NIL	5 Log reduction
Candida albicans	01×10^6 - 01×10^2	6.0 – 2.0	4 Log reduction
Aspergillus niger	04×10^6 - 01×10^2	6.0 – 2.0	4 Log reduction

- **BATCH NO. OPT/TIM/T-06** (BAK – 0.022% v/v)

Log reduction at 6th hours

Escherichia coli	02×10^5- 02×10^3	5.3- 3.3	2 Log reduction
Pseudomonas aeruginosa	01×10^5- 01×10^3	5.0- 3.0	2 Log reduction
Staphylococcus aureus	02×10^5- 02×10^3	5.3- 3.3	2 Log reduction
Candida albicans	01×10^6 - 01×10^6	6.0 – 6.0	0 Log reduction
Aspergillus niger	02×10^5- 01×10^5	5.3- 5.0	0 Log reduction

Log reduction at 24th hours

Escherichia coli	02×10^5- NIL	5.3- 0.0	5 Log reduction
Pseudomonas aeruginosa	01×10^5- NIL	5.0- 0.0	5 Log reduction
Staphylococcus aureus	02×10^5- NIL	5.3- 0.0	5 Log reduction
Candida albicans	01×10^6 - 01×10^5	6.0 – 5.0	1 Log reduction
Aspergillus niger	02×10^5- 01×10^4	5.3- 4.0	1 Log reduction

Log reduction at 7th day

Escherichia coli	02×10^5- NIL	5.3- 0.0	5 Log reduction
Pseudomonas aeruginosa	01×10^5- NIL	5.0- 0.0	5 Log reduction
Staphylococcus aureus	02×10^5- NIL	5.3- 0.0	5 Log reduction
Candida albicans	01×10^6 - 01×10^4	6.0- 4.0	2 Log reduction
Aspergillus niger	02×10^5- 01×10^3	5.3- 3.0	2 Log reduction

Log reduction at 14th day

Escherichia coli	02×10^5- NIL	5.3- 0.0	5 Log reduction
Pseudomonas aeruginosa	01×10^5- NIL	5.0- 0.0	5 Log reduction
Staphylococcus aureus	02×10^5- NIL	5.3- 0.0	5 Log reduction
Candida albicans	01×10^6 - 01×10^3	6.0- 3.0	3 Log reduction
Aspergillus niger	02×10^5- 02×10^2	5.3- 2.3	3 Log reduction

Log reduction at 21th day

Escherichia coli	02×10^5- NIL	5.3- 0.0	5 Log reduction
Pseudomonas aeruginosa	01×10^5- NIL	5.0- 0.0	5 Log reduction
Staphylococcus aureus	02×10^5- NIL	5.3- 0.0	5 Log reduction
Candida albicans	01×10^6 - 03×10^2	6.0- 2.4	4 Log reduction
Aspergillus niger	02×10^5- 02×10^1	5.3- 1.3	4 Log reduction

Log reduction at 28th day

Escherichia coli	02×10^5- NIL	5.3- 0.0	5 Log reduction
Pseudomonas aeruginosa	01×10^5- NIL	5.0- 0.0	5 Log reduction
Staphylococcus aureus	02×10^5- NIL	5.3- 0.0	5 Log reduction
Candida albicans	01×10^6 - 02×10^2	6.0- 2.3	4 Log reduction
Aspergillus niger	02×10^5- 02×10^1	5.3- 1.0	4 Log reduction

- **Summary of PET results :**

Results for Bacterial log reduction with different concentration of Benzalkonium chloride are summarized in following table 22.

Table 22: Log Reduction in Bacterial Growth.

BAK CONCENTRATION	OBSERVATION (LOG REDUCTION)					
	6 HRS	24 HRS	7th DAY	14th DAY	21th DAY	28th DAY
0.0%	0	0	1	1	1	1
0.010 %	1	2	5	5	5	5
0.012 %	2	3	5	5	5	5
0.016 %	2	3	5	5	5	5
0.020 %	2	5	5	5	5	5
0.022 %	2	5	5	5	5	5
Acceptance criteria	Min 2 Log Reduction	Min 3 Log Reduction	-	-	-	No Recovery

Results for Fungal log reduction with different concentration of Benzalkonium chloride are summarized in following table 23.

Table 23: Log Reduction in Fungal Growth.

BAK CONCENTRATION	OBSERVATION (LOG REDUCTION)					
	6 HRS	24 HRS	7th DAY	14th DAY	21th DAY	28th DAY
0.0%	0	0	0	0	0	1
0.010 %	0	0	1	1	1	1
0.012 %	0	0	1	1	1	1
0.016 %	0	0	1	2	2	2
0.020 %	0	1	2	3	3	3
0.022 %	0	1	2	3	4	4
Acceptance criteria	-	-	Min 2 Log Reduction	-	-	No Recovery

The results obtained from the experiment, in which

1) Benzalkonium chloride (0.0%v/v) failed for 2 log reduction at 6 hours and 3 log reductions at 24 hours for bacteria. In case of fungi, Benzalkonium chloride (0.0%v/v) showed zero log reduction at 7^{th} day whereas criteria states that reduction must be of 2 log at 7^{th} day.

2) Benzalkonium chloride (0.01%v/v) failed for 2 log reduction at 6 hours and 3 log reductions at 24 hours for bacteria. In case of fungi, Benzalkonium chloride (0.01% v/v) showed only 1 log reduction at 7^{th} day whereas criteria states that reduction must be of 2 log at 7^{th} day.

3) Benzalkonium chloride 0.012 ml (0.012% v/v) showed 2 log reduction at 6 hours and 3 log reductions at 24 hours for bacteria. There are 5 log reductions at 28 days. In case of fungi, Benzalkonium chloride (0.012% v/v) showed only 1 log reduction at 7^{th} day whereas criteria states that reduction must be of 2 log at 7^{th} day.

4) Benzalkonium chloride (0.016% v/v) passed criteria for bacteria for 2 log and 3 log reductions at 6 hours and 24 hours respectively. There were 5 log reductions at 28^{th} day means there was no recovery of bacteria. For fungi Benzalkonium chloride (0.016% v/v) failed to reduce fungus count by 2 log at 7^{th} day. It showed only 1 log reduction. Hence Benzalkonium chloride (0.016% v/v) passes only for bacteria but not for fungi.

5) Benzalkonium chloride (0.02% v/v) showed 2 log reductions at 6 hours and 5 log reductions at 24 hours and no recovery at 28^{th} day for bacteria. For fungi it showed log reduction as stated in criteria, 2 log reductions at 7^{th} day and no recovery at 28^{th} day.

6) Benzalkonium chloride (0.024% v/v) passed criteria for bacteria for 2 log and 5 log reductions at 6 hours and 24 hours respectively. There were 5 log reductions at 28^{th} day means there was no recovery of bacteria. For fungi Benzalkonium chloride (120% w/v strength) reduced fungus count by 2 log at 7^{th} day. It also showed 4 log reductions at 28^{th} day. Hence Benzalkonium chloride (0.024% v/v) passes both for bacteria but and for fungi.

➢ RESULTS OF STABILITY/COTAINER COMPATIBILITY STUDY

In stability/container compatibility study drug product was evaluated for assay of Timolol maleate and Benzalkonium chloride at initially, stress condition and at accelerated condition. Analysis was done by using HPLC. Other evaluated parameters are pH, Osmolality, Drop size and Water loss.

- The chromatograms for assay of Timolol maleate at each time point of the stability are as shown below.

1. Initial

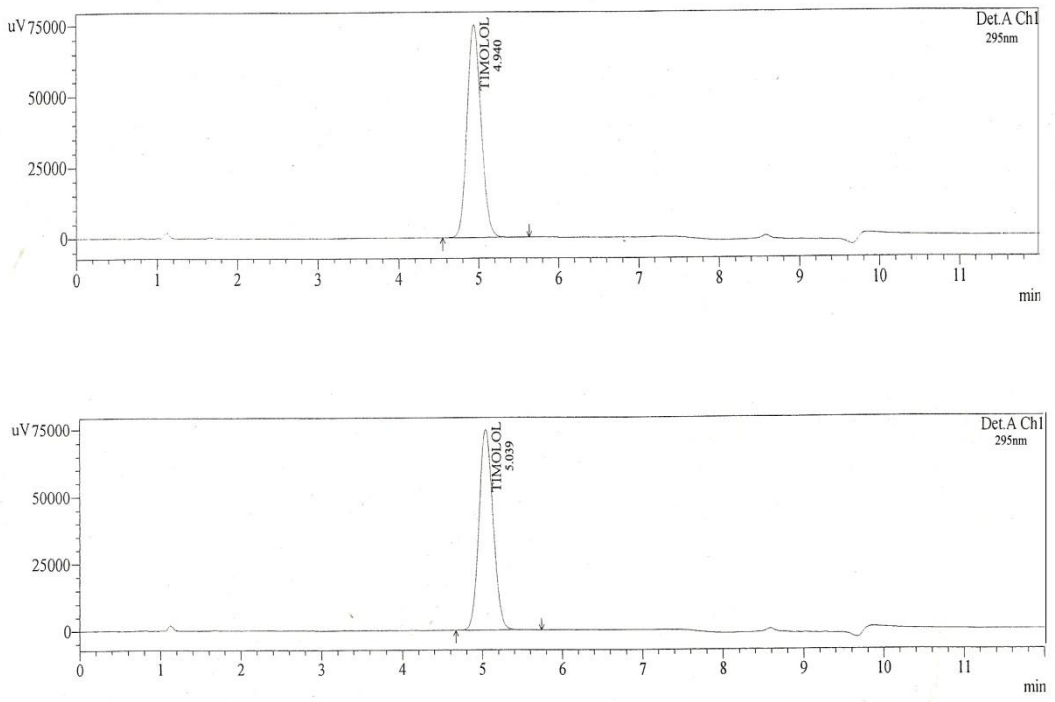
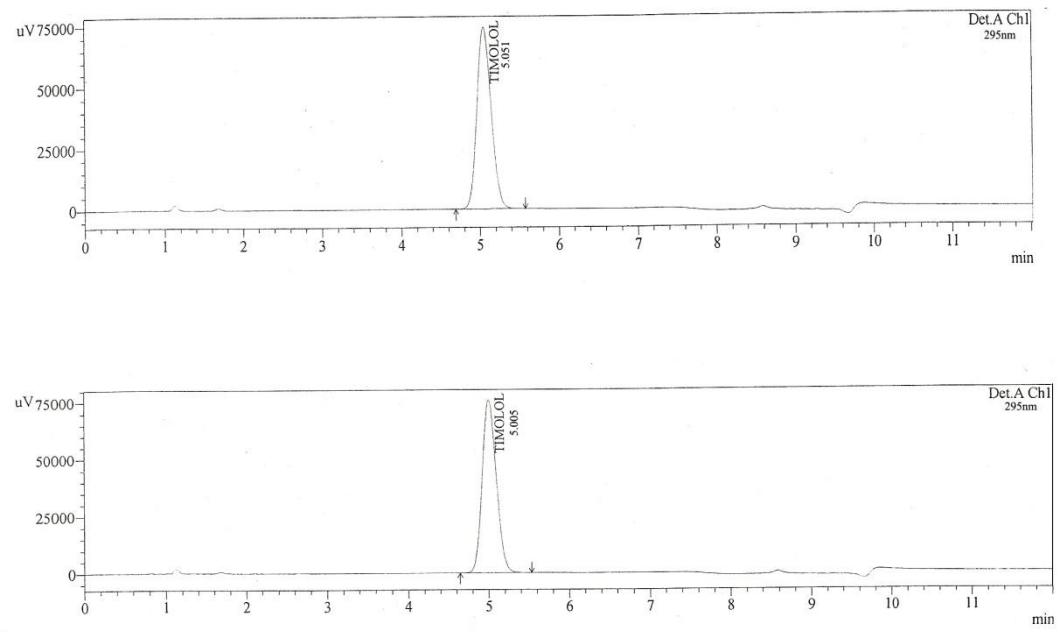


Fig 7: Chromatograms for Timolol maleate assay at Initial

2. Stress condition

2.1 Three piece container

1week



2 weeks

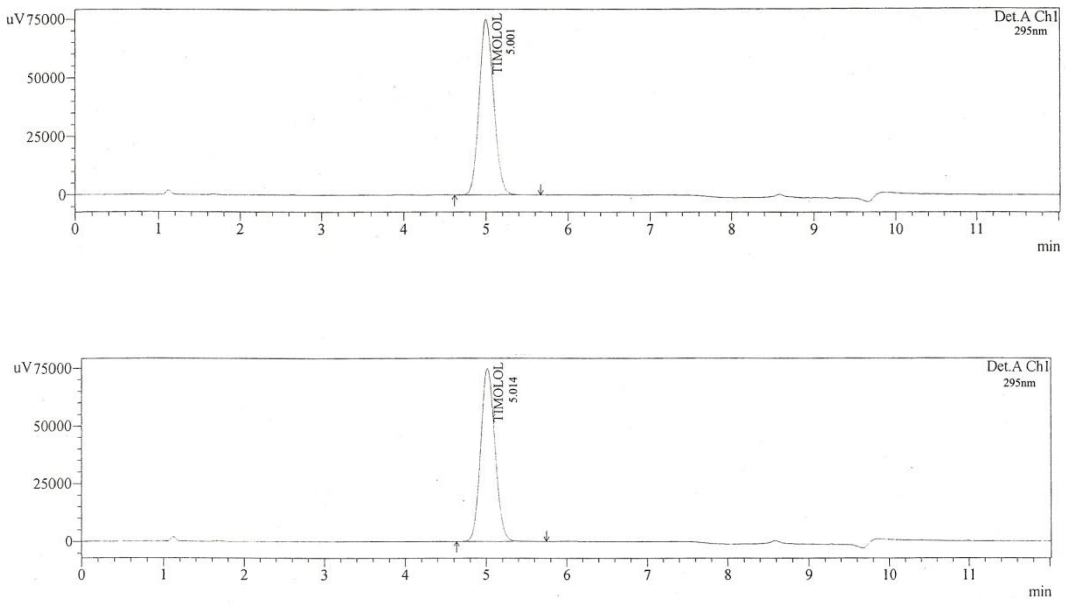
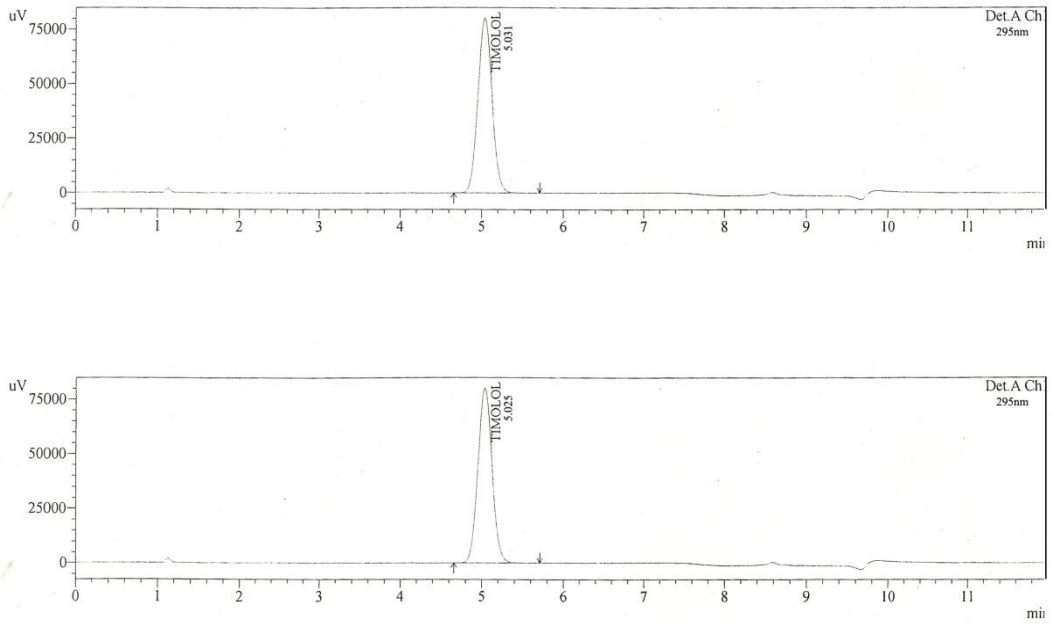


Fig 9: Chromatograms for Timolol maleate assay at Stress condition in Three piece containers.

2.2 BFS container

1 week



2 weeks

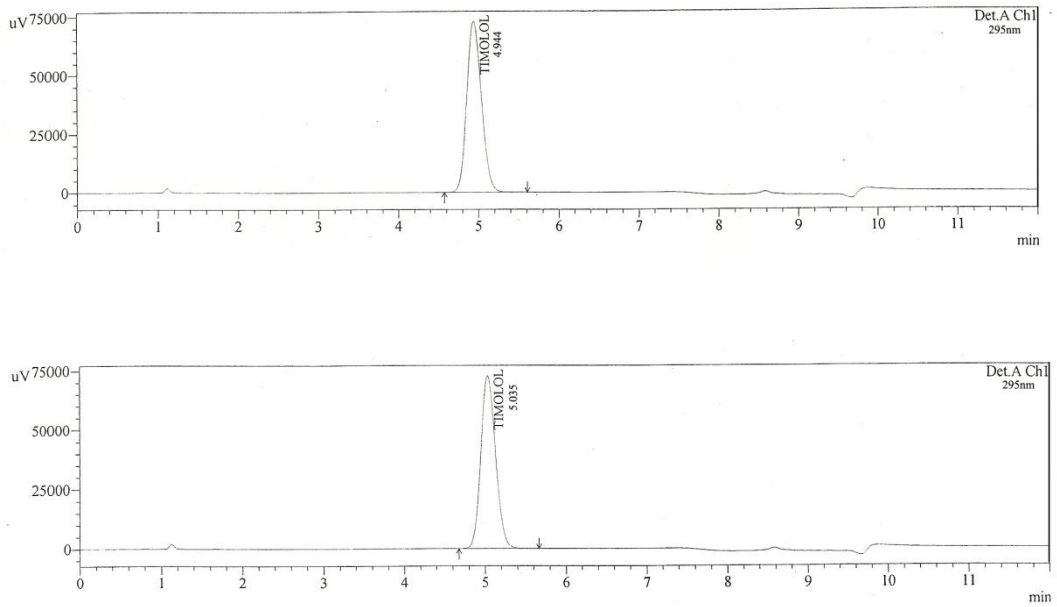
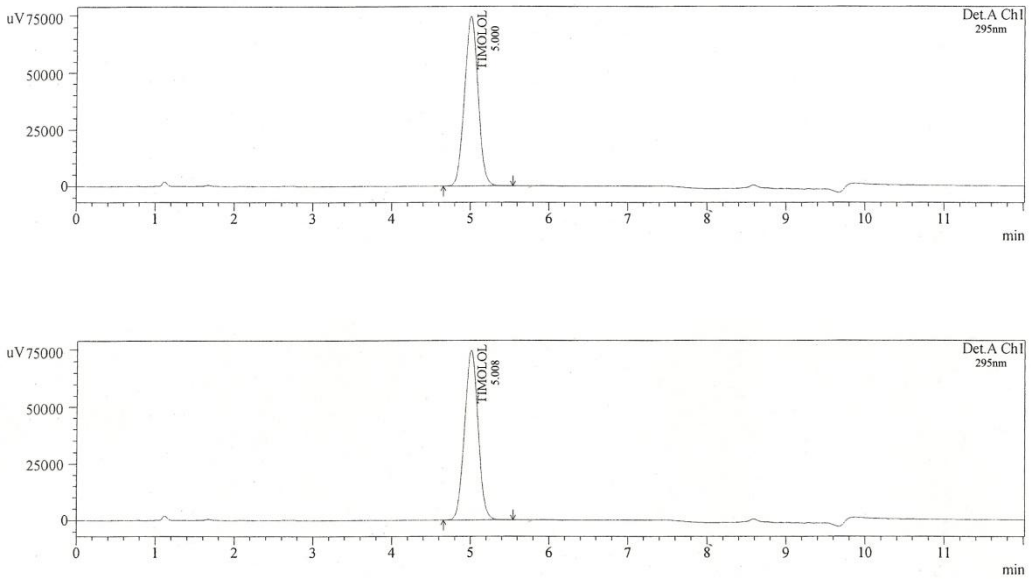


Fig 10: Chromatograms for Timolol maleate assay at Stress condition in BFS containers.

2.3 Amber Colored Glass Containers

1 week



2 weeks

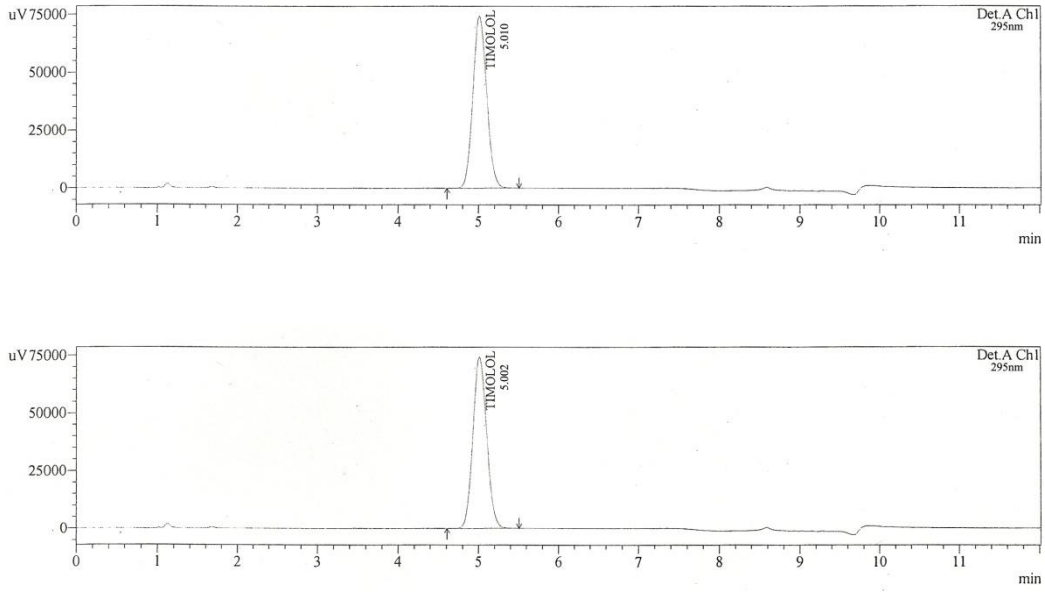
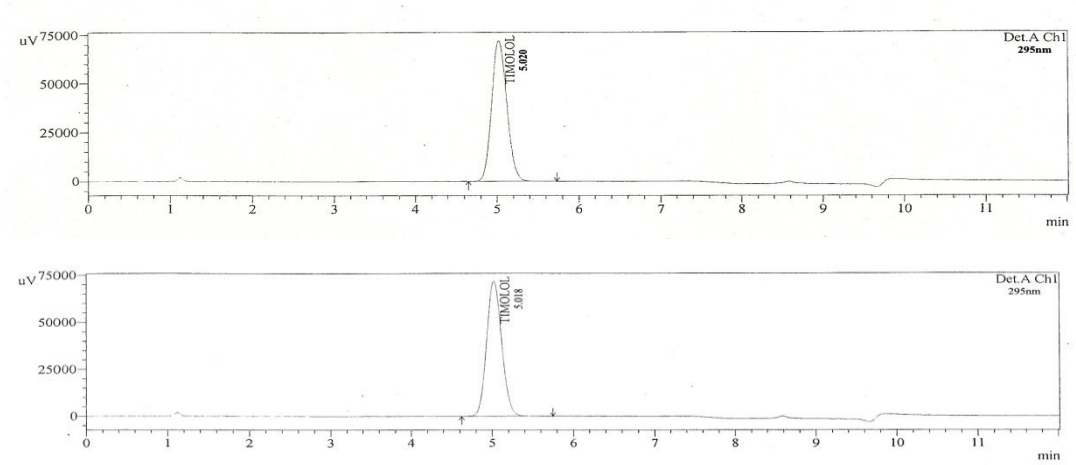


Fig 11: Chromatograms for Timolol maleate assay at Stress condition in Glass containers.

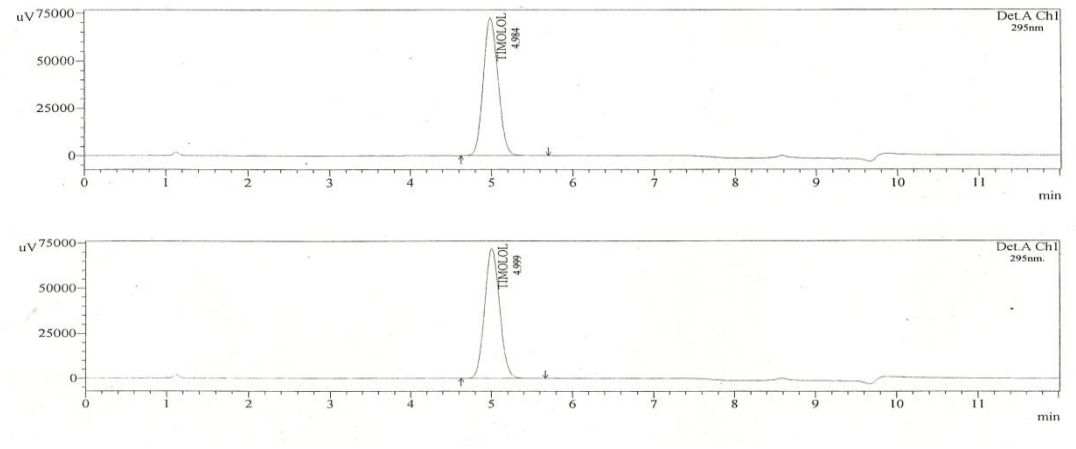
3. Accelerated Condition

3.1 Three Piece Container

1 month



2months



3 months

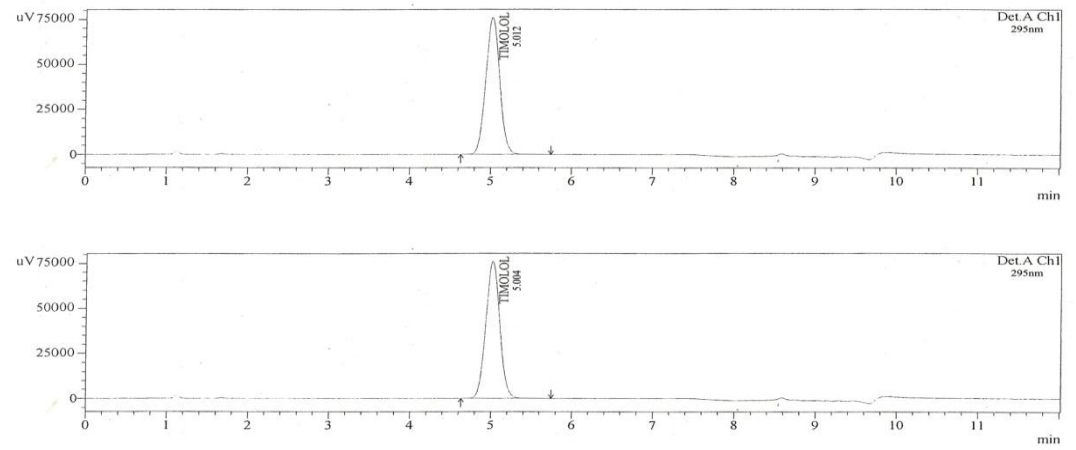
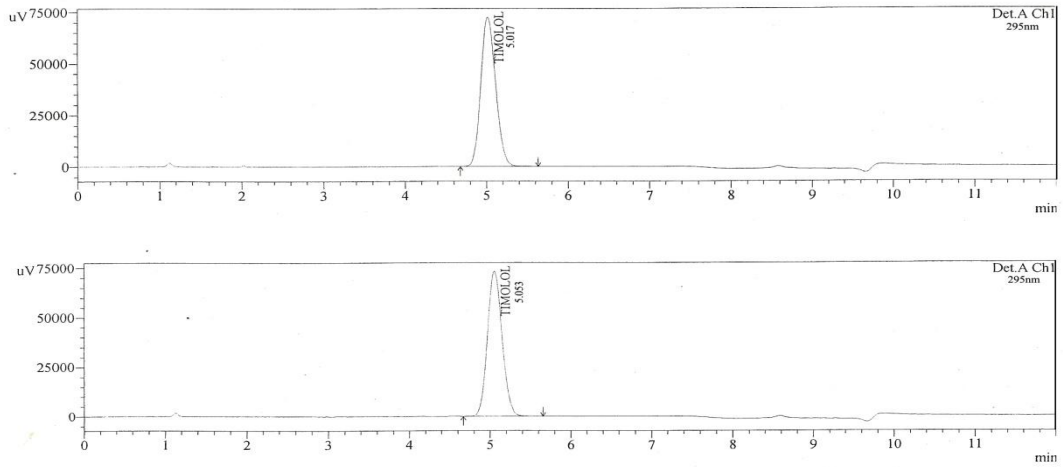


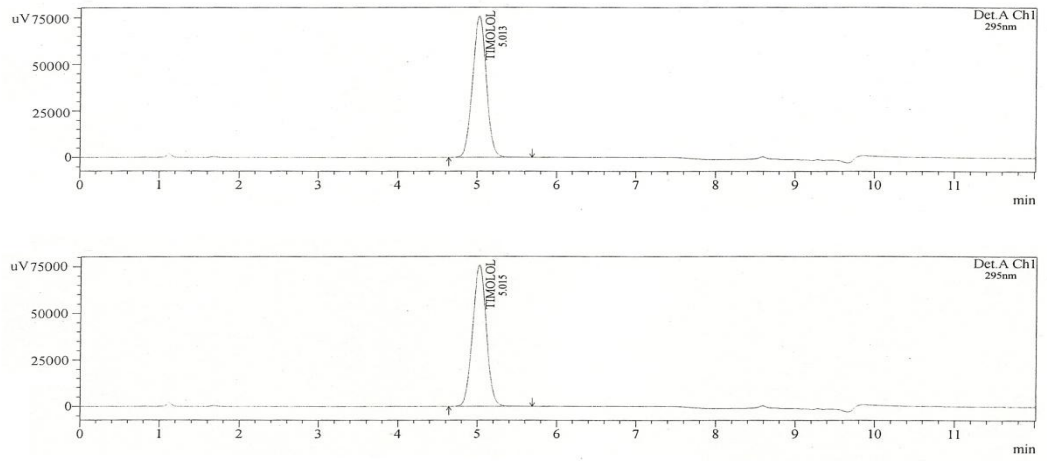
Fig 12: Chromatograms for Timolol maleate assay at accelerated condition in Three piece containers.

3.2 BFS container

1 month



2 months



3 months

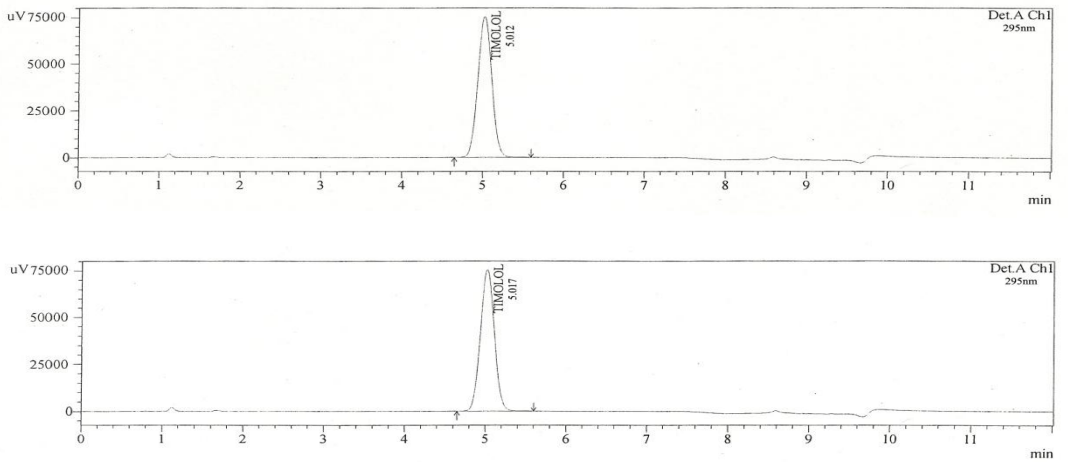
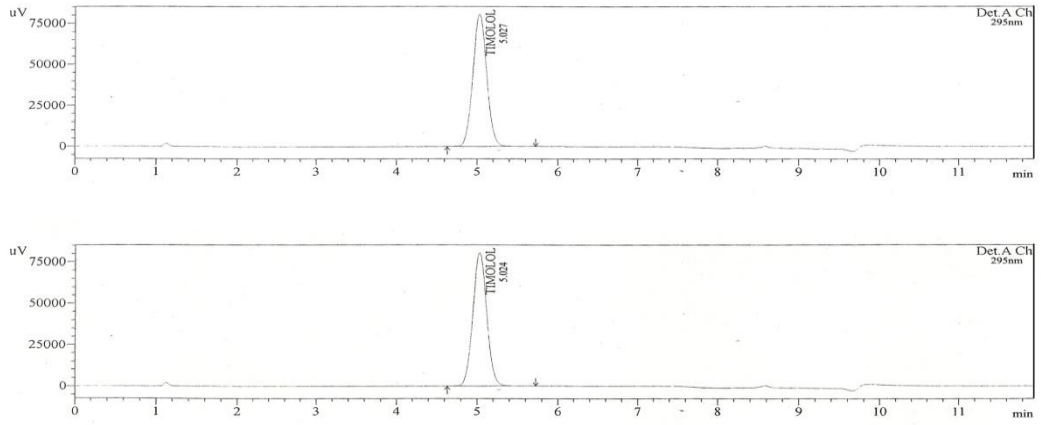


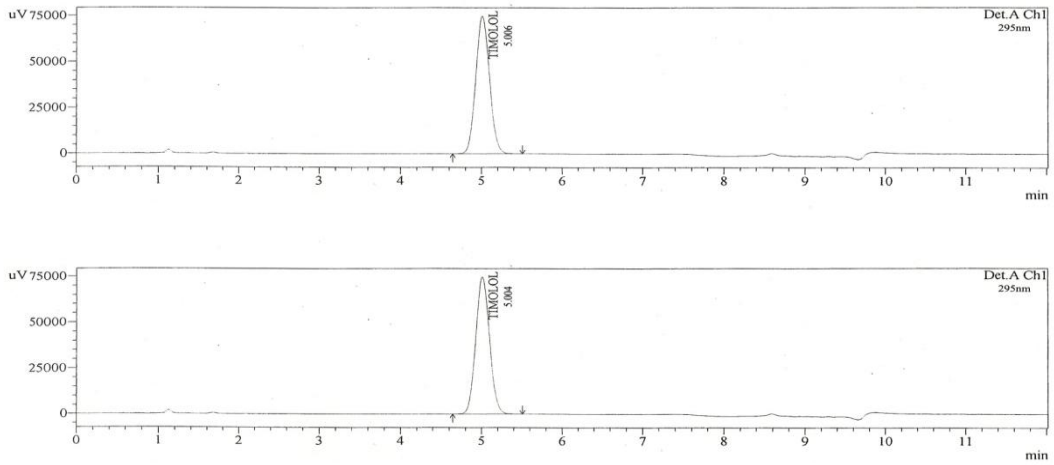
Fig 13: Chromatograms for Timolol maleate assay at accelerated condition in BFS containers.

3.3 Amber Colored Glass Containers

1 month



2 months



3 months

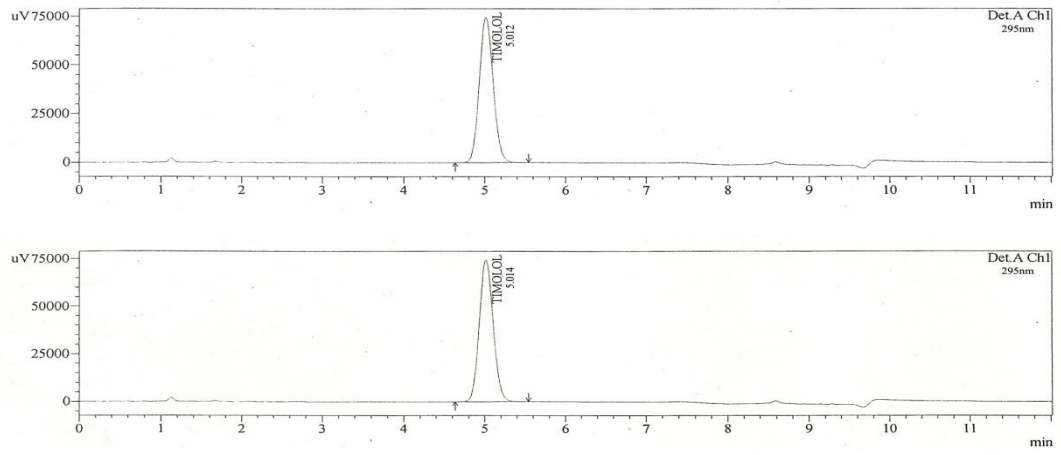
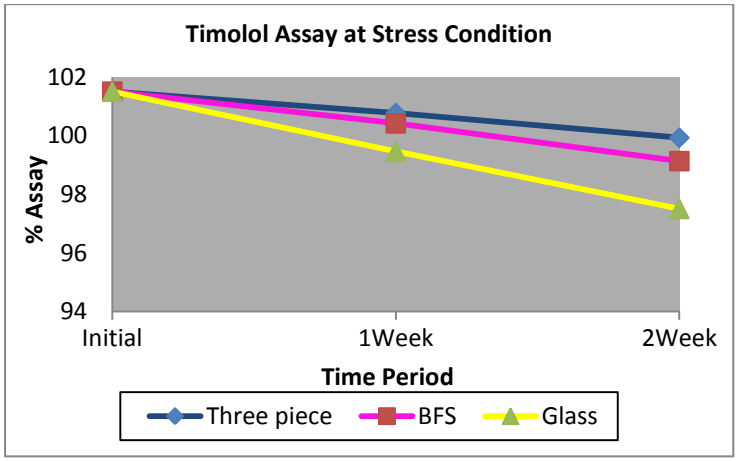


Fig 14: Chromatograms for Timolol maleate assay at accelerated condition in Glass containers.

Results for Timolol maleate assay are depicted in the Table 24

Table 24: Assay of Timolol maleate

S. No.	Containers	Specification	Initial	Stress condition		Accelerated condition		
				1Week	2Weeks	1Month	2Months	3Months
1	Three piece	90- 110	101.5	100.77	99.93	100.95	100.14	99.94
2	BFS	90- 110	101.5	100.42	99.13	100.3	99.52	97.11
3	Glass	90- 110	101.5	99.46	97.5	99.67	98.50	96.61



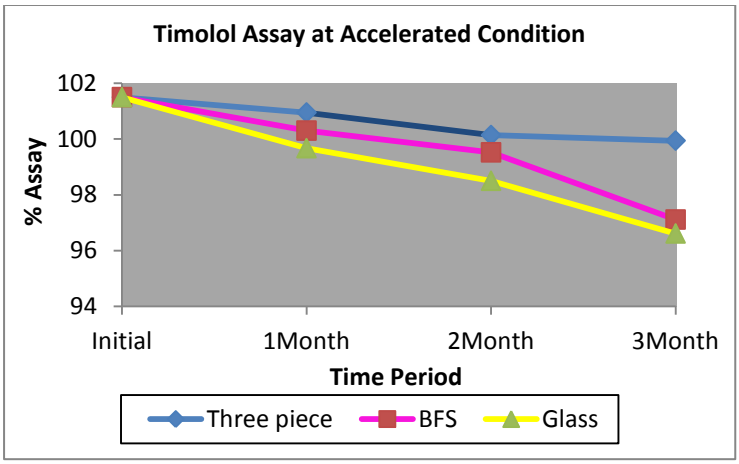


Fig 15: Graphical representation of Assay of Timolol maleate

Assay of Timolol maleate was evaluated at initial, at stress condition and at accelerated condition. Initially assay of Timolol maleate was found to be 101.5 %. At stress condition of temperature (60^0C) up to two week assay decreased to 99.93%

in Three piece container; to 99.13% in BFS container; to 97.50% in amber colored glass container. At accelerated condition of temperature and relative humidity (40°C± 2°C/ NMT 25 % RH for plastic and 40°C± 2°C/ NMT 75 % RH for glass container) assay was estimated up to three months. Assay decreased to 99.94% in Three piece container, to 97.11 in BFS container and to 96.11% in Glass container up to the three month.

Significant loss in assay was found in glass and BFS (3020 D) container as compared to Three piece container (PE 1840 H). Loss of Timolol maleate may be due to chemical interaction of cross linking present in the MOC of container with the components of drug product. Possibility is that the attachment of carbon atoms from Timolol maleate to long polymeric chain of carbon present in MOC. Another possible reason is that entrapment of Timolol maleate molecule in to the complex entanglement of polymer chain. In case of glass containers, interaction of rubber closure with product may responsible for the loss of drug by adsorption and/or chemical reaction.

- The chromatograms for assay of Benzalkonium chloride are as shown below.

1. Initial

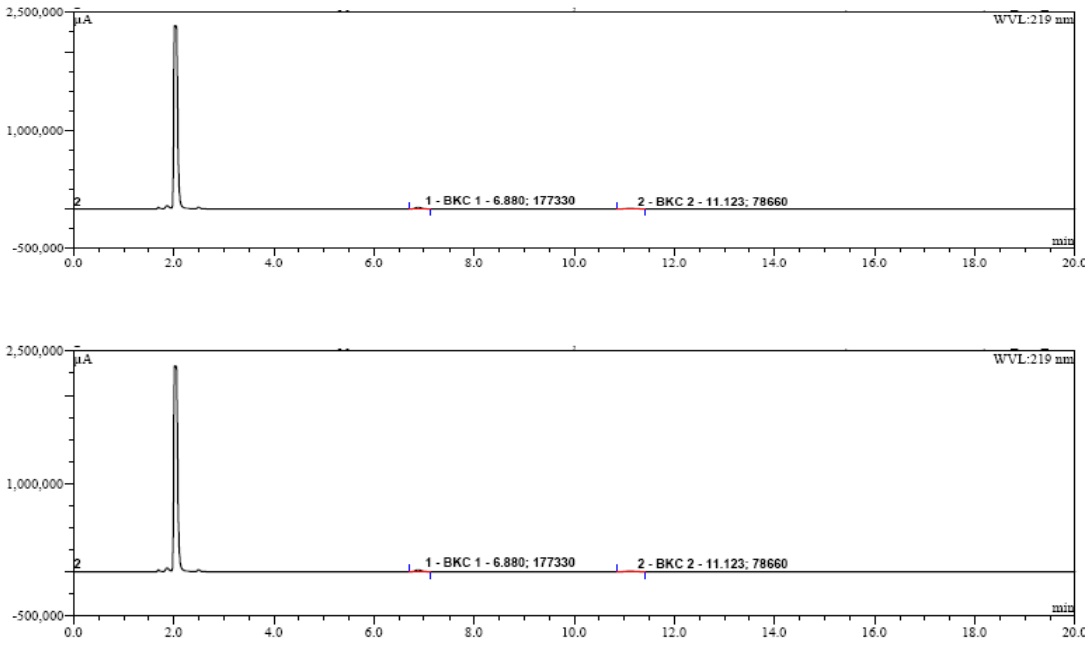
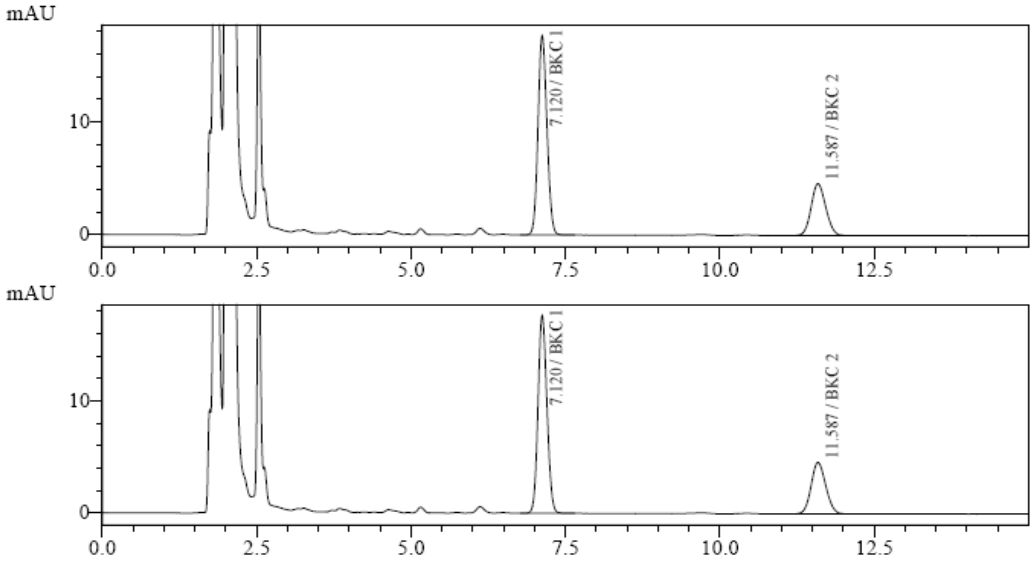


Fig 16: Chromatograms for Benzalkonium chloride assay at Initial

2. Stress condition

2.1 Three piece container

1 week



2weeks

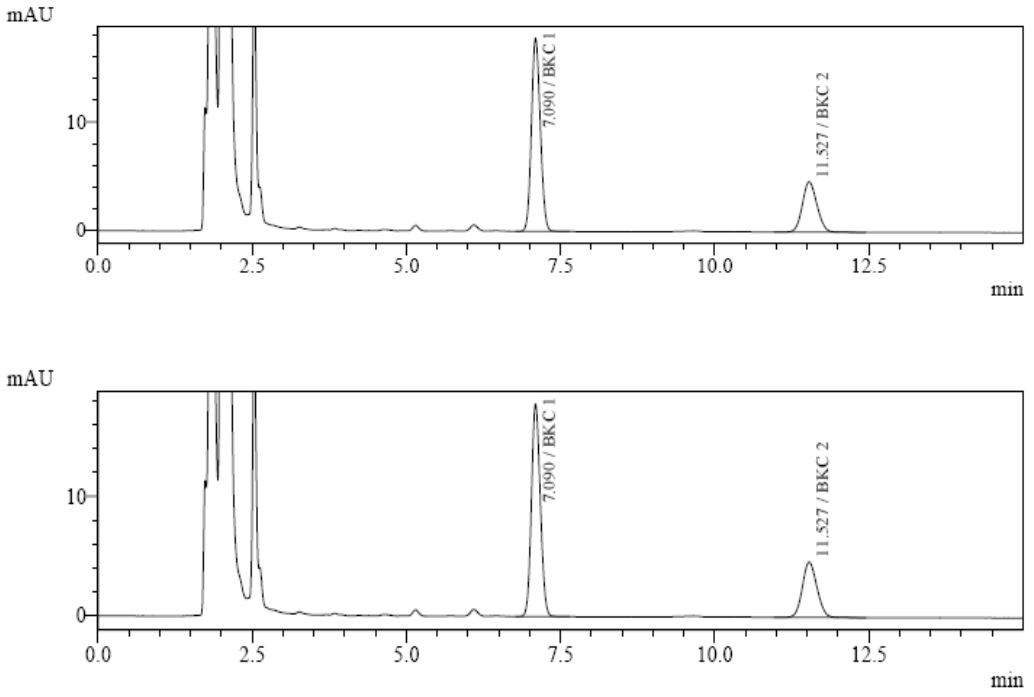
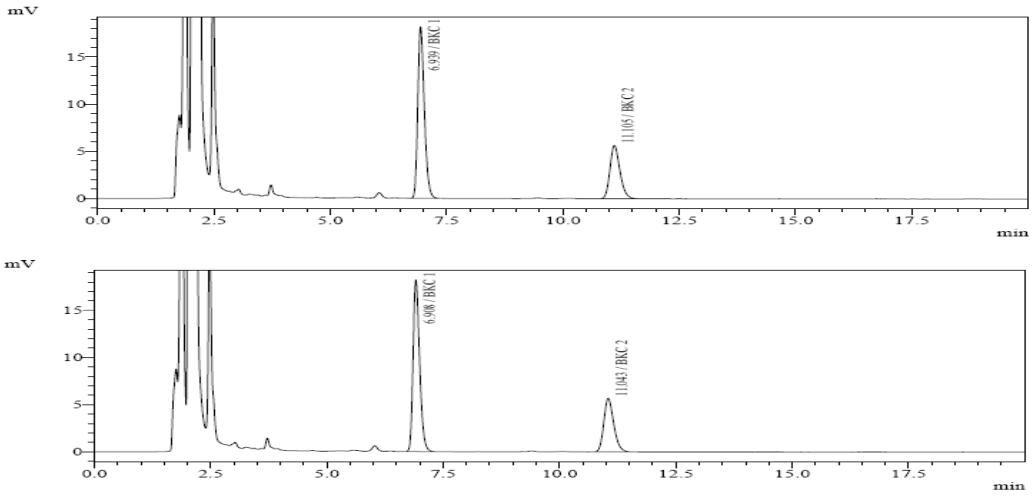


Fig 17: Chromatograms for Benzalkonium chloride assay at Stress condition in Three piece containers.

2.2 BFS container

1 week



2weeks

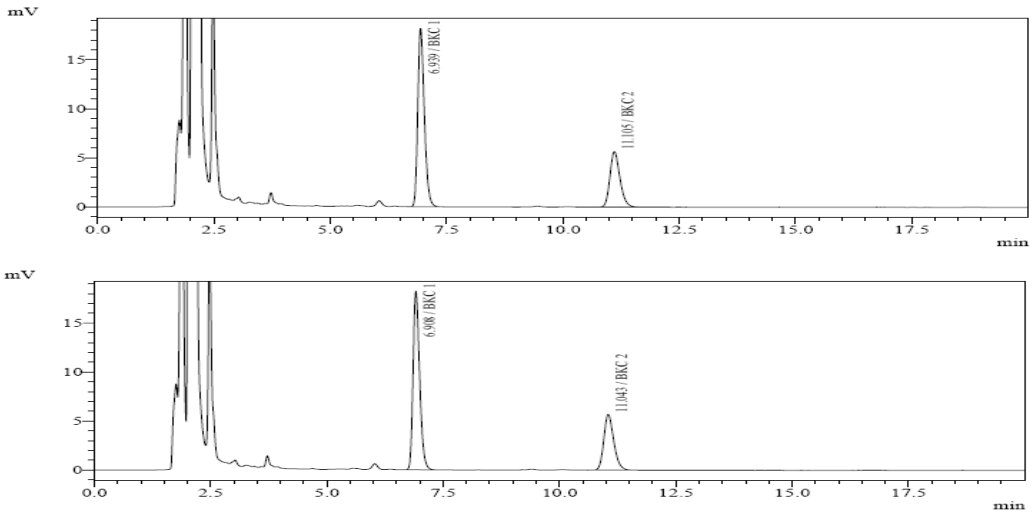
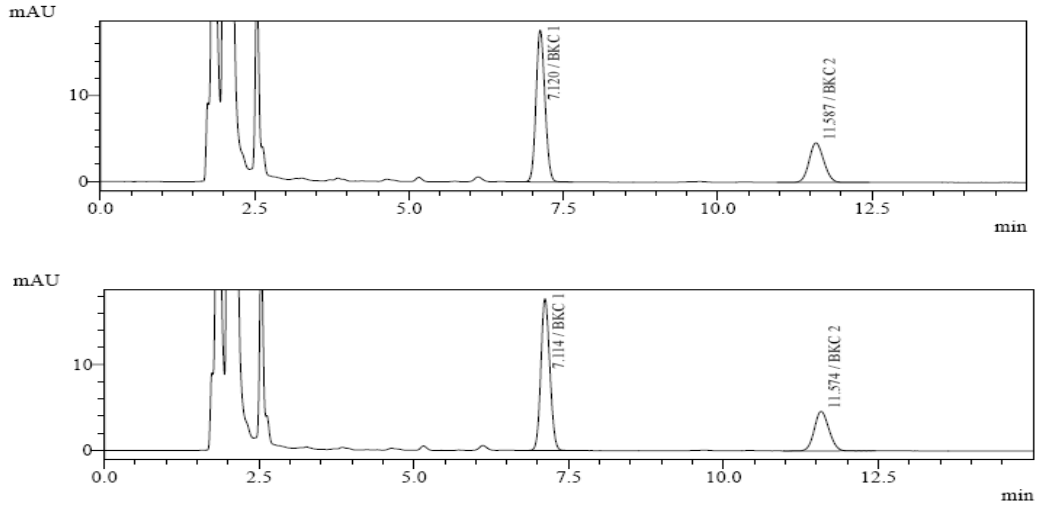


Fig 18: Chromatograms for Benzalkonium chloride assay at Stress condition in BFS containers.

2.3 Glass container

1week



2 weeks

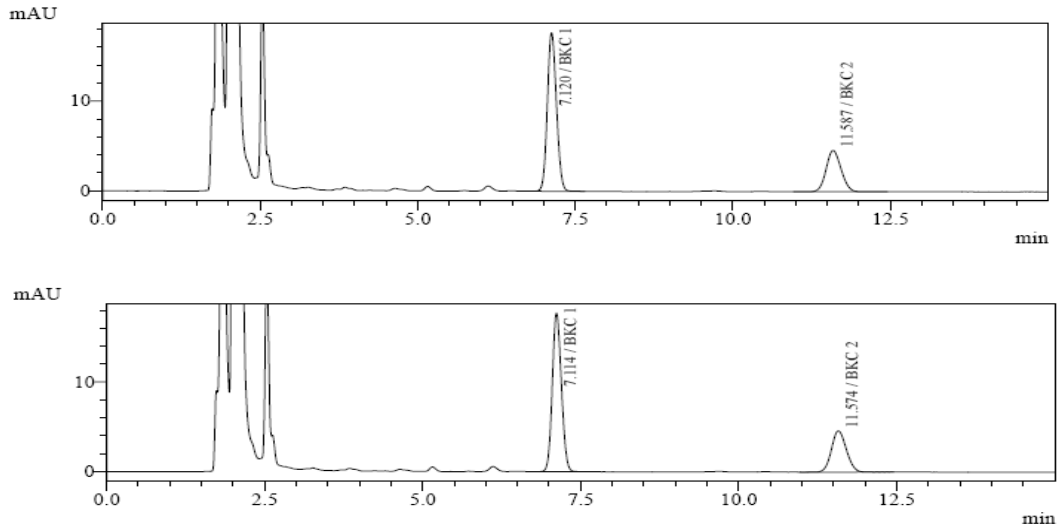
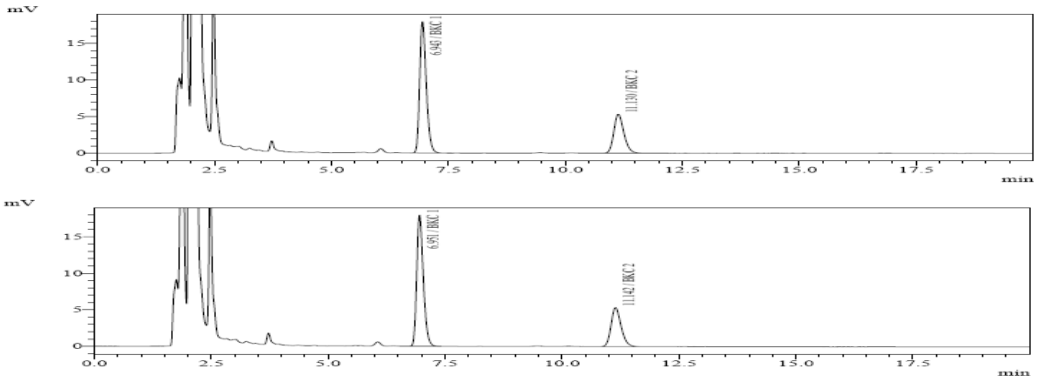


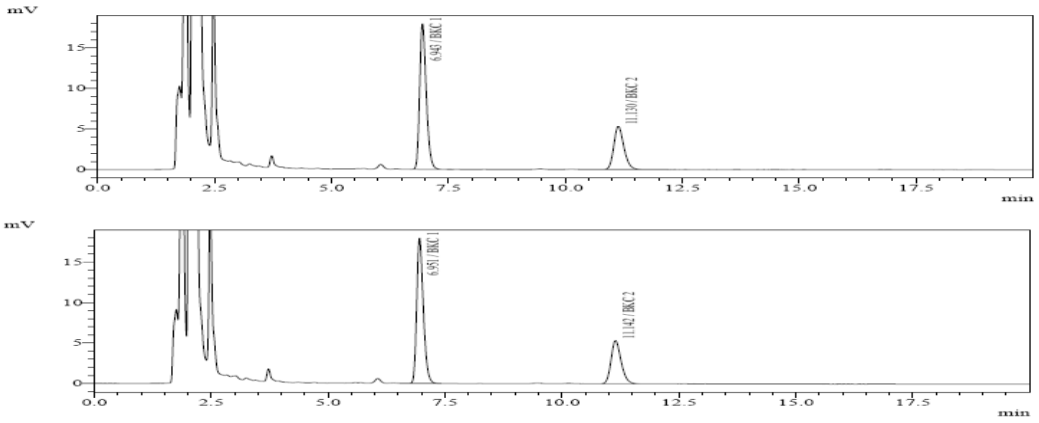
Fig 19: Chromatograms for Benzalkonium chloride assay at Stress condition in Glass containers.

3. Accelerated condition

3.1 Three piece container 1month



2months



3months

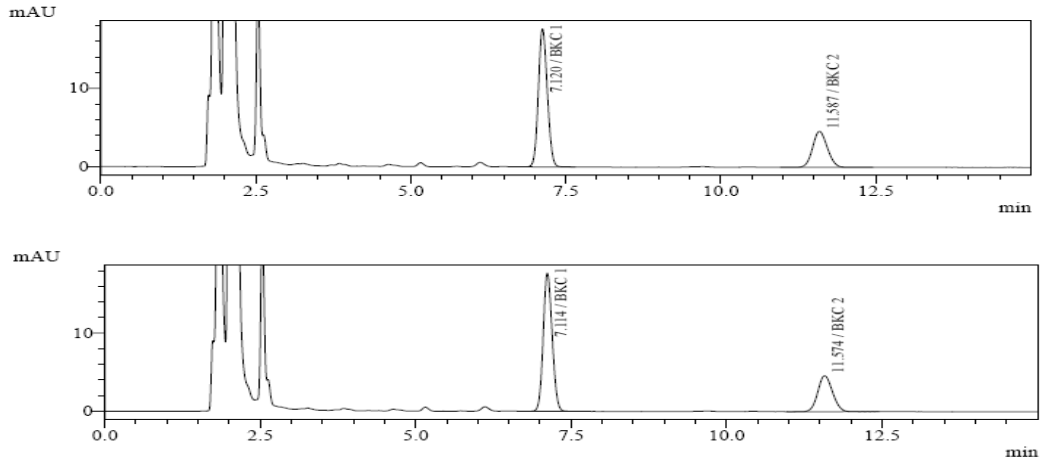
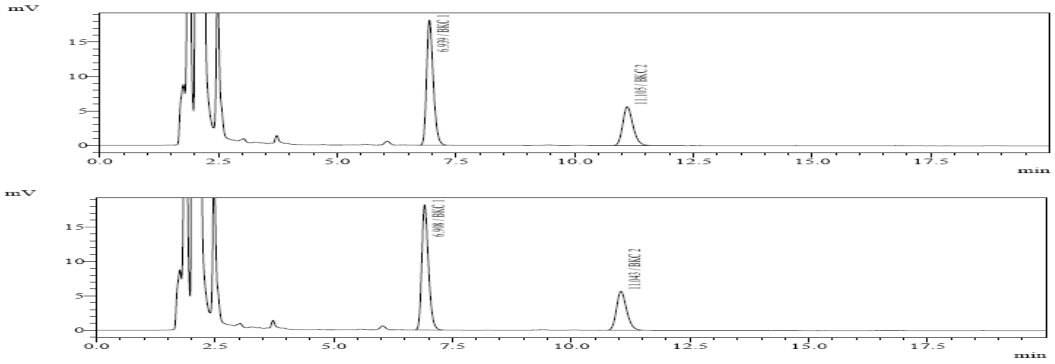


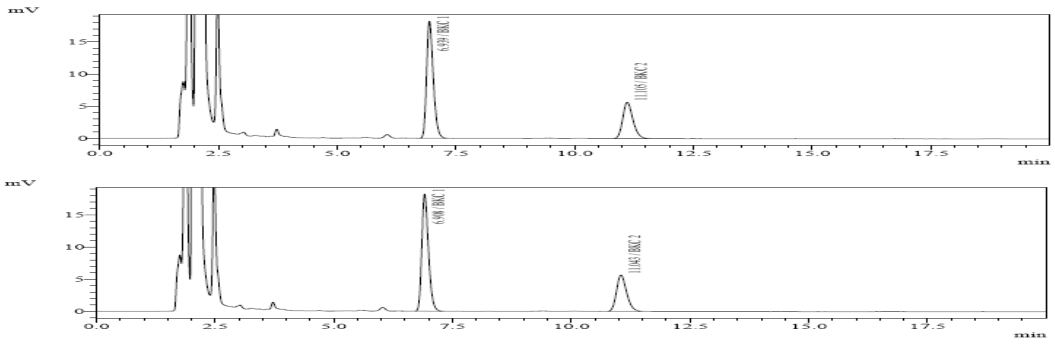
Fig 20: Chromatograms for Benzalkonium chloride assay at accelerated condition in Three piece containers.

3.2 BFS containers

1month



2months



3months

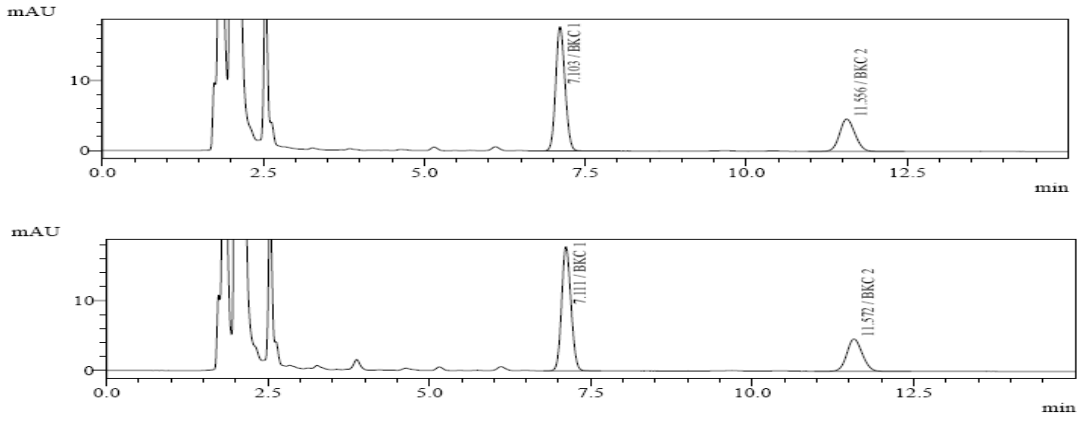
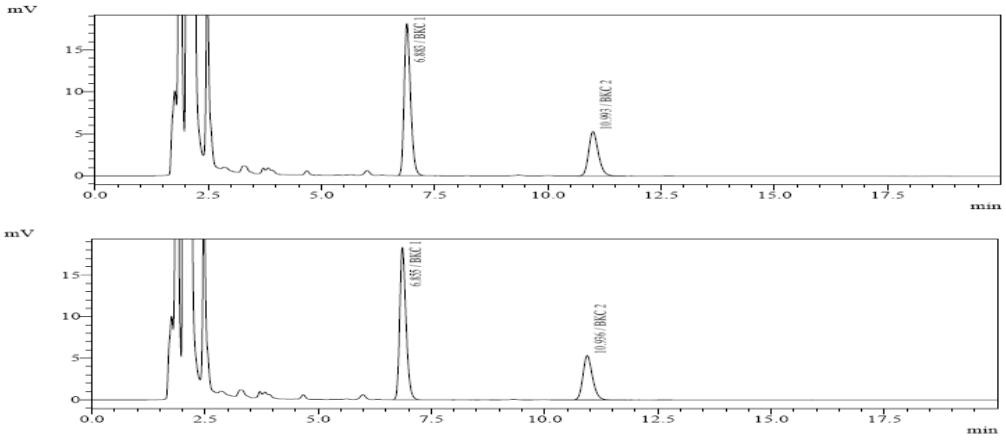


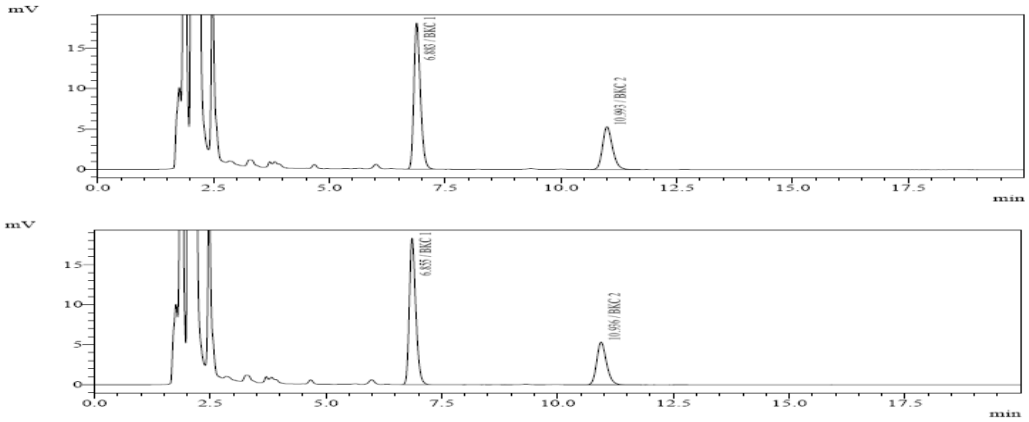
Fig 21: Chromatograms for Benzalkonium chloride assay at accelerated condition in BFS containers.

3.3 Glass containers

1 month



2 months



3 months

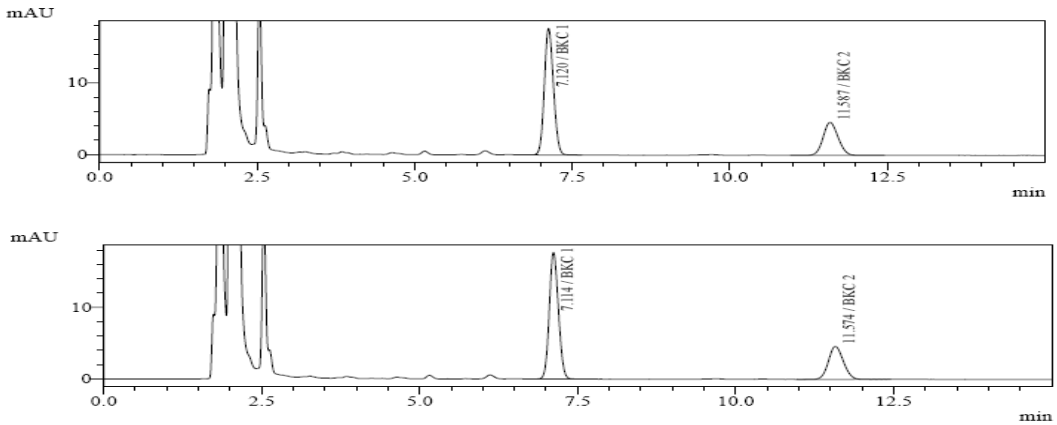


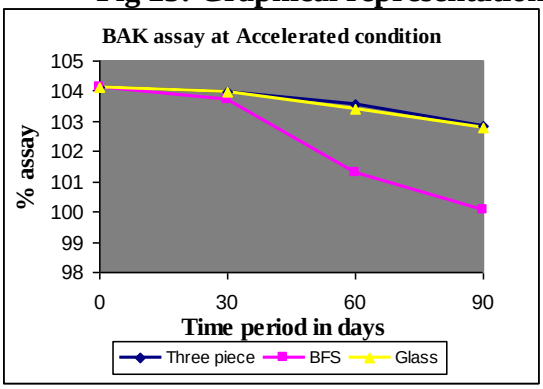
Fig 22: Chromatograms for Benzalkonium chloride assay at accelerated condition in Glass containers.

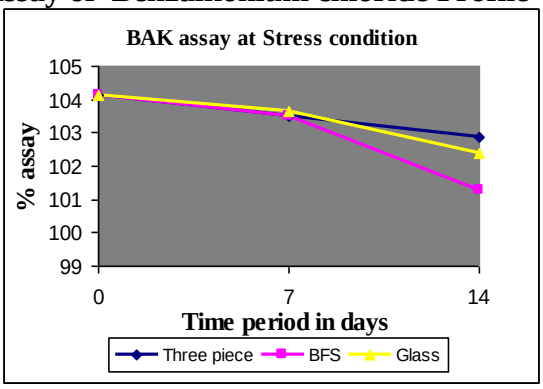
Results for Benzalkonium chloride assay are depicted in Table 25.

Table 25: Assay of Benzalkonium chloride

Sr. No.	Containers	Specification	Initial	Stress condition		Accelerated condition		
				1Week	2Weeks	1Month	2Months	3Months
1	Three piece	80-120	104.15	103.52	102.89	103.96	103.57	102.84
2	BFS	80-120	104.15	103.48	101.27	103.70	101.30	100.05
3	Glass	80-120	104.15	103.63	102.4	103.95	103.38	102.77

Fig 23: Graphical representation of assay of Benzalkonium chloride Profile





Similar to assay of Timolol maleate, assay of preservative Benzalkonium chloride was evaluated at initial, at stress condition and at accelerated condition. Initially assay of Benzalkonium chloride was found to be 104.15%. At stress condition of temperature (60^0C) up to two week assay decreased to 102.89 % in Three piece container; to 101.27% in BFS container; to 102.24 in amber colored glass container. At accelerated condition of temperature and relative humidity ($40^{\circ}C\pm 2^{\circ}C$/ NMT 25 % RH for plastic and $40^{\circ}C\pm 2^{\circ}C$/ 75 ± 5% RH for glass container) assay was estimated up to three months.

Significant loss in assay was found in glass and BFS container (100.05) as compared to Three piece container. Benzalkonium chloride has the tendency for adsorption on to the surface of plastic. Loss of preservative may be due to chemical adsorption of C^{12} – C^{18} chain of macro molecule of Benzalkonium chloride. Hydrophobic or anionic surface of container polymer exhibits significant adsorption of cationic molecule of preservative. Amount adsorption makes the difference in assay of preservative. Preservative may also be lost to inhibit microbial growth. In case of glass containers interaction of rubber closure with product may responsible for the loss of drug by adsorption and/or chemical reaction.

- **pH:**

  pH of the solution was determined by using Cyber scan®510PC Eutech pH meter. Initial pH was found to be 6.82. After charging to stability /container compatibility study pH was determined for each type of container at every station of the stability. The results are tabulated as follows.

Table 26: pH observations

S. No.	Containers	Specification	Initial	Stress condition		Accelerated condition		
				1Week	2Weeks	1Month	2Months	3Months
1	Three piece	6.8 – 7.0	6.82	6.83	6.80	6.80	6.38	6.35
2	BFS	6.8 – 7.0	6.82	6.80	6.76	6.73	6.35	6.28
3	Glass	6.8 – 7.0	6.82	6.82	6.80	6.76	6.38	6.30

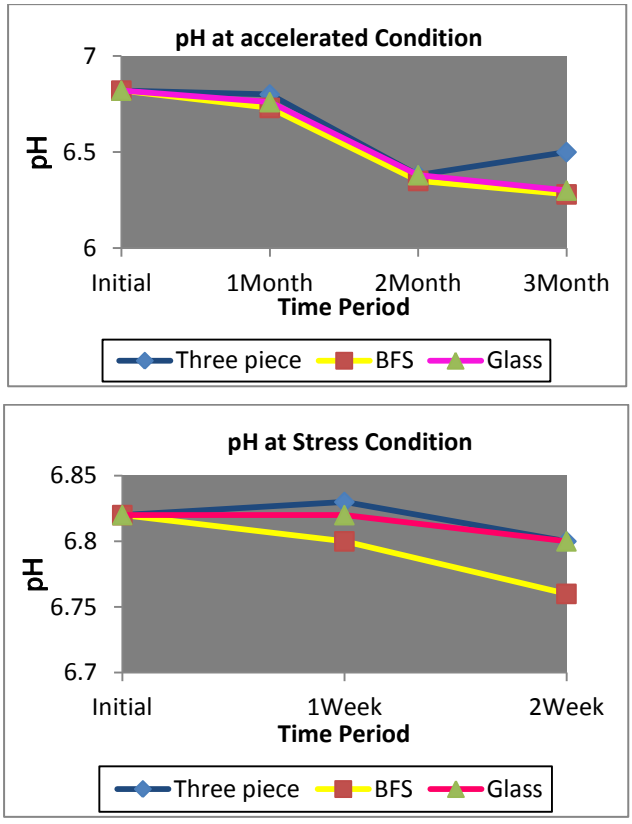


Fig 24: Graphical representation of pH profile

From the results it is found that, in accelerated condition up to the three month solution pH in Three piece container was decreased from 6.82 to 6.35. Solution pH in BFS container decreased to 6.28 and in glass container decreased to 6.30. At stress condition nearly same fall in pH is observed. Migration of some excipients

such as antioxidants, stabilizers, catalysts, plasticizers, lubricants, solvents and/or dyes from container to the drug product may hamper the pH of the solution. Though pH of solution in all containers was in specification range but it seems more stable in Three piece containers as compared to others. Decrease in the pH may cause the precipitation of drug, irritation of eye surface when applied. Ultimately it will affect shelf life of the product.

- **Osmolality :**

Osmolality is the function of the number of particles present in the solution. Any deviation in osmolality will reflect in the breakdown of drug molecule or any other excipients. It may be the reflection of leachables that may be added to solution from the wall of container. The results for the osmolality are tabulated as follows.

Table 27: Osmolality observations

S. No.	Container	Specification	Initial	Stress condition		Accelerated condition		
				1Week	2Weeks	1Month	2Months	3Months
1	Three piece	274 mOsm/kg	292	304	316	297	299	310
2	BFS	274 mOsm/kg	292	307	320	316	334	339
3	Glass	274 mOsm/kg	292	302	311	317	319	324

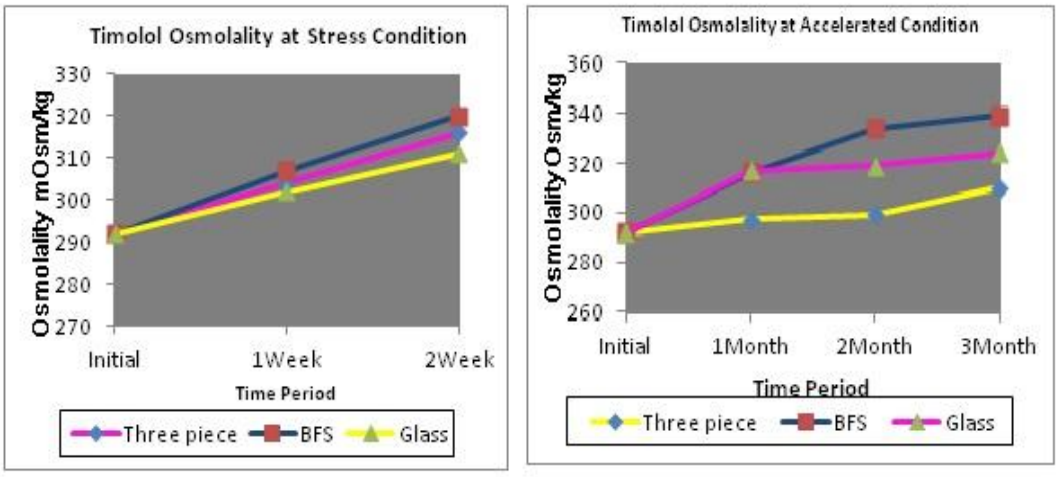


Fig 25: Graphical representation of Osmolality profile

From the above results and graphical representation it is observed that there was increase in the osmolality of solution in each type of container. Results show that at accelerated condition osmolality of solution in Three piece container was increased from 292 mOsm (initial) to 310 mOsm at 3 month. In BFS containers it was

increased to 339 mOsm and in glass container it was increased to 324 mOsm. At stress condition Osmolality in BFS container is more than the Osmolality at accelerated condition. This indicates that BFS container may not withstand higher temperature.

In BFS and glass containers there may be more decomposition of the formulation components or leaching from the containers. Timolol maleate ophthalmic solution formulation contains Sodium hydroxide. This Sodium hydroxide may attack on cross linkage of polymer. This alkylic reaction causes the breakdown of the component of polymer which migrates into the solution. This phenomenon may be the reason behind the increase in the Osmolality.

- **Drop size :**

The drop size was estimated on average weight basis of drops of ophthalmic solution by using the CPA224S, Sartorius balance. The results obtained are tabulated as follows.

Table 28: Drop size of Three Piece and BFS container observations

Sr. No.	Containers	Initial	Accelerated condition		
			1Month	2Months	3Months
1	Three piece	50	49	45	48
2	BFS	50	48	52	53

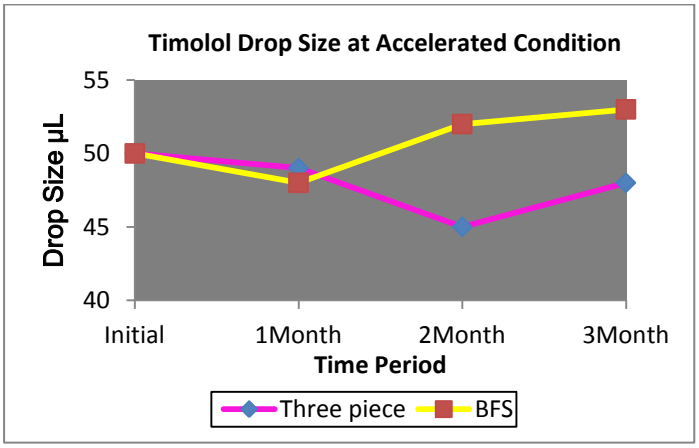


Fig 26: Graphical representation of Drop size

From the above results it has been found that up to third month in accelerated condition drop size of BFS containers (53 µl) was comparatively more increased than Three piece container(48 µl). Increase in drop size may be caused due to widening of the nozzle aperture or thinning of the solution. In case of BFS container whole structure is intact made of same composition of polymer. Polymer used in

BFS container was less rigid as compared to three piece container. In case of three piece container nozzles are made up of same polymer used for body but contains additional ingredients which increases the rigidity of nozzle. Thus at accelerated condition of stability softening and widening of nozzle aperture easily takes place in case of BFS container. Hence there was an increase in the drop size of BFS container.

It has been suggested that a decrease in drop size, would reduce the amount of overflow, the rate of drug loss through the drainage, the incidence of systemic side effects and the cost of therapy.

- **Water Loss Study:**

Water loss study was performed for LDPE containers as they are semi - permeable in nature. Percentage water loss from semi permeable containers is the function of loss of aqueous phase of formulation under the condition of temperature and humidity i.e. 40^0C/25% RH. 5 containers of each type i.e. Three piece and BFS were placed in upright position for water loss. The results obtained are as follows.

Table 29: Water Loss Study- Three Piece Containers

Container No.	Initial weight of the container(g)	One Month	% Loss	Two Months	% loss	Three months	% Loss
1	10.5390	10.4796	0.5636	10.4707	0.6481	10.4562	0.7857
2	10.5321	10.4700	0.5896	10.4613	0.6722	10.4492	0.7871
3	10.4951	10.4521	0.4097	10.4433	0.4936	10.4308	0.6127
4	10.6479	10.5686	0.7447	10.5580	0.8443	10.5428	0.9870
5	10.4737	10.4016	0.6883	10.3929	0.7715	10.3808	0.8870
Average	10.5376	10.4744	0.5992	10.4652	0.6859	10.4520	0.8119

Table 30: Water Loss Study- BFS Containers

Container No.	Initial weight of the container(g)	One Month	% Loss	Two Months	% loss	Three months	% Loss
1	8.3646	8.2941	0.8428	8.2867	0.9313	8.2768	1.0497
2	7.7248	7.6633	0.7961	7.6547	0.9075	7.6431	1.0576
3	7.9372	7.8680	0.8718	7.8571	1.0092	7.8422	1.1969
4	8.0603	7.9944	0.8175	7.8922	2.0855	7.5732	6.0432
5	8.1179	8.0543	0.7834	8.0352	1.0187	8.0226	1.1739
Average	8.0410	7.9748	0.8223	7.9452	1.1904	7.8716	2.1043

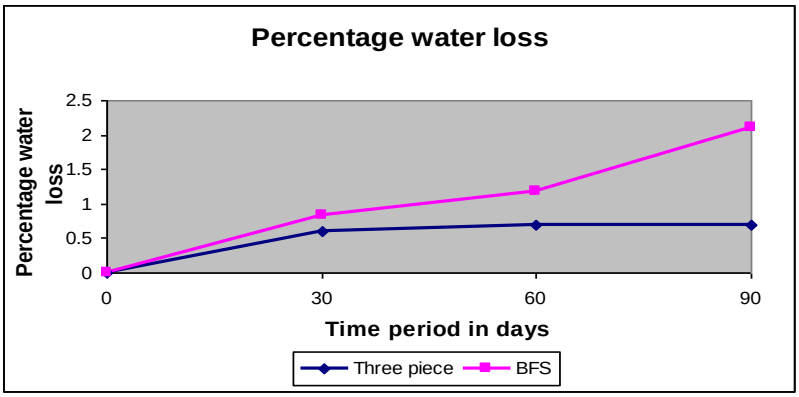


Fig 27: Graphical representation of Water loss from containers

From the above results it was found that for three piece containers average percentage water loss at one month, two month, and three month was 0.5992%, 0.6859%, 0.8119% respectively where as for BFS containers water loss was 0.8223%, 1.1904%, and 2.1043 % respectively.

Though the both type of containers passed the criteria of water loss i.e. not more than 5.0%. BFS containers are showing the more water loss up to 2.1043% and three piece containers are showing water loss just up to 0.8119%. Environmental stress cracking resistance (ESCR) number of Three piece container (PE 1840 H) is higher than ESCR number of BFS (PE 3020 D) container.

Hence there may be more cracking and increased permeability in case of BFS container as compared to the Three piece container.

Hence it can be concluded that the MOC of BFS container is more semi permeable as compared to the Three piece container's MOC. Water loss from the semi- permeable containers may hamper the drug content and preservative content.

8. SUMMARY AND CONCLUSION

I. SUMMARY

Ophthalmic preparations are specialized dosage forms designed to be instilled onto the external surface of eye (topical), administered inside (intraocular), adjacent to the eye (periocular) or used in conjunction with any special device.

Ophthalmic preparations are similar to parenteral dosage form in their requirements for sterility as well as consideration for osmotic pressure (tonicity), preservation, and tissue compatibility, avoidance of pyrogens and particulate matter and suitable packaging. Ophthalmic solutions are most often multidose product containing suitable preservative(s) to meet compendial Preservative Efficacy Test (USP, Ph Eu, JP) requirements.

There are several ophthalmic preparations, but ophthalmic solution was selected for study because solutions are most widely dosage form among the ophthalmics. Ophthalmic solution has several advantages like easy manufacturing, low cost, better dose uniformity, more ocular bioavailability, Improved ratio of local activity versus systemic effects, not induce a foreign-body sensation, long-lasting blurring, or a very bad aftertaste, sterilizable at industrial scale by a recognized process, compatible with an efficient antimicrobial preservative, or packaging.

Drug selected for the study, Timolol is a first-generation Beta blockers have effective action by the reduction of intra ocular pressure in Chronic open angle glaucoma, as well as in the treatment of Hypertension. Compared with other β-blockers, this drug has broader clinical applications in the treatment of Glaucoma.

Important factors to be considered in formulating an ophthalmic solution includes Clarity, Sterility, Osmolarity, pH, buffering, preservative, Solubility, Stability in appropriate vehicle. Viscosity, Suitable packaging and storage of finished product.

Benzalkonium chloride, a popular preservative for pharmaceutical products, is a complex mixture since the alkyl portion of the molecule is derived from natural sources. The chain lengths are principally C12 to C16, however the antimicrobial activity increases with the proportion of longer chain lengths.

Unfortunately the tendency to adsorb plastics also increases with chain length i.e. the most effective constituents of the mixture may be preferentially adsorbed.

Method preferred for sterilization of the ophthalmic solution was filtration sterilization.

Containers commonly used for ophthalmic products include glass containers, and polyethylene containers. Glass containers and polyethylene containers are said to be superior in maintaining stability of ophthalmic preparations.

Amber glass containers are often used where the product is suspected of being a light sensitive. The amber color is imparted by addition of iron and manganese oxides.

Plastic dropper bottle have been favored because they weight loss, are more resistant to shock and other mechanical influences, cost less and offer more design possibilities. Polyethylenes, that is, low density polyethylene with or without additives.

BFS containers are manufactured by Blow-Fill-Seal (BFS) technology.

Three Piece Container name itself indicates system contains three components viz... body, nozzle, and cap. Out of which, body and nozzle are made up of various grades of polymers of low density polyethylene whereas caps are made up of high density polyethylene.

The aim of the present study was to formulate a formulation for Timolol maleate (0.5%) ophthalmic solution using different concentration of Benzalkonium chloride as preservative. While reducing the concentration of Benzalkonium chloride it must be keep in mind that added quantity of preservative must meet compendial requirement of *Preservative Efficacy Testing.*

The present research work was also planned to provide the data about the selection of suitable primary packaging material for Timolol maleate (0.5%) ophthalmic solution to achieve the better stability during the shelf life of the product. As there are several factors responsible for the incompatibility of packaging material with the product, most suitable packaging material must be selected.

The proposed formula was optimized by varying the concentration of Benzalkonium chloride. The quantities of Timolol maleate and other excipients were kept constant. As the aim of the present study was to optimize the concentration of BKC in formulation for Timolol maleate (0.5%) ophthalmic solution. Batches were planned by taking different concentrations viz.0.0 % v/v, 0.01%,0.012%, 0.016%,

and 0.02% ,0.024 % v/v of Benzalkonium chloride. For all six batches *Preservative Efficacy Testing* was carried out according to British Pharmacopoeia.

After performing Preservative efficacy testing, optimized batch was filled into three types of container and were subjected to accelerated conditions.

Accelerated condition for semi-permeable containers (40ºC ± 2ºC/ NMT 25%) and for glass containers (40ºC ± 2ºC/ 75% ± 5% RH) were chosen for storage for stability/compatibility study. Finished product was analyzed for the parameters Appearance, Assay of Timolol maleate and Benzalkonium chloride, pH, Osmolality, drop size, and water loss.

II. CONCLUSION

1. From the results of Preservative efficacy test, it was found that Benzalkonium chloride (0.02% v/v) and (0.024% v/v) showed 2 log reductions at 6 hours and 5 log reductions at 24 hours and no recovery at 28^{th} day for bacteria. For fungi it showed log reduction as stated in criteria, 2 log reductions at 7^{th} day and no recovery at 28^{th} day. Both these concentrations passed the criteria according to British Pharmacopoeia. As the aim of study was to minimize the concentration, it will be preferable to use 0.02% v/v concentration of BKC in Timolol maleate 0.5% ophthalmic solution.

2. In case of container compatibility study, results for Assay of Timolol maleate and Benzalkonium chloride, pH, Osmolality, drop size and water loss are within range of specifications for Three piece containers, BFS containers and Glass containers. But Three piece containers showed better results for compatibility with Timolol ophthalmic solution as compared to the BFS and Glass containers. Therefore Three piece containers (Low density polyethylene, *PE 1840 H*) are the best containers for Timolol maleate (0.5%) ophthalmic solution.

III. FUTURE SCOPE

Future scope for the present dissertation work is as follows

1. Eye irritation test
2. Formulation development with newer preservative system like ‘SOFZIA’ (Containing propylene glycol, sorbitol, boric acid and zinc oxide).
3. Formulation development with drug release retarding agent.
4. Use of Three Piece container using different grades of low density polyethylene polymer.
5. Introduction of calculated dose dispensing container.
6. Intermediate and long term stability study with Three Piece containers.

9. BIBLIOGRAPHY

1. Chowhan M,.Weiner AL,Bhagat H. Encyclopedia of pharmaceutical technology, Drug Delivery: Ophthalmic Route, 3rd ed; USA: Informa Healthcare; 2007. vol 2 p.1220-21
2. Macha S, Mitra AK, Hughes PM. Ophthalmic drug delivery system: Overview of Ocular Drug Delivery, 2nd ed; Newyork, USA: Marcel Dekker; 2003. p. 1-10.
3. Lang JC, Roches RE, Jani R. Ophthalmic preperations. In: Remington the pharmaceutical science and practice of pharmacy. 21st ed. Philadelphia: Lipincott Williams and Wilkins 2005 p.850
4. Gibson M. Pharmaceutical Preformulation and Formulation: Parenteral Dosage Forms, First Indian Reprint, Inerpharm/CRC, 2008 p. 331-54.
5. ASHP [Online] 15 June 2008, [Cited 2008 July 04]; Available from URL : http://pharmalabs.unc.edu/ophthalmics/text.htm
6. Ding S, Tien WL. United States Patent. No. 5474979; Date of patent Dec 12, 1995. Available from URL: www.freepatentsonline.com
7. March WE, Stewart RM, Mandell AI. et al. Arch Ophthalmol,1982,100 p.1270.
8. Shell JW, Baker RW. Arch Ophthalmol,1975;7 p.1037
9. Gaudana R, Jwala J, Boddu H.S, Mitra AK; Recent Perspectives in Ocular Drug Delivery; Pharmaceutical research(2008) DOI:10.1007/s11095-008-9694-0
10. Freemantle N, Cleland J, Young P, Mason J, Harrison J (June 1999). "beta Blockade after myocardial infarction: systematic review and meta regression analysis". *BMJ* 318 (7200): 1730–7. PMC 31101. PMID 10381708.
11. Shen, Howard (2008). Illustrated Pharmacology Memory Cards: PharMnemonics. Minireview. pp. 15. ISBN 1-59541-101-1.
12. OFKA. [Onine]. 2007 May 22 [cited 2008 August 13] Available from URL: www.aishikapharma.com/products.htm
13. Michel JH. Ophthalmic solution: The preparation and sterilization of ophthalmic solutions. California medicine; 71 (06); 414-6
14. Drug distribution and control: Preparation and handling - Guidelines; ASHP Guidelines on Pharmacy prepared ophthalmic products. Page 32-33

15. USP 31 NF 26, 2008, General Chapters:<789> Particulate matter in ophthalmic solutions.Inc. official 8/1/08 - 11/30/08
16. Lachman L., Libermann HA., Kanig JL., The Theory And Practice of Industrial Pharmacy, Sterile Products, 3rd ed, Varghese Publishing House, Mumbai, 1991, p. 639-77.
17. Osmolarity-Wikipedia the free encyclopedia. Available from URL: http://en.wikipedia.org/wiki/osmolarity
18. Chowhan M,.Weiner AL,Bhagat H. Encyclopedia of pharmaceutical technology, Drug Delivery: Ophthalmic Route, 3rd ed; USA: Informa Healthcare; 2007. vol 2 p.1272
19. USP 31 NF 26, 2008, General Chapters <785> Osmolality and Osmolarity. Inc. official 4/1/06-7/31/06
20. Mayer DR. and Mc Culley.; pH tolerance of rabbit corneal epithelium in tissue culture. J ocu toxicol,19;11 (1)
21. Siddiqui et al, United States Patent. Patent No. 6,120,758. Date of patent Sep 19, 2000.
22. Pflugfelder S. Ophthalmic preservatives: The past, present, and future. Continuing medical education (CME) supported by an unrestricted educational grant from Alcon Laboratories Inc
23. Microbiological Aspects of Stability Testing; Quality Pharma Services, [Online] 18 June 2008, [Cited 12 July 2008]; Available from URL: www.qualitypharmaservices.co.uk/microbiological_aspects
24. Pharmaceutical solutions III ; Ophthalmic Solutions ASHP [Online] 15 June 2008, [Cited 04 July 2008]; Available from URL : http://pharmalabs.unc.edu/ophthalmics/text.htm
25. Banker GS, Rhodes CT. Modern pharmaceutics; Parenteral products: 4th ed. NewYork:Marcel Dekker, Inc.2002
26. Filter Integrity Test Methods. [Online], Available from URL: www.millipore.com
27. Bandyopadhyay P, Banker DS, et al. Medicinal products packaging; United states patent no. WO/2004/19837; Date of patent March 11, 2004 [Online] Available from URL: www.wipo.int/pctdb/en/wo.jsp

28. Weiner AL, Airy SC, et al. Prostaglandin products United States. Patent No. 6,235,781 Date of Patent May 22, 2001 [Online] Available from URL: www.wipo.int/pctdb/en/wo.jsp
29. Lachman L, Lieberman HA, et al. Phamaceutical dosage form; Parenteral medicament : Formulation of small volume parenterals.vol.- I Marcel Dekker Inc., p. 227-8.
30. Santvliet LV, Ludwig A. Determinants of eye drop size. Survey of opthalmol;49(2), March-April 2004, p. 197-213
31. ICH Guidelines; 3AQ10a; plastic primary packaging materials.
32. Vaczwk D. Blow-Fill-Seal, Pharmaceutical and medical packaging news. [Online] cited on 07 Sept 2008. Available at URL: www.devicelink.com/pmpn/archive.html
33. LDPE polymers. [Online] Cited on 22 Oct 2008; Available at URL: www.basell.com
34. Aulton ME. Pharmaceutics:The Science of Dosage Form Design, Packs and Packaging, 2nd ed, Churchil livingstone, Edinburgh, p .558-62.
35. Friedlander MH, Amoozgar B, Senchyna B. The dilution of benzalkonium chloride in the tear film; Advances in therapy, 23 (6): 835-41.
36. Okabe K, Kimura H, et al. Effect of benzalkonium chloride on transcleral drug delivery; Inv ophthalmol and vis sci, 2005; 46: 703-08.
37. Al-Hiti MMA. Changes in Preservative Sensitivity for the USP Antimicrobial Agents Effectiveness Test Micro-Organisms; J Applied Bacteriology, 1980; 49,119-126
38. Pfister RR and Burstein AL. The effects of ophthalmic drugs, vehicles, and preservatives on corneal epithelium: A scanning electron microscope study. Inv Ophthalmol; April 1976; 15(4): 246-49.
39. Chung HS.The impact of short-term exposure of commercial eye drops preserved with benzalkonium chloride on precorneal mucin; Molecular vision 2006; 12:415-42.
40. Hogan MJ. The Preparation and Sterilization of Ophthalmic Solutions; California medicine; 71 (06):414 Available at URL: http://www.pubmedcentral.nih.gov/articlerender.fcgi?article1520227
41. Santvliet LV, Ludwig A. Determinants of Eye Drop Size, Survey of Ophthalmol 49; 2: 197-213

42. Abelson MB, Kate F. How to Handle BAK Talk; Review of ophthalmology 2002; 09:12.
43. Hourcade F. Sautov – Miranda V.et al. Compatibility of granisetron towards glass and plastics and its stability under various storage conditions; Int J pharm 154(1997) 95-102.
44. Beitz C, Bersch T. et al. Compatibility of plastics with cytotoxic drug solutions – comparison of polyethylene with other container materials. Int J Pharm. 185(1999) 113-21
45. Guidance for Industry: "Container Closure System for Packaging of Human Drugs and Biologics" U.S. Department of Food and Drug administration. Available from URL: www.fda.gov/cvm/guidance/published.htm
46. Howard Schenker et al,. Patient Preference, Efficacy, and Compliance with Timolol Maleate Ophthalmic Gel-Forming Solution Versus Timolol Maleate Ophthalmic Solution in Patients with Ocular Hypertension or Open-Angle Glaucoma; Clinical therapeutics 1999; 0149-2918/99.
47. Masayo Higashiyama et al., Improvement of the ocular bioavailability of timolol by sorbic acid. International Journal of Pharmacuetics 2003; 272 (2004) 91–98
48. K. S. Rathore, Development and *In vivo In vitro charecterization* of Timolol maleate In-situ gels. International Journal of Pharma and Bio Sciences 2011; ISSN 0975-6299.
49. Hyun Jung Jung et al., Glaucoma therapy by extended release of timolol from nanoparticle loaded silicone-hydrogel contact lenses. Journal of Controlled Release; 165 (2013) 82–89.
50. Pfister RW. Inv Opththalmol and Vis Sci. April 1976; 15,(4): 246-59, 41.
51. Burstein et al., Inv Opththalmol and Vis Sci. October 1977 16:10: 899-911.
52. Solomun L., Svetlana I.et al. The impact of primary packaging on the quality of parenteral products. J Pharm biomed ana; 48(2008) 744-48
53. USP 29 NF 24, 2008, Monograph:<Page no. 2153> Timolol Maleate. Inc. official 8/1/08 - 11/30/08
54. Bruton LL, Lazo JS, Parker KL. Goodman and Gilman's the Pharmacological basis of therapeutics: Timolol and β Blockers in the treatment of hypertension and Glaucoma, 11th ed, McGraw Hill Medical Publishing Division, Chicago 2006, p.1119.

55. Rowe RC, Sheskey PJ, Owen SC. Handbook of Pharmaceutical excipients. Pharmaceutical press, Great Britain. Page 61-63.
56. Rowe RC, Sheskey PJ, Owen SC. Handbook of Pharmaceutical excipients. Pharmaceutical press, Great Britain. Page 656-658.
57. Rowe RC, Sheskey PJ, Owen SC. Handbook of Pharmaceutical excipients. Pharmaceutical press, Great Britain . Page 659-661.
58. Rowe RC, Sheskey PJ, Owen SC. Handbook of Pharmaceutical excipients. Pharmaceutical press, Great Britain. Page 648.
59. Timolol maleate, British Pharmacopoeia 2009, Monograph 1455
60. Efficacy of antimicrobial Preservative, Appendix XVI C, British Pharmacopoeia 2008
61. Ledrer C, Harold R. Drop size of commercial glaucoma medications; Amer J ophthalmol, 1986; 101:691-4.
62. USP 29 NF 4, 2008 Monograph; Timolol maleate ophthalmic solution. Pg 2152
63. USP 31 NF 26, 2008, Monograph: Benzalkonium chloride solution. Inc. official 4/1/06 - 7/31/06
64. Ledrer C, Harold R. Drop size of commercial glaucoma medications; Amer J ophthalmol, 1986; 101:691-4.